Aus dem

Charité Centrum für Neurologie, Neurochirurgie und Psychiatrie

Klinik für Neurologie mit Experimenteller Neurologie

Direktor: Prof. Dr. med. Matthias Endres

Habilitationsschrift

Advanced imaging in acute ischemic stroke of unknown onset

zur Erlangung der Lehrbefähigung für das Fach Experimentelle Neurologie

vorgelegt dem Fakultätsrat der Medizinischen Fakultät

Charité-Universitätsmedizin Berlin

von

Dr. Ivana Galinović

Eingereicht: Februar 2023

Dekan: Prof. Dr. Joachim Spranger

1. Gutachter/in: Frau Prof. Dr. Jennifer Linn

2. Gutachter/in: Frau Prof. Dr. Elke R. Gizewski

Abbreviations

Apparent diffusion coefficient (ADC)

Central image reading board (CIRB)

Computed tomography (CT)

Confidence interval (CI)

Diffusion weighted imaging (DWI)

Disability-adjusted life-years (DALYs)

Electronic case report form (eCRF)

Fluid attenuated inversion recovery (FLAIR)

FLAIR hyperintense vessels (FHV)

Intravenous tissue-type plasminogen activator (IV tPA)

Magnetic resonance imaging (MRI)

Middle cerebral artery (MCA)

Modified Rankin Score (mRS)

National Institute of Health Stroke Scale (NIHSS)

Odds ratio (OR)

Perfusion “weighted” imaging (PWI)

Region of interest (ROI)

Relative signal intensity (rSI)

Symptomatic intracranial hemorrhage (sICH)

Table of contents

1. Introduction

1.1. Synopsis / purpose of the work

The purpose of this cumulative thesis was to present the author's own work pertaining to the Efficacy and Safety of MRI-Based Thrombolysis in WAKE UP Stroke (WAKE UP) trial as well as the changes in clinical practice resulting from it. Part of the work was conducted in preparation of the trial to enable its commencement and part following the trial's termination. The thesis is structured accordingly, to offer a chronologically meaningful narrative.

WAKE UP was an investigator-initiated, multicenter, randomized, double-blind, placebo-controlled clinical trial investigating the safety and efficacy of thrombolytic therapy in patients with acute ischemic stroke of unknown onset time. It started recruiting patients in the fall of 2011 and ended in spring of 2017, having involved over 70 hospitals in eight European countries. It used advanced imaging in the form of magnetic resonance based "tissue clocking" to screen patients for eligibility, thereby testing a novel imaging criterion believed to be able to allocate patients into the approved time window for thrombolysis. This criterion, dubbed the DWI-FLAIR mismatch, was defined as an acute ischemic stroke already visible on diffusion-weighted imaging (DWI) but not yet visible on fluid-attenuated-inversion-recovery (FLAIR), hence the 'mismatch'. Although previously tested on large retrospective datasets and showing promise regarding estimation of time from stroke onset, this criterion was also not univocally defined and worryingly subjective. As such, it was difficult but imperative to the smooth running of the trial to ensure a homogeneous understanding of the imaging criteria across all centers and investigators. The thesis describes my contribution to this effort, from precise fine-tuning of the image evaluation to a seldom-used strategy of online-based investigator training as well as an elaborate quality control process of continuous monitoring of local centers by the central image reading board. The discussion opens with a synopsis of the trial's results and its influence on existing guidelines and clinical practice. Additionally, the safety and efficacy of the "tissue clocking" concept is presented in various patient subpopulations as well as in the context of evidence stemming from other clinical trials. Last but not least, the larger umbrella topic of advanced imaging used for patient selection in both the unknown and the extended time window of ischemic stroke is discussed, closing with the currently prevailing recommendations and controversies.

1.2. Acute ischemic stroke and its therapeutic options

Simply put, acute ischemic stroke is a medical condition occurring when a clot blocks blood flow through a brain artery. The clots themselves can be divided into thrombi and emboli, depending on where in the body they were formed. A thrombus forms directly within one of the many brain arteries and subsequently blocks the local bloodstream. An embolus is a clot (or piece of plaque which breaks off from an artery wall) originating from elsewhere in the cardiovascular system, such as the heart or the large vessels of the neck supplying the brain, which is carried through the bloodstream to eventually become lodged in a narrower vessel inside the brain, again blocking the local bloodstream. In either case, this acute occlusion of a brain vessel leads to reduced perfusion (hypoperfusion) coupled with a lower-to-absent oxygen supply of the downstream brain areas. Within seconds, brain cells seize to function and can die off. Whether the deficit will remain limited to a transient lack of function or lead to permanent destruction of tissue, as well as the volume of the affected brain area, depend on the size and position of the blocked artery and the duration and severity of the ischemia. Existing therapy aims, in the acute stage, to achieve reperfusion of the brain through partial to complete and timely dissolution or removal of the clot. Further treatment options revolve around preventing post-stroke complications (such as brain edema, pneumonia, urinary tract infections, seizures, depression, bedsores or deep vein thrombosis) and offering neurorehabilitation. Equally important is also a battery of diagnostic tests, serving to unearth (and subsequently treat) the cause of the stroke but also to identify individual predisposing factors in order to tailor prevention of future ischemic events.

Stroke is the second leading cause of death worldwide[1], a ranking that it has upheld for a good number of years. Ischemic stroke is also associated with vast healthcare costs, not just in terms of direct costs for inpatient hospital care but also indirect expenditures linked to post-stroke disability. Further sources of hidden costs derive from subclinical cerebrovascular disease, including so-called silent infarction and ischemic white matter disease, which play a significant role in causing functional disability[1]. Combating known risk factors for cerebrovascular disease such as high blood pressure, smoking, obesity, diabetes mellitus, atrial fibrillation, dyslipidemia and lack of physical activity, either as part of primary (before a stroke happens) or secondary (after the first stroke, in order to prevent future strokes) prevention, seems to have contributed to the recent decrease in stroke incidence, mortality and disability-adjusted life-years (DALYs) in high income countries. However, the absolute numbers of stroke incidence, survivors and stroke related deaths as well as DALYS lost has increased worldwide, partly due to expanding population numbers and ageing. An additional reason for the increase of the global burden of stroke is the increased incidence in low- and middle-income countries[2] due to an increased prevalence of many modifiable stroke risk factors in those regions[1]. The global impact of this disease certainly highlights the importance of investing effort in improving primary and secondary prevention as well as acute stroke treatment and neurorehabilitation.

Going back to the year 2011 when this particular journey began, there were three proven effective means of therapy for acute stroke: acetylsalicylic acid, monitoring of patients in dedicated stroke units and thrombolysis. Two large clinical trials conducted in the 1990s (the International Stroke Trial[3] and the Chinese Acute Stroke Trial[4]) have shown that early use of aspirin following an ischemic stroke produces a small but real reduction in deaths and recurrent strokes. Equally, multiple studies have shown that stroke patients who receive organized inpatient care in a stroke unit were more likely to be alive, independent, and living at home one year after the stroke[5]. Thrombolysis with intravenous tissue-type plasminogen activator (IV tPA) as a fibrin(ogen)olytic agent came under investigation as a potential treatment for ischemic stroke in the late 1980s. In 1995, the National Institute of Neurological Disorders and Stroke (NINDS) study group reported that patients with acute ischemic stroke who received IV tPA with alteplase within 3 hours of symptom onset were at least 30% more likely to have minimal or no disability at 3 months post-stroke than those who received placebo[6]. Through subsequent trials and the pooling of their data a clear association between treatment efficacy and the interval between the onset of symptoms and administration of the thrombolytic agent emerged[7]. In 2008 Werner Hacke and colleagues published the results of the ECASS III trial, proving that the efficacy of alteplase safely extends to a time window of 3 to 4.5 hours after the onset of stroke symptoms[7] and this was still the current state of guidelines for the acute treatment of stroke in 2011. So, unlike the use of aspirin or patient management in a dedicated stroke unit, arguably the most efficient therapy for stroke, IV tPA, was firmly attached to a rigidly defined time window of 4.5 hours following observed symptom onset. Clearly, this ruled out IV tPA as an approved option for a large percentage of patients. For some of these patients, the exact time of stroke could not be ascertained and for others, due to delays in recognizing the symptoms or reaching a hospital, a longer timespan than 4.5h would elapse. Hence, there was a pronounced need in the community of stroke researchers and clinicians to either find new therapies or extend the existing ones to cover a larger segment of patients suffering from acute ischemic stroke.

1.3. Stroke of unknown onset and its assessment using magnetic resonance imaging

One such (sub)population, estimated to account for up to 20% of all acute strokes, were patients in whom the time point of symptom onset is unknown. This happens either if patients wake up with stroke symptoms or if they are otherwise unable to state the exact time of onset (for example, due to aphasia or a severely impaired level of consciousness) and their stroke was not witnessed by a third party. The prevailing belief in the stroke community was that a large proportion of such patients might still benefit from reperfusion treatment. What was needed was proof that the time window as a

selection criterion could either be abolished or safely replaced by alternative parameters that are able to reliably estimate stroke onset.

An idea emerged that magnetic resonance imaging (MRI), through the use of sequences that are sensitive to different aspects of tissue pathophysiology, could provide the key to dating a stroke. Some of the groundwork was laid as early as the mid-1990s[8,9], showing that diffusion weighted imaging (DWI) is capable of depicting early ischemic changes taking place within minutes of stroke, whereas signal changes on T2-weighted MRI images evolve slowly enough (over the subsequent several hours) as to potentially provide an estimate of lesion age. This is due to the fact that the signal properties of these two sequences are affected by different pathophysiological processes, which happen sequentially. The early response to serious ischemia, namely cytotoxic edema, occurs due to a redistribution of water already present in brain tissue. A shift of Na+ and Cl- ions from the extracellular to the intracellular space draws water molecules into cells and causes cell swelling. This leads to an overall reduction in the diffusivity of water molecules and can be measured on DWI as a drop in the apparent diffusion coefficient (ADC) or signal increase on trace DWI[10]. In the hours to follow, the blood-brain-barrier breaks down, "leaking" new water into the ischemic tissue. This net increase of water, a process called vasogenic edema, then becomes visible as an increase in signal on T2 weighted imaging[10] (Figure 1).

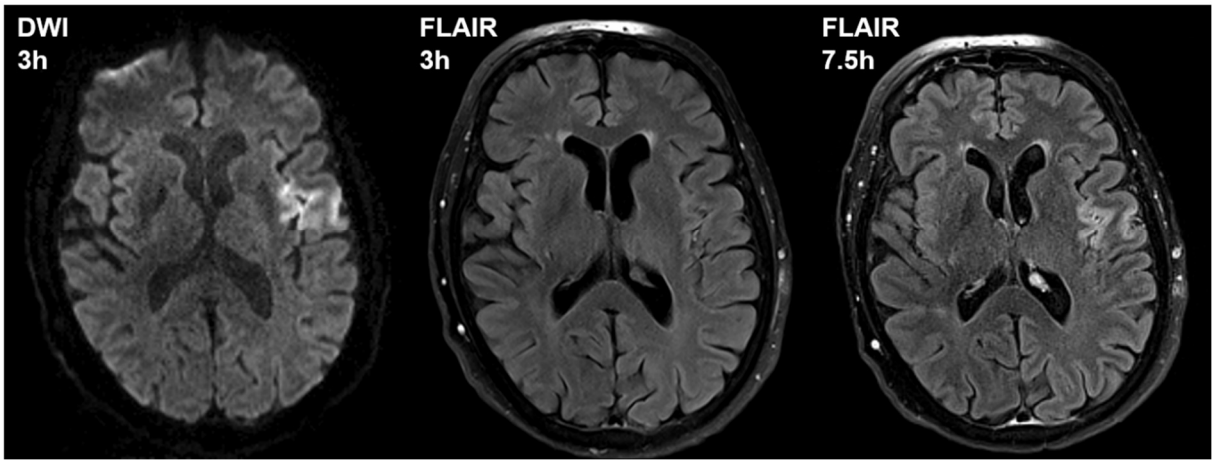

Figure 1. Two consecutive scans (3 hours and 7.5 hours after symptom onset, respectively) in a patient with a left-sided middle cerebral artery (MCA) stroke. DWI clearly shows the largely cortical ischemia at the earlier time point (signal increase in the left insula and operculum) whereas it is not yet visible on the corresponding FLAIR acquired during the same session (DWI-FLAIR mismatch), requiring several hours more for the T2 signal to evolve and become a DWI-FLAIR match.

Yet it wasn't until the mid-2000s that the first human studies were conducted, trying to precisely correlate the conspicuity of signal on fluid attenuated inversion recovery (FLAIR, a type of T2-weighted imaging with nulled cerebrospinal fluid signal) to time from symptom onset. In 2009, as part of my master's thesis, I examined a cohort of nearly 100 acute stroke patients, assessing the sensitivity and

specificity of the DWI-FLAIR mismatch to correctly allocate patients into the 4.5h time window for thrombolysis[11]. DWI-FLAIR mismatch was taken to signify an acute stroke lesion visible on DWI but not yet visible on FLAIR, whereas conversely DWI-FLAIR match would mean an acute stroke visible both on DWI and FLAIR. Subsequent efforts using a large pooled dataset further investigated the DWI-FLAIR mismatch concept. The multicenter observational study (PRE-FLAIR)[12], published in 2011, showed that the method was able to discriminate between patients within the 4.5h time window (for which thrombolysis is safe and effective) and those beyond with high specificity and positive predictive value. These findings lent support to the use of the DWI-FLAIR mismatch for selection of patients that might benefit from IV tPA despite the uncertainties stemming from their unknown stroke onset and subsequently led to the launch of a clinical trial.

