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Advances in Ischemic Stroke: The Role of Imaging Biomarkers in Improving Diagnosis, Prognosis, and Therapeutic Decision-making

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Abbreviations

ARWMH - age-related white matter hyperintensities

BA - basilar artery

BAO - basilar artery occlusion

CSVD - cerebral small vessel disease

CT – computed tomography

DWI – diffusion-weighted imaging

EVT - endovascular therapy

FHV - FLAIR hyperintense vessels

FLAIR – fluid-attenuated inversion recovery

IQR - interquartile range

MCA - middle cerebral artery

MCAO - middle cerebral artery occlusion

MRI - magnetic resonance imaging

OR - odds ratio

PET – positron emission tomography

PWI – perfusion-weighted imaging

RCT- randomized controlled trial

ROC - receiver operating characteristic

tPA - tissue plasminogen activator

TOAST - Trial of ORG 10172 in Acute Stroke Treatment

TP - thrombus perviousness

VSI - vessel size imaging

WMH - white matter hyperintensities

1. INTRODUCTION

BACKGROUND

Ischemic stroke is one of the leading causes of death and disability worldwide¹. Due to higher life expectancies and global population growth, patients with permanent disabilities following stroke are expected to rise substantially in the next years². There have been significant advances in the treatment of acute stroke in recent years that have led to decreases in stroke mortality and morbidity e.g. specialized facilities (stroke units), and the increase in the use of recanalizing therapies (e.g. thrombolysis and thrombectomy, even in extended time-windows)³. Nevertheless, most patients - even those who receive such acute treatments - are left with lasting neurological deficits and are at risk of recurrent vascular events which places a substantial burden on the healthcare system. Therefore, there is a continued search for clinical and imaging parameters that carry potential diagnostic and prognostic value to better guide our treatment decisions for ischemic stroke and ultimately improve long-term outcome.

An ischemic stroke is a result of a blood clot hindering tissue perfusion predominately caused by small vessel arteriosclerosis, cardioembolism, or large artery athero-thromboembolism⁴. Rapid initiation of acute imaging is a crucial first step in evaluating a suspected stroke primarily because it dictates acute treatment decisions, e.g. eligibility for thrombolysis or endovascular therapy (EVT). The aim of thrombolysis and EVT is arterial recanalization and reperfusion of the ischemic penumbra, however, these recanalizing therapies can lead to serious complications, such as symptomatic intracerebral hemorrhage. Although treatment decision is based primarily on time due to a clear association between treatment efficacy and the interval between symptom onset and initiation of the recanalizing therapy⁵, advances in imaging-based management of ischemic stroke provide crucial information such as infarct core, degree of collaterals, vessel occlusion site, etc. that can help select the best candidates for reperfusion therapy⁶. These advances have led to substantial changes in the treatment concept of acute ischemic stroke in recent years, in which we are increasingly

moving away from a rigid time-based approach to a more imaging-based approach based on patient individual risk-benefit profiles.

While non-contrast computed tomography (CT) remains the primary imaging modality for the initial evaluation of patients with suspected stroke, multiparametric magnetic resonance imaging (MRI) has become increasingly available and has gained standing in the standard diagnostic work-up following stroke. Both imaging modalities provide valuable information on the individual stroke at hand. An advanced understanding of the complexities of individual stroke pathology based on these acute imaging modalities enables physicians to better assess patient risk and make more targeted, individualized treatment decisions not only in the acute phase but also for guiding secondary prevention strategies.

With this context in mind, the focus of my research has been to identify imaging biomarkers on CT and MRI that may aid in optimizing stroke care at various points of contact, for example in selecting the best secondary prevention therapies and in identifying patients most likely to benefit from recanalizing therapies. In the following paragraphs, I will introduce several studies I initiated to address this central research objective.

IMAGING BIOMARKERS OF STROKE CAUSE

Accurate identification of stroke cause is essential for initiating effective secondary prophylactic therapies to reduce the risk of recurrent vascular events. It is particularly crucial to identify a stroke of cardioembolic source because this often requires more intensive anticoagulant therapy⁷. Although rapid and accurate identification of stroke etiology is essential following an ischemic stroke, stroke cause remains unknown in up to one-third of patients despite extensive diagnostic work-up⁴.

Infarct pattern (in combination with selected clinical markers) was suggested as a potential imaging biomarker to identify patients who would benefit from anticoagulant therapy compared to platelet inhibitors after ischemic stroke⁸. However, two clinical trials failed to show an advantage of anticoagulants versus

antiplatelet inhibitors in reducing recurrent stroke in patients with so-called embolic stroke of undetermined source^{9,10}.

There are several recent studies that suggest that detailed categorization of the thrombus within the occluded vessel can provide indirect clues to its histological composition which varies depending on stroke source^{11,12}. While cardioembolic strokes are associated with fibrin-rich thrombi, large-artery thromboembolism strokes have a higher concentration of red blood cells; these factors influence the thrombus permeability to contrast agent as well as other thrombus characteristics^{13,14}. In other words, there is increasing evidence that an advanced imaging analysis of the thrombus may offer promising potential biomarkers indicative of stroke etiology.

A recent study found that a CT-based imaging biomarker called 'Thrombus Perviousness' (TP) – which estimates the thrombus' permeability to contrast agent - not only correlates directly with histological clot composition but also was able to distinguish between cardioembolic and non-cardioembolic strokes with high specificity¹¹. However, it remained unclear whether this imaging biomarker is influenced by time-to-imaging, medication status, or selected laboratory parameters at the time of stroke.

Therefore, we set out to validate the accuracy of TP as an imaging biomarker to identify stroke etiology in two independent cohorts of ischemic stroke patients with different arterial occlusion sites, namely in i) patients with occlusion of the middle cerebral artery (MCA)¹⁵ and ii) in patients with basilar artery occlusion (BAO)¹⁶. Furthermore, we set out to determine whether clinical and laboratory parameters affect TP measurements on acute CT images to determine whether these factors should be considered when applying this novel imaging biomarker in future studies¹⁵.

IMAGING SELECTION CRITERIA FOR ACUTE STROKE TREATMENTS

In 1996, the first systemic treatment for acute ischemic stroke - tissue plasminogen activator (tPA) - was licensed for intravenous use within a strict therapeutic time

window of 3 hours (of symptom onset). Following the ECASS II trial in 2008, the therapeutic time window was extended to 4 hours¹⁷. Until this point, time was the driving factor for therapeutic decision-making in the setting of acute stroke, automatically excluding patients with unknown time of symptom onset which compromises up to 30% of acute ischemic strokes¹⁸.

Within the past two decades, several pivotal randomized controlled trials (RCTs) have demonstrated the efficacy of using imaging criteria to select eligible patients for reperfusion therapies. For example, the WAKE-UP trial used an MRI criterion as a so-called 'tissue-clock' to select patients with an unknown time of symptom onset who would still likely benefit from intravenous tPA¹⁹. The imaging criterion was a so DWI-FLAIR mismatch which refers to a demarcation of the ischemic lesion on diffusion-weighted imaging (DWI) but not yet on fluid-attenuated inversion recovery (FLAIR). The WAKE-UP trial showed that in patients with unknown time of symptom onset, intravenous tPA guided by a DWI-FLAIR mismatch resulted in a significantly better 3-month functional outcome compared to placebo. Subsequently, this imaging selection criterion has since been integrated into treatment guidelines for acute ischemic stroke.¹⁹ The DAWN and DEFUSE-III trials have further illustrated the importance of multimodal imaging in identifying patients with salvageable brain tissue, and hereby could extend the time window for EVT to up to 24 hours after symptom onset^{20,21}. The mentioned trials represent just a selection of the many pivotal trials that have taken place over the past 20 years and illustrate recent advancements in ischemic stroke treatment concepts in terms of using imaging criteria for guiding acute therapeutic decision-making.²²

Apart from FLAIR hyperintensities corresponding to the ischemic lesion, FLAIR hyperintensities within the vessels are observed in up to 80% of acute ischemic stroke patients²³. FLAIR hyperintense vessels (FHV) are focal, linear, or serpentine hyperintensities visible distal to the main occluded artery. Although preliminary studies have suggested that FHVs indicate collateral status, their clinical relevance has been long disputed. While some studies have shown that

FHVs are associated with increased collateralization and improved long-term functional recovery, others have found FHVs to be associated with a poor functional outcome²⁴⁻²⁶. These apparent discrepancies are likely due to different methodologies used in the assessment of FHVs and heterogeneous patient cohorts regarding acute treatments and time to MRI. If FHVs are indeed representative of collateral status and are predictive of lesion progression and outcome, this easily recognizable, contrast-free MRI biomarker could be promising to select patients most likely to benefit from recanalizing therapies.

Therefore, we initiated two studies to investigate: i) whether the extent of FHVs visible on acute MRI is associated with collateralization and functional recovery in a multicenter cohort of patients with MCA occlusion who received EVT²⁷, and ii) whether the extent of FHVs modifies the treatment efficacy of thrombolysis in a comprehensive cohort of patients stemming from the abovementioned WAKE-UP trial²⁸.

IMAGING BIOMARKERS FOR RISK STRATIFICATION

CT and MR not only provide insights into the acute stroke at hand but also reveal accompanying cerebral pathologies that are crucial for assessing long-term patient risk²⁹. Cerebral small vessel disease (CSVD) is the most prevalent comorbidity detected on acute stroke imaging and is known to substantially increase the risk of first-ever stroke and contribute to cognitive decline and dementia³⁰⁻³². Although there are several imaging biomarkers of CSVD including small subcortical infarcts, lacunes, enlarged perivascular spaces, and microbleeds, white matter hyperintensities (WMH) visible on FLAIR are the most common manifestation of CSVD³³. WMH mostly result from chronic ischemia caused by small vessel disease, which can stem from long-term smoking, diabetes mellitus, arterial hypertension, and other well-known vascular risk factors³⁴. In other words, a detailed assessment of underlying chronic CSVD in the setting of acute stroke is likely to aid in patient-specific risk stratification, helping to evaluate individual cerebrovascular risk profiles and potentially even the risk of recurrent stroke and long-term cognitive decline.

Therefore, we aimed to investigate the relationship between latent or 'borderline' risk profiles (such as glucose intolerance without overt diabetes) and WMH burden in a comprehensive cohort of patients with first-ever stroke. Additionally, we examined whether a high WMH burden was linked to an increased risk of recurrent vascular events, cognitive impairment, and depression up to three years post-stroke³⁵. Our ultimate goal was to evaluate whether WMH could serve as a valuable imaging biomarker for assessing long-term risk to guide secondary prevention treatment strategies.

ADVANCES IN IMAGING FOR ISCHEMIC STROKE

There have been significant advancements in the field of diagnostic imaging of ischemic stroke with the development of new MRI techniques in recent years. For example, arterial spin labeling and dynamic susceptibility contrast perfusion offer detailed insights into cerebral hemodynamics, enabling a more accurate identification of the ischemic penumbra.³⁶ Moreover, the role of molecular imaging techniques such as positron emission tomography (PET) is being increasingly explored for assessing individual stroke pathology including inflammation and neurovascular remodeling etc. Although these advancements are promising, the clinical utility of new imaging modalities has yet to be determined, with many studies still in the exploratory stage. Nonetheless, investigating the potential of new imaging techniques is crucial for deepening our understanding of stroke pathology and optimizing imaging-based treatment strategies.

In collaboration with the Department of Neuroradiology (Center for Stroke Research), we explored the diagnostic and prognostic potential of a novel MR sequence called vessel size imaging (VSI) in the setting of ischemic stroke. Preclinical studies show that vascular remodeling in and around the ischemic tissue is crucial for tissue recovery and the regaining of function³⁷. VSI allows for an in-vivo assessment of microvascular morphology, providing insights into changes in vascular density and vessel size³⁸. While studies in rat stroke models have shown that VSI measurements highly correlate with histological changes in vessel morphologies³⁹, the clinical relevance of VSI remains unclear.

Therefore, we initiated an exploratory analysis to identify the potential diagnostic value of imaging biomarkers derived from VSI, such as vessel size, density, and relative tissue perfusion, within different regions of interest, including the infarct core and perilesional space⁴⁰. Our goal was to determine the role of these biomarkers in infarct progression and functional recovery in a cohort of subacute ischemic stroke patients stemming from a RCT.

OBJECTIVE

In summary, the works that will be summarized below focus on exploring the potential prognostic and diagnostic value of selected imaging biomarkers on CT and MRI in ischemic stroke. First, two studies will be summarized that investigate whether a CT-based biomarker (TP) can be used to determine stroke etiology to guide secondary prevention strategies. Second, several studies on selected MRI-based biomarkers (FLAIR hyperintense vessels, presence of WMH) and their role in acute stroke management and long-term risk stratification will be discussed. Lastly, we will introduce a study that explores the diagnostic potential of a novel MR technique called VSI in ischemic stroke. The studies report findings stemming from five different prospective stroke registries and two RCTs. Taken together, these works touch on several ways that we can maximize the use of available imaging modalities in ischemic stroke to optimize all-around stroke care.

2. OWN WORK

2.1 THROMBUS PERVIOUSNESS IN PATIENTS WITH MCA OCCLUSION

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We performed a retrospective analysis of a prospectively maintained registry of patients with large vessel occlusion that received EVT at the Charité. Patients who received both non-contrast CT and CT angiography before EVT and demonstrated an isolated occlusion of the M1 segment of the MCA were included. Thrombus perviousness (TP) was determined by comparing signal intensities in the thrombus region in co-registered non-contrast CT and CT angiography images to estimate the thrombus' permeability to contrast agent.

We demonstrated that thrombi in patients with confirmed cardioembolic stroke exhibited significantly higher TP than those in patients with atherosclerotic stroke etiology. TP was significantly associated with stroke cause according to TOAST criteria (Trial of ORG 10172 in Acute Stroke Treatment; $p < 0.001$). Additionally, receiver operating characteristic (ROC) curve analysis indicated that TP was a significant indicator to identify cardioembolic stroke with an area under the curve of 0.75 (95% CI 0.63-0.87); a TP value above 6.23 Hounsfield units could detect cardioembolic stroke with a specificity of 100%. Applying our calculated TP thresholds to thrombi from patients with unknown stroke etiology suggested that up to 62% might have been due to cardiac embolism. The biomarker proved robust against potential confounding variables such as pre-treatment medication, altered blood coagulation values, and time delay to imaging. There was no association between TP and recanalization success following EVT. Our results indicate that thrombus characteristics in acute imaging are associated with stroke etiology. If this association is confirmed in multicenter cohorts, the potential use of TP as a therapy-guiding biomarker is promising.

CLINICAL AND POPULATION SCIENCES

Association Between Thrombus Perviousness Assessed on Computed Tomography and Stroke Cause

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BACKGROUND AND PURPOSE: A recent study proposed that thrombus perviousness (TP)—the degree to which contrast agents penetrate the thrombus in an occluded vessel measured on noncontrast computed tomography (NCCT) and CT angiography—may be associated with cardioembolic stroke cause with high specificity. Our aim was to investigate which clinical and laboratory parameters affect measures of TP and to validate its diagnostic accuracy in an independent cohort of patients with acute ischemic stroke.

METHODS: Seventy-five patients from a prospectively maintained database with proximal occlusions of the middle cerebral artery (M1) were retrospectively analyzed. Thrombi were segmented on coregistered noncontrast computed tomography and CT angiography to determine the thrombus attenuation increase and void fraction (attenuation increase relative to contralateral side).

RESULTS: TP measures were significantly higher in patients with cardioembolic stroke compared to patients with stroke attributed to large artery atherosclerosis (median thrombus attenuation increase [interquartile range], 2.79 [−3.54 to 8.85] versus −5.11 [−11.23 to −1.47]; $P=0.001$). In linear regression analysis for TP including age, time to scan, prior medication with antiplatelets or anticoagulants, and selected laboratory parameters, only stroke cause was significantly associated with TP. In multivariable binary logistic regression analysis for dichotomized stroke cause (ie, cardioembolic versus noncardioembolic stroke), only thrombus attenuation increase was independently associated with cardioembolic stroke (odds ratio of 1.12 [95% CI, 1.04–1.22]; $P=0.004$). Receiver operating characteristic analysis indicated that TP can identify cardioembolic stroke with an area under the curve of 0.75 (95% CI, 0.63–0.87) for thrombus attenuation increase. With a cutoff value of 6.23 Hounsfield units, cardioembolic strokes were identified with 100% specificity. Results for void fraction were similar.

CONCLUSIONS: The assessment of TP on baseline noncontrast computed tomography/CT angiography in patients with M1 occlusion may aid in determining cardioembolic stroke cause and guide secondary prevention. Selected clinical and laboratory parameters other than stroke cause did not affect TP measures.

Key Words: angiography ■ atherosclerosis ■ middle cerebral artery ■ secondary prevention ■ tomography

Acute ischemic stroke with large vessel occlusion can result from different causes.¹ In recently published series of patients treated with mechanical thrombectomy, the most commonly identified underlying causes were cardiogenic embolism (cardioembolic) in ≈50% of

patients and large artery atherosclerosis (LAA) in ≈20% of patients.^{2–4} Knowledge of stroke cause is important because it guides medical therapy for secondary prevention of recurrent ischemic stroke, in particular, the choice between anticoagulation and platelet function inhibition.⁵

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Stroke is available at www.ahajournals.org/journal/str

Nonstandard Abbreviations and Acronyms

CTA	computed tomography angiography
HU	Hounsfield Unit
LAA	large artery atherosclerosis
NCCT	noncontrast computed tomography
ROC	receiver operating characteristic
TAI	thrombus attenuation increase
TOAST	Trial of ORG 10172 in Acute Stroke Treatment
TP	thrombus perviousness

However, despite its significance for the determination of recommended long-term medical therapy, stroke cause remains undetermined after diagnostic workup in up to one-third of patients with stroke with large vessel occlusion.^{2–4}

It has been suggested that among patients with acute ischemic stroke of undetermined cause, clinical markers and infarct pattern could be used to identify patients who might benefit from treatment with oral anticoagulants as compared to platelet function inhibitors.⁵ However, 2 large clinical trials comparing either rivaroxaban or dabigatran with aspirin in patients with so-called embolic stroke of undetermined source failed to demonstrate a benefit in terms of reducing recurrent stroke rates.^{7,8}

Alternative strategies to determine stroke cause have recently been proposed, such as histological characterization of clot composition following endovascular therapy and detailed categorization of thrombi using advanced imaging techniques.⁹ Among the imaging characteristics, thrombus perviousness (TP), a computed tomography (CT)-based measure that quantifies the degree to which contrast agents in the blood penetrate the thrombus, was found in a recent study to distinguish between patients with presumed cardioembolic and noncardioembolic strokes with high specificity.¹⁰ It is known that thrombus composition and structure, and therefore, thrombus imaging characteristics, are influenced by the time between symptom onset and imaging.^{11,12} Similarly, cellular and noncellular determinants of thrombus formation in the blood such as the concentration of erythrocytes,^{13,14} leukocytes,¹⁵ and platelets,¹⁶ and coagulation parameters at the time of stroke could affect thrombus characteristics. However, it has not yet been investigated how these factors assessed during clinical routine influence TP, and whether TP is associated with cardioembolic stroke when these possible confounders are taken into consideration.

AIM

In the current study, we aimed (1) to analyze how clinical and laboratory parameters assessed during clinical

routine affect measures of TP as assessed on CT, and (2) to validate the accuracy of TP to determine stroke cause in an independent cohort of patients with acute ischemic stroke with a particular focus on its potential to accurately identify cardioembolic stroke cause and to distinguish cardioembolic from LAA strokes.

METHODS

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request. The data are not publicly available as they contain information that could compromise patient privacy.

Study Design and Patient Population

We conducted a retrospective analysis of a prospectively maintained registry of patients with consecutive acute stroke with large vessel occlusion that received mechanical thrombectomy at 2 of the 4 hospital sites of our tertiary medical center Charité – Universitätsmedizin Berlin. Patients who received both noncontrast computed tomography (NCCT) and CT angiography (CTA) before thrombectomy demonstrating isolated occlusion of the middle cerebral artery in any part of the M1 segment with or without extension in one or more M2-branches were eligible for this analysis. NCCT and CTA were acquired at our institution in a single session without repositioning the patient. Patients transferred from an external hospital were not included. If patients received treatment with intravenous thrombolysis, it was started after completion of both NCCT and CTA and therefore did not affect TP measures. Patients with concomitant occlusion of the ipsilateral internal carotid artery were excluded due to difficulties in defining the precise proximal extent of the thrombus. Patients with insufficient image quality, for example, due to motion artifacts that prohibited registration of images or thrombus segmentation and patients in whom stroke cause was not documented in the registry were also excluded from the study.

Based on the data by Berndt et al,¹⁰ we estimated that an analysis of 75 patients would have 95% power to detect an absolute difference in thrombus attenuation increase (TAI; see below) between cardioembolic and noncardioembolic strokes of 8 Hounsfield units (HU) with an assumed SD within each group of 7 HU (effect size, 1.43) and an allocation ratio of 5 given a 2-sided alpha level of 0.05.

Included patients received treatment between October 2015 and December 2019. For all patients, the following data were extracted from the database: baseline demographic, clinical, and imaging parameters; standard laboratory results from the time of admission (blood samples usually taken before acute imaging); radiological (reperfusion success quantified by Thrombolysis in Cerebral Infarction score¹⁷), clinical (National Institutes of Health Stroke Scale score at 24 hours and at discharge), and functional (modified Rankin Scale score at 3 months) outcome parameters, and presumed stroke cause (according to the TOAST [Trial of ORG 10172 in Acute Stroke Treatment] classification¹⁸). Stroke cause by TOAST criteria

was determined by the in-hospital treating physician based on routine workup (ie, Doppler-sonography of the cervical arteries, at least 24-hour rhythm monitoring, and echocardiography) during the hospital stay following acute admission. All patients were treated according to current guidelines on certified stroke units. Extracted laboratory values included cell counts of erythrocytes, leukocytes, and platelets, as well as hemostasis parameters (Quick and activated partial thromboplastin time).

Image Acquisition and Analysis

CT imaging was performed at different scanners from different manufactures (GE Healthcare, Canon Medical Systems, and Siemens Healthineers). NCCT was acquired with standard head protocols (120 kV, fixed mA values 280 to 300 mA, reconstructed slice thickness 1.0 mm). CTA was done immediately after NCCT following intravenous injection of 70 to 120 mL of a nonionic contrast media at 3 to 5 mL/s using standard CTA protocol settings (120 kV, automatic dose modulation, reconstructed slice thickness 0.65–1.0 mm).

For the assessment of TP, we analyzed images from NCCT and CTA scans performed at the time of acute presentation. First, NCCT and CTA scans were transformed to a common coordinate system. For this, we performed threshold-based bone extraction of both NCCT and CTA volumes. The resulting binary volumes were coregistered using a rigid transformation model based on intensity values. The transformation matrix was then applied to the original CTA volume. Complete 3-dimensional thrombus segmentation was performed manually on multiple (≥ 3) sequential axial NCCT-slices. Thrombus perviousness was based on complete thrombus segmentation evaluated on NCCT and CTA together; the distal thrombus border was considered to be either (1) reuptake of contrast on CTA in combination with density change on NCCT or (2) branching of the M1 into the M2-segments. The resulting segmentation was used to extract mean intensity values of the thrombus in both NCCT and CTA. In addition, we also segmented the contralateral M1-segment (using the resliced CTA volume as guidance) to calculate mean intensity values in both NCCT and CTA in a nonoccluded vessel (blood) as reference. Segmentations were performed by 2 raters with >3-year experience with neuroimaging (Drs Kufner and Schlemm) blinded to all clinical data; in uncertain cases, a consensus was reached. TP was assessed by calculating the difference in mean signal intensity (MSI) of the thrombus between CTA and NCCT, that is, TAI as given below:

$$TAI = MSI_{CTA}^{Thrombus} - MSI_{NCCT}^{Thrombus}$$

In addition, the void fraction ϵ of the thrombus was calculated as given below:

$$\epsilon = (MSI_{CTA}^{Thrombus} - MSI_{NCCT}^{Thrombus}) / (MSI_{CTA}^{Blood} - MSI_{NCCT}^{Blood})$$

Figure 1 shows a representative example of the methodology. Image analysis was done using FSL¹⁹ and ITK-SNAP v3.8.²⁰

Statistical Analysis

Categorical data are presented as absolute numbers and proportions and continuous data as median and interquartile

range. For between-group comparisons, we used Fisher exact tests and nonparametric Kruskal-Wallis tests followed by pairwise post hoc comparisons with Dunn-Bonferroni adjustment. In addition, we performed linear regression analysis for TAI and ϵ to identify selected clinical and serological parameters that may modify measurements of TP. Covariates for the multivariable analysis were defined a priori and included stroke cause according to TOAST criteria and parameters hypothesized to alter TP based on a systematic literature search (age, prestroke medication with antiplatelets or anticoagulants, and concentration of erythrocytes and thrombocytes on admission).

To investigate the potential ability of TP to predict cardioembolic stroke, we ran 2 binary logistic regression analyses: one for the detection of cardioembolic stroke (cardioembolic versus LAA+stroke of other determined cause; exclusion of patients with unknown cause), and one for distinction of cardioembolic stroke from LAA stroke (exclusion of patients with other determined cause and unknown cause). In addition to TP (TAI or void fraction ϵ), the multivariable model included the following covariates: age, sex, and history of arterial hypertension. Atrial fibrillation was not included in logistic regression models due to absence or small numbers in noncardioembolic groups.

Receiver operating characteristic (ROC) analyses were performed for both dichotomizations of stroke cause. Youden index was used to determine optimal cutoff thresholds to identify cardioembolic stroke and to distinguish cardioembolic from LAA stroke. Tables and figures for TAI are presented in the main text, detailed results for void fraction ϵ (which were similar to those for TAI) are available online (Figures I and II and Tables I and II in the [Data Supplement](#)). All statistical analyses were performed in STATA/SE 12.1. A *P* value of <0.05 was considered statistically significant.

Institutional Review Board Approval and Informed Consent

The study was designed and conducted in agreement with laws and regulations in the Federal State of Berlin (§25 Landeskrankenhausgesetz Berlin) according to which ethics committee approval and patient consent were not required for this retrospective study.

RESULTS

Descriptive Data of Patient Population

In total, 75 patients with isolated M1 occlusion that underwent thrombectomy were included in the analysis (Table 1). Median age was 71 years (interquartile range, 62–79), median National Institutes of Health Stroke Scale was 16 (interquartile range, 12–19), and 49% had a prestroke history of atrial fibrillation. Patients who had cardioembolic strokes ($n=39$) were older, more often female, and had higher rates of atrial fibrillation and arterial hypertension compared to patients with LAA stroke ($n=18$). Of 3 patients with cardioembolic strokes without atrial fibrillation, 2 were diagnosed with dilative cardiomyopathy and one with filiform thrombi of the aortic valve (Lambd excrescences). Patients with undetermined stroke cause ($n=13$) and patients with other determined

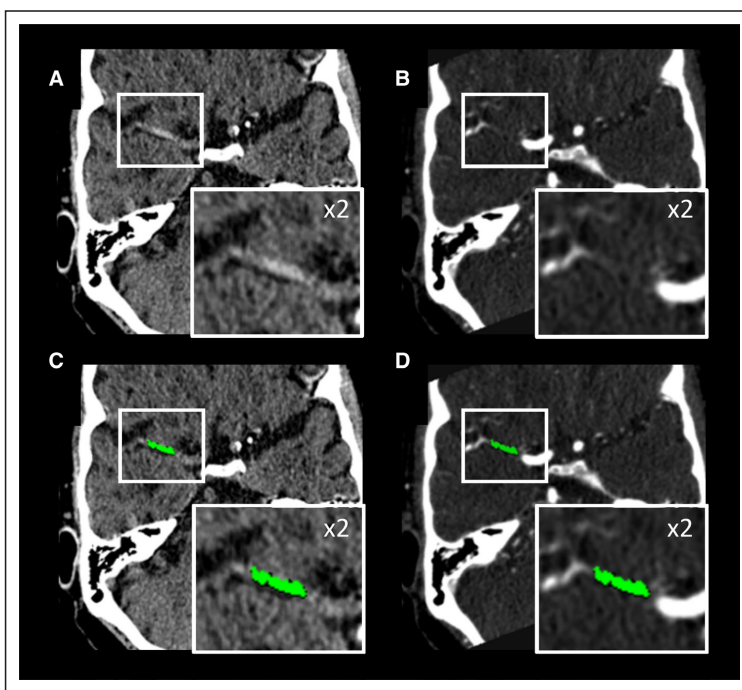


Figure 1. Example of thrombus segmentation for the assessment of thrombus perviousness.

Shown is a representative axial slice of a patient with proximal M1 occlusion without (**A** and **B**) and with (**C** and **D**) overlaid thrombus segmentation. Segmentation was done in ≥ 3 sequential axial slices after coregistration of noncontrast computed tomography (**A** and **C**) and computed tomography angiography (**B** and **D**). For assessment of the void fraction ϵ , the M1 segment contralateral to the thrombus was also segmented in each patient (not shown). Best viewed in color.

cause ($n=5$; 2 internal carotid artery dissections, 3 paradoxical embolisms due to persistent foramen ovale) tended to be younger compared with patients with cardioembolic stroke. No patients were classified in TOAST category 3 (small vessel occlusion).

