

Aus dem Institut/der Klinik für Neurologie
der Medizinischen Fakultät der Charité Universitätsmedizin Berlin

DISSERTATION

**Blood-brain barrier disruptions in the acute phase
of ischemic stroke in human patients**

zur Erlangung des akademischen Grades
Doctor of Philosophy (PhD)

vorgelegt der Medizinischen Fakultät
Charité Universitätsmedizin Berlin

von

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Datum der Promotion: 04.09.2015

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Abstract

Introduction: In acute ischemic stroke, magnetic resonance imaging (MRI) is a valuable tool for diagnosis, treatment decisions, and assessment of outcome. Baseline and early follow-up MRI examinations can help us predict and understand tissue fate. Until now, blood-brain barrier (BBB) permeability changes after stroke have been poorly studied in humans. However, they might be a risk factor for worse outcome and hemorrhagic transformation, especially after thrombolytic therapy.

Methods: Acute stroke patients received an MRI examination with contrast agent based perfusion imaging within 24 h after stroke onset, as well as on day 2. In a subgroup of patients, repetitive MRI examinations every two hours were performed on the first day. Volumes of hypoperfusion were assessed automatically with three different software packages and compared to a rater-based approach. Development of lesion size and vessel status was assessed in thrombolysed patients who received follow-up MRI examinations after therapy. On fluid-attenuated inversion recovery (FLAIR) images, the natural signal-intensity-time course of the lesion within the first 24 h of stroke onset was determined relative to the contralateral side. Furthermore, the hyperintense acute reperfusion marker (HARM) was evaluated as a sign for BBB disturbances.

Results: We found that vessel recanalization up until 24 h after treatment is still associated with a good clinical outcome and only moderate lesion growth. We also found that volumes of hypoperfused tissue were substantially overestimated when using an automated approach. We were able to show, that the natural time course of relative signal intensities on FLAIR images is significantly influenced by the presence of HARM, since HARM seems to go along with markedly increased contrast agent enhancement in the brain parenchyma. In a proportion of stroke patients, HARM was seen as early as a few hours after stroke onset.

Conclusions: MRI is a suitable and highly sensitive tool to monitor tissue fate in the acute phase of cerebral ischemia. We showed that HARM as a marker for blood-brain barrier permeability changes is a frequent and early occurring phenomenon. It might be associated with worse clinical outcome and is therefore worth monitoring.

Abstract

Einleitung: Im Fall eines akuten ischämischen Schlaganfalls ist Magnetresonanztomographie (MRT) ein wertvolles diagnostisches Instrument und gut geeignet, um Therapieentscheidungen zu vereinfachen und die Entwicklung der Schlaganfallläsion zu überwachen. MRT Untersuchungen in der akuten Phase und als Verlaufskontrollen können dabei helfen, das Schicksal des betroffenen Gewebes vorherzusehen und zu verstehen. Darüber hinaus kann man mit MRT Permeabilitätsstörungen der Blut-Hirn-Schranke untersuchen. Diese könnten einen Risikofaktor für schlechteres Outcome und hämorrhagischen Transformationen darstellen.

Methodik: Akute Schlaganfallpatienten wurden im MRT mit Kontrastmittel-unterstützter Perfusionsbildgebung bis zu 24 h nach dem Einsetzen der Schlaganfallsymptomatik untersucht. Eine Subgruppe der Patienten wurde repetitiv alle zwei Stunden am ersten Tag gemessen; alle Patienten erhielten jedoch mindestens eine Verlaufuntersuchung an Tag 2. Die Größe des minderperfundierten Gewebes wurde in einem automatisierten Ansatz mit drei verschiedenen Softwarepaketen bestimmt und mit einem manuellen Ansatz verglichen. Die Entwicklung von Läsionsgröße und Gefäßstatus wurde für thrombolysierte Patienten bestimmt, die eine frühe Verlaufskontrolle nach der Therapie bekommen hatten. Die natürliche Entwicklung der Signalintensitäten in den ersten 24 h nach dem Symptombeginn wurde auf fluid-attenuated inversion recovery (FLAIR) Bildern relativ zur Gegenseite bestimmt. Darüber hinaus wurde der "hyperintense acute reperfusion marker" (HARM) als Zeichen für Störungen der Blut-Hirn-Schranken beurteilt.

Ergebnisse: Wir konnten zeigen, dass eine Rekanalisation des betroffenen Gefäßes bis zu 24 h nach Therapie mit einem guten Outcome und geringen Läsionswachstum assoziiert ist. Die Volumina des minderperfundierten Gewebes wurden mit den automatisierten Ansätzen grob überschätzt. In unserer Studie war die natürliche Entwicklung von FLAIR Signalintensitäten signifikant durch das Auftreten von HARM beeinflusst, da HARM offenbar zusätzlich mit einem Kontrastmittelübertritt in das Gewebe einher geht. Bei einigen Patienten konnte HARM schon wenige Stunden nach dem Beginn der Schlaganfallsymptomatik nachgewiesen werden.