1.4. The imaging requirements of the WAKE UP trial

The proposal was submitted to the EU Framework Programme 7 Health-2011 under the category „investigator-driven clinical trials for the management of cardiovascular diseases" in early 2011 and granted funding. Under the acronym WAKE UP[13], the Efficacy and Safety of MRI-Based Thrombolysis in WAKE UP Stroke trial was conceptualized and run as an investigator-initiated, multicenter, randomized, double-blind, placebo controlled trial designed to test efficacy and safety of MRI-based intravenous thrombolysis in patients with stroke of unknown symptom onset. This was to be a large-scale collaborative project, including 12 partner institutions from 7 EU countries, coordinated by Prof. Christian Gerloff and Götz Thomalla from the Universitaetsklinikum Hamburg-Eppendorf (UKE).

My team was chosen to head the so-called "workpackage 02" which consisted of defining the imaging standards and carrying out the training of local centers. The WAKE UP trial[13] relied heavily on imaging criteria for the randomization of patients; concretely the confirmation of an acute stroke involving less than 1/3 of the MCA territory (or 1/2 of the anterior or posterior cerebral artery territory) as well as the always necessary exclusion of hemorrhage. This, in and of itself, was not unusual for a clinical stroke trial. What however was rare was the use of MRI as the only allowed screening modality. One criterion in particular was pivotal to the success of the study - the DWI-FLAIR mismatch - as the very premise of the trial was that this MRI marker would be capable of patient allocation into treatment groups. The issue we were facing was that visual ratings of lesion conspicuity on FLAIR were subjective, with modest interrater agreement[14] and requiring a fair amount of expertise. This is understandable when one takes into consideration the pathophysiological nature of the process we were trying to binarize, which is the gradual buildup of vasogenic edema leading to an increase in signal intensity on FLAIR. Additionally, non-physiological parameters (such as the contrast and brightness settings inside the viewing software and the overall image quality) also influence the outcome of visual ratings. Some

studies suggested that the size of the acute stroke as well as the personality and experience of the rater might equally play a role[12]. In the clinical setting, a dichotomy emerges, where agreement regarding DWI-FLAIR mismatch is high for lesions that are clearly absent or clearly present on FLAIR, but low for patients presenting with lesions that show subtle/emerging conspicuousness on FLAIR. As the DWI-FLAIR mismatch was the main randomization criterion for the WAKE UP trial, assuring the same interpretation of FLAIR positivity across all participating centers was important. To this end, multiple steps were taken. Firstly, I put together an illustrated imaging manual as mandatory reading for local investigators, complemented by a booklet showing borderline cases and offering guidance on how to judge them. These materials were distributed, electronically as well as in a paper copy, to all the recruiting centers. I then investigated whether we could offer even more support to recruiting centers. I assembled five raters to go over a sample of 45 cases with a "gold standard" rating (defined as consensus of two experienced neuroradiologists) of either FLAIR-negative or FLAIR-positive cases. The raters delineated a representative area within each acute ischemic lesion (and then the mirrored contralateral healthy tissue) by manually placing a small circular region of interest (ROI) within the lesion[15]. This so-called "hot spot" method was then used to assess relative signal intensity (rSI) values (Figure 2).

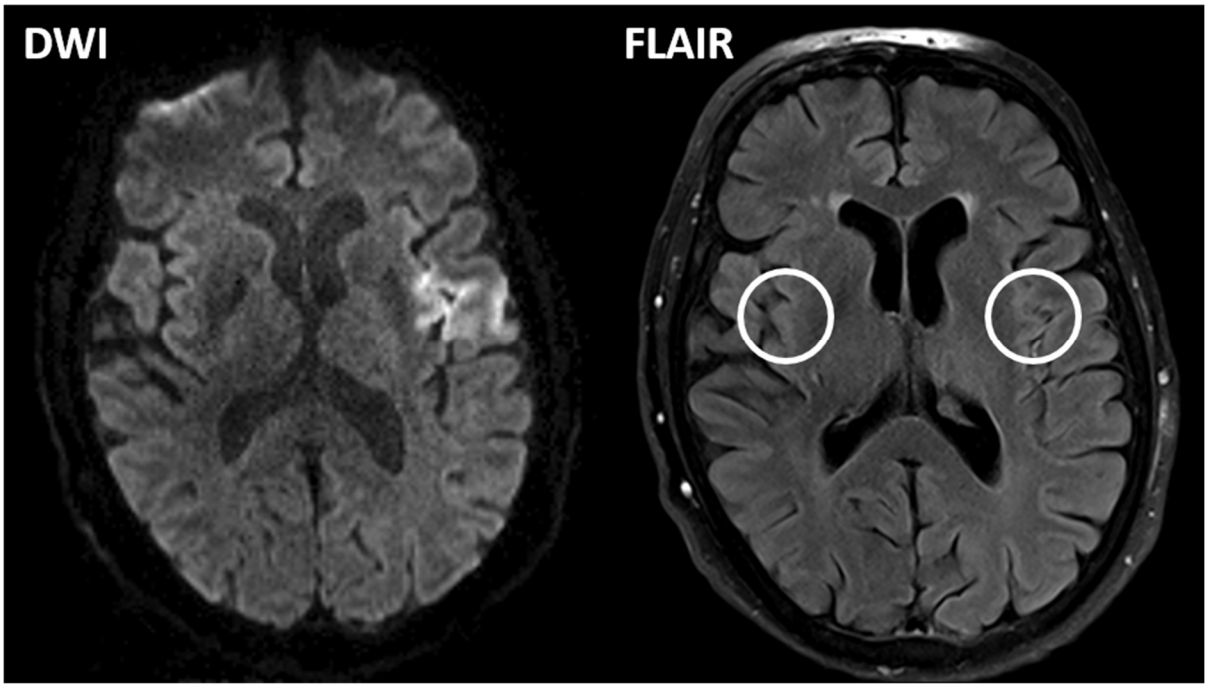

Figure 2. Using the example images from Figure 1, we visually identify the area on the FLAIR corresponding to the acute ischemic lesion on DWI and then place the ROI in the segment which we perceive as brightest (hot spot, shown here as white circle). The ROI is then duplicated and mirrored onto the contralateral hemisphere. The ratio between the signal intensity of the ROI on the infarcted side and the one on the unaffected side is calculated as rSI.

This strategy has been previously shown to give comparable results to the much more time-consuming method of measuring signal intensity in the entirety of the acute ischemic lesion[16]. The guiding idea of the analysis was to find an rSI cut-off value which best correlates with the turning point of the expert verdict of a DWI-FLAIR mismatch into a DWI-FLAIR match. The resulting threshold value was 1.2, which I then tested in a separate cohort to see whether it might improve interrater consensus[15]. Unfortunately, neither using the hot spot method alone nor combining it with a visual rating increased interrater agreement. However, I did find that in a significant number of cases even experienced raters signaled a need for "objective" help in judging FLAIR lesion conspicuity and reported that using the hot spot reassured them in making the final call. This is why the hot spot method, with the 1.2 threshold of rSI, was recommended for prospective use in the WAKE UP trial, in any and all situations in which a local investigator felt unsure of their visual assessment. A great strength of the method was that its implementation was possible and straightforward in any radiological viewer program and could be done in seconds, which gave us hope that it would be utilized as backup by radiologists and neurologists without reservations. An analysis of the frequency of its actual use was carried out towards the end of the trial, showing that the ROI method, despite its imperfections, had in fact been used in 27% of all screened patients, which points to its feasibility and acceptance in the real-life setting[17]. It warrants pointing out that even extensive analysis of signal behavior done after the conclusion of the WAKE UP trial was unable to find an alternative, better suited method to aid visual evaluation of DWI-FLAIR (mis)match[18].

1.5. Investigator training in the WAKE UP trial

However, the hot spot method was only an auxiliary helping tool aiding the evaluation of a single imaging parameter whereas the success of the screening process for the WAKE UP trial depended upon the simultaneous and homogenous assessment of several imaging criteria. As many as sixty centers in seven European countries were originally expected to participate in the WAKE UP study, actively recruiting patients. Imaging-based patient selection is known to work well in small studies with expert investigators, but scaling up to a multicenter setting, it often suffers from a significant number of protocol violations[19]. We knew that some of the criteria were more robust in their identification, such as the presence or absence of intracranial hemorrhage or an acute ischemic lesion. Others, such as the evaluation of the extent of infarction, and the DWI-FLAIR mismatch of course, were more pronouncedly subjective and likely to cause a high level of heterogeneity. To smooth the process and homogenize the results of judging various imaging inclusion and exclusion criteria, it was decided to develop a dedicated software to train local investigators (Figure 3).

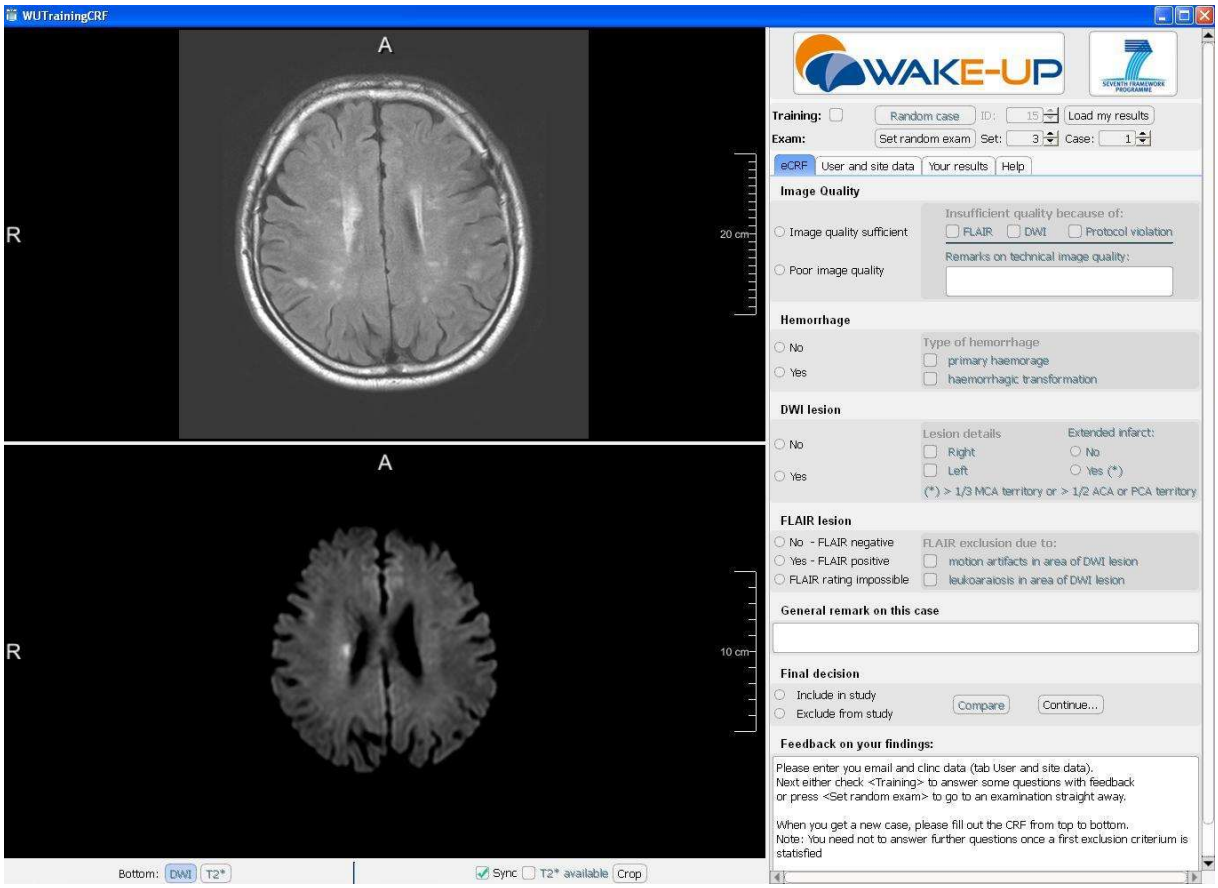

Figure 3. The graphic user interface of the WAKE UP investigator training software. The left side of the screen hosts the imaging panel with two windows for parallel and synchronized viewing of two different series (for example DWI and FLAIR, as shown here). The right side of the screen hosts the electronic case report form (eCRF) with questions pertaining to the different inclusion and exclusion criteria. The bottom of the eCRF offers feedback to the investigators regarding their answers and therefore serves a teaching function. When ready to put their knowledge to the test, investigators would switch from the training to the exam mode with a simple click in the checkbox at the top left corner of the eCRF.

