Aus der Klinik für Neurologie
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DISSERTATION

PREDICT STROKE: predicting response to thrombolysis in acute ischemic stroke patients using multiparametric MRI

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Abstract

Background: Tissue plasminogen activator remains the only licensed pharmaceutical treatment available for acute ischemic stroke. However due to the narrow therapeutic time window, overall rates of thrombolytic intervention are low. Currently, treatment decision is based primarily on time, however studies show that infarction rate is highly variable among patients depending on individual stroke pathology. Therefore, there is a continued search for parameters that may predict response to treatment. An enhanced understanding of the individual stroke at hand may aid in making more personalized treatment decisions. Here we investigate (i) whether a rapid diameter-based method for estimating lesion volume can be applied to guide treatment (ii) whether presence of FLAIR hyperintense vessels (FHVs) carries diagnostic and prognostic value in terms of collateral status, and (iii) whether cerebrovascular risk factors influence treatment efficacy in patients suffering from ischemic stroke.

Methods: All investigations were retrospective cohort analyses of data from three large studies registered under www.clinicaltrial.gov: a multicenter randomized trial (AXIS-2; NCT00927836), a multicenter observational study (PRE-FLAR; NCT01021319), and a single center observational study (1000Plus; NCT00715533).

Results: (i) Diameter-based measurement of lesion volume on diffusion-weighted-imaging (DWI) accurately estimates lesion size; 1-axis cut-off of 3.5cm predicts lesion of ≥15mL with 98% sensitivity and 51% specificity, analogously a 2-axis orthogonal and a 3-axis ABC/2 method predicts a lesion >70mL with high sensitivity and specificity (89% and 93%, and 94% and 86%, respectively). (ii) FHVs predict vessel occlusion with 86% specificity and 76% sensitivity and are associated with severe hypoperfusion and larger infarct growth following treatment. (iii) In multivariable analysis, smoking had an odds ratio of 4 (95% confidence interval 1-16; p=0.03) for recanalization and 6 (95% CI 1-30; p=0.05) for reperfusion. Smokers had a better outcome (modified Rankin Score 0-2) compared to non-smokers (77% vs. 55%; p=0.05).

Conclusion: Here we (i) demonstrated that diameter-based volumetric methods can estimate DWI lesion size with high specificity and sensitivity, (ii) showed that FHVs can predict vessel occlusion with high diagnostic accuracy and most likely represent severe ischemia resulting in larger infarct growth, and (iii) found that smoking leads to enhanced treatment efficacy in terms of recanalization and reperfusion rates. Based on these results, we see that selected MR imaging criteria (DWI lesion volume and FHVs), as well as knowledge of clinical risk factors (smoking) can aid in the assessment of individual stroke severity, and take us one step closer to making more personalized treatment decisions.

Zusammenfassung

Einleitung: Die Verwendung von Gewebeplasminogenaktivator (tPA) stellt zurzeit das einzige lizensierte Arzneimittel zur Behandlung von akuten ischämischen Schlaganfällen dar. Wegen des engen therapeutischen Zeitfensters kommt tPA nur in wenigen Fällen zur Anwendung. Derzeit wird die Therapieentscheidung primär vom Zeitfaktor abhängig gemacht. Studien zeigen jedoch, dass Infarktprogression stark variieren kann. Die Identifizierung klinischer Parameter, die einen Therapieerfolg prognostizieren, ist deswegen von großem Interesse. Ein genaueres Verständnis über die Pathophysiologie des einzelnen Schlaganfalls könnte es ermöglichen personalisierte Behandlungsentscheidungen zu treffen. Das Ziel unserer Untersuchungen war es deshalb herauszufinden, (i) ob eine schnelle Methode zur Bestimmung des Läsionsvolumens eine Behandlungsentscheidung stützen kann, (ii) ob das Vorhandensein von hyperintensen Gefäßen auf FLAIR-Sequenzen (FHVs) diagnostische Informationen über den Status der Kollateralen liefern kann und (iii) ob zerebrovaskuläre Risikofaktoren die Effizienz von Behandlungen beeinflussen.

Methoden: Alle Untersuchungen waren retroperspektive Kohortenanalysen ausgewählter Studien (registriert unter www.clinicaltrial.gov): eine randomisierte multizentrische Studie (AXIS-2; NCT00927836), eine multizentrische Beobachtungsstudie (PRE-FLAR; NCT01021319) und eine monozentrische Beobachtungsstudie (1000Plus; NCT00715533).

Ergebnisse: (i) Messungen mittels Diffusions-Tensor-Bildgebung (DWI) können das Volumen der Läsion mit hoher Sensitivität und Spezifität abschätzen: uniaxialer Querschnitt von 3.5cm (98% und 51% für Läsionen ≥15mL), eine 2-axis orthogonal Methode (89% und 93% für Läsionen >70mL) und eine 3-axis ABC/2 Methode (94% und 86% für Läsionen >70mL). (ii) FHVs detektieren Gefäßverschlüsse mit hoher Spezifität (86%) und Sensitivität (76%) und sind mit einer schwerwiegenden Hypoperfusion und einer größeren Infarktentwicklung assoziiert. (iii) Eine multivariate Analyse konnte zeigen, dass Raucher signifikant höhere Raten an Rekanalisierung (Odds Ratio [OR] 4 [95%-Konfidenzintervall: 1-16; p=0.03]) und Reperfusion aufweisen (OR 6 [95% KI: 1-30; p=0.05]). Raucher wiesen verglichen mit Nichtrauchern einen höheren Behandlungserfolg auf (Modifizierte Rankin Skala 0-2; 77% vs. 55%; p=0.05).

Schlussfolgerung: Wir konnten zeigen, dass (i) durchmesserbasierte volumetrische Methoden Läsionsgrößen mit hoher Genauigkeit abschätzen können, (ii) dass FHVs in der Lage sind, Gefäßverschlüsse zu erkennen und mit schwerwiegender Hypoperfusion und anschließenden größeren Infarkten einhergehen. Des Weiteren konnten wir zeigen dass (iii) Rauchen zu einer erhöhten Behandlungseffizienz führt. Aufgrund dieser Ergebnisse sind wir der Ansicht, dass ausgewählte MRT-Methoden (DWI Läsionsvolumen und FHVs), und ein Verständnis über klinische Risikofaktoren (z.B. Rauchen), helfen können personalisierte Behandlungsentscheidungen zu treffen.