Associations Between Clinical Parameters and Thrombus Characteristics

In the entire cohort ($N=75$), median TAI was 0.91 (-6.09 to 4.91), and median void fraction ϵ was 0.003 (-0.02 to 0.02). Both measures of TP were significantly associated with stroke cause according to TOAST criteria ($P<0.001$ for TAI and $P=0.002$ for void fraction ϵ). In post hoc pairwise comparisons, TAI was significantly higher in cardioembolic strokes compared with LAA strokes (median, 2.79 [-3.54 to 8.85] versus -5.11 [-11.23 to -1.47]; $P=0.001$). Similarly, strokes of undetermined cause also displayed significantly higher TAI values than LAA strokes (median, 2.57 [-0.18 to 6.30] versus see above; $P=0.005$). However, TAI did not differ significantly between cardioembolic strokes and strokes of undetermined cause ($P=1.00$). TAI in cardioembolic stroke was

also significantly higher than in the combined group of LAA and stroke of other determined cause (noncardioembolic stroke; median 2.79 [-3.54 to 8.85] versus -4.14 [-11.12 to -1.25]; $P=0.001$). The distribution of TAI according to stroke cause is presented in Figure 2.

In univariable linear regression analysis for TAI, only stroke cause was associated with TAI. In comparison to cardioembolic strokes, LAA strokes but not strokes with undetermined or other determined cause were associated with significantly lower TAI values ($P<0.001$). Age, prestroke medication with antiplatelets or anticoagulants, time from onset to acute imaging, and selected laboratory parameters (concentration of erythrocytes, platelets, leucocytes, Quick value, and activated partial thromboplastin time) did not show a significant association with TAI. In multivariable linear regression analysis, stroke cause was the only parameter independently associated with TAI ($P=0.001$; Table 2). Results for void fraction ϵ were similar (see Figure I and Table I in the [Data Supplement](#)).

In additional exploratory analyses, we found no association between stroke cause and mean signal intensity on NCCT (thrombus density, Table III in the [Data Supplement](#)).

Table 1. Baseline Demographics and Clinical Parameters

	All Patients (N=75)	Cardioembolic (n=39)	LAA (n=18)	Other Determined (n=5)	Undetermined (n=13)
Age, median (IQR)	71 (62–79)	73 (69–82)	72.5 (62–76)	50 (44–50)	64(57–74)
Sex, %female (n)	54.7 (41)	59 (23)	44.4 (8)	20 (1)	69.2 (9)
Cerebrovascular risk factors					
Atrial fibrillation, % (n)	50 (37)	92.3 (36)	0 (0)	0 (0)	8.3 (1)
Diabetes mellitus, % (n)	15 (11)	18.4 (7)	16.7 (3)	0 (0)	8.3 (1)
Hypertension, % (n)	82.4 (61)	86.8 (33)	77.8 (14)	40 (2)	92.3 (12)
Hyperlipidemia, % (n)	50.7 (37)	52.6 (20)	66.7 (12)	40 (2)	25 (3)
Current smoker, % (n)	26.6 (17)	16.1 (5)	23.5 (4)	40 (2)	54.6 (6)
Prior medication; antiplatelet or anticoagulants					
None, % (n)	45.2 (33)	39.5 (15)	33.3 (6)	80 (4)	66.7 (8)
Aspirin only, % (n)	31.5 (23)	23.7 (9)	50 (9)	20 (1)	33.3 (4)
Dual antiplatelet therapy, % (n)	2.7 (2)	0 (0)	11.1 (2)	0 (0)	0(0)
Vitamin-K-antagonist, % (n)	6.8 (5)	13.2 (5)	0 (0)	0 (0)	0(0)
Oral anticoagulants, % (n)	10.9 (8)	21.1 (8)	0 (0)	0 (0)	0(0)
Other, % (n)	2.7 (2)	2.6 (1)	5.6 (1)	0 (0)	0(0)
Known time of symptom onset, % (n)	53.3 (40)	38.5 (15)	72.2 (13)	80 (4)	61.5 (8)
Time onset to CT scan in minutes, median (IQR)	61 (53–78)	63 (55–80)	67 (56–70)	65 (36–161.5)	51.5 (44.5–71)
Time LSW to CT scan in minutes, median (IQR)	251 (148–448)	207 (150–430)	184 (81–311.5)	N/A	534 (309–844)
NIHSS on admission, median (IQR)	16 (12–19)	17 (12–20)	14.5 (11–17)	10 (7–11)	16.5 (14.5–18.5)
r-tPA, % (n)	60 (45)	53.9 (21)	61.1 (11)	80 (4)	69.2 (9)
ASPECTS at baseline, median (IQR)	8 (6–9)	8 (6–9)	9 (7–10)	7 (7–8)	8 (6–9.5)

ASPECTS indicates Alberta Stroke Program Early CT Score; CT, computed tomography; IQR, interquartile range; LAA, large artery atherosclerosis; LSW, last seen well; N/A, not applicable; NIHSS, National Institutes of Health Stroke Scale; and r-tPA, recombinant tissue-type plasminogen activator.

Use of Thrombus Perviousness to Determine Cardioembolic Stroke Cause

In univariable regression analysis for detection of cardioembolic stroke including all patients with cardioembolic and noncardioembolic stroke (LAA+stroke of other determined cause), TAI, age, and sex were independently associated with cardioembolic stroke. Following adjustment for confounders, only TAI was independently associated with cardioembolic stroke (odds ratio [OR], 1.12 [95% CI, 1.04–1.22]; $P=0.004$; Table 3).

Both in univariable and multivariable regression analysis for distinction of cardioembolic stroke from LAA stroke, only TAI was an independently associated with cardioembolic stroke (adjusted OR, 1.16 [95% CI, 1.05–1.27]; $P=0.002$; Table 3). Replacing TAI with void fraction ϵ in the model yielded similar results (Table II in the [Data Supplement](#)).

ROC-Analysis

ROC-analysis indicated that measurements of TP (TAI and void fraction ϵ) are both significant indicators to identify cardioembolic stroke (cardioembolic versus LAA+other) with an area under the curve of 0.75 (95% CI, 0.63–0.87) for TAI and 0.74 (95% CI, 0.61–0.86) for ϵ (Figure 3A, Figure IIA in the [Data Supplement](#)). According

to the ROC-analysis, the optimal TAI cutoff value to categorize a thrombus as cardioembolic was 0.09 HU, which was associated with a specificity of 78.3% and sensitivity of 69.2%. A TAI cutoff value of >6.23 HU achieved 100% specificity (at 33.3% sensitivity).

Similarly, ROC-analysis for the distinction of cardioembolic from LAA strokes could identify both TP parameters as significant indicators of cardioembolic stroke with area under the curves of 0.79 (95% CI, 0.68–0.91) and 0.78 (95% CI, 0.67–0.90), respectively. A cutoff value for TAI of >0.60 HU maximized the Youden index and could distinguish a cardioembolic stroke from LAA stroke with 94.4% specificity and 64.1% sensitivity (Figure 3B, Figure IIB in the [Data Supplement](#)). With a cutoff value of 1.93 HU, specificity was 100% (sensitivity, 53.8%).

Test characteristics including sensitivity, specificity, positive and negative predictive value, and accuracy for selected cutoff values of TAI to detect cardioembolic stroke and distinguish cardioembolic from LAA stroke are presented in Table IV in the [Data Supplement](#).

Thrombus Perviousness and Recanalization Success

There was no difference in TP between recanalizers (modified Thrombolysis in Cerebral Infarction 2b/3,

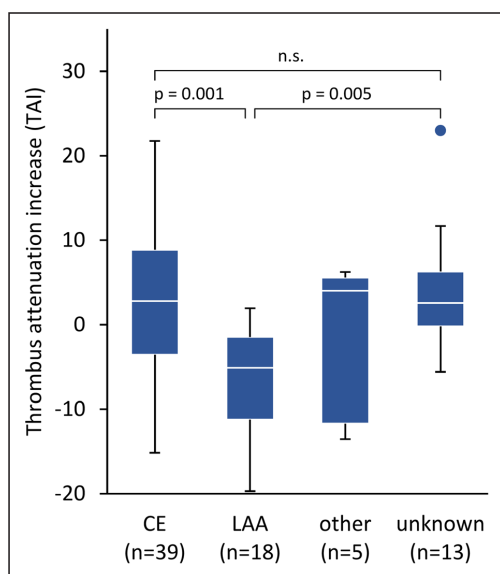


Figure 2. Boxplot of thrombus attenuation increase (TAI) assessed on baseline computed tomography imaging according to stroke cause.

Across all 4 groups, TAI was associated with stroke cause (Kruskal-Wallis test; $P=0.001$). P values presented in the figure are derived from post hoc pairwise comparisons with Dunn-Bonferroni adjustment. Pairwise comparisons involving group 3 (other determined cause) were not statistically significant (n.s.). CE (cardioembolic) stroke (TOAST category 1); LAA (large artery atherosclerosis) stroke (TOAST 2); other, stroke of other determined cause (TOAST 4); unknown, stroke of undetermined cause (TOAST 5).

$N=68$) and nonrecanalizers ($N=6$) in univariate analysis (median, 0.76 [−5.8 to 4.9] versus median, 0.27 [−8.4 to 4.1]; respectively $P=0.722$). In binary logistic regression analysis for recanalization status, TP had a crude OR of 1.02 (95% CI, 0.93–1.1, $P=0.660$). Following adjustment for covariates (including age, stroke cause, time from onset to first imaging) TP had an adjusted OR of 1.15 (95% CI 0.91–1.5, $P=0.231$) for recanalization. Results were similar for the void fraction ϵ ; there was no difference in ϵ between recanalizers and nonrecanalizers in univariate analysis (median 0.003 [0.021–0.024] versus 0.002 [0.29–0.026], $P=0.812$). Crude and adjusted OR for ϵ were 1.0 (95% 0.84–1.3, $P=0.782$) and 1.1 (95% CI, 0.86–1.5, $P=0.361$), respectively.

DISCUSSION

TP measured on baseline CT/CTA in patients with M1 occlusion is independently associated with cardioembolic stroke cause. Age, prestroke medication with antiplatelets or anticoagulants, time to imaging, and selected laboratory parameters do not modify measures of TP in terms of TAI or void fraction ϵ . Selected

threshold values of TAI can accurately distinguish cardioembolic from LAA thrombi with high specificity and positive predictive value.

As expected, patients who had cardioembolic strokes were more often female and more often had atrial fibrillation and a history of hypertension compared with patients with LAA strokes. However, in multivariable analyses that included these unevenly distributed baseline parameters, TP was the only parameter independently associated with cardioembolic stroke.

In line with previous results by Berndt et al,¹⁰ we found that measurements of TP were significantly higher in cardioembolic strokes and strokes with undetermined cause compared to LAA strokes. Absolute values of TAI reported in this analysis are lower than those reported previously (0.91 HU versus ≈ 9.6 HU), whereas differences of median TAI between cardioembolic and LAA strokes are similar (7.9 HU versus 9.3 HU, respectively). This apparent variation in absolute numbers most likely reflects different methodologies for thrombus delineation, namely complete 3-dimensional thrombus segmentation on multiple sequential axial slices in our study versus segmentation on a single best-fitting axial slice in previous reports. Whereas our approach has the advantage of capturing the signal from a larger volume of thrombus material, it is also more susceptible to small misalignments between NCCT and CTA after coregistration which could result in artificially low mean thrombus signal in the CTA due to overlap with low-intensity voxels of cerebrospinal fluid. Because of this effect, negative TAI values in our study were anticipated whenever the true thrombus signal was very similar in NCCT and CTA; therefore, cases with negative values were not excluded nor set to zero in our analysis. The observation that 2 differing methodologies in TP assessment (in Berndt et al¹⁰ and in the current analysis) yield similar results is a testament to the robustness of the method.

Erythrocytes, platelets, and fibrin are found in thrombi in different concentrations⁹ and can affect thrombus signal on NCCT.²¹ In addition, it has been suggested that thrombus structure and imaging characteristics change over time due to remodeling processes as the thrombus matures.^{11,12} This is the first study to assess how selected laboratory parameters (including platelet count, etc) assessed in clinical routine influence TP; in our study, we found no association between these parameters and TP measures. Although the sample size of our study may have been too small to detect associations of small magnitude and larger studies in the future should explore this topic further, our findings indicate that additional consideration of these factors is not likely to improve the ability of TP to identify cardioembolic stroke cause, simplifying its application in clinical practice.

Our results indicate that TP measures may be of clinical value as a novel imaging biomarker for the etiological classification of strokes of undetermined origin. In

Table 2. Linear Regression Models Showing Associations Between Clinical Parameters and Thrombus Attenuation Increase

	Univariable Model		Multivariable Model*	
	Beta (95% CI)	P Value	Beta (95% CI)	P Value
Stroke cause				
Cardioembolic	Reference		Reference	
LAA	-8.68 (-13.24 to -4.12)	<0.001†	-10.69 (-16.21 to -5.18)	<0.001
Stroke of undetermined cause	1.65 (-3.48 to 6.77)	0.523	0.32 (-5.66 to 6.30)	0.915
Stroke of other determined cause	-3.96 (-11.56 to 3.64)	0.303	-4.87 (-14.23 to 4.49)	0.302
Age	0.027 (-0.13 to 0.18)	0.723	0.005 (-0.18 to 0.19)	0.955
Prior medication				
None	Reference		Reference	
Antiplatelets	-1.48 (-6.24 to 3.27)	0.536	0.66 (-4.24 to 5.57)	0.788
Anticoagulants	-1.72 (-7.30 to 3.87)	0.542	-3.03 (-8.82 to 2.75)	0.299
Erythrocytes	-1.84 (-5.60 to 1.92)	0.332	-0.83 (-4.53 to 2.88)	0.657
Thrombocytes	0.01 (-0.11 to 0.03)	0.341	0.01 (-0.007 to 0.03)	0.211
Leucocytes	-0.03 (-0.64 to 0.58)	0.925	N/A	N/A
aPTT	-0.04 (-0.13 to 0.05)	0.352	N/A	N/A
Quick	0.003 (-0.01 to 0.10)	0.948	N/A	N/A
Time from symptom onset to CT in minutes	0.0006 (-0.04 to 0.04)	0.976	N/A	N/A

aPTT indicates activated partial thromboplastin time; CT, computed tomography; LAA, large artery atherosclerosis; and N/A, not applicable.

*Covariates for the multivariable model include stroke cause, age, premedication with antiplatelets or anticoagulants, and serological concentration of erythrocytes and thrombocytes on admission.

particular, the high positive predictive value to distinguish cardioembolic strokes from LAA strokes offers an opportunity to identify a subset of patients with undetected cardiac embolism who may benefit from treatment with anticoagulation instead of platelet inhibitors. Before such a strategy could be tested in prospective clinical trials, multicenter retrospective studies validating our findings in larger samples are required. Such studies should also include subgroup analyses such as the impact of pre-treatment with intravenous thrombolysis, a wider range

of occlusions sites, and the effect of different scanning parameters. Of note, based on our 2 conservative TAI cutoff values with 100% specificity (1.93 HU for distinction of cardioembolic stroke from LAA and 6.23 HU for detection of cardioembolic stroke versus non-cardioembolic stroke), 8 of 13 (62%) or 3 of 13 (23%) patients with undetermined stroke cause would be classified as potential cardioembolic strokes, respectively. TP is derived from routinely acquired imaging data and could be calculated in clinical practice without time delay

Table 3. Binary Logistic Regression Models Showing Associations Between Thrombus Attenuation Increase, Clinical Parameters, and Cardioembolic Stroke Cause

	Univariable Model		Multivariable Model	
	Odds Ratio (95% CI)	P Value	Odds Ratio (95% CI)	P Value
Detection of cardioembolic stroke*				
TAI, per unit increase	1.13 (1.04–1.22)	0.002	1.12 (1.04–1.22)	0.004
Age, per year increase	1.05 (1.00–1.10)	0.031	1.03 (0.98–1.09)	0.222
Male sex	0.45 (0.16–1.28)	0.134	0.78 (0.22–2.83)	0.710
Arterial hypertension	2.89 (0.79–10.53)	0.108	1.59 (0.30–8.51)	0.590
Distinction of cardioembolic stroke from large artery atherosclerotic stroke*				
TAI, per unit increase	1.16 (1.05–1.27)	0.002	1.16 (1.05–1.27)	0.002
Age, per year increase	1.02 (0.97–1.08)	0.375	0.98 (0.92–1.05)	0.746
Male sex	0.57 (0.18–1.72)	0.308	0.71 (0.17–3.01)	0.640
Arterial hypertension	1.89 (0.44–8.09)	0.393	1.54 (0.24–10.09)	0.651

TAI indicates thrombus attenuation increase.

*Two separate binary logistic regression models were created. One for the detection of cardioembolic stroke (vs the combined group of large artery atherosclerosis stroke and stroke of other determined cause). And one for the distinction of cardioembolic stroke from large artery atherosclerosis stroke (excluding strokes with other determined cause).

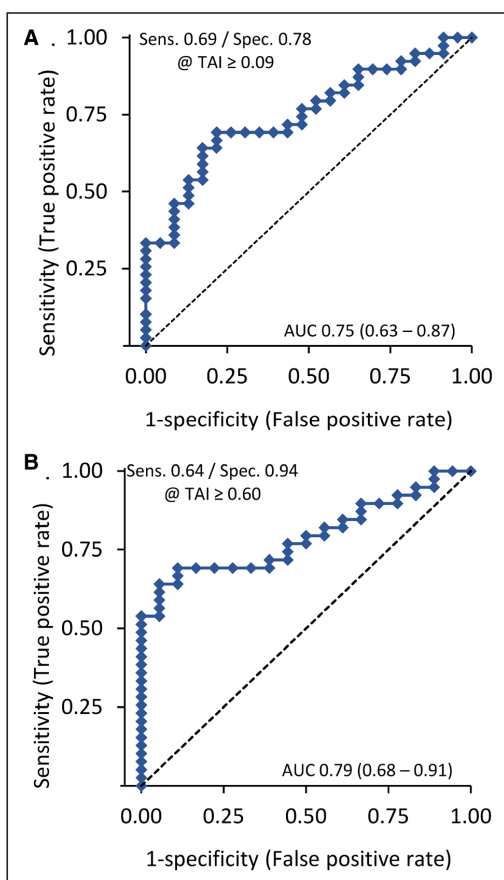


Figure 3. Receiver operating characteristics curves of the association between thrombus attenuation increase (TAI) and stroke cause.

A. Detection of cardioembolic strokes (vs the combined group of large artery atherosclerosis strokes and strokes with other determined cause). **B.** Distinction of cardioembolic strokes from large artery atherosclerosis strokes (excluding stroke with other determined cause). AUC indicates area under the curve; Sens., sensitivity associated with use of the displayed TAI cutoff value; and Spec., specificity associated with use of the displayed TAI cutoff value.

and at minimal cost, ensuring wide availability. Although the specificity was high, sensitivity and negative predictive value of TP to identify cardioembolic strokes in our study were only moderate, indicating that low TP measurements in patients with stroke of undetermined origin should not be used to argue against more extensive workup and monitoring for the detection of potential sources of embolism if clinically indicated.

The focus of the current analysis was to assess the potential role of TP measures for the determination of stroke cause. Additional potential applications of TP are (1) improvement of prognostic accuracy to

predict functional outcome and (2) TP-guided anticipatory planning of the optimal endovascular technique to be employed by the interventionalist, such as use of an aspiration catheter or stent retriever. Although previous studies have shown that TP is associated with successful recanalization and favorable clinical outcome after stroke^{22–24} and that stroke cause modifies the effect of endovascular treatment,²⁵ we are not aware of any studies that investigated how TP measures can be used to guide therapeutic decision making in the acute stage.

We are aware of the following limitations of our study. First, stroke cause according to TOAST was determined after routine workup during the acute hospital admission by the treating physicians; no central ascertainment of TOAST category was performed. However, all patients were treated according to standardized protocols on certified stroke units with continuous quality control. Cerebral magnetic resonance imaging and transesophageal echocardiography were only ordered if considered to be of additional diagnostic value by the treating neurologist; an otherwise covert cardioembolic source might have been detected in some patients if these tests had been done as part of a standard operating procedure. Second, all CT studies used in our analysis were done as acute stroke protocols including NCCT and subsequent CTA. However, scans were performed at 2 different sites, at different scanners, and at different times and therefore with slightly different parameters. Although this heterogeneity may have introduced additional variation in our data, it more closely reflects current clinical reality with a large number of scanners from different manufacturers and diverse protocols. Third, we did not maintain a screening log and therefore do not know what proportion of patients in an unselected population of stroke patients with large vessel occlusion would fulfill the eligibility criteria of our study; test statistics were calculated with reference to patients included in our study. Fourth, it is important to note that our analysis was limited to patients with M1 occlusion without concomitant ipsilateral internal carotid artery occlusion. Therefore, our results cannot be generalized to patients with occlusions of the carotid or basilar artery or isolated occlusions of the M2 segment of the middle cerebral artery. Additional studies are essential to investigate the role of TP measures in these cases. Fifth, the assessment of laboratory values only included routine parameters available in the emergency department; we did not perform functional assessments of coagulation or platelet function, which could also affect TP measures. Detailed results of cardiac workup in patients with cardioembolic stroke, in particular, cardiac output due to reduced ejection fraction, which could impact CTA was not available. However, reduced cardiac output would be expected to lead to numerically lower TP measures in cardioembolic

stroke patients as opposed to higher values observed in our study. Sixth, our sample size was too small to investigate the relationship between TP and long-term outcome among patients with undetermined stroke cause. We also do not know which patients (if any) of those with undetermined stroke cause at time of discharge were found to have an embolic source such as intermittent atrial fibrillation in subsequent follow-up examinations. Because of these limitations, TP measures should currently not be used as sole criterion to recommend anticoagulation for patients with stroke of undetermined cause.

In conclusion, assessment of TP on baseline NCCT/CTA in patients with M1 middle cerebral artery occlusion may be helpful to determine stroke cause and guide secondary prevention. Larger, multicenter, independent studies are warranted to confirm the predictive value of TP and to identify potential confounders before translation into clinical practice could be attempted.

ARTICLE INFORMATION

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Supplemental Materials

Online Tables I–IV
Online Figures I–II

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2.2 THROMBUS PERVIOUSNESS IN PATIENTS WITH BA OCCLUSION

Kufner A, Endres M, Scheel M, Leithner C, Nolte CH, Schlemm L. No Association Between Thrombus Perviousness and Cardioembolic Stroke Etiology in Basilar Artery Occlusion Stroke. *Front Neurol* **12**, 712449, (2021).

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Basilar artery (BA) occlusion occurs in approximately 10% of all patients with large vessel occlusion⁴¹. For these patients too, a timely and accurate identification of stroke cause is crucial to guide long-term secondary prevention to prevent recurrent events. However, in up to 35% of patients with BAO, no cause can be identified.

In 80 BAO patients stemming from a prospective clinical database of patients who underwent EVT at all three Charité Campus sites, TP was not associated with stroke etiology. Binary logistic regression analysis did not identify TP as an independent indicator of cardioembolic stroke (adjusted OR 1.0 95%CI 0.95 – 1.0, p=0.75). There was no association with successful recanalization (adjusted OR 1.4 95% CI 0.70–2.7, p = 0.35) or favorable outcome (adjusted OR 1.1 95%CI 0.94–1.2, p= 0.30). Although previous studies suggested that TP in BAO patients may aid in differentiating between BAO with and without underlying basilar stenosis and response to EVT, in the current study we could not validate these findings.



No Association Between Thrombus Perviousness and Cardioembolic Stroke Etiology in Basilar Artery Occlusion Stroke

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Background: Thrombus perviousness (TP) quantified by thrombus attenuation increase (TAI) assessed on acute non-contrast computed tomography (NCCT) and CT angiography (CTA) may be associated with stroke etiology in anterior circulation ischemic stroke. We investigated whether TP is associated with stroke etiology and recanalization after mechanical thrombectomy in patients with acute basilar artery occlusion (BAO).

Methods: Eighty patients with complete BAO and in-house acute imaging from a prospectively maintained database were included. Two raters independently segmented the complete thrombus on co-registered NCCT and CTA to determine TAI in Hounsfield units (HU_{CTA}–HU_{NCCT}); averaged values of the raters were used for analysis. Recanalization to modified treatment in cerebral ischemia (mTICI) score 2b/3 was considered successful, and 90-day modified Rankin Scale score 0–2 was considered favorable.

Results: TAI did not differ between patients with different stroke etiologies; median TAI in patients with cardioembolic stroke ($n = 36$) was -0.47 (interquartile range -4.08 to 7.72), 1.94 (-8.14 to 10.75) in patients with large artery atherosclerosis (LAA; $n = 25$), and -0.99 (-6.49 to 5.40) in patients with stroke of undetermined origin ($n = 17$; $p = 0.955$). Binary logistic regression analyses did not identify TAI as an independent indicator of cardioembolic stroke (adjusted odds ratio [OR] vs. LAA stroke: 1.0 [95% CI: 0.95 – 1.0], $p = 0.751$). There was no association with successful recanalization (adjusted OR 1.4 [0.70 – 2.7], $p = 0.345$) or favorable outcome (adjusted OR 1.1 [95% CI: 0.94 – 1.2], $p = 0.304$).

Conclusion: In contrast to proximal middle cerebral artery occlusions, TP in BAO patients is not associated with cardioembolic stroke etiology. Larger confirmatory studies to establish the potential role of TP for clinical applications should focus on patients with anterior circulation stroke.

Keywords: basilar artery occlusion, ischemic stroke, computed tomography, thrombus, perviousness

INTRODUCTION

Basilar artery (BA) occlusion (BAO) is less frequent than anterior circulation stroke and occurs in approximately 10% of all patients with large vessel occlusions (1, 2). While outcome following BAO has been generally poor in the past (3), with the availability of endovascular therapies (EVTs), up to one-third of BAO patients achieve functional independence (modified Rankin Scale [mRS] score 0–2) (2, 4, 5). Similar to anterior circulation stroke, accurate etiological classification and identification of a cardioembolic source are essential to guide long-term secondary prevention in patients with BAO. However, despite its significance for therapeutic decision making, no cause can be identified after diagnostic workup in 22–35% of patients with BAO (2).

In patients with acute stroke and occlusion of the proximal M1 segment of the middle cerebral artery (MCA), thrombus perviousness (TP)—quantified by thrombus attenuation increase (TAI) on computed tomography (CT) angiography (CTA) relative to non-contrast CT (NCCT)—is associated with cardioembolic stroke etiology (6, 7). In the current study, we analyzed whether TP on admission CT is also associated with cardioembolic stroke etiology, recanalization rates, and functional outcome following EVT in patients with BAO.

METHODS

The raw data are not publicly available, as they contain information that could compromise patient privacy; anonymized summary data can be provided by the corresponding author upon reasonable request.

We performed an investigator-initiated retrospective study including patients from three different sites of our university hospital. Acute ischemic stroke with BAO enrolled in our prospectively maintained in-house database of consecutive patients undergoing EVT between 2015 and 2020 were eligible for inclusion. Inclusion criteria were as follows: NCCT and CTA on admission in a single session, showing complete BAO, and no administration of intravenous thrombolysis before imaging. Clinical data including demographic baseline data, presumed stroke etiology according to the trial of ORG 10172 in acute stroke treatment (TOAST; determined by local investigators unaware of TP who treated patients on certified stroke units according to current guidelines), and recanalization status were extracted from the database. Recanalization to modified treatment in cerebral ischemia (mTICI) score $\leq 2b/3$ was considered successful, and 90-day mRS score 0–2 was considered favorable.

Image analysis was performed as described previously (7). Briefly, NCCT and CTA scans were co-registered using a rigid transformation based on high-intensity signals. With the use of information from both NCCT and CTA, thrombi in the BA were segmented on consecutive axial slices. The proximal and distal ends of the thrombus were defined as (a) abrupt change of signal intensity in the CTA and/or (b) hyperdense signal in the NCCT. Thrombus material in the vertebral or P1 segment of the posterior cerebral arteries was not included in the segmentation (Figure 1). All segmentations were performed

independently by AK and LS and averaged for further analyses. After confirming approximate normal distributions of voxel intensities within individual thrombi by visual inspection of intensity histograms, TP was calculated as mean thrombus signal intensity increase in the CTA relative to NCCT. Image analysis was done in ITK-SNAP v3.8 (8) and MRICroGL (<https://www.nitrc.org/projects/mricron>).

TP was compared between groups using the Kruskal–Wallis test; proportions were compared using analysis of (co-)variance. We calculated multivariable binary logistic regression models including age, sex, and arterial hypertension [variables differed between cardioembolic and large artery atherosclerosis (LAA) groups in a univariate analysis; atrial fibrillation could not be included due to no cases of LAA patients] to test for a significant association between cardioembolic stroke etiology and outcome. Concordance between readers was quantified as intraclass correlation and determined using a two-way mixed model.