Schlussfolgerung: MRT ist eine gut geeignete und hochempfindliche Untersuchungsmethode, um Gewebeschicksale bei akuter zerebraler Ischämie zu untersuchen. HARM als Indikator für Permeabilitätsstörungen der Blut-Hirn-Schranke ist ein häufiges und früh auftretendes Phänomen. Die klinische Relevanz von HARM ist nicht genau geklärt, da aber eine Assoziation mit schlechterem Outcome angenommen werden kann, sind genaue Untersuchungen angebracht.

Introduction

Acute ischemic stroke and blood-brain barrier disruptions

Acute ischemic stroke (AIS) is clinically defined as a focal neurological deficit caused by a lack of blood supply to a part of the brain. A thrombus or embolus blocking an intracranial artery, as well as severe atherosclerosis are the underlying causes [1]. AIS is a major cause of disability and death worldwide, and will become of greater importance in the next decades, considering the demographic development towards an ageing society. Thrombolysis with tissue plasminogen activator (tPA) is the only valid and proven therapeutic option. However, it can only be administered if certain criteria are met. One criterion is the time from symptom onset, which should not exceed 4.5 h [2].

However, even if patients are carefully selected for thrombolytic treatment, the outcome can never be estimated precisely. A lot of different pathological processes take place on a cellular level in the first hours after an acute ischemic stroke, such as edema formation, excitotoxicity, apoptosis and inflammation. It has been proposed that changes in the permeability of the blood-brain barrier (BBB) might influence the disease progression additionally and can be disadvantageous when tPA is applied. The BBB is composed of the endothelial cells of the cerebral vessels, the surrounding astrocytic end-feet, and pericytes. In contrast to other organs the endothelial cells in the brain are connected via tight junctions, that prevent blood-borne substances from passing the BBB and entering the brain [3]. A complex system of transporters and channels is needed to ensure the specific uptake of nutrients into the brain. Permeability changes of the BBB can occur in several neurological diseases, including stroke [3]. This could be problematic, as an open BBB might pose a risk for bleedings in patients that received thrombolytic therapy. Apart from that, even in non-treated patients, potentially dangerous substances could enter the brain and influence stroke progression. In animal models of stroke, BBB changes have been shown to occur in a significant proportion of animals. Often, a biphasic pattern of BBB openings after recanalization is reported [4].

Magnetic resonance imaging in stroke

In the clinical setting, brain imaging is crucial for diagnosis and treatment decisions. Computed tomography (CT) has been the standard for several decades, however, magnetic resonance imaging (MRI) has recently been proven a more sensitive method in the setting of acute cerebral ischaemia. MRI is increasingly used to predict and monitor tissue fate, with a wide variety of sequences. On diffusion-weighted images (DWI), a stroke lesion can be seen within minutes after the stroke [5]. Blood-sensitive T2*-weighted sequences allow us to exclude hemorrhagic strokes, and the status of the blood vessels can be visualized with contrast or even non-contrast time-of-flight-MR angio-graphy (TOF-MRA). Perfusion imaging (PI) has found its place in estimating

the hypoperfused but still salvageable tissue. Here the mismatch concept states that DWI shows irreversibly damaged tissue while perfusion imaging visualizes the inadequately perfused tissue with the mismatch area between the two representing tissue that can still be saved if treatment is rapidly applied. Therefore, PI is an additional help for treatment decisions in stroke [6]. Especially an automated calculation of perfusion maps and analysis of perfusion deficit volumes would be beneficial in everyday clinical work. As a first project during this PhD, we therefore compared three different perfusion software packages and their performance in an automated approach to determine volumes of hypoperfused tissue.

In addition to being a highly sensitive and multifunctional tool for acute diagnostics, imaging has also been proven suitable to assess recanalization, reperfusion, infarct growth and secondary hemorrhages as outcome parameters after thrombolysis. In the "Acute stroke imaging research roadmap" [7] it was recommended to perform follow-up MRI examinations 4 h and 24 h after thrombolytic therapy to assess these outcome parameters. However, it has been shown that recanalization can be delayed in a proportion of patients. As long as recanalization happens within 24 h, this does not necessarily signify worse clinical outcome [8]. Therefore, in order to analyze whether early follow-up provides important additional information in terms of infarct development, we compared different imaging parameters 4 h and 24 h after thrombolysis in a cohort of acute stroke patients. We determined the vessel status at every time point and compared the imaging and clinical outcome parameters of patients with different time points of recanalization. The results are presented in the second section of the results part.

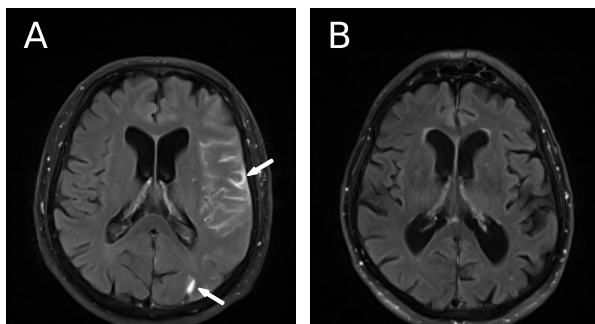


Figure 1: Example of a FLAIR image with visible hyperintensities in the sulci (**A**), which would be considered HARM positive, and a FLAIR image without HARM (**B**). (Adapted from [9]).