Introduction:

Background

Stroke is the second most common cause of death and disability worldwide and due to an increase in the aging population, the burden of stroke is expected to rise within the next decades. Although many advances have been made in terms of the prevention and treatment of stroke, tissue plasminogen activator (tPA) remains the only licensed medical treatment available in the acute setting of ischemic stroke. The aim of tPA is arterial recanalization and reperfusion of the ischemic penumbra. However, even when administered within the therapeutic time window of 4.5 hours of symptom onset, thrombolysis can lead to serious complications, such as symptomatic intracerebral hemorrhage (sICH)[1]. Consequently, due to the narrow therapeutic time window and often high rates of unknown time of symptom onset, overall rates of thrombolytic intervention are relatively low, ranging from 2-10%[2].

Currently, treatment decision is based primarily on time due to a clear association between treatment efficacy and the interval between symptom onset and tPA administration[1]. However, the progressive integration of advanced imaging techniques - such as multiparametric magnetic resonance imaging (MRI) – now allow for a more accurate assessment of individual stroke pathology[3]. Studies show that the rate of infarction is highly variable among patients depending on selected parameters i.e. cerebrovascular risk factors[4] and collateral status[5]. As a result, we see a high amount of variability among patients in terms of response to treatment and recovery, irrespective of time. Identification of those parameters that can predict - for example rate of infarction and treatment efficacy – would be tremendously valuable in terms of identifying patients most likely to benefit from thrombolysis. In other words, although today the familiar phrase ‘time is brain’ appropriately guides decision to treat, there is a continued search for predictive tools that would allow for a more targeted treatment decision based on the stroke at hand. Considering that only a minority of eligible stroke patients benefits from thrombolysis, this approach could potentially increase the yield of favorable outcomes and decrease rates of sICH following treatment.

In the search for parameters that may guide individualized treatment decisions, there are several fundamental aspects that should to be considered. For example, predictive imaging parameters need to be able to be assessed rapidly and

efficiently in the time-sensitive setting of acute stroke. Furthermore, the identification of factors that can predict rate of infarction or treatment efficacy outside the therapeutic time window would be particularly valuable, especially in those patients with unknown time of symptom onset. Finally, the identification of simple clinical risk factors with substantial prognostic value would be very practical in terms of rapidly assessing the stroke at hand and guiding personalized treatment decisions.

Hence, the aim of this project is to investigate whether well defined imaging parameters and selected risk factors can aid in the selection of patients most likely to benefit from thrombolysis in an acute setting. First, we focus on two well-established MRI sequences, namely diffusion-weighted imaging (DWI) and fluid-attenuated inversion recovery (FLAIR), in terms of their diagnostic and prognostic value. Finally we investigated whether the knowledge of cerebrovascular risk factors influence treatment efficacy.

Diffusion-weighted imaging (DWI)

DWI is a standard sequence applied in stroke MRI protocols and allows for the early detection of pathological changes in ischemic tissue. In fact, cerebral infarction can be detected on DWI within minutes following a persisting ischemic attack[3]. DWI lesion volume is often used in stroke trials as an inclusion criterion for enrollment primarily because previous studies have shown that response to thrombolysis and functional recovery differ depending on lesion size[6]. The gold standard for the measurement of lesion size is currently the manual delineation of infarct borders on all DWI slices. However, this method is rather time-consuming and consequently not suitable for the time-sensitive setting of acute stroke. Therefore, there is a need for an efficient and reliable method to assist in the estimation of infarct volume.

Alternative methods have been proposed for the rapid assessment of lesion size and have been used as an inclusion criterion for trials. For example, one of the main inclusion criteria for enrollment of a recent multicenter trial (AXIS-2[7]) was a baseline infarct volume of ≥15mL; a single measurement of the largest infarct diameter was used to predict lesion size in which a cut-off value of 3cm was applied. Alternative methods include assessment of orthogonal DWI lesion diameters[8] (od-values; 2-axis measurement of largest diameter and perpendicular diameter) and the so-called ABC/2 method[9] (3-axis measurement, similar to od-value only in which slice thickness is taken into account). However, these methods often lead to

overestimation of lesion size and therefore may mistakenly exclude patients eligible for treatment.

Therefore, we set out to retrospectively determine the sensitivity and specificity of the single largest diameter method for estimating lesion volume applied in the abovementioned trial. Furthermore, we aimed to apply distinct methodologies used to estimate lesion size (i.e. od-value and ABC/2) in the same population of patients to compare the accuracy of the latter two techniques. The purpose of this investigation was to determine which method most accurately estimates lesion size – an important determinant of response to thrombolysis – and to identify the advantages and disadvantages of the abovementioned techniques. The ultimate goal is to identify the most robust tool for harmonization of a study cohort in multicenter trials, as well as to determine the most efficient method for estimating lesion size in an acute setting.

Fluid-attenuated inversion recovery (FLAIR)

FLAIR sequences are also an integral part of stroke MRI; yet contrary to DWI, hyperintensities on FLAIR usually develop within hours following an ischemic event. The temporal discrepancy between the development of DWI and FLAIR hyperintensities has been applied to predict lesion age. A so-called DWI-FLAIR mismatch, in which an ischemic lesion appears hyperintense on DWI yet invisible on FLAIR, predicts lesion age of ≤4.5 hours with a high specificity and sensitivity[10]. This is now the foundation for an on-going randomized controlled trial investigating thrombolysis in patients with unknown time of symptom onset based on the presence of a DWI-FLAIR mismatch (WAKE-UP; EudraCT 2011-005906-32).

Apart from parenchymal FLAIR hyperintensities, hyperintense vessels on FLAIR are observed in up to 80% of acute ischemic stroke patients[11,12] (Figure 1). Despite the high prevalence of FLAIR hyperintense vessels (FHV) on acute MRI, results on the underlying pathophysiology are seemingly divided. While most studies agree that FHV are highly associated with large vessel occlusion, some attribute FHV to be indicative of inadequate collateralization[12], while others suggest precisely the opposite; namely, that FHVs represent increased leptomeningeal collateral flow[11].