Project partners from Fraunhofer MEVIS: Institute for Digital Medicine and I created the training tool in 2011. I was in charge of image acquisition and annotation as well as the design of the electronic case report form (eCRF), whereas colleagues from Fraunhofer MEVIS were tasked with software development and implementation. In its final version, the training tool included 65 cases selected to cover all the imaging criteria of the trial and offered two modes of operation: a training mode and an exam mode. Investigators were presented relevant image series (DWI, FLAIR and T2*-weighted images) and asked to form an opinion with respect to the WAKE UP imaging criteria, subsequently documenting their choices in an eCRF. Whilst using the training mode, as part of the learning procedure, a button was available to provide the investigators feedback on their answers. Once having switched to the exam mode, feedback was disabled and investigators were presented with a fresh batch of 13 cases which they needed to judge. The eCRFs with the individual exam results were sent

to Fraunhofer MEVIS for a centralized evaluation. Only those investigators who passed the exam (11 correctly judged cases out of 13) were considered eligible to partake in the WAKE UP study. All investigators were required to successfully complete this certification program prior to recruiting patients into the trial. Due to the multicenter nature of the trial with continuous personnel fluctuations over the years, the training and certification process, which started in June 2012, ran uninterrupted until the trials conclusion, with a total of 461 participants taking part in it. It was well accepted in the community of WAKE UP local centers, with the average exam taking only 15 minutes and nearly 75% of all examinees passing the exam on their first attempt[20]. A questionnaire sent out to the investigators after the conclusion of the trial revealed the validity of our belief in a structured training process, as approximately 90% of investigators reported that having done the image training helped them when screening patients and that it especially increased their confidence with regards to the DWI-FLAIR mismatch criterion[20]. This is of particular significance, as unsurprisingly 62% of the questioned investigators said that the DWI-FLAIR mismatch was the most difficult of all the imaging criteria to judge[20]. To ensure quality control and support to the recruiting centers, the central image reading board (CIRB) continuously monitored the MRI screening in local centers using a two-tiered evaluation process. MRI exams of all patients, both only screened and randomized, were sent in parallel to two blinded members of the CIRB for their independent assessment of the WAKE UP imaging criteria. In case of any discrepancies between the verdict of the CIRB and the local center an additional senior member of the CIRB was called upon to adjudicate. For any case in which the final opinion of the CIRB regarding randomization into the trial was divergent from that of the local center, a round of communication with the recruiting center was instigated to ensure a common understanding of the protocol violation.

In conclusion, it can be said that great effort was invested, both during the preparation and during the conduct of the WAKE UP trial, to guarantee the success of the heavily imaging-based patient selection. Nevertheless, following the trials conclusion, we were still positively surprised to find that the resulting agreement for assessing lesion conspicuity on FLAIR surpassed our expectations, with a kappa value (κ) of 0.60, which signifies substantial agreement[20]. The trial also saw very few protocol violators. When one takes into consideration that this was, in fact, a reflection of an achieved common understanding of hundreds of investigators who participated in the WAKE UP study, many of whom arguably did not have prior experience in assessing acute stroke conspicuity on FLAIR, it is nothing short of astounding. We therefore strongly believe that the efforts invested into the various aspects of assisting local investigators were instrumental to this high rate of agreement between the recruiting centers and the central image reading board that we saw throughout the trial and certainly played a role in the trial's overall success.

2. Publications

2.1. Visual and region of interest-based interrater agreement of the DWI-FLAIR mismatch

The following text corresponds to the abstract of the original paper:

Galinovic I, Puig J, Neeb L, Guibernau J, Kemmling A, Siemonsen S, Pedraza S, Cheng B, Thomalla G, Fiehler J, Fiebach JB. Visual and region of interest-based inter-rater agreement in the assessment of the diffusion-weighted imaging- fluid-attenuated inversion recovery mismatch. Stroke. 2014 Apr;45(4):1170-1172

https://doi.org/10.1161/STROKEAHA.113.002661

"Background and purpose: WAKE UP is a randomized, placebo-controlled MRI-based trial of thrombolysis in WAKE UP stroke using the mismatch between a lesion's visibility in diffusion-weighted imaging and fluid-attenuated inversion recovery (FLAIR) sequences as its main imaging inclusion criterion. Visual judgment of lesion conspicuity on FLAIR is however methodically limited by moderate inter-rater agreement. We therefore sought to improve rating homogeneity by incorporating quantitative signal intensity measurements.

Methods: One hundred forty-three data sets of patients with acute ischemic stroke were visually rated by 8 raters with respect to WAKE UP study inclusion and exclusion criteria, and inter-rater agreement was calculated. A subanalysis was performed on 45 cases to determine a threshold value of relative signal intensity (rSI) between the ischemic lesion and contralateral healthy tissue which best corresponded to a visually established verdict of FLAIR positivity. The usefulness of this threshold in improving inter-rater agreement was evaluated in an additional sample of 50 patients.

Results: Inter-rater agreement for inclusion into the WAKE UP trial was 73% with a free-marginal κ of 0.46. A threshold of rSI which best correlated with the visual rating of lesions as FLAIR positive was 1.20. The addition of rSI measurements to visual evaluation did not change the inter-rater agreement.

Conclusions: Introducing a semiquantitative measure for FLAIR rSI did not improve the agreement between individual raters. However, enhancing visual assessment with rSI measurements can provide reassurance to local investigators in cases of uncertainty."

2.2. A report from the WAKE UP study:

The role of investigator training in the assessment of imaging criteria

The following text corresponds to the abstract of the original paper:

Galinovic I, Dicken V, Heitz J, Klein J, Puig J, Guibernau J, Kemmling A, Gellissen S, Villringer K, Neeb L, Gregori J, Weiler F, Pedraza S, Thomalla G, Fiehler J, Gerloff C, Fiebach JB; WAKE UP Investigators. Homogeneous application of imaging criteria in a multicenter trial supported by investigator training: A report from the WAKE UP study. Eur J Radiol. 2018 Jul;104:115-119.

https://doi.org/10.1016/j.ejrad.2018.05.011

"Background and purpose: WAKE UP is a randomized, placebo-controlled trial of thrombolysis in stroke with unknown time of symptom onset using magnetic resonance imaging criteria to determine patients' eligibility. As it is a multicenter trial, homogeneous interpretation of criteria is an important contributor to the trial's success. We describe the investigator image training as well as results of the quality control done by the central image reading board (CIRB).

Methods: Investigators at local centers were given an imaging manual and passed a software-based image training prior to being allowed to judge images in the trial. Throughout the trial, the CIRB gave feedback to recruiting centers in cases of disagreement regarding a patient's randomization. We evaluated the investigators performance in the image training and analyzed results of this quality control from the first 1069 screened patients. Additionally, we obtained feedback from investigators regarding their experiences with the trial.

Results: Four-hundred-and-sixty physicians from eight European countries took part in the image training, of whom 436 (95%) successfully completed it. In the trial, agreement rates between the local investigators and members of the CIRB were high for the presence of an acute ischemic lesion (94%, $\kappa = 0.87$) as well as for the judgment of infarct extent (93%, $\kappa = 0.87$). Agreement for the criterion of DWI-FLAIR mismatch was 74%, $\kappa = 0.60$. The majority of investigators reported that the DWI-FLAIR mismatch was the hardest imaging criterion to evaluate. Ninety-one percent of investigators who responded to our survey stated that the image training specifically increased their confidence when assessing the DWI-FLAIR mismatch.

Conclusions: Despite its multicenter design, the WAKE UP study has demonstrated a high level of homogeneity amongst raters in interpreting the various imaging criteria for patient randomization, including the novel criterion of DWI-FLAIR mismatch. Systematic image training increased the confidence of investigators in applying imaging criteria."

2.3. The WAKE UP study:

MRI-guided thrombolysis for stroke with unknown time of onset

The following text corresponds to the abstract of the original paper:

Thomalla G, Simonsen CZ, Boutitie F, Andersen G, Berthezene Y, Cheng B, Cheripelli B, Cho TH, Fazekas F, Fiehler J, Ford I, Galinovic I, Gellissen S, Golsari A, Gregori J, Günther M, Guibernau J, Häusler KG, Hennerici M, Kemmling A, Marstrand J, Modrau B, Neeb L, Perez de la Ossa N, Puig J, Ringleb P, Roy P, Scheel E, Schonewille W, Serena J, Sunaert S, Villringer K, Wouters A, Thijs V, Ebinger M, Endres M, Fiebach JB, Lemmens R, Muir KW, Nighoghossian N, Pedraza S, Gerloff C; WAKE UP Investigators. MRI-Guided Thrombolysis for Stroke with Unknown Time of Onset. N Engl J Med. 2018 Aug 16;379(7)611-622.

https://doi.org/10.1056/NEJMoa1804355

"Background: Under current guidelines, intravenous thrombolysis is used to treat acute stroke only if it can be ascertained that the time since the onset of symptoms was less than 4.5 hours. We sought to determine whether patients with stroke with an unknown time of onset and features suggesting recent cerebral infarction on magnetic resonance imaging (MRI) would benefit from thrombolysis with the use of intravenous alteplase.

Methods: In a multicenter trial, we randomly assigned patients who had an unknown time of onset of stroke to receive either intravenous alteplase or placebo. All the patients had an ischemic lesion that was visible on MRI diffusion-weighted imaging but no parenchymal hyperintensity on fluid-attenuated inversion recovery (FLAIR), which indicated that the stroke had occurred approximately within the previous 4.5 hours. We excluded patients for whom thrombectomy was planned. The primary end point was favorable outcome, as defined by a score of 0 or 1 on the modified Rankin scale of neurologic disability (which ranges from 0 [no symptoms] to 6 [death]) at 90 days. A secondary outcome was the likelihood that alteplase would lead to lower ordinal scores on the modified Rankin scale than would placebo (shift analysis).

Results: The trial was stopped early owing to cessation of funding after the enrollment of 503 of an anticipated 800 patients. Of these patients, 254 were randomly assigned to receive alteplase and 249 to receive placebo. A favorable outcome at 90 days was reported in 131 of 246 patients (53.3%) in the alteplase group and in 102 of 244 patients (41.8%) in the placebo group (adjusted odds ratio, 1.61; 95% confidence interval [CI], 1.09 to 2.36; P=0.02). The median score on the modified Rankin scale at 90 days was 1 in the alteplase group and 2 in the placebo group (adjusted common odds ratio, 1.62; 95% CI, 1.17 to 2.23; P=0.003). There were 10 deaths (4.1%) in the alteplase group and 3 (1.2%) in the placebo group (odds ratio, 3.38; 95% CI, 0.92 to 12.52; P=0.07). The rate of symptomatic intracranial hemorrhage was 2.0% in the alteplase group and 0.4% in the placebo group (odds ratio, 4.95; 95% CI, 0.57 to 42.87; P=0.15).

Conclusions: In patients with acute stroke with an unknown time of onset, intravenous alteplase guided by a mismatch between diffusion-weighted imaging and FLAIR in the region of ischemia resulted in a significantly better functional outcome and numerically more intracranial hemorrhages than placebo at 90 days. (Funded by the European Union Seventh Framework Program; WAKE UP ClinicalTrials.gov number, NCT01525290; and EudraCT number, 2011-005906-32.)."

2.4. A report from the WAKE UP study:

Outcome of intravenous thrombolysis in infratentorial infarcts

The following text corresponds to the abstract of the original paper:

Galinovic I, Boutitie F, Fiebach JB, Villringer K, Cheng B, Ebinger M, Endres M, Fiehler J, Ford I, Thijs V, Lemmens R, Muir KW, Nighoghossian N, Pedraza S, Simonsen CZ, Roy P, Gerloff C, Thomalla G. Post-hoc Analysis of Outcome of Intravenous Thrombolysis in Infarcts of Infratentorial Localization in the WAKE UP Trial. Front Neurol. 2019 Sep 11;10:983.

doi: 10.3389/fneur.2019.00983.

"Introduction: In WAKE UP (Efficacy and Safety of MRI-based Thrombolysis in WAKE UP Stroke), patients with an acute stroke of unknown onset time were randomized to treatment with intravenous alteplase or placebo, guided by MRI.

Methods: In this exploratory post-hoc secondary analysis we compared clinical and imaging data, as well as treatment effects and safety of intravenous thrombolysis between patients with infra- vs. supratentorial stroke.

Results: Forty-eight out of 503 randomized patients (9.5%) presented with a stroke involving the cerebellum or brainstem. Patients with infratentorial stroke were younger compared to patients with supratentorial stroke (mean age 60 vs. 66 years), more frequently male (85 vs. 62%), and less severely affected (median NIHSS 4.5 vs. 6.0). There was no heterogeneity for treatment effect between supratentorial (OR 1.67 95% CI 1.11-2.51) and infratentorial (OR 1.31 95% CI 0.41-4.22) sub-groups (test for interaction p = 0.70). In patients with infratentorial stroke, favorable outcome [a score of 0-1 on the modified Rankin scale (mRS) at 90 days] was observed in 12/22 patients (54.5%) in the alteplase group and in 13/25 patients (52.0%) in the placebo group (p = 0.59). The primary safety endpoint (death or mRS 4-6 at day 90) occurred in three patients of the alteplase group (13.6%) and three patients in the placebo group (12.0%); p = 0.74.