In order to get a more precise idea about tissue fate in AIS, we also have to monitor BBB permeability changes. The BBB permeability can be studied with different contrast-enhanced imaging techniques. Common MRI contrast agents, like Gadolinium, are used in chelate molecules with a high molecular weight, which are unlikely to pass the intact BBB. Therefore, if leakage of Gadolinium into cerebrospinal fluid (CSF) space or brain parenchyma can be visualized with MRI sequences, this is considered a sign of BBB disturbances. The fluid-attenuated inversion recovery (FLAIR) sequence is for this purpose of special importance, since on FLAIR images the CSF signal is actively suppressed and thus appears hypointense. As little as a few Gadolinium molecules in the CSF are able to omit this suppression and lead to a hyperintense signal in the sulci and

ventricles (see Figure 1). This phenomenon was termed hyperintense acute reperfusion marker (HARM) by Warach and Latour [10] and was proven to be caused by Gadolinium molecules in the CSF by Köhrmann in 2012 [11]. In previous studies, HARM was associated with older age, as well as reperfusion. It was shown to be a risk factor for hemorrhagic transformation by some groups [10, 12], while others could not find such an association [13].

Goals

During this project we wanted to show that MRI can predict, monitor and potentially explain tissue fate in acute ischemic stroke patients. We applied an automated approach to determine volumes of hypoperfused tissue in the first part of the PhD. To assess infarct development on DWI, we examined stroke patients 4 h and 24 h after thrombolytic treatment, and additionally determined their vessel status. Furthermore, we wanted to characterize BBB changes in AIS patients in more detail, focussing on two main points:

- the time-course of BBB leakage in AIS patients; how early it can occur and whether it shows a similar temporal pattern as in animal models
- the association of HARM with signal intensities and enhancement in the parenchyma on FLAIR images.

To address these points we conducted a prospective longitudinal study, in which AIS patients were repeatedly examined with MRI within the first two days after stroke onset. Patient data on frequently repetitively examined patients are scarce and hard to collect. In our facility we had the fortunate environmental prerequisites necessary to conduct such a project, namely a dedicated research MRI scanner located in close proximity to the stroke unit of the Benjamin Franklin Hospital. The results presented in the third results section stem from analyses of this patient cohort.

Methods

Patients and image acquisition

Data for this PhD project were acquired within the prospective, single-center observational studies "1000Plus" ([14], ClinicalTrials.gov identifier: NCT00715533) and "LOBI" (ClinicalTrials.gov identifier: NCT02077582), conducted at the Benjamin Franklin Hospital of the Charité Universitätsmedizin Berlin. We screened all patients that were admitted to the hospital with clinical signs of an AIS and received an MRI examination at our facility within 24 h from symptom onset. All patients for whom we could confirm an acute ischemic (and not hemorrhagic) stroke and who were able to give written informed consent were asked to participate in the study. Patients that were examined within 4.5 h from symptom onset and clinically stable, were additionally included in the substudy "FLAIR B". For this substudy, we scanned the patients repetitively every two hours on the first day. All patients, irrespectively of the substudy, received an MRI examination on day 2 and day 5-7 after stroke. MRI examinations were performed on a 3 Tesla Siemens Tim Trio MRI Scanner. The acute stroke MRI protocol included DWI (TE=93 ms, TR=8,000 ms, 2.5 mm slice thickness without interslice gap); FLAIR (TE=100 ms, TR=8,000 ms, 5.0 mm slice thickness); T2*-weighted imaging (TE=20 ms, TR=620 ms, 5.0 mm slices thickness); TOF-MRA and perfusion imaging (PI, TE = 29 ms; TR = 1,390 ms ; 5 mm slice thickness; interslice gap =0.5 mm). For PI a fixed dosage of 5 ml Gadovist (Gadobutrol, 1 M, Bayer Schering Pharma AG, Berlin, Germany) followed by a 20 ml intravenous saline flush was used. Clinical severity of symptoms was measured with the National Institutes of Health Stroke Scale (NIHSS) score at admission and discharge. Long-term clinical outcome was measured via telephone interview with the modified Rankin Scale (mRS) after 3 months.

Image analysis

Vessel occlusion and recanalization were determined on TOF-MRA, hemorrhagic transformations (HT) and parenchymal hemorrhages (PH) were inspected on T2* and FLAIR images. For the analysis of automated perfusion software we compared three different software packages: Stroketool (Digital Image Solutions, Germany), PMA (v3.2.0.4 ASIST, Japan) and Perfscape/Neuroscape (Olea Medical SAS, France). With each software mean transit time (MTT), cerebral blood flow (CBF) and Tmax maps were calculated and three different thresholds were applied. For the automated approach the resulting regions of interest (ROIs) were saved after the thresholds were applied. Perfscape/Neuroscape provided a built-in filtering tool to remove scalp and CSF-filled space. For maps produced in PMA and Stroketool we additionally applied a brain mask created using SPM8 (Wellcome Trust Centre for Neuroimaging, UK) for the purposes of eliminating CSF. To compare the automated performance of the software packages, we also manually delineated perfusion deficits on the already thresholded maps and compared volumes

determined with both methods [15].