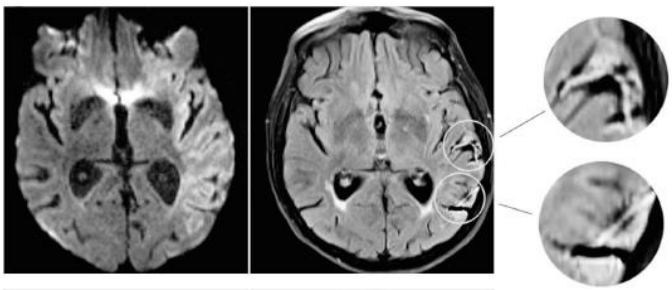

Figure 1 Diffusion-weighted imaging (DWI, left) and fluid-attenuated inversion recovery (FLAIR, right) sequences of a patient with acute ischemic stroke. FLAIR hyperintense vessels (FHV) are visible (magnification) in the corresponding area of diffusion restriction. Figure modified from Ebinger, Kufner et al. 2012.

Collateral status is a well-known predictor of the development of infarct size and response to thrombolysis[5], however assessment of collateralization in an acute setting is problematic[13]. For example, the use of imaging techniques like digital subtraction angiography (DSA) is only justifiable in cases in which intra-arterial thrombolysis is required for treatment. Nevertheless, a recent study investigated the use of perfusion-weighted-imaging (PWI) - a commonly applied MRI sequence requiring contrast agent – for estimating collateral flow[14]. PWI allows for the evaluation of microvascular circulation by calculating blood flow per unit time for a given voxel. In the presence of good collateral flow, perfusion distal to the site of vessel occlusion will be enhanced and infarction rate will be reduced. Bang and coworkers applied this concept and investigated whether a so-called hypoperfusion intensity ratio (HIR; time-to-maximum blood flow [Tmax] ≥ 8 seconds/Tmax≥2 seconds) correlated with collateral status and indeed, excellent and intermediate collateral grades were highly associated with lower HIRs and smaller infarct progression[14].

The use of HIR as a surrogate marker of collateralization in an acute setting may be unsuitable due to the time-consuming nature of its calculation and the requirement of using contrast agent. Therefore, we set out to determine whether presence of FHVs can be used as a surrogate marker of collateral status using HIR. First, we investigated which clinical and imaging parameters are associated with FHVs in a multicenter dataset of acute ischemic stroke patients. Secondly, we investigated whether the extent of FHVs is associated with severity of hypoperfusion (HIR) and infarct growth and whether this plays a role in terms of response to thrombolysis.

If FHVs do indeed represent collateral status, this easy-to-identify MRI sign may provide a rapid tool for the assessment of hypoperfusion, which plays a crucial role in infarction rate and hence treatment efficacy. If FHVs are identified as an accurate surrogate marker of collateral status and rate of infarction, FLAIR

sequences would not only carry temporal but also prognostic value in terms of the efficient assessment of individual stroke pathology.

Assessment of cerebrovascular risk factors (CVRF)

Patients that suffer from an ischemic event, including minor stroke or transient ischemic attack, are at risk of a second, recurrent stroke. Rapid, standard assessment scales have been established to identify patients who are at high risk of recurrent stroke in order to maximize patient care and treatment. A crucial part of risk assessment scales are determining cerebrovascular risk factors (CVRF)[15]. Well-known risk factors for ischemic stroke include arterial hypertension, atrial fibrillation (AF), smoking, diabetes mellitus, and hyperlipoproteinemia. Not surprisingly, studies have shown that the presence of the above-mentioned CVRFs not only increase the risk of a cerebrovascular event, but can also lead to a worse functional recovery post-stroke[4,16]. While one might expect that the presence of AF and arterial hypertension negatively affect the benefit of thrombolysis as previous studies have shown[16], the effect of smoking status on treatment efficacy has yielded controversial results. Somewhat counter intuitively, studies published up to two decades ago hint at a possible enhanced thrombolysis efficacy in smokers following myocardial infarction[17,18].

Regardless, most CVRF at the time of the index event can be rapidly assessed in an acute setting. Therefore, the identification of selected clinical parameters that carry prognostic value in terms of treatment efficacy could have tremendous value in selecting patients most likely to respond to treatment. We set out to determine which clinical parameters - specifically CVRF - were associated with increased recanalization and reperfusion rates following thrombolysis in a retrospective cohort analysis (1000plus[19]). We believe a more profound understanding of the impact of certain risk factors in response to thrombolysis may aid making a more knowledgeable treatment decision for high-risk patients.

Objective

A combination of easy-to-use MRI features - such as DWI lesion volume and FHVs on acute FLAIR – and knowledge of CVRF may allow for better-adapted patient selection for thrombolytic treatment. Multiparametric MRI provides extensive information on stroke severity, collateral status and salvageable tissue-at-risk of infarction. A more enhanced understanding of the clinical and pathophysiological relevance of selected risk factors and imaging criteria may optimize patient selection

for treatment thereby potentially maximizing functional outcome and minimizing treatment complications.

Here we investigate (i) whether an alternative, more rapid method for estimating DWI lesion volume can be applied to guide treatment decision (ii) whether the presence of FHVs on acute FLAIR carry diagnostic and prognostic value in terms of individual stroke pathology and collateral status, and (iii) whether selected CVRF influence treatment efficacy in patients suffering from ischemic stroke.

Methods

All investigations were retrospective cohort analyses of data from three distinct large studies described in detail in Table 1. The type of study, number of patients enrolled in the original investigation, primary hypothesis and endpoints are listed, as well as corresponding retrospective analyses for the current project with respective publications. All studies are registered under www.clinicaltrial.gov. For a detailed description of the methods applied for individual analyses and cohorts, refer to the selected publications listed in Table 1 (available in Annexes).