The study was designed and conducted in agreement with laws and regulations in the Federal State of Berlin according to which ethics committee approval and patient consent were not required for this retrospective study.

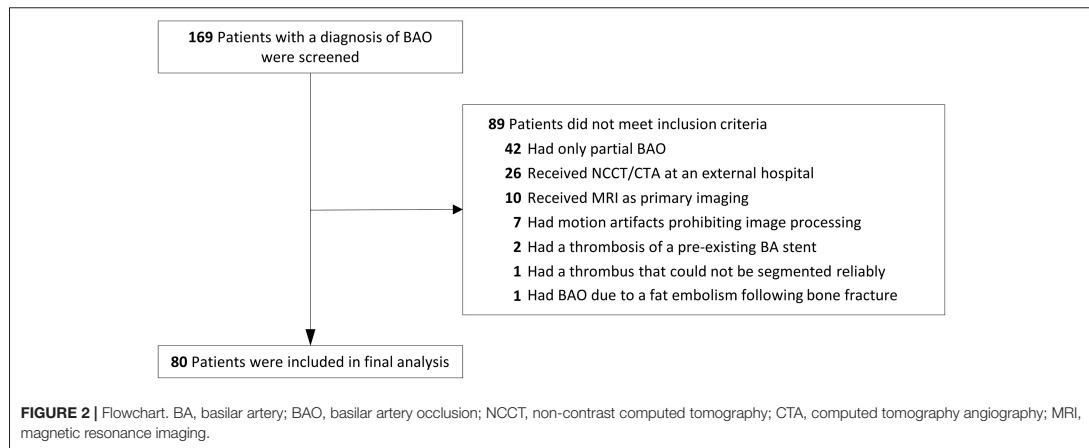
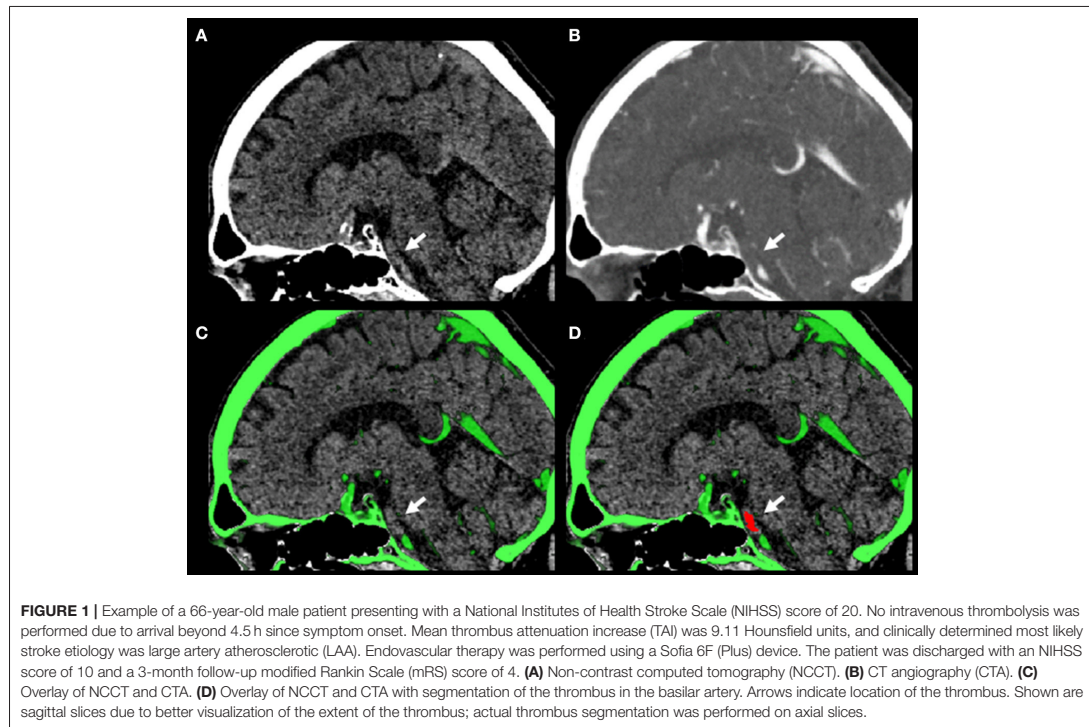
RESULTS

One hundred sixty-nine patients with a diagnosis of BAO were screened, of which 80 were included in the final analysis (Figure 2). Descriptive data of the patient population according to stroke etiology are presented in Table 1. Overall TAI values ranged from -20.8 to 29.1 Hounsfield units (HU); agreement between raters was excellent (intraclass correlation 0.97). Patients with cardioembolic stroke etiology ($N = 36$) were older and had higher rates of atrial fibrillation and arterial hypertension. Two patients had stroke of other determined etiology (one case was caused by dissection of the vertebral artery, and the other was due to a paraneoplastic clotting disorder that led to a paradoxical embolism and subsequent BAO). Intravenous thrombolysis with recombinant tissue plasminogen activator at a dose of 0.9 mg per kg body weight was administered in 50% of all patients. In all patients, endovascular clot removal was attempted, mostly with a Sofia 6F (Plus), a Sofia 5F, or a Trevo device.

There was no association between TP and stroke etiology; median (interquartile range [IQR]) values of TAI in patients with cardioembolic stroke, LAA stroke, stroke of undetermined origin, and stroke of other determined etiology were -0.47 (-4.08 to 7.72), 1.94 (-8.14 to 10.75), -0.99 (-6.49 to 5.40), and -0.47 (-1.86 to 0.92), respectively ($p = 0.955$; Figure 3).

In a multivariable binary logistic regression model for the distinction of cardioembolic stroke from LAA stroke etiology, TAI was not independently associated with cardioembolic stroke etiology (odds ratio [OR] 1.0 [95 CI: 0.95–1.0], $p = 0.751$; Table 2).

Mean thrombus density ranged from 22.1 to 74.7 HU (median 49.8, IQR = 41.6–55.8). There was no association between thrombus density and stroke etiology ($p = 0.341$). Median thrombus volume was 45.3 mm³ (IQR = 27.0–68.4) and did not differ between groups ($p = 0.942$).



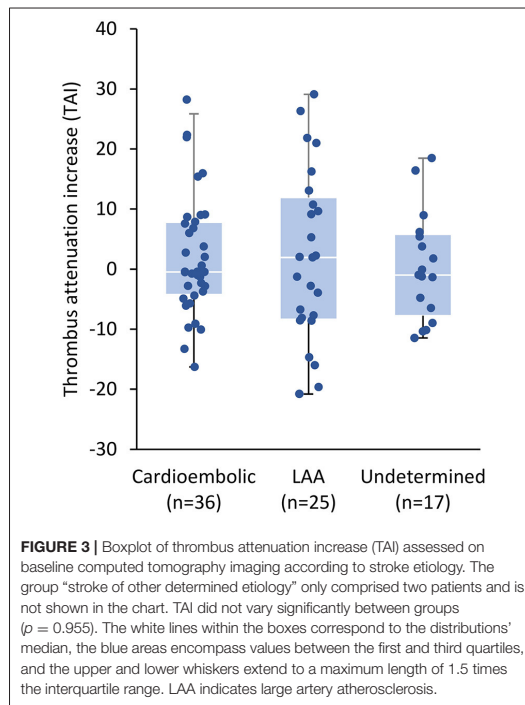
Seventy-three patients were successfully recanalized by treatment. TP was nominally lower in recanalizers compared with non-recanalizers (median -0.49 , IQR $[-6.1$ to $7.9]$ vs. 2.0 $[-0.80$ to $21.0]$; $p = 0.155$). Following adjustment for age, sex, and stroke etiology, TP had a crude OR of 1.5 (95% CI:

0.89 to 2.6 ; $p = 0.115$) and adjusted OR of 1.39 (0.70–2.7), $p = 0.345$) for successful recanalization. In a subgroup of 24 patients with available 90-day mRS, TP had a crude and adjusted OR for favorable outcome (mRS ≤ 2) of 1.05 (95% CI: 0.97–1.1, $p = 0.205$) and 1.1 (95% CI: 0.94–1.2, $p = 0.304$).

TABLE 1 | Baseline demographics and clinical parameters.

	All patients (n = 80)	Cardioembolic (n = 36)	LAA (n = 25)	Undetermined (n = 17)	Other (n = 2)
Age, median (IQR)	72.8 (12)	75.9 (9.5)	72.5 (8)	69.7 (17.1)	46 (12.7)
Sex, % female (n)	42.5 (34)	47.2 (17)	40 (10)	41.2 (7)	0 (0)
Cerebrovascular risk factors					
Atrial fibrillation, % (n)	44.3 (35)	88.9 (32)	0 (0)	18.8 (3)	0 (0)
Diabetes, % (n)	11.5 (9)	2.9 (1)	24 (6)	12.5 (2)	0 (0)
Hypertension, % (n)	80 (20)	93.3 (14)	71.4 (5)	33.3 (1)	0 (0)
Hyperlipidemia, % (n)	35.4 (28)	33.3 (12)	44 (11)	31.3 (5)	0 (0)
Current smoker, % (n)	14.1 (10)	5.6 (2)	13 (3)	49 (4)	50 (1)
Prior medication; antiplatelet or anticoagulants					
None, % (n)	44.2 (34)	34.3 (12)	52.2 (12)	47.1 (8)	100 (2)
Aspirin only, % (n)	24.7 (19)	8.6 (3)	43.5 (10)	35.3 (6)	0 (0)
Dual antiplatelet therapy, % (n)	1.3 (1)	0 (0)	4.4 (1)	0 (0)	0 (0)
Vitamin K antagonist, % (n)	9 (7)	20 (7)	0 (0)	0 (0)	0 (0)
Oral anticoagulants, % (n)	19.5 (15)	34.3 (12)	0 (0)	17.7 (3)	0 (0)
NIHSS on admission, median (IQR)	20 (12–30)	18 (12–28)	24 (11–30)	26.5 (17–32)	12.5 (10–15)
rTPA, % (n)	50 (40)	27.8 (10)	76 (19)	56.3 (9)	100 (2)

LAA, large artery atherosclerosis; IQR, interquartile range; NIHSS, National Institutes of Health Stroke Scale; rTPA, recombinant tissue plasminogen activator.



DISCUSSION

In the current analysis, TP assessed on acute NCCT/CTA in patients with BAO was not associated with underlying stroke etiology.

Absolute values of TAI reported in this study were markedly lower than those reported previously in patients with BAO (mean 2.2 HU vs. ~18.9 HU) (9); this is most likely due to differing methodologies as described in detail previously (7). Briefly, here, an entire 3D thrombus segmentation across multiple consecutive slices was applied compared with previous reports in which spherical regions of interest (ROIs) were placed within thrombi on a best-fitting axial slice and negative values were set to zero (6, 9, 10). Despite the discrepancy of absolute values reported, we also observed higher TAI measurements in patients with LAA stroke compared with non-LAA stroke, although this difference was not statistically significant.

Interestingly, previous studies found that high TAI values in patients with M1 occlusion were associated with cardioembolic stroke etiology (6, 7). Therefore, one might have expected a similar observation in BAO patients; however, this was not the case. In contrast to the MCA, the BA can be perfused antegrade as well as retrograde in the case of occlusion, depending on the local perfusion pressures. Furthermore, a much higher rate of LAA BAO occurs due to local thrombosis of underlying stenosis, whereas M1 occlusions are more often caused by embolism from proximal arteries (11). These pathophysiological differences likely contribute to altered clot characteristics and may affect TAI depending on occlusion site and characteristics of the circle of

TABLE 2 | Binary logistic regression analysis for the distinction of cardioembolic stroke from large artery atherosclerosis stroke.

	Univariate model		Multivariate model	
	Odds ratio (95% CI)	p-value	Odds ratio (95% CI)	p-value
TAI, per unit increase	0.99 (0.96–1.04)	0.997	0.99 (0.95–1.04)	0.751
Age, per year increase	0.96 (0.90–1.02)	0.158	0.96 (0.89–1.04)	0.892
Male sex	1.34 (0.48–3.78)	0.577	1.05 (0.34–3.26)	0.928
Arterial hypertension	0.50 (0.12–2.09)	0.342	0.80 (0.15–4.29)	0.792

TAI, thrombus attenuation increase.

Willis. Also, the close proximity of the BA to the skull base might affect perviousness measurements. However, a comprehensive analysis on effect of thrombus location on TP is still lacking.

It was recently suggested that TP might differentiate between BAO with and without underlying basilar stenosis (9). In our study, information on basilar stenosis as assessed on digital subtraction angiography was not available. Instead, we examined the relationship between TP and stroke etiology defined by TOAST criteria and found no significant association, neither in a univariable analysis nor in a multivariable analysis. These results are in line with findings from a recent study, which—in contrast to patients with M1 occlusions (6)—found no association between histological thrombus composition and stroke etiology in patients with BAO (12). The available evidence therefore suggests that the potential role of TP measurements to guide determination of stroke etiology might be limited to occlusions in the anterior circulation.

The primary limitations of this study include its retrospective nature and relatively small sample size, which increase the likelihood of type II errors. Nonetheless, considering BAO makes up only ~2% of all acute ischemic strokes (2), we believe that these results of a consecutive cohort of 80 BAO patients are valuable to further our understanding of the diagnostic value of TP as a novel imaging marker. Secondly, there was no central assessment of stroke etiology; rather, stroke cause was determined by the treating physicians on certified stroke units at the admitting hospital. Thirdly, we did not perform histological analyses of the retrieved thrombi and therefore could not assess any relationship between thrombus composition and TP measures or outcome. Lastly, clinical follow-up was only available for a subset of patients.

CONCLUSION

In conclusion, we found no association between TP assessed on acute NCCT/CTA in patients with BAO and stroke etiology. Additional studies are required to identify the mechanisms by which thrombus location affects the relationship between TP and stroke etiology. In the meantime, confirmatory studies to establish the potential role of TP for

clinical applications should focus on patients with anterior circulation stroke.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available in anonymized form from the corresponding author upon reasonable request. The data are not publicly available due to their containing information that could compromise the privacy of research participants.

ETHICS STATEMENT

Ethical review and approval was not required for this retrospective study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

LS, AK, and CN conceived and designed the study. LS developed the pipeline for image analysis. AK and LS performed thrombus segmentations and prepared the manuscript. AK performed the statistical analyses. All authors contributed to acquisition, analysis of the data and critical revision of the manuscript, and figures for intellectual content.

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Conflict of Interest: ME reports grants from Bayer and fees paid to the Charité from Bayer, Boehringer Ingelheim, BMS, Daiichi Sankyo, Amgen, Sanofi, Novartis, and Pfizer, all outside the submitted work. MS reports no conflicts of interest related to this work. He is a patent holder and shareholder of PhantomX a company producing 3D models for CT. CL reports personal fees from Edwards Lifesciences and fees paid to Charité from Bard, Zoll, and Pfizer, all outside the submitted work. CN reports personal fees from Boehringer Ingelheim, Pfizer Pharma, Britsol-Myers Squibb, Abbott, and Portola, outside the submitted work. LS reports personal fees from Daiichi Sankyo, outside the submitted work.

The remaining author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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2.3 FLAIR HYPERINTENSE VESSELS, COLLATERALIZATION, AND OUTCOME

Nave AH, **Kufner A**, Bücke P, Siebert E, Kliesch S, Grittner U, Bätzner H, Liebig T, Endres M, Fiebach JB, Nolte CH, Ebinger M, Henkes H. Hyperintense Vessels, Collateralization, and Functional Outcome in Patients With Stroke Receiving Endovascular Treatment. *Stroke*. 2018 Mar;49(3):675-681.

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In a multicenter cohort of 116 patients with isolated M1 occlusions following EVT, we investigated whether FHVs assessed on acute MRI were associated with collateral status (assessed on baseline angiography performed during EVT), rates of vessel recanalization, as well as 3-month functional outcome (modified Rankin Score; mRS)²⁷. All patients included in this study received an MRI within 12 hours of symptom onset and had visible FHVs on at least one FLAIR slice. Interrater reliability was high for FHV assessment (Cronbach $\alpha = 0.9$).

Patients with extensive FHVs had significantly higher rates of good collateral grading scores (83% versus 57%, $p=0.025$), assessed by two experienced interventional neuroradiologists and graded according to the American Society of Interventional and Therapeutic Neuroradiology Score. Extensive FHVs were also associated with better functional outcomes 3 months post-stroke (median mRS 2 IQR 0-5 versus 4 IQR 3-6, $p=0.015$). Multiple logistic regression analysis of the pooled cohort confirmed that extensive FHVs were independently associated with good collateral status (OR 4.4 95%CI 1.1-18.3; $p=0.03$) and good functional outcome (mRS \leq 2; OR 5.0 95% CI 1.8-13.9) after adjustment for relevant confounders.

Based on this study, we can conclude that FHV assessment is a useful, easy-to-rate MR-parameter that serves as a relevant surrogate for collateralization in the acute setting of ischemic stroke. In other words, assessment of FHVs might be a valuable guide for future patient selection for EVT.

Hyperintense Vessels, Collateralization, and Functional Outcome in Patients With Stroke Receiving Endovascular Treatment

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Background and Purpose—Fluid-attenuated inversion recovery hyperintense vessels (FHV) are frequently observed on magnetic resonance imaging in acute stroke patients with proximal vessel occlusion. Whether FHV can serve as a surrogate for the collateral status and predict functional outcome of patients is still a matter of debate.

Methods—Acute ischemic stroke patients with M1-middle cerebral artery occlusion who received magnetic resonance imaging before endovascular treatment in 3 hospitals in Germany between January 2007 and June 2016 were eligible. Quantification of FHV was performed using an FHV–Alberta Stroke Program Early CT Score (ASPECTS) rating system. Functional outcome was evaluated with the modified Rankin Scale 3 months after stroke. Collateral status of patients was graded on baseline angiography using the American Society of Interventional and Therapeutic Neuroradiology grading system. Odds for good outcome (modified Rankin Scale score, 0–2) were determined using logistic regression analyses.

Results—Overall, 116 patients were analyzed (median age, 74; interquartile range [IQR], 64–79; median National Institutes of Health Stroke Scale, 14; IQR, 10–19). The median FHV-ASPECTS was 2 (IQR, 1–3). Good collateral status (American Society of Interventional and Therapeutic Neuroradiology grade 3–4) on angiography was more frequently observed in patients with FHV-ASPECTS ≤ 2 (83% versus 57%; $P=0.025$). Patients with an FHV-ASPECTS ≤ 2 had a better functional outcome after 3 months (median modified Rankin Scale score, 2; IQR, 0–5), compared with patients with an FHV-ASPECTS > 2 (median modified Rankin Scale score, 4; IQR, 3–6; $P=0.015$). In multiple regression analyses, FHV-ASPECTS ≤ 2 was independently associated with good functional outcome (adjusted odds ratio, 5.3; 95% confidence interval, 1.5–18.2).

Conclusions—Low FHV-ASPECTS is associated with both better collateral status and better 3-month functional outcome in acute stroke patients with M1 vessel occlusion. (*Stroke*. 2018;49:675–681. DOI: 10.1161/STROKEAHA.117.019588.)

Key Words: angiography ■ logistic models ■ magnetic resonance imaging ■ stroke ■ thrombectomy

Endovascular treatment (EVT) can effectively reduce disability of patients with anterior circulation stroke and proximal vessel occlusion after 3 months.¹ Baseline collateral status of patients with stroke is considered to play a crucial role for functional recovery² and risk of hemorrhagic transformation after recanalization.³ Good collaterals are critical regarding the extension of treatment times by sustaining the ischemic penumbra and limiting infarct growth.⁴ For example, a computed tomography (CT)-based collateral grading was used as a selection criterion in 1⁵ of the 5 recent thrombectomy trials.^{6–9} However, noncontrast CT or magnetic resonance

imaging (MRI)-based approaches to assess collateral status in acute stroke are lacking and require direct comparison with angiography—the gold standard for cerebral collateralization assessment.

Fluid-attenuated inversion recovery hyperintense vessels (FHV) have been suggested as possible surrogate markers of collateral status. FHV are frequently observed on fluid-attenuated inversion recovery (FLAIR) sequence scans in the affected hemisphere of patients with acute stroke.¹⁰ Although it is believed that slow blood flow in cerebral arteries explains the visibility of FHV,¹¹ the pathophysiological interpretation of

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FHV varies.^{10,12–14} Although most studies agree that the presence of FHV in stroke is associated with large vessel occlusion,¹⁴ results on the clinical relevance of this MRI sign are inconsistent. Some studies found that FHV is associated with more severe cerebral hypoperfusion and worse functional recovery poststroke and postulated that FHV represents insufficient collateralization.^{14–16} However, these studies consisted of small heterogeneous cohorts, including large and distal vessel occlusions, questioning the generalizability of such findings. Other studies have described the correlation of FHV with collateral status of conventional angiography in smaller pilot studies.¹⁷ Further, a recent study by Liu et al¹⁸ demonstrated that FHV–Alberta Stroke Program Early CT Score (ASPECTS) graded outside of the diffusion-weighted imaging (DWI) lesion was associated with good clinical outcome at discharge and postulated that FHV is indicative of sufficient collateralization.

Thus, the question remains whether FHV can be used as a surrogate for collateral status and whether the presence of FHV carries prognostic value in terms of functional recovery after ischemic stroke.

Therefore, in this study, we assessed whether FHV is independently associated with functional outcome after 3 months in a homogenous cohort of acute stroke patients with M1 vessel occlusion. Furthermore, we investigated whether FHV is associated with cerebral collateral status.

Materials and Methods

Data Sources, Study Design, and Patient Selection

The data that support the findings of this study are available from the corresponding author on reasonable request. For this study, we pooled 2 large, prospective registries of patients with acute stroke from 3 medical centers (Campus Benjamin Franklin of the Charité-Universitätsmedizin, Berlin, and Katharinenhospital and Bürgerhospital of the Klinikum Stuttgart) in Germany. The databases registered consecutive patients with acute ischemic stroke who received EVT (ie, thrombectomy or intra-arterial thrombolysis, or both) from 2008 (Charité-Universitätsmedizin Berlin) and 2007 (Klinikum Stuttgart), respectively, until June 2016. All ischemic stroke patients with an acute M1-middle cerebral artery (MCA) occlusion who received an MRI before EVT were included in this study. Patients with MRI lacking FLAIR imaging, coronal FLAIR imaging only, or strong motion artifacts making proper rating impossible were excluded from the analysis. Stroke severity was assessed with the National Institutes of Health Stroke Scale (NIHSS) score on admission. Patient's clinical outcome was assessed with the NIHSS score and the modified Rankin Scale (mRS) at time of discharge. Clinical follow-up of patients was performed by trained raters blinded to the imaging data using the mRS 3 months after stroke via a standardized telephone interview. The primary end point was a good functional outcome 3 months after stroke defined as mRS 0 to 2. Secondary objectives include the association of FHV with collateral status on angiography at time of mechanical thrombectomy and clinical outcome at discharge from hospital, respectively. The study was performed in accordance to the declaration of Helsinki. The local ethics committee approved patient data assessment and analyses (EA4/061/14, EA4/019/08, and F-2012–077). Informed consent was obtained from patients.

Imaging Analysis

Patients received an MRI including transversal FLAIR sequence within 12 hours of stroke onset before EVT. MRI scans were performed with a 3.0-Tesla scanner (Tim Trio; Siemens AG, Germany) in Berlin and a 3.0-Tesla scanner (MAGNETOM Skyra; Siemens

AG, Germany) or 1.5-Tesla scanner (AERA; Siemens AG, Germany) in Stuttgart. Two raters with multiple years of clinical and scientific stroke imaging experience independently performed MRI ratings in Berlin (A.H.N. and A.K.) and in Stuttgart (A.H.N. and P.B.). If grading differed between raters, a consensus decision was made.

FHVs were defined as circular or serpentine hyperintensities present on at least 2 consecutive slices and quantified on transversal FLAIR images using a FHV-ASPECTS grading system, as described previously.^{16,19} In brief, the presence of FHV was evaluated on 2 standardized slices, together representing 7 vascular territories of the MCA (M1–3 segments and the insular ribbon next to the basal ganglia, and M4–6 segments, superior to the basal ganglia and next to the lateral ventricles). The detection of FHV in each MCA-ASPECTS territory was added up and the sum then subtracted from 7. Thus, FHV-ASPECT scores range from zero (FHV visible in all territories) to 7 points (no FHV visible). Examples of different FHV-ASPECTS ratings are illustrated in Figure 1. The median FHV-ASPECTS was used to dichotomize into low and high FHV-ASPECTS. Additional MRI ratings included FLAIR demarcation of the DWI lesion according to the WAKE-UP trial criteria,²⁰ as well as DWI-ASPECTS grading to assess infarct size.²¹

Two experienced interventional neuroradiologists (E.S. and S.K.) reviewed baseline angiography data of all included patients. Revascularization success was graded using the Modified Thrombolysis in Cerebral Ischemia (mTICI) score (zero=complete occlusion to 3=complete revascularization). Good mTICI was defined as mTICI 2b–3. The collateral grading score, endorsed by the American Society of Interventional and Therapeutic Neuroradiology (ASITN) and Society of Interventional Radiology,²² was used to grade the collateral status (ASITN grade zero=no collaterals to 4=complete and rapid collateral perfusion of the ischemic territory). Good collateral status was defined by ASITN grade 3–4.² Collateral status assessment was performed in the Stuttgart cohort only because only this data set met the special requirements of a full diagnostic angiogram for a valid grading of the collateral status.²³ For patients with wake-up stroke, the last-seen-well time point, if available, was chosen as a proxy for stroke onset to calculate treatment times.

Statistical Analysis

FHV-ASPECTS was analyzed as a dichotomous variable, categorized at the median value of the pooled cohort. Collateral status was dichotomized in ASITN <3 versus ≥3. We performed univariate analyses using the Mann–Whitney *U* test for the comparison of continuous variables. The χ^2 test was used for the comparisons of frequencies between groups, as appropriate. Bivariate and multiple logistic regression analyses were performed to determine how FHV-ASPECTS and other characteristics were associated with good outcome at 3 months after stroke. A limited number of possible confounders were included in the multiple regression models and selected if they were clinically relevant cardiovascular risk factors or showed some relation to functional outcome after stroke. The first model (model 1) included demographics and comorbidities: FHV-ASPECTS, age, sex, diabetes mellitus, and hypertension as independent variables. The second model (model 2) included covariates of the first model and variables of stroke severity and revascularization success: DWI-ASPECTS, NIHSS, and mTICI score. An exploratory multivariable regression analysis of the Stuttgart cohort was performed to assess the association of collateral status with low FHV-ASPECTS and with good functional outcome after 3 months, respectively. Covariates defined as clinically relevant and possible confounders were included into the model: low FHV-ASPECTS, good collateral status, age, sex, NIHSS, diabetes mellitus, and hypertension. Interobserver reliability for FHV-ASPECTS rating was assessed using Cronbach α .

Statistical analyses were performed with IBM SPSS Statistics 22 (SPSS, Inc, Chicago). Results were considered significant at a 2-sided α level of 0.05.

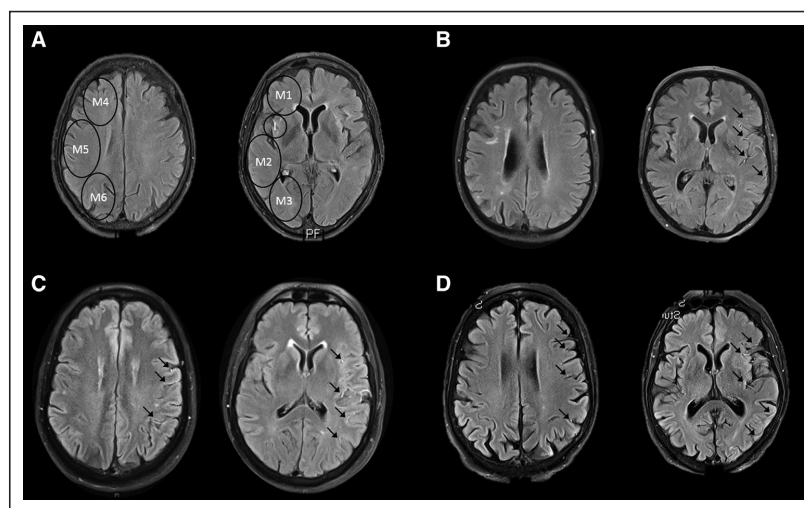


Figure 1. Examples of fluid-attenuated inversion recovery hyperintense vessel–Alberta Stroke Program Early CT Score (FHV-ASPECTS) ratings. FHVs were defined as linear or serpentine hyperintensities corresponding to a typical arterial course on at least 2 consecutive axial slices. FHV-ASPECTS score was evaluated on 2 standardized slices (A), together representing 7 vascular territories of the middle cerebral artery (M1–3 segments and the insular ribbon, and M4–6 segments). The detection of FHV (black arrows) in each middle cerebral artery–ASPECTS territory was added up and the sum then subtracted from 7. Panels represent different rating examples: (A) no FHV visible; FHV-ASPECTS=7. B, FHV visible in M1, M2, and I; FHV-ASPECTS=4. C, FHV visible in M2, M3, M5, M6, and I; FHV-ASPECTS=2. D, FHV visible in all territories; FHV-ASPECTS=0.