DWI lesion volumes for day 1 and 2, as well as FLAIR lesion volumes for day 5-7 were determined using either MRlcro (version 1.4, ©1999-2005, Chris Rorden) or AnToNIa (Analysis Tool for Neuro Image Data, Institute for Computational Neuroscience, Hamburg, Germany). To determine FLAIR signal intensities relative to the contralateral side, the software AnToNIa was used to roughly encircle the DWI lesions. These ROIs were refined by an automated algorithm reducing the volumes to those voxels that were two standard deviations more hyperintense compared to the contralateral hemisphere. The refined DWI-ROIs were mirrored to the contralateral side and voxels containing CSF were generously deleted by using apparent diffusion coefficient (ADC) images [9]. The final ROIs were superimposed on the FLAIR image. FLAIR relative signal intensities (rSI) were calculated as

$$rSI = \frac{\text{meanSI}_{\text{ipsilateral}}}{\text{meanSI}_{\text{contralateral}}} \quad (1)$$

For the last project we also examined different aspects of the BBB in AIS. Therefore, we used HARM on post-contrast FLAIR images as a marker for BBB permeability changes. HARM was evaluated on the FLAIR images acquired subsequent to the baseline examination and compared to the pre-contrast baseline image. We used a simple rating system, as illustrated in Table 1.

Rating	Description
HARM 0	no hyperintensity in the CSF-filled space on FLAIR in comparison to baseline image
HARM 1	hyperintensity in CSF unclear, visible on less than 3 slices compared to baseline image, artifacts and cortical hyperintensity may confound the visibility of HARM
HARM 2	hyperintensity in CSF subtle, visible on 3 to 5 slices compared to the baseline image
HARM 3	hyperintensity in CSF clear, visible on more than 5 slices compared to baseline image

Table 1: The HARM rating scheme as used in the last project

For statistical reasons patients were afterwards dichotomized into patients rated HARM 0-1 (HARM negative) and HARM 2-3 (HARM positive) [9].

Statistics

Statistical analysis was performed using IBM SPSS Statistics (version 19). Metric data was tested for significant differences in distribution between groups with the non-parametric Mann-Whitney U test. For ordinal and categorical data, the Chi-square or Fisher's exact test was used. Differences in variance between two groups was calculated with the Levene's test. For correlations between two variables, the non-parametric Spearman's rho was used.

Results

Automated interpretation of perfusion MRI

In this first project we wanted to analyze the automated interpretation of perfusion maps with three different software packages. Using Stroketool, PMA and Perfscape/Neuroscape, perfusion maps of CBF, MTT and Tmax were calculated for 145 patients imaged within 24 h of stroke symptom onset. For each parameter map three thresholds were applied. The median final lesion volume in this cohort was 6.55 ml (interquartile range (IQR) 0.8-31.6 ml) [15]. With the automated approach the volumes of hypoperfusion were overestimated, regardless of the software used. Compared to the manual approach, volumes derived automatically were in median up to 210 ml larger in Perfscape/Neuroscape, 123 ml larger in PMA and 135 ml larger in Stroketool [15]. When we applied filtering of CSF to the automated volumes calculated in PMA and Stroketool, the median difference between the automatically and manually derived volumes were reduced to a maximum of 64.91 ml and 67.28 ml, respectively [15]. Volumes of perfusion deficit had a higher correlation with radiological and clinical outcome when determined manually in comparison with the automatically determined volumes, with Tmax being the best predictor in all three programs [15].

MRI follow-up as surrogate parameter for treatment success

The purpose of this second project was to obtain an overview about lesion development in the first hours after a stroke and subsequent thrombolytic therapy. We particularly wanted to take into consideration the vessel status of the patients. For this study we included 40 ischemic stroke patients (13 females) with a median age of 71 years and a median NIHSS score of 8. The patients had a MRI examination before treatment, a first follow-up MRI examination after 1.8-6.5 h (median 4.4 h) and a second follow-up after 23.5 h. Perfusion imaging was available for post-processing for 103 examinations, and FLAIR on day 5 for 32 patients [16]. At baseline, the median DWI lesion volume was 2.8 ml (IQR 0.6-11.4), at the first follow-up it was 4.8 ml (IQR 1.5-14.9) and at the second follow-up it had increased to 5.3 ml (IQR 3.0-27.5). The median perfusion deficit at baseline was 48.2 ml (IQR 11.1-116.8), 16.1 ml (IQR 0.7-91.9) at follow-up 1 and 1.5 ml (IQR 0.0-23.6) at follow-up 2. We were able to detect hemorrhagic transformation at follow-up 1 in two patients and at follow-up 2 in 14 patients [16].