Title	NCT number	Type of study	No. Patients in original study	Protocol	Primary hypothesis	Primary endpoints	Retrospective analysis for current project	No. of patients included in retrospective analysis	Resulting publication
AXIS-2[7]	00927836	Multinational, multicenter, randomized, placebo-controlled	328	Clinical examination and multiparametric MRI within 10h of symptom onset	To investigate efficacy of Granulocyte colony-stimulating factor as a neuroprotective agent in treatment of acute stroke	mRS and NIHSS at day 90	DWI lesion volume estimation	238	Kufner et al. Stroke 2015 (Annex A)
PRE-FLAIR[10]	01021319	Multicenter, observational	543	Clinical examination and multiparametric MRI within 12h of symptom onset	To investigate whether a DWI-FLAIR mismatch can be used to detect patients within recommended time window for thrombolysis	Not applicable	Clinical and imaging parameters associated with FHVs	516	Cheng et al. Stroke 2012 (Annex B)
1000Plus[19]	00715533	Single-center, prospective, observational	1472	Clinical examination and multiparametric MRI on day 1, day 2, and day 5-7	To investigate whether PWI-DWI mismatch is predictive of final lesion size and functional recovery	Infarct growth (DWI)	Diagnostic and prognostic value of smoking status and FHVs	148 62	Kufner et al. Stroke 2013 (Annex C) Kufner et al. AJNR 2015 (Annex D)

MRI indicates magnetic resonance imaging; mRS, modified Rankin Score; NIHSS, National Institute of Health Stroke Scale; DWI, diffusion-weighted imaging; FLAIR, fluid-attenuated inversion recovery; FHVs, FLAIR hyperintense vessels; PWI, perfusion-weighted imaging.

Table 1: The three large studies (AXIS-2, PRE-FLAIR, and 1000Plus) used for retrospective analyses in the current project are listed in detail, along with the corresponding projects and resulting publications.

Results

DWI – diameter-based volume estimation

In the AXIS-2 trial, 328 patients were enrolled. Due to partial image incompatibility with our imaging software, only 238 patients were included for the final analysis. Measurement of the largest infarct diameter on baseline DWI was assessed for predictability of infarct volume ≥15mL. A 1-axis cut-off value of 3cm measuring the largest infarct diameter on baseline DWI predicted lesion volume ≥15mL with a 98.8% sensitivity and a 33.3% specificity (Figure 2). The use of a cut-off value of 3.5cm predicted infarct volume of ≥15mL with 96.8% sensitivity and 50.6% specificity (Kufner et al. Annex A).

The specificity of the previously described 2-axis od-value method (cut-off of 32 for lesion >70mL and cut-off of 42 for lesion >100mL[8]) for predicting infarct volume of >70mL and >100mL was significantly higher than the 3-axis ABC/2 method (93% vs. 86%; p<0.01 and 97% vs. 93%; p<0.01, respectively). However, the sensitivity of od-value calculation was lower than for the ABC/2 method for the prediction of lesion size of >70mL (88% vs. 94%, p=0.25) and >100mL (70% vs.95%, p<0.01). While use of the ABC/2 method led consistently to higher rates of overestimation compared to od-values estimation (11% vs. 5% for >70mL and 6% vs. 3% for >100mL), the methods were similar in terms of their accuracy for predicting lesions >70mL and >100mL (87% vs. 92% and 93% vs. 93%, respectively [unpublished results]).

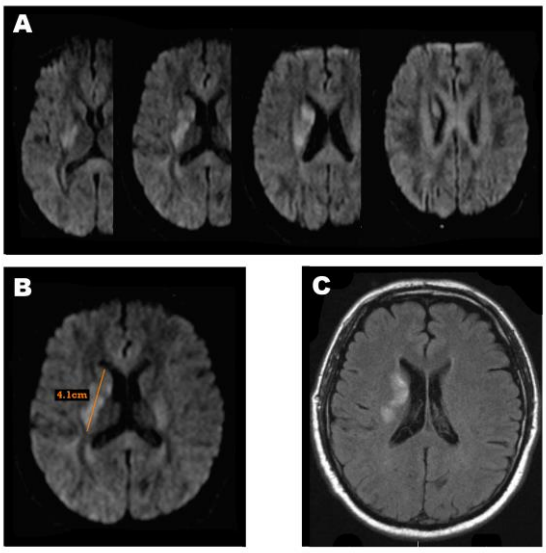

Figure 2 An example of a patient enrolled in AXIS-2 trial based on single diameter volumetric assessment. The diffusion restriction spanned three slices (A), and maximum infarct diameter measured 4.1 centimeters (B). However, manual delineation of infarct volume revealed a volume of 3.6mL. Final lesion is depicted on follow-up fluid-attenuated inversion recovery sequence (C). Figure modified from original in Kufner et al. (Annex A).

FLAIR hyperintense vessels; association with clinical and imaging parameters

Data of 516 patients included in the previously published PRE-FLAIR study were included for analysis. Presence of FHVs was identified in 47% of patients (N=240). Patients with FHVs had larger baseline lesion volumes (median, 12.3 vs. 4.9mL; p<0.001) and a more severe clinical impairment (median National Institutes of Health Stroke Scales [NIHSS] 10.5 vs. 6; p<0.001). In a sub-group analysis of those patients who received MR-angiography (N=198), 80% of patients with vessel occlusion had FHVs on acute FLAIR images. A multivariable logistic regression analysis showed that FHVs are an independent predictor of vessel occlusion (OR 22, 95% confidence interval 9.6-49.9; p<0.001), with a specificity of 86% and sensitivity of 76% (Cheng, Ebinger, Kufner et al. Annex B).

FLAIR hyperintense vessels; hemodynamic correlates and response to thrombolysis

Data of 62 patients with proven vessel occlusion enrolled in 1000Plus were included for final analysis. FHVs were present in 87% of patients (N=54). Patients with extensive FHVs (FHVs visible on >4 slices) had higher NIHSS (median 14.5, interquartile range [IQR] 11-18 vs. 5 IQR 4-13; p<0.001), larger baseline lesion volumes (median 8.1mL IQR 2.4-20.6 vs. 1.6 IQR 0.3-4.9; p<0.01), and more severe hypoperfusion (HIR median 0.61 IQR 0.44-0.73 vs. 0.28 IQR 0.07-0.47; p<0.01) compared to patients with less extensive FHVs (≤4 slices). Despite similar vessel occlusion sizes, patients with extensive FHVs had significantly larger infarct growth on follow-up DWI (median 12.8mL IQR 2.5-43.9 vs. 1.2mL IQR 0.2-12.7; p<0.01); refer to Figure 3 (Kufner et al. Annex D). Although patients with extensive FHVs showed non-significantly higher rates of recanalization following thrombolysis (74.2% vs. 53.3%; p=0.11), they showed lower rates of a favorable outcome (modified Rankin Score [mRS] <2) three months post-stroke (38.7% vs. 66.7%; p=0.02 [Kufner et al. Annex D).

