EARLY DIAGNOSIS AND COMPLICATIONS OF ACUTE ISCHEMIC STROKE

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A thesis submitted in partial fulfillment of the requirements for the Doctoral Degree in Medicine at Faculdade de Ciências Médicas | NOVA Medical School of NOVA University Lisbon

JULY, 2023
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OF ACUTE ISCHEMIC STROKE

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July, 2023
The content of this thesis is published in:


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Abstract

Stroke is the first cause of death and disability in Portugal, and the second cause of death and third cause of disability worldwide.

In patients with stroke, treatment interventions are time-dependent, meaning that shorter time from stroke onset to treatment is associated with better outcomes. Blood-based biomarkers represent a potential alternative to neuroimaging for early and rapid stroke diagnosis. Accurate and timely differentiation between patients with acute ischemic stroke (AIS), intracerebral hemorrhage (ICH) and stroke mimics (SM) could improve prehospital pathways and potentially allow earlier treatment administration.

We conducted an exploratory prospective observational study on untargeted blood biomarkers in consecutive patients with suspected stroke, collecting blood samples at hospital admission. Quantitative analysis from mass spectrometry data was performed and biomarker-based prediction models were developed to differentiate AIS from ICH and from ICH and SM. Biomarker-based prediction models including intercellular adhesion molecule-2, plasminogen like-A, complement component 3, syntaxin binding protein-5 and immunoglobulin heavy variable 3-64 showed between 75 to 88% sensitivity at 100% specificity for identifying patients with AIS.

In addition to the timing of diagnosis and treatment, early stroke recurrences influence long-term prognosis. Stroke patients ideally are hospitalized in stroke units in order to prevent stroke recurrence and other in-hospital complications. However, the impact of being in a stroke unit at the time of an early recurrent stroke has not previously been
studied. In a retrospective analysis, we showed that in patients with an in-stroke unit stroke, the exact time of stroke onset was more frequently known than in other stroke patients, that treatment opportunities were less often missed than in other patients with in-hospital stroke, and that the endovascular treatment rate was higher than in patients with community-onset stroke. Finally, patients with in-stroke unit stroke had better functional outcomes than those with other in-hospital strokes or community-onset strokes.

Another complication in patients with AIS is early worsening of arterial patency, either due to stroke recurrence or progressive clot formation. However, no study so far has described the frequency, associated factors and outcome of patients with worsening of arterial patency. We conducted a retrospective study showing that 3% of patients with AIS experienced worsening of arterial patency within the first 24 hours (with or without preceding revascularisation treatment). History of hypertension, initial stroke severity, intracranial and extracranial stenosis, and good collaterals were identified as independent predictors, and worsening of arterial patency was associated with a six-fold higher likelihood of poorer functional outcome.

In patients undergoing endovascular treatment (EVT), reocclusion after initial successful recanalization is another possible complication. This phenomenon has only seldom been studied. In our retrospective study, we showed that 6.6% of patients experienced reocclusion after successful EVT. Preadmission statin therapy, intracranial internal carotid artery occlusion, number of passes during EVT, transient reocclusion during EVT, and atherosclerotic stroke etiology were identified as independent predictors, and its
occurrence was associated with a five times higher rate of unfavorable functional outcome.

Using a combination of translational and clinical research, this PhD thesis attempts to add new data to reduce the global burden of stroke. We explored a new blood-based biomarker strategy for stroke diagnosis, investigated the protective role of stroke units on early stroke recurrence, and studied predictors and outcome of patients with worsening of arterial patency and reocclusion after EVT. The final discussion of this thesis integrates the potential applications of the results and proposes future research questions.
Resumo

O acidente vascular cerebral (AVC) é primeira causa de morte e incapacidade em Portugal, e segunda causa de morte e a terceira causa de incapacidade em todo o mundo.

Em doentes com AVC, as intervenções terapêuticas estão dependentes do tempo, ou seja, menor tempo entre início de sintomas e tratamento associa-se a melhor prognóstico funcional. Os biomarcadores séricos representam uma alternativa à neuroimagem para o rápido diagnóstico de doentes com AVC. Uma precoce e precisa diferenciação de doentes com AVC isquémico, AVC hemorrágico e mimetizadores de AVC poderá contribuir para a melhoria das redes de referenciação pré-hospitalar e potencialmente permitir a instituição precoce de terapêutica dirigida.

Conduzimos um estudo exploratório, observacional e prospetivo, de biomarcadores séricos em doentes consecutivos com suspeita de AVC, com colheita de amostras à admissão hospitalar. Foi realizada uma análise quantitativa por espectrometria de massa e foram desenvolvidos modelos de predição baseados em biomarcadores séricos para diferenciar AVC isquémico de AVC hemorrágico, e AVC isquémico de AVC hemorrágico e mimetizadores de AVC. Um modelo de predicação baseado em biomarcadores séricos incluindo a molécula de adesão intercelular tipo 2, plasminogénio tipo-A, componente 3 do complemento, proteína de ligação à sintaxina-5 e variável 3-64 da cadeia pesada de imunoglobulina, demonstrou uma sensibilidade entre 75 a 88% na identificação de doentes com AVC isquémico, com uma especificidade de 100%.
Para além do diagnóstico e tratamento precoce, o AVC recorrente intra-hospitalar também tem um importante impacto no prognóstico a longo prazo. Idealmente, os doentes com AVC devem ser internados em unidades de AVC pelo seu impacto na prevenção de recorrência e de outras complicações hospitalares. Contudo, o impacto de estar internado numa unidade AVC aquando de AVC recorrente intra-hospitalar nunca foi investigado. Conduzimos um estudo retrospetivo que demonstrou que doentes com AVC durante internamento em unidade AVC, mais frequentemente apresentaram uma hora de início de sintomas conhecida em comparação com outros doentes com AVC. Apresentaram também menos oportunidades perdidas de tratamento de fase aguda em comparação com outros doentes com AVC intra-hospitalar, e maior frequência de tratamento endovascular em comparação com doentes com AVC na comunidade. Os doentes com AVC durante internamento em unidade AVC apresentaram melhor prognóstico funcional do que outros doentes com AVC intra-hospitalar ou AVC na comunidade.

Outra complicação em doentes com AVC isquémico é a deterioração de patência arterial. Esta pode resultar de evento recorrente ou progressão da trombose arterial. No entanto, nenhum estudo descreveu a frequência, fatores associados e prognóstico de doentes com deterioração da patência arterial. Realizámos uma análise retrospetiva na qual se identificou que 3% de doentes com AVC isquémico apresentaram deterioração da patência arterial às 24 horas (com ou sem tratamento de recanalização prévio). Hipertensão arterial prévia, gravidade de AVC à admissão, estenose intra-/extracraniana e bons colaterais foram identificados como preditores, e a presença de deterioração de patência arterial associou-se a uma probabilidade seis vezes superior de pior prognóstico funcional.
No subgrupo de doentes com AVC isquémico submetidos a tratamento endovascular (TEV), a reoclusão após recanalização é outra possível complicação. Este fenómeno foi pouco estudado. Realizámos outra análise retrospectiva em que identificamos que 6.6% dos doentes com recanalização após TEV apresentam reoclusão. Tratamento prévio com estatinas, oclusão da artéria carótida intracraniana, número de passagens durante TEV, reoclusão transitória durante TEV, e etiologia aterosclerótica foram identificados como preditores, e a sua ocorrência associada a uma probabilidade cinco vezes superior de mau prognóstico funcional.

Ao combinar investigação translacional e clínica, a presente tese de doutoramento procura adicionar informação que seja relevante na redução do impacto global do AVC. Explorámos uma nova estratégia de biomarcadores séricos para o diagnóstico de AVC, investigámos o papel protetor das unidades AVC no AVC recorrente, e estudámos os preditores e prognóstico de doentes com deterioração da patência arterial e reoclusão após TEV. A discussão final desta tese integra as potenciais aplicações dos seus resultados, e propõe novas perguntas de investigação.
List of abbreviations

AIS – Acute Ischemic Stroke
ASTRAL – Acute STroke Registry and Analysis of Lausanne
ASPECTS – Alberta Stroke Program Early CT Score
C3 – Complement component 3
CI – Confidence Interval
COS – Community-Onset Stroke
CT – Computed Tomography
CTA – Computed Tomography Angiography
CTP – Computed Tomography Perfusion
END – Early Neurological Deterioration
EVT – Endovascular Treatment
GFAP – Glial Fibrillary Acid Protein
ICA – Internal Carotid Artery
ICAM-2 – Intercellular Adhesion Molecule 2
ICH – Intracerebral Hemorrhage
IGHV3-64 – Immunoglobulin Heavy Variable 3-64
IHS – In-Hospital Stroke
IQR – Interquartile Range
IS – Ischemic Stroke
ISUS – In-Stroke Unit Stroke
IVT – Intravenous Thrombolysis
LVO – Large Vessel Occlusion
MCA – Middle Cerebral Artery
MRA – Magnetic Resonance Angiography
MRI – Magnetic Resonance Imaging
mRS – Modified Rankin Scale
MSU – Mobile Stroke Unit
MT – Mechanical Thrombectomy
NIHSS – National Institutes of Health Stroke Scale
NPV – Negative Predictive Value
NT-proBNP – N-Terminal pro-B-Type Natriuretic Peptide
OR – Odds Ratio
PLGLA – Plasminogen like A
POCT – Point-Of-Care Tests
RBP4 – Retinol-Binding Protein 4
SM – Stroke Mimics
STROBE – Strengthening the Reporting of Observational Studies in Epidemiology
STXBP5 – Syntaxin Binding Protein 5
TIA – Transient Ischemic Attack
TICI – Thrombolysis in Cerebral Infarction
TOAST – Trial of ORG 10172 in Acute Stroke Treatment
Chapter 1 – Introduction

1.1 – Stroke: Definition, subtypes, and disease burden

Stroke is defined as an acute neurological deficit attributed to a focal injury of the central nervous system (brain, retina, or spinal cord) of presumed vascular cause.\(^1\) Hemiparesis, facial palsy, hemisensory deficits, ataxia, dysarthria, aphasia and visual field deficits are the most frequent stroke manifestations.

The most common stroke subtype is ischemic stroke, which accounts for between 62–87% of all stroke cases.\(^2\)–\(^5\) In patients with acute ischemic stroke (AIS), there is a reduction in blood flow to specific areas of the brain generally caused by an arterial occlusion or stenosis. This will result in rapid, progressive, and irreversible neuronal cell death. The acutely ischemic brain tissue can be divided in two regions: (i) the ischemic core, center of the ischemic territory with a severe reduction of blood flow that rapidly results is cell necrosis and tissue loss; and (ii) the ischemic penumbra, a surrounding rim of hypoperfused tissue that may remain viable for several hours or even days.

The reduction in blood flow to the brain means that it receives fewer substrates, particularly oxygen and glucose, which impairs its ability to produce energy through oxidative phosphorylation. This leads to depletion of energy, loss of membrane potential, and depolarization of neurons and glial cells. Voltage-dependent calcium channels become activated and excitatory amino acids are released into the extracellular space. Calcium overload and glutamate excitotoxicity are between the main pathophysiological mechanism implicated in cell death in the ischemic penumbra. The complex interaction
of additional pathophysiological processes such as oxidative stress, inflammation and apoptosis will further contribute to progressive infarct growth.\textsuperscript{6,7}

Without treatment, it has been estimated that in each minute, a mean of 1.9 million neurons, 14 billion synapses, and 12 km of myelinated fibers are destroyed.\textsuperscript{8} An interindividual variability of infarct growth rate was latter shown, meaning that in patients with AIS the loss of neurons can range from less than 35000 to above 27 million per minute.\textsuperscript{9} The more widespread the tissue loss, the higher are the odds of stroke-related disability and mortality. The primary aim of current acute stroke interventions is to prevent the progression of the ischemic penumbra to established infarct.

Figure 1. Imaging of patients with ischemic stroke

Legend.
A – Non-contrast computed tomography without visible infarct at hospital admission. B – Computed tomography angiography showing an intracranial vessel occlusion on the right hemisphere (proximal right middle cerebral artery occlusion). C – Digital subtraction angiography showing a proximal right middle cerebral artery occlusion (coronal view). D – Non-contrast computed tomography showing a severe established infarct in the right middle cerebral artery vascular territory (untreated intracranial occlusion). Source: Department of Neurology, Centro Hospitalar Lisboa Ocidental.
The remaining 13–38% of strokes are hemorrhagic, resulting from the rupture of cerebral arteries, and can be intracerebral or subarachnoid. The most common type of hemorrhagic stroke is intracerebral hemorrhage (ICH). Most commonly traumatic, both subdural and epidural hematomas are not classified as strokes.

Figure 2. Imaging of patients with intracerebral hemorrhage.

Legend.
A – Non-contrast computed tomography showing a lobar intracerebral hemorrhage. B – Non-contrast computed tomography showing a deep intracerebral hemorrhage with a mild intraventricular extension. C – Non-contrast computed tomography showing a severe deep intracerebral hemorrhage with a significant intraventricular extension and relevant midline shift. Source: Department of Neurology, Centro Hospitalar Lisboa Ocidental.

Finally, an infrequent stroke subtype is the cerebral venous thrombosis. It is caused by an occlusion of cerebral veins or venous sinuses leading to ischemic and/or hemorrhagic venous infarction. This stroke subtype shares very few vascular risk factors with AIS or ICH, and affects predominantly young woman under estrogen therapy, patients with cancer or with other hypercoagulable state, or patients with head and neck infections.
Stroke is a common disease and affects one in four people over their lifetime.³ Millions of people have a stroke every year. In 2019, there were an estimated 12.2 million incident cases of stroke and 101 million prevalent cases of stroke worldwide.⁴ Stroke is the second cause of death worldwide (11.6% of total deaths), corresponding to 6.55 million deaths in 2019.⁴ Stroke case-fatality ranges from 10–40%.¹⁰ Stroke is also the third leading cause of disability in adults (5.7% of total disability-adjusted life-years)⁴ with approximately 60% of stroke survivors having neurological symptoms, and between 5% to 50% requiring at least some assistance with basic activities of daily living.¹¹,¹² Worldwide, particularly in low-income countries and younger patients, stroke incidence, prevalence, and related deaths and disability, have increased in the last two decades.⁴,¹³ The increasing aging and growth of the population are expected to translate into a higher stroke burden, especially in the elderly.¹⁴

In Portugal, numbers from 1998 to 2000 showed a high stroke incidence in rural and urban Northern regions in comparison with other western European territories.¹⁵ Data from 2009 to 2011 indicated a reduction in stroke incidence, including disabling and fatal strokes, probably due to the implementation of stroke awareness campaigns, close surveillance of vascular risk factors and improvement in stroke care. In 2019, Portugal had similar stroke incidence compared to other Western European countries, but still a higher stroke-related disability-adjusted life-years.⁴
1.2 – Stroke care pathway

In order to reduce stroke burden, health systems and health care professionals play an essential role in different steps of the stroke care pathway, from prevention to post-stroke care (Figure 3).

Figure 3. Stroke care pathway

A first step in reducing stroke burden is lowering its incidence. For this purpose, the implementation of primordial prevention through health policies that improve lifestyle and provide better education of the general population are essential to reduce stroke burden and of other cardiovascular diseases. In addition, major coordinated efforts are necessary, particularly by primary care health services, to identify modifiable risk factors such as hypertension, diabetes, hyperlipidemia, smoking, obesity and physical inactivity, and to optimize primary prevention strategies.
When a stroke occurs, time-to-treatment delays are of major prognostic significance, and it is crucial to ensure rapid stroke diagnosis and hospital admission. In this sense, educational campaigns are of utmost importance, so patients and bystanders know how to recognize stroke symptoms and activate the emergency medical system. Also, the implementation of educational stroke programs for health care professionals and the optimization of prehospital referral systems are essential. Finally, the organization of stroke networks (group of hospitals with different level of stroke treatment capabilities and expertise working together in a specific region) and improvements of institutional stroke protocols have shown to be determinant in improving stroke care.\textsuperscript{16}

Every stroke case should be identified at the prehospital setting, first by the patient itself or bystanders, and subsequently by healthcare professionals. The latter should trigger a prehospital notification to the receiving hospital, known as “stroke code”, informing them about the incoming patient. This procedure allows the appropriate hospital to mobilize resources before patient arrival. In an alternative scenario, a stroke code can be activated at the time of hospital admission during triage.

Then, a stroke-trained physician should swiftly evaluate the patient, while nurses, laboratory/radiology technicians and other health professionals are responsible for monitoring vital signs, blood sample collection and preparing the patient for immediate brain imaging [Computed tomography (CT) or Magnetic Resonance Imaging (MRI)]. If the stroke physician confirms the clinical diagnosis and there is no evidence of intracranial hemorrhage, the diagnosis of AIS is generally established, even in the absence of an ischemic lesion on brain imaging. If there are no contraindications, immediate
revascularization treatment can be delivered in patients with disabling deficits and limited bleeding risk.

For AIS, two revascularization therapies are approved. The first, intravenous thrombolysis (IVT), converts plasminogen to plasmin, which can breakdown fibrin and dissolve the thrombus causing the stroke. This treatment is available in every stroke-ready hospital and uses recombinant tissue plasminogen activator (or more rarely tenecteplase). The second, endovascular treatment (EVT), implies passing an intraarterial catheter from a peripheral puncture site into an intracranial artery and removing an occluding thrombus by entrapping it on a stent or by suction with an aspiration device. Particularly in early arriving patients, IVT and EVT are usually combined. For selecting patients, non-invasive vessel imaging [Computed tomography angiography (CTA) or magnetic resonance angiography (MRA)] is usually performed to confirm the presence of a treatable vessel occlusion. If this treatment is not available in the hospital receiving the patient initially, immediate transfer to an EVT-capable hospital must be sought.
Figure 4. Patient with ischemic stroke due to large vessel occlusion and successful endovascular treatment

Legend.

IVT and EVT have been shown to improve patients’ outcomes in a time-dependent manner, with shorter time-to-treatment leading to better outcomes.\textsuperscript{17,18} With IVT, each minute of onset-to-treatment time saved grants on average 1.8 days of extra healthy life, while in EVT, the benefit increases to 4.2 days.\textsuperscript{19,20} Indeed, rapid successful vessel recanalization is the cornerstone of AIS treatment and is a strong determinant of better functional outcomes.\textsuperscript{21,22}

In the case of ICH, although there is no specific approved treatment, acute blood pressure lowering likely improves functional outcome, with earlier interventions being associated with reduced hematoma growth.\textsuperscript{23,24} New treatments for ICH like intraventricular
fibrinolysis also seem to be time-dependent.\textsuperscript{25} ICH guidelines recommend that stroke recognition tools should be used like in AIS to reduce time-to-diagnosis and treatment.\textsuperscript{26}

Figure 5. Schematic representation of the acute stroke care pathway

Regardless of age, sex, stroke subtype, severity or treatment received, it has been shown that every stroke patient will benefit from an admission in a specialized ward.\textsuperscript{27,28} Stroke units are organized in-hospital facilities dedicated to care for patients with stroke. Stroke patients admitted to stroke units have lower rates of mortality and disability, and higher odds of living at home after stroke.\textsuperscript{27,28} Stroke units include continuous monitoring of vital parameters and are staffed by a core interdisciplinary team with stroke expertise including physicians, nurses, occupational therapists, physiotherapists, speech-language pathologists, social workers, and clinical nutritionists. The existence of stroke unit is the foundation on which stroke interventions are being organized and delivered.
The benefits of stroke unit admission in improving patients’ outcome stem from three major achievements.\textsuperscript{27,28} (Figure 6).

Figure 6. Benefits of stroke unit care

![Diagram of benefits of stroke unit care]

In patients with AIS receiving revascularization treatment, a repeat brain CT or MRI is usually performed at 12-24 hours, or earlier if the patient worsens. This allows to assess infarct size, to exclude relevant intracranial hemorrhage and initiate antithrombotic therapy in most patients.

During the further hospital stay a thorough work-up is usually performed to define stroke etiology and to better optimize secondary stroke prevention strategies. Depending on each patient’s needs, additional rehabilitation may be offered after hospital discharge, either in an inpatient rehabilitation facility or at home. Rehabilitation can include motor, speech, swallowing, occupational and cognitive therapy. After hospital discharge, follow-up
appointments with a stroke specialist may translate into better management of post-stroke sequelae and complications, and enhanced secondary stroke prevention strategies.

Even with the major developments in every step of the stroke care pathway implemented in the last decades, results from the recent pivotal clinical trials still show high proportions of disability and mortality after AIS.29-31 Because time is such a major determinant of prognosis in patients with AIS, even in those not receiving reperfusion therapies,32,33 new strategies for early stroke diagnosis, referral and treatment are warranted. In addition, exploring seldom studied stroke complications and their impact on prognosis, may help anticipate, prevent and treat them, which ultimately can translate to better long-term outcomes.
1.3 – Early diagnosis: The potential role of blood-biomarkers

Regarding time-to-diagnosis and to treatment, different strategies have been explored over the past years. One proposal is the implementation of mobile stroke units (MSU) as a new tool to promptly identify patients with AIS and deliver early IVT in the prehospital setting. A MSU is an ambulance that incorporates a CT scanner, point-of-care tests (POCT), and telemedicine communication with a stroke-capable hospital. To operate, it needs a specialized health professional team including a physician, nurse, CT radiology technician and paramedic or other ambulance staff. A neuroradiologist can validate the CT findings by telemedicine. Compared to usual care, MSU have shown to reduce treatment delays and to improve patients’ outcome. Nevertheless, MSU strategies are not easy to implement due to licensing and legislation procedures, that together with financial and technical limitations, will affect widespread applicability.

As an alternative, blood-based biomarkers represent an opportunity to develop a simple diagnostic test, similar to an electrocardiogram in acute coronary syndromes, that could be reproducible and generalized. If blood-based biomarkers achieve satisfactory sensitivity and specificity in the discrimination between AIS and ICH, its implementation could guide prehospital stroke management, including very early targeted stroke treatments and patient transfers optimization, without the need of prehospital imaging. In addition, blood-based biomarkers could also contribute to the diagnosis of conditions that can simulate a stroke, namely stroke mimics (SM). Previous studies found that 20 to 40% of stroke code activations at the prehospital level are SM. In patients receiving IVT, about 5% are latter diagnosed with a SM. Although relatively safe, a potential harmful effect of IVT in stroke mimics exists. In this sense, new methods to accurately
discriminate between AIS and SM would also be important, not just in terms of safety but also in resources utilization, such as emergency transfers, admission to stroke units and stroke treatments.

Many different biomarkers or biomarker panels have been studied. The glial fibrillary acid protein (GFAP) is an intermediate filament found almost exclusively in astrocytic cells within the central nervous system. This protein is typically not detectable in the plasma of healthy individuals as it is not actively secreted from cells.\textsuperscript{39} In an acute ICH, immediate astrocyte necrosis and destruction of the blood-brain barrier will translate into instantaneous release of GFAP into the blood.\textsuperscript{40} This contrast with patients with AIS, in which GFAP levels only peak after 2-3 days.\textsuperscript{41} As such, GFAP has been identified as one of the most promising biomarkers for the differentiating AIS from ICH.\textsuperscript{42–44}

Retinol-binding protein 4 (RBP4) delivers retinol (vitamin A) from the liver to peripheral tissues. In the brain, retinoic acid plays an important role in neurogenesis and neuroplasticity, and acts as an anti-apoptotic agent since it has been found to reduce cerebral ischemic injury by regulating inflammation.\textsuperscript{43} N-Terminal Pro-B-Type Natriuretic Peptide (NT-proBNP) is released into the circulation from myocytes in response to a stretch stimulus such as increased wall tension and volume/pressure overload. NT-proBNP levels are associated with the presence of AF\textsuperscript{45} and cardioembolic stroke\textsuperscript{46,47} Both RBP4 and NT-proBNP levels were found to be increased in patients with AIS in comparison with patients with ICH and SM.\textsuperscript{43,44,48}

Additional biomarkers such as endostatin, caspase-3, D-dimer, S100 calcium-binding protein B, soluble receptor for advanced glycation end products, secretagogin and matrix metalloproteinase 9 also have demonstrated some level of accuracy in the differential diagnosis of stroke in the acute setting.\textsuperscript{48–50}
Although previous studies showed promising results, to date, no biomarker or biomarker panel with a clinically significant predictive accuracy for AIS or ICH diagnosis has been validated. Of note, the studied biomarkers were identified based on former experimental and clinical data, and assessed by immunoassay techniques.

Disclosing new potential biomarkers by using new research approaches, such as mass spectrometry for proteomics, may improve the reliability of the molecular diagnosis of stroke and contribute to the applicability of blood-based biomarkers in stroke care. Mass spectrometry for proteomics is a high-throughput method to screen blood samples to identify candidate proteins. To date, there is only one pilot study aiming at differentiating ICH and IS based on label-free proteomics of plasma from three patients with IS and four patients with ICH, and not in the acute setting. Herein, additional studies, using proteomics in a larger number of patients and in the acute setting, can present as an opportunity to disclose additional potential biomarkers for the early diagnosis of patients with suspected stroke.
1.4 – Stroke unit care and in-hospital stroke

As mentioned above, stroke unit care is fundamental to reduce long-term disability and mortality. This is partially due to its impact on the prevention, early detection and treatment of in-hospital complications which are frequent after AIS and associated with worse outcomes. These complications can be grouped as follows: 1) Related to patients’ comorbidities, such as atrial fibrillation and other arrhythmias, heart failure and acute myocardial infarction; 2) Medical complications, such as pneumonia, urinary tract infection, venous thromboembolism, pressure ulcers, falls, delirium, pain and constipation; 3) Stroke-related complications, such as hemorrhagic transformation, cerebral edema, and seizures; 4) Treatment complications, such as orolingual angioedema, intracranial and extracranial bleeding, vessel perforation and puncture site hematoma/pseudoaneurysm; 5) Early stroke recurrences; and 6) Early neurological deterioration without evident cause.

Complications related to patients’ comorbidities, medical complications, stroke-related and treatment complications are well known, have established predictors and defined approaches. On the contrary, in-hospital stroke recurrence and mechanisms involved in early neurological deterioration need further study.

Although infrequent, in-hospital stroke recurrence is known to be associated with worse outcomes. Despite the demonstrated reduction of early stroke recurrence risk by admitting patients to stroke units, the impact of being in a stroke unit on the diagnosis, recanalization treatment rates, treatment metrics, and outcome of recurrent stroke has not been previously studied. This is also true for other patients with in-hospital stroke (IHS) [e.g.: after a transient ischemic attack (TIA) or ICH]. In many hospitals or health-systems
worldwide, patients with stroke are monitored in a stroke unit for the first few days, and then transferred to a Neurology, Internal Medicine or Rehabilitation wards, without the same level of care.