Results

Pooled Study Cohort

Overall, information of 116 acute stroke patients (47 from Berlin and 69 from Stuttgart) with M1 vessel occlusion and MRI before angiography was pooled for this analysis. One hundred and six patients received MR imaging within 6 hours after stroke onset, and all patients were scanned within 12 hours. One hundred and four patients received mechanical thrombectomy, 6 patients received intra-arterial thrombolysis only, and 6 had a diagnostic angiography without intervention after IV thrombolysis. The median age of the cohort was 74 years (interquartile range [IQR], 64–79), and 45% of patients were men. The median NIHSS score was 14 (10–19). The median DWI-ASPECTS was 7 (5–8), and 17% of the DWI lesions were positive on FLAIR imaging. All patients included in the analysis had visible FHV on baseline FLAIR. Table I in the [online-only Data Supplement](#) displays the patient characteristics of the study cohort.

Cohorts from both cities did not differ significantly in most patient characteristics. The Stuttgart cohort had significantly higher proportions of diagnosed atrial fibrillation (58% versus 36%), whereas hypercholesterolemia was more prevalent in the Berlin cohort (25% versus 49%). The median onset-to-groin time was 209 minutes in Berlin, compared with 224 minutes in Stuttgart. The frequency of good recanalization (mTICI 2b–3) was 81% in the pooled cohort, as displayed in Table I in the [online-only Data Supplement](#). Analyses of baseline angiography data from Stuttgart revealed that 46 of 63 patients (73%) had a good collateral status (ASITN grade

3–4). The median FHV-ASPECTS was 2 (IQR, 1–3) in both the Berlin and the Stuttgart cohort. Cronbach α for interobserver reliability of FHV-ASPECTS rating was 0.9 for the Berlin cohort and 0.91 for the Stuttgart cohort. In the pooled analysis, prevalence of risk factors was similar between patients with low FHV-ASPECTS (FHV-ASPECTS ≤ 2) and high FHV-ASPECTS (FHV-ASPECTS > 2), as displayed in Table 1.

Association of Low FHV-ASPECTS With Collateral Status and Clinical Outcome at Discharge

In the Stuttgart cohort, good ASITN collateral grading scores were found in 83% of patients with low FHV-ASPECTS, compared with 57% in high FHV-ASPECTS patients ($P=0.025$). Low FHV-ASPECTS was associated with good collateral status (unadjusted odds ratio [OR], 3.6; 95% confidence interval, 1.1–11.6; $P=0.029$). The association remained statistically significant with an OR=4.4 (1.1–18.3) in additional multivariable analyses after adjusting for possible confounders (Table II in the [online-only Data Supplement](#)).

Clinical outcome parameters at discharge did not differ in both cohorts. In the pooled cohort, patients with lower FHV-ASPECTS were more likely to have a lower NIHSS score (median NIHSS, 4; IQR, 1–9) at discharge, compared with patients with higher FHV-ASPECTS (median NIHSS, 8; IQR, 3–15). Frequency of good functional outcome at discharge was significantly higher in low FHV-ASPECTS patients than in high FHV-ASPECTS patients (43% versus 23%; $P=0.032$; Table 1).

Table 1. Patient Characteristics Stratified by High vs Low FHV-ASPECTS Dichotomized at the Median

Variable	FHV-ASPECTS ≤2 (n=75)	FHV-ASPECTS >2 (n=41)	P Value
Age, y	74 [67–79]	72 [62–79]	0.24*
Sex, male	47 (35/75)	42 (17/41)	0.59†
NIHSS at hospitalization (miss=5)	15 [9–18]	14 [12–19]	0.89*
Right hemispheric infarction	41 (31/75)	49 (20/41)	0.44†
Hypertension	76 (57/75)	81 (33/41)	0.58†
Atrial fibrillation	49 (37/75)	49 (20/41)	0.96†
CAD	13 (10/75)	24 (10/41)	0.13†
Diabetes mellitus	11 (8/75)	22 (9/41)	0.10†
Hypercholesterolemia	29 (22/75)	44 (18/41)	0.12†
Smoking	15 (11/75)	22 (9/41)	0.32†
DWI-ASPECTS (miss=1)	7 [6–8]	7 [4–7]	0.57*
FLAIR demarcation, positive	19 (14/75)	15 (6/41)	0.35†
ASITN collateral grade (miss=6)‡			
Good ASITN grade (3–4)‡	83 (33/40)	57 (13/23)	0.025†
mTICI			
Good mTICI (2b–3)	83 (62/75)	78 (32/41)	0.54†
OTGT, min (miss=17)	212 [183–302]	218 [195–383]	0.77*
OTIT, min (miss=19)	87 [62–148]	85 [60–165]	0.94*
OTRT, min (miss=16)	317 [252–411]	304 [245–456]	0.83*
NIHSS at discharge (miss=21)	4 [1–9]	8 [3–15]	0.009*
Good mRS at discharge (0–2)	43 (32/75)	23 (9/40)	0.032†
Good mRS at 3 mo (0–2)	51 (38/74)	24 (10/41)	0.005†

Values are given as median [IQR] for continuous data and as frequency in percentage (n) for categorical data. ASITN indicates American Society of Interventional Therapeutic Neuroradiology; ASPECTS, Alberta Stroke Program Early CT score; CAD, coronary artery disease; DWI, diffusion-weighted imaging; FHV, fluid-attenuated inversion recovery hyperintense vessel; FLAIR, fluid-attenuated inversion recovery; IQR, interquartile range; Miss, missing values; mRS, modified Rankin Scale; mTICI, Modified Treatment in Cerebral Ischemia; NIHSS, National Institutes of Health Stroke Scale; OTGT, onset to groin time; OTIT, onset to imaging time; and OTRT, onset to recanalization time.

*Mann–Whitney *U* test for the comparison of medians.

† χ^2 test was used for the comparison of categorical variables between groups.

‡Only available in the Stuttgart cohort.

Low FHV-ASPECTS, Collateral Status, and Functional Outcome After 3 Months

Functional outcome assessment at 3 months after stroke was available from 115 of 116 patients of the pooled cohort (1 patient declined the follow-up interview). Good functional outcome was observed in 48 patients (41%) in total. Age, baseline NIHSS score, onset-to-groin time, and FHV-ASPECTS were lower in patients with good functional outcome, compared with patients with poor outcome after 3 months (Table 2). Onset-to-groin time was on average 30 minutes longer in patients with poor outcome ($P=0.008$; Table 2). Patients with good outcome more frequently demonstrated

Table 2. Univariate Analysis for the Association of Patient Characteristics With Good Functional Outcome (mRS, 0–2) 3 mo After Stroke

Variable	mRS, 0–2 (n=48)	mRS, 3–6 (n=67)	P Value
Age, y	70 [57–74]	76 [70–82]	0.001*
Sex, male	50 (24/48)	40 (27/67)	0.30†
NIHSS at hospitalization (miss=5)	11 [8–15]	18 [13–21]	<0.001*
Right hemispheric infarction	52 (25/48)	39 (26/67)	0.16†
Hypertension	71 (34/48)	84 (56/67)	0.10†
Atrial fibrillation	48 (23/48)	51 (34/67)	0.77†
CAD	13 (6/48)	21 (14/67)	0.24†
Diabetes mellitus	8 (4/48)	19 (13/67)	0.10†
Hypercholesterolemia	35 (17/48)	33 (22/67)	0.77†
Smoking	15 (7/48)	18 (12/67)	0.64†
DWI-ASPECTS (miss=1)	7 [6–8]	7 [4–8]	0.85*
FLAIR demarcation, positive	21 (10/48)	15 (10/67)	0.51†
FHV-ASPECTS	2 [1–2]	2 [1–4]	0.009*
ASITN collateral grade (miss=6)‡			
Good ASITN grade (3–4)‡	89 (25/28)	60 (21/35)	0.009†
mTICI (miss=8)			
Good mTICI (2b–3)	96 (46/48)	72 (48/67)	0.001†
OTGT, min (miss=17)	200 [184–230]	230 [195–350]	0.008*
OTIT, min (miss=19)	67 [53–134]	100 [68–179]	0.10*
OTRT, min (miss=16)	282 [239–384]	342 [252–478]	0.35*

Values are given as median [IQR] for continuous data and as frequency in percentage (n) for categorical data. ASITN indicates American Society of Interventional Therapeutic Neuroradiology; ASPECTS, Alberta Stroke Program Early CT score; CAD, coronary artery disease; DWI, diffusion-weighted imaging; FHV, fluid-attenuated inversion recovery hyperintense vessel; FLAIR, fluid-attenuated inversion recovery; IQR, interquartile range; Miss, missing values; mRS, modified Rankin Scale; mTICI, Modified Treatment in Cerebral Ischemia; NIHSS, National Institutes of Health Stroke Scale; OTGT, onset to groin time; OTIT, onset to imaging time; and OTRT, onset to recanalization time.

*Mann–Whitney *U* test for the comparison of medians.

† χ^2 test was used for the comparison of categorical variables between groups.

‡Only available in the Stuttgart cohort.

low FHV-ASPECTS on MRI (79% versus 54%; $P=0.005$) and good mTICI scores (96% versus 72%; $P=0.001$).

Patients with an FHV-ASPECTS ≤2 had a better functional outcome after 3 months (median mRS, 2; IQR, 0–5), compared with patients with an FHV-ASPECTS >2 (median mRS, 4; IQR, 3–6; $P=0.015$). Multivariable, logistic regression analyses could demonstrate that FHV-ASPECTS ≤2 was independently associated with good functional outcome with an adjusted OR=5.0 (95% confidence interval, 1.8–13.9) after adjustments for age, sex, diabetes mellitus, and hypertension in model 1 and OR=5.3 (95% confidence interval, 1.5–18.2) after additional adjustment for DWI-ASPECTS, NIHSS, and good mTICI scores in model 2 (Table 3). A post hoc analysis demonstrated a linear association between FHV-ASPECTS (with scores 5–7 combined) and good functional outcome after

Table 3. Multiple Binary Logistic Regression Analysis*

Variable	Unadjusted (n=115)	Model 1 (n=115; r ² =0.29)	Model 2 (n=109; r ² =0.52)
FHV-ASPECTS ≤2	3.27 (1.40–7.63)	5.01 (1.81–13.87)	5.25 (1.52–18.15)

Model 1: model with FHV-ASPECTS ≤2 and age, additionally adjusted for sex, arterial hypertension, and diabetes mellitus. Model 2: model 1+DWI-ASPECTS, NIHSS pretreatment, and mTICI. ASPECTS indicates Alberta Stroke Program Early CT score; DWI, diffusion-weighted imaging; FHV, fluid-attenuated inversion recovery hyperintense vessel; mTICI, Modified Treatment in Cerebral Ischemia; NIHSS, National Institutes of Health Stroke Scale; and r², Nagelkerkes R squared.

*Odds ratios and 95% confidence interval, of binary logistic regression analysis of the pooled cohort for the association of patient characteristics with good functional outcome (mRS, 0–2) 3 mo after stroke.

3 months (Figure 2; linear trend test: *P*=0.008). Sensitivity analyses using a cutpoint of ≤3 for FHV-ASPECTS dichotomization revealed similar results as the used cutpoint of 2.

In the Stuttgart cohort, 89% of patients with good functional outcome had good ASITN collateral grades, opposed to only 60% of patients with poor functional outcome (*P*=0.009). Exploratory multivariable logistic regression analysis did not show a statistically significant association between good collateral grade and good functional outcome after 3 months (*P*=0.07; Table III in the [online-only Data Supplement](#)).

Discussion

The main results of this study are 3-fold. First, hyperintense vessels on FLAIR imaging are a frequent finding in acute stroke patients with proximal M1-MCA occlusion presenting to the MRI within 12 hours after stroke onset. Second, greater extent of FHV quantified by FHV-ASPECTS is associated with better collateral grades assessed by the ASITN collateral grading system in patients with proximal M1-MCA occlusion. Third, in these patients, the extent of FHV is associated with

good functional outcome at 3 months after stroke in 2 independent stroke registries. This is the first multicenter study to demonstrate an association of FHV quantification, cerebral collateral status, and functional outcome at 3 months post-stroke in a large cohort of stroke patients with M1 occlusions receiving EVT.

Although numerous studies have been performed investigating the diagnostic and prognostic value of FHV, results on their clinical relevance are heterogeneous. Although some studies found that FHV are associated with poor functional recovery suggesting insufficient collateralization,^{14–16,24} others found precisely the opposite suggesting FHV to be indicative of high collateral grades and favorable treatment response.^{10,13,18} Interestingly, those studies that found FHV to be associated with poor functional recovery were performed in smaller, heterogeneous cohorts and used merely clinical parameters or perfusion imaging to assume collateral status. A direct association between FHV and collateral status assessed via angiography was lacking in these studies, justifying the comparison in a large and homogenous stroke cohort in relation to functional outcome data. The results of our study clearly demonstrate an association between FHV, cerebral collateralization, and functional outcome in stroke patients with M1 vessel occlusion. Previous studies that used advanced imaging techniques (such as digital subtraction angiography) in selected stroke cohorts with homogenous vessel occlusion also found FHV to be associated with high collateral status.^{13,25} Thus, we think that this apparent discrepancy can be explained by comparison of heterogeneous stroke patients with various vessel occlusions, lack of systematic FHV quantification systems, and incomprehensive imaging data to assess collateral status.

Stroke registries of 3 hospitals from Berlin and Stuttgart were pooled for this study. Presence of FHV was observed in all patients; this is consistent with previous studies that show

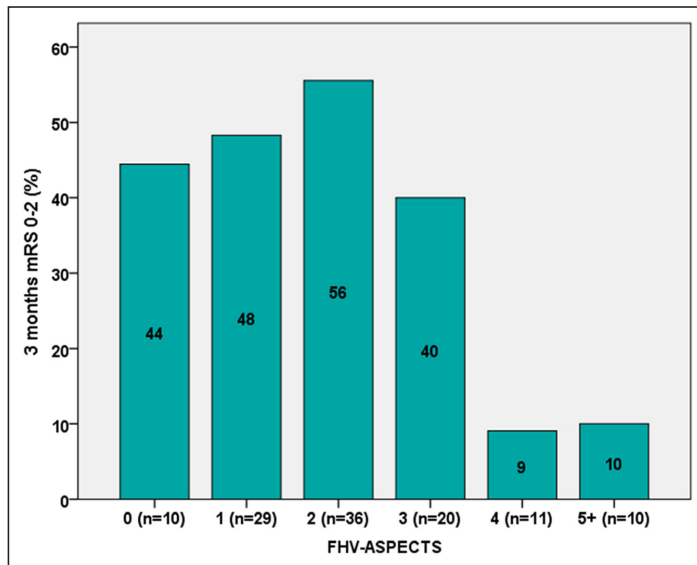


Figure 2. Distribution of fluid-attenuated inversion recovery hyperintense vessel–Alberta Stroke Program Early CT Score (FHV-ASPECTS) scores in relation to functional outcome. Association between FHV-ASPECTS and good functional outcome 3 mo after stroke, defined as modified Rankin Scale (mRS) score 0 to 2. FHV-ASPECTS 5 to 7 were combined. *P*=0.008 in linear trend test.

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a high association of visible FHV and large vessel occlusion,¹² but lower rates were reported in a previous study using a 1.5T and 3.0T MRI.²⁵ The majority of patients included in this study were examined on a 3.0T MRI. It remains to be shown whether field strength has an impact on FHV detection and whether this is of clinical significance. Patient characteristics from both stroke cohorts were comparable (Table I in the [online-only Data Supplement](#)). We interpreted the longer onset-to-recanalization time in Stuttgart to be because of implementing a full angiogram within the intervention and the substantial proportion of long-distance referrals from the surrounding area of Stuttgart.²⁶

In this study, FHV was quantified with the FHV-ASPECTS, and collateral status was graded in the Stuttgart cohort using a well-established collateral flow grading system recommended by ASITN.²³ Similar to a previous study, we could demonstrate FHV to be independently associated with better collateral grades with this approach (Table II in the [online-only Data Supplement](#)).²⁵ We found that a low FHV-ASPECTS was not only associated with better collateral status but also with good functional outcome 3 months after stroke (Table 3). Good collateral status was also associated with good functional outcome 3 months after stroke (unadjusted OR, 5.6; 95% confidence interval, 1.4–22.0). An exploratory multivariable analysis in the Stuttgart cohort only failed to demonstrate that good collateral status was associated with good functional outcome after 3 months (Table III in the [online-only Data Supplement](#)), presumably because of lack of power. Of note, adding good ASITN collateral grades as a covariate to the model attenuated the OR of low FHV-ASPECTS in the Stuttgart cohort. This finding strengthens the indication that a low FHV-ASPECTS acts as a surrogate for good collateral status, but these results need to be validated in other large cohorts.

Multiple logistic regression analysis of the pooled cohort confirmed that an FHV-ASPECTS ≤ 2 was associated with good functional outcome after adjustment for potential confounders. These results stand in line with previous studies investigating the prognostic value of FHV in patients with proximal anterior circulation vessel occlusion.^{18,23} Low FHV-ASPECTS indicate good collateral status being present in patients with acute M1 vessel occlusion. However, treatment decisions based on these associations are not yet justified.

In this study, MRI was routinely available in patients with acute suspected stroke. However, given our stringent inclusion and exclusion criteria, only 16% of all M1 occlusions entered this study. Comparing patients who received acute MRI versus acute CT before EVT during the study period revealed that the latter were older (76 versus 73 years; $P=0.02$), had higher NIHSS scores on admission (17 versus 12; $P<0.01$), and were more likely to have atrial fibrillation (59.6% versus 48.8%; $P=0.05$). This finding is in line with a previous study from Berlin comparing clinical characteristics of patients who received CT versus MRI in the acute setting of stroke demonstrating higher rates of cardiac comorbidities in stroke patients with acute CT imaging.²⁷

The strength of this study is that it is the first multicenter study to investigate the prognostic value of quantified FHV in terms of functional outcome at 90 days poststroke in patients with proximal MCA occlusion receiving EVT. In addition, 2 blinded raters independently performed imaging ratings, highlighting the reliability of results.

However, this study has some limitations. Most importantly, this is a retrospective, observational study, and, therefore, the presence of potential selection bias has to be acknowledged limiting the generalizability of results. This study analyzed only patients who received an acute MRI and were considered suitable for EVT post-imaging. Furthermore, full angiograms to assess the ASITN collateral grading score were only available for the subset of patients from the Stuttgart clinics; therefore, the multicenter nature of this study only applies to the functional outcome assessment.

In conclusion, FHV-ASPECTS was found to be an easily applicable and useful rating system to quantify the extent of FHV in acute stroke patients with M1 vessel occlusion. Therefore, we think that FHV-ASPECTS emerges as a clinically relevant contrast agent-independent surrogate for cerebral collateralization in the acute setting of stroke. The independent association of low FHV-ASPECTS and good functional outcome 3 months after stroke supports the idea that FHV-ASPECTS might be a valuable guide for future patient selection for endovascular therapy, especially when conventional angiography or contrast-based vascular imaging is not accessible.

Conclusions

The extent of FHV quantified by FHV-ASPECTS is associated with better collateral status and better functional outcome after 3 months in acute stroke patients with M1 vessel occlusion. FHV-ASPECTS represents an easy tool to estimate collateral status and outcome based on native FLAIR imaging.

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Disclosures

Dr Henkes is a coinventor and patent co-owner of Solitaire FR; cofounder and shareholder of phenox GmbH; serves as proctor, consultant, and board speaker for phenox; and is cofounder and shareholder of femtos GmbH. He has received a stroke research grant from Covidien. The other authors report no conflicts.

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2.4 FLAIR HYPERINTENSE VESSELS AND EFFICACY OF THROMBOLYSIS

Grosch AS*, **Kufner A***, Boutitie F, Cheng B, Ebinger M, Endres M, Fiebach JB, Fiehler J, Königsberg A, Lemmens R, Muir KW, Nighoghossian N, Pedraza S, Siemonsen CZ, Thijs V, Wouters A, Gerloff C, Thomalla G, Galinovic I. Extent of FLAIR Hyperintense Vessels May Modify Treatment Effect of Thrombolysis: A Post hoc Analysis of the WAKE-UP Trial. *Front Neurol.* 2021 Feb 4;11:623881.

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In a follow-up study on FHV, we explored whether the extent of FHV on acute baseline MRI influences the efficacy of thrombolysis and recanalization rates in patients with mild to moderate acute ischemic stroke of unknown symptom onset time. The study used data from the WAKE-UP Trial (ClinicalTrials.gov, NCT01525290), which evaluated thrombolysis safety and efficacy based on a DWI-FLAIR mismatch (ischemic lesions visible on DWI but not yet on FLAIR, indicating symptom onset likely within 4.5 hours)¹⁹.

Our study included 165 patients with confirmed unilateral single-vessel occlusion. This was the first to longitudinally assess FHV on follow-up MRI (22-36 hours after admission) compared to baseline. While FHV did not affect lesion progression or long-term recovery, a reduction in FHV was independently associated with successful recanalization (adjusted OR 5.82, 95% CI 2.00–16.92; $p = 0.001$, adjusted for treatment group and age). Additionally, patients with fewer FHV showed better functional outcomes (3-month mRS) with thrombolysis compared to placebo (OR 5.3, 95% CI 1.2–24.0). Conversely, placebo patients with more pronounced FHV had higher chances of good clinical outcomes. These results suggest that prominent FHV may offer protective benefits in the initial hours after occlusion due to better collateral circulation. In other words, patients with fewer FHV may rely more on therapy for achieving favorable functional outcomes.



Extent of FLAIR Hyperintense Vessels May Modify Treatment Effect of Thrombolysis: A *Post hoc* Analysis of the WAKE-UP Trial

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Background and Aims: Fluid-attenuated inversion recovery (FLAIR) hyperintense vessels (FHVs) on MRI are a radiological marker of vessel occlusion and indirect sign of collateral circulation. However, the clinical relevance is uncertain. We explored whether the extent of FHVs is associated with outcome and how FHVs modify treatment effect of thrombolysis in a subgroup of patients with confirmed unilateral vessel occlusion from the randomized controlled WAKE-UP trial.

Methods: One hundred sixty-five patients were analyzed. Two blinded raters independently assessed the presence and extent of FHVs (defined as the number of slices with visible FHV multiplied by FLAIR slice thickness). Patients were then separated into two groups to distinguish between few and extensive FHVs (dichotomization at the median <30 or ≥30).

Results: Here, 85% of all patients ($n = 140$) and 95% of middle cerebral artery (MCA) occlusion patients ($n = 127$) showed FHVs at baseline. Between MCA occlusion patients with few and extensive FHVs, no differences were identified in relative lesion growth

($p = 0.971$) and short-term [follow-up National Institutes of Health Stroke Scale (NIHSS) score; $p = 0.342$] or long-term functional recovery [modified Rankin Scale (mRS) <2 at 90 days poststroke; $p = 0.607$]. In linear regression analysis, baseline extent of FHV (defined as a continuous variable) was highly associated with volume of hypoperfused tissue ($\beta = 2.161$; 95% CI 0.96–3.36; $p = 0.001$). In multivariable regression analysis adjusted for treatment group, stroke severity, lesion volume, occlusion site, and recanalization, FHV did not modify functional recovery. However, in patients with few FHVs, the odds for good functional outcome (mRS) were increased in recombinant tissue plasminogen activator (rtPA) patients compared to those who received placebo [odds ratio (OR) = 5.3; 95% CI 1.2–24.0], whereas no apparent benefit was observed in patients with extensive FHVs (OR = 1.1; 95% CI 0.3–3.8), p -value for interaction was 0.11.

Conclusion: While the extent of FHVs on baseline did not alter the evolution of stroke in terms of lesion progression or functional recovery, it may modify treatment effect and should therefore be considered relevant additional information in those patients who are eligible for intravenous thrombolysis.

Clinical Trial Registration: Main trial (WAKE-UP): ClinicalTrials.gov, NCT01525290; and EudraCT, 2011-005906-32. Registered February 2, 2012.

Keywords: ischemic stroke, FLAIR hyperintensities, thrombolysis, wake-up stroke, prognosis, MRI, hyperintense vessel

INTRODUCTION

The fluid-attenuated inversion recovery (FLAIR) hyperintense vessel (FHV) sign is commonly observed on magnetic resonance imaging (MRI) of acute ischemic stroke patients and is represented by ipsilateral linear or serpentine hyperintensities on FLAIR sequences distal to the vessel occlusion (1–6). FHVs have been shown to be an independent predictor of large vessel occlusion. However, studies investigating the underlying pathophysiology and prognostic value of FHVs have yielded contradictory results (7).

While some have shown that FHVs are associated with increased collateralization, decreased lesion growth, and improved long-term functional recovery (6, 8–11), others have shown that patients with extensive FHVs have increased lesion growth and worse functional outcome 3 months poststroke (2, 4, 12, 13). The apparent discrepancies in previous studies regarding the diagnostic and prognostic value of FHVs may be due the use of different methodologies in the assessment of FHVs and inhomogeneous cohorts of patients in terms of treatment in the acute setting and time to MRI.

The aim of the present study was to investigate whether the extent of FHVs has an effect on stroke evolution in terms of lesion progression and long-term functional recovery in a cohort of acute ischemic stroke patients with middle cerebral artery (MCA) occlusion and unknown time of onset from the randomized controlled WAKE-UP trial (14). Furthermore, we

investigated whether the extent of FHVs on baseline imaging modifies the treatment effect of thrombolysis and recanalization rates on follow-up imaging.

METHODS

Patients

This is a retrospective study including patients who were enrolled in the multicenter, randomized, double-blind, placebo-controlled WAKE-UP trial (14). Trial patients were randomized to either treatment with alteplase or placebo. For this analysis, 165 patients with confirmed, unambiguous, unilateral, and single-vessel occlusion on time-of-flight magnetic resonance angiography (MRA-TOF) were included. Patients were excluded from final analysis if baseline FLAIR was not available or not ratable due to poor image quality.

Clinical Assessment

Demographic data included age, gender, and presence or previous history of the following cardiovascular risk factors: smoking, alcohol consumption, arterial hypertension, atrial fibrillation, hypercholesterolemia, diabetes mellitus type II, coagulation disorder, transient ischemic attack, ischemic stroke, and/or intracranial hemorrhage. Clinical assessment comprised the National Institutes of Health Stroke Scale (NIHSS) on admission and follow-up (5–9 days poststroke, or if this data point was not available 22 to 36 h poststroke, considered short-term outcome in our analysis) as well as good long-term outcome defined as modified Rankin Scale (mRS) <2 at 90 days poststroke.

Radiological Assessment

A central image-reading committee reviewed all images acquired for patient enrollment in the WAKE-UP trial and

Abbreviations: DWI, diffusion-weighted imaging; FLAIR, fluid-attenuated inversion recovery; FHV, FLAIR hyperintense vessel; MCA, middle cerebral artery; ICA, internal carotid artery; MRI, magnetic resonance imaging; mRS, modified Rankin Scale; NIHSS, National Institutes of Health Stroke Scale (score); OR, odds ratio; TOF, time-of-flight; PWI, perfusion-weighted imaging.

reevaluated imaging inclusion/exclusion criteria assessed by local investigators. A detailed description of image assessment within the trial (i.e., measurement of lesion volumes) has been previously published (14). For the current analysis, all acquired images were retrospectively reevaluated by two independent raters (ASG and IG) at the Center for Stroke Research Berlin at Charite University Hospital Berlin. In this subsample of the WAKE-UP trial, diffusion-weighted imaging (DWI) and FLAIR were available for all patients ($n = 165$) on hospital admission and in 154 patients (93%) at follow-up (22–36 h after hospital admission). Lesion volumes were derived from baseline and follow-up DWI imaging to determine relative (follow-up divided by baseline DWI lesion volume) and absolute lesion growth (follow-up subtracted by baseline DWI lesion volume).

We also assessed the evolution of FHV from baseline to follow-up FLAIR. We defined that a reduction in FHVs was present if there was a drop of more than one slice affected by FHV between baseline and follow-up imaging. Dynamic susceptibility contrast perfusion MRI [perfusion-weighted imaging (PWI)] of diagnostic quality was available in 66 of all patients (40%), and volumes of hypoperfusion were calculated using RAPID (<https://www.rapidai.com>) with a threshold of $T_{max} > 6$ s. PWI–DWI mismatch was defined as an absolute mismatch volume of > 10 ml and a mismatch ratio between PWI and DWI of > 1.2 . Occlusion site was evaluated on MRA-TOF. For MCA occlusion analyses, we only included the occlusions sites ICA+M1, ICA+M2, and M3/M4. Recanalization status was classified into either complete or no/partial recanalization on follow-up compared to baseline imaging.