In a stepwise backward multivariate regression analysis for HIR (including stroke etiology, age, perfusion deficit, baseline lesion volume, smoking, and extent of FHVs), extensive FHVs were independently associated with severe hypoperfusion (OR 6.8; 95% CI 1.1-42.7; p=0.04). High HIR was an independent predictor of a worse functional outcome three months post-stroke (OR 0.2, 95% CI 0.5-0.6; p<0.01 [Kufner et al. Annex D).

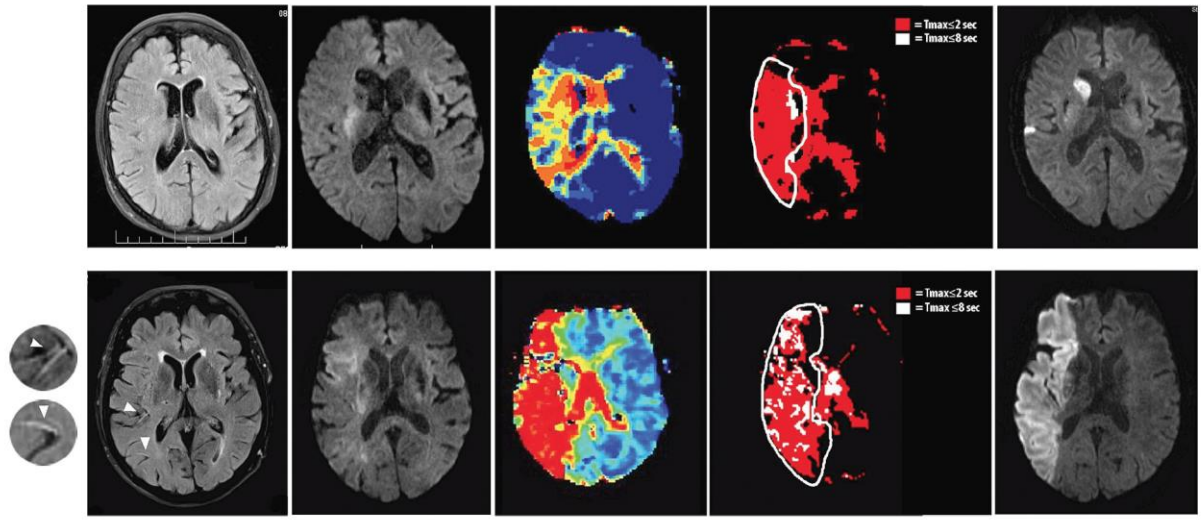

Figure 3 MR imaging (left to right: baseline FLAIR, baseline DWI, acute Tmax perfusion map, dichotomized Tmax for calculation of hypoperfusion intensity ratio, follow-up DWI showing infarct growth) of two patients (top and bottom row) with identical M1 middle cerebral artery occlusions. Patient on the top row had FLAIR hyperintense vessels on only 3 slices (FHV≤4; not visible on depicted slice) and patient on the bottom row had FHVs on 8 slices (arrows; FHV>4). Figure modified from original in Kufner et al. (Annex D).

CVRF and treatment efficacy

A total of 148 patients from the 1000Plus study were included for the final analysis. A total of 47 patients (47%) recanalized and 35 patients reperfused (36.8%); 79 patients (59%) had a favorable outcome (mRS≤2 90days post-stroke). Recanalization was associated with larger baseline perfusion deficits, higher rates of perfusion-diffusion mismatch, higher rates of arterial occlusion and smoking in univariate analysis. Reperfusion was associated with larger baseline perfusion deficits, smoking, and a favorable outcome three months post-stroke in univariate analysis. Other CVRF, such as arterial hypertension, diabetes mellitus, hyperlipoproteinemia and atrial fibrillation did not differ among groups. A favorable outcome three months after treatment was associated with younger age, lower rates of atrial fibrillation, smoking, lower NIHSS scores on admission, smaller baseline DWI lesion volumes, and higher rates of reperfusion (Kufner et al. Annex C).

In a backward stepwise regression analysis (including age, sex, hypertension, serum glucose levels, perfusion deficit and current smoking status), smoking had an odds ratio of 4 (95% CI 1.2-16; p=0.03) for reperfusion and 6 (95% CI 1.0-30; p=0.05) for recanalization (regression analysis for latter also included localization of vessel occlusion). Smokers had a better outcome compared to non-smokers three months post-stroke (77% vs. 55%; p=0.05). In a stepwise backwards regression

analysis for favorable outcome following treatment (including age, sex, atrial fibrillation, smoking, NIHSS on admission and baseline lesion volume), the following variables remained in the model: age, NIHSS on admission and DWI lesion volume (Table 2 [Kufner et al. Annex C).

Recanalization (N=68)	Odds Ratio	95% Confidence Interval	*P* Value	Univariate r^2
First step (multivariate r^2=0.33)				
Age (>70 y)	1.2	0.21–6.30	0.88	0.01
Sex (female)	0.91	0.28–3.00	0.87	0.00
Serum glucose levels (>6.7 mmol/L)	0.86	0.27–2.80	0.81	0.00
Arterial hypertension	0.64	0.07–5.00	0.64	0.02
Perfusion deficit	1.0	0.99–1.00	0.28	0.11
Smoking	5.1	0.92–28.50	0.06	0.17
Localization of arterial occlusion	2.2	1.2–4.3	0.015	0.17
Last step (multivariate r^2=0.31)				
Age (>70 y)	0.71	0.2–2.8	0.63	
Smoking	5.6	1.0–30.4	0.05	
Localization of arterial occlusion	2.3	1.2–4.2	0.009	
Reperfusion (N=93)				
First step (multivariate r^2=0.20)				
Age (>70 y)	0.98	0.26–3.60	0.97	0.03
Sex (female)	1.7	0.63–4.70	0.28	0.00
Serum glucose levels (>6.7 mmol/L)	1.5	0.55–4.00	0.43	0.00
Arterial hypertension	0.81	0.22–3.10	0.76	0.01
Perfusion deficit	1.0	0.99–1.00	0.08	0.09
Smoking	4.6	1.2–17.3	0.03	0.12
Last step (multivariate r^2=17)				
Age (>70 y)	1.3	0.42–3.80	0.68	
Smoking	4.3	1.2–16.1	0.03	
Perfusion deficit	1.0	1.00–1.01	0.06	
Favorable outcome (N=127)				
First step (multivariate r^2=0.33)				
Age (>70 y)	0.50	0.18–1.40	0.12	0.04
Sex (female)	0.61	0.26–1.50	0.27	0.05
Atrial fibrillation	0.50	0.2–1.2	0.13	0.07
Smoking	2.0	0.53–7.50	0.31	0.04
NIHSS on admission	0.90	0.83–0.97	0.007	0.22
Baseline DWI lesion volume	0.95	0.90–0.99	0.05	0.10
Last step (multivariate r^2=0.29)				
Age (>70 y)	0.33	0.13–0.85	0.02	
NIHSS on admission	0.88	0.82–0.95	0.001	
Baseline DWI lesion volume	0.96	0.91–1.00	0.06	