It is known that patients with in-hospital ischemic stroke (IHS) (e.g.: hospitalized in internal medicine or surgical wards) have longer time-to-imaging and treatment, lower intravenous thrombolysis rates and worse outcomes compared to community-onset stroke (COS).62-66

Knowing if being hospitalized in a stroke unit improves the diagnosis, treatment and outcome of an IHS could impact the allocation of hospital resources by making more beds available for stroke patients and avoid their early transfer to other wards.
1.5 – Worsening of arterial patency

Some patients experience neurological clinical worsening, defined previously as “stroke progression”\(^{67,68}\). One of its main mechanisms are infarct growth (progression of penumbra to core) and lesion growth beyond initial penumbra. These processes seem to be related to the presence of proximal vessel occlusion and to the absence of vessel recanalization at 24h\(^{69,70}\). Still, a significant proportion of patients experience early neurological deterioration without a clear cause.

Given that acute vessel occlusion is a dynamic process, changes in vessel patency could represent another explanation for early neurological deterioration. After initial imaging, recanalization can occur spontaneously\(^{21,71}\) or as a result of recanalization treatment (IVT or EVT)\(^{21,22,29,71}\). On the contrary, worsening of arterial patency can arise from thrombus extension or new occlusions, either spontaneously or after treatment\(^{71,72}\). Nevertheless, no study described the frequency, characteristics, associated factors and outcome of patients with worsening of arterial patency independently of whether acute revascularization treatment was performed. Recognizing these risk factors may allow more targeted interventional or drug-based preventive strategies. Furthermore, the magnitude of the clinical impact of this type of arterial deterioration on short and long-term outcomes is insufficiently known. Besides being implicated in early neurological deterioration, worsening of arterial patency can also explain early stroke recurrences.
1.6 – Reocclusion after successful endovascular treatment

In the particular subset of patients achieving partial or complete recanalization after acute recanalization treatment, arterial reocclusion can occur, with rates ranging from 2% to 41%.\textsuperscript{73–79} This complication may partially explain why about half of patients with successful recanalization after EVT do not achieve favorable functional outcome.\textsuperscript{29} Indeed, arterial reocclusion was associated with unfavorable outcomes.\textsuperscript{74,77–79} Of the five randomized trials that established mechanical thrombectomy (MT) as the standard treatment for large vessel occlusion in AIS,\textsuperscript{29} four documented recanalization rates at 24h, but only one reported the rate of reocclusion after successful recanalization in a secondary analysis.\textsuperscript{77} Predictors and clinical impact of reocclusion after successful MT were seldom studied, and with conflicting results.\textsuperscript{78,79} Suggested predictors included higher platelets levels, prestroke functional dependence, stroke of undetermined cause or other specified pathogenesis according to the TOAST (Trial of ORG 10172 in Acute Stroke Treatment) classification, and pretreatment collateral flow.\textsuperscript{78,79} Knowing potential predictors of this complication and its impact on outcome could allow physicians to take specific monitoring and treatment decisions in high-risk patients. A better understating of arterial reocclusion after successful MT would also shed light on an additional potential mechanism of early stroke recurrence and early neurological deterioration.
1.7 – Aims and research setting

The goals of this PhD thesis are to explore new possibilities on how to achieve an early diagnosis of stroke patients, and to better understand, prevent and treat some of the early stroke complications. Combined together, better understanding of these topics may have a positive impact on patients’ outcome. Given these challenges, the specific aims of this PhD thesis are:

AIM #1: To identify new potential blood-based biomarkers in patients with suspected stroke, by using mass spectrometry analysis.

AIM #2.1: To investigate the recognition, treatment and outcome of patients with in-stroke unit stroke.

AIM #2.2: To study the frequency, associated factors and outcome of worsening of arterial patency.

AIM #2.3: To assess the frequency, associated factors and outcome of reocclusion after successful mechanical thrombectomy.
The described research projects were developed in two different settings:

For AIM#1, funding was obtained from a grant from Fundação para a Ciência e a Tecnologia (FCT) (PTDC/MEC-NEU/28750/2017) in order to develop an ambitious project conducted at a tertiary university hospital in Portugal: Centro Hospitalar Lisboa Ocidental.

Centro Hospitalar Lisboa Ocidental stroke unit functions as a primary stroke center with a primary catchment area of 240,000 people. It is integrated in a network of four stroke centers located in the Lisbon metropolitan area, organized to deliver continuous access to ischemic stroke EVT in Lisboa e Vale do Tejo, Alentejo and Algarve regions (approximately 4 million people).

In order to implement this project, collaborations between different hospital departments, namely Department of Neurology, Emergency Department, Department of Internal Medicine and Department of Clinical Pathology, were needed. Also, a straight collaboration with different research groups from NOVA was established, namely with the Applied Molecular Biosciences Unit from NOVA School of Science and Technology and with the Computational and Experimental Biology Research Group from NOVA Medical School.

For AIM#2.1 to 2.3, I had the opportunity to develop the planned research projects at the Lausanne University Hospital stroke center, known for its clinical and research excellence, and for having one of the most comprehensive stroke registries. The Acute STroke Registry and Analysis of Lausanne (ASTRAL) is a prospective single-centre registry with all consecutive AIS patients admitted to the Lausanne University Hospital within 24 hours of last-seen-well time. Lausanne University Hospital stroke unit is a
comprehensive stroke center that delivers state-of-the-art stroke care, continuously updated according to the latest scientific data and national and international guidelines. This stroke unit serves a primary population of approximately 270,000, and is a reference center for 15 referral hospitals in the canton of Vaud and for parts of 3 neighboring cantons, bringing the total population served to approximately 1 million. In ASTRAL, a comprehensive dataset of epidemiological, clinical, metabolic, multimodal imaging, therapeutic, etiologic, and outcome data is registered. The quality and completeness of ASTRAL, together with the implemented standard procedure of performing 24-hour vessel imaging, were fundamental for the feasibility of the developed projects. Because patients’ demographics, burden of vascular risk factors, and access and delivery of acute stroke care (stroke unit admission, IVT and EVT) are similar between Portugal and Switzerland, the results obtained in these analyses can be translated to the Portuguese population.4,80
Chapter 2 – Proteomics to Identify New Blood Biomarkers for Diagnosing Patients with Acute Stroke

This chapter is based on the following manuscript:
2.1 – Abstract

Background and purpose

Blood biomarkers are a potential tool for early stroke diagnosis. We aimed to perform a pilot and exploratory study on untargeted blood biomarkers in patients with suspected stroke by using mass spectrometry analysis.

Methods

Prospective observational study of consecutive patients with suspected stroke admitted within 6-hours last-seen-well. Blood samples were collected at admission. Patients were divided into three groups: Ischemic stroke (IS), intracerebral hemorrhage (ICH) and stroke mimics (SM). Quantitative analysis from mass spectrometry data was performed using a supervised approach. Biomarker-based prediction models were developed to differentiate IS from ICH and ICH+SM. Models were built aiming to minimize misidentification of patients with ICH as having IS.

Results

We included 90 patients, one-third within each subgroup. Median age was 71 (IQR 57–81) years and 49 (54.4%) were female. In quantitative analysis, complement component 3 (C3), intercellular adhesion molecule-2 (ICAM-2), plasminogen like-A (PLGLA), syntaxin binding protein-5 (STXBP5) and immunoglobulin heavy variable 3-64 (IGHV3-64) were the five most significantly dysregulated proteins for both comparisons. Biomarker-based models showed 88% sensitivity and 89% negative predictive value (NPV) for differentiating IS from ICH, and 75% sensitivity and 95% NPV for differentiating IS from ICH+SM. ICAM-2, STXBP5, PLGLA, C3 and IGHV3-64
displayed the highest importance score in our models, being the most informative for identifying stroke patients.

Conclusion

In this proof-of-concept and exploratory study, our biomarker-based prediction models, including ICAM-2, STXBP5, PLGLA, C3, and IGHV3-64, showed 75 to 88% sensitivity for identifying patients with IS, while aiming to minimize misclassification of ICH. Although our methodology provided an internal validation, these results still need validation in other cohorts and with different measurement techniques.
2.2 – Introduction

Stroke is a leading cause of disability and mortality worldwide. For ischemic stroke (IS) treatment, two recanalization therapies, namely intravenous thrombolysis with recombinant tissue plasminogen activator (tPA) and mechanical thrombectomy (MT), have been demonstrated to improve patients’ outcomes. Treatment effect is time-dependent, with a shorter time from stroke onset to recanalization associated with better outcomes. In the case of intracerebral hemorrhage (ICH), early blood pressure lowering may reduce hematoma growth and improve clinical outcomes. For early tPA treatment, mobile stroke units allow the administration of tPA at the prehospital level, reducing time-to-treatment and improving patients’ outcomes. However, the need for an ambulance equipped with a computed tomography scanner to accurately rule out ICH, together with a specialized health professional team, raises relevant financial and technical limitations that affect its widespread applicability.

Blood-based biomarkers represent a potential alternative for early and rapid stroke diagnosis. Accurate and timely differentiation of IS, ICH, and stroke mimics (SM) could improve prehospital pathways and referral systems, and potentially allow early treatment administration.

Different biomarkers or biomarker panels have shown promising results. Glial fibrillary acid protein (GFAP), retinol-binding protein 4 (RBP4), N-Terminal Pro-B-Type Natriuretic Peptide (NT-proBNP), endostatin, caspase-3 and D-dimer have demonstrated some level of accuracy in the differential diagnosis of stroke in the acute setting. These biomarkers were identified based on previous experimental and clinical data, but to date, no biomarker or biomarker panel with a clinically significant predictive accuracy for IS or ICH diagnosis has been validated.
Disclosing new potential biomarkers by using different new research approaches, such as mass spectrometry for proteomics, may improve the reliability of the molecular diagnosis of stroke, contributing to the applicability of blood-based biomarkers in stroke care.

In this sense, there is only one pilot study aimed at differentiating ICH from IS based on label-free proteomics of plasma from three patients with IS and four patients with ICH, and not in the acute setting.\textsuperscript{52}

Herein, we performed a pilot and exploratory study on untargeted blood biomarkers in patients with suspected stroke. Our aim was to find new potential biomarkers that can help improve the sensitivity and specificity of blood-based biomarkers in the diagnosis of suspected acute stroke patients.
2.3 – Methods

Study Design, Patient Selection and Study Variables

This was a prospective, observational study of consecutive patients with a suspected stroke admitted to the Centro Hospitalar Lisboa Ocidental Emergency Department as stroke code patients, from November 2018 to December 2019. Patients were screened if they met the following criteria: 1) Age ≥ 18 years; 2) Admitted within 6 hours of symptom onset; 3) No history of a cerebrovascular or major vascular event within the 3 months (myocardial infarction, systemic embolism, venous thrombosis). Patients without stroke code activation were not included, nor were patients transferred from other hospitals. After admission, a definitive clinical and imaging-supported diagnosis was established, and patients were divided into three groups: (1) IS; (2) ICH; and (3) SM. IS diagnosis was established according to the World Health Organization definition, together with neuroimaging confirmation of a new ischemic brain lesion in a clinically relevant area. ICH was defined as an acute neurological syndrome caused by a confirmed brain parenchymal hemorrhage. SM diagnosis was established by trained neurologists and supported by ancillary tests deemed necessary in each case. Patients without a definitive diagnosis at hospital discharge, without good quality samples for mass spectrometry analysis or without signed informed consent were further excluded.

For the purpose of our exploratory study, we established a target of enrolling 30 patients within each subgroup. Therefore, recruitment for each subgroup was stopped when 30 patients met the inclusion criteria, had established definitive diagnoses, and provided good quality blood samples. For patients’ inclusion flow chart see Figure 7.
Figure 7. Patients’ inclusion flow chart

The following variables were collected: age, sex, vascular risk factors, admission blood pressure, and stroke severity at admission (accessed by the National Institutes of Health Stroke Scale [NIHSS]). Additionally, clinical variables at hospital admission, previously studied to differentiate IS from ICH (headache, vomiting, decreased level of consciousness, epileptic seizure)\(^{82}\) and IS from SM (isolated sensory deficit, facial palsy, history of seizures)\(^{83,84}\) were collected. For patients with IS, presence of intracranial vessel occlusion, recanalization treatment and stroke etiology were recorded. For patients with ICH probable etiology was assessed. For SM, final diagnosis was defined. In stroke patients, outcome was evaluated at 3 months by the modified Rankin Scale (mRS) and defined as favorable if mRS \(\leq 2\). Finally, time from symptom onset to blood collection was registered for all patients.
Standard Protocol Approvals, Registrations, and Patient Consents

The study protocol was approved by the Centro Hospital Lisboa Ocidental (Registry number: 20170700050) and the NOVA Medical School Ethics Committee (Registry number: 85/2021/CEFCM). All patients or relatives signed a written informed consent, before or after blood sample collection. As stated, if informed consent was not obtained, patients were excluded from this study.

Statistical analysis of clinical variables

We summarized continuous variables as median values with an interquartile range and categorical variables as absolute numbers and percentages. We compared baseline characteristics between the three groups using the Pearson's Chi-squared test and Fisher exact test for categorical variables and Kruskal–Wallis test for continuous variables, as appropriate. The Shapiro-Wilk test was used to assess the normality of continuous variables. All tests were two-sided and p-values \( \leq 0.05 \) were considered significant. Bonferroni correction was used for adjusting all p-values in multiple comparisons. We performed statistical analysis with R statistical software, version 4.0.3.

Blood Collection and processing

Blood samples were collected at hospital admission before any therapeutical intervention or brain imaging, into EDTA tubes. Blood samples were centrifuged at 1500 g for 15 minutes at 4°C, and plasma samples were aliquoted in the presence of protease inhibitors and stored at −80°C until further processing. The 14 most abundant proteins in plasma were eliminated using a commercially available kit (Agilent Human 14 Multiple Affinity Removal System Spin Cartridges for the High-Abundant Proteins from Human Proteomic Samples) following the manufacture instructions.
Plasma samples were further treated for protein digestion. Protein solutions containing sodium dodecyl sulfate (SDS) and dithiothreitol (DTT) were loaded onto filtering columns and washed exhaustively with 8M urea in HEPES buffer. Proteins were reduced with DTT and alkylated with (iodoacetamide) IAA. Protein digestion was performed by overnight digestion with trypsin sequencing grade (Promega).

*Mass Spectrometry Analysis*

Each sample was analyzed in duplicate except for one, which was analyzed as triplicate. Peptide samples were analyzed by nano-LC-MS/MS (Dionex RSLCnano 3000) coupled to an Exploris 480 Orbitrap mass spectrometer (Thermo Scientific, Hemel Hempstead, UK). In brief, the samples (5 µL) were loaded onto a custom-made fused capillary precolumn (2 cm length, 360 µm OD, 75 µm ID) packed with ReproSil Pur C18 5.0 µm resin (Dr. Maish, Ammerbuch-Entringen, Germany) with a flow of 5 µL per minute for 6 minutes. Trapped peptides were separated on a custom-made fused capillary column (25 cm length, 360 µm outer diameter, 75 µm inner diameter) packed with ReproSil Pur C18 1.9-µm resin (Dr. Maish, Ammerbuch-Entringen, Germany) with a flow of 250 nL per minute using a linear gradient from 89% A (0.1% formic acid) to 32% B (0.1% formic acid in 80% acetonitrile) over 30 minutes. Mass spectra were acquired in positive ion mode applying automatic data-dependent switch between one Orbitrap survey MS scan in the mass range of 350–1200 m/z followed by higher-energy collision dissociation (HCD) fragmentation and Orbitrap detection of fragment ions with a cycle time of 2 s between each master scan. MS and MS/MS maximum injection time were set to “Auto”, and HCD fragmentation in the ion routing multipole was performed at normalized collision energy of 30%, and ion selection threshold was set to 10,000 counts. For 30 s, selected sequenced ions were dynamically excluded.
Protein Identification

The obtained data from the 181 LC-MS runs were searched using Virtual Expert Mass Spectrometrist (VEMS). A standard human proteome database from UniProt (3AUP000005640) including permutated protein sequences, where Arg and Lys were not permutated, was used as sequence database. For trypsin cleavages, a maximum of four missed cleavages were permitted. Carbamidomethyl cysteine was included as fixed modification. Methionine oxidation and N-terminal protein acetylation were included as variable modifications. 10 ppm mass accuracy was specified for precursor ions and 0.01 m/z for fragment ions. The false discovery rate (FDR) for protein identification was set at 1% for peptide and protein identifications. No restriction was applied to the minimal peptide length for VEMS search. Identified proteins were divided into evidence groups as previously defined. To avoid sample-related biases in the biomarker analysis, we used reference proteomes of erythrocytes, platelets, and plasma to check for potential biases.

Quantitative Analysis

Quantitative data from VEMS were analyzed in the R statistical programming language. Intensity-based absolute quantification (iBAQ) and protein spectral counts from the VEMS were preprocessed by three approaches: 1) removing common MS contaminants followed by log2(x + 1) transformation, 2) removing common MS contaminants followed by log2(x + 1) transformation and quantile normalization, 3) removing common MS contaminants followed by log2(x + 1) transformation, quantile normalization and abundance filtering to optimize overall Gaussian distribution of the quantitative values. Quantitative values from technical replicas were averaged. After confirming similar results from the three data processing approaches described above, quantitative data from
iBAQ data was used for statistical analysis using the R package limma after removing common MS contaminants, followed by log2(x + 1) transformation and quantile normalization. The distribution of the quantitative values across individual samples is depicted as a boxplot (Supplemental Material – Figure I). Contrasts between “IS and ICH”, “IS and SM” and “ICH and SM” were calculated. Correction for multiple testing was applied using the method of Benjamini & Hochberg.90

Partial Least Squares Comparator Models

The R package “caret” was used to generate six supervised partial least-squares (PLS) comparator models to test potential classification performance based on different input data.91 Three models were built based on separating patients with IS from patients with ICH and three models were built for separating patients with IS from those with ICH and SM. For each comparison, models were based on only clinical data, 10 most significant proteins based on training data, and all proteins. For both clinical models (differentiating IS from ICH and IS from ICH and SM) the following variable were used: vomiting, headache, epileptic seizure, decreased level of consciousness, DBP ≥ 110mmHg; SBP; history of stroke/TIA. In the model for differentiating IS from ICH and SM, age <50, atrial fibrillation, history of epileptic seizures, isolated sensory deficit and facial palsy were also included.

The data was randomly split for all models, using 75% of the cases to train the models and 25% of the data as an unseen validation set. The data was preprocessed by removing zero variance cases, then scaled, centered and log2 transformed. Statistical analysis based on the R package limma was used as a feature selection method based only on the training data.92 Models were built aiming to minimize misidentification of patients with ICH as having IS due to safety reasons (e.g.: treating a patient with ICH with tPA).
PLS was chosen after testing for multiple classifiers with preliminary results showing high classification accuracy without significant difference compared to other models. PLS regression finds a set of new variables, referred to as latent variables or components, that correlate with the class labels and capture the majority of crucial information in the predictor variables. The initial predictor variables and the class labels are then combined to create these latent variables iteratively. PLS can handle high-dimensional data and capture nonlinear correlations between the predictor variables and the class labels, thus rendering it an appropriate strategy for binary classification in general. Overall, PLS can also be beneficial when there are correlations between the predictor variables because it can minimize the data's dimensionality and boost the classifier's stability.

ROC curves were estimated by the R package plotROC\textsuperscript{93} which estimates exact confidence intervals based on Clopper and Pearson exact method.\textsuperscript{94} The 95% confidence intervals in the ROC curves were estimated at the point of maximum sensitivity where specificity was 100%. The varImp function from caret R package was applied calculation of variable importance scores based on the permutation method, which assesses the impact of permuting the values of each predictor variable on the model's performance. The resulting importance scores are scaled between 0 and 100, with higher scores indicating greater importance.
2.4 – Results

*Participant characteristics and clinical data*

According to our goal, we have included 90 patients, one-third within each subgroup. Median age was 71 (interquartile range [IQR] 57–81) years and 49 (54.4%) were female. Patients with ICH had higher systolic and diastolic blood pressure, more frequently had headache, and less frequently atrial fibrillation, than patients with IS. Patients with IS had higher prevalence of hypertension, dyslipidemia, atrial fibrillation, smoking and coronary artery disease, and higher NIHSS in comparison with SM. SM had higher prevalence of history of seizures, and more frequently presented with isolated sensory symptoms, without facial palsy, and with acute seizures than patients with IS. Time from symptom onset to blood collection was similar across the three subgroups. (Table 1)
Table 1. Baseline characteristics

<table>
<thead>
<tr>
<th>Demographics</th>
<th>Total (n=90)</th>
<th>IS (n=30)</th>
<th>ICH (n=30)</th>
<th>SM (n=30)</th>
<th>p-value</th>
<th>p-value*</th>
<th>p-value¥</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>71 (57–81)</td>
<td>73 (69–83)</td>
<td>70 (60–80)</td>
<td>62 (51–76)</td>
<td>0.120</td>
<td>1.000</td>
<td>0.127</td>
</tr>
<tr>
<td>Female sex</td>
<td>49 (54.4%)</td>
<td>13 (43.3%)</td>
<td>16 (53.3%)</td>
<td>20 (66.7%)</td>
<td>0.200</td>
<td>0.606</td>
<td>0.119</td>
</tr>
<tr>
<td>Vascular risk factors and medical history</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>69 (76.7%)</td>
<td>26 (86.7%)</td>
<td>27 (90.0%)</td>
<td>16 (53.3%)</td>
<td>0.001</td>
<td>1.000</td>
<td>0.005</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>23 (25.6%)</td>
<td>9 (30.0%)</td>
<td>8 (26.7%)</td>
<td>6 (20.0%)</td>
<td>0.700</td>
<td>1.000</td>
<td>0.552</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>35 (38.9%)</td>
<td>18 (60.0%)</td>
<td>9 (30.0%)</td>
<td>8 (26.7%)</td>
<td>0.014</td>
<td>0.037</td>
<td>0.009</td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>16 (17.8%)</td>
<td>11 (36.7%)</td>
<td>2 (6.7%)</td>
<td>3 (10.0%)</td>
<td>0.004</td>
<td>0.013</td>
<td>0.002</td>
</tr>
<tr>
<td>Smoking</td>
<td>16 (17.8%)</td>
<td>10 (33.3%)</td>
<td>4 (13.3%)</td>
<td>2 (6.7%)</td>
<td>0.019</td>
<td>0.125</td>
<td>0.010</td>
</tr>
<tr>
<td>Coronary artery disease</td>
<td>11 (12.2%)</td>
<td>7 (23.3%)</td>
<td>3 (10.0%)</td>
<td>1 (3.3%)</td>
<td>0.072</td>
<td>0.299</td>
<td>0.023</td>
</tr>
<tr>
<td>Previous stroke/TIA</td>
<td>8 (8.9%)</td>
<td>4 (13.3%)</td>
<td>0 (0.0%)</td>
<td>4 (13.3%)</td>
<td>0.120</td>
<td>0.038</td>
<td>1.000</td>
</tr>
<tr>
<td>History of seizures</td>
<td>7 (7.8%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>7 (23.3%)</td>
<td>&lt;0.001</td>
<td>1.000</td>
<td>0.005</td>
</tr>
<tr>
<td>Admission characteristics</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NIHSS</td>
<td>9 (3–17)</td>
<td>14 (6–19)</td>
<td>12 (9–22)</td>
<td>3 (2–5)</td>
<td>&lt;0.001</td>
<td>0.443</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Presence of facial palsy</td>
<td>64 (71.1%)</td>
<td>29 (96.7%)</td>
<td>29 (96.7%)</td>
<td>6 (20%)</td>
<td>&lt;0.001</td>
<td>1.000</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Isolated sensory symptoms</td>
<td>5 (5.6%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>5 (16.7%)</td>
<td>0.010</td>
<td>1.000</td>
<td>0.020</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>161 (134–188)</td>
<td>157 (146–177)</td>
<td>186 (160–220)</td>
<td>136 (126–163)</td>
<td>&lt;0.001</td>
<td>0.008</td>
<td>0.319</td>
</tr>
<tr>
<td>SBP ≥150mmHg</td>
<td>55 (61.1%)</td>
<td>20 (66.7%)</td>
<td>25 (83.3%)</td>
<td>10 (33.3%)</td>
<td>&lt;0.001</td>
<td>0.233</td>
<td>0.017</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>86 (74–100)</td>
<td>83 (70–94)</td>
<td>102 (88–117)</td>
<td>79 (73–86)</td>
<td>&lt;0.001</td>
<td>0.002</td>
<td>1.000</td>
</tr>
<tr>
<td>DBP ≥110mmHg</td>
<td>15 (16.7%)</td>
<td>2 (6.7%)</td>
<td>13 (43.3%)</td>
<td>0 (0.0%)</td>
<td>&lt;0.001</td>
<td>0.001</td>
<td>0.491</td>
</tr>
<tr>
<td>Decreased level of consciousness</td>
<td>13 (14.4%)</td>
<td>4 (13.3%)</td>
<td>9 (30.0%)</td>
<td>0 (0.0%)</td>
<td>0.003</td>
<td>0.209</td>
<td>0.112</td>
</tr>
<tr>
<td>Headache</td>
<td>9 (30.0%)</td>
<td>0 (0.0%)</td>
<td>12 (40.0%)</td>
<td>3 (10.0%)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.237</td>
</tr>
<tr>
<td>Vomiting</td>
<td>14 (15.6%)</td>
<td>3 (10.0%)</td>
<td>9 (30.0%)</td>
<td>2 (6.7%)</td>
<td>0.052</td>
<td>0.104</td>
<td>1.000</td>
</tr>
<tr>
<td>Acute seizures</td>
<td>7 (7.8%)</td>
<td>0 (0.0%)</td>
<td>1 (3.3%)</td>
<td>6 (20.0%)</td>
<td>0.015</td>
<td>1.000</td>
<td>0.010</td>
</tr>
<tr>
<td>Onset to blood collection, min</td>
<td>90 (72–131)</td>
<td>97 (76–129)</td>
<td>77 (64–108)</td>
<td>112 (75–151)</td>
<td>0.119</td>
<td>0.151</td>
<td>1.000</td>
</tr>
</tbody>
</table>

Values are presented as median (interquartile range) or as numbers (proportions). IS, Ischemic stroke; ICH, Intracerebral hemorrhage; SM, Stroke mimics; TIA, transient ischemic attack; NIHSS, National Institutes of Health Stroke Scale; SBP, systolic blood pressure; DBP, Diastolic blood pressure. *Comparison between AIS and ICH; ¥Comparison between IS and SM.
Half of the patients with IS presented with an identifiable intracranial vessel occlusion, seven (23.3%) patients received isolated intravenous thrombolysis, seven (23.3%) patients received bridging therapy and six (20%) patients were treated with direct MT. Regarding etiology, four (13.3%) were due to large artery disease, 15 (50%) due to cardioembolism, eight (26.7%) due to small vessel disease, and three (10.0%) of undetermined cause. In patients with ICH, 24 (80.0%) patients were classified as hypertensive, four (13.3%) were considered to be in the context of amyloid angiopathy and in two (6.7%) due to an arteriovenous malformation. In three (10.0%) patients, the ICH was also associated with anticoagulation. Regarding outcomes, it was considered favorable in 14 (46.7%) patients with IS and 12 (40.0%) patients with ICH. In the case of SM, the final diagnosis was functional disorder in nine (30%) of the patients, epileptic seizure in seven (23.3%), migraine in four (13.3%), metabolic and syncope both in three (10%), and encephalitis, peripheral nerve disease, and peripheral vertigo in the remaining patients.
Protein identification

In total, 1606 protein isoforms were identified, which summed to 748 protein coding genes (Figure 8–A). The total number of proteins was higher in patients with ICH compared to patients with IS or SM (Figure 8–A). Of note, unique proteins identified within each group may be detected only in a subset of patients. Patients with IS and ICH shared more common protein identifications than they shared with SM when analyzing the total identified proteins across all samples (Figure 8–A). However, when analyzing the protein identifications at the individual LC-MS run level across sample groups, it was not significant (Figure 8–B).