Discussion: WAKE UP was underpowered for demonstrating treatment effect in subgroup analyses however, based on our current results, there is no evidence to recommend withholding MRI-guided thrombolysis in patients with unknown onset stroke of infratentorial localization."

frontiers in Neurology

BRIEF RESEARCH REPORT
published: 11 September 2019
doi: 10.3389/fneur.2019.00983

Post-hoc Analysis of Outcome of Intravenous Thrombolysis in Infarcts of Infratentorial Localization in the WAKE-UP Trial

Ivana Galinovic[1], Florent Boutitie[2,3,4], Jochen B. Fiebach[1], Kersten Villringer[1], Bastian Cheng[5], Martin Ebinger[1,6], Matthias Endres[1,7,8], Jens Fiehler[9], Ian Ford[10], Vincent Thijs[11,12], Robin Lemmens[13,14,15], Keith W. Muir[16], Norbert Nighoghossian[17], Salvador Pedraza[18], Claus Z. Simonsen[19], Pascal Roy[2,3,4], Christian Gerloff[5] and Götz Thomalla[5] on behalf of the WAKE-UP Investigators*

[1] Center for Stroke Research Berlin (CSB), Charité-Universitätsmedizin Berlin, Berlin, Germany, [2] Hospices Civils de Lyon, Service de Biostatistique, Lyon, France, [3] Université Lyon 1, Villeurbanne, France, [4] Laboratoire de Biométrie et Biologie Evolutive, Equipe Biostatistique-Santé, Villeurbanne, France, [5] Klinik und Poliklinik für Neurologie, Kopf- und Neurozentrum, University Medical Center Hamburg-Eppendorf, Hamburg, Germany, [6] Neurologie der Rehaklinik Medical Park Humboldtmühle, Berlin, Germany, [7] Klinik und Hochschulambulanz für Neurologie, Charité-Universitätsmedizin Berlin, Berlin, Germany, [8] German Center for Neurodegenerative Diseases Within the Helmholtz Association, Partner Site Berlin, Bonn, Germany, [9] Department of Diagnostic and Interventional Neuroradiology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany, [10] Robertson Centre for Biostatistics, University of Glasgow, Glasgow, United Kingdom, [11] Stroke Theme, Florey Institute of Neuroscience and Mental Health, University of Melbourne, Heidelberg, VIC, Australia, [12] Austin Health, Department of Neurology, Heidelberg, VIC, Australia, [13] Department of Neurology, University Hospitals Leuven, Leuven, Belgium, [14] Department of Neurosciences, Experimental Neurology, KU Leuven–University of Leuven, Leuven, Belgium, [15] VIB, Laboratory of Neurobiology, Center for Brain and Disease Research, Leuven, Belgium, [16] Institute of Neuroscience and Psychology, University of Glasgow, Glasgow, United Kingdom, [17] Department of Stroke Medicine, Université Claude Bernard Lyon 1, Lyon, France, [18] Department of Radiology, Hospital Dr. Josep Trueta, Institut de Diagnostic per la Image (IDI), Institut d'Investigació Biomèdica de Girona (IDIBGI), Girona, Spain, [19] Department of Neurology, Aarhus University Hospital, Aarhus, Denmark

Introduction: In WAKE-UP (Efficacy and Safety of MRI-based Thrombolysis in Wake-Up Stroke), patients with an acute stroke of unknown onset time were randomized to treatment with intravenous alteplase or placebo, guided by MRI.

Methods: In this exploratory *post-hoc* secondary analysis we compared clinical and imaging data, as well as treatment effects and safety of intravenous thrombolysis between patients with infra- vs. supratentorial stroke.

Results: Forty-eight out of 503 randomized patients (9.5%) presented with a stroke involving the cerebellum or brainstem. Patients with infratentorial stroke were younger compared to patients with supratentorial stroke (mean age 60 vs. 66 years), more frequently male (85 vs. 62%), and less severely affected (median NIHSS 4.5 vs. 6.0). There was no heterogeneity for treatment effect between supratentorial (OR 1.67 95% CI 1.11–2.51) and infratentorial (OR 1.31 95% CI 0.41–4.22) sub-groups (test for interaction $p = 0.70$). In patients with infratentorial stroke, favorable outcome [a score of 0–1 on the modified Rankin scale (mRS) at 90 days] was observed in 12/22 patients (54.5%) in the alteplase group and in 13/25 patients (52.0%) in the placebo group ($p = 0.59$). The primary safety endpoint (death or mRS 4–6 at day 90) occurred in three patients of the alteplase group (13.6%) and three patients in the placebo group (12.0%); $p = 0.74$.

OPEN ACCESS

Edited by:
Bruce Campbell,
The University of Melbourne, Australia

Reviewed by:
James Evans,
Gosford Hospital, Australia
Miriam Priglinger-Coorey,
Royal North Shore Hospital, Australia

***Correspondence:**
Ivana Galinovic
ivana.galinovic@charite.de

Specialty section:
This article was submitted to Stroke, a section of the journal Frontiers in Neurology

Received: 12 July 2019
Accepted: 28 August 2019
Published: 11 September 2019

Citation:
Galinovic I, Boutitie F, Fiebach JB, Villringer K, Cheng B, Ebinger M, Endres M, Fiehler J, Ford I, Thijs V, Lemmens R, Muir KW, Nighoghossian N, Pedraza S, Simonsen CZ, Roy P, Gerloff C and Thomalla G (2019) Post-hoc Analysis of Outcome of Intravenous Thrombolysis in Infarcts of Infratentorial Localization in the WAKE-UP Trial. Front. Neurol. 10:983. doi: 10.3389/fneur.2019.00983

Discussion: WAKE-UP was underpowered for demonstrating treatment effect in subgroup analyses however, based on our current results, there is no evidence to recommend withholding MRI-guided thrombolysis in patients with unknown onset stroke of infratentorial localization.

Keywords: infratentorial infarct, infratentorial stroke, intravenous thrombolysis, alteplase, MRI, WAKE-UP

INTRODUCTION

The WAKE-UP trial (a randomized, double-blind, placebo-controlled trial, ClinicalTrials.gov number, NCT01525290) (1) was an investigator-initiated, multicenter, randomized, double-blind, placebo-controlled clinical trial which provided evidence of clinical benefit of MRI-guided treatment with intravenous alteplase in acute stroke patients with an unknown time of symptom onset. The study was based on the concept of DWI-FLAIR mismatch, with lesions visible on diffusion-weighted imaging (DWI) but not clearly visible on fluid-attenuated-inversion-recovery (FLAIR) identifying patients within 4.5 h of stroke onset. This concept was established through previous studies reporting a high specificity (78%) and positive predictive value (83%) of the DWI-FLAIR mismatch in identifying hyperacute stroke patients (2). Since previous studies have shown that FLAIR signal changes might develop more slowly in infratentorial than in supratentorial stroke (3, 4), there remained uncertainty about the safety and efficacy of thrombolysis based on DWI-FLAIR mismatch in this cohort. In WAKE-UP, patients were randomized irrespective of the localization of the acute ischemic stroke, providing us with an opportunity to perform a subgroup analysis of patients with brainstem and cerebellar strokes. The objective of the current study was to investigate the safety and efficacy of intravenous alteplase administered based on the presence of a DWI-FLAIR mismatch in patients with infratentorial strokes.

MATERIALS AND METHODS

Study Design

The national competent authorities and ethics committees in all participating countries approved the study. WAKE-UP was registered at https://www.clinicaltrials.gov. Unique identifier: NCT01525290. URL: https://www.clinicaltrialsregister.eu. Unique identifier: 2011-005906-32. Informed consent was obtained from all patients prior to enrollment into the trial. Patients were included in this substudy if the sole location of their acute ischemic lesion (based on baseline DWI) was in one or more of the following brain regions: the pons, medulla oblongata, cerebellum, or mesencephalon. We examined demographic characteristics, clinical, and imaging data at baseline and follow-ups for this subgroup of patients and compared them to patients with a supratentorial stroke.

Outcome Measures

The primary efficacy endpoint was favorable outcome defined as a score of 0–1 on the modified Rankin scale (mRS) at final follow up (90 days post-stroke). As a secondary efficacy endpoint we evaluated an ordinal analysis of the mRS ("shift analysis"). The primary safety endpoint was death or dependence (defined as a score of 4–6 on the mRS at 90 days post-stroke), additional safety outcomes were the incidence of symptomatic intracerebral hemorrhage (SICH) according to the protocols of SITS-MOST, ECASS II, ECASS III, NINDS, and parenchymal hemorrhage type 2 (PH-2) on follow-up imaging 22–36 h after treatment (5–8).

Statistical Analysis

Statistical analyses of treatment effects were performed in the intention-to-treat population. To investigate the interaction between stroke location (i.e., infra- vs. supratentorial lesion) 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. The interaction term was tested with the Wald-Chi-squared test, and the treatment effect (odds ratio [OR]) and its 95% confidence interval (CI) was estimated for each category of the stroke location. We furthermore repeated the analysis of primary and secondary endpoints as in the original trial analysis in the subpopulation of patients with infratentorial strokes. The main efficacy variable as well as the safety endpoints were assessed using an unconditional logistic regression analysis, fitted to estimate the OR and its 95% CI interval. The categorical shift in the distribution of mRS scores was analyzed by fitting a proportional-odds logistic regression model. All analyses were adjusted for the stratification parameters age and NIHSS. All tests were carried out with a two-sided alpha level of 5% without correction for multiple comparisons.

RESULTS

Comparison of Infra- vs. Supratentorial Strokes in the Screened Population

Of 1,362 patients screened for WAKE-UP, 84 (6%) had a cerebellar and/or brainstem stroke. These patients were younger (mean 62.5 years, SD 12.0) when compared to patients with supratentorial strokes (mean 65.3 years, SD 11.8; $p = 0.02$), and were more often male (64/84, 76% as opposed to 769/1,278, 60%; $p = 0.004$). In addition, the NIHSS score at baseline (median; IQR) was lower in patients with infratentorial stroke (5; 3–6) than in patients with supratentorial stroke (6; 4–11), $p < 0.001$. We did not identify a difference in cardiovascular risk factors e.g., arterial hypertension, diabetes mellitus, hypercholesterolemia, or history of ischemic stroke between groups. However, there was a higher prevalence of atrial

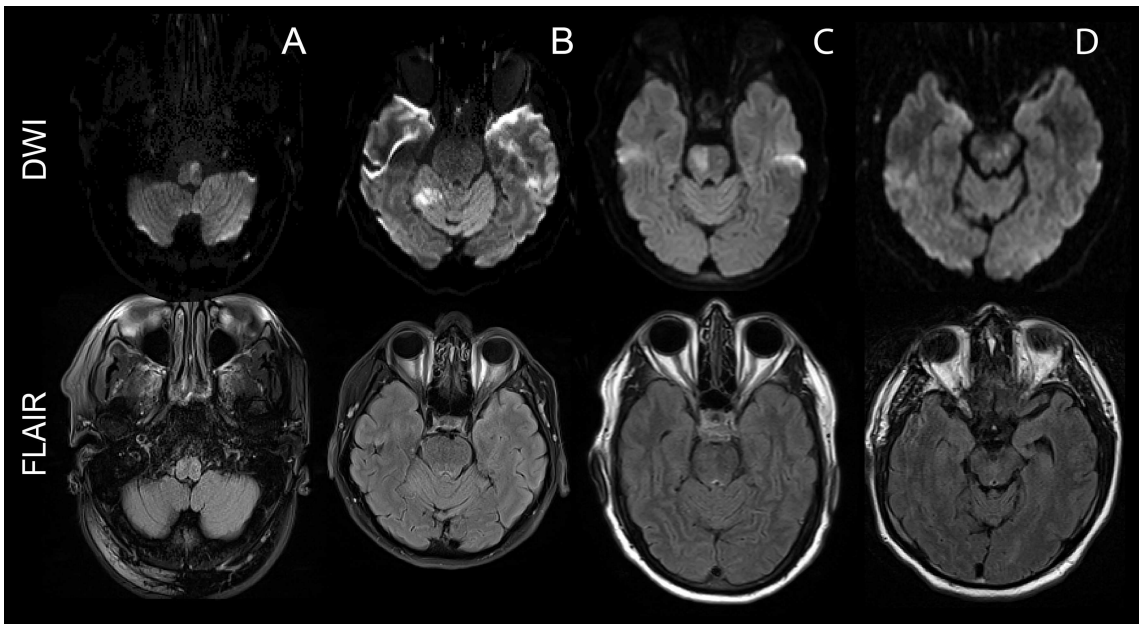

FIGURE 1 | Examples of infratentorial strokes in the intention-to-treat WAKE-UP cohort. The upper row shows diffusion-weighted images depicting the acute ischemic stroke. The bottom row shows a FLAIR image of the corresponding slice, depicting a lack of signal hyperintensity in the area of the acute stroke. The different columns offer examples for the different stroke locations included into this substudy: **(A)** depicts a stroke of the ventral left portion of the medulla oblongata, **(B)** a right-sided cerebellar stroke in the feeding territory of the superior cerebellar artery, **(C)** a right-sided stroke in the pons, and **(D)** a focal mesencephalic stroke.