Eleven patients showed no vessel occlusion at the baseline examination. From the patients with initial vessel occlusion 19 had patent vessels already at 1-6 h (early recanalizers), and 6 patients had patent vessels at 24 h (late recanalizers). Only four patients did not show signs of recanalization at 24 h (non-recanalizers). Age, gender and time to treatment did not differ between the recanalization subgroups. Non-recanalizers had significantly larger perfusion deficits at baseline than recanalizers ($p = 0.011$) and patients with no initial occlusion had significantly

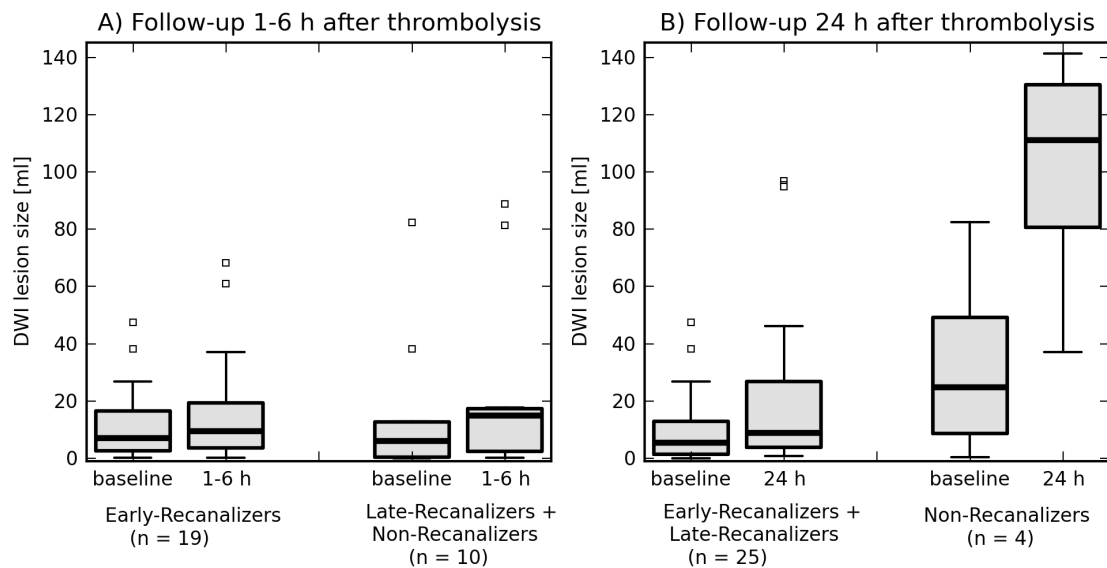


Figure 2: Radiological outcome at the two follow-up time points. **A)** Outcome 1-6 h after therapy. Neither patients with nor without recanalization show considerable change in DWI deficit until that time point. **B)** Outcome 24 h after therapy. Only non-recanalizers showed a substantial increase in DWI lesion size. (Adapted from [16].)

smaller DWI and hypoperfusion volumes than patients with occlusion. Median lesion growth from baseline to 1-6 h did not differ between the groups ($p = 0.167$). Median lesion growth from baseline to 24 h was however significantly larger in the group of non-recanalizers compared to the other groups (71.4 vs. 2.8 ml, $p < 0.05$) [16]. An illustration of the differences in lesion size between recanalizers and non-recanalizers at the two follow-up time points can be seen in Figure 2. Perfusion deficit volumes at 24 h as well as final lesion size on FLAIR did not differ between patients with early and late recanalization. However, both these volumes were significantly increased in non-recanalizers. A good outcome, measured by a modified Rankin Scale score of 0-2 at 3 months was reached by 66% of the recanalizers. None of the non-recanalizers were functionally independent at 3 months ($p = 0.02$) [16].

Occurrence of HARM and time course of FLAIR signal intensity

For the third project we examined the time course of BBB changes in the first two days after a stroke and the influence of HARM on FLAIR rSI. We analyzed imaging data from those patients who received at least 3 to 5 MRI examinations on the first day and 1 to 2 examinations on the second day following a stroke. Eighteen patients (5 females, 27.8%) with a median age of 69 years (IQR 62-74) and a median NIHSS score at admission of 5 (IQR 4-8) were included. Eight of the patients (44.4%) showed HARM on at least one examination [9]. In the following, this subgroup of patients will be called HARM positive.

From all HARM positive patients, six showed HARM on the second examination, at the earliest 3.5 h after symptom onset. A single patient showed HARM on only one examination