Figure 8. Total number of identifications across sample groups and in individual runs.

Legend

A) Venn diagram of overlapping proteins identified in IS, ICH and SM. B) Boxplot of number of identifications in individual LC-MS runs versus sample groups. Boxplots indicate the median and quartiles, with whiskers indicating the 1.5 interquartile ranges. The dotted lines indicate the global medians.
Quality control

Preliminary linear discriminant analysis (LDA) based on the iBAQ values was able to provide strong separation between all sample groups (Figure 9).

Figure 9. Linear discriminant analysis based on all quantitative iBAQ proteomics data.

LDA is a supervised classification method, and although all data was applied for training in this preliminary LDA analysis, it suggested that reasonable classification into correct clinical sample groups was achievable based on mass spectrometry data. No significant differences in the identification of bias proteins were observed across the three subgroups (Supplement Material – Figure II), supporting the hypothesis that the statistical differences observed between groups were not caused by blood collection or experimental artifacts.
Protein quantitation

In quantitative analysis, the comparison between samples of patients with IS and ICH or IS and SM resulted in higher number of significantly regulated proteins compared to the comparison between ICH and SM (Figure 10). Comparison between samples of patients with ICH and SM resulted in no significantly differences after correction for multiple testing (Figure 10).

Figure 10. Volcano plots based on the three pairwise comparisons performed by limma R package.

Legend
A) IS versus ICH, B) IS versus SM, and C) ICH versus SM. Dotted horizontal line indicates (green) adjusted p-value 0.05 – proteins above the horizontal line are significantly regulated. Dotted vertical lines correspond to a fold change of 1.5 – proteins at the right side of the right vertical line are upregulated, proteins at the left side of the left vertical line are downregulated. Colored data points indicate potential bias proteins (blood coagulation, erythrocyte and platelet proteins).

Focusing on differentiating patients with IS from those with ICH and SM we looked at the five most significantly dysregulated proteins. From the two comparisons, the same five proteins were identified – Complement component 3 (C3), Intercellular adhesion...
molecule 2 (ICAM-2), Plasminogen Like A (PLGLA), Syntaxin Binding Protein 5 (STXBP5) and immunoglobulin heavy variable 3-64 (IGHV3-64) (Figure 11). In patients with IS, C3, PLGLA, STXBP5 and IGHV3-64 were upregulated and ICAM-2 was downregulated.

Figure 11. Boxplot of the five top most significantly dysregulated proteins obtained for the differentiating ischemic stroke (IS) from intracerebral hemorrhage (ICH) and stroke mimics (SM).

Legend
C3, Complement component 3; ICAM-2, Intercellular adhesion molecule 2; PLGLA, Plasminogen Like A; STXBP5, Syntaxin Binding Protein 5; IGHV3-64, Immunoglobulin heavy variable 3-64
Classification

As a proof of concept, PLS classifier was built to differentiate patients with IS from patients with ICH and SM. The first three models were designed to differentiate patients with IS and ICH. As comparator, a first model using exclusively clinical data showed a 12% sensitivity and 53% negative predictive value (NPV) for diagnosis of IS. The most important variables were diastolic blood pressure of 110 mmHg, the presence of a headache, and a decreased level of consciousness (Figure 12).
Figure 12. Partial least square classifier using clinical data for differentiating ischemic stroke (IS) and intracerebral hemorrhage (ICH).

Legend
A) Tables indicating number of samples used for training versus test data set. B) Receiver operating characteristics and area under the curve with confusion matrix embedded. Metrics of classification performance on the validation data set are provided on the right. The cross indicates the cut of threshold used to calculate the exact confidence interval. C) The lower panel depicts the most important predictor variables for the PLS model. The X-axis indicates the importance score scaled from 0-100 provided by the R package caret.
In a second model, based on top ten regulated proteins in the training data set (Figure 13), sensitivity and NPV largely improved to 88% and 89%, respectively. ICAM-2, STXBP5, PLGLA, C3, and IGHV3-64 were, in this order, the most relevant proteins in the model.

Figure 13. Partial least square classifier using PLS model based on a maximum of 10 proteins for differentiating ischemic stroke (IS) from intracerebral hemorrhage (ICH)

Legend
A) Tables indicating number of samples used for training versus test data set. B) Receiver operating characteristics and area under the curve with confusion matrix embedded. Metrics of classification performance on the validation data set are provided on the right. The cross indicates the cut of threshold used to calculate the exact confidence interval. C) The lower panel depicts the most important predictor variables for the PLS model. The X-axis indicates the importance score scaled from 0-100 provided by the R package caret.
To evaluate the full potential of the plasma proteome, a third model, using all proteins, showed improved results and additional potential useful biomarkers for stroke diagnosis (Supplement Material – Figure III).

To differentiate patients with IS from those with ICH and SM, three additional models were built, using the same three variable categories (Figure 14 and Supplement Material – Figure IV–V). As with the first three models, protein-based models showed better performance than the model only using clinical data. In a model based on top ten regulated proteins in training data (Figure 14) sensitivity and negative predictive value were 75% and 95%, respectively. ICAM-2, PLGLA, C3 and IGHV3-64 were, in this order, the most important proteins in the model.
Figure 14. Partial least square classifier using PLS model based on a maximum of 10 proteins for differentiating ischemic stroke (IS) from intracerebral hemorrhage (ICH) and Stroke Mimics (SM).

Legend
A) Tables indicating number of samples used for training versus test data set. B) Receiver operating characteristics and area under the curve with confusion matrix embedded. Metrics of classification performance on the validation data set are provided on the right. The cross indicates the cut of threshold used to calculate the exact confidence interval. C) The lower panel depicts the most important predictor variables for the PLS model. The X-axis indicates the importance score scaled from 0-100 provided by the R package caret.
The model using the full proteome showed perfect discrimination with 100% sensitivity and negative predictive value. (Supplemental Material – Figure V)

To elucidate the complexity of the decision boundary of the top markers used in these models, data from all samples were visualized in a scatter plot with pairwise expression values. Figure 15 displays an example of a scatter plot of iBAQ intensity values, in this case for ICAM2 and PLGLA. The results suggest that a simple classifier based on thresholds for 2-3 protein markers may provide a suitable separation of IS from ICH and SM. The linear decision boundaries provide additional evidence that the two plotted variables ICAM2 and PLGLA have a relevant discriminatory power and influence the classification outcome.

Figure 15. Scatterplot of iBAQ values for ICAM2 and PLGLA.

Legend
Dashed lines indicate area containing exclusively IS samples. iBAQ, Intensity-based absolute.
2.5 – Discussion

In our pilot and exploratory study, using a proteomics analysis approach in suspected acute stroke patients, we identified new potential targets for identifying patients with IS. Using a limited number of proteins, our models showed a sensitivity of 88% and NPV of 89% for separating patients with IS and ICH, and a sensitivity of 75% and NPV of 95%, for differentiating patients with IS and ICH or SM. All models were created aiming to minimize misclassification of ICH as IS, which could potentially lead to harmful treatment (e.g.: giving tPA to a patient with ICH). To our knowledge, no previous blood-based biomarker analysis showed these high levels of sensitivity and specificity for identifying patients with acute IS.

Our models using the full proteome showed better results, including 100% sensitivity for differentiating patients with IS from patients with ICH or SM. Contrary to the models above, full proteome analysis would be more difficult to translate to clinical practice. Nevertheless, the results of these analyses can also suggest additional potential biomarkers for stroke diagnosis that can be further assessed in future studies.

Five proteins were identified as being particularly relevant in differentiating patients with IS from patients with ICH and SM, namely C3, ICAM-2, PLGLA, STXB5 and IGHV3-64.

C3 is the most abundant protein of the complement system, which is involved in multiple physiological functions and pathophysiological processes. After cerebral ischemia, C3 activation is involved in the mechanism of brain injury, and inhibition of complement activation may reduce the volume of cerebral infarction. Previous studies have found increased C3 levels after IS, while there is conflicting data about the association between C3 levels and IS outcome. In ICH, C3 is associated with inflammation and
microglia activation, contributing to ICH-induced brain injury.\textsuperscript{100} One study has assessed C3 levels in patients with ICH and found no association between C3 levels and ICH rebleeding or functional outcome.\textsuperscript{101}

ICAM-2 is a transmembrane glycoprotein expressed on the surface of endothelial cells, platelets and a variety of leucocyte subsets. It is expressed in the blood–brain barrier endothelium and contributes to both leucocyte diapedesis and extravasation from blood vessels into surrounding tissues.\textsuperscript{102,103} ICAM-2 has overlapping characteristics and functions with ICAM-1, which in contrary to ICAM-2, has been fairly well studied in patients with IS. Previous reports showed increasing ICAM-1 levels in patients with IS,\textsuperscript{104–106} with higher ICAM-1 levels associated with worse prognosis.\textsuperscript{106,107} Due to the potential role of ICAM-1 in IS pathophysiology, a randomized clinical trial using ICAM-1 antibody was conducted but failed to show beneficial results.\textsuperscript{108} In patients with ICH, there is conflicting data regarding the association between ICAM-1 levels and functional outcome.\textsuperscript{101,109}

PLGLA functions have been rarely studied. Because nucleotide sequence comparisons showed a high degree of homology with plasminogen\textsuperscript{110} it has been hypothesized that PLGLA is involved in the same functions as plasminogen. Besides its role in fibrinolysis, plasminogen also plays a role in synaptic plasticity and increased blood-brain barrier permeability.\textsuperscript{111} In proteomic-based studies, higher plasminogen levels were described in patients with IS in comparison with ICH,\textsuperscript{52} while there is conflicting data regarding comparison with controls.\textsuperscript{52,112,113}

STXBP5 is a protein involved in endothelial exocytosis and platelet secretion. In particular, STXBP5 regulates the secretion of von Willebrand factor (VWF), a glycoprotein with an important role in hemostasis, especially by promoting platelet adhesion.\textsuperscript{114} STXBP5 mutations were associated with reduced plasma VWF levels and
VWF activity,\textsuperscript{114,115} while STXBP5 genetic variations may increase the risk of thromboembolic disease.\textsuperscript{115} STXBP5 also seems to promote the release of tPA from endothelial cells.\textsuperscript{116} To our knowledge, there is no study investigating the role of STXBP5 in stroke patients or measuring its levels.

IGHV3-64 corresponds to a particular variable domain in a heavy-chain immunoglobulin, a polypeptide subunit of an antibody. Being part of an antibody structure, IGHV3-64 is involved in different functions such as antigen binding activity, activation of the immune response, and integrating immunoglobulin complexes. However, its specific role, as well as that of other variable domains in heavy chain immunoglobulin, remains to be elucidated. Inflammation has a significant role in stroke, and different inflammatory proteins have been shown to be associated with stroke risk, diagnosis, etiology and prognosis.\textsuperscript{48,52,96–101,104–107,109,117} In one proteomic study, other variable domains of immunoglobulin heavy chains were found to be significantly increased in patients with IS and ICH in comparison with healthy controls.\textsuperscript{52}

Previous studies, with other pre-selected biomarkers, have shown promising results for the identification of patients with IS.\textsuperscript{43,44,48,50} In particular, a blood based-biomarker panel using GFAP, RBP4 and NT-proBNP showed a sensitivity rate of 51.5% at 100% specificity for IS diagnosis.\textsuperscript{44} Although its moderate sensitivity rate, this blood-based biomarker panel was studied in rapid POCT, making it closer to clinical application. Potentially, a combination of previously tested and newly disclosed biomarkers such as those presented in our study could contribute to better performance of blood-based biomarker panels, increasing the likelihood of their use in stroke care.

Only one previous study has used label-free proteomics for differentiating patients with IS and ICH, including a total of only seven patients\textsuperscript{52} and not in the acute phase. Nine candidate blood biomarkers were identified, none of which overlapped with our results.
nor with previous studied biomarkers.\textsuperscript{42-44,48,50} Time intervals of up to 15 days between symptom onset and blood collection are one of the possible explanations for these differences.

Proteomics has also been explored in the clot characterization of patients with IS, disclosing different biomarker profile according to stroke etiology.\textsuperscript{118,119} While being different from our results, the described proteome profiles can work together with our findings by suggesting unexplored pathways implicated in stroke pathophysiology, and potentially in stroke treatment.

As previously suggested,\textsuperscript{44} if accurate blood-based biomarker panels were available for diagnosing patients with acute ischemic stroke with feasible rapid POCT, this could allow safe prehospital thrombolysis, in selected patients, without the need of pre-hospital imaging. Albeit preliminary, our results can add to a potential future biomarker panel, that after validation in large cohort studies and subsequent testing in well-conducted trials, could be implemented with this goal. Our exploratory study revealed a new blood biomarker combination, that, while aiming to minimize false-positives, can correctly identify at least four out of five patients with IS that could be immediately treated, with the remaining patients still having the opportunity to be treated after hospital admission.

In a hospital setting, after imaging confirms the absence of an intracranial hemorrhage, our results could help in guiding the decision to administer intravenous thrombolysis by assisting in the differentiation of IS from stroke mimics. This can be especially relevant in patients with risk factors for bleeding, in which the decision to administer thrombolysis becomes particularly challenging.

However, the application of biomarkers in this clinical setting is not devoid of challenges. First, because ischemic stroke is a heterogeneous disease, involving different pathophysiological processes and infarcts ranging from large embolic lesions to small
lacunar strokes, the goal of having a blood-based biomarker panel that integrates all dimensions of patients with stroke is challenging. Indeed, as previously discussed, the different biomarker profile between patients with suspected stroke, may result not from the acute event, but from the differences in patients’ medical conditions.\textsuperscript{42,44,97} Also, the presence of concomitant systemic medical conditions, such as metabolic and infectious diseases, or cancer, can influence the biomarker measurement. Because a kinetic component of blood-based biomarkers cannot be excluded, there is the possibility that some biomarkers will only be useful for diagnostic purposes in a limited time window, as shown with GFAP.\textsuperscript{120} The applicability of prehospital biomarkers will require trained paramedics and prehospital health workers, whose level of expertise is very asymmetric across countries. While conceiving the idea of treating patients without neuroimaging, additional benefits from imaging such as, identifying potential contraindications for tPA or imaging findings that stratify the risk of hemorrhagic transformation, would be waived. Although selecting patients for thrombolysis with a blood-based biomarker will be difficult in clinical practice, the applicability of blood-based biomarkers for diagnosing stroke patients is not limited to this goal. In patients with severe neurological deficits, blood-based biomarker could be used to better select patients for swift referral to a comprehensive stroke center in a mothership approach, increasing the probability of correctly selecting a candidate for endovascular treatment. As such, blood biomarkers could also help select patients for a direct transfer to an angiography suite approach in this subset of patients, a strategy with growing evidence and promising results.\textsuperscript{121} Our study has limitations. Being an exploratory study designed to disclose new potential biomarkers, the full dimension of clinical heterogeneity as well as our biomarker analysis are limited by our small sample size. Although clinically relevant, the decision to build our models aiming to minimize misidentification of patients with ICH as having IS,
additionally limits our analysis. Due to the large confidence intervals, we cannot exclude the possibility of misclassifications with our methodology.

Because alternative classifiers based on fewer than 10 markers provided similar but slightly lower performance, further validation should not only be restricted to the top five highlighted proteins. The data presented needs to be replicated in different and larger cohorts and clinically validated using alternative measurement techniques. Furthermore, in a clinical and practical scenario, serum samples instead of plasma samples would need to be used for such biomarkers’ detection. Likewise, MS analysis require sample processing that could influence our analysis and contribute to different results compared with orthogonal methods. Also, since we cannot exclude that the identified biomarkers will have a particular kinetic profile over time, our results may not be applicable to suspected stroke patients arriving after six hours.

Nonetheless, this is the first study to use MS on samples obtained in an acute setting to diagnose patients with suspected stroke. It can also add information on IS-related acute adaptation mechanisms that could potentially be applied to new neuroprotective targets. Our prospective and consecutive enrollment of patients with acutely suspected stroke and the robustness of our training and testing approach are important strengths of our approach.
2.6 – Conclusions

In this proof-of-concept exploratory study, our proteomics analysis identified potential new biomarkers for diagnosing acute IS patients with good to excellent sensitivity while aiming at avoiding the misclassification of ICH as IS. Although our approach provided an internal validation, our results still need to be confirmed in other cohorts and with different measurement techniques.
Chapter 3 - Stroke in the Stroke Unit: Recognition, treatment and outcomes in a single-center cohort

This chapter is based on the following manuscript:

Stroke in the stroke unit: Recognition, treatment and outcomes in a single-centre cohort.
3.1 – Abstract

Background and purpose

In-hospital strokes (IHS) are associated with longer diagnosis times, treatment delays, and poorer outcomes. Strokes occurring in the stroke unit have seldom been studied. Our aim was to assess the management of in-stroke unit ischemic stroke (ISUS) by analyzing ISUS characteristics, delays in diagnosis, treatments and outcomes.

Methods

Consecutive patients from the ASTRAL registry, from January 2003 to June 2019, were classified as ISUS, other-IHS or community-onset stroke (COS). Baseline and stroke characteristics, time-to-imaging and -to treatment, missed treatment opportunities, treatment rates and outcomes were compared using multivariate analysis with adjustment for relevant clinical, imaging and laboratory data available in ASTRAL.

Results

Among the 3456 patients analysed, 138 (4.0%) were ISUS, 214 (6.2%) other-IHS and 3104 (89.8%) COS. In multivariate analysis, patients with ISUS more frequently had known stroke onset-time than other-IHS (adjusted odds ratio [aOR] 2.44; 95% confidence interval [CI] 1.39–4.35) or COS (aOR 2.56; 95% CI 1.59–4.17), had less missed treatment opportunities than other-IHS (aOR 0.22; 95% CI 0.06–0.86) and higher endovascular treatment rates than COS (aOR 3.03; 95% CI 1.54–5.88). ISUS were associated with a favorable shift in the modified Rankin Scale at 3-months in comparison with other-IHS (aOR 1.73; 95% CI, 1.11–2.69) or COS (aOR 1.46; 95% CI, 1.00–2.12).
Conclusion

ISUS more frequently had known stroke onset-time than other-IHS or COS, less missed treatment opportunities than other-IHS and a higher endovascular treatment rate than COS. This readiness to identify and treat patients in the stroke unit may explain the better long-term outcome of ISUS.
3.2 – Introduction

Patients with in-hospital ischemic stroke (IHS) are known to have longer times to imaging and treatment, lower intravenous thrombolysis treatment rates and worse outcomes compared with community-onset stroke (COS).\textsuperscript{62,63,65,66,122} Stroke units are the cornerstones of stroke care, reducing mortality and disability.\textsuperscript{27,28} Of the IHS, in-stroke unit ischemic strokes (ISUS) seem to have shorter onset-to-needle times.\textsuperscript{123} However, the impact of being in a stroke unit on the diagnosis, treatment metrics and outcome of IHS has not been previously studied. In-hospital stroke recurrence after a first community-onset stroke or transient ischemic attack is relatively rare, but associated with a higher in-hospital mortality.\textsuperscript{124} Acute recanalization treatment rates and long-term outcome of these patients are yet to be assessed.

In this study our aim was to compare patient and stroke characteristics, times to investigation and treatment, access to acute recanalization treatments and long-term outcome between: 1) ISUS and IHS occurring in other hospital departments and 2) ISUS and COS.
3.3 – Methods

Study Design and Patient Selection

The Acute Stroke Registry and Analysis of Lausanne (ASTRAL) is a single-center-based cohort study of all consecutive AIS patients admitted to the stroke unit and intensive care unit of Lausanne University Hospital within 24 hours of last-seen-well time, as published previously.125

For the current retrospective analysis, we selected all patients from January 2003 to June 2019 having an IHS occurring in the Lausanne University Hospital or a COS within the hospital primary referral area. Patients with IHS occurring in the Emergency Department were excluded. We included in-hospital ischemic stroke recurrences; in cases of multiple in-hospital stroke recurrences, only the first event was considered.

ASTRAL follows institutional regulations for clinical and research databases. All data collected stem from routine clinical and radiological management, and were anonymized before analysis following the principles of the Swiss Human Research Act. Given that only anonymized data were used, there was no need for ethics committee approval or patient consent according to the Swiss Human Research Act. The reporting of this observational study is in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines.126

ASTRAL Variables

This registry incorporates a large set of pre-specified parameters including, demographics (age and sex), medical history and vascular risk factors (prestroke mRS, previous stroke or TIA, hypertension, diabetes mellitus, dyslipidemia, smoking, atrial fibrillation, coronary artery disease, active cancer), current medications (antiplatelets, anticoagulants,
antihypertensives and lipid-lowering drugs), process-oriented data (prehospital and door-to-treatment times), if stroke onset was witnessed, clinical symptoms and neurological signs, stroke localization, stroke severity at admission and 24 hours (per NIHSS, performed or supervised by NIHSS-certified personnel), vital signs (body temperature, blood pressure), metabolic parameters, acute and subacute multimodal brain-imaging, acute recanalization treatments performed and their characteristics, stroke mechanism and long-term clinical outcome.

If an acute recanalization treatment is not performed, we record systematically contraindications or other specified reasons, including missed stroke diagnosis and missed treatment opportunities. A missed treatment opportunity was considered when a particular patient did not receive an acute recanalization treatment according to the define institutional criteria at that time. Stroke mechanism was classified according to TOAST classification.¹²⁷

**In-hospital ischemic stroke and stroke recurrence definition**

The ASTRAL registry indicates where the index-stroke occurred (COS vs. IHS) and specifies in-hospital ischemic stroke recurrence. In all IHS and in-hospital ischemic stroke recurrence cases, available data (medical notes, discharge letters, neuroimaging and radiology reports) were reviewed by a vascular neurologist in order to ascertain the event.

We defined both index and recurrent ischemic stroke according to the historical World Health Organization definition.⁸¹ In addition, for the definition of recurrent events, the following criteria were used in order to differentiate them from worsening of a previous (recent) stroke: 1) onset of new neurological signs suggesting the involvement of initially unaffected vascular territories; or 2) onset of new neurological signs related to the same
territory as the initial stroke with i) imaging confirming a new ischemic lesion corresponding to the new neurological signs and not translating infarct growth; or ii) new neurological signs not attributable to previously identified brain lesion(s),\(^{53}\) presented on waking or established within 30 minutes of their onset, and exclusion of other potential causes of the new neurological signs such as intracranial hemorrhage, cerebral edema, seizures, infection, acute confusional state or other systemic disorders.

We considered IHS cases as ISUS if the patient was hospitalized in the stroke unit or in the intensive care unit for a recent ischemic or hemorrhagic stroke at the time of the new stroke. They were considered as other-IHS if the patient was hospitalized in another department or ward, including rehabilitation, general neurology, or in the intensive care unit for a reason other than a recent cerebrovascular event. For the purpose of this study, all cases of IHS and in-hospital ischemic stroke recurrences were reviewed.

**Revascularization Treatments**

Acute revascularization treatments (IVT and EVT) and acute ischemic stroke management were performed according to hospital criteria at the time of admission. These were based on Swiss national\(^{128}\) and European guidelines\(^{129,130}\) and updated according to new positive randomized trials. In detail, IVT treatment was performed up to 3 hours after last-seen-well before 2008 and if the NIHSS was \(\geq 6\), and up to 4.5 hours for any disabling stroke, thereafter. EVT was performed up to 2014 for anterior circulation strokes if treatment could be initiated within 6 hours, with NIHSS \(\geq 6\), computed tomography angiography (CTA) disclosing a proximal intracranial vessel occlusion and CT-perfusion (CTP) showing \(> 50\%\) of penumbra. Late arriving patients and unknown-onset patients were treated in selected cases, if CTP showed \(>50\%\) penumbra, and with informed consent. After 2014, for patients arriving within 6 hours, CTP criteria were replaced by
Alberta Stroke Program Early CT Score (ASPECTS) ≥5 and the lower NIHSS limit was replaced by the presence of a disabling deficit. From May 2017, we treated patients with these same criteria, but up to 8 hours. After 8 hours, treatment was offered if the NIHSS ≥10 and core <50 mL, or NIHSS <10 and core <30 mL. After January 2018, late treatment was alternatively based on any NIHSS, core <70 mL and the ratio of ischemic tissue volume to initial infarct volume ≥1.8. For posterior circulation stroke in specific cases of basilar artery occlusion, patients were treated with EVT up to 6 hours in the absence of extensive brainstem infarct. From May 2017, the treatment window was extended to 8 hours if posterior circulation ASPECTS (pc-ASPECTS) ≥7, and up to 24 hours if no transverse irreversible brainstem ischemia (MRI) was present or pc-ASPECTS was ≥8. For all EVT cases, the upper age limit of 80 years was abandoned in 2012.

**Outcome Analysis**

For short-term outcome, we assessed the need to perform hemicraniectomy and in-hospital mortality. For long-term outcome, modified Rankin Scale (mRS)-certified medical personnel evaluated the patient’s 3-month mRS either in the outpatient stroke clinic or by a structured telephone interview.