Assessment of FLAIR Hyperintense Vessels

Blinded to clinical and radiological outcomes, two raters (ASG and IG) independently rated baseline and follow-up FLAIR images for the presence and extent of FHV. FHV were defined as linear or serpentine hyperintensities distal to the site of the occluded vessel (Figure 1). Due to different FLAIR slice thicknesses of the participating medical centers, the extent of FHV was defined as the number of slices with visible FHV multiplied by FLAIR slice thickness. Inter-rater agreement for the presence of FHV was 95.76% with a free marginal kappa of 0.92 [95% confidence interval (CI) 0.85–0.98] at baseline and 88.49% with a free marginal kappa of 0.77 (95% CI 0.67–0.87) at follow-up. The two raters agreed on the extent of FHV (up to a maximum difference of one slice) in 52% of all cases. Consensus was reached for discrepant cases. For further analysis, only patients with MCA occlusion were separated into two groups to distinguish between few and extensive FHV (dichotomization at the median < 30 or ≥ 30) (1, 2).

Statistical Analysis

Spearman's rank correlation coefficient was used for correlation analyses. Based on the scale level of the variables, Mann–Whitney-U test, Fisher's exact test, or chi-square test were applied for two-group analyses. Binary logistic regression analyses were performed for recanalization (adjustment for reduction in FHV, treatment group, and age) as well as for good outcome defined as mRS < 2 at 90 days poststroke (adjustment for

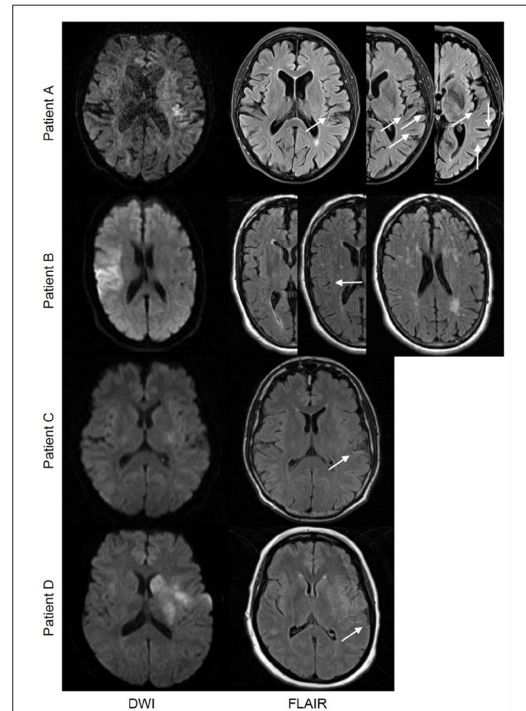


FIGURE 1 | Patient A and Patient B represent two cases at different ends of the spectrum of the extent of FLAIR hyperintense vessels (FHVs). Patient A is a 69-year-old female with a left-sided M2 branch occlusion and baseline FHV extent of 60 (multiple linear and serpentine vessels visible surrounding the operculum and temporal lobe on all three images). Patient B is a 70-year-old male with a right-sided occlusion in the M2 branch of the middle cerebral artery whose initial extent of FHVs at baseline was 13 (a single serpentine vessel is visible between the operculum and the temporal lobe on the middle image). Both were treated with placebo; the modified Rankin Scale (mRS) at 90 days was 0 for patient A and 3 for patient B. Patients C and D represent cases with comparable FHV patterns but different stroke extent and severity at baseline. A comparison of two patients, one a 44-year-old male (patient C) and the other a 46-year-old female (patient D), both with a left-sided occlusion of the mainstem middle cerebral artery (MCA). The baseline extent of FHVs was 30 for both cases with a comparable distribution of vessels, yet the stroke volumes and distributions were different. Patient C showed only small scattered lesions in the insula, tip of the putamen, as well as the temporal and parietal lobes (total volume of 3 ml), while patient D showed an infarction encompassing the entire putamen and nucleus caudatus as well as portions of the insula and operculum, with additionally some scattered lesions in the frontal and parietal lobes (total volume of 15 ml). Their baseline National Institutes of Health Stroke Scale (NIHSS) score was also different (6 for patient C and 20 for patient D). At follow-up, both patients recanalized [patient C received recombinant tissue plasminogen activator (rtPA) and patient D received placebo]. They had a similar dynamics of FHVs showing a reduction in their extent (a complete reduction to zero in patient C and a partial reduction to 12 in patient D). Their mRS outcome at 90 days was 1 for patient C and 3 for patient D.

well-known predictors of outcome including baseline NIHSS score, recanalization status, treatment group, baseline lesion volume, occlusion site, FHV group, hours from last seen well to

treatment). Linear regression analysis was performed for volume of hypoperfused tissue (adjustment for baseline extent of FHV) as well as NIHSS score at follow-up (adjustment for baseline NIHSS score, recanalization status, treatment group, baseline lesion volume, occlusion site, FHV group). To investigate the interaction between the extent of FHV and treatment effect on the primary endpoint, we used an unconditional logistic regression model, relating the log-odds of the primary outcome with the covariate of interest, the treatment group, and their interaction, with adjustment on NIHSS score at baseline. The interaction term was tested with the Wald–chi-square test, and the treatment effect [odds ratio (OR)] and its 95% CI were estimated for each category. Statistical analysis was performed using IBM SPSS (www.ibm.com, version 24) and $p \leq 0.05$ were considered significant.

RESULTS

Entire Patient Cohort

Out of 503 patients enrolled in the WAKE-UP trial, 165 met all inclusion criteria (328 were excluded due to absence of vessel occlusion, two due to poor image quality, three due to bilateral vessel occlusion, five due to unavailable imaging data). The mean age of this subgroup of patients was 64.2 years, 47% were female, median NIHSS score at baseline was 9.0 [interquartile range (IQR) 6.0–15.0]. In total, 85% ($n = 140$) had FHV visible on baseline FLAIR, and median extent of FHV was 30.0 (IQR 21.3–39.0). Of the 25 patients without baseline FHV, four had an occlusion of the internal carotid artery (ICA) (16%), three of M2 or ICA+M2 (12%), four of M3 or M4 (16%), seven of the posterior cerebral artery (PCA) (28%), and seven of other vessels (28%).

Patients With Middle Cerebral Artery Occlusion

In patients with MCA occlusion ($n = 134$, 81%), 95% had FHV at baseline ($n = 127$), and the median extent of FHV was 30.0 (IQR 24.0–40.0). Patients with extensive FHV did not differ from patients with few FHV in terms of baseline DWI lesion volumes (9.7 vs. 17.5 ml; $p = 0.218$) and baseline NIHSS scores (12.0 vs. 9.0; $p = 0.147$). Baseline extent of FHV (defined as a continuous variable) was highly associated with the volume of hypoperfused tissue ($\beta = 2.161$; 95% CI 0.96–3.36; $p = 0.001$), with patients with extensive FHV having significantly larger hypoperfused areas at baseline. The occlusion site also differed significantly between few and extensive FHV, with extensive FHV being associated with proximal vessel occlusions ($p < 0.001$). Patients with few and extensive FHV revealed no differences in the time between last seen well to MRI ($p = 0.261$), last seen well to treatment ($p = 0.301$), and MRI to treatment ($p = 0.271$). Likewise, continuous extent of FHV did not correlate with any of the abovementioned variables. In terms of outcome, there were no differences in relative lesion growth ($p = 0.971$) or short-term ($p = 0.342$) or long-term functional recovery ($p = 0.607$) between groups (Table 1).

Middle Cerebral Artery Occlusion Patients: Functional Recovery and Treatment Effect

Univariate regression analysis of long-term functional recovery revealed merely baseline NIHSS score and recanalization as predictors. Treatment group, baseline DWI lesion volume, occlusion site, dichotomized extent of FHV, and hours from last seen well to treatment were not identified as independent predictors in this subgroup analysis. Multivariable regression analysis confirmed baseline NIHSS score and recanalization as independent predictors for long-term functional recovery (Table 2).

When patients were separated into groups based on treatment, there was a clear trend pointing to the extent of FHV as a factor that modifies treatment effect. In patients with FHV extent <30 , only 14% of individuals with a proximal occlusion (M1 segment of the MCA) and 10% with a more distal occlusion (M2, M3, or M4 segments of the MCA) had good outcome if treated with placebo, whereas 25 and 46% of patients (with proximal and distal occlusions, respectively), had good outcome if given recombinant tissue plasminogen activator (rtPA). Accordingly, in patients with FHV extent <30 , the odds for good outcome were increased by 5.3 in rtPA-treated patients as compared to those treated with placebo (OR = 5.3; 95% CI 1.2–24.0), whereas no apparent benefit of rtPA was observed in patients with FHV extent ≥ 30 (OR = 1.1; 95% CI 0.3–3.8), p -value for interaction = 0.11. There were no differences in baseline clinical or radiological parameters (including occlusion site) between patients who received placebo and those who received rtPA. When the extent of FHV was treated as a continuous variable in tPA-treated patients, the probability of good outcome was relatively stable across the entire range of FHV. However, in patients receiving placebo, there was a very low likelihood of a good outcome with less prominent FHV, with chances improving parallel to increasing FHV extent (Figure 2).

Recanalization and Reduction in FLAIR Hyperintense Vessels

Overall, the majority of patients (64%; $n = 82$) experienced a reduction in FHV between baseline and follow-up; the median relative reduction was 50% (ICR 15–100%). In MCA occlusion patients, the relative extent of reduction was significantly more pronounced in patients who recanalized as compared to non-recanalizers (86 vs. 31%; $p = 0.001$). In binary logistic regression of MCA occlusion patients, a reduction in FHV had an adjusted OR of 5.82 (adjusted for treatment group and age; 95% CI 2.00–16.92; $p = 0.001$) for successful recanalization on follow-up. There were only five patients who recanalized but did not show a reduction in FHV on follow-up, whereas 33 patients showed a reduction in FHV despite persistent vessel occlusion. Among these non-recanalizers, there was no difference in terms of absolute lesion progression (21.0 vs. 13.1 ml; $p = 0.589$), follow-up NIHSS score (9.0 vs. 7.0; $p = 0.917$), or 3-month mRS (3.0 vs. 3.0; $p = 0.497$) between patients who showed a reduction in FHV and those who did not (Figure 1).

TABLE 1 | Demographic data, baseline and follow-up clinical and radiological data for all patients, MCA occlusion patients, MCA occlusion patients with few FHVs, and MCA occlusion patients with extensive FHVs.

	All patients (n = 165)	MCA occlusion patients (n = 134)	MCA occlusion patients with few FHVs (n = 53)	MCA occlusion patients with extensive FHVs (n = 74)	P-value few vs. extensive FHVs
Age, mean (SD)	64.2 (11.9)	64.5 (11.7)	63.9 (11.6)	64.9 (11.9)	0.514
Female sex, % (n)	47% (77)	49% (66)	38% (20)	54% (41)	0.049
Previous history of CVRF, % (n)					
- Arterial hypertension	49% (80)	49% (65)	48% (25)	48% (35)	0.988
- Atrial fibrillation	17% (27)	19% (25)	9% (5)	25% (18)	0.036
- TIA	3% (5)	3% (4)	6% (3)	1% (1)	0.307
- Ischemic stroke	10% (16)	9% (12)	9% (5)	7% (5)	0.741
- Intracranial hemorrhage	0% (0)	0% (0)	0% (0)	0% (0)	1.000
- Hypercholesterolemia	36% (56)	37% (47)	42% (21)	33% (23)	0.306
- Diabetes mellitus type II	13% (21)	15% (19)	15% (8)	13% (9)	0.792
- Coagulation disorder	0% (0)	0% (0)	0% (0)	0% (0)	1.000
- Gastrointestinal bleeding	2% (3)	2% (3)	6% (3)	0% (0)	0.066
- Current smoking	31% (48)	31% (40)	22% (11)	38% (27)	0.080
- Alcohol	39% (59)	41% (52)	41% (20)	44% (31)	0.707
FHV on admission					
- % (n)	85% (140)	95% (127)	100% (53)	100% (74)	1.000
- Median extent (IQR)	30.0 (21.3–39.0)	30.0 (24.0–40.0)	22.0 (17.5–25.0)	39.0 (30.0–48.0)	<0.001
Reduction in FHVs between baseline and follow-up imaging					
- Absolute, median (IQR)	15.0 (5.0–25.0)	15.0 (5.0–25.0)	14.0 (2.7–24.0)	15.0 (6.0–30.0)	0.059
- Relative, median (IQR)	50% (15%–100%)	50% (16%–100%)	100% (14%–100%)	40% (16%–83%)	0.040
ASPECTS mismatch, % (n)	73% (97)	73% (93)	59% (31)	84% (62)	0.002
NIHSS score					
- Baseline, median (IQR)	9.0 (6.0–15.0)	10.0 (6.0–15.5)	9.0 (6.0–14.0)	12.0 (7.0–16.0)	0.147
- Follow-up, median (IQR)	6.0 (2.0–13.3)	6.0 (1.5–13.0)	6.0 (1.0–10.0)	8.0 (2.0–15.0)	0.342
MRS at 90 days					
- Median (IQR)	3.0 (1.0–4.0)	3.0 (1.0–4.0)	3.0 (1.0–3.0)	3.0 (1.0–4.0)	0.255
- Good outcome, % (n)	26% (42)	27% (36)	39% (16)	26% (19)	0.607
DWI lesion volume in ml					
- Baseline, median (IQR)	9.8 (3.1–24.0)	10.4 (4.7–25.9)	17.5 (4.8–31.8)	9.7 (4.4–22.1)	0.218
- Follow-up, median (IQR)	21.9 (5.6–59.0)	23.1 (6.2–57.6)	29.7 (5.7–60.0)	21.3 (6.1–54.7)	0.653
DWI lesion growth in %					
- Absolute, median (IQR)	10.6 (1.1–38.3)	12.2 (1.9–36.2)	11.8 (1.7–36.2)	12.8 (1.6–37.0)	0.746
- Relative, median (IQR)	121% (37–320%)	121% (28–254%)	114% (14–253%)	121% (31–276%)	0.971
Treatment with rtPA, % (n)	50% (83)	50% (67)	57% (30)	45% (33)	0.186
Occlusion site, % (n)					<0.001
- ICA	6% (9)	0% (0)	0% (0)	0% (0)	
- ICA+M1 or M1	39% (64)	48% (64)	28% (15)	66% (49)	
- ICA+M2 or M2	27% (45)	34% (45)	45% (24)	24% (18)	
- M3/M4	15% (25)	19% (25)	26% (14)	10% (7)	
- PCA	9% (14)	0% (0)	0% (0)	0% (0)	
- Other	5% (8)	0% (0)	0% (0)	0% (0)	
Recanalization, % (n)	36% (49)	41% (45)	46% (19)	34% (21)	0.204
PWI-DWI mismatch, % (n)	65% (42)	78% (38)	72% (18)	83% (19)	0.499
PWI volume, median (IQR)	50.7 (26.0–89.7)	64.4 (30.2–95.1)	49.0 (29.3–72.3)	74.8 (50.3–109.2)	0.047
Hours from LSW to MRI, median (IQR)	10.0 (6.8–11.8)	10.1 (6.9–11.9)	10.1 (6.3–11.5)	10.2 (7.3–12.9)	0.261
Hours from LSW to treatment, median (IQR)	10.5 (7.4–12.4)	10.6 (7.5–12.5)	10.5 (6.9–12.1)	10.7 (7.6–13.4)	0.301
Hours from MRI to treatment, median (IQR)	0.4 (0.3–0.6)	0.4 (0.3–0.6)	0.4 (0.2–0.6)	0.5 (0.3–0.6)	0.271

P-values are given for group comparisons between patients with few and extensive FHVs.

MCA, middle cerebral artery; FHV, FLAIR hyperintense vessel; SD, standard deviation; n, number; CVRF, cardiovascular risk factors; IQR, interquartile range; ASPECTS, Alberta stroke program early CT score; NIHSS, National Institutes of Health Stroke Scale; mRS, modified Rankin Scale; DWI, diffusion-weighted imaging; rtPA, recombinant tissue plasminogen activator; ICA, internal carotid artery; M1, M1 segment of the MCA; M2, M2 segment of the MCA; M3/M4, M3 or M4 segment of the MCA; PCA, posterior cerebral artery; PWI, perfusion-weighted imaging; LSW, last seen well. The bold values indicate the statistical significance (i.e., $p < 0.05$).

TABLE 2 | Univariate and multivariable regression analyses for good outcome (mRS <2) 3 months poststroke in MCA occlusion patients.

	Univariable logistic regression		Multivariable logistic regression	
	Crude odds ratio (95% CI)	P-value	Adjusted odds ratio (95% CI)	P-value
Baseline NIHSS score	0.768 (0.687; 0.858)	<0.001	0.753 (0.647; 0.878)	<0.001
Recanalization	3.873 (1.578; 9.508)	0.003	3.922 (1.147; 13.404)	0.029
Treatment group	1.618 (0.746; 3.506)	0.223	1.948 (0.584; 6.494)	0.278
Small baseline DWI lesion volume	0.971 (0.942; 1.000)	0.051	1.000 (0.958; 1.043)	0.988
Occlusion site (more distal)	1.405 (0.856; 2.306)	0.178	0.665 (0.272; 1.626)	0.371
FHV group (few vs. extensive)	0.814 (0.371; 1.785)	0.607	1.123 (0.308; 4.091)	0.861
Hours from LSW to treatment	0.973 (0.897; 1.054)	0.498	1.039 (0.922; 1.170)	0.528

NIHSS, National Institutes of Health Stroke Scale; 95% CI, 95% confidence interval; DWI, diffusion-weighted imaging; FHV, FLAIR hyperintense vessel; mRS, modified Rankin Scale; LSW, last seen well; MCA, middle cerebral artery. The bold values indicate the statistical significance (i.e., $p < 0.05$).

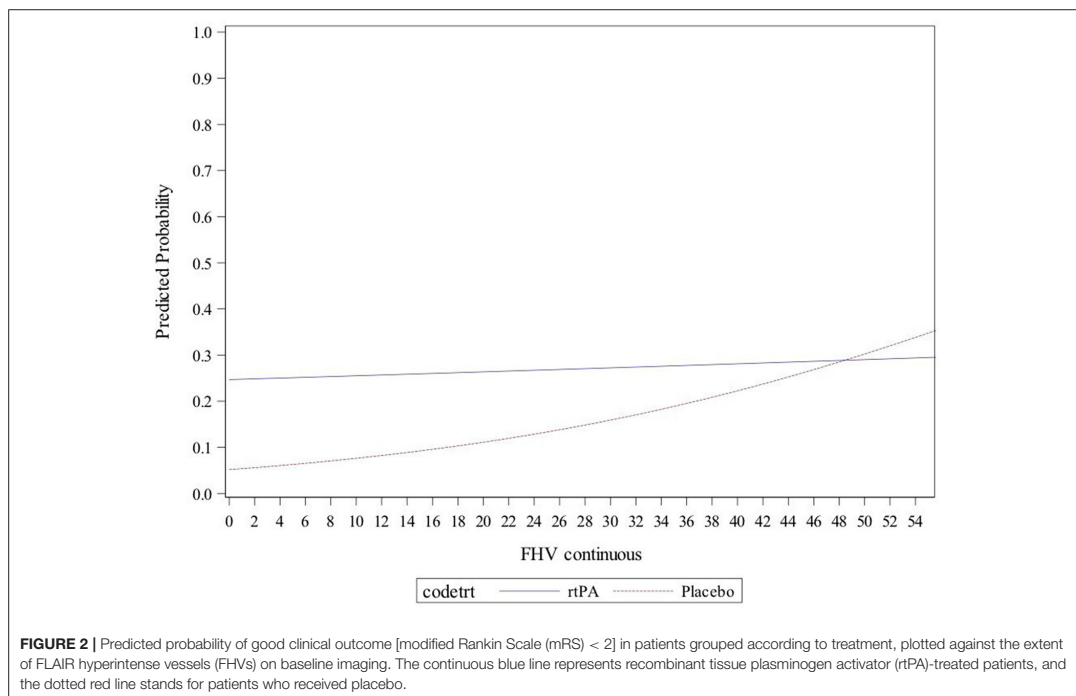


FIGURE 2 | Predicted probability of good clinical outcome [modified Rankin Scale (mRS) < 2] in patients grouped according to treatment, plotted against the extent of FLAIR hyperintense vessels (FHV) on baseline imaging. The continuous blue line represents recombinant tissue plasminogen activator (rtPA)-treated patients, and the dotted red line stands for patients who received placebo.

DISCUSSION

In the current study, the extent of FHV on baseline imaging did not alter stroke progression in terms of initial stroke severity, lesion growth, or long-term functional recovery in patients with MCA occlusion and unknown time of symptom onset. However, patients with less pronounced FHV had higher odds of achieving a good outcome following treatment with rtPA. In other words, the extent of FHV assessed on acute imaging may modify the treatment effect of thrombolysis.

In line with previous studies (2, 4, 11), here, 85% of ischemic stroke patients with proven vessel occlusion presented with FHV ipsilateral to the ischemic lesion on baseline imaging. Extent of

FHV correlated directly with the volume of hypoperfused tissue. This is likely in part due to the higher rates of proximal occlusions observed in patients with extensive FHV (Table 1). Similar results were previously reported, showing an association between FHV and more severe hypoperfusion (2) and identifying FHV as an independent predictor of a perfusion–diffusion mismatch in the case of vessel occlusion (15, 16).

In our study, the extent of FHV had no effect on clinical stroke severity or lesion size on admission, nor did it modify lesion progression or functional recovery (Table 1). This matches the results of a recently published systematic review of FHV in ischemic stroke (7); in a pooled sample of over 3,000 patients, there was no association between functional outcome and extent

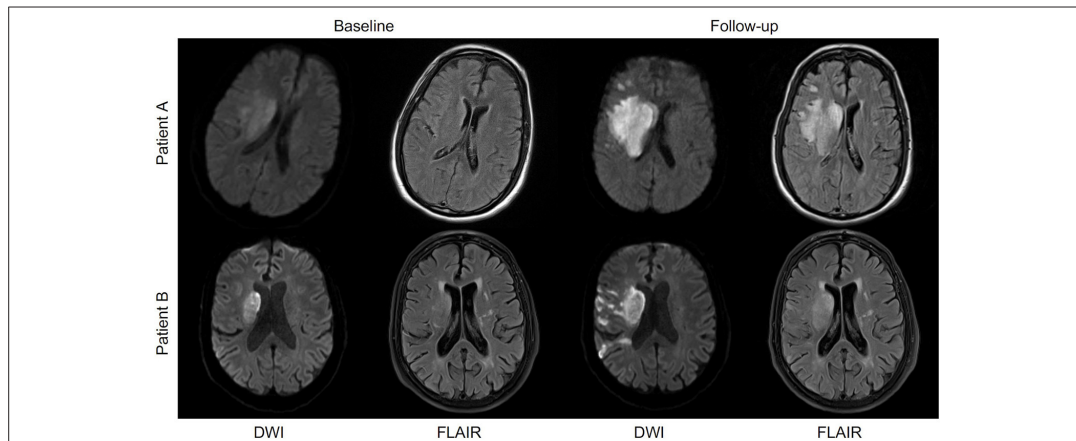


FIGURE 3 | Patients A and B represent cases with comparable stroke evolution yet differing evolution of FLAIR hyperintense vessels (FHV) between baseline and follow-up imaging. Both 64-year-old males with a right-sided M1 occlusion. Patient A additionally had an occlusion of the ipsilateral internal carotid artery (ICA). The baseline lesions were comparable in terms of volume and pattern (predominantly basal ganglia involvement and lesion size up to 20 ml). Their baseline National Institutes of Health Stroke Scale (NIHSS) score was also comparable (15 for patient A and 14 for patient B). The baseline extent of FHV was different between the patients (30 in patient A and 50 in patient B). Both patients received placebo, and neither of them experienced a recanalization by the time of follow-up. There was a pronounced difference in the dynamics of FHV between the patients, with patient A showing no more visible FHV at follow-up, whereas the extent of FHV in patient B actually increased from 50 to 55. Their outcome, however, was the same (both had a 90-day mRS of 4), and their final lesion volumes were almost identical (patient A 52 ml and patient B 59 ml).

of FHV. To further illustrate this, in our cohort, we found examples of patients with matching occlusions, similar lesion extent, and severity of stroke who presented with very different extents of FHV at baseline as well as the opposite (patients with identical occlusions and similarly pronounced FHV yet different clinical and imaging stroke severities) (Figure 3).

Interestingly, the extent of baseline FHV modified treatment effect, with thrombolysis being more effective in patients with fewer visible collaterals, and especially so if they had a more distally placed vessel occlusion. Although patients with large vessel occlusions still benefit from intravenous thrombolysis, previous studies have shown that the presence of a proximally placed vessel occlusion is associated with worse outcome following intravenous thrombolysis (17) (additional REF). At the same time, for patients receiving placebo, higher likelihoods of good clinical outcome were found in individuals with more pronounced FHV (Figure 2). This might point to a protective component of prominent FHV, at least in the initial hours after occlusion occurs, with patients who are unable to quickly recruit an extensive collateral network being that much more dependent on therapy for a chance at good functional outcome. The generalizability of these results to different patient populations, i.e., to ischemic stroke patients with large vessel occlusion eligible for endovascular therapy should be viewed with caution. According to the clinical and radiological criteria of the DAWN and DEFUSE 3 trials, these patients would be candidates for direct endovascular therapy (18, 19). However, a better understanding of rtPA efficacy in patients with unknown symptom onset and extensive FHV could be particularly valuable in selecting patients who might

benefit from a bridging therapy with rtPA before endovascular therapy. Larger independent cohort analyses on this topic are warranted to validate our findings.

In this study, treatment with tPA did not reach statistical significance for good outcome 90 days poststroke in the overall cohort (Table 2). This is most likely due to the smaller sample size of the current study; point estimates for treatment were similar in this analysis (crude OR of 1.62) to those reported in the original trial analysis (crude OR 1.6) (14).

It is known that FHV are a transient MRI phenomenon and typically disappear by 36 h poststroke (5, 20, 21). Similar to previous studies, we observed an overall reduction of FHV over time in ~64% of patients (5, 22), and this reduction was independently associated with successful recanalization. In other words, early reduction in FHV may be a surrogate marker of successful recanalization and hence be associated with less stroke progress and better functional recovery. However, in the case of persistent vessel occlusion, a reduction in FHV was not associated with a smaller lesion growth or better functional recovery (Figure 1).

Interestingly, there were significantly more females in the group of patients with MCA occlusion and extensive FHV (Table 1). Previous studies have described sex-specific differences in cerebrovascular parenchymal hyperintensities on FLAIR (23) (additional REF). However, to the best of our knowledge, previous studies on FHV have not observed sex-specific differences in terms of the extent of FHV in the setting of acute stroke. Future analyses on this topic would be of great interest.

Based on previous studies and our current analysis, it is clear that FHV are radiological markers of proximal vessel occlusion.

They most likely represent arteries distal to the occlusion site exhibiting slow flow (owing to a collateral circulation that is sufficient enough to provide retrograde flow but insufficient to achieve the extent of perfusion present prior to the stroke and therefore associated with the size of the perfusion deficit (2, 5, 12). This, however, is not to say that FHV indicate hypoperfusion below the ischemia threshold leading to tissue infarction, as their presence and magnitude seem to confer a certain protective advantage to the tissue—an advantage that, as many studies have shown (7), is neither unequivocal nor easy to understand. In this they are not alone, as other MRI markers (for example, dynamic susceptibility contrast MRI, also known as perfusion imaging) have failed to deliver an indisputable parameter and/or threshold that reliably predicts tissue fate (24). The reason might lie in the highly dynamic evolution of an acute ischemic stroke; the timely unfolding of several factors, such as treatment, changes in antegrade flow (the extent of recanalization), and retrograde flow (the continuous improvement of collateral circulation), but also different tissue susceptibilities to ischemia collectively play crucial roles in determining tissue fate. Therefore, any given MRI must be seen as a snapshot of the current situation, which is inevitably destined to undergo change and can hence only partially be predictive of future outcome.

There are several limitations of this study. First, due to its retrospective nature and small numbers, we run the risk of type II error in our analysis. Furthermore, PWI was only available in a limited number of our patients, and no gold standard information on collateral status exists for this cohort. In addition, information pertaining to stroke etiology as well as thrombus composition is also lacking in our cohort. In addition, this cohort comprises patients treated with tPA and placebo, and adjustment for treatment group in multivariable regression analyses only partially compensates for this limitation of a heterogeneous cohort. However, this is the first study to investigate the diagnostic and prognostic value of FHVs in a cohort of patients stemming from a multinational, randomized, placebo-controlled trial.