DWI indicates diffusion-weighted imaging; IQR, interquartile range; and NIHSS, National Institutes of Health Stroke Scale.

Table 2 Stepwise-backwards multivariable regression analyses for recanalization, reperfusion and a favorable outcome (modified Rankin Score ≥2; 90 days post-stroke); all analyses were performed with forced inclusion of age. Table modified from original in Kufner et al (Annex C).

Discussion

In the current study we were able to (i) identify rapid and efficient diameter-based volumetric methods to estimate lesion size with high specificity and sensitivity (Annex A), (ii) show that FHVs most likely represent severe ischemia due to insufficient collateralization resulting in larger infarct growth (Annex B/D), and (iii) demonstrate that smoking status plays a significant role in terms of recanalization and reperfusion rates following thrombolysis indicating enhanced tPA efficacy in patients with this risk factor (Annex C).

DWI – diameter-based volume estimation

Diameter-based methods for estimating lesion size are efficient and accurate and can be applied to estimate DWI lesion volume with - for example - a minimum infarct volume for inclusion (≥15mL), as well as a maximum infarct volume for exclusion (>70mL and >100mL). Patients in the AXIS-2 trial were enrolled based on single measurement of infarct diameter used to predict lesion size of ≥15mL[7]. We found retrospectively that use of the 3am cut-off identified patients with a volume of ≥15mL with high sensitivity. However, specificity remained low (33%) due to an overestimation of infarct volume in 23% of cases. Use of a cutoff value of 3.5cm led to an increase in specificity (51%), while sensitivity remained high (97%; Kufner et al. Annex A). In other words, use of a 3.5cm cut-off may lead to a more accurate estimation of lesion size, however this requires confirmation in alternative datasets.

On the other hand, many trials apply maximum limits to infarct volume to exclude patients from treatment due to an observed increased risk of sICH following thrombolysis in patients with large baseline infarction[6]. Two perpendicular maximum-lesion-diameter methods have recently been proposed for the rapid estimation of lesion size, namely a 2-axis od-value cut-off[8] and a 3-axis ABC/2 calculation[9]. Similar to a previous study[8], we found that the two methods are comparable in terms of their accuracy in estimating lesion sizes of both >70mL and >100mL. While the sensitivities of the two techniques are similar, the ABC/2 technique tends to overestimate lesion size resulting in a significantly lower specificity. While the tendency of the ABC/2 method to overestimate lesion size has previously been reported[9], this is the first study to confirm the accuracy of these 2-diameter methods for estimating large infarction in a multicenter database.

In conclusion, lesion diameter measurements for estimating lesion size are accurate and efficient techniques and may enable multicentric patient recruitment

with pre-specified minimum and maximum infarct volumes. In the time-sensitive setting of acute stroke, diameter-based infarct estimation is a reliable tool for harmonization of a study cohort and is recommended and preferable to manual delineation in an acute setting. However, overestimation should be considered.

Diagnostic and prognostic value of FLAIR hyperintense vessels

FHVs are frequently observed in acute stroke, however the underlying pathophysiology and the clinical implications of FHVs remain a matter of debate. Angiographic studies have shown that FHVs most likely represent slow blood flow in leptomeningeal collaterals resulting in loss of the so-called “flow void” phenomenon in vessels, resulting in the observed hyperintensies on FLAIR[11]. Despite numerous studies on this MRI feature, it remains unclear whether slow flow in FHVs represents adequate collateralization or indicates the insufficiency of collaterals to maintain ischemic tissue viable until reperfusion is achieved[11,12].

In the largest study of stroke patients investigating FHVs yet (PRE-FLAIR), we found presence of FHVs to be associated with larger lesion volumes and more severe clinical impairment. Not only was arterial occlusion an independent predictor of presence of FHVs, but FHVs can also predict vessel occlusion with high specificity and sensitivity (86% and 76%, respectively), demonstrating the substantial diagnostic accuracy of this MRI feature (Kufner et al. Annex B). The findings from this multicenter study laid the foundation for the follow-up study, in which we investigated the hemodynamic correlates of FHVs in patients with proven vessel occlusion.

In a retrospective 1000Plus analysis of 62 patients with arterial occlusion, 87% of patients had FHVs visible on at least one FLAIR slice. Due to the high prevalence of FHVs in patients with vessel occlusion, patients were grouped based on the extent of FHVs across FLAIR slices (≤ or > 4 slices). Similar to results from the previously mentioned study, we found patients with extensive FHVs (>4 slices) had more severe clinical impairment and larger baseline lesion volumes. However, in the 1000Plus study we found that extensive FHVs (>4 slices) was an independent predictor of severe hypoperfusion, resulting in larger infarct growth following treatment (Figure 3). Although extent of FHVs was not independently associated with functional recovery, severe hypoperfusion was an independent predictor of a worse outcome 90 days following treatment (Kufner et al. Annex D).

Severity of hypoperfusion was defined using a Tmax HIR introduced by Bang and coworkers in 2009[14]. They found similar results in terms of clinical impairment

and lesion size in association with HIR. Interestingly, they found that high HIR (severe hypoperfusion) was associated with poor collateral grades. This finding is in line with the hypothesis that FHVs most likely indicate severe hypoperfusion as a result of insufficient collateralization; this may explain the larger infarct growth observed in these patients (Figure 3).