TABLE 1 | Group comparison between patients with an infra- and a supratentorial localization of the acute stroke in the intention-to-treat population.

	Supratentorial stroke *N* = 455	Infratentorial stroke *N* = 48	
Mean age (years) (SD)	65.8 (11.3)	59.9 (12.2)	$p < 0.001$
Gender (male), *N* (%)	284 (62.4%)	41 (85.4%)	$p = 0.001$
Median symptom recognition to start of treatment (hours) (IQR)	3.1 (2.5–3.9)	3.2 (2.7–3.9)	$p = 0.227$
Arterial hypertension, *N* (%)	241 (53.0%)	25 (52.1%)	$p = 0.912$
Diabetes mellitus, *N* (%)	72 (15.8%)	10 (20.8%)	$p = 0.680$
Atrial fibrillation, *N* (%)	58 (12.8%)	1 (2.1%)	$p = 0.049$
Hypercholesterolemia, *N* (%)	160 (35.2%)	18 (37.2%)	$p = 0.387$
Median NIHSS at baseline (IQR)	6.0 (4.0–10.0)	4.5 (3.0–6.0)	$p = 0.001$
Median NIHSS at 7 days post-stroke (IQR)	2.0 (1.0–6.0)	2.0 (0.0–6.0)	$p = 0.156$
Median stroke volume at baseline (ml) (IQR)	2.6 (0.9–9.6)	0.8 (0.3–1.8)	$p < 0.001$
Median stroke volume at follow up (ml) (IQR)	3.5 (1.1–19.5)	0.8 (0.3–3.1)	$p < 0.001$

Follow up was 22–36 h after treatment.

fibrillation in patients with supratentorial strokes (106/1,278, 8%) as compared to patients with infratentorial strokes (1/84, 1%; $p = 0.03$). In infratentorial stroke patients, there were numerically fewer FLAIR positive lesions compared to the group with a supratentorial stroke localization (23/84, 27% as opposed to 479/1,278, 38%; $p = 0.09$).

Comparison of Infra- vs. Supratentorial Strokes in the Intention-to-Treat Population

Of the 503 patients who were randomized into the trial, 48 (9.5%) presented with a stroke in an infratentorial brain region. Twenty-six patients (54%) were assigned to placebo with one patient not having received infusion and 22 patients received alteplase (46%). Twenty-eight patients had an ischemic lesion in the pons (58%), nine patients had cerebellar stroke (19%), seven patients presented with an infarct in the medulla oblongata (15%), two patients had a stroke in the mesencephalon (4%), and two patients (4%) had strokes in more than one location (brainstem plus cerebellum); see **Figure 1** for examples. The distribution of lesion localization was extremely uniform between the placebo and the alteplase group. As in the overall screened population, patients randomized with infratentorial strokes were also younger, more often male and less severely affected at admission to hospital than those with supratentorial strokes (**Table 1**). However, at day 7 post-stroke, there was no longer a difference in the NIHSS scores between the groups (**Table 1**). As expected, the median baseline volume of infratentorial strokes was smaller than that of supratentorial strokes (0.8 vs. 2.6 ml; $p < 0.001$). There was no statistically significant difference in the percentage of patients reaching the primary efficacy endpoint in infratentorial stroke (25/47, 53%) vs. supratentorial stroke (208/443, 47%); $p = 0.45$. In addition, the rate of reaching the primary safety endpoint did not differ, with 6/47 (13%) patients in the infratentorial group and 72/443 (16%) patients in the supratentorial group ($p = 0.23$). There were no symptomatic

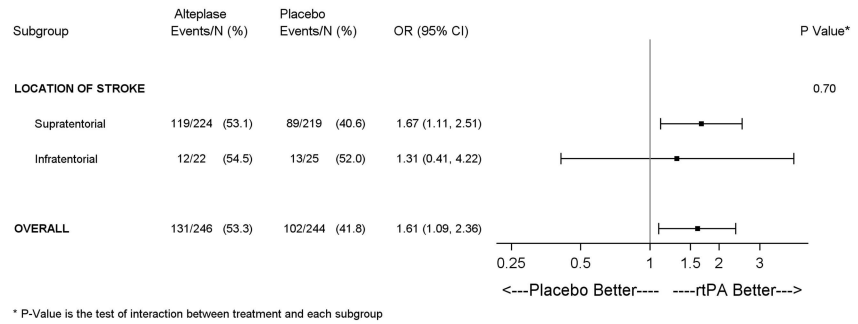

FIGURE 2 | Forest plots depicting the effect (adjusted OR) of alteplase on favorable outcome in patients with a supra- and infratentorial stroke with no evidence of a significant interaction between stroke localization and treatment effect.

intracerebral hemorrhages in the infratentorial patient group and only three patients experienced petechial hemorrhagic transformation (HI-1 and HI-2) (6%, as compared to 73/455 or 16% in the supratentorial group).

Treatment with alteplase was associated with higher odds of favorable outcome with no significant heterogeneity of treatment effect for stroke subtype (infratentorial vs. supratentorial). The adjusted OR for favorable outcome with alteplase was 1.31 (95% CI 0.41–4.22) in patients with infratentorial infarct and 1.67 (95% CI 1.11–2.51) in patients with supratentorial infarct (test for interaction, $p = 0.70$; see **Figure 2**).

Results in the Subpopulation of Infratentorial Strokes

Baseline parameters were comparable for patients randomized to receiving alteplase or placebo (**Table 2**). Favorable outcome was observed in 12 out of 22 patients (55%) in the alteplase group and in 13 out of 25 patients (52%) in the placebo group (adjusted OR, 1.38; 95% CI 0.42–4.56; $p = 0.60$). The 90 day distributions of mRS scores for the remaining categories were, for the alteplase and the placebo arm, respectively, five patients (23%) vs. seven patients (28%) with mRS of 2, two patients each (9 vs. 8%) for mRS of 3, two patients (9%) vs. one patient (4%) for mRS of 4 and one patient (5%) vs. two patients (8%) with an mRS of 5 or 6. We were unable to show a trend for a shift toward better outcomes in those infratentorial stroke patients treated with alteplase as compared to those who received placebo (adjusted common OR 1.19; 95% CI 0.41–3.42; $p = 0.75$). There were no SICH or deaths in the group of patients with infratentorial strokes, regardless of the administered treatment. The primary safety endpoint (death or mRS score 4–6 at day 90) occurred in three patients of the alteplase group (14%) and three patients in the placebo group (12%); $p = 0.74$. Petechial hemorrhagic transformation (HI-1 and HI-2) occurred in two patients (9%) who have received alteplase and one patient (4%) who received placebo ($p = 0.59$).

TABLE 2 | Comparison between patients who received alteplase and those who received placebo in the group of patients with an infratentorial stroke localization.

	Alteplase *N* = 22	**Placebo *N* = 26**	
Mean age (years) (SD)	62.6 (10.3)	57.7 (13.5)	$p = 0.230$
Gender (male), *N* (%)	20 (90.9%)	21 (80.8%)	$p = 0.429$
Median symptom recognition to start of treatment (hours) (IQR)	3.1 (2.6–3.6)	3.5 (2.9–4.0)	$p = 0.272$
Arterial hypertension, *N* (%)	12 (54.6%)	13 (50.0%)	$p = 0.780$
Diabetes mellitus, *N* (%)	7 (31.8%)	3 (11.5%)	$p = 0.152$
Atrial fibrillation, *N* (%)	1 (4.6%)	0 (0.0%)	$p = 0.205$
Hypercholesterolemia, *N* (%)	11 (50.0%)	7 (26.9%)	$p = 0.138$
Median NIHSS at baseline (IQR)	5.0 (3.0–8.0)	4.0 (3.0–5.0)	$p = 0.295$
Median NIHSS at 7 days post-stroke (IQR)	1.5 (0.0–6.0)	2.0 (1.0–4.0)	$p = 0.580$
Median stroke volume at baseline (ml) (IQR)	0.8 (0.2–1.9)	0.7 (0.3–1.3)	$p = 0.820$
Median stroke volume at follow up (ml) (IQR)	0.8 (0.3–3.1)	0.9 (0.4–3.1)	$p = 0.656$

Follow up was 22–36 h after treatment.

DISCUSSION

WAKE-UP demonstrated a clear clinical benefit of treatment with intravenous alteplase in patients with an acute ischemic lesion visible on DWI but not yet evidently visible on FLAIR imaging. In this secondary *post hoc* analysis, we focused on the treatment effect and further elucidated the clinical characteristics and outcome in a subpopulation of WAKE-UP patients with a brainstem or cerebellar DWI lesion. We did not observe heterogeneity of the treatment effect based on stroke localization. There was no difference in death or dependence between the two treatment arms and no symptomatic intracerebral hemorrhages occurred in our cohort of patients with infratentorial ischemic lesions. Thus, we do not recommend excluding patients with infratentorial stroke of unknown time of symptom onset with a DWI/FLAIR mismatch from intravenous thrombolysis. However, the analysis was unable to prove benefit of thrombolysis in the alteplase arm, presumably due to the fact that the trial was underpowered to demonstrate superiority in this context. An additional limitation of the study was the inclusion of patients with relatively mild strokes, making the generalization of the findings on patients suffering severe stroke difficult.

There were some clinical differences between the subgroups. Infratentorial stroke patients were younger and more frequently male as compared to patients with supratentorial stroke, which corresponds to trends reported in literature (4, 9). They also had lower baseline NIHSS scores and smaller stroke volumes, results which are logical and equally in line with previous observations (4, 9). The higher prevalence of atrial fibrillation in patients with supratentorial as opposed to infratentorial stroke, as found in our study, has similarly been previously reported (9). These findings could in part be explained by the younger age and male predominance in our cohort, as there is a known higher prevalence of atrial fibrillation in older women leading to an increased risk of severe cardioembolic strokes in the anterior circulation in females (10).

In the context of stroke of unknown onset, previous research has shown that ischemic lesions in infratentorial brain regions likely take longer to develop a FLAIR hyperintense signal (4) which subsequently implies that a proportion of patients treated based on the presence of a DWI-FLAIR mismatch are likely beyond the conventional 4.5 h time window for rt-PA. Justifiably, at the time of the study's conduct, this raised potential safety concerns. Our current analysis suggests that these concerns were unfounded as no deaths and no parenchymal hemorrhages (symptomatic or otherwise) occurred in our subgroup of patients with infratentorial strokes. This may in part be the effect of the mild stroke severity in our cohort, but is also in line with previous large cohort studies which have reported very low SICH rates in patients with isolated brainstem and cerebellar strokes (9, 11). Also befitting the literature (9, 11), the percentage of observed hemorrhagic transformations was lower in the subgroup of infratentorial as compared to supratentorial stroke patients, which comes to no surprise as it is often not associated with thrombolysis but rather dependent on stroke size and severity (12). This underlines the safety of patient selection for intravenous thrombolysis based on the DWI-FLAIR mismatch approach in infratentorial, mild to moderate stroke. Some previous studies have pointed to a lesser importance of the time to treatment (with regards to developing intracranial hemorrhage or unfavorable outcome) in posterior circulation strokes as compared to anterior circulation strokes (13). However, it is also conceivable that the stage of tissue damage depicted by a positive DWI but negative FLAIR signifies a condition in which thrombolysis is still safe, irrespective of the actual time which elapsed since the onset of ischemia. Other recent studies have equally pointed to the safety (as well as efficacy) of acute stroke treatment in the unknown and extended time window in carefully selected patient cohorts, further moving evidence away from time-based and toward tissue-based models and individually tailored therapy (14).

Our analysis did not show a difference between rt-PA and placebo on the treatment effect in patients with unknown onset stroke of infratentorial localization. There is a general lack of information on this subject in the literature, as very few randomized, controlled trials or phase IV studies evaluating the safety and efficacy of iv tPA in posterior circulation strokes are available (15, 16). Hence our findings, notwithstanding the small cohort size, represent knowledge novel and relevant to the field. Within the WAKE-UP study population itself, the percentage of infratentorial alteplase treated patients who reached favorable outcome was slightly higher than in the overall study cohort (54.5 vs. 53.3%) but the placebo group of infratentorial patients did remarkably well with 52% reaching favorable outcome (as opposed to only 41.8% of patients in the complete WAKE-UP population). This is not surprising as some preexisting studies have shown that up to 60% of patients with a stroke in the posterior circulation recover to the point of being able to carry out all usual duties and activities despite lack of treatment (16). This same study (16) identified a cutoff baseline NIHSS score of 5 or below as one with a high sensitivity and specificity for predicting favorable outcome in untreated patients with a posterior circulation stroke (and 75% of the placebo cohort in our current substudy had a baseline NIHSS of 5 or less). Hence, a high response rate in the placebo group of our study was to be expected, a fact which has arguably undermined the potential to detect treatment effect in some other previously conducted trials (17).