In summary, FHVs may serve as a surrogate marker of large vessel occlusion and successful activation of collaterals to increase blood flow to hypoperfused tissue; in turn, early reduction of FHVs is also an independent predictor of successful recanalization. Although there is no clear clinical relevance for the extent of FHV alone in terms of functional recovery, FHVs may modify treatment effect of thrombolysis. In other words, patients with less pronounced FHVs on acute imaging seem to profit from rtPA more. We maintain that this frequently observed MRI parameter should not guide treatment decisions based on current findings and that a validation in a larger independent cohort is warranted. However, FHVs may serve as an additional piece of information in selecting patients with confirmed vessel occlusion for intravenous thrombolysis or bridging therapy before endovascular treatment.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the WAKE-UP trial protocol was approved by the national regulatory authority in each of the six participating countries (Belgium, Denmark, France, Germany, Spain, and United Kingdom). The trial was approved by the respective national or local ethics committees or institutional review boards of all participating centers. Patients or their legal representatives provided written informed consent according to national and local regulations. The main trial was conducted according to the principles laid down in the Declaration of Helsinki in its version of Seoul, 2008; the EU Clinical Trial Directive 2001/20/EC; the Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95 of January 17, 1997); the applicable national drug laws, e.g., German Drug Law (Arzneimittelgesetz, 15. Novelle, AMG); and the GCP-Regulation from August 9, 2004. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR'S NOTE

Ischemic stroke is one of the leading causes of death and disability worldwide. Rapid administration of tissue plasminogen activator (i.e., thrombolysis) within 4.5 h of symptom onset has been shown to greatly increase the likelihood of achieving a good outcome (functional independence) following an ischemic stroke. However, not all patients benefit from thrombolysis; therefore, there is a continued search for clinical and imaging parameters that can identify those most likely to achieve a good outcome following treatment. Here, we investigated the diagnostic and prognostic value of a frequently observed MRI sign (so-called hyperintense vessels) in acute ischemic stroke patients with unknown time of symptom onset. Previous studies on the topic have yielded contradictory results. Here, we found that although this MRI sign does not necessarily predict stroke progression or outcome, the degree to which this MRI vessel sign is expressed might modify the treatment effect of thrombolysis. This could be of particular importance in an acute clinical setting when selecting patients who are eligible for thrombolysis. Although these results need to be validated in independent cohorts, they take us closer to understanding individual stroke pathology using clinical routine diagnostics.

AUTHOR CONTRIBUTIONS

CG and GT conceived and designed the WAKE-UP trial. FB performed the data analysis. SP and JBF were part of the central image reading board. IG and AG performed imaging analysis specific to this analysis. AKu and IG conceived and designed the current *post hoc* analysis. AKu, AG, and IG wrote the first draft of the manuscript. All authors were involved in patient recruitment, interpreted the data, reviewed and edited the manuscript, and approved the final version of the manuscript.

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Conflict of Interest: JBF reports consulting and advisory board fees from BioClinica, Cerevast, Abbvie, AC Immune, Artemida, Brainomix, Biogen, BMS, Daiichi-Sankyo, Guerbet, Ionis Pharmaceuticals, Julius Clinical, Eli Lilly, Tau Rx, and EISA outside the submitted work. MEEn reports grants from Bayer and fees paid to the Charité from Bayer, Boehringer Ingelheim, BMS, Daiichi Sankyo, Amgen, GSK, Sanofi, Covidien, Novartis, and Pfizer, all outside the submitted work.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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2.5 WHITE MATTER HYPERINTENSITIES FOR RISK STRATIFICATION

Ali HF, Fast L, Khalil A, Siebert E, Liman T, Endres M, Villringer K, **Kufner A**. White matter hyperintensities are an independent predictor of cognitive decline 3 years following first-ever stroke—results from the PROSCIS-B study. *J Neurol*. 2023 Mar;270(3):1637-1646. Epub 2022 Dec 6. PMID: 36471099; PMCID: PMC9971076.

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We performed an exploratory analysis of 431 patients from the PROSpective Cohort with Incident Stroke (PROSCIS⁴²) who received an MRI within seven days of their first-ever ischemic stroke. WMH burden was assessed using the Age-Related White Matter Changes (ARWMC) score. The study aimed to explore the association between selected cerebrovascular risk factors and WMH load, as well as the effect of increased WMH burden on recurrent vascular events, cognitive impairment, and depression up to three years post-stroke.

Distribution analysis revealed no clear association between cerebrovascular risk profiles and WMH load. High WMH lesion load (ARWMC score ≥ 10) was found to be significantly associated with cognitive impairment (adjusted OR 1.05 [95% CI 1.00–1.11]; $p < 0.02$). Kaplan–Meier survival analysis showed a visible increase in the risk of recurrent vascular events following stroke in patients with high WMH burden (adjusted HR 1.5 [95% CI 0.76–3]; $p = 0.18$), though this finding was not statistically significant. There was no significant association between WMH burden and depression one year post-stroke (adjusted OR 0.72 [95% CI 0.31–1.64]; $p = 0.44$).

In summary, increased WMH burden was identified as an independent predictor of cognitive decline three years following the first-ever stroke in this cohort. Further research is necessary to validate these findings and explore the potential of WMH burden as a biomarker for tailored secondary prevention strategies in stroke patients.



White matter hyperintensities are an independent predictor of cognitive decline 3 years following first-ever stroke—results from the PROSCIS-B study

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Abstract

Background White matter hyperintensities (WMH) are the result of cerebral small vessel disease and may increase the risk of cognitive impairment (CI), recurrent stroke, and depression. We aimed to explore the association between selected cerebrovascular risk factors (CVRF) and WMH load as well as the effect of increased WMH burden on recurrent vascular events, CI, and depression in first-ever ischemic stroke patients.

Methods 431 from the PROSpective Cohort with Incident Stroke (PROSCIS) were included; Age-Related White Matter Changes (ARWMC) score was used to assess WMH burden on FLAIR. The presence of CVRF (defined via blood pressure, body-mass-index, and serological markers of kidney dysfunction, diabetes mellitus, and hyperlipoproteinemia) was categorized into normal, borderline, and pathological profiles based on commonly used clinical definitions. The primary outcomes included recurrent vascular events (combined endpoint of recurrent stroke, myocardial infarction and/or death), CI 3 years post-stroke, and depression 1-year post-stroke.

Results There was no clear association between CVRF profiles and WMH burden. High WMH lesion load (ARWMC score ≥ 10) was found to be associated with CI (adjusted OR 1.05 [95% CI 1.00–1.11]; $p < 0.02$) in a mixed-model analysis. Kaplan–Meier survival analysis showed a visible increase in the risk of recurrent vascular events following stroke; however, after adjustment, the risk was non-significant (HR 1.5 [95% CI 0.76–3]; $p = 0.18$). WMH burden was not associated with depression 1-year post stroke (adjusted OR 0.72 [95% CI 0.31–1.64]; $p = 0.44$).

Conclusion Higher WMH burden was associated with a significant decline in cognition 3 years post-stroke in this cohort of first-ever stroke patients.

Keywords White matter hyperintensities · Cerebrovascular risk factors · Cognitive impairment · Recurrent cerebrovascular events · First-ever ischemic stroke · Depression

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Introduction

Cerebral small vessel disease (CSVD) is one of the most prevalent pathologies that neurologists and radiologists encounter in routine clinical practice. Not only does the presence of CSVD substantially increase the risk of stroke, but it is known to contribute to cognitive decline and dementia [1–3]. The increased availability of magnetic resonance imaging (MRI) in the clinical routine, has substantially increased the detection rates of CSVD; especially as an incidental finding in asymptomatic cases [2,4,5].

Imaging biomarkers of CSVD include small subcortical infarcts, lacunes, white matter hyperintensities (WMH), enlarged perivascular spaces, cerebral microbleeds, and global cerebral atrophy [4]. The presence of WMH are the most common imaging biomarker of CSVD, and are most easily detected on so-called fluid-attenuated inversion recovery (FLAIR) sequences. Although possible underlying pathologies behind WMH are heterogenous, histological studies suggest that WMH are most likely a result of chronic ischemia leading to demyelination and ultimately axonal loss [6–8]. WMH are so prevalent, that nearly 80% of healthy 60-year-olds and approximately 95% of people aged 90 years or older were found to have developed WMH [9,10]. Within the past decades, numerous modifiable risk factors for the development of WMH have been identified, including smoking, diabetes mellitus, arterial hypertension, hyperlipidemia, chronic kidney disease, and the presence of metabolic syndrome [11–13]. While CSVD and ultimately the presence of WMH can remain asymptomatic and is often merely an incidental finding on routine MRI [2], previous studies suggest that progressive WMH likely leads to increased stroke risk and early cognitive decline depending on distribution and localization within the brain [3].

Interestingly, recent studies suggest that selected cerebrovascular risk factors lead to different WMH distribution patterns within the brain [3]. For example, a cohort study found that patients with chronic hypo- and hypertension present with different patterns and distributions of WMH (periventricular versus deep white matter) on MRI, with a history of hypertension leading to increased periventricular WMH burden [14]. Moreover, smoking has been linked to low cerebral blood flow, leading to increased WMH volumes across all age groups [15]. Furthermore, a recent study reported a strong association between type 2 diabetes with increased whole-brain WMH volume [16]. Most recently, a large population-based study found that obesity and associated low-grade systemic inflammation led to primarily deep WMH burden and comparatively less periventricular WMH volumes [17]. In other words, the presence or absence of selected risk factors is likely to influence WMH distribution

patterns and ultimately may contribute to recurrent stroke risk and risk of dementia.

As the individual risk of stroke recurrence varies considerably among patients [18], patient-specific risk stratification is of enormous clinical importance. This is the case especially in younger patients with first-ever stroke, as early detection and initiation of secondary prevention could subsequently reduce recurrent cerebrovascular events. Therefore, we set out to investigate the relationship between latent or 'borderline' risk profiles (e.g., glucose intolerance without manifest diabetes mellitus) of common and well-known cerebrovascular risk factors and WMH burden in a comprehensive cohort of first-ever stroke patients. More specifically, we aimed to investigate the association between serological markers of selected cerebrovascular risk factors (i.e., HbA1c for diabetes mellitus, low-density lipoprotein (LDL) for hypolipoproteinemia, and glomerular filtration rate (GFR) for kidney function) as well as clinical parameters (body mass index [BMI] and blood pressure) with WMH lesion load using the Age-related white matter changes (ARWMC) score. Subsequently, we investigated whether high WMH burden (defined as an ARWMC score ≥ 10) was associated with increased recurrent vascular events (including recurrent stroke, MI, and/or death) and cognitive impairment 3 years and depression one year following the first-ever stroke in a prospectively collected cohort of ischemic stroke patients.

Methods

Data availability statement

The data that supports the findings of this study are available upon reasonable request from the corresponding author [AK]. Raw imaging data are not publicly available as they contain information that could compromise patient privacy.

Cohort characteristics

This is a retrospective analysis of the single-center prospective study *PROSpective Cohort with Incident Stroke (PROSCIS)* conducted at the Center for Stroke Research Berlin, Charité University Hospital. Patients aged ≥ 18 years with first-ever ischemic stroke were recruited between 2010 and 2013 after providing written informed consent. The exclusion criteria included any prior stroke, patients with a brain tumor or brain metastasis, and/or participation in an intervention study. A detailed description of the inclusion and exclusion criteria of the PROSCIS study can be found in the previously published study protocol [19]. The ethics committee approved the study for all recruiting centers in Berlin according to the Declaration of Helsinki and the study was registered in clinicaltrials.org (NCT01363856). For this

study, patients with at least one MRI during standard clinical care following the index event (within 7 days following stroke onset) were included in the analysis.

MRI assessment

The MRI protocol included the following sequences: T2*, diffusion-weighted imaging (DWI), and fluid-attenuated inversion recovery (FLAIR) images. All MRI examinations were conducted on a 3.0 Tesla or 1.5 Tesla Siemens MRI scanner. Post-processing of MRI images was performed offline with MRICron Software from the Center for Advanced Brain Imaging (University of South Carolina, Chris Rordan, USA).

The ARWMC visual rating scale developed by Wahlund and team [20] was used to rate WMH on FLAIR images of all patients included in this study. Rating was performed independently by two evaluators (neurologist A.K. and senior neuroradiologist K.V.). The ARWMC score ranges from 0 to 30 with an assessment of both sides of the brain and pre-specified regions of the brain, which includes frontal, parieto-occipital, temporal, basal ganglia, and infra-tentorial. Each region's grading ranges from 0 to 3 where grade 0 is occasional or non-punctate WMH; grade 1, multiple punctate WMH; grade 2, bridging of punctate WMH into confluent lesions; and grade 3, widespread confluent WMH [20]. For this study, ARWMC score was dichotomized with a cut-off of < 10 representing a lower WMH load and ≥ 10 as a higher WMH load [21].

Clinical assessment

All patients included in the study were examined and interviewed within 7 days after the onset of stroke symptoms. Clinical parameters included basic patient demographics, education status, smoking status, history of diabetes mellitus, hypertension, atrial fibrillation, and hyperlipoproteinemia. Clinical parameters documented on admission (BMI and systolic and diastolic blood pressure) as well as serological parameters (HbA1c, LDL, and GFR) were used in this analysis.

Clinical examinations included stroke severity and functional outcome on admission. Stroke severity was assessed using the National Institutes of Health Stroke Scale (NIHSS), a 15-item scale ranging from 0 to 42, with higher values reflecting a more severe neurological deficit^{[[22]]}. Functional outcome was assessed using the modified Rankin Scale (mRS) score, ranging from 0 to 6. mRS is a measure of disability where 0 reflects no symptoms; 5, severe disability leading to nursing aid; and 6, death^{[[23]]}.

Cerebrovascular risk factors assessment

Diabetic status was evaluated by analyzing the HbA1c levels, which were categorized into normal as 4.5–5.7%, borderline as 5.8–6.4%, and pathological as $\geq 6.5\%$ [24]. LDL was categorized into normal as ≤ 70 mg/dL; borderline as 71–139 mg/dL; and pathological as ≥ 140 mg/dL. BMI was divided into normal as ≤ 25 , borderline as 26–29, and pathological as ≥ 30 [25]. Blood pressure readings were analyzed separately for systolic and diastolic readings. The mean of three separate blood pressure measurements on admission was calculated and then categorized into the following profiles: systolic blood pressure was categorized into hypotension (≤ 120 mmHg), normal (121–139 mmHg) and hypertension (≥ 140 mmHg). Similarly, diastolic blood pressure readings were categorized into hypotension (≤ 80 mmHg), normal (81–89 mmHg), and hypertension (≥ 90 mmHg) [26]. Kidney function was analyzed using the estimated glomerular filtration rate (eGFR), which was categorized into normal as ≥ 90 mL/min; borderline as 61 mL/min to 89 mL/min; and pathological as ≤ 60 mL/min [27]. The definitions and cut-offs of the selected cerebrovascular risk factors included in this analysis are summarized in Supplementary Table 1.

Primary endpoints

All three primary endpoints were assessed at 1-, 2-, and 3 years post-stroke.

Cognitive assessment

Cognitive status was evaluated at baseline using the Mini-Mental State Examination (MMSE) [28]. During follow-up, cognitive status was assessed using the Modified Telephone Interview for Cognitive Status (TICS-M), which is a modified variant of the MMSE which has been validated for use via telephone interviews [29,30]. The MMSE is a 30-point questionnaire for cognitive impairment to screen for dementia where a cut-off of ≥ 24 indicates normal cognition and ≤ 23 indicates cognitive impairment. The TICS-M is an 11-test-item questionnaire yielding a total score of 50 points where a cut-off of ≤ 31 represents cognitive impairment and ≥ 32 represents normal cognition.

Depression assessment

Depression status was assessed using the Center for Epidemiologic Studies Depression Scale (CES-D) [31]. Depression is an inherently difficult endpoint to assess as it can fluctuate for individual patients over time and is likely influenced by many factors including mobility status, stroke severity etc. For this reason, depression scores at only 1-year

follow-up were used in the analysis. CES-D is a 20-item questionnaire comprising six scales that reflect significant facets of depression based on self-reported information on depressive mood, feelings of guilt and worthlessness, helplessness and hopelessness, psychomotor retardation, loss of appetite, and sleep disturbance. The cut-off value of ≥ 16 represents clinical depression [32]. To account for missing scores we applied inverse probability-weighted estimation of death as part of a sensitivity analysis.

Combined endpoint of recurrent vascular events

The combined endpoint of recurrent vascular events included the first of either recurrent stroke, myocardial infarction (MI), or death by any cause. Incidences of recurrent vascular events were assessed using Rose Angina Questionnaire for cardiovascular events and Stroke Symptom Questionnaire for cerebrovascular events [33,34]. Requested measures were collected at baseline and follow-up of 1-, 2-, and 3 years post-stroke. The follow-up was carried out via a structured telephonic questionnaire or mail. In case of any positive vascular outcome event, the information was validated by the admitting hospital or the treating physician.

Statistical analysis

Baseline characteristics of categorical data is represented in absolute and relative frequencies, and the distribution of continuous data is described as median and interquartile ranges (IQR). The selection of covariates for our analyses was evaluated by Directed Acyclic Graphs (DAGs). DAGs, a derivative of causal diagrams in epidemiology, provide a graphical representation of rigorous mathematical methodology for mapping all a priori assumptions surrounding a causal question [35]. In our study, a separate DAG was created for each causal relationship of interest to identify the potential variables of confounding while avoiding colliders and intermediate pathways using the online tool, Dagitty [<http://dagitty.net/>] [36]. All DAGs used to inform our analytic strategy have been uploaded onto an open repository and can be accessed via the following link: [<https://doi.org/10.6084/m9.figshare.20152487>].

Logistic regression (unadjusted and adjusted analysis) was performed to assess the association of borderline vs. pathological cerebrovascular risk factors with dichotomized ARWMC score; interactions between CVRF categories were not considered in the analysis. The models were adjusted based on DAG graphs created for each variable as described above. The model for HbA1c was adjusted for age, sex, smoking, BMI, and hyperlipidemia; models for systolic and diastolic blood pressures were adjusted for age, sex, diabetes, smoking, BMI, and hyperlipidemia; LDL model was adjusted for age, sex, BMI, and smoking; BMI model for

age, sex, and smoking; and GFR model for age, sex, diabetes, BMI, hypertension, hyperlipidemia, smoking, and atrial fibrillation.

Kaplan–Meier survival analysis was performed to analyze the association of WMH lesion load (ARWMC score ≥ 10) on the recurrent vascular events within 3 years post-stroke. We report unadjusted and adjusted hazard ratios (HR) with 95% CI obtained from Cox proportional hazards model, and adjustment was made for age, sex, and hypertension.

The effect of WMH lesion load on cognition status (dichotomized at ≤ 23) 3 years post-stroke was analyzed by (non-linear) mixed-model analysis followed by additional sensitivity analysis which excluded all deaths post-stroke within three years. Adjustments were made for age, sex, education status, smoking, hypertension, diabetes, atrial fibrillation, and hyperlipidemia. All models included subject ID as random effects and WMH and cognition scores as fixed effects.

Depression at 1-year post-stroke relative to WMH severity was analyzed by a logistic regression model followed by weighted analysis for probability of death. An adjustment was made for age, sex, CI at baseline, NIHSS, and mRS status at 1-year post-stroke.

For all models, a 2-sided p -value < 0.05 was considered statistically significant. All analyses were performed using the software STATA IC version 15 (StataCorp, College Station, Texas, USA).

Results

Cohort description

627 patients in total are included in PROSCIS, of which 431 patients presenting with first-ever ischemic stroke with an available MRI following the index event were analyzed. The mean age of this cohort was 66.8 years (standard deviation [SD] 13.2), of which 244 (38.9%) were females, with baseline NIHSS of (median NIHSS 2 [IQR 01–05]). The 196 patients who did not receive an MRI at the baseline had a mean age of 69.6 years [SD 12.42], and median baseline NIHSS of 3 (IQR 2–5). Baseline characteristics of the entire analyzed cohort are described in Table 1.

Cerebrovascular risk factors and WMH

Logistic regression analysis found no significant association across cerebrovascular risk factor categories (normal, borderline, and pathological as defined in Supplementary Table 1), with ARWMC score ≥ 10 (Table 2). For a graphical depiction of ARWMC score distribution (as a continuous variable) across cerebrovascular risk factor categories,

Table 1 Baseline clinical and imaging characteristics of the PROS-CIS-B cohort.

Total patient cohort	
Demographics	
Age, mean (\pm SD)	66.9 (\pm 13.2)
Sex, female, <i>n</i> (%)	244 (38.9%)
Education level \geq 10 years, <i>n</i> (%)	173 (28.8%)
Cardiovascular risk factors	
Hypertension, <i>n</i> (%)	409 (65.2%)
Diabetes, <i>n</i> (%)	138 (22%)
Hyperlipidemia, <i>n</i> (%)	128 (22.7%)
Atrial fibrillation, <i>n</i> (%)	135 (21.5%)
BMI, Mean (SD)	27.5 (4.9)
Current smoking, <i>n</i> (%)	173 (28.0%)
Stroke etiology	
Large artery atherosclerosis, <i>n</i> (%)	169 (26.9%)
Cardioembolic stroke, <i>n</i> (%)	149 (23.8%)
Small vessel occlusion, <i>n</i> (%)	96 (15.3%)
Other, <i>n</i> (%)	22 (3.5%)
Unknown, <i>n</i> (%)	191 (30.5%)
Baseline clinical characteristics	
NIHSS at admission, Median [IQR]	2 [1–5]
mRS at admission, Median [IQR]	2 [1–5]
Infarct pattern	
Territorial with subcortical and cortical, <i>n</i> (%)	110 (17.5%)
Subcortical, <i>n</i> (%)	112 (17.9%)
Scattered infarct, <i>n</i> (%)	118 (18.8%)
Lacunar, <i>n</i> (%)	2 (0.3%)
Infratentorial, <i>n</i> (%)	107 (17.1%)
Watershed, <i>n</i> (%)	33 (5.3%)
ARWMC score	
ARWMC score, Median [IQR]	5 [3–9]

SD standard deviation, IQR interquartile range, BMI body mass index, NIHSS National Institute of Health Stroke Scale, mRS modified Rankin Score, ARWMC Age-Related White Matter Changes

please refer to the violin plots depicted in Supplementary Fig. 1.

Pathological HbA1c values had an odds ratio (OR) of 1.21; (95% CI 0.69–2.09) for high WMH load. Pathological diastolic blood pressure values had an adjusted OR of 1.57 (95% CI 0.74–3.29). Neither systolic blood pressure, high LDL, BMI, nor chronic kidney disease defined by GFR showed a significant association with increased WMH load (Table 2). Due to the fact that systolic and diastolic blood pressure values may vary substantially at the time of acute stroke (irrespective as to whether a true diagnosis of hypertension exists), we performed an additional logistic regression analysis including the history of hypertension (yes/no); here we found a significant association with WMH load and history of hypertension with an adjusted OR of 2.44 (95% CI 1.30–4.57; $p=0.00$).

For a visual depiction of the results of the regression analysis (unadjusted and adjusted OR with 95% CI) for all cerebrovascular risk factors and ARWMC score, please refer to Supplementary Fig. 2.

WMH and recurrent vascular events

In the Kaplan–Meier survival analysis for the combined vascular endpoint (recurrent stroke, MI, and/or death) patients with high ARWMC scores had visibly lower survival rates when analyzed 3 years post-stroke (Fig. 1). In the cox-regression analysis, patients with a ARWMC score \geq 10 had an unadjusted HR of 1.6 (95% CI 0.79–3.13; $p=0.20$) and adjusted HR of 1.5 (95% CI 0.76–3.03; $p=0.18$) for the combined vascular endpoint 3 years post-stroke.

WMH and cognitive impairment

A significant association between higher WMH load and cognitive impairment 3 years post-stroke was found in the mixed model analysis with an unadjusted OR of 2.22 (95% CI 1.20–4.08; $p=0.01$) and an adjusted OR of 1.05 (95% CI 1.00–1.11; $p=0.02$). The sensitivity analysis produced similar results. The models were adjusted for age, sex, education status, smoking, hypertension, diabetes, atrial fibrillation, and hyperlipidemia (Table 3).

WMH and depression

In logistic regression analysis, high WMH load had an adjusted OR of 0.83 (95% CI 0.39–1.77; $p=0.64$), following adjustment for age, sex, cognitive impairment at baseline, NIHSS, and mRS at 1-year post-stroke. An additional inverse probability weighted analysis also showed no significant association between increased WMH load and depression 1-year post-stroke with an adjusted OR of 0.72 (95% CI 0.31–1.64; $p=0.44$).

Discussion

The main finding of this study is that increased WMH load (defined as ARWMC score \geq 10) was significantly associated with cognitive impairment after 3 years in first-ever stroke patients. In addition, a higher WMH load at the time of stroke is likely associated with recurrent vascular events including recurrent stroke, MI, and/or death in patients with first-ever ischemic stroke. One of the primary aims of the current study was to assess whether selected cardiovascular risk profiles affect the WMH burden in first-ever stroke patients. While a history of hypertension was significantly associated with increased WMH load, the current study found no apparent differences in WMH load across so-called

Table 2 Logistic regression analysis for effects of borderline and pathological clinical/serological risk profiles and white matter hyperintensities (ARWMC score ≥ 10)

	n (%)	Unadjusted			Adjusted		
		Odds ratio	95% Confidence interval	p-value	Odds ratio	95% Confidence interval	p-value
HbA1c^a							
Normal	259 (43.9%)	Ref					
Borderline	152 (25.8%)	1.50	0.86–2.56	0.15	0.99	0.52–1.91	0.99
Pathological	179 (30.3%)	1.21	0.69–2.09	0.49	0.93	0.49–1.78	0.84
Systolic BP^b							
Normal	116 (19.3%)	Ref					
Borderline	205 (34.1%)	0.81	0.39–1.66	0.57	0.57	0.25–1.28	0.17
Pathological	280 (46.6%)	1.44	0.75–2.76	0.26	1.03	0.49–2.16	0.93
Diastolic BP^b							
Normal	398 (66.1%)	Ref					
Borderline	99 (16.4%)	1.07	0.58–1.95	0.82	1.21	0.58–2.49	0.60
Pathological	105 (17.4%)	0.91	0.49–1.71	0.78	1.57	0.74–3.29	0.23
LDL^c							
Normal	49 (8.2%)	Ref					
Borderline	362 (61.2%)	0.83	0.35–1.96	0.67	1.60	0.55–4.60	0.38
Pathological	180 (30.5%)	1.05	0.43–2.56	0.91	2.25	0.75–6.68	0.14
BMI^d							
Normal	261 (42.3%)	Ref					
Borderline	206 (33.4%)	0.96	0.58–1.60	0.88	1.00	0.57–1.75	0.99
Pathological	150 (24.3%)	0.74	0.40–1.35	0.33	0.99	0.51–1.90	0.98
eGFR^e							
Normal	193 (32.2%)	Ref					
Borderline	282 (47.1%)	2.35	1.32–4.18	0.00	0.91	0.43–1.92	0.79
Pathological	124 (20.7%)	3.32	1.66–6.64	0.00	0.91	0.35–2.36	0.84

BP blood pressure, LDL low-density lipoprotein, BMI body mass index, GFR glomerular filtration rate

^aAdjusted for- age, sex, smoking, BMI, hyperlipidemia

^bAdjusted for- age, sex, diabetes, smoking, BMI, hyperlipidemia

^cAdjusted for- age, sex, BMI; smoking

^dAdjusted for- age, sex, smoking

^eAdjusted for- age, sex, diabetes, BMI, hypertension, hyperlipidemia, smoking, atrial fibrillation

borderline and pathological cerebrovascular risk profiles assessed via clinical and serological markers on admission.

Similar to previous studies, we found that a history of arterial hypertension is associated with an increased burden of WMH [9,14]. However, we found no association between baseline systolic or diastolic blood pressure values and increased WMH load in the current analysis. A previously published meta-analysis of four trials has shown that anti-hypertensive medication could significantly slow the progression of WMH [37]. Unfortunately, in the current study, medication status was not available and could not be considered. This may have affected our categorizations and ultimately our results. For example, patients with a long-term history of hypertension and a recent start of anti-hypertensive medication may have normotensive systolic blood pressure at the time of the index event. The

same might be true for serological markers of hypercholesterolemia and diabetes mellitus with LDL cholesterol and HbA1c, respectively. An earlier study found a significant association of diabetes mellitus with increased WMH severity, particularly in terms of increased whole-brain WMH volume in a population-based sample of 99 patients with diagnosed diabetes mellitus type 2 [16]. These patients had an average of nearly 9 years of diabetes duration, therefore generalizability of these findings to a cohort of first-ever stroke patients where diagnostic work-up following stroke often leads to first diagnosis of cerebrovascular risk factors is limited. Although previous population-based studies also suggest high BMI to be significantly associated with the development of increased deep WMH lesions [17], here we also failed to find a clear association between high BMI and WMH burden.