We know that vessels become hyperintense on FLAIR due to slow flow, however it remains unclear which vessels are actually being depicted as FHVs. While some hypothesize that FHVs are a direct representation of sluggish blood flow in distal leptomeningeal collaterals[11], an alternative postulation is that FHVs merely represent slow flow in vessels distal to the site of occlusion. Regardless, the results of our two studies suggest that FHVs most likely reflect slow flow in distal collateral pathways distributed over a large ischemic area, resulting in extensive infarct progression.

Although HIR was an independent predictor of functional recovery, we observed no independent associations of FHVs and outcome; the heterogeneity of vessel occlusion size may account for this observation. Baseline infarct volume accounted for 21% of variance in terms of functional recovery; this is to be expected because initial infarct volume may reflect collateral status (Kufner et al. Annex D). The direct prognostic value of FHV remains uncertain based on this study alone.

Limitations of this study include its retrospective nature, the lack of digital subtraction angiography to allow for direct assessment of collateral grade, and relatively small cohort numbers. Nevertheless, this is the first study to investigate the hemodynamic correlates of FHVs based on multiparametric MRI including comprehensive perfusion data. In conclusion, this frequently observed MRI sign most likely indicates severe ischemic due to the insufficiency of established collaterals to maintain ischemic tissue viable before recanalization is achieved. Therefore, FHVs may be a useful tool to assess the severity of hypoperfusion as a result of poor collateralization. The prognostic value of this MRI sign in terms of functional recovery warrants larger cohort analyses with homogenous vessel occlusion sizes. Furthermore, it is important to note that - unlike parenchymal FLAIR hyperintensities - FHVs are not associated with time from symptom onset (Kufner et al. Annex B); therefore their temporal value remains unclear as well. Nevertheless, mere observation of the extent of FHVs across FLAIR images may allow for a more comprehensive understanding of individual stroke pathology.

Smoking status and treatment efficacy

We investigated which clinical parameters - specifically CVRF - were associated with recanalization and reperfusion rates following thrombolysis in a retrospective cohort of 1000plus patients. All CVRF were taken into account, yet only smoking status at the time of the index event revealed prognostic effects in our study, aside from age. Smokers showed a seven-fold increase in recanalization and a four-fold increase in reperfusion rates following thrombolytic treatment (Table 2). The observed reduction in relative infarct growth among smokers supports the hypothesis that tPA acts more aggressively in smokers due to increased blood fibrinogen levels caused by smoking[20]. For treatment of acute stroke, recombinant tPA is given to dissolve the blood clot causing ischemia in the brain; interestingly tPA is an endogenous protein, released rather continuously from the endothelium of blood vessels. Smoking impairs endogenous tPA release and causes circulating fibrinogen levels to rise[20]. While this causes blood to become more hypercoaguable (hence increased risk of stroke and myocardial infarction), the resulting fibrin-rich thrombus may be more susceptible to exogenously applied recombinant tPA. In other words, tPA used for treatment of acute stroke may act more specifically in smokers.

As expected, smoking was associated with younger age and less severe clinical impairment; these baseline differences between smokers and non-smokers may contribute to the improved functional recovery. Previous studies have questioned the accuracy of this so-called smoking-thrombolysis paradox and attributed the effect to the low clinical risk profiles (i.e. younger age) of these patients[17]. Although we observed similar differences in baseline parameters, we forced age into all multivariable regression analyses and still, smoking accounted for 17% of the variance for recanalization and 12% of variance for reperfusion. Although smoking was not an independent predictor of outcome in this study, a favorable outcome was positively associated with reperfusion, suggesting that increased tPA efficacy in smokers may partially contribute to the observed smoking-paradox phenomenon. After all, it is well known that reperfusion leads to an improved outcome following stroke[5]. Of note, smokers presented with slightly higher rates of arterial occlusion and with larger baseline perfusion deficits. In other words, smokers may be more likely to benefit from reperfusion therapy in the first place.

Although the principle mechanism underlying the smoking thrombolysis paradox remains a matter of debate, there is most likely an additive effect of various factors leading to this observation - namely, younger age, lower risk profiles and

more aggressive thrombolytic effect (Kufner et al. Annex D). Nevertheless, assessment of smoking status at the time of the index event takes essentially no time and provides us with substantial prognostic information and may aid in making a more targeted treatment decision, especially in high-risk patients.

Summary and conclusions

We have confirmed that diameter-based estimation of lesion volume is an accurate and reliable tool for the rapid assessment of infarct size for including and/or excluding patients from treatment, especially in the setting of harmonized multicenter stroke trials. Furthermore, we have demonstrated that easy-to-identify FHVs on acute FLAIR images not only predict vessel occlusion with high diagnostic accuracy, but also represent severe hypoperfusion and predict larger infarct growth. Finally, we were able to show an enhanced tPA efficacy in terms of recanalization and reperfusion rates in smokers following thrombolytic treatment.

In summary, we have demonstrated that well-defined imaging criteria and knowledge of selected risk factors carry substantial prognostic and diagnostic value and may aid in the identification of patients most likely to benefit from thrombolysis. Based on these studies alone, we by no means recommend withholding treatment based merely on the presence/absence on the diagnostic parameters discussed above. However, these parameters may help sway a decision to treat in certain patient groups, i.e. patients with relative contraindications to tPA. The increase in thrombolysis rates in Germany in recent years is in part due to an increase in off-label use of tPA[2] (i.e. patients >80 years of age). If a number of clinical/imaging parameters suggest a high probability of good treatment response, the treating physician may be inclined to choose thrombolysis despite the presence of relative contraindications, such as advanced age or severe clinical symptoms.

In conclusion, multiparametric MRI and clinical examination provide us with extensive information about the stroke at hand and individual risk; an enhanced understanding of the pathophysiology underlying frequently observed MRI features will not only allow us to assess individual stroke pathology in an acute setting, but take us one step closer to making more personalized treatment decisions. Although without a doubt ‘time is brain’, tools that help us understand the pathological state of each stroke at hand can only aid in making superior treatment decisions.