DATA AVAILABILITY

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the national competent authorities and ethics committees in all participating countries approved the study. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

IG: literature research, conception and design of the study, and manuscript drafting. FB: statistical analysis. IG, JBF, KV, BC, MEb, MEn, JF, IF, VT, RL, KM, NN, SP, CS, PR, CG, and GT: protocol development of the WAKE-UP study and patient recruitment. GT: ethical approval. All authors reviewed, edited, and approved the final manuscript.

ACKNOWLEDGMENTS

WAKE-UP Steering Committee: CG (Chair), GT (Coordinating Investigator), MEb, CS, SP, RL, NN, KM, IF, and MEn. This study was previously presented as an e-poster at the European Stroke Organization Conference 2019 in Milan, Italy. We acknowledge support from the German Research Foundation (DFG) and the Open Access Publication Fund of Charité–Universitätsmedizin Berlin. WAKE-UP received funding from the European Union Seventh Framework Program [FP7/2007–2013] under grant agreement no. 278276 (WAKE-UP).

REFERENCES

1. Thomalla G, Simonsen CZ, Boutitie F, Andersen G, Berthezene Y, Cheng B, et al. mri-guided thrombolysis for stroke with unknown time of onset. *N Engl J Med*. (2018) 379:611–22. doi: 10.1056/NEJMoa1804355
2. Thomalla G, Cheng B, Ebinger M, Hao Q, Tourdias T, Wu O, et al. DWI-FLAIR mismatch for the identification of patients with acute ischaemic stroke within 4·5 h of symptom onset (PRE-FLAIR): a multicentre observational study. *Lancet Neurol*. (2011) 10:978–86. doi: 10.1016/S1474-4422(11)70192-2
3. Aoki J, Kimura K, Iguchi Y, Shibazaki K, Sakai K, Iwanaga T. FLAIR can estimate the onset time in acute ischemic stroke patients. *J Neurol Sci*. (2010) 293:39–44. doi: 10.1016/j.jns.2010.03.011
4. Grosse-Dresselhaus F, Galinovic I, Villringer K, Audebert H, Fiebach JB. Difficulty of MRI based identification of lesion age by acute infra-tentorial ischemic stroke. *PLoS ONE*. (2014) 9:e92868. doi: 10.1371/journal.pone.0092868
5. National Institute of Neurological Disorders and Stroke rt_PA Stroke Study Group. Tissue plasminogen activator for acute ischemic stroke. *N Engl J Med*. (1995) 333:1581–7. doi: 10.1056/NEJM199512143332401
6. Wahlgren N, Ahmed N, Dávalos A, Ford GA, Grond M, Hacke W, et al. Thrombolysis with alteplase for acute ischaemic stroke in the Safe Implementation of Thrombolysis in Stroke-Monitoring Study (SITS-MOST): an observational study. *Lancet*. (2007) 369:275–82. doi: 10.1016/S0140-6736(07)60149-4
7. Larrue V, von Kummer RR, Mueller A, Bluhmki E. Risk factors for severe hemorrhagic transformation in ischemic stroke patients treated with recombinant tissue plasminogen activator: a secondary analysis of the European-Australasian Acute Stroke Study (ECASS II). *Stroke*. (2011) 32:438–41. doi: 10.1161/01.STR.32.2.438
8. Hacke W, Kaste M, Fieschi C, von Kummer R, Davalos A, Meier D, et al. Randomised double-blind placebo-controlled trial of thrombolytic therapy with intravenous alteplase in acute ischaemic stroke (ECASS II). Second European-Australasian Acute Stroke Study Investigators. *Lancet*. (1998) 352:1245–51. doi: 10.1016/S0140-6736(98)08020-9
9. Yoshimura S, Lindley RI, Carcel C, Sato S, Delcourt C, Wang X, et al. NIHSS cut point for prediction outcome in supra- vs infratentorial acute ischemic stroke. *Neurology*. (2018) 91: e1695–701. doi: 10.1212/WNL.0000000000006437
10. Reeves MJ, Bushnell CD, Howard G, Gargano JW, Duncan PW, Lynch G, et al. Sex differences in stroke: epidemiology, clinical presentation, medical care and outcomes. *Lancet Neurol*. (2008) 7:915–26. doi: 10.1016/S1474-4422(08)70193-5
11. Dornak T, Kral M, Hazlinger M, Herzig R, Veverka T, Burval S, et al. Posterior vs. anterior circulation infarction: demography, outcomes and frequency of hemorrhage after thrombolysis. *Int J Stroke*. (2015) 10:1224–8. doi: 10.1111/ijs.12626
12. Trouillas P, Kummer Rv. Classification and pathogenesis of cerebral hemorrhages after thrombolysis in ischemic stroke. *Stroke*. (2006) 37:556–61. doi: 10.1161/01.STR.0000196942.84707.71
13. Dornak T, Kral M, Sedlackova Z, Sanak D, Cechakova E, Divisova P, et al. Predictors of intracranial hemorrhage following intravenous thrombolysis in posterior circulation stroke. *Transl Stroke Res*. (2018) 9:582–8. doi: 10.1007/s12975-018-0608-0
14. Nogueira RG, Jadhav AP, Haussen DC, Bonafe A, Budzik RF, Bhuva P, et al. Thrombectomy 6 to 24 hours after stroke with a mismatch between deficit and infarct. *N Engl J Med*. (2018) 378:11–21. doi: 10.1056/NEJMoa1706442
15. Sarikaya H, Arnold M, Engelter ST, Lyrer PA, Mattle HP, Georgiadis D, et al. Outcomes of intravenous thrombolysis in posterior versus anterior circulation stroke. *Stroke*. (2011) 42:2498–502. doi: 10.1161/STROKEAHA.110.607614
16. Sato S, Toyoda K, Uehara N, Toratani C, Yokota H, Moriwaki H, et al. Baseline NIH Stroke Scale Score predicting outcome in anterior and posterior circulation strokes. *Neurology*. (2008) 70:2371–7. doi: 10.1212/01.wnl.0000304346.14354.0b
17. Hacke W, Furlan AJ, AL-Rawi Y, Davalos A, Fiebach JB, Gruber F, et al. Intravenous desmoteplase in patients with acute ischemic stroke selected by MR perfusion-diffusion weighted imaging or perfusion CT (DIAS-2): a prospective, randomized, double-blind, placebo-controlled study. *Lancet Neurol*. (2009) 8:141–50. doi: 10.1016/S1474-4422(08)70267-9

Conflict of Interest Statement: FB reports grants from University Medical Center Hamburg-Eppendorf during the conduct of the study. CG, GT, IG, JBF, KM, MEb, MEn, SP, and VT report grants from European Union Seventh Framework Program during the conduct of the study.

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.

2.5. A report from the WAKE UP study:

Influence of the extent of FLAIR hyperintense vessels on treatment effect

The following text corresponds to the abstract of the original paper:

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.

doi: 10.3389/fneur.2020.623881.

"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 (β = 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."

frontiers in Neurology

ORIGINAL RESEARCH
published: 04 February 2021
doi: 10.3389/fneur.2020.623881

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

Anne Sophie Grosch[1†], Anna Kufner[1,2,3†], Florent Boutitie[4,5,6], Bastian Cheng[7], Martin Ebinger[1,8], Matthias Endres[1,2,3,9,10,11], Jochen B. Fiebach[1], Jens Fiehler[12], Alina Königsberg[7], Robin Lemmens[13,14,15], Keith W. Muir[16], Norbert Nighoghossian[17], Salvador Pedraza[18], Claus Z. Siemonsen[19], Vincent Thijs[20,21], Anke Wouters[13,14,15], Christian Gerloff[7], Götz Thomalla[7] and Ivana Galinovic[1] on behalf of the WAKE-UP Investigators*

[1] Center for Stroke Research Berlin, Charité-Universitätsmedizin Berlin, Berlin, Germany, [2] Klinik und Hochschulambulanz für Neurologie, Charité-Universitätsmedizin Berlin, Berlin, Germany, [3] Berlin Institute of Health (BIH), Berlin, Germany, [4] Hospices Civils de Lyon, Service de Biostatistique, Lyon, France, [5] Université Lyon 1, Villeurbanne, France, [6] Centre National de la Recherche Scientifique, Unité Mixte de Recherche 5558, Laboratoire de Biométrie et Biologie Evolutive, Equipe Biostatistique-Santé, Villeurbanne, France, [7] Department of Neurology, Head and Neurocenter, University Medical Center Hamburg-Eppendorf, Hamburg, Germany, [8] Department of Neurology, Medical Park Berlin Humboldtmühle, Berlin, Germany, [9] German Centre for Cardiovascular Research (DZHK), Berlin, Germany, [10] German Center for Neurodegenerative Diseases (DZNE), Berlin, Germany, [11] Excellence Cluster NeuroCure, Charite-Universitätsmedizin Berlin, Berlin, Germany, [12] Department of Diagnostic and Interventional Neuroradiology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany, [13] Department of Neurology, University Hospitals Leuven, Leuven, Belgium, [14] Department of Neurosciences, Experimental Neurology, Katholieke Universiteit Leuven-University of Leuven, Leuven, Belgium, [15] Laboratory of Neurobiology, Center for Brain & Disease Research, Flanders Institute for Biotechnology, Leuven, Belgium, [16] Institute of Neuroscience & Psychology, University of Glasgow, Glasgow, United Kingdom, [17] Department of Stroke Medicine, Claude Bernard University Lyon 1, CREATIS National Center for Scientific Research Mixed Unit of Research 5220-National Institute of Health and Medical Research U1206, National Institute of Applied Sciences of Lyon, Lyon Civil Hospices, Lyon, France, [18] Department of Radiology, Girona Institute of Biomedical Research, Institute of Diagnostic Imaging, Dr. Josep Trueta Hospital, Girona, Spain, [19] Department of Neurology, Aarhus University Hospital, Aarhus, Denmark, [20] Stroke Theme, Florey Institute of Neuroscience and Mental Health, University of Melbourne, Heidelberg, VIC, Australia, [21] Department of Neurology, Austin Health, Heidelberg, VIC, Australia

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

OPEN ACCESS

Edited by:
Maurizio Acampa, Siena University Hospital, Italy

Reviewed by:
Raffaele Ornello, University of L'Aquila, Italy
Svetlana Lorenzano, Sapienza University of Rome, Italy
Manuel Cappellari, Integrated University Hospital Verona, Italy

***Correspondence:**
Ivana Galinovic
ivana.galinovic@charite.de

[†] These authors have contributed equally to this work

Specialty section:
This article was submitted to Stroke, a section of the journal Frontiers in Neurology

Received: *30 October 2020*
Accepted: *29 December 2020*
Published: *04 February 2021*

Citation:
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 and Galinovic I (2021) Extent of FLAIR Hyperintense Vessels May Modify Treatment Effect of Thrombolysis: A Post hoc Analysis of the WAKE-UP Trial. Front. Neurol. 11:623881. doi: 10.3389/fneur.2020.623881

($p = 0.971$) 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.

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.

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

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 FHVs 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 FHVs 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 Tmax >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 FHVs. FHVs 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 FHVs was defined as the number of slices with visible FHVs multiplied by FLAIR slice thickness. Inter-rater agreement for the presence of FHVs 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 FHVs (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 FHVs (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 FHVs, treatment group, and age) as well as for good outcome defined as mRS <2 at 90 days poststroke (adjustment for 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

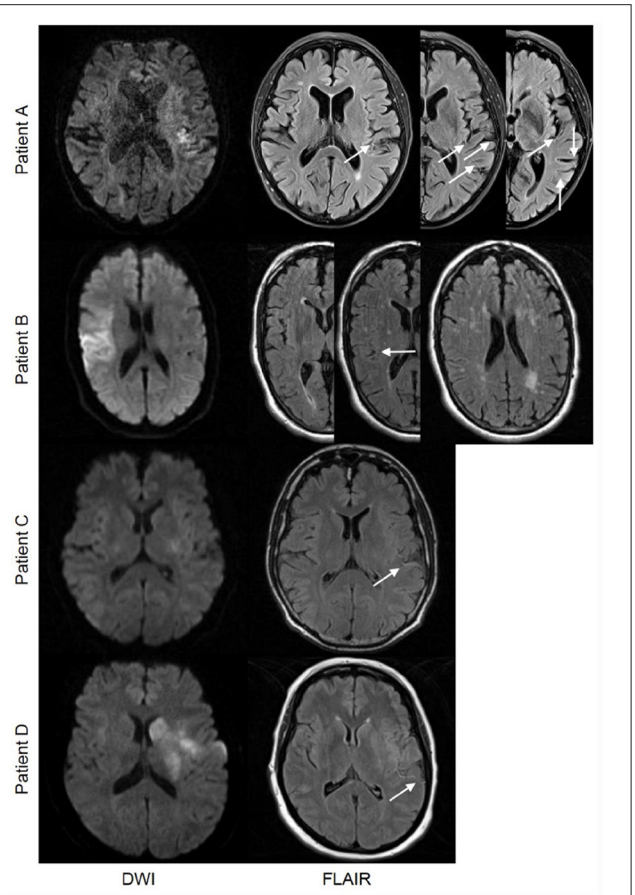

FIGURE 1 | Patient A and Patient B represent two cases at different ends of the spectrum of the extent of FLAIR hyperintense vessels (FHVs). Patient A is a 69-year-old female with a left-sided M2 branch occlusion and baseline FHV extent of 60 (multiple linear and serpentine vessels visible surrounding 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.

treatment). Linear regression analysis was performed for volume of hypoperfused tissue (adjustment for baseline extent of FHVs) 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 FHVs 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 FHVs visible on baseline FLAIR, and median extent of FHVs was 30.0 (IQR 21.3–39.0). Of the 25 patients without baseline FHVs, 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 FHVs at baseline ($n = 127$), and the median extent of FHVs was 30.0 (IQR 24.0–40.0). Patients with extensive FHVs did not differ from patients with few FHVs 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 FHVs (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 FHVs having significantly larger hypoperfused areas at baseline. The occlusion site also differed significantly between few and extensive FHVs, with extensive FHVs being associated with proximal vessel occlusions ($p < 0.001$). Patients with few and extensive FHVs 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 FHVs 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 FHVs, 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 FHVs 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 FHVs was treated as a continuous variable in tPA-treated patients, the probability of good outcome was relatively stable across the entire range of FHVs. However, in patients receiving placebo, there was a very low likelihood of a good outcome with less prominent FHVs, 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 FHVs 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 FHVs 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 FHVs on follow-up, whereas 33 patients showed a reduction in FHVs 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 FHVs 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).