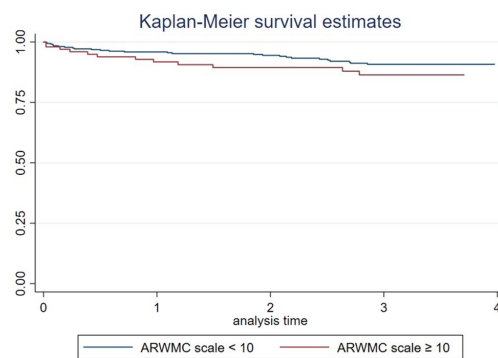


Fig. 1 Kaplan–Meier survival analysis curve showing association of white matter hyperintensities with the incidence of recurrent stroke, myocardial infarction, and/or death on follow-up of 1-, 2-, and 3-years post-stroke

While arterial hypertension, diabetes mellitus, hyperlipoproteinemia, obesity, and poor kidney function are all well-known cerebrovascular risk factors and likely do play an important role in the development of WMH [11,14–18,38,39], we did not see relevant associations with our selected risk profiles and higher ARWMC scores in this cohort of first-ever stroke patients. It is important to note that the aforementioned studies are all population-based studies; in other words, patients were selected based on the presence of the risk factor of interest. Therefore, generalizability to a cohort of first-ever stroke patients is likely limited. Furthermore, the current cohort has a relatively mild WMH burden (median ARWMC score of 5 [IQR 3–9]). The visual assessment of WMH lesion load via the ARWMC score may not be sensitive enough to detect associations of risk factors and very early manifestations of ARWMC in first-ever stroke patients. An analysis using a quantitative assessment of WMH volume is certainly warranted to explore this topic further.

The Kaplan–Meier survival analysis for recurrent vascular events showed a visible increase in risk in patients with ARWMC score ≥ 10 across 3 years following stroke (Fig. 1); however, these results were not statistically significant in adjusted cox-regression analysis (HR 1.52 95% CI

0.76–3.03; $p=0.18$). This may be due to the small sample size in the current study. Literature has shown that the risk of stroke recurrence varies considerably among patients and depends on age, sex, and the presence of co-morbidities [40,41]. In line with our observation, a recent Danish Stroke Registry-based observational study including 832 patients reported a significant association between WMH and increased risk of recurrent stroke (HR 5.28; 95% CI [1.98–14.07]) [42] in a cohort of incident ischemic stroke patients.

We found a significant association of increased WMH lesion load with CI 3 years following first-ever stroke. Additional sensitivity analysis also yielded a similar result (adjusted OR of 1.1 95% CI 1.0–1.1; $p=0.03$). Previous studies on the effect of WMH on cognitive decline have yielded somewhat controversial results; while several studies found no clear association between WMH and cognitive decline [43,44], others have found an increase in WMH volume to be an independent predictor of CI in both stroke cohorts as well as population-based studies [18,45]. Recently, a study reported that increased WMH volume was associated with cognitive decline in patients < 80 years of age following a minor stroke or TIA [44]. Since WMH are very commonly seen on MRI even in healthy subjects, the interpretation of these as a proxy for risk of CI can be complex and should still be reported and applied with caution. However, there is increasing evidence for a causal relationship between increased WMH load and the risk of the development of cognitive decline which could have important clinical implications i.e., early identification of an increased risk of CI could play a major role in initiating secondary prevention strategies in patients at risk.

We found no association between high WMH load and depression post-stroke in this analysis. There are conflicting results reported in the literature on the effect of WMHs on the development of depression. Although several population-based studies have found that WMH progression is associated with depression and antidepressants can even slow WMH progression [46], the effects of WMH burden in stroke patients on post-stroke depression remains unclear. As of yet, different WMH patterns such as peri-ventricular WMH and deep WMH are found to affect depression at baseline or a yearlong follow-up in stroke patients, respectively [47]. Depression is an inherently difficult endpoint to

Table 3 Mixed model analysis showing the effect of white matter hyperintensities burden with long-term cognitive function

ARWMC Score	Unadjusted OR	[95% CI]	<i>p</i> -value	Adjusted OR ^a	[95% CI]	<i>p</i> -value
Cognitive impairment	2.22	1.21–4.08	0.01	1.05	1.00–1.11	0.02
Sensitivity analysis model						
Cognitive impairment	1.88	0.98–3.56	0.05	1.05	1.00–1.11	0.03

^aAdjusted for age, sex, education status, smoking, hypertension, diabetes, atrial fibrillation, and hyperlipidemia

assess in stroke patients, as depression can fluctuate over time for an individual patient and is affected by many factors (medication, functional status post-stroke, and co-morbidities), for which we could not account for in the current analysis. Nonetheless, very few studies have explored the association between WMH and depression in stroke patients, and we recommend more comprehensive studies in independent cohorts to further explore this interesting and clinically highly relevant topic.

To the best of our knowledge, this is the first study to analyze the association of selected clinical and serological cardiovascular risk profiles (borderline vs. pathological) with WMH burden in a comprehensive cohort of first-ever stroke patients. However, our study has several limitations that warrant discussion. First and foremost, this is a retrospective, exploratory analysis of a prospective cohort that was not designed to address our primary research questions, therefore we did not adjust for multiple testing. Furthermore, data on patients' pre-stroke medication was not available for this cohort, therefore we could not account for the possible influence of pre-stroke medications on baseline clinical and serological risk profiles like blood pressure or HbA1c. This may partially explain why we failed to see a clear association between selected risk profiles and WMH burden. Similarly, we cannot exclude the possible presence of pre-stroke cognitive impairment in this cohort; the use of the mixed-model analysis for cognitive impairment in this analysis only partially compensates for this limitation. Additionally, we could only include patients within PROSCIS that received an MRI, which introduces a potential bias into our cohort because patients with contraindications for an MRI were automatically excluded from the current analyses. This may be reflected by the relatively mild strokes of the patients included in our analysis (median NIHSS at the admission of 2 [IQR 1–5]). Therefore, generalizability to more severely affected stroke cohorts is limited.

A fundamental limitation of this study is that we applied a qualitative visual method for assessing WMH burden (i.e., the ARWMC score). This did not allow us to determine the WMH burden in more detail, i.e., total WMH volume or anatomical distribution (peri-ventricular versus deep white matter). ARWMC score may not be sensitive enough to detect very early manifestations of ARWMC and a ceiling effect at higher scores is to be expected. However, the ARWMC score is a well-known and well-established scoring system for assessing WMH burden in the clinical setting. The advantage of applying the ARWMC score as done in this study is that it increases the clinical applicability of the results. Nonetheless, a more detailed analysis of WMH distribution pattern and volume in terms of cerebrovascular risk profiles and long-term outcomes is undoubtedly warranted to increase our understanding of the prognostic value of early WMH progression. Finally, we can not rule out entirely the

possibility that some patients included in the current analysis had other less common causes of WMH – for example, amyloid angiopathy, previous radiation exposure, or genetic disorders. The further assessment of additional imaging biomarkers of CSVD (lacunes, microbleeds, cerebral atrophy, and enlarged perivascular spaces) was beyond the scope of the current study but would also be essential to fully comprehend the causal effect of CSVD on recurrent vascular events, cognitive decline, and depression in first-ever stroke patients with CSVD.

Conclusion

In summary, our study found no clear associations between selected cerebrovascular risk profiles and WMH burden; this may be due to the small sample size and the methodology applied (i.e., ARWMC score vs. quantitative assessment of WMH including volume and anatomical distribution). However, our study supports the hypothesis that WMH burden leads to a significantly higher risk of cognitive decline and possibly even recurrent vascular events following the first-ever stroke which may allow us to identify patients at risk and initiate early patient-individualized preventative treatment strategies if validated in further studies.

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Declarations

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2.6 VESSEL SIZE IMAGING OF ISCHEMIC STROKE

Kufner A, Khalil AA, Galinovic I, et al. Magnetic resonance imaging-based changes in vascular morphology and cerebral perfusion in subacute ischemic stroke. *Journal of Cerebral Blood Flow & Metabolism*. 2021;41(10):2617-2627.



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We performed an exploratory analysis in 62 patients with subacute ischemic stroke who received VSI in addition to a standard stroke MRI within 40 days of stroke onset from the BAPTISe cohort ('Biomarkers and Perfusion – Training-induced changes after stroke'). BAPTISe was a pre-planned prospective observational study embedded within a RCT which aimed to assess the effect of physical fitness training on functional recovery in moderate to severely affected subacute stroke patients.

Vessel size (VS) and vessel density (Q), as well as measurements of cerebral perfusion within the ischemic lesion, were significantly altered compared to the mirrored lesion on the contralateral side. Specifically, relative VS was increased, and Q was decreased within the ischemic lesion. There was no significant difference in vessel morphology within the perilesional space, although reduced cerebral perfusion measurements were observed in this area. Larger vessel size values within the ischemic lesion were associated with larger lesion volumes (β 34, 95% CI 6.2–62; $p = 0.02$) and more severe strokes (β 3.0, 95% CI 0.49–5.3; $p = 0.02$), findings that align with preclinical VSI studies in rodent stroke models. However, lesion VSI measurements were not predictive of six-month functional outcome assessed via mRS. In summary, the use of VSI as a minimally invasive imaging technique can aid in assessing in-vivo cerebral microvasculature in the subacute phases of ischemic stroke. While VSI may carry prognostic value in terms of lesion progression and stroke severity, the role of VSI in long-term functional recovery requires further investigation. Time-to-MRI did not significantly affect VS or Q within two months post-stroke, highlighting the need for larger, longitudinal studies to validate these findings.

Magnetic resonance imaging-based changes in vascular morphology and cerebral perfusion in subacute ischemic stroke

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Abstract

MRI-based vessel size imaging (VSI) allows for *in-vivo* assessment of cerebral microvasculature and perfusion. This exploratory analysis of vessel size (VS) and density (Q; both assessed via VSI) in the subacute phase of ischemic stroke involved sixty-two patients from the BAPTISE cohort ('Biomarkers And Perfusion--Training-Induced changes after Stroke') nested within a randomized controlled trial (intervention: 4-week training vs. relaxation). Relative VS, Q, cerebral blood volume (rCBV) and –flow (rCBF) were calculated for: ischemic lesion, perilesional tissue, and region corresponding to ischemic lesion on the contralateral side (mirrored lesion). Linear mixed-models detected significantly increased rVS and decreased rQ within the ischemic lesion compared to the mirrored lesion (coefficient[standard error]: 0.2[0.08] p = 0.03 and –1.0[0.3] p = 0.02, respectively); lesion rCBF and rCBV were also significantly reduced. Mixed-models did not identify time-to-MRI, nor training as modifying factors in terms of rVS or rQ up to two months post-stroke. Larger lesion VS was associated with larger lesion volumes (β 34, 95%CI 6.2–62; p = 0.02) and higher baseline NIHSS (β 3.0, 95%CI 0.49–5.3; p = 0.02), but was not predictive of six-month outcome. In summary, VSI can assess the cerebral microvasculature and tissue perfusion in the subacute phases of ischemic stroke, and may carry relevant prognostic value in terms of lesion volume and stroke severity.

Keywords

Angiogenesis, ischemic stroke, microvasculature, perfusion, vessel size imaging

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Introduction

Vessel size imaging (VSI) is a magnetic resonance imaging (MRI) sequence that has emerged as a promising technique able to non-invasively assess microvasculature morphology and cerebral perfusion in rodents and humans.^{1–3} VSI is based on a multi-contrast MR-acquisition that includes using a contrast agent, which leads to susceptibility changes in the vasculature, which are influenced by morphological properties of the vascular network. By means of analytical modeling, this method allows for the *in-vivo* assessment of the vascular density (reflected by Q), average vessel size (VS) in μm , and relative tissue perfusion.¹

Previous studies in rat stroke models have shown that quantitative measurements derived from VSI highly correlate with the observed histological changes in vessel morphologies.^{4,5} Subsequent studies in humans - including ischemic stroke patients - confirmed that following an acute ischemic stroke there is an increase in the mean VS and decrease in vessel density within the ischemic lesion.^{6,7} In the acute phase following ischemia, an increase in lesion VS is likely a reflection of vessel dilation in a presumed attempt to increase tissue perfusion; an alternative hypothesis suggests that the compression of small capillaries following focal cytotoxic edema leads to an overall shift in calculated average VS to larger values.⁷ In subacute phases of stroke, there is discussion whether VSI may aid in the assessment of vascular remodeling in the context of angiogenesis; the formation of abnormal, bulky vessels in post-ischemic areas may lead to increased lesion VS measurements.⁸ Although only a handful of preclinical studies have applied VSI to evaluate vascular remodeling in subacute stages of ischemia,^{9,10} clinical VSI analyses in subacute and chronic stages following stroke in patients are still lacking.

Angiogenesis and vascular remodeling following ischemic stroke play a crucial role in recovery of damaged tissue and post-stroke functional recovery.^{11,12} *In-vivo* visualization of early vascular remodeling may aid in identifying those patients most likely to regain function post-stroke,¹³ if a clear clinical benefit of angiogenesis is observed in patients in future studies. Although pre-clinical murine stroke studies have well characterized the complex physiological cascade leading to changes in the neighboring vascular bed following ischemia,^{4,12–15} studies investigating potential angiogenesis in ischemic stroke patients are still generally lacking.¹¹ The use of VSI may allow for the *in-vivo* assessment of ischemia-induced changes in the microvasculature in subacute ischemic stroke patients and could help us to better understand vascular remodeling and functional recovery post-stroke.

Aim

This is a pre-planned, exploratory analysis of ischemic stroke patients with prospectively acquired MRI data as part of the observational BAPTISE study ('Biomarkers And Perfusion – Training-Induced changes after Stroke', clinicaltrials.gov identifier: NCT01954797), which aimed to study potential markers (including MRI parameters) of angiogenesis.¹⁶ All patients were enrolled in the randomized, controlled trial PHYS-STROKE ('Physical Fitness Training in Sub-acute Stroke' clinicaltrials.gov identifier: NCT01953549).^{17,18}

The primary aim of the current study was to investigate vessel size and density (assessed via VSI) and cerebral perfusion (assessed via VSI or dynamic susceptibility contrast perfusion weighted-imaging [DCS-PWI]) in the subacute phase following an ischemic stroke in selected regions of interest, including the ischemic lesion and perilesional space. Furthermore, our aim was to assess whether vessel size within the ischemic lesion is associated with time from stroke onset to imaging and stroke progression (i.e. stroke severity, lesion size, and functional recovery at six months after stroke).

Materials and methods

Data availability statement

Raw imaging data are not publicly available as they contain information that could compromise patient privacy; program code of the imaging processing pipeline can be shared upon request. Numerical data has been uploaded onto an open repository and can be accessed via the following link: <https://doi.org/10.6084/m9.figshare.13176647.v1>.

Patients and study design

All patients participated in the BAPTISE study nested within the randomized controlled PHYS-STROKE trial; a detailed description of all inclusion and exclusion criteria of PHYS-STROKE and BAPTISE are listed in the Supplemental files. All participants provided informed consent. The study was approved by the institutional review board of Charité–Universitätsmedizin Berlin (EA1/138/13). The original PHYS-STROKE trial was conducted in line with the CONSORT extension for non-drug treatments, and study procedures were carried out in accordance with the Declaration of Helsinki.

Patients enrolled in PHYS-STROKE were randomized to four weeks of aerobic physical fitness training or four weeks of relaxation courses (in addition to standard rehabilitation) and patients were eligible to participate in BAPTISE if they experienced an ischemic stroke and had no MRI contraindications. In

BAPTISE, all patients received an MRI before (v01) and after (v02) the intervention. Patients received a clinical follow-up for six months after stroke; for the current analysis of functional outcome we included the assessment of stroke severity via the National Institute of Health Stroke Scale (NIHSS; at time of stroke onset, v01, and v02) and functional recovery via the modified Ranking Scale (mRS; at six months post-stroke). Detailed study protocols were published previously.^{12,17} For the current analysis, patients had to receive at least one MRI either before or after intervention, which included either VSI and/or DSC-PWI. Only patients with non-lacunar supratentorial strokes were included in this analysis to allow for accurate VSI analysis of the ischemic lesion.

Imaging

All studies were performed using a 3-Tesla clinical MRI scanner (Tim Trio, Siemens Healthineers, Erlangen, Germany). The MR protocol included a FLAIR sequence (TE = 100 ms, TR = 8000 ms, TI = 2370.5 ms, field-of view [FOV] = 220 mm², matrix = 256 × 232, 5-mm slice thickness with a 0.5-mm interslice gap) and diffusion-weighted imaging (echo time [TE] = 93.1 ms, repetition time [TR] = 7600 ms, 6 spatial directions, b = 0 and 1000 s/mm², FOV = 230 mm², matrix = 192 × 192, 2.5-mm slice thickness with no interslice gap, 25 slices).¹⁶

In addition, VSI measurements were acquired using a combined spin-echo (SE) and gradient-echo (GRE) single-shot 2D Echo Planar Imaging (EPI) sequence (TEGRE = 22 ms, TESE = 85 ms, TR = 1890 ms, Flip Angle = 90°, FOV = 230 mm, matrix size 64 × 64, 16 slices, slice thickness = 5 mm, no gap, Bandwidth = 2111 Hz/Pixel, 60 repetitions). A total of 0.13 mL/kg body weight of contrast agent (Gadolinium) was injected intravenously (5 mL/second) 5 seconds after the scan was started. Perfusion parameters including cerebral blood flow (CBF) and cerebral blood volume (CBV) were calculated from the GRE measurement acquired from VSI. If VSI could not be performed, conventional DSC-PWI was performed using a single-shot 2D Echo Planar Imaging (EPI) sequence (TE = 22 ms, TR = 1880 ms, Flip Angle = 60°, FOV = 230 mm, matrix = 80 × 80, 16 slices, 5-mm slice thickness, no gap, Bandwidth = 1690 Hz/Pixel, GRAPPA Acceleration Factor = 2, 60 time points); the identical dose of 0.13 mL/kg body weight of contrast agent with Gadolinium was injected with a 5 second delay.

Data processing

All data were managed, annotated, and processed using a local instance of the medical-imaging platform

NORA (www.nora-imaging.org, University Medical Center Freiburg). VSI was calculated using a MATLAB-based software package, which was embedded into NORA. Maps of apparent diffusion coefficient (ADC) were calculated from diffusion-weighted images via monoexponential fitting. Data processing consisted of the following steps: Gibbs ringing artifact removal,¹⁹ co-registration of all image contrasts using SPM8, extraction of the first bolus²⁰ and conversion from gradient- and spin-echo (GRE, SE) signals to the corresponding relaxation rates ΔR_{2GE} and ΔR_{2SE} , respectively. From these curves, following the approach described previously,¹⁹ vessel density index (reflected by Q [s^{-1/3}]), and vessel size (defined by VS [μ m]) were calculated as defined in Kiselev et al.:¹

$$Q \approx \Delta R_{2SE} / \Delta R_{2GE}^{2/3}$$

$$VS = 1.734 \times (ADC \times CBV)^{1/2} / Q^{3/2}$$

The formula requires absolute values for CBV. Since DSC-MRI in general only yields relative CBV (rCBV) values, we constructed pseudo-quantitative but consistent values by scaling rCBV to a literature value of 3.2% in healthy tissue²¹ i.e. the contralateral hemisphere. CBV and CBF were calculated from ΔR_{2GE} following the general tracer-kinetic-approach as previously described in detail.²² Here, the post-processing consisted of initial motion correction, bivariate filtering, brain extraction and a deconvolution of the tissue curves with an automatically selected arterial input function (AIF); all automatically selected AIFs were visually checked for anomalies. Due to the software upgrade to VB19 at the MRI scanner, patients scanned between November 2013 and June 2014 (N = 23) could not be measured with combined spin- and gradient-echo, and hence no VSI calculation was possible. For these patients perfusion parameters were calculated from a gradient-echo DSC-PWI.

Regions of interest selection

The region of interest (ROI) of the ischemic lesion was manually delineated by a single experienced rater (AK) for each patient on DWI with reference to correlating ADC and FLAIR images, while blinded to VSI and perfusion images. The perilesional space ROIs were created via standardized dilation of the ischemic lesion ROI by two voxels in 3D space with subsequent subtraction of inner ischemic lesion ROI to create a “border zone” for the perilesional space (Figure 1). Furthermore, the ischemic lesion ROI was mirrored to the contralateral healthy side (mirrored lesion).

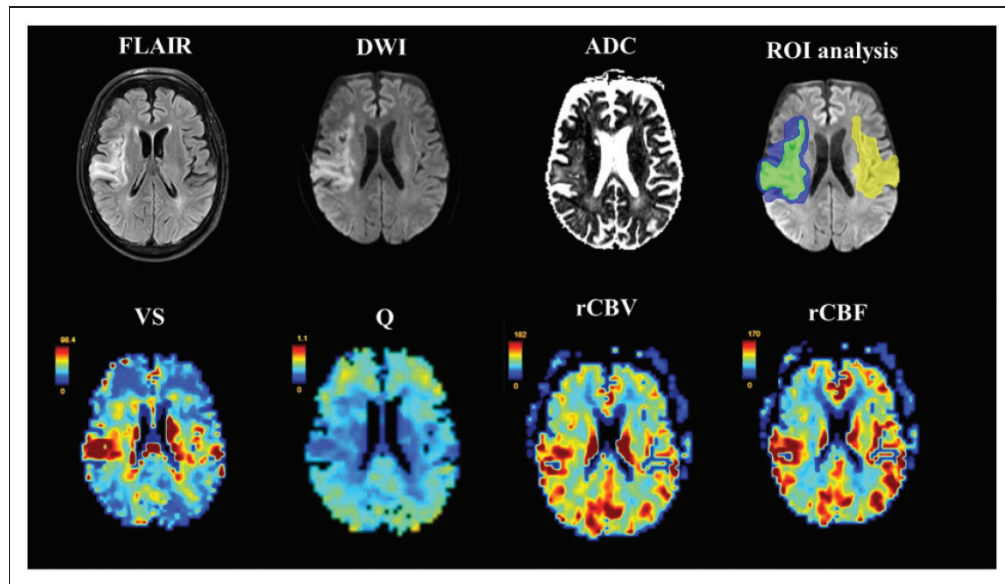


Figure 1. Top row from left to right: FLAIR, diffusion weighted imaging (DWI), and apparent diffusion coefficient (ADC) images with schematic depiction of selected ROI (region of interest) delineations on baseline DWI (green=lesion; blue=perilesional space; yellow=contralateral healthy). Bottom row from left to right: corresponding VS (vessel size; [μm]), Q (vessel density index; [$\text{s}^{-1/3}$]), and relative cerebral blood volume (rCBV) and blood flow (rCBF) maps derived from contrast-enhanced vessel size imaging.

For all ROIs, CBV and CBF are reported as relative values (rCBV and rCBF = normalized to contralateral hemisphere). Similarly, VS and Q are reported as relative values (rVS and rQ); the supratentorial segment of the contralateral healthy hemisphere was manually delineated for each patient for intra-individual normalization of VS and Q to healthy contralateral tissue (i.e. relative VS expressed as a ratio defined by median VS within lesion/median VS contralateral hemisphere). All derived ROIs (ischemic lesion, perilesional space, and mirrored lesion) were superimposed onto VSI and perfusion maps for evaluation.

Statistical analysis

Categorical data are presented as absolute numbers and proportions, continuous data as median and interquartile range (IQR) or mean and standard deviation (SD), depending on the respective distribution of data. Normality of distributions of continuous variables was assessed using Shapiro-Wilk test.

In order to assess differences in VSI and perfusion parameters across selected ROIs, we performed four linear mixed-models in which the dependent variables were defined as the MRI parameter of interest (rVS, rQ, rCBV, and rCBF). All models included subjects as

a random effect and intervention group, time points (v01 vs. v02), as well as time-to-MRI (in days) as fixed effects.

A linear regression analysis was used to assess the association of rVS within the ischemic lesion at v01 and lesion size (assessed on v01 MRI) and stroke severity (assessed at time of acute stroke, and time of v01 and v02 MRI). A univariate and multivariate logistic regression analysis for good outcome (defined as $\text{mRS} \leq 2$ at six months post stroke) was performed with adjustment for the following parameters (age, rVS within ischemic lesion at v01, stroke severity at time of acute stroke, and lesion volume on v01 MRI). All statistical analyses were performed using STATA (StataCorp 2015 Software release 14).

Results

Description of cohort and data

The mean age of the cohort was 68 (SD 11) years, median NIHSS at time of acute stroke was 10 (IQR 6–14). The median time from stroke onset to first MRI (v01) was 27 (IQR 15–34) days. Refer to Table 1 for description of patient demographics.

Fifty-one patients received both v01 and v02 MRIs. Thirty-one patients had available VSI on v01 MRI and 20 had available VSI on v02 MRI (only 17 had VSI at both v01 and v02). Perfusion maps were available for 53 patients on v01 MRI and 34 patients on v02 MRI.

Table 1. Patient demographics and clinical parameters of entire patient cohort.

	All patients (N = 62)
Age in years, mean (SD)	67.9 (10.6)
Male sex, %(n)	56.5 (35)
Cerebrovascular risk factors	
Smoking, %(n)	35.5 (22)
Arterial hypertension, %(n)	79.0 (49)
Diabetes mellitus, %(n)	29.9 (26)
Atrial fibrillation, %(n)	21.0 (13)
Hypolipoproteinemia, %(n)	46.8 (29)
Stroke etiology	
Large-artery atherosclerosis, %(n)	33.9 (21)
Cardioembolism, %(n)	30.7 (19)
Small-vessel occlusion, %(n)	14.5 (9)
Stroke of other determined etiology, %(n)	6.5 (4)
Stroke of undetermined etiology, %(n)	12.9 (8)
NIHSS time of acute stroke, mean (SD)	10 (6–14)
Time from stroke onset to first MRI (v01) in days, median (IQR)	27 (15–34)
Time from onset to second MRI (v02) in days, median (IQR)	61 (47–67)
NIHSS at v01, median (IQR)	6 (3–9)
NIHSS at v02, median (IQR)	4 (2–6)
Wahlund score assessed v01, median (IQR)	5 (3–8)
Lesion volume in mL on v01, median (IQR)	32.2 (9.7–86.4)
Treatment group physical fitness, %(n)	49.2 (30)

SD= standard deviation; IQR=interquartile range; MRI =magnetic resonance imaging; v01 = time point first MRI, v02 = time point second MRI.

Table 2. Absolute values of vessel size (VS) and vessel density index (Q) before normalization to contralateral hemisphere, and markers of cerebral perfusion (including relative cerebral blood flow and cerebral blood volume) on first (v01) and second (v02) MRI in selected regions of interest.

	VS (μm)	Q ($\text{s}^{-1/3}$)	rCBF (%)	rCBV (%)
	N = 31		N = 53	
v01 MRI				
Lesion	55.9 (± 15.1)	0.27 (± 0.04)	61.7 (± 20.3)	71.1 (± 19.2)
Perilesional	50.7 (± 12.3)	0.29 (± 0.05)	68.5 (± 13.6)	81.2 (± 16.1)
Mirrored lesion	50.0 (± 15.9)	0.30 (± 0.06)	86.9 (± 19.2)	86.2 (± 17.1)
	N = 20		N = 34	
v02 MRI				
Lesion	55.8 (± 19.9)	0.28 (± 0.07)	54.1 (± 15.4)	66.5 (± 17.9)
Perilesional	47.4 (± 14.1)	0.30 (± 0.05)	61.7 (± 13.6)	78.3 (± 16.4)
Mirrored lesion	45.2 (± 5.7)	0.31 (± 0.03)	86.0 (± 25.7)	83.0 (± 17.5)

Q=vessel density index, VS=vessel size, CBF=cerebral blood flow, CBV=cerebral blood volume.

On v01 MRI, mean VS within the ischemic lesion was $56 \mu\text{m}$ ($\text{SD} \pm 15 \mu\text{m}$), mean Q was $0.27 \text{ s}^{-1/3}$ ($\text{SD} \pm 0.04 \text{ s}^{-1/3}$). For a comprehensive description of quantitative values of VS and Q, as well as rCBF and rCBV on v01 and v02 scan in selected ROIs, refer to Table 2. For a visual depiction of the distribution of rVS, rQ, rCBF and rCBV values across selected ROIs on v01 and v02 scans combined, refer to the violin plots in Supplemental Figure 1. Correlation matrices for all MR variables of interest within the lesion and contralateral healthy ROI are available in Supplemental Figure 2.

Mean ADC within the ischemic lesion was $961 \text{ mm}^2/\text{s}$ ($\text{SD} \pm 280 \text{ mm}^2/\text{s}$) and $1135 \text{ mm}^2/\text{s}$ ($\text{SD} \pm 353 \text{ mm}^2/\text{s}$) on v01 and v02 MRI, respectively (comprehensive description of ADC values across ROIs at both scanning time-points available in Supplemental Table 2).