**Statistical Analysis**

Continuous data were summarized as median values and interquartile range (IQR) and categorical data as absolute numbers and percentages. Multivariate multinomial regression was performed to identify potential factors associated with ISUS with all the relevant clinical, radiological and biological data available in ASTRAL, as described above. Imputation of the missing values of the independent covariates studied was carried out with the method of chained equations, generating five imputed data sets. We then
performed multinomial logistic regression analysis to identify factors associated with ISUS on each imputed data set and final results were derived by combining the output of the five imputed multiple analyses. We implemented stepwise methods on each imputed data set to identify significant main effects. Variables with imputed values and respective numbers are detailed in Supplemental Material – Table I.

For the adjusted clinical outcome, we performed an ordinal logistic regression analysis (shift-analysis towards favorable outcome) of the 3-month mRS. This model was adjusted for the same variables included in the multinomial logistic regression analysis for factors associated with ISUS. In all analyses, the level of significance was set at 5%. Statistical analyses were performed with the Statistical Package R (version 4.1.1).
3.4 – Results

In our study we included 3456 patients with a median age of 75.5 (IQR, 19.6) years, of whom 1595 (46.2%) were female. Of the included patients, 138 (4.0%) were ISUS, 214 (6.2%) other-IHS and 3104 (89.8%) COS, as indicated in the inclusion flow-chart (Figure 16).

Figure 16. Flow-chart for patient inclusion

Legend.
ASTRAL, The Acute Stroke Registry and Analysis of Lausanne; CHUV, Centre Hospitalier Universitaire Vaudois; ISUS, In-Stroke Unit Ischemic Stroke; Other-ISUS, Other In-Hospital Stroke; Community-Onset Stroke.
ISUS patients were mainly admitted for ischemic stroke (70.3%) followed by TIA (24.6%), hemorrhagic stroke (3.6%) or elective procedures (1.4%). For other-IHS, the main reasons for hospital admission were elective surgical procedures (26.6%) and acute cardiac diseases (25.7%). Median time from admission to ischemic stroke were two (IQR 4) and three (IQR 6) days for ISUS and other-IHS, respectively. Ninety-six (69.6%) ISUS patients and three (1.4%) other-IHS patients corresponded to in-hospital ischemic stroke recurrences. Detailed information on reasons for hospital admission and departments in which other-IHS occurred are presented in Table 2.
Table 2. Reasons for hospital admission and corresponding departments of other-IHS

<table>
<thead>
<tr>
<th>Reason for hospital admission</th>
<th>ISUS (n=138)</th>
<th>Other-IHS (n=214)</th>
<th>Departments of other-IHS</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ischemic stroke</td>
<td>93 (67.4%)</td>
<td>4 (1.9%)</td>
<td>Internal Medicine</td>
<td>45 (21.1%)</td>
</tr>
<tr>
<td>TIA</td>
<td>34 (24.6%)</td>
<td>3 (1.4%)</td>
<td>Cardiology</td>
<td>43 (20.1%)</td>
</tr>
<tr>
<td>ICH/SAH</td>
<td>5 (3.6%)</td>
<td>4 (1.9%)</td>
<td>Cardiac and Thoracic Surgery</td>
<td>16 (7.5%)</td>
</tr>
<tr>
<td>Elective procedure</td>
<td>2 (1.4%)</td>
<td>57 (26.6%)</td>
<td>Vascular Surgery</td>
<td>29 (13.6%)</td>
</tr>
<tr>
<td>Infection</td>
<td>1 (0.7%)†</td>
<td>30 (14.0%)</td>
<td>Orthopaedics</td>
<td>19 (8.9%)</td>
</tr>
<tr>
<td>Trauma</td>
<td>0 (0.0%)</td>
<td>20 (9.3%)</td>
<td>Visceral Surgery</td>
<td>12 (5.6%)</td>
</tr>
<tr>
<td>Cancer</td>
<td>1 (0.7%)†</td>
<td>13 (6.1%)</td>
<td>Urology and Plastic Surgery</td>
<td>10 (4.7%)</td>
</tr>
<tr>
<td>Cardiac diseases</td>
<td>1 (0.7%)†</td>
<td>55 (25.7%)</td>
<td>Neurosurgery and Otorhinolaryngology</td>
<td>13 (6.1%)</td>
</tr>
<tr>
<td>Other</td>
<td>1 (0.7%)†</td>
<td>28 (13.1%)</td>
<td>Intensive Care Unit</td>
<td>12 (5.6%)</td>
</tr>
</tbody>
</table>

†First stroke outside of the Stroke Unit followed by ischemic stroke recurrence in the Stroke Unit. ISUS, n-stroke unit ischemic stroke; IHS, in-hospital stroke.

In the univariate comparison, patients with ISUS were younger, had a higher pre-stroke dependency and more frequently had a history of previous ischemic stroke or TIA. In addition, ISUS patients more often had a known stroke onset-time and strokes were more frequently due to large vessel atherosclerosis (Table 3).
Table 3. Baseline and stroke characteristics.

<table>
<thead>
<tr>
<th></th>
<th>All cohort (n=3456)</th>
<th>ISUS (n=138)</th>
<th>other-IHS (n=214)</th>
<th>COS (n=3104)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>75.5 (19.6)</td>
<td>69.8 (20.7)</td>
<td>75.5 (14.9)</td>
<td>75.7 (19.7)</td>
<td>0.003</td>
</tr>
<tr>
<td>Female sex</td>
<td>1595 (46.2%)</td>
<td>69 (50.0%)</td>
<td>83 (38.8%)</td>
<td>1443 (46.5%)</td>
<td>0.058</td>
</tr>
<tr>
<td>Pre-stroke mRS≥2</td>
<td>1037 (30.1%)</td>
<td>88 (65.7%)</td>
<td>105 (49.1%)</td>
<td>844 (27.2%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Prehospitalization mRS≥2</td>
<td>982 (28.5%)</td>
<td>36 (26.1%)</td>
<td>102 (47.6%)</td>
<td>844 (27.2%)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Vascular risk factors

<p>| | | | | | |</p>
<table>
<thead>
<tr>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertension</td>
<td>2578 (74.6%)</td>
<td>99 (71.7%)</td>
<td>168 (78.5%)</td>
<td>2311 (74.5%)</td>
<td>0.301</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>668 (19.3%)</td>
<td>27 (19.6%)</td>
<td>65 (30.4%)</td>
<td>576 (18.6%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>2587 (74.9%)</td>
<td>110 (79.7%)</td>
<td>144 (67.3%)</td>
<td>2333 (75.3%)</td>
<td>0.017</td>
</tr>
<tr>
<td>Smoking</td>
<td>774 (22.5%)</td>
<td>34 (24.6%)</td>
<td>51 (23.8%)</td>
<td>689 (22.4%)</td>
<td>0.742</td>
</tr>
<tr>
<td>Alcohol</td>
<td>369 (10.8%)</td>
<td>19 (13.8%)</td>
<td>32 (15.0%)</td>
<td>318 (10.3%)</td>
<td>0.070</td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>1044 (30.2%)</td>
<td>33 (23.9%)</td>
<td>86 (40.2%)</td>
<td>925 (29.8%)</td>
<td>0.002</td>
</tr>
<tr>
<td>Ischemic heart disease</td>
<td>667 (19.3%)</td>
<td>26 (18.8%)</td>
<td>88 (41.1%)</td>
<td>553 (17.9%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Heart valves</td>
<td>130 (3.8%)</td>
<td>3 (2.2%)</td>
<td>24 (11.2%)</td>
<td>103 (3.3%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Previous stroke or TIA</td>
<td>722 (20.9%)</td>
<td>137 (99.3%)</td>
<td>49 (22.9%)</td>
<td>536 (17.3%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Active cancer</td>
<td>216 (6.3%)</td>
<td>19 (13.8%)</td>
<td>29 (13.6%)</td>
<td>168 (5.4%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Chronic kidney disease</td>
<td>554 (16.0%)</td>
<td>12 (8.7%)</td>
<td>62 (29.0%)</td>
<td>480 (15.5%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Depression</td>
<td>154 (4.5%)</td>
<td>12 (8.7%)</td>
<td>11 (5.1%)</td>
<td>131 (4.2%)</td>
<td>0.074</td>
</tr>
</tbody>
</table>

Pre-stroke therapy

<p>| | | | | | |</p>
<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>Antiplatelets</td>
<td>1353 (39.1%)</td>
<td>75 (54.3%)</td>
<td>109 (50.9%)</td>
<td>1169 (37.7%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Anticoagulants</td>
<td>487 (14.1%)</td>
<td>31 (22.5%)</td>
<td>62 (29.0%)</td>
<td>394 (12.7%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Antihypertensives</td>
<td>2137 (62.0%)</td>
<td>89 (64.5%)</td>
<td>156 (73.2%)</td>
<td>1892 (61.1%)</td>
<td>0.001</td>
</tr>
<tr>
<td>Statins</td>
<td>1050 (30.4%)</td>
<td>98 (71.0%)</td>
<td>96 (44.9%)</td>
<td>867 (28.0%)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Stroke characteristics

<p>| | | | | | |</p>
<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>Known onset-time</td>
<td>2218 (64.2%)</td>
<td>102 (73.9%)</td>
<td>138 (64.5%)</td>
<td>1978 (63.7%)</td>
<td>0.044</td>
</tr>
<tr>
<td>Anterior circulation</td>
<td>2445 (70.7%)</td>
<td>103 (74.6%)</td>
<td>163 (76.2%)</td>
<td>2179 (70.2%)</td>
<td>0.098</td>
</tr>
<tr>
<td>NIHSS</td>
<td>5.0 (9.0)</td>
<td>9.0 (12.2)</td>
<td>11.5 (13.0)</td>
<td>5.0 (8.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Decreased level of consciousness</td>
<td>378 (11.0%)</td>
<td>45 (32.6%)</td>
<td>58 (27.1%)</td>
<td>275 (8.9%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Admission SBP, mmHg</td>
<td>153 (35)</td>
<td>149 (32)</td>
<td>136 (31)</td>
<td>154 (34)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Admission glucose, mmol/L</td>
<td>6.6 (2.3)</td>
<td>6.5 (2.7)</td>
<td>7.1 (3.0)</td>
<td>6.5 (2.2)</td>
<td>0.033</td>
</tr>
</tbody>
</table>

Imaging

<p>| | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
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<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukoaraiosis</td>
<td>1126 (36.3%)</td>
<td>54 (39.1%)</td>
<td>66 (30.8%)</td>
<td>1006 (36.6%)</td>
<td>0.182</td>
</tr>
<tr>
<td>Chronic stroke lesions</td>
<td>1138 (36.7%)</td>
<td>84 (60.9%)</td>
<td>64 (29.9%)</td>
<td>990 (36.0%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Baseline ASPECTS</td>
<td>10 (9–10)</td>
<td>9 (8–10)</td>
<td>10 (8–10)</td>
<td>10 (9–10)</td>
<td>0.089</td>
</tr>
<tr>
<td>Site of most proximal vessel occlusion†</td>
<td>223 (7.6%)</td>
<td>11 (9.2%)</td>
<td>24 (12.7%)</td>
<td>188 (7.4%)</td>
<td>0.004</td>
</tr>
<tr>
<td>ICA</td>
<td>281 (9.6%)</td>
<td>13 (10.8%)</td>
<td>28 (14.8%)</td>
<td>240 (9.1%)</td>
<td></td>
</tr>
<tr>
<td>M1</td>
<td>388 (13.2%)</td>
<td>20 (16.7%)</td>
<td>31 (16.4%)</td>
<td>337 (12.8%)</td>
<td></td>
</tr>
</tbody>
</table>
ISUS less frequently had a missed diagnosis, had lower rates of missed opportunities to receive reperfusion therapy and lower times from stroke recognition-to-imaging and -to EVT in comparison with other-IHS (Table 4).
Table 4. Diagnosis, treatment and outcomes.

<table>
<thead>
<tr>
<th>Diagnosis and treatment</th>
<th>All cohort (n=3456)</th>
<th>ISUS (n=138)</th>
<th>other-IHS (n=214)</th>
<th>COS (n=3104)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Missed stroke diagnosis</td>
<td>130 (3.8%)</td>
<td>8 (5.8%)</td>
<td>19 (8.9%)</td>
<td>103 (3.3%)</td>
<td>0.001</td>
</tr>
<tr>
<td>Stroke recognition-to-imaging, min†</td>
<td>24 (36)</td>
<td>54 (66)</td>
<td>96 (132)</td>
<td>24 (18)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Acute reperfusion treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Intravenous thrombolysis (isolated)</td>
<td>564 (16.3%)</td>
<td>15 (10.9%)</td>
<td>42 (19.6%)</td>
<td>507 (16.3%)</td>
<td></td>
</tr>
<tr>
<td>EVT or bridging therapy</td>
<td>235 (6.8%)</td>
<td>18 (13.0%)</td>
<td>41 (19.2%)</td>
<td>176 (5.7%)</td>
<td></td>
</tr>
<tr>
<td>Stroke recognition-to-thrombolysis, min (door-to-thrombolysis in community-onset stroke)</td>
<td>48 (36)</td>
<td>80 (36)</td>
<td>84 (60)</td>
<td>42 (36)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Stroke recognition-to-puncture, min (door-to-puncture in community-onset stroke)</td>
<td>114 (84)</td>
<td>150 (264)</td>
<td>210 (96)</td>
<td>114 (60)</td>
<td>0.011</td>
</tr>
<tr>
<td>Missed opportunity to treat</td>
<td>100 (2.9%)</td>
<td>4 (2.9%)</td>
<td>17 (7.9%)</td>
<td>79 (2.5%)</td>
<td>0.001</td>
</tr>
<tr>
<td>Outcomes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Symptomatic hemorrhagic transformation‡</td>
<td>69 (2.1%)</td>
<td>7 (5.4%)</td>
<td>7 (3.6%)</td>
<td>55 (1.9%)</td>
<td>0.023</td>
</tr>
<tr>
<td>In-hospital mortality</td>
<td>338 (9.8%)</td>
<td>29 (21.0%)</td>
<td>63 (29.4%)</td>
<td>246 (7.9%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hemicraniectomy</td>
<td>22 (0.6%)</td>
<td>5 (3.6%)</td>
<td>3 (1.4%)</td>
<td>14 (0.5%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>3-month mRS§</td>
<td>2.0 (3.0)</td>
<td>3.0 (4.0)</td>
<td>4.0 (4.0)</td>
<td>2.0 (2.0)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are presented as median (interquartile range) or as numbers (proportions). ISUS, in-stroke unit ischemic stroke; IHS, in-hospital stroke; COS, community-onset stroke; EVT, Endovascular treatment; mRS, modified Rankin scale;
†In those clinically eligible for acute reperfusion therapy at stroke onset.
‡Available in 3291 patients (95.3%), 129 (93.5%) ISUS, 195 (91.1%) other-IHS and 2967 (95.6%) COS.
§Available in 3308 patients (95.7%), 136 (95.6%) ISUS, 211 (98.6%) other-IHS and 2961 (95.4%) COS.
Detailed contraindications or reasons for withholding acute reperfusion therapies in ISUS and other-IHS are presented in Table 5.

Table 5. Contraindication or reason for withholding acute reperfusion therapies

<table>
<thead>
<tr>
<th>Reason for Withholding Acute Reperfusion Therapies</th>
<th>In-stroke unit ischemic stroke (n=138)</th>
<th>Other in-hospital stroke (n=214)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outside of treatment time-window</td>
<td>26 (18.9%)</td>
<td>60 (28.0%)</td>
</tr>
<tr>
<td>Pre-stroke dependency</td>
<td>4 (2.9%)</td>
<td>18 (8.4%)</td>
</tr>
<tr>
<td>Mild stroke</td>
<td>21 (15.2%)</td>
<td>26 (12.1%)</td>
</tr>
<tr>
<td>Established infarct</td>
<td>6 (4.3%)</td>
<td>15 (7.0%)</td>
</tr>
<tr>
<td>Elevated INR or thrombocytopenia</td>
<td>6 (4.3%)</td>
<td>13 (6.1%)</td>
</tr>
<tr>
<td>Therapeutic anticoagulation</td>
<td>18 (13.0%)</td>
<td>37 (17.3%)</td>
</tr>
<tr>
<td>Recent surgical intervention</td>
<td>0 (0%)</td>
<td>52 (24.3)</td>
</tr>
<tr>
<td>Intracranial hemorrhage (current or past)</td>
<td>8 (5.8%)</td>
<td>1 (0.5%)</td>
</tr>
<tr>
<td>Recent ischemic stroke</td>
<td>68 (49.3%)</td>
<td>2 (0.9%)</td>
</tr>
<tr>
<td>Missed stroke/ no good reason to withhold treatment</td>
<td>4 (2.9%)</td>
<td>17 (7.9%)</td>
</tr>
<tr>
<td>Other</td>
<td>-</td>
<td>3 (1.4%)</td>
</tr>
<tr>
<td>Patients with more than one contraindication or reason for withholding acute reperfusion therapies</td>
<td>41 (29.7%)</td>
<td>74 (34.6%)</td>
</tr>
</tbody>
</table>

In multivariate analysis, patients with ISUS were younger, more frequently female, had a higher pre-stroke dependency, a lower prevalence of diabetes and chronic kidney disease, and higher rates of pre-stroke statin therapy compared with other-IHS or COS. In addition, they presented more often with known stroke onset-time and more frequently with large artery atherosclerosis as stroke mechanism. In comparison with other-IHS, ISUS had a lower prevalence of atrial fibrillation and prosthetic heart valves, lower rates of IVT and of missed opportunities to receive acute reperfusion therapies. When compared to COS, ISUS had higher rates of pre-stroke antithrombotic therapy, more frequently a decreased level of consciousness at presentation, more frequently other known causes as stroke mechanism and higher rates of EVT (Table 6).
Table 6. Multivariate multinomial regression models for variables associated with ISUS in comparison with other-IHS (Model 1) and with COS (Model 2)

<table>
<thead>
<tr>
<th></th>
<th>Model 1</th>
<th>Model 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>aOR (95% CI)</td>
<td>p-value</td>
</tr>
<tr>
<td>Age</td>
<td>0.96 (0.93–0.98)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Female sex</td>
<td>2.00 (1.19–3.33)</td>
<td>0.008</td>
</tr>
<tr>
<td>Known stroke onset-time</td>
<td>2.44 (1.39–4.35)</td>
<td>0.002</td>
</tr>
<tr>
<td>Pre-mRS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.72 (0.80–3.70)</td>
<td>0.165</td>
</tr>
<tr>
<td>2</td>
<td>2.78 (1.28–5.88)</td>
<td>0.010</td>
</tr>
<tr>
<td>3</td>
<td>2.86 (1.19–7.14)</td>
<td>0.019</td>
</tr>
<tr>
<td>4</td>
<td>5.88 (2.13–16.67)</td>
<td>0.001</td>
</tr>
<tr>
<td>5</td>
<td>20.00 (4.35–100.00)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Pre-stroke statin therapy</td>
<td>2.44 (1.33–4.35)</td>
<td>0.004</td>
</tr>
<tr>
<td>Pre-stroke antiplatelets</td>
<td>N.S</td>
<td></td>
</tr>
<tr>
<td>Pre-stroke anticoagulation</td>
<td>N.S</td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>0.36 (0.19–0.67)</td>
<td>0.001</td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>0.36 (0.19–0.68)</td>
<td>0.001</td>
</tr>
<tr>
<td>Prosthetic heart valve</td>
<td>0.14 (0.03–0.60)</td>
<td>0.008</td>
</tr>
<tr>
<td>Chronic kidney disease</td>
<td>0.26 (0.12–0.56)</td>
<td>0.001</td>
</tr>
<tr>
<td>Large artery atherosclerosis</td>
<td>3.13 (1.39–7.14)</td>
<td>0.006</td>
</tr>
<tr>
<td>Other known stroke cause</td>
<td>N.S</td>
<td></td>
</tr>
<tr>
<td>Intravenous thrombolysis</td>
<td>0.46 (0.22–0.97)</td>
<td>0.042</td>
</tr>
<tr>
<td>Endovascular treatment</td>
<td>N.S</td>
<td></td>
</tr>
<tr>
<td>Missed opportunity to treat</td>
<td>0.22 (0.06–0.86)</td>
<td>0.030</td>
</tr>
</tbody>
</table>

aOR, adjusted odds ratio; CI, confidence interval; mRS, modified Rankin Scale; N/A, Non-applicable; N.S, Non-significant
In the 3-month mRS shift analysis, univariate analysis showed no differences between ISUS and other-IHS (OR 1.21; 95% CI 0.84–1.79) and an unfavorable shift in ISUS compared with COS (OR, 2.70; 95% CI, 2.00–3.70) (Figure 17).

Figure 17. Disability score proportions on the modified Rankin Scale

Legend.
Distribution of disability scores according to the modified Rankin scale at 3 months for the in-stroke unit ischemic stroke (ISUS), other in-hospital stroke (other-IHS) and community-onset stroke (COS)

However, after adjustment, ISUS was associated with a favorable shift in the distribution of functional outcomes in the 3-month mRS in comparison to other-IHS (adjusted odds ratio, 1.73; 95% CI, 1.11–2.69; P=0.016) or COS (adjusted odds ratio, 1.46; 95% CI, 1.00–2.12; P=0.048) (Table 7).
Table 7. Final multivariate model on 3-month modified Rankin Scale. Adjusted odds ratios and p-values for a favorable shift in the distribution of functional outcomes

<table>
<thead>
<tr>
<th>Site of stroke-onset (ISUS as reference)</th>
<th>aOR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Other-HIS</td>
<td>0.58 (0.37–0.90)</td>
<td>0.016</td>
</tr>
<tr>
<td>COS</td>
<td>0.68 (0.47–0.99)</td>
<td>0.048</td>
</tr>
<tr>
<td>Age</td>
<td>0.98 (0.97–0.99)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Female sex</td>
<td>0.84 (0.74–0.96)</td>
<td>0.001</td>
</tr>
<tr>
<td>Pre-mRS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.57 (0.49–0.69)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>2</td>
<td>0.19 (0.15–0.23)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>3</td>
<td>0.08 (0.06–0.10)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>4</td>
<td>0.07 (0.04–0.12)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>5</td>
<td>0.16 (0.06–0.42)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>0.79 (0.67–0.92)</td>
<td>0.002</td>
</tr>
<tr>
<td>Ischemic heart disease</td>
<td>0.81 (0.66–0.91)</td>
<td>0.013</td>
</tr>
<tr>
<td>Active cancer</td>
<td>0.61 (0.46–0.82)</td>
<td>0.001</td>
</tr>
<tr>
<td>NIHSS</td>
<td>0.87 (0.85–0.88)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Decreased level of consciousness</td>
<td>0.70 (0.53–0.91)</td>
<td>0.008</td>
</tr>
<tr>
<td>Blood glucose</td>
<td>0.95 (0.93–0.98)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ASPECTS</td>
<td>1.11 (1.06–1.16)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Intracranial vessel occlusion</td>
<td>0.62 (0.52–0.75)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Large artery atherosclerosis</td>
<td>0.76 (0.61–0.93)</td>
<td>0.008</td>
</tr>
<tr>
<td>Other known stroke cause</td>
<td>0.60 (0.45–0.76)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Intravenous thrombolysis</td>
<td>1.33 (1.11–1.61)</td>
<td>0.002</td>
</tr>
<tr>
<td>Endovascular treatment</td>
<td>2.00 (1.51–2.63)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

aOR, adjusted odds ratio; CI, confidence interval; ISUS, In-Stroke Unit Ischemic Stroke; Other-IHS, Other In-Hospital Stroke; Community-Onset Stroke; mRS, modified Rankin Scale; NIHSS, National Institutes of Health Stroke Scale; ASPECTS, Alberta Stroke Program Early CT score.
3.5 – Discussion

In this single-center cohort study, patients with ISUS more frequently had a known stroke onset-time than other-IHS or COS, had less missed opportunities to receive acute reperfusion therapies than other-IHS, and had higher rates of EVT than COS. In those with clinical criteria for any acute reperfusion therapy at stroke onset, ISUS had a lower time from stroke recognition-to-imaging and -to EVT compared with other-IHS. In comparison to both other subgroups, ISUS was associated with a significant shift in the distribution of the 3-month mRS towards favorable outcome.

To our knowledge, no previous study has characterized patients with ischemic stroke in the stroke unit and compared them with patients with an ischemic stroke in other clinical settings, namely other in-hospital strokes or community-onset strokes. While the benefits of admitting patients to stroke units are well-known in reducing death, disability, infections and other stroke-related complications, our study also shows the benefits extend to recognizing and treating ischemic stroke within stroke units (as opposed to other wards; the other-IHS comparison group). Our results reveal that ISUS more often have a known onset-time, shorter times to imaging and treatment and less missed treatment opportunities, which speak to the value of continuous or frequent clinical monitoring in the stroke unit. Together with the stroke team’s advanced knowledge, this likely explains the improved adjusted long-term outcomes in ISUS.

As in previous studies, IHS (ISUS and other-IHS) were more severe than COS. In addition, we found a higher prevalence of intracranial vessel occlusion. These differences presumably explain the higher rate of EVT for both IHS subgroups in comparison with COS, which has also been shown previously.
The lower IVT rates in ISUS patients compared with other-IHS probably comes from the high rate of recent preceding strokes in the ISUS subgroup, making them ineligible for such treatment. Of note, IVT rates in the other-IHS patients in our institution were higher than previously reported in the literature.\textsuperscript{132}

The high number of patients with in-hospital stroke recurrences or recent TIA in the ISUS group likely explains the higher frequency of large artery atherosclerosis as stroke mechanism in this group, as acute symptomatic stenosis has been associated with higher early recurrence rates.\textsuperscript{133,134} Similarly, other known stroke causes, including cancer-related hypercoagulable state, were more frequent in our ISUS patients, which is probably due to the high rate of recurrence.\textsuperscript{135,136}

Our study further shows that in-hospital ischemic stroke recurrences can be considered eligible for acute reperfusion therapies, in particular for EVT, and for some, repeated IVT and EVT, as also previously investigated.\textsuperscript{137,138}

Our study has some limitations. Due to its retrospective design, information bias cannot be excluded. On the other hand, we have collected data in a prespecified manner, and our study does not suffer from consent bias given that consent was not required according to the Swiss Human Research Act. The data registered and used for analysis may not include some of the comorbidities and clinical and laboratory variables needed for complete adjustment, and we cannot exclude that additional specific medical and surgical clinical data would explain the differences in stroke care and outcomes. Given the single-center quality assurance nature of this project, the results may not be generalizable to other institutions. Patients with severe medical conditions and mild strokes requiring dedicated care in their specific departments may not have been transferred to our stroke unit and were therefore not captured in our registry. Given that the severity of IHS in our cohort
was similar or lower than in previous studies\textsuperscript{65,122} while the prevalence of IHS was higher than previously described\textsuperscript{65,66} this is likely not a major bias. Patients with ISUS while being hospitalized in the intensive care unit, may have received a different level of care in comparison with patients in regular stroke unit beds. Routine visits by neurology, stroke and neurorehabilitation specialists to the intensive care unit, should have minimized this potential difference. With a relevant number of ISUS patients having stroke recurrences, we acknowledge the difficulty in distinguishing neurological deterioration from a stroke recurrence in some cases. We minimized this issue by applying a standard stroke definition and by reviewing all potential in-hospital stroke recurrences. Only four in-hospital stroke recurrences could not be confirmed by new identifiable parenchymal lesions or vessel occlusion. Of these, only one was considered to be in the same arterial territory as the index event.
3.6 – Conclusion

ISUS more frequently had a known stroke onset-time, lower time-to-imaging and -to-endovascular treatment and less missed opportunities to receive acute reperfusion therapies than other IHS outside of the stroke unit. This preparedness to identify and treat patients by a dedicated stroke team probably explains the better adjusted long-term outcomes of ISUS in comparison with ischemic stroke patients in other clinical settings.
Chapter 4 – Associated factors and long-term prognosis of 24-hour worsening of arterial patency after ischemic stroke

This chapter is based on the following manuscript:

4.1 – Abstract

Background and Purpose

Early arterial recanalization in acute ischemic stroke is strongly associated with better outcomes. However, early worsening of arterial patency was seldom studied. We investigated potential predictors and long-term prognosis of worsening of arterial patency at 24-hours after stroke onset.