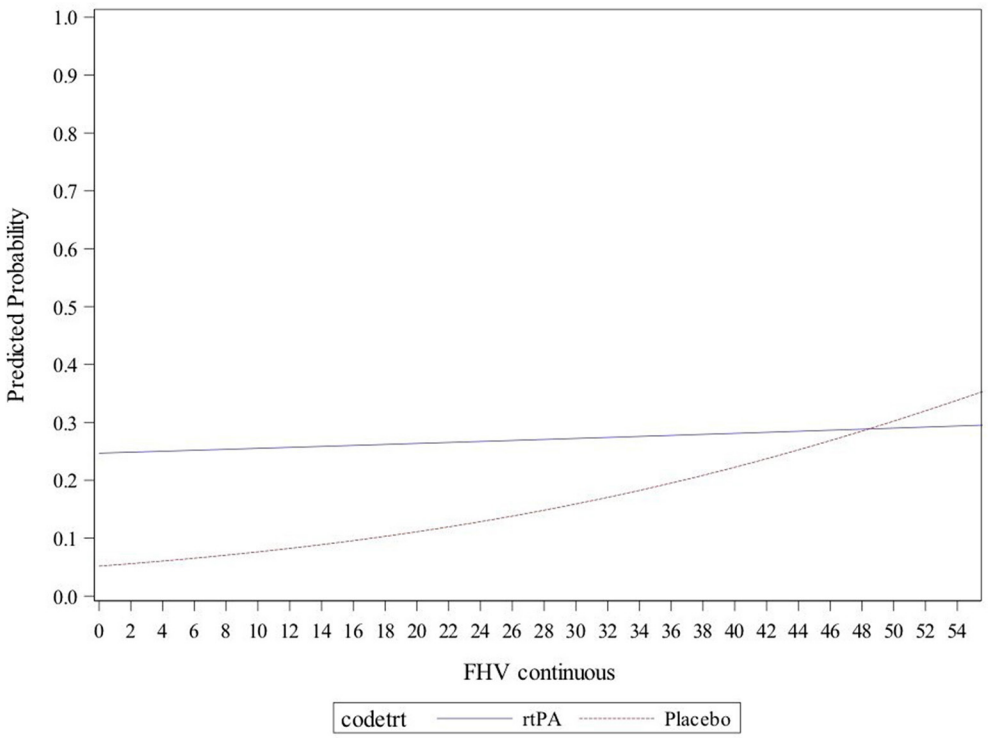

FIGURE 2 | Predicted probability of good clinical outcome [modified Rankin Scale (mRS) < 2] in patients grouped according to treatment, plotted against the extent of FLAIR hyperintense vessels (FHVs) on baseline imaging. The continuous blue line represents recombinant tissue plasminogen activator (rtPA)-treated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

DISCUSSION

In the current study, the extent of FHVs 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 FHVs had higher odds of achieving a good outcome following treatment with rtPA. In other words, the extent of FHVs 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 FHVs ipsilateral to the ischemic lesion on baseline imaging. Extent of FHVs 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 FHVs (**Table 1**). Similar results were previously reported, showing an association between FHVs 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 FHVs 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 FHVs in ischemic stroke (7); in a pooled sample of over 3,000 patients, there was no association between functional outcome and extent

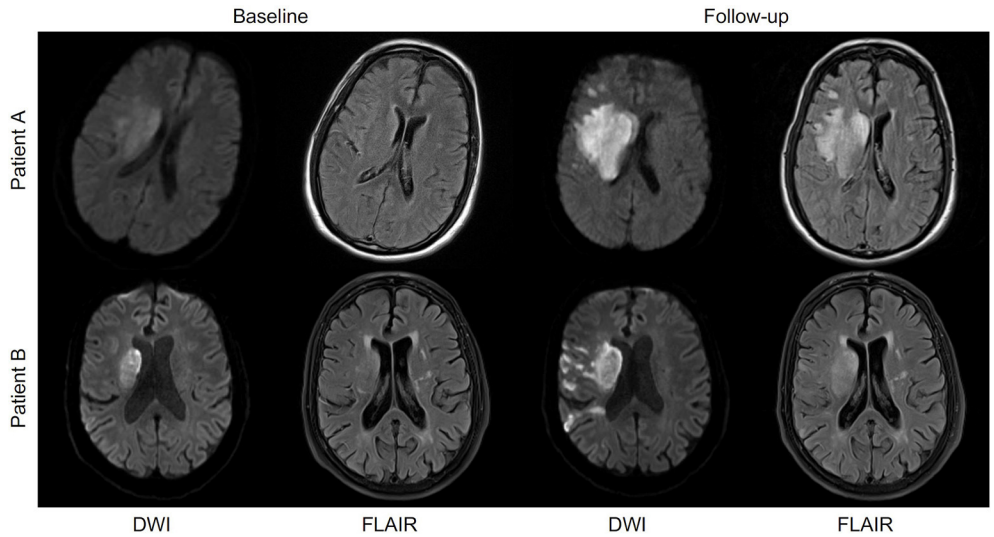

FIGURE 3 | Patients A and B represent cases with comparable stroke evolution yet differing evolution of FLAIR hyperintense vessels (FHVs) between baseline and follow-up imaging. Both 64-year-old males with a right-sided M1 occlusion. Patient A additionally had an occlusion of the ipsilateral internal 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 FHVs 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 FHVs between the patients, with patient A showing no more visible FHVs at follow-up, whereas the extent of FHVs 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 FHVs. 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 FHVs at baseline as well as the opposite (patients with identical occlusions and similarly pronounced FHVs yet different clinical and imaging stroke severities) (**Figure 3**).

Interestingly, the extent of baseline FHVs 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 FHVs (**Figure 2**). This might point to a protective component of prominent FHVs, 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 FHVs 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 FHVs 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 FHVs over time in ∼64% of patients (5, 22), and this reduction was independently associated with successful recanalization. In other words, early reduction in FHVs 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 FHVs 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 FHVs (**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 FHVs have not observed sex-specific differences in terms of the extent of FHVs 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 FHVs 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 FHVs 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.

FUNDING

MEn received funding from DFG under Germany's Excellence Strategy–EXC-2049–390688087, BMBF, DZNE, DZHK, EU, Corona Foundation, and Fondation Leducq. The main clinical trial, WAKE-UP, was funded by a grant from the European Union Seventh Framework Program (278276). AKu is a participant in the BIH-Charité Junior Clinical Scientist Program funded by the Charité–Universitätsmedizin Berlin and the Berlin Institute of Health. We acknowledge support from the German Research Foundation (DFG) and the Open Access Publication Fund of Charité–Universitätsmedizin Berlin. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

REFERENCES

1. Nave AH, Kufner A, Bücke P, Siebert E, Kliesch S, Grittner U, et al. Hyperintense vessels, collateralization, and functional outcome in patients with stroke receiving endovascular treatment. *Stroke.* (2018) 49:675–81. doi: 10.1161/STROKEAHA.117.019588
2. Kufner A, Galinovic I, Ambrosi V, Nolte CH, Endres M, Fiebach JB, et al. Hyperintense vessels on FLAIR: hemodynamic correlates and response to thrombolysis. *Am J Neuroradiol.* (2015) 36:1426–30. doi: 10.3174/ajnr.A4320
3. Yoshioka K, Ishibashi S, Shiraishi A, Yokota T, Mizusawa H. Distal hyperintense vessels on FLAIR images predict large-artery stenosis in patients with transient ischemic attack. *Neuroradiology.* (2013) 55:165–9. doi: 10.1007/s00234-012-1092-y
4. Cheng B, Ebinger M, Kufner A, Köhrmann M, Wu O, Kang D-W, et al. Hyperintense vessels on acute stroke fluid-attenuated inversion recovery imaging: associations with clinical and other MRI findings. *Stroke.* (2012) 43:2957–61. doi: 10.1161/STROKEAHA.112.658906
5. Hohenhaus M, Schmidt WU, Brunecker P, Xu C, Hotter B, Rozanski M, et al. FLAIR vascular hyperintensities in acute ICA and MCA infarction: a marker for mismatch and stroke severity? *Cerebrovasc Dis.* (2012) 34:63–9. doi: 10.1159/000339012
6. Olindo S, Chausson N, Joux J, Saint-Vil M, Signate A, Edimonana-Kaptue M, et al. Fluid-attenuated inversion recovery vascular hyperintensity: an early predictor of clinical outcome in proximal middle cerebral artery occlusion. *Arch Neurol.* (2012) 69:1462–8. doi: 10.1001/archneurol.2012.1310
7. Zhou Z, Malavera A, Yoshimura S, Delcourt C, Mair G, Al-Shahi Salman R, et al. Clinical prognosis of FLAIR hyperintense arteries in ischaemic stroke patients: A systematic review and meta-analysis. *J Neurol Neurosurg Psychiatry.* (2020) 91:475–482. doi: 10.1136/jnnp-2019-322625
8. Huang X, Liu W, Zhu W, Ni G, Sun W, Ma M, et al. Distal hyperintense vessels on flair: a prognostic indicator of acute ischemic stroke. *Eur Neurol.* (2012) 68:214–20. doi: 10.1159/000340021
9. Pérez De La Ossa N, Hernández-Pérez M, Domènech S, Cuadras P, Massuet A, Millán M, et al. Hyperintensity of distal vessels on FLAIR Is associated with slow progression of the infarction in acute ischemic stroke. *Cerebrovasc Dis.* (2012) 34:376–84. doi: 10.1159/000343658
10. Liu W, Xu G, Yue X, Wang X, Ma M, Zhang R, et al. Hyperintense vessels on FLAIR: a useful non-invasive method for assessing intracerebral collaterals. *Eur J Radiol.* (2011) 80:786–91. doi: 10.1016/j.ejrad.2010.09.043
11. Lee KY, Latour LL, Luby M, Hsia a. W, Merino JG, Warach MpS. Distal hyperintense vessels on FLAIR: an MRI marker for collateral circulation in acute stroke? *Neurology.* (2009) 72:1134–9. doi: 10.1212/01.wnl.0000345360.80382.69
12. Ebinger M, Kufner A, Galinovic I, Brunecker P, Malzahn U, Nolte CH, et al. Fluid-attenuated inversion recovery images and stroke outcome after thrombolysis. *Stroke.* (2012) 43:539–42. doi: 10.1161/STROKEAHA.111.632026
13. Azizyan A, Sanossian N, Mogensen MA, Liebeskind DS. Fluid-attenuated inversion recovery vascular hyperintensities: an important imaging marker for cerebrovascular disease. *Am J Neuroradiol.* (2011) 32:1771–5. doi: 10.3174/ajnr.A2265
14. Thomalla G, Simonsen CZ, Boutitie F, Andersen G, Berthezene Y, Cheng B, et al. MRI-Guided thrombolysis for stroke with unknown time of onset. *N Engl J Med.* (2018) 16;379:611–22. doi: 10.1056/NEJMoa1804355
15. Gawlitza M, Gragert J, Quäschling U, Hoffmann KT. FLAIR-hyperintense vessel sign, diffusion-perfusion mismatch and infarct growth in acute ischemic stroke without vascular recanalisation therapy. *J Neuroradiol.* (2014) 41:227–33. doi: 10.1016/j.neurad.2013.10.004
16. Haussen DC, Koch S, Saraf-Lavi E, Shang T, Dharmadhikari S, Yavagal DR. FLAIR distal hyperintense vessels as a marker of perfusion-diffusion mismatch in acute stroke. *J Neuroimaging.* (2013) 23:397–400. doi: 10.1111/j.1552-6569.2012.00784.x
17. Rai A, Cline B, Williams E, Carpenter J, Roberts T. Intravenous thrombolysis outcomes in patients presenting with large vessel acute ischemic strokes-CT angiography-based prognosis. *J Neuroimaging.* (2015) 25:238–42. doi: 10.1111/jon.12126
18. Nogueira RG, Jadhav AP, Haussen DC, Bonafe A, Budzik RF, Bhuva P, et al. Thrombectomy 6 to 24 hours after stroke with a mismatch between deficit and infarct. *N Engl J Med.* (2018) 378:11–21. doi: 10.1056/NEJMoa1706442
19. Albers GW, Marks MP, Kemp S, Christensen S, Tsai JP, Ortega-Gutierrez S, et al. Thrombectomy for stroke at 6 to 16 hours with selection by perfusion imaging. *N Engl J Med.* (2018) 378:708–18. doi: 10.1056/NEJMoa1713973
20. Maeda M, Koshimoto Y, Uematsu H, Yamada H, Kimura H, Kawamura Y, et al. Time course of arterial hyperintensity with fast fluid-attenuated inversion-recovery imaging in acute and subacute middle cerebral arterial infarction. *J Magn Reson Imaging.* (2001) 13:987–90. doi: 10.1002/jmri.1142
21. Seo K-D, Lee KO, Choi Y-C, Kim W-J, Lee K-Y. Fluid-attenuated inversion recovery hyperintense vessels in posterior cerebral artery infarction. *Cerebrovasc Dis Extra.* (2013) 3:46–54. doi: 10.1159/000350459
22. Sakuta K, Saji N, Aoki J, Sakamoto Y, Shibazaki K, Iguchi Y, et al. Decrease of hyperintense vessels on fluid-attenuated inversion recovery predicts good outcome in t-PA patients. *Cerebrovasc Dis.* (2016) 41:211–8. doi: 10.1159/000443533
23. Fatemi F, Kantarci K, Graff-Radford J, Preboske GM, Weigand SD, Przybelski SA, et al. Sex differences in cerebrovascular pathologies on FLAIR in cognitively unimpaired elderly. *Neurology.* (2018) 90:e466–73. doi: 10.1212/WNL.0000000000004913
24. Galinovic I, Ostwaldt AC, Soemmer C, Bros H, Hotter B, Brunecker P, et al. Search for a map and threshold in perfusion MRI to accurately predict tissue fate: a protocol for assessing lesion growth in patients with persistent vessel occlusion. *Cerebrovasc Dis.* (2011) 32:186–93. doi: 10.1159/000328663