Factors associated with vessel size, density, and perfusion

The results of the linear mixed-models are presented in Table 3. The rVS in the ischemic lesion was higher compared to contralateral mirrored lesion (coefficient 0.21, standard error [SE] ± 0.08 , $p = 0.01$) and rQ measured in the ischemic lesion was lower than the contralateral mirrored lesion (coefficient -0.10 , $\text{SE} \pm 0.03$, $p < 0.01$), following adjustment for repeated and within-subject measurements. There was no difference in rVS nor rQ in the perilesional space compared to the contralateral mirrored lesion. There was no significant association between time-to-MRI (in days) and rVS (coefficient -0.001 , $\text{SE} \pm 0.004$, $p = 0.83$) or rQ (coefficient -0.001 , $\text{SE} \pm 0.002$, $p = 0.48$).

Mixed-model analyses for rCBF and rCBV similarly identified the ROI as the main modifying effect; perfusion parameters differed across all selected ROIs including lesion and perilesional space in comparison

Table 3. Linear mixed-models for each dependent variable (relative vessel size and Q, rCBV, and rCBF).

Dependent variable: relative vessel size			
Fixed-effects	Coefficient	Std. Error	p-value
Region of interest			
Contralateral healthy	- reference -	- reference -	-
Perilesional space	0.030	0.080	0.705
Lesion	0.206	0.080	0.010
Time to MRI in days	-0.001	0.004	0.831
Time point of MRI (v01 vs. v02)	-0.086	0.140	0.534
Intervention group (training)	-0.136	0.109	0.211
Random-effects			
	Estimate	Std. Error	95% CI
Subject ID	0.250	0.048	0.170-0.362
Dependent variable: relative vessel density			
Fixed-effects	Coefficient	Std. Error	p-value
Region of interest			
Contralateral healthy	- reference -	- reference -	-
Perilesional space	-0.037	0.032	0.255
Lesion	-0.099	0.032	0.002
Time to MRI in days	-0.001	0.002	0.477
Time point of MRI (v01 vs. v02)	0.079	0.057	0.165
Intervention group (training)	-0.046	0.045	0.308
Random-Effects			
	Estimate	Std. Error	95% CI
Subject ID	0.104	0.020	0.072-0.151
Dependent variable: relative cerebral blood volume			
Fixed-effects	Coefficient	Std. Error	p-value
Region of interest			
Contralateral healthy	- reference -	- reference -	-
Perilesional space	-6.71	2.68	0.012
Lesion	-17.69	2.67	<0.001
Time to MRI in days	-0.251	0.128	0.050
Time point of MRI (v01 vs. v02)	2.50	4.26	0.557
Intervention group (training)	-3.87	3.634	0.288
Random-effects			
	Estimate	Std. Error	95% CI
Subject ID	11.21	1.59	8.49-14.8
Dependent variable: relative cerebral blood flow			
Fixed-effects	Coefficient	Std. Error	p-value
Region of interest			
Contralateral healthy	- reference -	- reference -	-
Perilesional space	-20.30	2.83	<0.001
Lesion	-27.58	2.81	<0.001
Time to MRI in days	-0.248	0.119	0.037
Time point of MRI (v01 vs. v02)	4.17	4.07	0.306
Intervention group (training)	-2.20	3.35	0.512
Random-effects			
	Estimate	Std. Error	95% CI
Subject ID	9.67	1.49	7.15-13.1

All models included subjects as a random effect and intervention group, time points (v01 vs. v02), as well as time-to-MRI (in days) as fixed effects.

to the contralateral healthy ROI as a reference. The time-to-MRI was identified as a significant fixed effect for both rCBV and rCBF; perfusion parameters were inversely correlated with time-to-MRI in days (Table 3). Descriptive two-way scatter plots depicting rVS, rQ, and perfusion measurements within selected ROIs based on time-to-MRI in days (v01 and v02 included) are shown in Figure 2.

Patient demographics were balanced between both intervention groups of the PHYS-STROKE trial. The aerobic fitness training intervention group was not identified as a main effect for any of the MRI parameters in the mixed-models (Table 3). Neither VSI, nor perfusion parameters in selected ROIs at v01 and v02, nor absolute changes of VSI and perfusion parameters between v01 and v02 scans differed significantly between intervention groups (for univariate analysis based on intervention group, refer to Supplemental Table 2).

Associations with stroke severity, lesion size, and functional outcome

Higher rVS values (at v01 MRI) within the ischemic lesion were associated with larger lesion sizes assessed

on both v01 MRI (β 34 95% CI 6.2 – 62; $p=0.018$) and v02 MRI (β 33 95%CI 5.7 – 60; $p=0.020$). Higher VS values within the lesion at v01 were also associated with more severe strokes at the time of symptom onset (β 2.9 95%CI 0.49 – 5.3; $p=0.02$), assessed by the NIHSS score. However, there was no association between lesion VS at v01 and NIHSS at the time of v01 MRI (β 0.46 95%CI $-1.9 - 2.9$, $p=0.70$) or v02 MRI (β 0.27 95%CI $-1.5 - 2.2$, $p=0.68$).

Patients who experienced a good outcome ($mRS \leq 2$) six months following enrollment had significantly lower lesion rVS measured on v01 (1.2, IQR 1.0 – 1.5 vs. 1.7, IQR 1.3–1.9; $p=0.04$) in univariate analysis compared to patients who had a $mRS > 2$. Lesion rQ did not differ between patients who experienced a good vs. poor outcome six months post-stroke (1.0, IQR 0.86 – 1.1 vs. 0.85, IQR 0.74 – 0.97; $p=0.28$). Multivariate analysis did not identify v01 lesion VS as an independent predictor of functional recovery six months post-stroke (adjusted OR 0.16 95% 0.01 – 5.6, $p=0.32$; Table 4).

Among 17 patients with consecutive VSI measurements, changes in rVS and rQ were not associated with lesion progression or outcome at six months after stroke in exploratory analyses (data not shown).

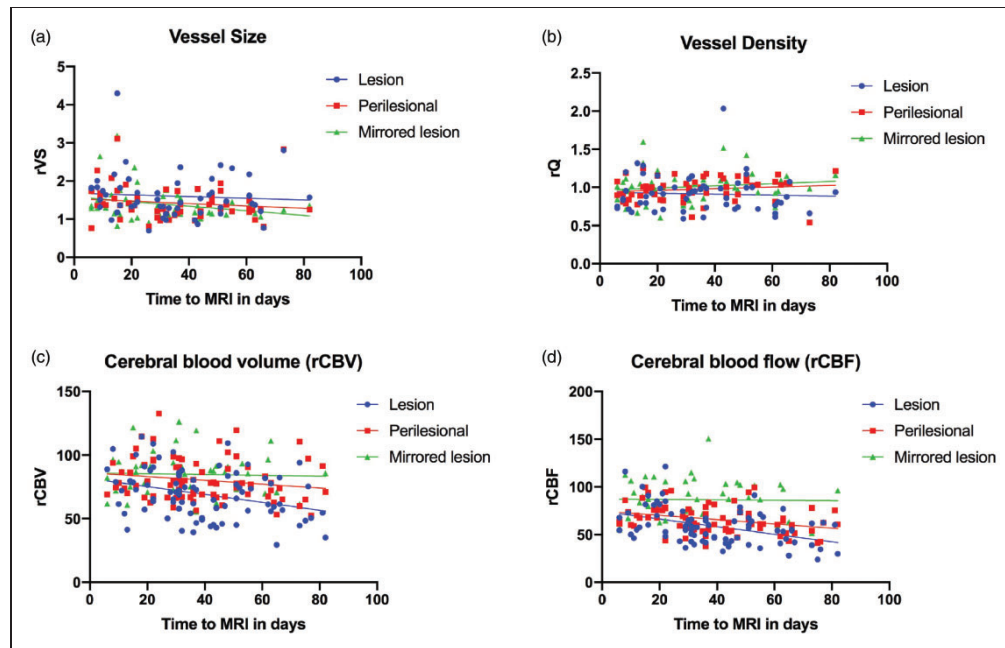


Figure 2. Two-way descriptive scatterplots depicting relative vessel size (rVS), relative vessel density index (rQ), rCBV and rCBF (Panels (a) to (d), respectively) within selected ROIs (ischemic lesion, perilesional space, and contralateral mirrored lesion) based on time to MRI in days. Measurements from v01 and v02 scans are included in all scatter-plots.

Table 4. Univariate and multivariate regression analysis for good outcome (modified Rankin Score ≤ 2) six months following enrollment presenting crude and adjusted odds ratios (ORs) with 95% confidence intervals (CI); N = 62.

Binary logistic regression analysis for good outcome (mRS ≤ 2) at six months					
	Number of cases	Univariate model		Multivariate model	
		Odds ratio (95% CI)	p-value	Odds ratio (95% CI)	p-value
Relative vessel size within lesion assessed on v01 MRI	31	0.14 (0.02–1.3)	0.09	0.16 (0.005–5.6)	0.32
Lesion volume in mL assessed on v01 MRI	57	0.99 (0.97–1.0)	0.05	0.97 (0.93–1.0)	0.14
Stroke severity (NIHSS at v01)	62	0.71 (0.57–0.88)	0.002	0.62 (0.40–0.98)	0.04
Age	62	0.94 (0.89–1.0)	0.03	0.91 (0.81–1.0)	0.12

Discussion

This is the first study to investigate the cerebral microvasculature in patients with subacute ischemic stroke using MRI-based VSI. Linear mixed-models identified an increase in vessel size (VS) with corresponding decrease in vessel density index (Q) within the ischemic lesion compared to corresponding healthy contralateral tissue. There was no difference in VS or Q within the perilesional space compared to the contralateral mirrored ROI, whereas rCBF and rCBV were significantly reduced. Time-to-MRI in days was not associated with VS or Q measured within the selected ROIs in this analysis. Although increased VS within the ischemic lesion was associated with larger lesion volumes and more severe strokes at the time of symptom onset, VS was not predictive of functional outcome at six months after stroke in multivariable analysis.

The findings of this study of patients with subacute ischemic stroke are in line with previous preclinical studies in the acute and subacute phase of stroke^{4,5,12} and one previous clinical study in the acute phase of stroke that also observed increased VS and reduced Q within the ischemic lesion.⁶ The absolute values of previously published Q values in acute stroke patients⁷ are similar to the values of Q reported in this analysis (mean $0.27 \text{ s}^{-1/3}$ vs. $0.30 \text{ s}^{-1/3}$; Table 2) corroborating the feasibility of this imaging modality in stroke research. In this analysis, VS was calculated as the mean vessel diameter averaged over the capillary population within an imaging voxel. The absolute values of VS within ischemic lesions reported in this analysis are higher compared to those reported previously, (mean $56 \mu\text{m}$ diameter vs. $18 \mu\text{m}$ radius).⁷ Although the post-processing pipelines of the VSI sequences were similar, differences in scan resolution and differing patient cohorts analyzed (i.e. differences in stroke severity, lesion size, and time after stroke) may explain this observed difference.

Linear mixed-models identified significant differences in measured rVS and rQ, as well as markers of cerebral perfusion (rCBF and rCBV) across selected

ROIs (Table 3). Interestingly, time-to-MRI in days was not identified as a modifying factor of VS or Q values in this analysis. These results may stand in line with a previously published longitudinal VSI study in a murine stroke model, in which early ischemia-induced changes in vascular morphology within and around the ischemic lesion remained constant over time into the subacute phase of stroke.⁵

Although time-to-MRI in days did not modify VSI measurements, time to scan was inversely associated with rCBF and rCBV; in other words these perfusion metrics decreased over time. In the case of large-scale and functional angiogenesis, one might expect to observe an increase in Q with corresponding increases in tissue perfusion. Although previous observations from Boehm-Sturm *et al.* described sparse histological correlates of perilesional angiogenesis four weeks post-stroke in rats, they did not observe MRI correlates in their translational VSI study.⁵ Similarly, in this explorative clinical study, we observed no MRI-based evidence for large-scale changes in vessel size or density within selected ROIs in the subacute phases following ischemic stroke. Still, our observations require validation in larger, longitudinal studies.²³

Previous pre-clinical studies suggest that increased physical fitness may enhance angiogenesis and influence functional recovery following stroke.^{8,24,25} These served as the foundation for the randomized controlled PHYS-STROKE trial and the accompanying observational biomarker study BAPTISE.¹⁷ The trial intervention (aerobic training) did not affect VS, Q, or perfusion measurements in linear mixed-model analyses in this cohort. Although this study was not powered to detect differences in absolute VS and Q values between intervention groups, we performed exploratory univariate analyses stratified by treatment allocation (4-weeks of aerobic fitness training vs. 4-weeks of relaxation); here, we also did not observe differences in VSI values or relative tissue perfusion within or surrounding the ischemic lesion (Supplemental Table 2).

Interestingly, the enlargement of microvessel diameter within the ischemic lesion measured on v01 MRI was associated with larger lesion volumes and more severe strokes at the time of the index event. There are several hypotheses as to why VS increases within an ischemic lesion: one being that severe cytotoxic edema leads to the compression of small vessels leading to an overall shift in the average vessel size per unit space,^{6,7} and another being that following ischemic tissue damage, failed vascular remodeling leads to the formation of larger, bulky, dysfunctional vessels.^{4,8} The observed decrease in Q is likely explained by widespread cell death following ischemia.^{4,26} Whether the observed increase in VS within the subacute ischemic lesions in this study is directly attributed to the loss of patent capillaries, to abnormal angiogenesis, or both is not entirely clear. Either way based on our results and what we know from preclinical studies, it is reasonable to assume that early large-scale changes in VS within the ischemic lesion are indicative of severe stroke, progression of lesion size, and potentially worse functional recovery. Although larger lesion VS was associated with a worse long-term functional outcome in univariate analysis, multivariate regression analysis did not identify VS within the stroke lesion to be independently associated with outcome (Table 4). However, due to the limited sample size of this study, a larger independent cohort analysis is warranted to investigate the prognostic value of VSI further.

This study has several limitations that should be considered. Firstly, this is a relatively heterogeneous cohort of stroke patients with respect to ischemic lesion pattern (pure subcortical strokes versus territorial strokes with cortical and subcortical affection), stroke etiology, and cerebrovascular risk profiles. However to the best of our knowledge there is no data available on how VS and Q are modified based on ischemic lesion location, cause of stroke, or presence of cerebrovascular risk factors such as long-term diabetes mellitus. These points should be addressed in future studies involving VSI. Furthermore, time from onset to first and second MRI was highly variable. This is due to the inclusion criteria of the randomized-controlled PHYS-STROKE trial. A longitudinal, long-term study with sequential MRIs at predefined time points after stroke should be performed in future analyses for a more precise MR-based quantification of ischemia-induced microvascular changes in the subacute to chronic phases of stroke. In the current analysis relative perfusion values (rCBV and rCBF) were analyzed; a possible limitation of this approach is that the effect of perfusion changes on the contralateral hemisphere cannot be entirely accounted for. Finally, we also observed a relatively high inter-

individual variability in terms of absolute VS values assessed within selected ROIs (Table 2; Supplemental Figure 1); previous studies with this sequence have also reported high standard errors within reported VSI measurements.^{5,7} However, it is important to consider that VSI does not allow for a direct visualization of the cerebrovascular morphology. Values of VSI are based on complex analytical models that allow for an accurate approximation of vessel size and vessel density indices based on a number of theoretical assumptions (i.e. intravascular signal and native blood paramagnetism are neglected).¹ These assumptions can result in errors up to an order of two,^{1,27,28} however, differences in actual and measured values follow a monotonic function, which preserves the given order of values and hence may even ease detection. In the current study, measured VSI values were normalized against the inter-individual contralateral healthy hemisphere in order to partially compensate for any additional patient individual variations caused by for example age.

Nonetheless, this is the first longitudinal analysis applying this MR-imaging technique to assess the cerebral microvasculature in a well-characterized cohort of subacute stroke patients, and the results support evidence that VSI may aid in the assessment of ischemia-induced changes following moderate to severe subacute stroke in the clinical setting. In comparison to the standard DSC-PWI, VSI has the advantage of having a similar spatial resolution and comparable acquisition time (albeit 2 min longer) while providing the additional valuable information about vessel size and density. Furthermore, VSI may have the advantage of more precisely assessing tissue-at-risk in the acute clinical setting compared to DSC-PWI.^{6,29} However, it is also important to note that there are alternative methods i.e. selected functional MRI (fMRI) sequences that can provide information on vessel size/density as well without the use of a contrast agent. However, the post-processing of fMRI for this purpose is still largely experimental, time-consuming, and therefore not always suitable for routine clinical diagnostics. VSI could therefore have substantial implications in the clinical setting far beyond the scope of ischemic stroke. The role of VSI in predicting stroke recovery however needs to be validated in larger independent cohort analyses.

Conclusion

In conclusion, the use of VSI as a minimally invasive imaging technique can aid in assessing the in-vivo cerebral microvasculature in the subacute phases following an ischemic stroke. Translating from preclinical

studies, we also observed an increase in VS and a decrease in vessel density with corresponding decreased perfusion within the ischemic lesion, compared to contralateral healthy brain. Time from stroke onset to MRI (up to two months post-stroke) did not alter the dynamic of VS or Q. Higher VS values within the ischemic lesion were associated with larger lesion volumes and more severe strokes, but the prognostic role of VSI in terms of long-term functional recovery requires further testing in larger, independent cohorts.

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Authors' contributions





M Ebinger, M Endres, A Flöel and AH Nave conceived and designed the PHYS-Stroke Trial and accompanying BAPTISe Study. AH Nave and T Rackoll were involved in

patient recruitment. A Kufner post-processed the MRI data, performed the statistical analysis and wrote the first draft of the manuscript. A Kufner, AA Khalil, I Galinovic, E Kellner, R Mekle, T Rackoll, P Boehm-Sturm, JB Fiebach, A Flöel, M Ebinger, M Endres and AH Nave were involved in conceiving and applying methodologies applied in the current study, interpretation of data, review and editing of the manuscript and approval of final version of the manuscript.

Supplementary material

Supplemental material for this article is available online.

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3. DISCUSSION

Acute imaging with either CT or MRI is indispensable in the case of a suspected stroke. Although there is increasing evidence that an MRI-based acute stroke concept is suitable, fast, and in some cases more beneficial than CT in terms of selecting patients for recanalizing therapies, currently there is no randomized controlled study that has unequivocally demonstrated the advantage of an MRI-based acute stroke concept in terms of improved patient outcomes⁴³. In either case, we must take advantage of the imaging modalities available at hand to gain a deeper understanding of individual stroke pathology and to improve all-around stroke care.

Based on the studies above, one might conclude that i) thrombus assessment on acute CT can identify stroke cause in the case of M1 occlusion^{15,16}, ii) selected MR-parameters can identify patients likely to benefit from recanalizing therapies and predict outcome^{27,28,40}, and iii) assessment of underlying small vessel disease can predict long-term vascular risk and cognitive decline after stroke³⁵. However, it is crucial to note that as of yet there is no Class I evidence for the use of any of these imaging criteria to make treatment decisions in the clinical setting. Nonetheless, these studies set the groundwork for further validation studies and bring us closer to a more imaging-based and patient-individualized stroke treatment concept.

THROMBUS PEROVIOUSNESS TO IDENTIFY STROKE CAUSE

Our study investigating whether TP can detect cardioembolic stroke on acute CT imaging in patients with M1 occlusion showed promising results: with a cutoff value of 6.23 Hounsfield units, cardioembolic strokes were identified with a 100% specificity¹⁵. Not only did we validate previous findings reported by Berndt et al that found TP-measurements to be associated with histological thrombus composition and stroke etiology¹¹, but we could show the robustness of TP-measurements regardless of time-to-scan, prior medication with antiplatelets or anticoagulants, and selected laboratory parameters. Although functional outcome data in our

studies was limited, recent works have since provided further evidence that TP measurements are predictive of EVT success, as well as functional outcome in patients with MCA occlusion^{44,45}. Further implications of TP are two-fold, namely improvement of prognostic accuracy to predict patient outcome and TP-guided anticipatory planning of the optimal EVT technique (e.g. stent retriever or aspiration catheter) to be used by the interventionalist.

Although TP has consistently shown promising results in detecting stroke etiology in anterior circulation stroke, studies on TP in posterior circulation stroke have been largely inconclusive^{16,46}. The negative findings in BAO in our study may be explained by the proximity of the BA to the skull which is likely to affect imaging analysis measurements due to increased artifacts. Furthermore, the BA can be perfused both antegrade and retrograde, unlike the MCA. Noteworthy is also that BAO is more often caused by large artery atherothrombosis due to local thrombosis of an underlying stenosis, whereas M1 occlusions are more often caused by embolism from proximal arteries. These substantial pathophysiological differences likely lead to altered clot composition and hereby affect TP values. Additional studies are also required to identify the mechanisms by which thrombus location affects the relationship between TP and stroke etiology. Ultimately, the utility of TP in the case of posterior circulation stroke has yet to be determined.

Crucial next steps for clinical translation of TP in MCAO include standardization of the imaging analysis pipelines across centers, improvement of our understanding of TP interaction with other thrombus characteristics (e.g. thrombus length and location), and ultimately validation of the diagnostic accuracy of TP in anterior circulation stroke in prospective, multicenter studies.

FLAIR HYPERINTENSE VESSELS AS A TREATMENT SELECTION CRITERION

Here, we also summarize two studies that investigated the prognostic and diagnostic value of a frequently observed imaging biomarker on FLAIR sequences, namely FHV. Although studies largely agree that FHV represent collateral circulation which becomes visible because of slow retrograde flow distal to the site of the occluded vessel, their clinical relevance has been long disputed. Therefore,

we set out to elucidate the diagnostic value of this imaging biomarker in two independent studies.

In a multicenter study including a homogenous cohort of patients with isolated MCAO occlusion who received EVT, we found that extensive FHV were directly associated with good collateral grades assessed on baseline angiography²⁷. Furthermore, FHV were independently associated with a 3-month better functional outcome. Interestingly, in an additional follow-up study in 165 patients with confirmed unilateral occlusion (albeit differing occlusion sites including anterior and posterior circulation strokes), we were unable to detect an association between the extent of FHV and stroke progression or functional recovery²⁸. However, we did find that the baseline extent of FHV modified the treatment effect of thrombolysis, making it more effective in patients with fewer collaterals. This can be explained by a protective effect of FHV (good collateral supply of hypoperfused tissue⁴⁷) in the hyperacute phase of stroke making patients who are unable to recruit a sufficient collateral network more dependent on thrombolysis to achieve a good functional outcome.

In 2020, a systematic review of FHV in ischemic stroke found no clear association between the extent of FHV and functional outcome²³. Ultimately, as long as methods for assessment and quantification of FHV differ across centers and studies, no universally valid insights regarding the clinical utility of FHV can be gained. Whether the extent of FHV on acute MRI can be used as a potential selection criterion for acute treatments has yet to demonstrate its usefulness. The studies presented on this topic can only be considered exploratory due to their retrospective nature and relatively small sample sizes and can therefore not conclusively answer this question.

ROLE OF WHITE MATTER HYPERINTENSITIES IN LONG-TERM RISK STRATIFICATION

Early risk stratification following an ischemic stroke is crucial to best initiate an appropriate secondary prevention strategy. Underlying CSVD is the most frequent incidental co-finding on acute imaging of ischemic stroke patients and is directly correlated with increased age and the presence of cerebrovascular risk factors.^{31,48}

In a comprehensive prospective cohort of 413 first-ever ischemic stroke patients, we demonstrated that an increased burden of WMH was predictive of three-year cognitive decline³⁵. Kaplan–Meier survival analysis showed a visible increase in the risk of recurrent vascular events following stroke in patients with high WMH burden, though this finding did not reach statistical significance. Our findings stand in line with a recently published meta-analysis including >70.000 ischemic stroke patients that found the severity of WMH to be strongly correlated with an increase in risk of dementia, stroke recurrence, and increased mortality³¹. Whether there is a causal link between WMH severity and post-stroke depression remains unclear⁴⁹.

We found no clear association between selected clinical and serological cardiovascular risk profiles (borderline versus pathological) and WMH burden. This may be explained by the sample size of the cohort and the crudeness of the WMH scoring system used. Although ARWMC score is a well-known and established scoring system to quantify WMH in the clinical setting (easing the clinical applicability of our findings), it may not be sensitive enough to detect early manifestations of CSVD. A more detailed assessment of WMH in terms of volume and anatomical distribution (peri-ventricular versus deep white matter) would further deepen our understanding of the true predictive potential of assessing markers of small vessel disease after stroke.

Taken together, these findings underscore the importance of additionally evaluating underlying cerebral pathologies to identify patients at risk. WMH burden is a promising biomarker of a patient-individual risk profile that can be used to guide preventative treatment strategies if validated in further studies.

VESSEL SIZE IMAGING OF ISCHEMIC STROKE

To the best of our knowledge, we performed the first exploratory study investigating the potential diagnostic and prognostic value of imaging biomarkers stemming from the new MR-sequence VSI in ischemic stroke patients⁴⁰. Interest in this sequence stemmed originally from preclinical murine stroke studies, which found that histological changes in the microvasculature corresponded to tissue

recovery following ischemia and in the regaining of function; these changes could be quantified with VSI^{39,50}. Therefore, a translation into the clinic was pursued to potentially identify new biomarkers that could predict recovery of damaged tissue and functional recovery based on microvascular correlates³⁸.

In our study, we observed that an increase in vessel size within the ischemic lesion was associated with larger lesion volumes and more severe strokes⁴⁰. Several hypotheses might explain this observation: one being that severe cytotoxic edema leads to compression of small vessels, resulting in an overall shift in the average vessel size, and another being that failed vascular remodeling following ischemia leads to the formation of bigger yet dysfunctional vessels⁵¹. The observed decrease in vessel density is likely explained by widespread cell death following ischemia. We did not observe an association between VSI parameters within or surrounding the lesion and functional outcome in this study.

Although this is the first longitudinal analysis applying this novel MR imaging technique to assess cerebral microvasculature in a well-characterized cohort of stroke patients, the cohort size was relatively small and heterogeneous in terms of time to imaging and ischemic lesion patterns, both of which are likely to affect VSI measurements. A longitudinal long-term study with sequential MRIs at predefined time points after stroke would allow for a more precise quantification of microvascular changes following stroke to truly uncover the diagnostic and prognostic potential of this new MR sequence; this is a planned follow-up study within our working group in collaboration within the Department of Radiology using data from the prospective LOBI-II cohort (EA4-056-18). Since the publication of our study, the diagnostic value of VSI beyond the field of scope has shown very preliminary yet promising results in terms of differentiating cerebral neoplasms⁵² as well as in the early diagnosis of cerebral small vessel disease⁵³.

Ultimately, VSI is emerging as a promising, minimally invasive imaging technique that can aid in the assessment of the in-vivo microvasculature in patients. Our exploratory study shows that the use of VSI is feasible in the setting of ischemic stroke and sets the stage for future validation studies to uncover the therapeutic potential of this sequence.

CONCLUSION

In conclusion, clinical imaging-focused research in the coming years will hopefully continue to address the discussed points above to ultimately identify suitable imaging selection criteria for acute treatments as well as for patient-tailored secondary prevention strategies for ischemic stroke patients. There is a notable paradigm shift in the treatment of ischemic stroke, in which we are increasingly moving away from a rigid time-based approach to a more imaging-based approach tailored to the patient's risk-benefit profile. We hope our findings are considered an important groundwork for future studies to pursue this promising direction and to further enhance the precision and effectiveness of stroke management.

4. SUMMARY

In summary, MRI and CT are excellent diagnostic tools in the acute phase of stroke, providing valuable information that is crucial for understanding individual stroke pathology. The discussed studies highlight the potential of various imaging biomarkers, including thrombus characteristics on CT, FLAIR hyperintense vessels, vessel size determined on VSI, and underlying small vessel disease, all of which provide imaging-based physiological information to assess the patient-specific risk-benefit profile.

There is still a strong need for the definitions of these imaging biomarkers to be standardized across centers and sites. Only in this way can the true diagnostic and prognostic value of these biomarkers be tested in a multicenter, controlled setting and the actual clinical utility be sufficiently assessed. Nonetheless, the multitude of information gained through different acute imaging modalities can be explored in smaller cohort studies like ours. These findings contribute to both our pathophysiological understanding of ischemic stroke and move us towards a more imaging-based and targeted treatment concept in all-around stroke care.

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7. DECLARATION

§ 4 Abs. 3 (k) der HabOMed der Charité

Hiermit erkläre ich, dass

- weder früher noch gleichzeitig ein Habilitationsverfahren durchgeführt oder angemeldet wurde,
- die vorgelegte Habilitationsschrift ohne fremde Hilfe verfasst, die beschriebenen Ergebnisse selbst gewonnen sowie die verwendeten Hilfsmittel, die Zusammenarbeit mit anderen Wissenschaftlern/Wissenschaftlerinnen und mit technischen Hilfskräften sowie die verwendete Literatur vollständig in der Habilitationsschrift angegeben wurden,
- mir die geltende Habilitationsordnung bekannt ist.

Ich erkläre ferner, dass mir die Satzung der Charité – Universitätsmedizin Berlin zur Sicherung Guter Wissenschaftlicher Praxis bekannt ist und ich mich zur Einhaltung dieser Satzung verpflichte.

Berlin, den 11. November 2024

Unterschrift