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Affidavit

I, Anna Kufner certify under penalty of perjury by my own signature that I have submitted the thesis on the topic PREDICT STROKE: predicting response to thrombolysis in acute ischemic stroke patients using multiparametric MRI I wrote this thesis independently and without assistance from third parties, I used no other aids than the listed sources and resources.

All points based literally or in spirit on publications or presentations of other authors are, as such, in proper citations (see "uniform requirements for manuscripts (URM)" the ICMJE www.icmje.org) indicated. The sections on methodology (in particular practical work, laboratory requirements, statistical processing) and results (in particular images, graphics and tables) correspond to the URM (s.o) and are answered by me. My contributions in the selected publications for this dissertation correspond to those that are specified in the following joint declaration with the responsible person and supervisor. All publications resulting from this thesis and which I am author of correspond to the URM (see above) and I am solely responsible.

The importance of this affidavit and the criminal consequences of a false affidavit (section 156,161 of the Criminal Code) are known to me and I understand the rights and responsibilities stated therein.

Date Signature

Declaration of any eventual publications

Anna Kufner had the following share in the following publications:

Annex A: Kufner A, Wouters A, Bracoud L, Laage R, Schneider A, Schäbitz WR, Hermier M, Thijs V, Fiebach JB. Infarct volume-based subgroup selection in acute ischemic stroke trials. Stroke. 2015;56.

Contribution in detail: Retrieval of data from trial team, MRI-post processing of diffusion-weighted imaging using MRICron®, data analysis including statistics, drafted and completed manuscript.

Annex B: Cheng B, Ebinger M, Kufner A, Köhrmann M, Wu O, Kang DW, Liebeskind D, Tourdias T, Singer OC, Christensen S, Warach S, Luby M, Fiebach JB, Fiehler J, Gerloff C, Thomalla G; Stroke Imaging Repository (STIR) Investigators. Hyperintense Vessels on Acute Stroke Fluid-Attenuated Inversion Recovery Imaging: Associations With Clinical and Other MRI Findings. Stroke. 2012;43:2957-2961.

Contribution in detail: Conceptualization of project, gathering of data from PRE-FLAIR databank, data analysis including, revision of manuscript.

Annex C: Kufner A, Nolte CH, Galinovic I, Brunecker P, Kufner GM, Endres M, Fiebach JB, Ebinger M. Smoking thrombolysis paradox: recanalization and reperfusion rates after IV-tPA in smokers with ischemic stroke. Stroke. 2013;44:407-413.

Contribution in detail: Conceptualization of project and hypotheses, gathering of data from 1000Plus databank, MRI-post processing of perfusion-weighted images using Stroketool®, MRI-post processing of diffusion-weighted imaging using MRICron®, creating statistical algorithem for data analysis, data analysis including statistics, drafted and completed manuscript.

Annex D: Kufner A, Galinovic I, Ambrosi A, Nolte CH, Endres M, Fiebach JB, Ebinger M. Hyperintense vessels on FLAIR – hemodynamic correlates and response to thrombolysis. Am J Neuroradiol. 2015.

Contribution in detail: Conceptualization of project, gathering of data, MRI-post processing of perfusion-weighted images using Stroketool®, MRI-post processing of diffusion-weighted imaging using MRICron®, rating of FLAIR images for FLAIR hyperintense vessels, data analysis including statistics, drafted and completed manuscript.

Signature, date and stamp of the supervising University teacher

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Signature of the doctoral candidate

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ANNEX A

Kufner A, Wouters A, Bracoud L, Laage R, Schneider A, Schäbitz WR, Hermier M, Thijs V, Fiebach JB. Infarct volume-based subgroup selection in acute ischemic stroke trials. *Stroke.* 2015;56.

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ANNEX B

Cheng B, Ebinger M, **Kufner A**, Köhrmann M, Wu O, Kang DW, Liebeskind D, TourdiasT, Singer OC, Christensen S, Warach S, Luby M, Fiebach JB, Fiehler J, Gerloff C, Thomalla G; Stroke Imaging Repository (STIR) Investigators. Hyperintense Vessels on Acute Stroke Fluid-Attenuated Inversion Recovery Imaging: Associations With Clinical and Other MRI Findings. Stroke. 2012;43:2957-2961.

DOI: 10.1161/STROKEAHA.112.658906

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ANNEX C

Kufner A, Nolte CH, Galinovic I, Brunecker P, Kufner GM, Endres M, Fiebach JB, Ebinger M. Smoking thrombolysis paradox: recanalization and reperfusion rates after IV-tPA in smokers with ischemic stroke. Stroke. 2013;44:407-413.

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ANNEX D

Kufner A, Galinovic I, Ambrosi A, Nolte CH, Endres M, Fiebach JB, Ebinger M. Hyperintense vessels on FLAIR – hemodynamic correlates and response to thrombolysis. *Am J Neuroradiol*. 2015.

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Publication List

1. [1]**Kufner A,** Galinovic I, Ambrosi A, Nolte CH, Endres M, Fiebach JB, Ebinger M. Hyperintense vessels on FLAIR – hemodynamic correlates and response to thrombolysis. *Am J Neuroradiol*. 2015. **3.59 Impact factor**

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5. [3]**Kufner** A, Nolte CH, Galinovic I, Brunecker P, Kufner GM, Endres M, Fiebach JB, Ebinger M. Smoking thrombolysis paradox: recanalization and reperfusion rates after IV-tPA in smokers with ischemic stroke. Stroke. 2013;44:407-413. **5.72 Impact factor**

6. [4]Cheng B, Ebinger M, **Kufner A**, Köhrmann M, Wu O, Kang DW, Liebeskind D, TourdiasT, Singer OC, Christensen S, Warach S, Luby M, Fiebach JB, Fiehler J, Gerloff C, Thomalla G; Stroke Imaging Repository (STIR) Investigators. Hyperintense Vessels on Acute Stroke Fluid-Attenuated Inversion Recovery Imaging: Associations With Clinical and Other MRI Findings. Stroke. 2012;43:2957-2961. **5.72 Impact factor**

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[1] Selected publication for PhD thesis: Annex D
[2] Selected publication for PhD thesis: Annex A
[3] Selected publication for PhD thesis: Annex C
[4] Selected publication for PhD thesis: Annex B

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