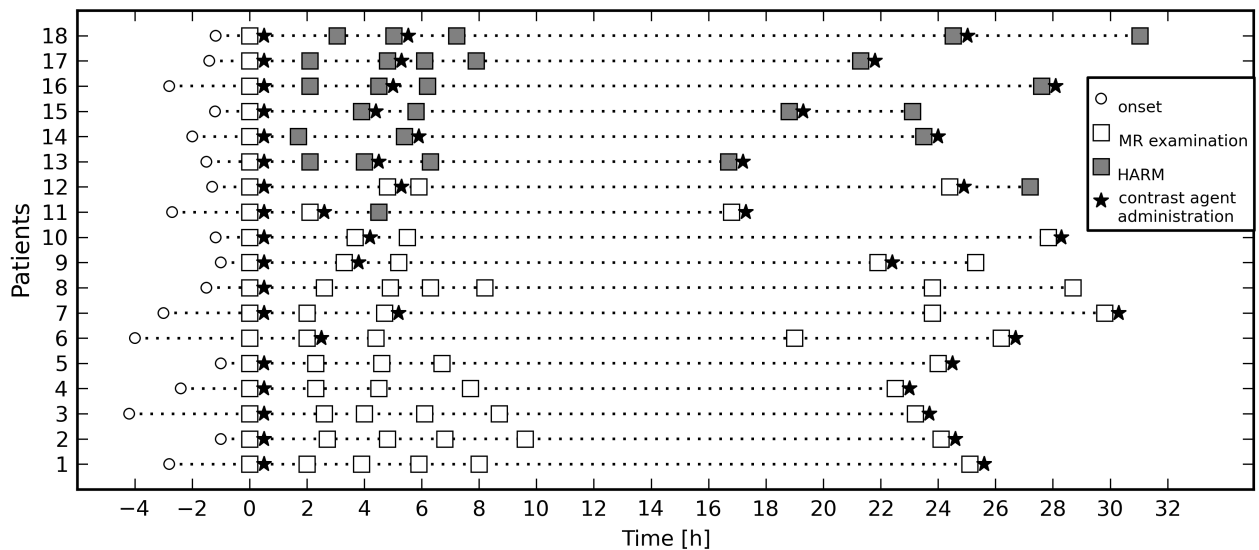


Figure 3: Detailed diagram of MRI examinations, contrast agent administration and occurrence of HARM in all 18 patients. (Adapted from [9].)

after 7.2 h and another patient showed HARM on one examination 28.5 h after symptom onset (Figure 3) [9].

All examinations acquired before the second administration of contrast agent were subject to more detailed analyses. FLAIR rSI in the DWI positive tissue was determined with the AnToNIa software with careful exclusion of CSF-filled space from the ROIs. We showed that HARM positive patients had considerably higher rSI values for infarcted tissue on FLAIR images compared to HARM negative patients from the second examination on (Figure 4) [9].

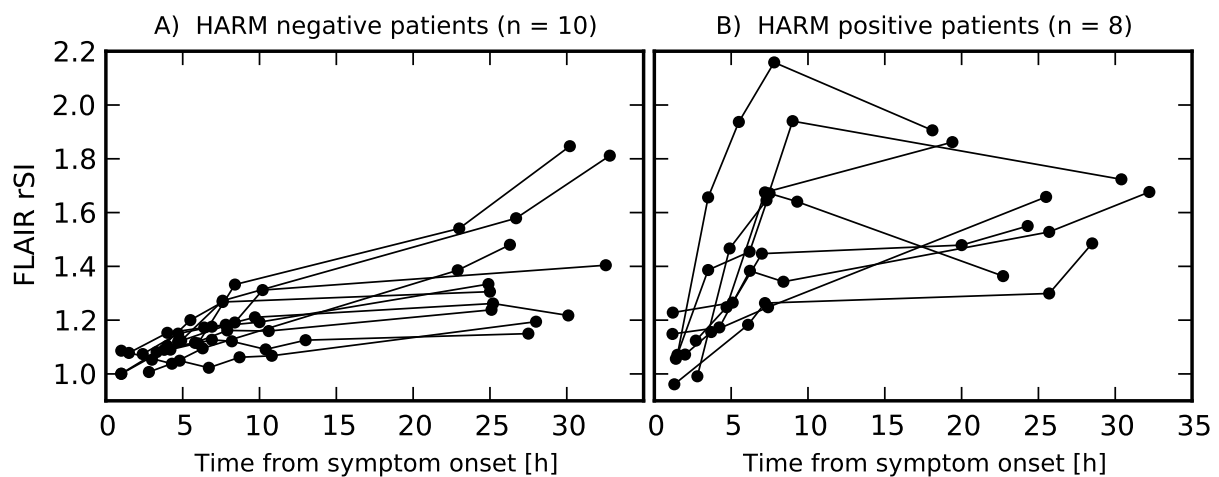


Figure 4: Development of FLAIR relative signal intensities (rSI) over time. **A)** Natural development in patients without HARM and **B)** in patients who showed HARM on at least one examination. All FLAIR rSI values were determined relative to the contralateral side. (Adapted from [9].)

The FLAIR rSI values in the stroke ROI were significantly higher in HARM positive patients

for the second and the third examination (median 4.31 h and 6.37 h from symptom onset, $p < 0.001$ and $p = 0.005$, respectively). The rSI determined on B0 and ADC images for the same ROIs did not differ significantly between groups. These sequences served as controls, since they are not sensitive to MR contrast agents [9].

Discussion

With this PhD project, we examined three different aspects of magnetic resonance imaging which are crucial for its usage in acute ischemic stroke:

- perfusion imaging as a diagnostic aid in the hyperacute phase,
- imaging parameters as surrogates for treatment success and
- HARM as a marker for blood-brain barrier disruptions and worse outcome after stroke.

We showed that MRI can be an important and valuable diagnostic and monitoring tool in all three of these phases.

For the acute phase, perfusion imaging is essential if patient selection is based on the DWI-PI mismatch concept. We showed that there is a lot of variation in median volume of the perfusion deficit when assessed with different software and different perfusion maps. Partly, this might be explainable by different calculation algorithms and preprocessing steps implemented in the software packages [15].