Methods

Patients from the ASTRAL registry including admission and 24-hour vascular imaging [Computed tomography angiography (CTA) or magnetic resonance angiography (MRA)] were included. Worsening of arterial patency was defined as a new occlusion and/or significant stenosis in any extra- or intracranial artery, comparing 24-hour with admission imaging. Variables associated with worsening of arterial patency were assessed by stepwise multiple logistic regression. The impact of arterial worsening on 3-month outcome was investigated with an adjusted modified Rankin Scale (mRS) shift analysis.

Results

Among 2152 included patients, 1387 (64.5%) received intravenous thrombolysis and/or endovascular treatment, and 65 (3.0%) experienced 24-hour worsening of arterial patency. In multivariable analysis, history of hypertension seemed protective (aOR 0.45, 95% CI 0.27–0.75), while higher admission NIHSS (aOR 1.06, 95% CI 1.02–1.10), intracranial (aOR 4.78, 95% CI 2.03–11.25) and extracranial stenosis (aOR 3.67, 95% CI 1.95–6.93), and good collaterals (aOR 3.71, 95% CI 1.54–8.95) were independent predictors of worsening of arterial patency. Its occurrence was associated with
a major unfavorable shift in the distribution of the mRS at 3 months (aOR 5.97, 95% CI 3.64–9.79).

Conclusion
Stroke severity and admission vascular imaging findings may help to identify patients at a higher risk of developing worsening of arterial patency at 24-hours. The impact of worsening of arterial patency on long-term outcome warrants better methods to detect and prevent this early complication.
4.2 – Introduction

In patients with AIS, prevalence of symptomatic arterial occlusion ranges from 22 to 46% and is associated with worse outcome.\textsuperscript{139–142} Vessel occlusion is a dynamic process. After initial imaging, recanalization can occur spontaneously\textsuperscript{21,71} or as a result of recanalization treatment (IVT or EVT)\textsuperscript{21,22,29,71}. On the contrary, worsening of arterial patency can arise from thrombus extension or new occlusions, either spontaneously\textsuperscript{71} or after treatment\textsuperscript{72}. Patients achieving partial or complete recanalization after acute treatment can additionally undergo arterial reocclusion, with rates ranging from 2% to 41%\textsuperscript{73–79}.

So far, there have been no studies describing the frequency, characteristics, associated factors and outcome of patients with worsening of arterial patency, independently of whether acute revascularization treatment was performed. Recognizing these risk factors may allow more targeted interventional or drug-based preventive strategies. Furthermore, the clinical impact of this type of arterial deterioration on short and long-term outcomes is insufficiently known.

Our aim was to assess the frequency, associated factors and impact on outcome of worsening of arterial patency in a large cohort of consecutive AIS patients.
### 4.3 – Methods

**Study design and patient selection**

The ASTRAL is a single center-based cohort study of all consecutive AIS patients admitted to the stroke unit and/or intensive care unit of the Lausanne University Hospital within 24 hours of last-seen-well time, as published previously.\(^\text{125}\)

For the current analysis, we retrospectively selected all AIS patients from January 2003 to August 2018 with: (i) good quality acute computed tomography angiography (CTA) or magnetic resonance angiography (MRA), performed within 24-hours of last-seen-well time; (ii) availability of a second good-quality arterial imaging (CTA or MRA) at 24 hours after admission (range: 12–48 hours) and allowing assessment of vessel patency. This time-point was selected as findings at 24-hours are stronger predictors for 3-month outcomes.\(^\text{75,77}\) In addition, imaging 24-hours after revascularization procedure is considered the standard time-point for radiological assessment in most stroke centers.

ASTRAL follows institutional regulations for clinical and research databases. All data collected stem from routine clinical and radiological management, and were anonymized before analysis following the principles of the Swiss Human Research Act. Given that only anonymized data were used, there was no need for ethics committee approval or patient consent according to the Swiss Human Research Act. The reporting of this observational study is in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines.\(^\text{126}\)

**ASTRAL variables**

This registry incorporates a large set of pre-specified parameters including, demographics (age and sex), medical history and vascular risk factors (prestroke mRS, previous stroke or TIA, hypertension, diabetes mellitus, dyslipidemia, smoking, atrial fibrillation,
coronary artery disease, active cancer), current medications (antiplatelets, anticoagulants, antihypertensives and lipid-lowering drugs), process-oriented data (prehospital and door-to-treatment times), stroke localization, stroke severity at admission and 24-hours (per NIHSS, performed or supervised by NIHSS-certified personnel), vital signs (body temperature, blood pressure), metabolic and hematologic parameters (glucose, creatinine, total cholesterol, C-reactive protein, white blood cell count, hemoglobin, platelet count), acute and subacute multimodal brain-imaging (including 24-hour vessel patency), acute recanalization treatments performed and their characteristics, stroke mechanism and long-term clinical outcome.

**Imaging in ASTRAL**

Acute multimodal brain imaging including arterial study of extra- and intracranial arteries is part of the institutional stroke protocol since 2003. Brain imaging was mainly based on CT-imaging until April 2018 (a 16-detector row CT until November 2005 and afterwards on a 64-detector row CT scanner) and mainly MRI-based since May 2018 (3 Tesla MRI).

For strokes involving the middle cerebral artery (MCA) territory, the ASPECTS was recorded, while pc-ASPECTS was calculated for posterior circulation strokes. In patients with MRI, ASPECTS or pc-ASPECTS were evaluated on Diffusion Weighted Imaging/ Apparent Diffusion Coefficient sequences. To compare ASPECT scores assessed with different imaging modalities (CT or MRI), in case of baseline MRI one additional point was added to the original ASPECT score. CTA or MRA were analyzed regarding the presence and location of significant stenosis and/or occlusion (definitions described below). For anterior circulation strokes, the Clot Burden Score\textsuperscript{143} was registered. The Tan collateral score\textsuperscript{143} was recorded in patients with CTA imaging and occlusion of the intracranial internal carotid artery (ICA), first segment of middle cerebral artery (MCA)
or proximal second segment of MCA. In detail, patients were considered to have poor collaterals if collateral supply was filling < 50% of the occluded MCA territory, partial collaterals if collateral supply was filling 50–99% of the occluded MCA territory and good collaterals if collateral supply was filling 100% of the occluded MCA territory. Patients not fulfilling the above criteria for collateral assessment were classified as “not applicable”.

Repeated brain imaging was performed at approximately 24 hours after treatment in essentially all patients receiving acute recanalization treatment. Imaging was also repeated if clinically indicated, including clinical worsening or etiological work-up, with the exception of palliative patients.

A senior board-certified vascular neurologist and senior, reviewed all imaging data with prior knowledge of the basic clinical findings. Difficult and controversial findings were reviewed at a weekly joint neuroradiology conference and consensus was reached. Interrater agreement on different imaging findings for 100 consecutive ASTRAL patients, were good to excellent, as reported previously. For the purpose of this research, all acute and subacute imaging from patients considered to have "worsening of arterial patency" were reviewed again by an experienced neuroradiologist.

**Definition of arterial vessel patency**

In CTA imaging, occlusion was defined as the absence of contrast medium filling the examined arterial segment on initial acquisition and in MRA imaging, as the absent signal in the examined arterial segment and absent immediate distal flow. For extracranial arteries, significant stenosis was defined as a caliber reduction of ≥70% for carotid arteries and ≥50% for vertebral arteries using the North American Symptomatic Carotid Endarterectomy Trial (NASCET) method. For intracranial
arteries, stenosis of ≥50% caliber reduction was defined using the Warfarin-Aspirin Symptomatic Intracranial Disease (WASID) method.\textsuperscript{146}

Definitions of worsening of arterial patency and vessel recanalization

For changes in arterial patency, we considered only CTA and MRA performed at approximately 24-hours (range 12-48 hours). If both gave good quality images, CTA-based imaging was preferred.

Worsening of arterial patency was defined as a new occlusion and/or stenosis, as defined above, in any artery, within or outside the initial ischemic territory, comparing subacute to acute vessel imaging. Each of the following arteries were considered as separate entities to assess worsening: ICA (extra- and intracranial combined), MCA, anterior cerebral artery, posterior cerebral artery, vertebral artery (extra- and intracranial combined) and basilar artery. Progression of previously documented stenosis from a lower to higher degree in the same artery was not considered as worsening of arterial patency. Similarly, an occlusion or stenosis extending to a new arterial segment, within the same artery already occluded at baseline, were not considered. Finally, an iatrogenic embolization to a previous normal territory during EVT was also not considered.

In cases of worsening, each patient was classified as: i) Type of worsening: new occlusion, new stenosis or both; ii) Localization of the worsening: intracranial, extracranial, or both; iii) Worsening occurring within or outside the same axis of a preexisting stenosis/occlusion. In cases of worsening within the same axis of a preexisting stenosis/occlusion, the site of worsening with respect to initial findings was registered (e.g.: proximal, at the site or distal to the initial stenosis/occlusion).
Revascularization treatments and secondary prevention

Acute revascularization treatment (IVT and/or EVT), AIS management and secondary prevention were performed according to hospital criteria at the time of admission. These were based on national and European guidelines and updated with recent positive randomized trial data. Regarding acute revascularizations treatments, IVT treatment was performed up to 3 hours after last-seen-well before 2008 and if the NIHSS was ≥6, and up to 4.5 hours for any disabling stroke, thereafter. EVT was performed up to 2014 for anterior circulation strokes if treatment could be initiated within 6 hours, with NIHSS ≥6, CTA disclosing a proximal intracranial vessel occlusion and CTP showing > 50% of penumbra. Late arriving patients and unknown-onset patients were treated in selected cases, if CTP showed >50% penumbra, and with informed consent. After 2014, for patients arriving within 6 hours, CTP criteria were replaced by ASPECTS ≥5 and the lower NIHSS limit was replaced by the presence of a disabling deficit. From May 2017, we treated patients with these same criteria, but up to 8 hours. After 8 hours, treatment was offered if the NIHSS ≥10 and core <50 mL, or NIHSS <10 and core <30 mL. After January 2018, late treatment was alternatively based on any NIHSS, core <70 mL and the ratio of ischemic tissue volume to initial infarct volume ≥1.8. For posterior circulation stroke in specific cases of basilar artery occlusion, patients were treated with EVT up to 6 hours in the absence of extensive brainstem infarct. From May 2017, the treatment window was extended to 8 hours if pc-ASPECTS ≥7, and up to 24 hours if no transverse irreversible brainstem ischemia (MRI) was present or pc-ASPECTS was ≥8. For all EVT cases, the upper age limit of 80 years was abandoned in 2012.
For antithrombotic therapy within the first 24-48 hours after hospital admission, the following institutional recommendations were considered:

1. No acute recanalization treatment: intravenous aspirin 250mg load followed by daily dose of clopidogrel 75mg (or rarely, aspirin 100mg). Since 2013, clopidogrel 300mg load was added in the presence of an extra- or intracranial stenosis, unstable plaque or recent vascular event in the same vascular territory. In patients with effective preadmission anticoagulation therapy, this therapy was maintained alone, unless large infarct volumes;

2. IVT (with or without additional EVT): antithrombotic therapy was withheld until major intracranial hemorrhage was excluded and thereafter according to point 1;

3. Direct EVT: intravenous aspirin 250mg load immediately before EVT, and thereafter according to point 2. If there was effective preadmission anticoagulation therapy, no antiplatelet therapy was performed;

4. Permanent extracranial stenting: if IVT, immediate intravenous aspirin 250mg load and if no hemorrhagic complications at 12 hours, clopidogrel 300mg load followed by daily dual antiplatelets. If no IVT, double antiplatelet load during intervention, followed by daily dual antiplatelets. If there was effective preadmission anticoagulation therapy, load with only one antiplatelet.

5. In addition to the above, immobilized and/or severe paretic patients were given immediate 40mg of low-molecular-weight heparin (LMWH) in the absence of IVT. LMWH was started in IVT-treated patients at 12-24 hours after exclusion of hemorrhagic complications.
**Stroke mechanism**

Stroke mechanism was classified according to TOAST\(^{127}\), with dissections recorded as additional mechanism and small vessel disease, undetermined or other specified causes grouped together for the multivariate analysis.

**Outcome analysis**

For short-term outcome, we assessed early neurological deterioration (END) at 24 hours, defined as NIHSS increase $\geq 4$ points compared to admission NIHSS.\(^{67}\) Also, early ischemic stroke recurrence (ERIS) up to 48 hours was recorded, with recurrent stroke defined according to the World Health Organization and after reviewing all clinical and radiological data to ascertain the event.\(^{81}\) For long-term outcome, mRS-certified medical personnel assessed mRS at 3 and 12 months, either at the outpatient stroke clinic or by structured telephone interview.\(^{131}\) Mortality at 7 days and at 3 and 12 months was also assessed.

**Statistical analysis**

Continuous data were summarized as median values and IQR and categorical data as absolute numbers and percentages. Logistic regression was performed to identify potential factors associated with worsening of arterial patency, with all relevant clinical, radiological and biological data included in the ASTRAL, as described above. In addition, association of worsening of arterial patency with the following outcomes was assessed: END, ERIS, 7-day, 3- and 12-month mortality, unfavorable outcome (defined as mRS $> 2$ and higher than prestroke mRS score) at 3 and 12 months. Imputation of the missing values of the covariates studied was carried out with the method of chained equations, generating five imputed data sets. Multiple logistic regression analysis for the
identification of factors associated with worsening of arterial patency was performed on each imputed data set and final results were derived by combining the output of the 5 imputed multiple analyses. Stepwise methods were implemented on each imputed data set to identify significant main effects. Variables with imputed values and respective numbers are detailed in Supplemental Material – Table II.

For adjusted clinical outcome, an ordinal logistic regression analysis (shift-analysis) of 3-month mRS was performed with levels 5 and 6 grouped together. This model was adjusted for the same variables included in the multiple logistic regression analysis for factors associated with worsening of arterial patency, including variables associated with 3-month mRS. A subgroup analysis of treated patients (IVT and/or EVT) to search for variables associated with worsening of arterial patency and its impact on outcome was also conducted. In all analyses, the level of significance was set at 5%. Statistical analyses were performed with the Statistical Package R (version 3.5.2).
4.4 – Results

From January 2003 to August 2018, 4972 patients entered the ASTRAL registry. Of these, 2152 fulfilled our inclusion criteria (Figure 18).

Figure 18. Inclusion flow-chart
In the selected cohort, median age was 70.5 (IQR 20.9) and 912 (42.4%) were female. Median admission NIHSS was 9.0 (IQR 12.0), 1369 (63.6%) patients had an intracranial vessel occlusion, 858 (39.9%) were treated with IVT only and 529 (24.6%) received EVT (with or without bridging). Worsening of arterial patency was observed in 65 (3.0%) patients: 60 had a new occlusion, three a new stenosis and two a new occlusion and stenosis. Three typical cases are shown in Figure 19.

Figure 19. Typical cases of worsening of arterial patency

Legend.

A – Admission CTA showing an extracranial left internal carotid occlusion at the site of carotid bifurcation (white arrow). Without intracranial occlusion at admission, a new left middle cerebral artery occlusion was identified at 24 hours (white arrowhead). B – Admission magnetic resonance angiography showing a basilar artery stenosis (black arrowhead). At 24 hours, basilar artery occlusion (black arrow). C – Admission CTA showing a right MCA occlusion (white arrow). At 24 hours, both right (white arrow) and left (white arrowhead) MCA occlusion.
Forty-three patients had intracranial worsening, 11 extracranial and 11 extra- and intracranial. In Figure 20 are presented the number of patients with worsening of arterial patency by localization and initial findings in the same vascular territory.

Figure 20. Worsening of arterial patency by localization and initial findings in the same vascular territory

Worsening occurred within a vascular axis with an initial stenosis/occlusion in 50 patients (77%), while in the remaining 15 patients (23%), it occurred in a vascular axis without preexisting stenosis/occlusion. In the former, 18 had worsening in an artery with previous stenosis, 20 distal and 8 proximal to preexisting stenosis/occlusion, while 4 had arterial worsening both at the site and distal to the previous stenosis.

Factors associated with worsening of arterial patency

Patients with worsening of arterial patency were younger and had a lower frequency of history of hypertension and of preadmission statin therapy. Admission NIHSS and white blood count were higher. In acute imaging, patients with worsening of arterial patency
had a lower frequency of leukoaraiosis and a higher frequency of both intracranial and extracranial stenosis and good collaterals. Regarding etiology, atherosclerosis and dissection were more frequently found in patients with worsening of arterial patency (Table 8).

Table 8. Baseline and stroke characteristics

<table>
<thead>
<tr>
<th></th>
<th>All cohort (n=2152)</th>
<th>No worsening of arterial patency (n=2087)</th>
<th>Worsening of arterial patency (n=65)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>70.5 (20.9)</td>
<td>70.6 (20.7)</td>
<td>65.9 (24.7)</td>
<td>0.013</td>
</tr>
<tr>
<td>Female sex</td>
<td>912 (42.4%)</td>
<td>882 (42.3%)</td>
<td>30 (46.2%)</td>
<td>0.535</td>
</tr>
<tr>
<td>Pre-stroke mRS&gt;2</td>
<td>151 (7.0%)</td>
<td>148 (7.1%)</td>
<td>3 (4.6%)</td>
<td>0.441</td>
</tr>
<tr>
<td><strong>Vascular risk factors</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>1461 (68.0%)</td>
<td>1428 (68.6%)</td>
<td>33 (50.8%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>381 (17.7%)</td>
<td>373 (17.9%)</td>
<td>8 (12.3%)</td>
<td>0.223</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>1631 (76.1%)</td>
<td>1586 (76.3%)</td>
<td>45 (69.2%)</td>
<td>0.199</td>
</tr>
<tr>
<td>Smoking</td>
<td>538 (25.4%)</td>
<td>520 (25.4%)</td>
<td>18 (27.7%)</td>
<td>0.673</td>
</tr>
<tr>
<td>Alcohol</td>
<td>242 (11.4%)</td>
<td>235 (11.4%)</td>
<td>7 (10.8%)</td>
<td>0.875</td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>643 (30.0%)</td>
<td>626 (30.1%)</td>
<td>17 (26.2%)</td>
<td>0.486</td>
</tr>
<tr>
<td>Ischemic heart disease</td>
<td>359 (16.8%)</td>
<td>349 (16.8%)</td>
<td>10 (15.4%)</td>
<td>0.759</td>
</tr>
<tr>
<td>Heart valves</td>
<td>80 (3.7%)</td>
<td>78 (3.7%)</td>
<td>2 (3.1%)</td>
<td>0.772</td>
</tr>
<tr>
<td>Active cancer</td>
<td>103 (4.8%)</td>
<td>100 (4.8%)</td>
<td>3 (4.6%)</td>
<td>0.939</td>
</tr>
<tr>
<td>Previous stroke or TIA</td>
<td>527 (24.7%)</td>
<td>510 (24.6%)</td>
<td>17 (26.6%)</td>
<td>0.729</td>
</tr>
<tr>
<td><strong>Preadmission therapy</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Antiplatelets</td>
<td>699 (32.6%)</td>
<td>681 (32.7%)</td>
<td>18 (27.7%)</td>
<td>0.387</td>
</tr>
<tr>
<td>Anticoagulants</td>
<td>234 (10.9%)</td>
<td>230 (11.0%)</td>
<td>4 (6.2%)</td>
<td>0.179</td>
</tr>
<tr>
<td>Antihypertensives</td>
<td>1176 (54.9%)</td>
<td>1150 (55.4%)</td>
<td>26 (40.0%)</td>
<td>0.014</td>
</tr>
<tr>
<td>Statins</td>
<td>639 (29.8%)</td>
<td>627 (30.2%)</td>
<td>12 (18.5%)</td>
<td>0.034</td>
</tr>
<tr>
<td><strong>Stroke characteristics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anterior circulation</td>
<td>1568 (73.9%)</td>
<td>1519 (73.9%)</td>
<td>49 (75.4%)</td>
<td>0.642</td>
</tr>
<tr>
<td>Admission NIHSS</td>
<td>9.0 (12.0)</td>
<td>9.0 (12.0)</td>
<td>12.0 (12.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Decreased level of consciousness</td>
<td>315 (14.9%)</td>
<td>295 (14.4%)</td>
<td>20 (30.8%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Onset-door time, min</td>
<td>132 (198)</td>
<td>132 (198)</td>
<td>120 (210)</td>
<td>0.743</td>
</tr>
<tr>
<td>Admission SBP, mmHg</td>
<td>150 (34)</td>
<td>150 (34)</td>
<td>150 (36)</td>
<td>0.767</td>
</tr>
<tr>
<td>Admission glucose, mmol/L</td>
<td>6.6 (2.2)</td>
<td>6.6 (2.1)</td>
<td>6.7 (2.1)</td>
<td>0.986</td>
</tr>
<tr>
<td><strong>Imaging</strong></td>
<td></td>
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<tr>
<td>Leukoaraiosis</td>
<td>581 (28.6%)</td>
<td>572 (29.1%)</td>
<td>9 (13.8%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Chronic stroke lesions</td>
<td>617 (30.4%)</td>
<td>603 (30.7%)</td>
<td>14 (21.5%)</td>
<td>0.105</td>
</tr>
<tr>
<td>Baseline ASPECTS</td>
<td>10 (8–10)</td>
<td>10 (8–10)</td>
<td>10 (8–10)</td>
<td>0.756</td>
</tr>
<tr>
<td></td>
<td>Clot Burden Score</td>
<td>Presence of intracranial vessel occlusion</td>
<td>Site of most proximal vessel occlusion</td>
<td>Collaterals</td>
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<td>-------------------------------</td>
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<tr>
<td></td>
<td>9.0 (4.0)</td>
<td>1369 (63.6%)</td>
<td>292 (21.3%)</td>
<td>Poor</td>
</tr>
<tr>
<td></td>
<td>9.0 (4.0)</td>
<td>1327 (63.6%)</td>
<td>277 (20.9%)</td>
<td>Partial</td>
</tr>
<tr>
<td></td>
<td>8.0 (5.0)</td>
<td>42 (64.6%)</td>
<td>434 (32.7%)</td>
<td>Good</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>299 (22.5%)</td>
<td>Not applicable</td>
</tr>
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<td></td>
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<td></td>
<td>87 (6.5%)</td>
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<td></td>
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<td></td>
<td>2 (4.8%)</td>
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Values are presented as median (interquartile range) or as number (proportions). mRS, modified Rankin score; TIA, transient ischemic attack; NIHSS, National Institutes of Health Stroke Scale; ASPECTS, Alberta Stroke Program Early CT score; ICA, internal carotid artery; M1/2/3/4, first, second, third and fourth segments of middle cerebral artery; ACA, anterior cerebral artery; BA, basilar artery; V4, fourth segment of vertebral artery; PCA, posterior cerebral artery; EVT, endovascular treatment

PhD thesis in Medicine – João Pedro Marto
In the multivariate analysis, history of hypertension, admission NIHSS, intracranial stenosis, extracranial stenosis and good collaterals were independently associated with worsening of arterial patency (Table 9).

Table 9. Multivariate logistic regression model for worsening of arterial patency

<table>
<thead>
<tr>
<th></th>
<th>aOR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>History of hypertension</td>
<td>0.45 (0.27–0.75)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Admission NIHSS</td>
<td>1.06 (1.02–1.10)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Intracranial stenosis</td>
<td>4.78 (2.03–11.25)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Extracranial stenosis</td>
<td>3.67 (1.95–6.93)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Good collaterals</td>
<td>3.71 (1.54–8.95)</td>
<td>&lt; 0.001</td>
</tr>
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</table>

aOR, adjusted odds ratio; CI, confidence interval; NIHSS, National Institutes of Health Stroke Scale.

In the subgroup of treated patients (IVT and/or EVT), extracranial stenosis and good collaterals, as well as atherosclerosis and dissection as stroke mechanism were associated with worsening of arterial patency (Table 10). In this analysis, the association between intracranial stenosis and worsening of arterial patency was not significant.
Table 10. Multivariate logistic regression model for worsening of arterial patency in the subgroup of treated patients (IVT and/or EVT)

<table>
<thead>
<tr>
<th></th>
<th>aOR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atherosclerosis</td>
<td>3.09 (1.29–8.03)</td>
<td>0.020</td>
</tr>
<tr>
<td>Dissection</td>
<td>6.14 (2.07–18.26)</td>
<td>0.001</td>
</tr>
<tr>
<td>Extracranial stenosis</td>
<td>2.73 (1.08–6.94)</td>
<td>0.035</td>
</tr>
<tr>
<td>Good collaterals</td>
<td>2.76 (0.98–7.77)</td>
<td>0.055</td>
</tr>
</tbody>
</table>

aOR, adjusted odds ratio; CI, confidence interval.

Association of worsening of arterial patency with outcome

Worsening of arterial patency was associated with both early neurological deterioration (20 [30.7%] vs 146 [7.0%]) and early recurrence of ischemic stroke (19 [29.2%] vs 14 [0.7%]). Mortality was higher at all three time-points (16 [25.4%] vs 69 [3.5%], 21 [32.3%] vs 206 [11.3%], and 21 [36.8%] vs 279 [17.6%], respectively at 7 days and 3 and 12 months) and unfavorable outcome at 3 months (49 [75.4%] vs 814 [39.0%]) and 12 months (41 [71.9%] vs 622 [39.3%]) was more frequent in patients with 24-hour worsening of arterial patency (Table 11).

Table 11. Univariate logistic regression model for the association between worsening of arterial patency and different outcomes

<table>
<thead>
<tr>
<th></th>
<th>END</th>
<th>ERIS</th>
<th>7-day mortality</th>
<th>3-month poor outcome</th>
<th>3-month mortality</th>
<th>12-month poor outcome</th>
<th>12-month mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Worsening of arterial patency</td>
<td>5.60 (3.16–9.64)</td>
<td>61.16 (29.10–131.76)</td>
<td>9.53 (5.02–17.33)</td>
<td>5.37 (2.70–8.49)</td>
<td>3.73 (2.14–6.33)</td>
<td>4.60 (2.20–7.11)</td>
<td>2.72 (1.54–4.70)</td>
</tr>
</tbody>
</table>

Results are presented as odds ratio and (95% confidence intervals). END, early neurological deterioration; ERIS, early recurrence of ischemic stroke. In all p-value<0.001.
In the 3-month mRS shift-analysis, worsening of arterial patency was associated with an unfavorable shift in the distribution of functional outcomes in the 3-month mRS (aOR 5.97, 95% CI 3.64–9.79; p-value<0.001) (Figure 21 and Table 12). The same association was found in the subgroup of patients undergoing IVT and/or EVT (aOR 6.14, 95% CI 3.36–11.20; p-value<0.001).

Figure 21. Distribution of disability scores on the modified Rankin scale at 3 months according to the presence of worsening of arterial patency
Table 12. Final multivariate model on 3-month modified Rankin Scale. Adjusted odds ratios and p-values for unfavorable shift in the distribution of functional outcomes

<table>
<thead>
<tr>
<th></th>
<th>aOR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>1.02 (1.02–1.03)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Pre-stroke mRS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.70 (1.38–2.09)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>2</td>
<td>3.80 (2.81–5.12)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>&gt;2</td>
<td>12.27 (8.59–17.54)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Admission NIHSS</td>
<td>1.11 (1.08–1.13)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Decreased level of consciousness</td>
<td>1.81 (1.35–2.43)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Anterior circulation</td>
<td>0.68 (0.55–0.84)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Admission glucose</td>
<td>1.08 (1.04–1.12)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>ASPECTS</td>
<td>0.85 (0.80–0.89)</td>
<td>&lt; 0.001</td>
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<tr>
<td>Leukoaraisis</td>
<td>1.44 (1.19–1.76)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Clot Burden Score</td>
<td>0.88 (0.84–0.93)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Partial collaterals</td>
<td>0.74 (0.57–0.97)</td>
<td>0.028</td>
</tr>
<tr>
<td>Intravenous thrombolysis</td>
<td>0.79 (0.64–0.98)</td>
<td>0.029</td>
</tr>
<tr>
<td>Endovascular treatment</td>
<td>0.64 (0.50–0.82)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Cardioembolism</td>
<td>0.74 (0.60–0.90)</td>
<td>0.002</td>
</tr>
<tr>
<td>Worsening of arterial patency</td>
<td>5.97 (3.64–9.79)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

aOR, adjusted odds ratio; CI, Confidence interval. mRS, modified Rankin score; NIHSS, National Institutes of Health Stroke Scale; ASPECTS, Alberta Stroke Program Early CT score.
4.5 – Discussion

In our cohort study of consecutive AIS patients, we found admission NIHSS, intracranial and extracranial stenosis and good collaterals to be independently associated with 24-hour worsening of arterial patency. History of hypertension seemed protective. Worsening of arterial patency was clinically relevant for both short- and long-term outcomes, with a significant effect on shifting the distribution of 3-month mRS towards unfavorable outcomes.

To our knowledge, no previous studies have documented presence of new occlusion or stenosis in AIS patients after comparing subacute with acute vessel imaging. In our cohort, 3% of patients experienced worsening of arterial patency, comparable to some studies on vessel reocclusion after complete recanalization. However, our numbers were considerably lower than a study on preprocedural worsening of arterial occlusion in AIS patients selected for EVT or in comparison to other studies of vessel reocclusion after different revascularization treatments. Different definitions and more restrictive inclusion criteria (mainly patients with large vessel occlusion) probably contributed to the discrepancies.

In our study, admission NIHSS was independently associated with worsening of arterial patency. Higher stroke severity at admission measured by the NIHSS is a well-known predictor of poor outcome after stroke. Previously, one study showed association between higher admission NIHSS and vessel reocclusion after IVT treatment. Admission NIHSS can be a surrogate for both proximal vessel occlusion and clot burden, meaning higher rates of incomplete clot dissolution, especially when EVT is not performed. Residual clots can act as a nidus for in-situ thrombosis or as a source of artery-to-artery embolization, therefore increasing the risk of worsening of arterial patency.
However, given that in our study worsening of arterial patency was not associated with presence of vessel occlusion or CBS, other unexplored mechanisms could be sought. Both intracranial and extracranial stenosis were associated with arterial worsening. Most commonly of atherosclerotic origin, stenosis can also result from an embolic thrombus or arterial dissection. Mechanisms by which stenosis can promote worsening of arterial patency are in-situ thrombosis, artery-to-artery embolization and distal hypoperfusion with blood stasis and increased thrombogenicity. Acutely symptomatic stenosis have been associated with high recurrence rates, risk of distal embolization and reocclusion.

Good collateral status is an established predictor of good outcome after AIS. Nevertheless, some studies have shown that better collateral status was associated with vessel reocclusion, new distal vessel occlusions and stroke recurrence. In our study, good collaterals were independently associated with worsening of arterial patency, which could be explained by various mechanisms. Higher collateral flow may compete with antegrade perfusion, resulting in a slower flow at the site of the culprit lesion and consequent increased local thrombogenicity. Alternatively, good collaterals may enhance flow distally to the occlusion, which could then more easily dislodge unstable parts of the occlusive clot and cause new distal occlusions. In our view, the role of collaterals in stroke recurrence or vessel status deterioration needs further clarification.

Hypertension is an established major risk factor for stroke and late stroke recurrence. In our study, after multivariate analysis, hypertension was negatively associated with worsening of arterial patency. One hypothesis for this association may come from the role of chronic hypertension as the main risk factor for small vessel disease stroke, which was never accompanied by worsening of arterial patency in our cohort. However, chronic hypertension also has an important effect in the pathogenesis of other
stroke mechanisms (e.g.: large artery disease and atrial fibrillation). Therefore, alternative hypotheses could be suggested and our observation should be verified in other cohorts. 24-hour worsening of arterial patency was associated with both short- and long-term outcome. Indeed, odds ratios above 5.0 were found for END, ERIS and unfavorable shift in the distribution of functional outcomes at 90 days. Previous studies, focusing on vessel reocclusion or other definitions of arterial patency deterioration, also showed an association with either short-or long-term outcome.

Given the clinical impact of worsening of arterial patency, efforts to detect and prevent its occurrence seem warranted. This could include careful early monitoring of neurological status and continuous or repeated evaluation by transcranial Doppler. If worsening of arterial patency is confirmed, both IVT and EVT have been described as options in patients with early recurrent stroke. In addition, some patients will benefit from early aggressive anti-platelet regimen and despite limited data, high-intensity statins may also be considered.

This study has some limitations. Due to our retrospective design, information bias cannot be excluded, although data was collected in a pre-specified manner. Being a single-center study, selection bias may have influenced our results. Nevertheless, our cohort includes primary and tertiary-referred patients. We included patients treated over large periods of time and as treatment criteria and techniques and patient management protocols have changed over the years, this may have biased our results. Additionally, our choice of assessing worsening arterial patency between admission and 24-hour imaging may not have captured the dynamics of recanalization and reocclusion, nor its precise time-point. On the other hand, this definition allowed for a uniform classification among a heterogeneous sample of patients, regardless of whether acute revascularization treatment
was performed. The exclusion of patients without 24-hour vessel imaging may have led to an underestimation of worsening and therefore biased our results.
4.6 – Conclusion

Higher stroke severity, presence of intra- and extracranial stenosis and good collaterals seem to predict 24-hour worsening of arterial patency in acute ischemic stroke. History of hypertension was negatively associated with its occurrence. The impact of worsening of arterial patency on long-term outcome may warrant better methods to detect and prevent it.
Chapter 5 – 24-hour reocclusion after successful mechanical thrombectomy: associated factors and long-term prognosis

This chapter is based on the following manuscript:

5.1 – Abstract

Background and Purpose

Early arterial recanalization is a strong determinant of prognosis in acute ischemic stroke. Nevertheless, reocclusion can occur after initial recanalization. We assessed associated factors and long-term prognosis of reocclusion after mechanical thrombectomy (MT).

Methods

Consecutive patients from the prospectively constructed ASTRAL cohort were included if treated by successful MT [modified treatment in cerebral infarction (mTICI) 2b–3] and if a 24-hour vascular imaging [Computed tomography angiography (CTA) or magnetic resonance angiography (MRA)] was available. Reocclusion at this time-point was defined as new intracranial occlusion within an arterial segment recanalized at the end of MT. Stepwise multivariate logistic regression was used to investigate associated factors and long-term prognosis (unfavorable at 3-months when mRS > 2). In a 4:1 matched-cohort, we also assessed the presence of residual thrombus or stenosis in the post-recanalization angiographic images as a potential factor associated with reocclusion.

Results

Among 473 patients with successful recanalization, 432 (89%) were included. 28 (6.6%) patients showed 24-hour reocclusion. Preadmission statin therapy (aOR 0.27, 95% CI 0.08–0.94), intracranial internal carotid artery occlusion (aOR 3.53, 95% CI 1.50–8.32), number of passes (aOR 1.31, 95% CI 1.06–1.62), transient reocclusion during MT (aOR 8.55, 95% CI 2.14–34.09) and atherosclerotic etiology (aOR 3.14, 95% CI 1.34–7.37) were independently associated with reocclusion. In the matched-cohort analysis, presence
of residual thrombus or stenosis was also associated with reocclusion (aOR 15.6, 95% CI 4.6–52.8). Patients experiencing reocclusion had a higher likelihood of an unfavorable outcome (aOR, 5.0; 95% CI 1.2–20.0).

Conclusion

Reocclusion within 24-hours of successful MT was independently associated with statin pre-treatment, occlusion site, more complex procedures, atherosclerotic etiology and presence of residual thrombus or stenosis after recanalization. Reocclusion impact on long-term outcome highlights the need to improve strategies to monitor and prevent this early complication.
5.2 – Introduction

Rapid vessel recanalization is the cornerstone of AIS treatment and is a strong determinant of better functional outcomes.\textsuperscript{21,22} Nevertheless, after successful recanalization, between 2 and 41\% of patients experience reocclusion of the treated vessel, \textsuperscript{73–79} which was associated with unfavorable prognosis.\textsuperscript{74,77–79} Of the five randomized trials that established EVT as the standard treatment for large vessel occlusion in AIS\textsuperscript{29}, four documented recanalization rates at 24-hours, but only one reported the rate of reocclusion after successful recanalization in a secondary analysis\textsuperscript{77}. Furthermore, predictors and clinical impact of reocclusion after successful MT are insufficiently known, as they were only investigated in two of the above-mentioned studies, with conflicting results.\textsuperscript{78,79}

Our aim was to add further knowledge on the frequency, associated factors and long-term prognosis of reocclusion after successful MT, in a prospectively collected cohort of consecutive AIS patients.
5.3 – Methods

Study design and patient selection

The ASTRAL is a single-center prospectively collected cohort of all consecutive AIS patients admitted to the stroke unit and/or intensive care unit of Lausanne University Hospital, within 24 hours after last-well time, as published previously. For this study, we retrospectively selected all AIS patients included in ASTRAL from January 2003 to August 2018, fulfilling the following inclusion criteria: (i) acute symptomatic intracranial vessel occlusion treated by MT with stent retriever and/or aspiration catheter; (ii) successful recanalization at the end of the procedure (defined as modified Thrombolysis in Cerebral Infarction [mTICI] score, 2b or 3); (iii) availability of good quality arterial imaging (CTA or MRA) 24 hours after the procedure (range: 12–48 hours) to assess vessel patency. This time-point was selected as findings at 24 hours are stronger predictors for 3-month outcomes. In addition, imaging 24 hours after revascularization procedure is considered the standard time-point for radiological assessment in most stroke centers.

ASTRAL follows institutional regulations for clinical and research databases. All data collected stem from routine clinical and radiological management, and were anonymized before analysis following the principles of the Swiss Human Research Act. Given that only anonymized data were used, there was no need for ethics committee approval or patient consent according to the Swiss Human Research Act. The reporting of this observational study is in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines.
ASTRAL variables

This registry incorporates a large set of pre-specified parameters including, demographics (age and sex), medical history and vascular risk factors (prestroke mRS, previous stroke or TIA, hypertension, diabetes mellitus, dyslipidemia, smoking, atrial fibrillation, coronary artery disease, active cancer), current medications (antiplatelets, anticoagulants, antihypertensives and lipid-lowering drugs), process-oriented data (prehospital and door-to-treatment times), stroke localization, stroke severity at admission and 24 hours (per NIHSS, performed or supervised by NIHSS-certified personnel), vital signs (body temperature, blood pressure), metabolic and hematologic parameters (glucose, creatinine, total cholesterol, C-reactive protein, white blood cell count, hemoglobin, platelet count), acute and subacute multimodal brain-imaging (including 24-hour vessel patency), acute recanalization treatments performed and their characteristics, stroke mechanism and long-term clinical outcome.

Imaging in ASTRAL

Acute multimodal brain imaging including arterial study of extra- and intracranial arteries and perfusion imaging is part of the institutional stroke protocol since 2003. Brain imaging was mainly based on Computed Tomography (CT)-imaging until April 2018 (a 16–detector row CT until November 2005 and afterwards a 64–detector row CT scanner) and mainly Magnetic Resonance Imaging (MRI)-based since May 2018 (3 Tesla MRI). For strokes involving the MCA territory, the ASPECTS was recorded, while pc-ASPECTS was calculated for posterior circulation strokes. In patients with MRI data, ASPECTS or pc-ASPECTS were evaluated on Diffusion Weighted Imaging/ Apparent Diffusion Coefficient sequences. We also recorded presence of chronic ischemic lesions and leukoaraiosis. To compare ASPECT scores assessed with different imaging
modalities (CT or MRI), in case of baseline MRI one additional point was added to the original ASPECT score.

Regarding arterial vessel imaging, (mainly CTA-based with multidetector-array technology in helicoidal mode, rarely contrast-enhanced MRA) we registered the presence of occlusion and/or stenosis, as well as its location. We considered occlusion if CTA showed absence of contrast medium filling the examined arterial segment on initial acquisition, while for MRA, absent signal in the examined arterial segment and absent immediate distal flow. For extracranial arteries, significant stenosis was defined as a caliber reduction of ≥70% for carotid arteries and ≥50% for vertebral arteries using the North American Symptomatic Carotid Endarterectomy Trial (NASCET) method. For intracranial arteries, stenosis of ≥50% caliber reduction was defined using the Warfarin-Aspirin Symptomatic Intracranial Disease (WASID) method. For anterior circulation stroke, Clot Burden Score (CBS) was registered as well as the Collateral Score, in patients with CTA imaging and proximal intracranial occlusion (intracranial internal carotid artery [ICA], MCA first segment and proximal second segment).

Repeated brain CT or MRI, including CTA or MRA, was repeated 24 hours (permitted range 12-48 hours) after acute recanalization treatment. If both good quality studies were available, we gave priority to CTA. If a hemorrhagic transformation was present, we classified it into radiological and clinically symptomatic groups according to ECASS-II.

A senior board-certified vascular neurologist and senior neuroradiologist, reviewed all imaging data. Difficult and controversial findings were reviewed at a weekly joint neuroradiology conference and consensus was reached. Inter-rater agreement on different imaging findings for 100 consecutive ASTRAL patients, were good to excellent, as reported previously.
Endovascular treatment variables in ASTRAL

In patients treated with MT, we collected the following variables: onset-to-groin puncture and onset-to-recanalization times, type of device used (stent retriever, aspiration, balloon angioplasty, permanent stent or balloon-guiding catheter), number of device passes, type of anesthesia technique (general anesthesia or conscious sedation), occurrence of procedural complications (embolization in previously normal territory [i.e. embolization in an artery without initial clinical and/or radiological ischemia], transient reocclusion during procedure and iatrogenic vessel perforation or dissection) and degree of reperfusion at the end of the procedure (according to mTICI score and classified in the angio-suite by the neurointerventionalist).

Definition of reocclusion after successful mechanical thrombectomy

As stated above, we assessed vessel patency at 24-hours (12-48 hours window) by CTA or MRA. Reocclusion was defined as a new intracranial occlusion on 24-hour imaging within an arterial segment recanalized at the end of MT. Embolization in previously normal territories during MT was not considered as reocclusion.

Additional angiographic imaging analysis

We reviewed angiographic images for assessing the presence of residual thrombus fragments or stenosis in the final angiographic series post-recanalization. We defined the presence of residual thrombus fragments or stenosis as an intraluminal focal filling defect or focal irregular arterial narrowing (>50%). Transient procedure-related vasospasm was differentiated from residual intracranial stenosis, since an additional angiographic image (after 10-15 minutes) was routinely performed by the neurointerventionalist.
We analyzed the angiographic images of all patients with reocclusion, and in a 4:1 control-group matched for the site of most proximal intracranial vessel occlusion and intervention period (before or after 2015). Two investigators evaluated subtracted cranial anteroposterior and lateral views of cerebral angiograms prior to and following final recanalization and in cases of discordance, presence of significant findings were obtained by consensus.

Revascularization treatments and secondary prevention

Acute revascularization treatment (IVT and/or EVT), AIS management and secondary prevention were performed according to hospital criteria at the time of admission. These were based on national\textsuperscript{128} and European guidelines\textsuperscript{129,130} and updated with recent positive randomized trial data. Regarding acute revascularizations treatments, IVT treatment was performed up to 3 hours after last-seen-well before 2008 and if the NIHSS was ≥6, and up to 4.5 hours for any disabling stroke, thereafter. EVT was performed up to 2014 for anterior circulation strokes if treatment could be initiated within 6 hours, with NIHSS ≥6, CTA disclosing a proximal intracranial vessel occlusion and CTP showing > 50% of penumbra. Late arriving patients and unknown-onset patients were treated in selected cases, if CTP showed >50% penumbra, and with informed consent. After 2014, for patients arriving within 6 hours, CTP criteria were replaced by ASPECTS ≥5 and the lower NIHSS limit was replaced by the presence of a disabling deficit. From May 2017, we treated patients with these same criteria, but up to 8 hours. After 8 hours, treatment was offered if the NIHSS ≥10 and core <50 mL, or NIHSS <10 and core <30 mL.\textsuperscript{30} After January 2018, late treatment was alternatively based on any NIHSS, core <70 mL and the ratio of ischemic tissue volume to initial infarct volume ≥1.8.\textsuperscript{31} For posterior circulation stroke in specific cases of basilar artery occlusion, patients were treated with EVT up to
6 hours in the absence of extensive brainstem infarct. From May 2017, the treatment window was extended to 8 hours if pc-ASPECTS ≥7, and up to 24 hours if no transverse irreversible brainstem ischemia (MRI) was present or pc-ASPECTS was ≥8. For all EVT cases, the upper age limit of 80 years was abandoned in 2012.

For antithrombotic therapy within the first 24-48 hours after hospital admission, the following institutional recommendations were considered:

1. Bridging EVT, with or without extra- or intracranial balloon angioplasty:
   a. Acute phase: no antithrombotic therapy before 12-24 hours control imaging.
   b. After exclusion of major intracranial hemorrhage at 12-24 hours control imaging:
      intravenous aspirin 250mg load followed from the next day by daily dose of clopidogrel 75mg (starting with oral 300 mg load)

2. Direct EVT, with or without extra- or intracranial balloon angioplasty:
   a. Acute phase: intravenous aspirin 250 mg load immediately before procedure.
   b. After exclusion of major intracranial hemorrhage at 12-24 hours control imaging:
      daily dose of clopidogrel 75mg (starting with oral 300 mg load)

3. Presence of unstable (extra- or intracranial) symptomatic atherosclerotic plaques based on radiological evaluation:
   a. Acute phase: 1.a and 2a.
   b. After control imaging at 12-24 hours: intravenous aspirin 250 mg load AND oral clopidogrel 300 mg load followed by daily dual antiplatelets (no aspirin load if already performed in acute phase, but oral aspirin 100 mg).

4. Permanent extra- or intracranial stenting, or local (extra- or intracranial) rethrombosis during EVT: if preceding IVT, immediate intravenous aspirin 250mg load. If no hemorrhagic complications at 12-24 hours, clopidogrel 300mg load
followed by daily dual antiplatelets (aspirin 100 mg and clopidogrel 75 mg daily). If no previous IVT, double antiplatelet load during intervention, followed by daily dual antiplatelets.

5. In all cases: if effective anticoagulation was documented at admission, this treatment was continued, thereby replacing the initial antiplatelet agent and its load.

6. For antithrombotic prevention of venous thrombosis, prophylactic dose of low-molecular-weight heparin was given immediately after admission in immobilized and/or patients with severe paresis. This therapy was withheld for 24 hours if IVT was performed.

The following stent retriever and aspiration catheters devices were used for mechanical thrombectomy treatment: Solitaire (Medtronic, Dublin, Ireland), Trevo (Stryker, Cork, Ireland), Catch (Balt, Montmorency, France), Merci (Penumbra, Alameda, CA), ACE (Penumbra, Alameda, CA), SOFIA (Microvention, Tustin, CA) and Catalyst (Stryker, Cork, Ireland).

*Stroke etiology*

We classified stroke etiology according to TOAST,\textsuperscript{127} with dissections recorded as additional mechanism and with both unknown and other specified causes grouped together.

*Outcome analysis*

For short-term outcome, early neurological deterioration (END) at 24 hours (NIHSS increase ≥ 4 compared to admission)\textsuperscript{67} and “delta 24h NIHSS” (difference between admission NIHSS and 24-hour NIHSS) were assessed. For long-term outcome, mRS was
assessed at 3 months by mRS-certified medical personnel, either at the outpatient stroke clinic or by structured telephone interview. Outcome was considered favorable if mRS was $\leq 2$ or equal to the prestroke mRS score if the prestroke mRS $>2$. 

**Statistical analysis**

We summarized continuous data as median value and IQR and categorical data as absolute numbers and percentage. We compared baseline variables and short-term outcomes between the reocclusion and non-reocclusion subgroups, using the Fisher’s exact tests for categorical variables, and Mann–Whitney U tests for continuous variables, as appropriate.

Demographic, clinical, imaging and MT procedural variables associated with 24-hour reocclusion in the univariate analyses (p-value $<0.10$), were entered as independent variables into a multivariable logistic model with 24-hour reocclusion as dependent variable, adopting a backward-stepwise approach using a removal criterion of p-value $>0.10$.

A second analysis was conducted on a cohort matched for occlusion site and treatment period (before and after 2015), using a 4:1 random matching. For patients included in this matched cohort we reviewed all angiographies for the presence of residual thrombus or stenosis in the final angiographic series post-recanalization. We then conducted a new multivariable logistic regression model, with this additional independent variable included among the other potential predictors of reocclusion on the matched cohort.

Since some patients underwent acute intracranial balloon angioplasty and/or permanent stenting, factors that could potentially increase the risk of reocclusion and therefore perceived as a source of bias, we performed a first sensitivity analysis on the matched cohort excluding patients undergoing intracranial stenting and a second one excluding
also those receiving balloon angioplasty. We also performed a sensitivity analysis excluding patients with posterior circulation stroke.

Finally, we performed a multivariable logistic regression analysis to assess the association between 24-hour reocclusion and 3-month outcome. In the multivariate models, results were displayed with aOR and 95% CI. For Statistical analysis, we used R statistical software (version 3.3.2, R Core Team [2016], R Foundation for Statistical Computing, Vienna, Austria).
5.4 – Results

From January 2003 to August 2018, 4972 patients entered the ASTRAL registry. Of these, 551 (11.1%) underwent mechanical thrombectomy for intracranial vessel occlusion and 423 satisfied the inclusion criteria of our study (Figure 22).

Figure 22. Inclusion flow-chart
In the study cohort, median age was 71.4 years (IQR 59.5–80.0) and 181 (42.8%) were female. At baseline, median NIHSS was 15 (IQR 9–19), median ASPECTS 9 (IQR 8–10), the most frequent site of arterial occlusion was the first segment of MCA (41.4%, n=175). IVT prior to MT was administered in 289 patients (68.4%) and median time from onset-to-groin puncture was 233 minutes (IQR 175–335). The majority of included patients were admitted after 2015 (73.7%, n=314). Reocclusion at 24 hours was present in 28 (6.6%) patients and was considered symptomatic in 20 of them (71.4%). 10 out of the 28 (35.7%) fulfilled criteria for END. Illustrative images of two patients with 24-hour reocclusion are shown in Figure 23. Detailed information on each patient with reocclusion is presented in Table 13.
Figure 23. Illustrative images of two patients with 24-hour reocclusion

Panel 1 – Admission CTA with a filling defect in the left MCA (white arrow head in A). DSA imaging disclosed a left M1 occlusion (white arrow head in B). Recanalization after mechanical thrombectomy with mTICI 3 (C). Final angiographic images after recanalization disclosed a small residual thrombus at the level of the lenticulostriate arteries (white arrow in D). 24-hour CTA shows a left M1 reocclusion (white arrow head in E). CT shows an ischemic infarct in the deep left MCA territory (F).