CSF filtering (either as implemented by the software or done with SPM) did not help against all of the typical artifacts seen in perfusion maps. This explains the larger volumes obtained with the automated delineations as compared to the manual ones. Considering the fact that even a lesion of a few milliliters can have substantial impact on patients outcome, we could not recommend using the software packages in an automated way in the acute clinical setting without additional postprocessing [15].

To assess outcome parameters after thrombolytic treatment, MRI follow-up examinations have been considered very useful [7]. We showed that for the evaluation of the response to rtPA in terms of lesion growth, recanalization and hemorrhagic transformation, a MRI examination 24 h after treatment is the most informative. The subgroup of patients that recanalized within this time span experienced a similar outcome as patients who recanalized within 6 h. By contrast, patients with a persistent vessel occlusion at 24 h after treatment showed considerably larger FLAIR volumes at day 5 and a poor outcome measured with mRS [16]. However, differences in baseline lesion volume represent an important factor for outcome as well. This might have contributed to smaller final lesion volumes even in late recanalizers as compared to the early recanalizers in this study. With the results of this study we do not suggest that the time point of treatment is unimportant. Clearly, treatment should always be applied as early as possible after stroke symptom onset. We only state that an early follow-up MRI examinations in a phase II clinical trial might not yield valuable additional information about treatment success. We would therefore like to suggest to put more effort into obtaining a 24 h follow-up examination

of good quality to identify all patients with persistent occlusion and potentially poor outcome [16].

In the final project, we were able to illuminate some imaging aspects of the pathological opening of the blood-brain barrier after acute ischemic stroke.

We showed that HARM is strongly associated with contrast agent enhancement in the brain parenchyma on FLAIR images. This implies that contrast agent does not only leak into the CSF filled space, but also into the tissue, and speaks for a more general concept of BBB permeability changes. This observed tissue enhancement severely influences the natural evolution of FLAIR lesion hyperintensities (Figure 3). Recently, certain imaging biomarkers have been proposed as a marker for the acuteness of an infarct lesion, therefore potentially identifying patients with symptom onset within 4.5 h. This information is crucial for patients whose time from symptom onset is unknown and for whom tPA treatment would otherwise be withheld. Especially lesion visibility on FLAIR images has lately been discussed as such a marker [17] and analyzed in a large clinical trial [18]. Our results suggest that tissue signal intensity on FLAIR is much more susceptible to contrast agent than previously thought. This is why we propose that post-contrast FLAIR images should always be interpreted with caution [9].

In this project we furthermore demonstrated that HARM can be found in a considerable proportion of AIS patients. We showed that HARM can occur as early as a few hours after stroke onset and can persist over many days. BBB changes should therefore be considered a frequently occurring phenomenon that affects even the hyperacute phase of stroke. However, a biphasic pattern, as observed in animal models, could not be detected with the number of examinations we obtained from our patient cohort [9].

Admittedly, in the last two projects the cohorts contained limited patient numbers and patients showed quite some heterogeneity concerning stroke severity. However, as pointed out, repetitive MRI examinations in acutely ill patients only hours after the stroke onset are not easy to obtain. We had a methodological bias towards including rather mildly affected patients, since they had to be able to give informed consent and endure the repetitive measurements on the first days. For these reasons we refrained from making any conclusions on the effect of HARM on the clinical outcome of stroke patients based on these patient cohorts. Instead, we decided to analyze a much larger cohort of AIS patients with only two MRI examinations to determine the association of HARM with several imaging and clinical parameters. In a sample of 529 ischemic stroke patients, we found that HARM is significantly associated with older age and poor kidney function. Also, HARM positive patients had a significantly higher mRS at three months. The results of this project are currently under review in a peer reviewed journal.

With this PhD project we were able to gain substantial knowledge about pathological processes occurring in the very early phases after AIS. These processes are clinically relevant and worth monitoring, especially regarding the potential association with a worse clinical outcome. We were able to show how well we can monitor tissue fate with different MRI sequences. Regarding the BBB disruptions after stroke, further studies should illuminate which other imaging and serum biomarkers are associated with HARM. Also the association of HARM with contrast agent extravasation on T1 images, typically seen in later stages of the stroke, should be examined.

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Eidstattliche Versicherung

“Ich, Ostwaldt, Ann-Christin, versichere an Eides statt durch meine eigenhändige Unterschrift, dass ich die vorgelegte Dissertation mit dem Thema: **“Blood-brain barrier disruptions in the acute phase of ischemic stroke in human patients”** selbstständig und ohne nicht offengelegte Hilfe Dritter verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel genutzt habe. Alle Stellen, die wörtlich oder dem Sinne nach auf Publikationen oder Vorträgen anderer Autoren beruhen, sind als solche in korrekter Zitierung (siehe “Uniform Requirements for Manuscripts (URM)” des ICMJE www.icmje.org) kenntlich gemacht. Die Abschnitte zu Methodik (insbesondere praktische Arbeiten, Laborbestimmungen, statistische Aufarbeitung) und Resultaten (insbesondere Abbildungen, Graphiken und Tabellen) entsprechen den URM (s.o) und werden von mir verantwortet. Meine Anteile an den ausgewählten Publikationen entsprechen denen, die in der untenstehenden gemeinsamen Erklärung mit dem/der Betreuer/in, angegeben sind. Sämtliche Publikationen, die aus dieser Dissertation hervorgegangen sind und bei denen ich Autor bin, entsprechen den URM (s.o) und werden von mir verantwortet. Die Bedeutung dieser eidesstattlichen Versicherung und die strafrechtlichen Folgen einer unwahren eidesstattlichen Versicherung (§156,161 des Strafgesetzbuches) sind mir bekannt und bewusst.”