Panel 2 – Patient with a basilar artery occlusion (white arrow head in A). DSA imaging confirmed the occlusion (white arrow head in B). Recanalization after MT with mTICI 2b (C). Final angiographic images after recanalization with intraluminal filling defect suggesting small intraluminal thrombus (with arrow head in D). 24-hour CTA shows a reocclusion of the basilar artery (with arrow head in E).
Table 13. Detailed description of patients with 24-hour vessel reocclusion

<table>
<thead>
<tr>
<th>Sex, age</th>
<th>Etiology</th>
<th>IVT</th>
<th>mTICI</th>
<th>Reocclusion during MT</th>
<th>Residual stenosis or luminal thrombus</th>
<th>Occlusion site</th>
<th>Reocclusion site</th>
<th>END</th>
<th>3-month mRS</th>
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<tbody>
<tr>
<td>M, 54</td>
<td>Dissection</td>
<td>Yes</td>
<td>3</td>
<td>No</td>
<td>No</td>
<td>ICA (intra) and M1</td>
<td>ICA (intra)</td>
<td>No*</td>
<td>1</td>
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<tr>
<td>F, 54</td>
<td>Mechanic heart valve</td>
<td>No</td>
<td>3</td>
<td>No</td>
<td>Yes</td>
<td>M2</td>
<td>M2</td>
<td>Yes</td>
<td>3</td>
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<tr>
<td>F, 59</td>
<td>LAA</td>
<td>Yes</td>
<td>2b</td>
<td>No</td>
<td>Yes</td>
<td>ICA (intra) and M1-2</td>
<td>ICA (intra)</td>
<td>No</td>
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<td>F, 78</td>
<td>LAA</td>
<td>No</td>
<td>2b</td>
<td>No</td>
<td>Yes</td>
<td>ICA (intra)</td>
<td>ICA (intra)</td>
<td>Yes</td>
<td>6</td>
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<td>M, 63</td>
<td>LAA</td>
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<td>2b</td>
<td>No</td>
<td>No</td>
<td>ICA (extra and intra)</td>
<td>ICA (extra and intra)</td>
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<td>M, 80</td>
<td>LAA</td>
<td>Yes</td>
<td>2b</td>
<td>No</td>
<td>Yes</td>
<td>ICA (extra and intra) and MCA</td>
<td>ICA (intra)</td>
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<tr>
<td>M, 66</td>
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<td>No</td>
<td>No</td>
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<td>ICA (extra and intra)*</td>
<td>No*</td>
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<tr>
<td>F, 26</td>
<td>ESUS</td>
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<td>3</td>
<td>No</td>
<td>No</td>
<td>M2</td>
<td>M2</td>
<td>No*</td>
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<td>M, 41</td>
<td>Vasculitis</td>
<td>Yes</td>
<td>2b</td>
<td>Yes</td>
<td>Yes</td>
<td>ICA (intra) and M1</td>
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<td>F, 72</td>
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<td>3</td>
<td>No</td>
<td>Yes</td>
<td>M1 and A1</td>
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<td>2b</td>
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<td>M2</td>
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<td>M1</td>
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<td>M, 65</td>
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<td>2b</td>
<td>No</td>
<td>Yes</td>
<td>V4 and BA</td>
<td>V4 and BA</td>
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<tr>
<td>M, 73</td>
<td>LAA and cardiac aneurysm</td>
<td>Yes</td>
<td>3</td>
<td>No</td>
<td>Yes</td>
<td>P1</td>
<td>P1</td>
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<td>LAA</td>
<td>Yes</td>
<td>3</td>
<td>No</td>
<td>Yes</td>
<td>V4 and BA</td>
<td>BA</td>
<td>No</td>
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<tr>
<td>F, 90</td>
<td>AF</td>
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<td>3</td>
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<td>M1</td>
<td>M1</td>
<td>Yes</td>
<td>6</td>
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<tr>
<td>M, 52</td>
<td>ESUS</td>
<td>Yes</td>
<td>2b</td>
<td>No</td>
<td>Yes</td>
<td>M2</td>
<td>M2</td>
<td>No</td>
<td>3</td>
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<tr>
<td>M, 21</td>
<td>PFO</td>
<td>Yes</td>
<td>3</td>
<td>No</td>
<td>Yes</td>
<td>M2</td>
<td>M2</td>
<td>No*</td>
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<td>F, 87</td>
<td>AF</td>
<td>Yes</td>
<td>3</td>
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<td>Yes</td>
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<td>No*</td>
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<td>No</td>
<td>ICA (extra and intra) and MCA</td>
<td>ICA (extra and intra)</td>
<td>No*</td>
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<td>M, 76</td>
<td>LAA</td>
<td>Yes</td>
<td>2b</td>
<td>No</td>
<td>No</td>
<td>ICA (extra and intra), MCA and ACA</td>
<td>M2</td>
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<td>LAA</td>
<td>Yes</td>
<td>2b</td>
<td>No</td>
<td>No</td>
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<td>ICA (extra and intra)</td>
<td>No</td>
<td>2</td>
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<tr>
<td>M, 49</td>
<td>AF</td>
<td>Yes</td>
<td>3</td>
<td>No</td>
<td>Yes</td>
<td>ICA (extra and intra) and M1-2</td>
<td>M2</td>
<td>No</td>
<td>4</td>
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<tr>
<td>M, 70</td>
<td>ESUS</td>
<td>No</td>
<td>3</td>
<td>No</td>
<td>Yes</td>
<td>M1-2</td>
<td>M2</td>
<td>No*</td>
<td>6</td>
</tr>
<tr>
<td>M, 52</td>
<td>LAA</td>
<td>No</td>
<td>2b</td>
<td>No</td>
<td>Yes</td>
<td>ICA (extra and intra) and M1-2</td>
<td>M1</td>
<td>Yes*</td>
<td>6</td>
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<tr>
<td>F, 55</td>
<td>Trousseau syndrome</td>
<td>Yes</td>
<td>3</td>
<td>No</td>
<td>Yes</td>
<td>M1-2</td>
<td>M2</td>
<td>No</td>
<td>1</td>
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<tr>
<td>M, 71</td>
<td>LAA</td>
<td>No</td>
<td>2b</td>
<td>No</td>
<td>Yes</td>
<td>Both V4 and BA</td>
<td>Both V4 and BA</td>
<td>Yes</td>
<td>5</td>
</tr>
<tr>
<td>M, 61</td>
<td>LAA</td>
<td>No</td>
<td>2b</td>
<td>Yes</td>
<td>Yes</td>
<td>VA4, BA and PCA</td>
<td>VA4</td>
<td>No</td>
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</tbody>
</table>

IVT – Intravenous thrombolysis; TICI – Thrombolysis in cerebral infarction; MT – Mechanical thrombectomy; END – Early neurological deterioration; mRS – modified Rankin Scale; F – Female; M – Male; MCA – Middle cerebral artery; ICA – Internal carotid artery; Intra – Intracranial; Extra – Extracranial; M1 – Middle cerebral artery first segment; M2 – Middle cerebral artery second segment; ACA – Anterior cerebral artery; V4 – Vertebral artery fourth segment; BA – Basilar artery; PCA – Posterior cerebral artery; P1 – Posterior cerebral artery first segment; LAA – Large artery atherosclerosis; ESUS – Embolic stroke of undetermined source; AF – Atrial fibrillation; NIHSS – National Institutes of Health Stroke Score;

* = clinical deterioration not captured by the END definition.

† = parenchymal hemorrhage type 2 per European Co-operative Acute Stroke Study-II (ECASS-II).

* = rescue EVT.
Factors associated with 24-hour vessel reocclusion

In univariate comparison, patients with reocclusion were younger and had a lower frequency of atrial fibrillation and preadmission statin therapy. Intracranial ICA occlusion was more frequent in patients with reocclusion. These patients also had longer onset-to-recanalization and groin puncture-to-recanalization times, and number of passes to achieve recanalization was higher. Balloon angioplasty and/or stenting were more frequently used in patients with reocclusion and both transient reocclusion during MT and embolization in a previously normal territory occurred more frequently. Finally, large artery atherosclerosis etiology was more frequent in patients with reocclusion, including either extracranial or intracranial site of atherosclerosis (Table 14).

Table 14. Baseline and stroke characteristics

<table>
<thead>
<tr>
<th>Demographics</th>
<th>Included patients (n=423)</th>
<th>No reocclusion (n=395)</th>
<th>Reocclusion (n=28)</th>
<th>p-value</th>
</tr>
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<tbody>
<tr>
<td>Age</td>
<td>71.4 (59.5–80.0)</td>
<td>71.7 (60.1–80.5)</td>
<td>63.7 (52.3–72.1)</td>
<td>0.010</td>
</tr>
<tr>
<td>Female sex</td>
<td>181 (42.8)</td>
<td>173 (43.8)</td>
<td>8 (28.6)</td>
<td>0.169</td>
</tr>
<tr>
<td>Caucasian</td>
<td>405 (95.7)</td>
<td>380 (96.2)</td>
<td>25 (89.3)</td>
<td>0.080</td>
</tr>
<tr>
<td>Pre-stroke mRS &gt; 2</td>
<td>28 (6.6)</td>
<td>25 (6.3)</td>
<td>3 (10.7)</td>
<td>0.367</td>
</tr>
<tr>
<td>Vascular risk factors</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>287 (67.8)</td>
<td>270 (68.3)</td>
<td>17 (60.7)</td>
<td>0.531</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>59 (13.9)</td>
<td>55 (13.9)</td>
<td>4 (14.3)</td>
<td>1.000</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>311 (74)</td>
<td>293 (74.7)</td>
<td>18 (64.3)</td>
<td>0.319</td>
</tr>
<tr>
<td>Smoking</td>
<td>119 (28.9)</td>
<td>107 (27.9)</td>
<td>12 (42.9)</td>
<td>0.140</td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>168 (39.9)</td>
<td>165 (42)</td>
<td>3 (10.7)</td>
<td>0.002</td>
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<td>Coronary artery disease</td>
<td>78 (18.6)</td>
<td>75 (19.2)</td>
<td>3 (10.7)</td>
<td>0.389</td>
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<tr>
<td>Active cancer</td>
<td>20 (4.8)</td>
<td>19 (4.8)</td>
<td>1 (3.6)</td>
<td>1.000</td>
</tr>
<tr>
<td>Previous cerebrovascular events</td>
<td>67 (15.8)</td>
<td>62 (15.7)</td>
<td>5 (17.9)</td>
<td>0.972</td>
</tr>
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<td>Preadmission therapy</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Antiplatelets</td>
<td>129 (30.5)</td>
<td>123 (31.1)</td>
<td>6 (21.4)</td>
<td>0.386</td>
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<td>Anticoagulants</td>
<td>77 (18.2)</td>
<td>74 (18.7)</td>
<td>3 (10.7)</td>
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<td>Statins</td>
<td>127 (30.2)</td>
<td>124 (31.6)</td>
<td>3 (10.7)</td>
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<td>Stroke characteristics</td>
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<td></td>
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<tr>
<td>Anterior circulation</td>
<td>351 (83.2)</td>
<td>328 (83.2)</td>
<td>23 (82.1)</td>
<td>0.872</td>
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<tr>
<td>Baseline NIHSS</td>
<td>15 (9–19)</td>
<td>15 (9–19)</td>
<td>12 (8–20)</td>
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<tr>
<td>Variable</td>
<td>Value 1 (Range)</td>
<td>Value 2 (Range)</td>
<td>Value 3 (Range)</td>
<td>P-value</td>
</tr>
<tr>
<td>----------------------------------</td>
<td>-------------------------</td>
<td>-------------------------</td>
<td>-------------------------</td>
<td>---------</td>
</tr>
<tr>
<td>Admission SBP</td>
<td>143 (128–160)</td>
<td>143 (129–160)</td>
<td>149 (127–169)</td>
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<tr>
<td>Admission temperature</td>
<td>36.1 (35.8–36.6)</td>
<td>36.1 (35.8–36.6)</td>
<td>36.5 (36.1–36.7)</td>
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<td>Admission glucose, mmol/L</td>
<td>6.9 (5.8–8.2)</td>
<td>6.9 (5.8–8.2)</td>
<td>7 (6.1–7.9)</td>
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<td>Onset-to-door, min</td>
<td>126 (69–245)</td>
<td>125 (68–244)</td>
<td>203 (84–289)</td>
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<td>Imaging</td>
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<tr>
<td>CT</td>
<td>401 (94.8)</td>
<td>376 (95.2)</td>
<td>25 (89.3)</td>
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<td>ASPECTS*</td>
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<td>ac-ASPECTS</td>
<td>9 (8–10)</td>
<td>9 (8–10)</td>
<td>9 (7–10)</td>
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<td>10 (10–10)</td>
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<td>103 (28.1)</td>
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<td>Leukoaraiosis</td>
<td>101 (25.6)</td>
<td>93 (25.3)</td>
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<td>Most proximal site of occlusion</td>
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<td>Intracranial ICA</td>
<td>90 (21.3)</td>
<td>77 (19.5)</td>
<td>13 (46.4)</td>
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<td>M1</td>
<td>175 (41.4)</td>
<td>170 (43)</td>
<td>5 (17.9)</td>
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<tr>
<td>M2/M3/A1</td>
<td>92 (21.8)</td>
<td>87 (22)</td>
<td>5 (17.9)</td>
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<td>BA/V4</td>
<td>42 (9.9)</td>
<td>38 (9.6)</td>
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<td>PCA</td>
<td>24 (5.7)</td>
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<td>Tandem pattern</td>
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<td>6 (4–8)</td>
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<td>Poor</td>
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<td>Partial</td>
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<td>Good</td>
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<td>41 (10.4)</td>
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<td>Baseline perfusion CT</td>
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<tr>
<td>Infarct core volume (ml)</td>
<td>97.6 (48.6–140.3)</td>
<td>97.6 (48.6–141.1)</td>
<td>76.6 (48.8–109.3)</td>
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<td>Penumbra volume (ml)</td>
<td>14.9 (3.8–52.4)</td>
<td>14.9 (3.9–57)</td>
<td>17.5 (2.6–39.5)</td>
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<td>82 (71–96)</td>
<td>84 (68–95.5)</td>
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<td>Total cholesterol, mmol/L</td>
<td>4.6 (3.7–5.6)</td>
<td>4.6 (3.7–5.6)</td>
<td>5.2 (4.2–6.1)</td>
<td>0.085</td>
</tr>
<tr>
<td>C-reactive protein, mg/L</td>
<td>4 (2–12)</td>
<td>4 (2–12)</td>
<td>3 (1.2–12.7)</td>
<td>0.494</td>
</tr>
<tr>
<td>White blood cell count, G/L</td>
<td>8.9 (7.2–11.4)</td>
<td>8.8 (7.1–11.4)</td>
<td>9.4 (8.8–12.5)</td>
<td>0.074</td>
</tr>
<tr>
<td>Hemoglobin level, g/L</td>
<td>136 (122–148)</td>
<td>135.5 (122–147)</td>
<td>147 (128–154)</td>
<td>0.069</td>
</tr>
<tr>
<td>Platelets, G/L</td>
<td>226 (184–273)</td>
<td>226 (184–273)</td>
<td>245 (183–286)</td>
<td>0.487</td>
</tr>
<tr>
<td>IV thrombolysis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bridging with IV thrombolysis</td>
<td>289 (68.3)</td>
<td>272 (68.9)</td>
<td>17 (60.7)</td>
<td>0.493</td>
</tr>
<tr>
<td>Onset-to-needle</td>
<td>115 (86–170)</td>
<td>115 (85–165)</td>
<td>154 (95–267)</td>
<td>0.369</td>
</tr>
<tr>
<td>Mechanical thrombectomy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment period (after 2015)</td>
<td>314 (74.2)</td>
<td>298 (75.4)</td>
<td>16 (57.1)</td>
<td>0.055</td>
</tr>
<tr>
<td>Onset-to-groin puncture</td>
<td>233 (175–335)</td>
<td>230 (173–330)</td>
<td>272 (197–418)</td>
<td>0.082</td>
</tr>
<tr>
<td>General anesthesia</td>
<td>383 (93.6)</td>
<td>357 (93.7)</td>
<td>26 (92.9)</td>
<td>1.000</td>
</tr>
</tbody>
</table>
| Number of device passes | 2 (1–3) | 2 (1–3) | 3 (2–5) | <0.001
|-------------------------|---------|---------|---------|--------
| Stent retriever device  | 344 (81.3) | 322 (81.5) | 22 (78.6) | 0.892
| Aspiration device      | 92 (21.8) | 83 (21.0) | 9 (32.1) | 0.253
| Balloon guide catheter  | 226 (53.4) | 209 (52.9) | 17 (60.7) | 0.546
| Balloon angioplasty or stenting | 28 (6.6%) | 22 (5.6%) | 6 (21.4%) | 0.004
| Permanent intracranial stent | 13 (3.1%) | 11 (2.8%) | 2 (7.1%) | 0.197
| mTICI 3                 | 264 (62.4) | 251 (63.5) | 13 (46.4) | 0.108
| Onset-to-recanalization, min | 299 (231–400) | 291 (227–398) | 360 (304–421) | 0.019
| Groin puncture-to-recanalization, min | 49 (32–79) | 47 (32–75) | 64 (43–99) | 0.011
| Complications           |         |         |         |        
| Transient reocclusion   | 13 (3.1) | 8 (2.0) | 5 (17.9) | <0.001
| Embolization in previously normal territory | 15 (3.5) | 11 (2.8) | 4 (14.3) | 0.008
| Iatrogenic dissection or perforation | 40 (9.5) | 38 (9.6) | 2 (7.1) | 0.921
| Acute antithrombotics   |         |         |         |        
| Antiplatelet therapy <24h | 101 (24.3) | 97 (25.0) | 4 (14.3) | 0.294
| Anticoagulant therapy <24h | 13 (3.1) | 12 (3.1) | 1 (3.6) | 1.000
| Stroke mechanism        |         |         |         |        
| Atherosclerosis         | 77 (18.2) | 64 (16.2) | 13 (46.4) | <0.001
| Cardioembolism          | 199 (47.0) | 194 (49.1) | 5 (17.9) |         
| Dissection              | 18 (4.3) | 16 (4.1) | 2 (7.1) |         
| Unknown/ other rare causes | 129 (30.5) | 121 (30.6) | 8 (17.9) |         
| Dissection other etiologies as reference |         |         |         |        
| Atherosclerosis mechanism by site^v |         |         |         |        
| Extracranial            | 57 (13.5%) | 48 (12.2%) | 9 (32.1%) | <0.001
| Intracranial            | 20 (4.7%) | 16 (4.0%) | 4 (14.3%) |         

Values are presented as median (quartiles) or numbers (proportions). mRS, modified Rankin score; NIHSS, National Institutes of Health Stroke Scale; SBP, systolic blood pressure; ICA, internal carotid artery; M1, first segment of middle cerebral artery; M2, second segment of middle cerebral artery; M3, third segment of middle cerebral artery; A1, first segment of anterior cerebral artery; BA, basilar artery; V4, fourth segment of vertebral artery; PCA, posterior cerebral artery; CT, computed tomography; Min, minutes; ASPECTS, Alberta Stroke Program Early CT score; WBC, white blood cell count; mTICI, modified Thrombolysis in Cerebral Infarction; *both anterior (ac) and posterior (pc) strokes grouped together. In case of baseline MRI one additional point was added to the original ASPECT score (6). ^vother etiologies as reference.
In multivariate analysis, intracranial ICA occlusion (aOR 3.53), number of device passes required to achieve recanalization (aOR 1.31), transient reocclusion during mechanical thrombectomy (aOR 8.55) and atherosclerotic stroke mechanism (aOR 3.14) remained significantly associated with reocclusion. On the other hand, preadmission statin therapy (aOR 0.27) was negatively associated with reocclusion (Table 15).

Table 15. Multivariate logistic regression models for 24h-reocclusion in the entire cohort and in a 4:1 occlusion site and treatment period matched-cohort

<table>
<thead>
<tr>
<th></th>
<th>Entire cohort</th>
<th>Matched cohort*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>aOR (95% CI)</td>
<td>p-value</td>
</tr>
<tr>
<td>Smoking</td>
<td>-</td>
<td>n.s.</td>
</tr>
<tr>
<td>Preadmission statin therapy</td>
<td>0.27 (0.08–0.94)</td>
<td>0.040</td>
</tr>
<tr>
<td>Intracranial ICA occlusion</td>
<td>3.53 (1.50–8.32)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Number of devices passes</td>
<td>1.31 (1.06–1.62)</td>
<td>0.010</td>
</tr>
<tr>
<td>Transient reocclusion during MT</td>
<td>8.55 (2.14–34.09)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Atherosclerotic stroke mechanism</td>
<td>3.14 (1.34–7.37)</td>
<td>0.010</td>
</tr>
<tr>
<td>Residual thrombus fragment or</td>
<td>Not assessed</td>
<td>-</td>
</tr>
<tr>
<td>stenosis</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

aOR, adjusted odds ratio; CI, confidence interval; ICA, internal carotid artery; MT, mechanical thrombectomy. n.s., not significant; variable excluded from the model in the backward stepwise logistic regression. *Matched for most proximal arterial occlusion site and period of treatment (before or after year 2015).

To assess the possibility of a different degree of association between intracranial and extracranial atherosclerosis with reocclusion, we performed a second multivariate logistic regression model with separate levels for atherosclerotic stroke mechanism sites (extra- or intracranial): both locations showed odds ratios similar to the original model (extracranial atherosclerosis aOR 3.20, 95% CI 1.23–8.32; intracranial atherosclerosis aOR 3.09 95% CI 0.65-14.65).
Association between residual thrombus or stenosis in the final angiographic series and 24-hour vessel reocclusion in the matched cohort

Results of the univariate analysis in the 4:1 comparison of patients matched for occlusion site and year of intervention (before or after 2015), showed that patients with subsequent vessel reocclusion more frequently had residual thrombus or stenosis in the final angiographic series post-recanalization (71.4% vs 20.5%) (Supplemental Material – Table III).

In the multivariate analysis, residual thrombus or stenosis in the final angiographic series remained associated with 24-hour vessel reocclusion (aOR 15.57, 95% CI 4.60–52.79).

Also, and in comparison, with the model for the whole cohort, smoking was associated with reocclusion but not site of occlusion (because used for matching) and transient reocclusion during MT (Table 15). The sensitivity analyses excluding patient undergoing permanent intracranial stenting (n=4) and/or balloon angioplasty (n=10) showed similar independent association between residual thrombus sign and reocclusion (aORs 14.77, 95% CI 4.23–51.57, and 11.75, 95% CI 3.27–42.28, respectively). Similar results were obtained in the additional analysis performed only in anterior circulation strokes (n=115): aOR 13.24, 95% CI 3.51–49.96.

Association of 24-hour vessel reocclusion with outcome

24-hour vessel reocclusion was associated with END (10 [35.7%] vs 26 [6.7%] patients, OR 7.88, 95% CI 3.31–18.81) and lower delta 24-hour NIHSS (0 [-6–1] vs 6 [2–11], p-value <0.001). In one of these patients with END, rescue mechanical thrombectomy was performed.
There were no differences regarding parenchymal hematoma (2 [7.1%] vs 26 [6.6%] patients, OR 1.09, 95% CI 0.25–4.85) or symptomatic hemorrhagic transformation (2 [7.1%] vs 16 [4.1%] patients, OR 1.82, 95% CI 0.40–8.35).

Regarding 3-month outcome, patients with 24-hour vessel reocclusion less frequently had a favorable prognosis (11 [29.3%] vs 264 [62.4%] patients, OR 0.32, 95% CI 0.15–0.71). After adjustment, this association remained significant (aOR 0.20, 95% CI 0.05–0.85) (Table 16).

Table 16. Multivariate logistic regression model for favorable outcome (entire cohort)

<table>
<thead>
<tr>
<th></th>
<th>aOR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.91 (0.88–0.94)</td>
<td>0.000</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>2.36 (1.12–4.94)</td>
<td>0.020</td>
</tr>
<tr>
<td>Admission NIHSS</td>
<td>0.95 (0.89–1.01)</td>
<td>0.080</td>
</tr>
<tr>
<td>Acute glucose</td>
<td>0.89 (0.82–0.96)</td>
<td>0.000</td>
</tr>
<tr>
<td>Acute creatinine</td>
<td>0.99 (0.97–1.00)</td>
<td>0.040</td>
</tr>
<tr>
<td>Acute WBC</td>
<td>0.89 (0.81–0.98)</td>
<td>0.020</td>
</tr>
<tr>
<td>Baseline ASPECTS</td>
<td>1.42 (1.14–1.75)</td>
<td>0.000</td>
</tr>
<tr>
<td>Chronic stroke lesion</td>
<td>0.43 (0.20–0.91)</td>
<td>0.030</td>
</tr>
<tr>
<td>Clot Burden Score</td>
<td>1.20 (1.05–1.36)</td>
<td>0.010</td>
</tr>
<tr>
<td>Good collaterals</td>
<td>2.32 (1.18–4.56)</td>
<td>0.010</td>
</tr>
<tr>
<td>IV thrombolysis</td>
<td>2.26 (1.03–4.93)</td>
<td>0.040</td>
</tr>
<tr>
<td>Onset-to-groin puncture*</td>
<td>0.86 (0.78–0.95)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Groin puncture-to-recanalization#</td>
<td>0.85 (0.77–0.94)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>24-hour vessel reocclusion</td>
<td>0.20 (0.05–0.85)</td>
<td>0.030</td>
</tr>
</tbody>
</table>

aOR, adjusted odds ratio; CI, confidence interval; NIHSS, National Institutes of Health Stroke Scale; WBC, white blood cell count; ASPECTS, Alberta Stroke Program Early CT score; IV, intravenous; *per 30 minutes; #per 10 minutes
5.5 – Discussion

In our study of a cohort of consecutive patients undergoing successful endovascular recanalization, 6.6% of patients experienced vessel reocclusion, which is in line with previously documented reocclusion rates of 2.3 to 13.0%, 24 to 48h following successful EVT.\(^77\,79\,158\) We found that intracranial ICA occlusion, transient reocclusion during MT, number of device passes, atherosclerosis as stroke mechanism and presence of residual thrombus or stenosis in the final angiographic series were positively associated with 24-hour vessel reocclusion, while preadmission statin therapy was negatively associated. Although vessel reocclusion was infrequent, it was highly relevant for the patients’ clinical outcome, with an adjusted odds ratio of 5.0 for unfavorable outcome at 3 months. Patients with AIS due to intracranial ICA occlusion, including those receiving EVT, are known to have worse clinical outcomes compared to other sites of intracranial occlusion.\(^29\,159\) Higher initial stroke severity, likely related to lower clot burden\(^143\) and collateral score are possible explanations. In addition, as found in our patients, more difficult endovascular procedures\(^160\) and a higher rate of reocclusion after successful recanalization might also be relevant.

The association between number of passes and 24-hour vessel reocclusion may indicate more challenging endovascular interventions. Alternatively, multiple passes may induce vessel wall damage,\(^161\)–\(^163\) promoting local thrombosis and increasing risk of reocclusion. We also found that transient reocclusion during MT was associated with later reocclusion. Previously, transient reocclusion during EVT was documented in patients with longer procedure times,\(^78\,164\) with use of rescue therapy (balloon angioplasty and/or permanent intracranial stenting)\(^164\)\,\(^165\) and intracranial atherosclerotic disease, as described mostly in Asian patients.\(^164\)\,\(^166\) Reocclusion during the procedure probably corresponds to a more
unstable occlusion site, and together with new attempts to achieve recanalization, will be associated with a high-risk of latter reocclusion.