Datum, Unterschrift

Anteilserklärung an den erfolgten Publikationen

Ann-Christin Ostwaldt hatte folgenden Anteil an den folgenden Publikationen:

Publikation 1: Ostwaldt AC, Rozanski M, Schmidt WU, Nolte CH, Hotter B, Jungehülsing GJ, Villringer K, Fiebach JB. Early time course of FLAIR signal intensity differs between acute ischemic stroke patients with and without Hyperintense Acute Reperfusion Marker. *Cerebrovasc Dis* 2014;37(2):141-146.

65%

Beitrag im Einzelnen: Die Erstautorin (Ann-Christin Ostwaldt) war bei einem Großteil der Patienten direkt an den wiederholten Untersuchungen beteiligt. Sie hat die Daten analysiert (Deliniierung der Läsionen, Analyse der Signalintensitäten) und war daran beteiligt, HARM auf den Bildern zu beurteilen. Sie hat die Statistik komplett selbst gerechnet. Sie hat das Manuskript verfasst und alle Abbildungen erstellt, sowie die Einreichung und Revision des Manuskripts durchgeführt.

Publikation 2: Ostwaldt AC, Galinovic I, Grosse-Dresselhaus F, Neeb L, Villringer K, Rocco A, Nolte CH, Jungehülsing GJ, Fiebach JB. MRI follow-up after 24 h is an accurate surrogate parameter for treatment success after thrombolysis. *Cerebrovasc Dis* 2013;36(5-6):464-465.

55%

Beitrag im Einzelnen: Die Erstautorin (Ann-Christin Ostwaldt) hat die Bilddaten analysiert (Berechnung der Perfusionskarten, Deliniierung der Läsionen). Sie hat die Statistik komplett selbst gerechnet. Sie hat das Manuskript verfasst und alle Abbildungen erstellt, sowie die Einreichung und Revision des Manuskripts durchgeführt.

Publikation 3: Galinovic I, Ostwaldt AC, Soemmer C, Bros H, Hotter B, Brunecker P, Fiebach JB. Automated vs manual delineations of regions of interest - a comparison in commercially available perfusion MRI software. *BMC Med Imaging* 2012;12(16).

20%

Beitrag im Einzelnen: Die Koautorin Ann-Christin Ostwaldt war signifikant an der Auswertung der Daten beteiligt (Berechnung und Weiterverarbeitung der Perfusionskarten). Sie hat beim Verfassen und Überarbeiten des Manuskripts geholfen.

Print versions of the selected publications

Ostwaldt et al. 2014

Early time course of FLAIR signal intensity differs between acute ischemic stroke patients with and without Hyperintense Acute Reperfusion Marker. *Cerebrovasc Dis*; 37:141-146

<http://dx.doi.org/10.1159/000357422>

Ostwaldt et al. 2013

MRI follow-up after 24 h is an accurate surrogate parameter for treatment success after thrombolysis. *Cerebrovasc Dis*; 36:464-465

<http://dx.doi.org/10.1159/000355498>

Galinovic et al. 2012

Automated vs manual delineation of regions of interest - a comparison in commercially available perfusion MRI software. BMC Medical Imaging; 12:16

<http://dx.doi.org/10.1186/1471-2342-12-16>

Curriculum Vitae

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

Complete list of publications

Ostwaldt AC, Rozanski M, Schaefer T, Ebinger M, Jungehülsing GJ, Villringer K, Fiebach JB. Hyperintense acute reperfusion marker is associated with higher contrast agent dosage in acute ischaemic stroke.

European Radiology 2015, doi: 10.1007/s00330-015-3749-5

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Ostwaldt AC, Rozanski M, Schmidt WU, Nolte CH, Hotter B, Jungehülsing GJ, Villringer K, Fiebach JB. Early time course of FLAIR signal intensity differs between acute ischemic stroke patients with and without Hyperintense Acute Reperfusion Marker.

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Stroke 2010;41(8):1823-1825

Acknowledgment

First and above all I would like to express my deepest gratitude to Jochen B. Fiebach, my supervisor, for being a constant source of support, guidance, and encouragement and for always being available for discussions and advice. I would also like to thank my colleagues and friends Peter Brunecker and Ivana Galinovic, who taught me a lot in the last years and whom I could always rely on for help. Thank you, Ahmed, for proofreading and for all the lively scientific discussions. My sincere thanks go to all my coauthors, especially Kersten Villringer and Michal Rozanski, for the invaluable constructive criticism and friendly advice during the writing of my manuscripts. Finally, I am very grateful for all the colleagues who worked at our MR scanner, since I know how much commitment this demands.