In our study, atherosclerosis as stroke mechanism was an independent risk factor for 24-hour reocclusion. In addition, after separating extracranial from intracranial atherosclerotic disease, both sites seemed associated with 24-hour reocclusion. As previously shown, atherosclerotic tandem lesions may decrease cerebral perfusion pressure and favour blood stasis, increasing the risk of future reocclusion. In intracranial atherosclerotic disease, vessel stenosis was previously recognized as unstable lesions with a high-risk of instant reocclusion. Regarding stroke mechanism, our findings are in discordance with the study from Mosimann et al, where stroke of undetermined or other specified causes was found to be associated with reocclusion.

In the matched-cohort analysis, residual thrombus fragments or stenosis in the final post-recanalization angiographic series was an additional major factor associated with 24-hour reocclusion, as previously described. 71.4% of our patients with 24-hour reocclusion had residual thrombus or stenosis, similar to the 81.3% found in the Mosimann et al study.

Residual thrombus fragments can act as a nidus of highly concentrated platelets and coagulation factors, while vessel stenosis can disrupt endothelial wall exposing tissue factor, therefore promoting reocclusion. In this matched comparison, smoking was additionally associated with vessel reocclusion and possibly, high serum fibrinogen levels and impaired endogenous fibrinolytic capacity contribute to this finding.

The negative association of preadmission statin therapy with 24-hour reocclusion is in line with previous observations showing more favorable outcomes in stroke patients with statin pre-treatment. In addition to statin effects on lowering cholesterol levels, anti-inflammatory and anti-oxidative properties that promote plaque stabilization as well as
antithrombotic and fibrinolytic effects,\textsuperscript{169,170} may explain the association between statins and reocclusion.

24-hour vessel reocclusion had a profound impact on both short- and long-term outcomes in our study and could be the mechanism that explains futile recanalization in some patients. These results highlight the need to identify patients at high-risk of reocclusion and to search for effective means to detect and prevent it. Rigorous angiographic re-evaluation at the end of an intervention is recommended to detect residual thrombus or stenosis that could eventually be corrected. Indeed, in our study, presence of residual thrombus or stenosis in the final angiographic images identified a group of patients at higher risk to develop future reocclusion. In identified high-risk patients, waiting 10-20 minutes to perform a final angiographic series may be advised to assure successful intervention.\textsuperscript{166} In addition, such patients may benefit from aggressive antiplatelet treatment strategy, or early prescription of high-intensity statins.\textsuperscript{171} Finally, more comprehensive monitoring, including repeated or continuous transcranial Doppler, may help to promptly identify patients with reocclusion that could benefit from a new endovascular procedure.\textsuperscript{138,155,172}

This study has limitations. First, its retrospective nature could have led to information bias, although the data was collected in a prespecified and standardized manner, using up-to-date scales, definitions and neurovascular imaging methods. Although we included a typical cohort of a comprehensive stroke center that contains both subjects from a primary and tertiary referral population, our single-center design could have induced selection bias. The small number of patients, especially in the re-occlusion group, may also limit our conclusions. Since we included patients treated during a large time period, EVT inclusion criteria, institutional patient management protocols and technical details have changed over the years, and these changes may have biased our results. To minimize
this fact, the treatment period variable (before or after 2015) was included in the analysis. The absence of independent angiographic and clinical-outcome adjudication could also be a source of bias. Even though a relevant number of patients were included in the matched-cohort analysis, not all angiographic images were re-evaluated to assess the presence of residual thrombus or stenosis, which may have biased our results in unpredictable ways. The exclusion of patients due to lack of 24h vessel imaging, mainly due to premature death and palliative decisions may have underestimated the percentage of patients with vessel reocclusion after successful MT.
5.6 – Conclusion

24-hour vessel reocclusion after successful mechanical thrombectomy was infrequent but clinically relevant for patient outcomes. Smoking, intracranial internal carotid artery occlusion, transient reocclusion during mechanical thrombectomy, number of device passes, atherosclerosis as stroke mechanism and presence of residual thrombus fragments or stenosis in the final angiographic series were independently associated with 24-hour vessel reocclusion. Preadmission statin therapy seemed protective. The impact on long-term outcome highlights the need to improve strategies to monitor and prevent reocclusion, especially in patients considered at high risk of developing it.
Chapter 6 – Discussion

Stroke is a leading cause of death and disability worldwide and better management strategies are needed. Stroke research is a critical resource for developing new diagnostic and treatment alternatives that ultimately can help reducing stroke global burden. The results presented address a new blood-based biomarker strategy for rapid stroke diagnosis, the protective role of stroke units in patients with in-hospital stroke, and unexplored mechanisms of early stroke complications. After further development and implementation, these shared findings may translate into better stroke care in the near future.

While the implementation of blood-based biomarkers in acute stroke care is sometimes perceived as intangible, they are already being used in certain clinical scenarios. As an example, American Heart Association/American Stroke Association stroke prevention guidelines make specific recommendations based on low-density lipoprotein-cholesterol and hemoglobin A1c target levels. Furthermore, stroke physicians can use NT-proBNP levels to stratify the risk of atrial fibrillation in patients with cryptogenic stroke. The potential role of blood-based biomarkers is also being investigated in other steps of the stroke care pathway. Blood-based biomarkers have shown some promising results in assessing initial stroke severity, establishing ischemic stroke etiology, anticipating ischemic stroke complications such as hemorrhagic transformation or cerebral edema, and predicting short- and long-term outcome.

In our first study, we analyzed blood-based biomarkers to diagnose patients with suspected stroke in the acute setting (less than 6 hours since symptom onset) by using...
mass spectrometry analysis. This was the first study to apply this sample analysis methodology in acute stroke patients while comparing patients with AIS, ICH and SM. Our findings showed that a new subset of biomarkers – C3, ICAM-2, PLGLA, STXBP5 and IGHV3-64 – can correctly identify at least four out of five patients with AIS in a cohort of patients including also ICH and SM, while aiming to avoiding false-positives. The population included in our study had similar characteristics to those included in other acute biomarker studies, and the excepted clinical differences between patients with AIS, ICH and SM were found.82–84

After validation with rapid and accurate POCT in prehospital cohorts of patients with suspected stroke, these results could potentially be applied for safe prehospital thrombolysis in selected patients, without the need of prehospital imaging. This approach could reduce time-to-treatment, which is well known to improve patients’ outcome.17,19 Although the concept of early prehospital thrombolysis has already shown a beneficial effect on patients’ prognosis,35 this was achieved with MSU – a strategy that represents significant challenges for its applicability, such as significant financial costs and technical expertise.

POCT devices have shown a good correlation with ELISA method in measuring stroke biomarkers, namely GFAP, RBP4 and NT-proBNP.44 Moreover, the BIOFAST study (Biomarkers for Initiating Onsite and Faster Ambulance Stroke Therapies) is a first study using biomarkers for stroke diagnosis in a prehospital setting, and has finished its recruitment (biofast.technology/; ClinicalTrials.gov Identifier: NCT04612218). To further improve the applicability of blood-based biomarker testing in stroke care, the development of more accurate POCT devices,176 and the combination of previously tested (GFAP, RBP4 and NT-proBNP)44 and newly identified biomarkers such as those presented in our study, needs to be explored.
One of the main reasons for caution and skepticism regarding the application of blood-based biomarkers in the prehospital setting is the risk of misclassifying ICH as AIS. This could potentially lead to harmful treatments such as erroneously giving tPA to a patient with ICH. For this purpose, and as done in our study, blood-based biomarkers panels should be built targeting 100% specificity for AIS. However, if perfect specificity for AIS could not be achieved in clinical practice, or if the additional benefits from are considered too relevant for treatment decisions imaging (e.g.: identifying potential contraindications for tPA or stratification of the risk of hemorrhagic transformation), blood-based biomarkers for diagnosing stroke patients in the prehospital setting could still be useful, namely in patients’ referral.

Prehospital transport for patients with a suspected stroke has traditionally consisted of referrals to the closest hospital. However, since EVT was approved, and because not every hospital can be an EVT-capable center, the question if a patient with suspect stroke should be transferred to the closest hospital or to the nearest EVT-capable center was raised. Two models have emerged: the "drip-and-ship" model, in which patients are taken to the closest hospital for initial treatment and then transferred to an EVT-capable center if necessary, and the "mothership" model, in which patients are taken directly to an EVT-capable center for treatment (Figure 24 A–B).
The mothership paradigm was proposed because EVT is largely associated with a better outcome in patients with large vessel occlusion (LVO) ischemic stroke, in a time-dependent manner.\textsuperscript{18,29} In this context, observational studies suggested that patients receiving EVT in a mothership model had better outcomes compared with those treated with drip-and-ship.\textsuperscript{177,178} These findings led to a redesign of prehospital stroke systems of
care in some countries or regions. In these places, patients with suspected LVO who are within a 30- to 60-minute transportation time to an EVT-capable hospital are recommended to bypass the closest stroke center to avoid delays inherent to interhospital transfers.\textsuperscript{179–181} Several prehospital scales were developed to identify patients with presumed LVO ischemic stroke on scene.\textsuperscript{182} However, the mothership model has also raised some concerns: delaying IVT or even its denial due to longer transport times, and unnecessary transport of patients without a LVO ischemic stroke. Indeed, futile admissions to EVT-capable centers could theoretically lead to inadequate resource utilization, overcapacity difficulties, and patient inconvenience. Because early therapeutical interventions also benefit patients with ICH\textsuperscript{23–26} and AIS patients not receiving any reperfusion treatments,\textsuperscript{32,33} the potential delay of stroke care in these situations cannot be overlooked.

To determine which prehospital system might be better, the RACE-CAT trial was conducted.\textsuperscript{183} This trial was stopped prematurely since an interim analysis showed that it was unlikely that one organizational model would be better than the other. This study raised the important issue of non-IS patients, since more than 30% of the included patients had an ICH or were SM. Additionally, patients with ICH had a trend towards higher mortality rate in the mothership arm. Of note, RACE-CAT trial results may not be applicable to other regions where interhospital transfers are longer, and may have been underpowered to detect statistically significant differences or harm in subgroup analysis.\textsuperscript{183}

Considering all the above, blood-based biomarker could help selecting a candidate for EVT, therefore contributing for swift and accurate referral to an EVT-capable center in a mothership model. Biomarkers could help avoid transferring non-IS patients and enhance
the prehospital detection of the subset of patients with LVO ischemic stroke. Figure 25 conveys a possible integration of the potential role of blood-based biomarkers with prehospital stroke treatment and referral system.

Figure 25. Potential role of blood-based biomarkers on prehospital stroke treatment and referral system.

Legend.
Additionally, biomarkers could also be of interest for selecting patients with EVT-criteria directly to the angiography suite in primary admissions to EVT-capable centers. Direct-to-angiography suite transfer has shown to reduce door-to-treatment times and improve patient’s outcome. However, because up to 28% of patients included in direct to angiography studies were not eligible for EVT (ICH, SM or no LVO), there is a considerable risk of overwhelming neurointerventional teams with false activations and unnecessary disruption of elective procedures may come as an unfortunate consequence of this approach. Before directing patients straight to the angiography suite in EVT-capable centers, screening with biomarkers could contribute to a more accurate patient selection method.

In acute stroke care, blood-based biomarkers may also be of interest if specific biomarkers could show a relationship between its blood concentration and time since stroke onset. Indeed, some biomarkers have shown to have a particular kinetic profile after stroke. The possibility that changes in expression of biomarkers over time would act as a “stroke clock” or as a marker of “salvageable penumbra” could increase treatment rates with IVT and EVT. This would be especially true in late-window patients since advanced imaging, recommended for patient selection, is not widespread available.

In a more global perspective, and outside of the scope of state-of-the-art acute stroke care, blood-based biomarkers diagnostic methods could also be a major asset for reducing stroke burden. Patients in developing countries have limited access to brain imaging, which will delay or even preclude stroke diagnosis. Without a timely diagnosis, simple stroke interventions including early secondary prevention with antithrombotic cannot be
implemented. Affordable and accessible blood-based biomarkers diagnostic methods could be explored as an alternative.

Finally, our exploratory approach can also add information on stroke pathophysiology and related acute adaptation mechanisms. This additional knowledge could potentially be applied to the development of new stroke therapies. Accordingly, is it interesting to highlight that biomarker research and MSU can be integrated in disclosing new and early biological processes activated in patients with stroke.¹⁸⁶

Within the last eight years, stroke physicians and researchers have witnessed major breakthroughs in acute reperfusion treatment and secondary prevention. Notwithstanding, patients with stroke continue to experience high rates of death and disability. Understanding why, and how to address some of the underlying factors, can ultimately lead to improved outcomes.

Moving from the prehospital to the in-hospital phase, the introduction of stroke units revolutionized stroke care.²⁷,²⁸ One major impact of stroke units is the prevention, early recognition and prompt treatment of early stroke complications.²⁷,²⁸ Stroke units are also associated with swift initiation of tailored secondary prevention therapies, reducing the risk of stroke recurrence and improving long-term outcome.¹⁸⁷,¹⁸⁸ Despite of the above, the impact of stroke units on the diagnosis, treatment metrics, recanalization treatment rates, and outcome of in-hospital stroke remained to be established.

In our study, we have shown that in comparison with other-IHS or COS, having a stroke while hospitalized in a stroke unit resulted in higher rates of witnessed stroke, shorter
times-to-imaging and to treatment, and less missed treatment opportunities. Altogether, these might be a consequence of better clinical monitoring and stroke team’s expertise, and explain the better outcomes registered in patients with in-stroke unit stroke. These results reinforce the additional benefits of offering stroke unit care during the entire hospitalization of patients with stroke. This contrasts with the real-world scenario where patients are often monitored in stroke units for a few days and then transferred to neurology, internal medicine or rehabilitation wards, without the same level of care. While addressing patients with minor strokes or TIA, these results can also help explaining the benefits of stroke unit admission. In addition to the benefit in reducing stroke recurrence, it now can be added that in the event of an early occurrence, being in a stroke unit will probably increase the chance of being treated in time and of having a good outcome.

With this study we also showed that many patients experiencing in-hospital stroke recurrences are eligible for acute reperfusion therapies, especially for EVT, as previously investigated.\textsuperscript{138,155,172,189–192} International guidelines caution against administering IVT to AIS patients with a prior ischemic stroke within 3 months, citing potential harm\textsuperscript{16} or insufficient evidence to make an evidence-based recommendation.\textsuperscript{193} Our study supports the results of other observational studies suggesting that IVT may be safe in selected patients.\textsuperscript{137,194–197} In line with the above, given the potential expansion of EVT criteria to include more distal vessel occlusion, paired with the increased capability to detect them,\textsuperscript{198–200} treatment eligibility in patients with in-hospital ischemic stroke recurrences can increase significantly in the coming years.
In our subsequent study, worsening of arterial patency over the first 24-hours (meaning the presence of new occlusion or stenosis comparing subacute with acute vessel imaging) was clinically relevant as it was associated with higher odds of early ischemic stroke recurrences and with unfavorable outcomes. As the addition of vascular assessment (mainly CTA) to admission imaging protocols has shown to be advantageous in the diagnosis and treatment of patients with stroke, repeated vascular imaging (for example at 24-hours when control parenchymal imaging is routinely performed) may help detecting early stroke recurrences in patients with subtle worsening of NIHSS or neurological deterioration attributed to other causes (e.g.: concomitant systemic infection). Repeated vessel imaging may also help clarifying why some patients experience otherwise unexplained neurological deterioration, or do not improve despite initial successful revascularization. Retreating patients with new or recurrent vessel occlusion is currently being studied, and is expected to improve outcomes, highlighting the importance of re-evaluating vessel patency.

Recognizing which patients have a higher risk of worsening of arterial patency and/or of early stroke recurrence would enable a more patient-tailored approach, avoiding the challenges of repeating 24-hour arterial imaging in every patient with AIS. In our study, both extracranial and intracranial stenosis were identified as independent predictors of worsening of arterial patency. These findings are supported by previous studies showing higher recurrence rates in patients with large artery atherosclerosis stroke. Also, they are in accordance with our stroke in the stroke unit analysis where patients with ISUS, largely composed of patients with recurrent ischemic stroke or new stroke after recent TIA, had a higher frequency of large artery atherosclerosis as stroke mechanism in comparison with the other subgroups. As patients with large artery atherosclerosis stroke
seem to benefit from more aggressive antithrombotic and lipid lowering strategies,\textsuperscript{204–206} different monitoring approaches may also be reasonable. While several predictors such as stroke severity and collateral status may influence the decision of reassessing vessel patency with CTA or MRA, other strategies such as continuous or repeated evaluation by transcranial Doppler can also be considered. Finally, in addition to vascular imaging, blood-based biomarkers could also help identifying patients at risk for reocclusion or early recurrence.\textsuperscript{174,207}

We also tried to understand the mechanisms of worsening of arterial patency, in particular, of arterial reocclusion, in the subgroup of EVT patients achieving initial successful recanalization. Although successful vessel recanalization is considered a strong determinant of better functional outcomes,\textsuperscript{21,22} only about half of patients who achieve successful recanalization after EVT actually experience favorable functional outcome.\textsuperscript{29} These so-called “futile recanalizations” may be related to pre-stroke disability, comorbid disease including preexisting chronic brain damage, blood pressure changes, poor collaterals, early infarct growth and post-procedural or systemic complications.\textsuperscript{208–210} Arterial reocclusion can additionally explain this phenomenon.

In our reocclusion study, we aimed to assess rates, predictors and clinical impact of reocclusion after successful EVT. This complication had been only investigated in a limited number of studies, with some conflicting results.\textsuperscript{77–79}

In line with the worsening of arterial patency study, large artery atherosclerosis as stroke mechanism, in both extracranial and intracranial localization, was associated with 24-hour reocclusion after EVT. Not only can proximal extracranial disease decrease cerebral perfusion pressure, promote blood stasis, and increase the risk of future reocclusion,\textsuperscript{74} but in-situ intracranial stenosis can disrupt endothelial wall and contribute to local
In this study, transient reocclusion during intervention and higher number of passes, more frequently seen in patients with intracranial atherosclerosis, were also independently associated with reocclusion. As such, our results show an intricate association of atherosclerotic disease with vessel reocclusion summarized in Figure 26.

Figure 26. Association between intracranial atherosclerosis, arterial reocclusion and related-mechanisms.

Our results also showed that vessel reocclusion after successful EVT was associated with a high likelihood of unfavorable long-term outcome. As with worsening of arterial patency, these results suggest a potential value of repeated arterial imaging in patient monitoring protocols. If not for every patient, repeated imaging could be recommended in patients with high risk of reocclusion and in those with otherwise unexplained neurological deterioration or without clinical improvement despite successful revascularization. As stated, the relevance of detecting subacute arterial reocclusion is emphasized by the opportunity of safely perform repeated EVT. Further data
showing the safety and benefits of EVT in large ischemic lesions\textsuperscript{211–213} and beyond 24 hours last-seen-well\textsuperscript{214} provides additional reassurance for the possible benefits of retreating such patients.

In order to prevent reooclusion after successful recanalization, patients may benefit from earlier and more aggressive antithrombotic strategies. As in other situations (e.g.: carotid stenting during EVT),\textsuperscript{215} patients with high risk of reocclusion could be considered for early control neuroimaging at 12 rather than 24 hours, followed by immediate initiation of antithrombotic treatment. Of note, although guidelines recommend follow-up imaging at 24 hours before starting antithrombotic agents after recanalization therapy,\textsuperscript{16} the risk of severe hemorrhagic transformation seems to mainly concentrate on the first 12 hours.\textsuperscript{216} Furthermore, in those studies raising safety concerns with early antithrombotic medication in the context of acute recanalization therapies, antithrombotics were administered during or immediately after IVT/EVT.\textsuperscript{217,218}

After our analysis of reooclusion after successful EVT, further evidence on this topic was published. Because across studies vessel reooclusion was assessed at different time-points and risk factors were not uniformly evaluated, prevalence and associated factors of reooclusion needed further evaluation. Moreover, the safety and effectiveness of treatment strategies for patients with reooclusion had not been systematically evaluated. To better understand the prevalence, associated factors and potential treatment possibilities of patients with reooclusion after successful EVT, a systematic review and meta-analysis of published studies was performed.\textsuperscript{219}
In this analysis, the rate of vessel reocclusion was 5%, and patients with reocclusion were three times more likely to have large artery atherosclerosis as stroke mechanism. Reocclusion was associated with a fourfold higher likelihood of unfavorable functional outcome. Regarding repeated EVT, large artery atherosclerosis etiology was particularly common within the first 24-hours after EVT. Successful reperfusion was achieved in 90% of patients and 8% had symptomatic ICH. A favorable outcome was achieved in 40% of patients at 3 months, and mortality rate was 18.9%. These results confirmed the findings of our initial study regarding reocclusion rates, potential predictors and clinical outcome, and reinforced the relevance of reocclusion diagnosis by further showing the possibility of successfully treating these patients. Nevertheless, the results of this systematic review and meta-analysis need to be interpreted with caution due to several limitations: heterogeneity between studies regarding the location of LVO, endovascular treatment criteria, reporting and definition of acute stroke and treatment complications; limited data for the specific repeated EVT analysis; limitations inherent to the retrospective design of included studies; and the possibility of selection bias, including publication bias.
Future directions

After the completion and publication of our four studies, further research questions arise and should be addressed.

In blood-based biomarker research, the development and validation of new biomarker panels integrating our five main biomarkers with other promising studied biomarkers\(^{44}\) could be explored. Also, investigating new POCT devices\(^{176,220,221}\) could help improving the diagnostic accuracy of blood-based biomarkers in the prehospital setting. Finally, as suggested by preliminary studies\(^{184,185}\) the role of blood-based biomarker in the identification of LVO ischemic stroke may be further studied, especially if integrated in diagnostic algorithms together with clinical variables.

Regarding in-hospital stroke recurrences, design and recruitment challenges, and probably lack of equipoise, would preclude conducting a randomized clinical trial in this setting. As such, a prospective, international, multicenter registry would be of great relevance. Understanding patients’ characteristics, outcomes, and potential effect modifiers of safety and outcome of acute reperfusion treatment would be the main objectives of such registry. Furthermore, the lack of evidence informing current guideline recommendations for IVT and EVT in patients with recent prior IS could be potentially addressed with comprehensive analysis of large datasets.

Based on the presented results and discussion, a prospective observational registry exploring the frequency and impact of routine 24-hour arterial imaging in patients with high risk of worsening of arterial patency or reocclusion after EVT would be of interest.
Pending the results, this new standard procedure could be compared with historical controls. To explore a potential association of vessel reocclusion with futile recanalization, routine assessment of vessel patency could also be relevant in every stroke patient with successful EVT.

Transient vessel reocclusion during EVT was identified as a potential predictor of subsequent reocclusion in one of our studies. This has only been studied in Asian cohorts where intracranial large artery atherosclerosis is more prevalent than in western populations. As such, investigating the rate, predictors and outcome of transient vessel reocclusion during EVT within a cohort of non-Asian patients would provide valuable information. Furthermore, understanding which technical and interventional antithrombotic strategies are safer and more efficacious would help define recommendations for these challenging patients.
**Personal reflection**

These past years were both exciting and challenging. I take great pride in the work and time I dedicated to my PhD thesis and related research. Balancing clinical work, research and familial life was demanding, but ultimately fulfilling. Inspired by my mentors, I approached all my tasks with rigor and delight.

The key to success was teamwork.

Engaging, connecting, and motivating all collaborators was pivotal in conducting our prospective blood-based biomarker study. Without the participation of Neurology residents, nurses from the Emergency Department, and technicians from the Department of Clinical Pathology, our project would not have succeeded.

In Lausanne, I learned how stroke care and research can be integrated in a perfect symbiosis. Clinical and research work were conducted with exceptional quality and efficiency, thanks to excellent preparation, organization, and discussion.

Now, I am trying to implement some of these lessons at my department by creating and maintaining our prospective stroke registry, and by encouraging residents to build their stroke research projects based on thoughtful research questions and strong methodological foundations.
My ultimate goal is to build on the work presented here and further develop it. For achieving this aim, I will have to be capable to engage others to join me in a collaborative, productive and positive research environment.
Acknowledgments

I am grateful for the path that brought me to this moment in my career.

I would like to start by thanking all my teachers for motivating me to study and pursue science, medicine and neurology.

I thank all patients and family members for their generosity, selflessness, and willingness to participate in medical research, for their gratitude, as well as for all that they have taught me throughout my career.

I would like to acknowledge the work and dedication put in by my fellow researchers, without whom these projects would not have been possible.

I thank all members from the Nursing Emergency Department team for their support in sample collection. I would also like to thank Dr. João Faro Viana and the Clinical Pathology Department team for their collaboration in blood samples processing and storage.

I am extremely grateful to my colleagues from the Department of Neurology and Stroke Unit in Centro Hospitalar Lisboa Ocidental for what they taught me and for collaborating on our research project.

My sincere appreciation goes to the Lausanne Stroke Team. I would like to acknowledge their welcoming spirit and their friendly approach in making me feel part of their group.
I extend a special thanks to Ashraf Eskandari for her affection and support, and to Davide Strambo for his countless hours of work and dedication to our projects.

I am deeply grateful to Professor Patrik Michel for sharing his expertise and knowledge with me. I am particularly thankful for his guidance on how to perfectly balance clinical and research work, and witness his way of leadership, placing a greater emphasis on the well-being of his team members and creating a positive and productive environment where everyone is motivated to contribute with their best work.

During my PhD journey I had the pleasure of knowing Prof. Doutora Helena L.A. Vieira, to whom I am deeply grateful. I thank her for her friendship and her generous contributions to my research. Additionally, I would like to express my appreciation for her advice, not only in the field of science but also in personal relationships and life.

I would like to express my sincere gratitude to Prof. Doutor Miguel Viana Baptista, my mentor since my early days in Neurology. I thank him for his invaluable guidance, support, and friendship throughout my clinical and research journey. I thank him for his aid in establishing the grounds on which my career has been built and for empowering me to pursue ambitious goals.

Finally, I am enormously grateful to have the unconditional love and support of my family. I thank my mother and father for supporting my decisions in life and for conveying the values of self-respect and perseverance.
I thank Leonor for being the best wife, friend and mother that I could imagine. I am grateful for her solidarity and encouragement in pursuing my goals, for guiding me in valuing the most important things in life, and for helping me in taking the right steps.

To Zé Francisco, Assunção and Joaquim, thank you for keeping me recharged and optimistic after those difficult days with your happiness, fun, and love.
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