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 EISAI outside the submitted work. MEn 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.

3. Discussion

3.1. The results of the WAKE UP trial

After more than a year of preparation, the WAKE UP trial enrolled its first patient at UKE Hamburg in October 2012. The trial was subsequently conducted over a period of nearly five years at 70 sites in eight European countries (Austria, Belgium, Denmark, France, Germany, Spain, the Netherlands and United Kingdom), screening a total of 1362 patients. Approximately two-thirds of the screened patients were not randomized into the trial, primarily due to a failure to pass the imaging criteria or due to the presence of a large vessel occlusion with planned endovascular treatment. Patients were assigned in a 1:1 ratio to either the treatment arm (0.9 mg of alteplase per kilogram of body weight) or the placebo arm. Randomization was further stratified according to age (≤60 or >60 years) and stroke severity as assessed on the National Institute of Health Stroke Scale (NIHSS) ≤10 or >10[21]. The last patient was enrolled on the 30th of June 2017, bringing the number of successfully randomized patients (deemed eligible for thrombolysis and judged as being approximately within 4.5 hours of symptom onset as based on the DWI-FLAIR mismatch) to 503. As individuals with large vessel occlusion were often excluded from the study, WAKE UP primarily recruited patients with mild to moderate stroke severity, with the median NIHSS at the time of the enrollment being 6 in both the placebo and the tPA group. The primary outcome was measured using the Modified Rankin Score (mRS), the most widely used metric in clinical trials involving stroke. The scores range from 0 to 6 and are defined as follows: 0 = no symptoms, 1 = symptoms yet no significant disability, 2 = slight disability but able to look after self, 3 = moderate disability requiring assistance, 4 = moderately severe disability with inability to walk unassisted, 5 = severe disability requiring continuous care and 6 = death. Favorable outcome, defined as a score of 0 or 1 on the mRS at 90 days, was reached by 131 of 246 patients (53.3%) in the alteplase group and in 102 of 244 patients (41.8%) in the placebo group, giving an adjusted odds ratio of 1.61 (95% confidence interval [CI], 1.09 to 2.36; P=0.02) in favor of treatment with tPA[21]. This translated to a rate of freedom from neurologic deficit or major disability 11.5% higher in the tPA group as compared to the placebo group. In addition, there was a clear shift toward better clinical outcomes in all categories on the mRS scale for patients treated with alteplase vs those who received placebo[21] (Figure 4).

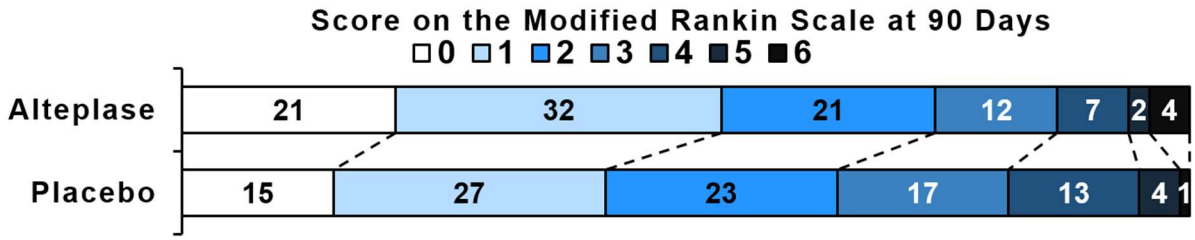

Figure 4. The distribution of mRS 90 days post-stroke for the intention-to-treat population. The numbers are not absolute patient numbers but expressed as percentages of patients. The difference in favor of the alteplase group in the overall distribution of scores was statistically significant (adjusted common odds ratio, 1.62; 95% confidence interval, 1.17 to 2.23; P=0.003). Reproduced with permission from (Thomalla G, Simonsen CZ, Boutitie F, Andersen G, Berthezene Y, Cheng B, Cheripelli B, Cho TH, Fazekas F, Fiehler J, Ford I, Galinovic I, Gellissen S, Golsari A, Gregori J, Günther M, Guibernau J, Häusler KG, Hennerici M, Kemmling A, Marstrand J, Modrau B, Neeb L, Perez de la Ossa N, Puig J, Ringleb P, Roy P, Scheel E, Schonewille W, Serena J, Sunaert S, Villringer K, Wouters A, Thijs V, Ebinger M, Endres M, Fiebach JB, Lemmens R, Muir KW, Nighoghossian N, Pedraza S, Gerloff C; WAKE UP Investigators. MRI-Guided Thrombolysis for Stroke with Unknown Time of Onset. N Engl J Med. 2018 Aug 16;379(7):611-622. doi: 10.1056/NEJMoa1804355. Epub 2018 May 16. PMID: 29766770), Copyright Massachusetts Medical Society.

The primary safety endpoint, death or inability to live independently (score on the mRS 4 to 6), occurred in 33 of 244 patients (13.5%) in the alteplase group and 44 of 241 patients (18.3%) in the placebo group (adjusted odds ratio, 0.68; 95% CI, 0.39 to 1.18; P=0.17)[21]. Deaths and symptomatic intracranial hemorrhage of the parenchymal hematoma type 2 (as defined by a bleed exceeding 30% of the infarct area on MRI) were more frequent in the alteplase group than in the placebo group, a finding that was consistent with many previous thrombolysis trials and is a known risk of this type of treatment. Because the trial was stopped prior to reaching its target number of recruited patients, the safety aspect of the study must be interpreted with caution, as the observed trend toward a higher rate of death in the alteplase group may have become significant with a larger sample size[21]. The WAKE UP trial did however prove the efficacy of using tPA in imaging-selected patients with minor or moderate acute ischemic stroke of unknown onset. The novelty and singular importance of the trial was in showing benefit of reperfusion treatment in patients who would otherwise not have been eligible for this type of acute stroke therapy.

3.2. DWI-FLAIR mismatch in patient subgroups

In the wake of the trial, its investigators have looked at several subgroups in order to ascertain the validity of the DWI-FLAIR mismatch concept for treatment selection in these subpopulations. The first such subgroup to be investigated was patients with lacunar infarctions. Half a year after the main trial results were published, Ewgenia Barow from UKE published a subanalysis in JAMA Neurology which showed more favorable outcomes in patients with lacunar strokes treated with tPA as compared to patients receiving placebo[22]. Several months later, my subanalysis of patients with an infra-tentorial stroke location followed, motivated by a previous finding[23] concerning the potential unreliability of using the DWI-FLAIR mismatch as a clock in acute ischemic infra-tentorial stroke. Although the sample of my study was small, it hinted at the possibility that lesions in this location take longer to develop a visible FLAIR signal than those located supra-tentorially, a finding that seemed to be more pronounced in brainstem than cerebellar stroke[23]. Irrespective of this, the decision of the WAKE UP consortium was to continue allowing patients with infra-tentorial infarcts to be randomized in the WAKE UP trial. At the trials conclusion, the final number of these patients was comparatively low (48 out of 503 randomized patients, or just under 10%), which was representative of an equally low percentage of patients with infra-tentorial strokes (6%) amongst the screened population[25]. In line with the suspicion that such patients may present with a DWI-FLAIR mismatch for longer, a higher percentage of patients in the screened cohort of WAKE UP with supra-tentorial strokes had their lesions already visible on FLAIR (DWI-FLAIR match) as compared to the group of infra-tentorial stroke patients (38% vs. 27%; p = 0.09)[24]. However, we did not observe any safety issues associated with thrombolysis in this cohort and there was no difference in death or dependence between the patients receiving tPA and those receiving placebo. Also, no patient developed symptomatic intracranial hemorrhage and the rate of petechial hemorrhagic transformation was lower in the infra-tentorial than in the supra-tentorial group[24]. Statistically, we found no significant heterogeneity of treatment effect based on stroke localization and treatment with tPA was associated with higher odds of favorable outcome (1.31; 95% CI 0.41–4.22), albeit with a wide confidence interval[24]. Consequently, we were unable to prove the efficacy of thrombolysis for this cohort, a result surely influenced by the small cohort size with a general lack of power for such an analysis as well as the high response rate in the placebo group.

WAKE UP spawned many other sub analyses too. These showed, for example, the association between tPA treatment and lower odds of post-stroke depression[25] and a potential benefit for health-related quality of life[26] as well as higher odds of favorable outcome despite chronic kidney disease[27] or the presence of cerebral microbleeds[28]. A pre-specified post-hoc subgroup analysis investigated the controversial "smoking paradox" which stipulates that there is a benefit of current smoking for functional outcome after ischemic stroke. Although current smokers in the WAKE UP population benefited from thrombolysis the same as non-smokers or ex-smokers, there was a trend for them to

have worse functional outcome than non-smokers in adjusted mRS shift analyses, thus casting doubt on the smoking paradox[29]. Another notable substudy revealed a similar incidence and impact of hemorrhagic transformation in patients with unknown-onset stroke as in trials conducted with a known early time window[30], proving the comparable safety of tPA in these two groups. Finally, additional published WAKE UP sub-analyses proved the cost-effectiveness[31] of MRI-guided tPA as well as treatment benefit in patients on antiplatelet therapy[32] or with polypharmacy[33] at the time of stroke.

Some subanalyses looked at additional imaging criteria that might serve to modify treatment response. For example, one such imaging biomarker was the PWI-DWI mismatch, defined as the presence of ischemic-but-not-yet-infarcted tissue on perfusion weighted imaging (PWI) surpassing the area of irreversibly infarcted tissue on DWI; an imaging criterion frequently used in recent clinical trials. One prespecified post hoc analysis of the WAKE-UP trial investigated both the PWI-DWI and the DWI-FLAIR mismatch paradigms in parallel. It found that in the cohort of 208 randomized WAKE UP patients who received PWI, the PWI-DWI mismatch status (defined as ischemic core volume < 70ml, mismatch volume > 10ml, and mismatch ratio > 1.2) did not modify treatment response[34], a result whose interpretation is, however, limited due to lack of power. It bears mentioning that of 431 screened patients with available PWI at baseline, a DWI-FLAIR mismatch was identified in 48% of patients vs only 26% of patients who had a PWI-DWI mismatch[34]. So, although using PWI-DWI mismatch as the sole inclusion criterion (instead of the DWI-FLAIR mismatch) would have led to a lower percentage of eligibility in the WAKE UP trial, a significant additional percentage of patients (13%) would have been eligible for treatment had the presence of either of the two MRI-based mismatch paradigms been accepted as inclusion criteria[34].

Another study, which I conducted, examined a further imaging biomarker which is frequently observed on MRI in acute ischemic stroke, the so-called FLAIR hyperintense vessels sign (FHV). FHV are linear or serpentine hyperintensities appearing on the FLAIR image distal to the site of an occluded vessel. They represent collateral circulation made visible through the effects of slow retrograde flow distal to an occluded vessel and are associated with areas of reduced cerebral perfusion[35] (Figure 5).

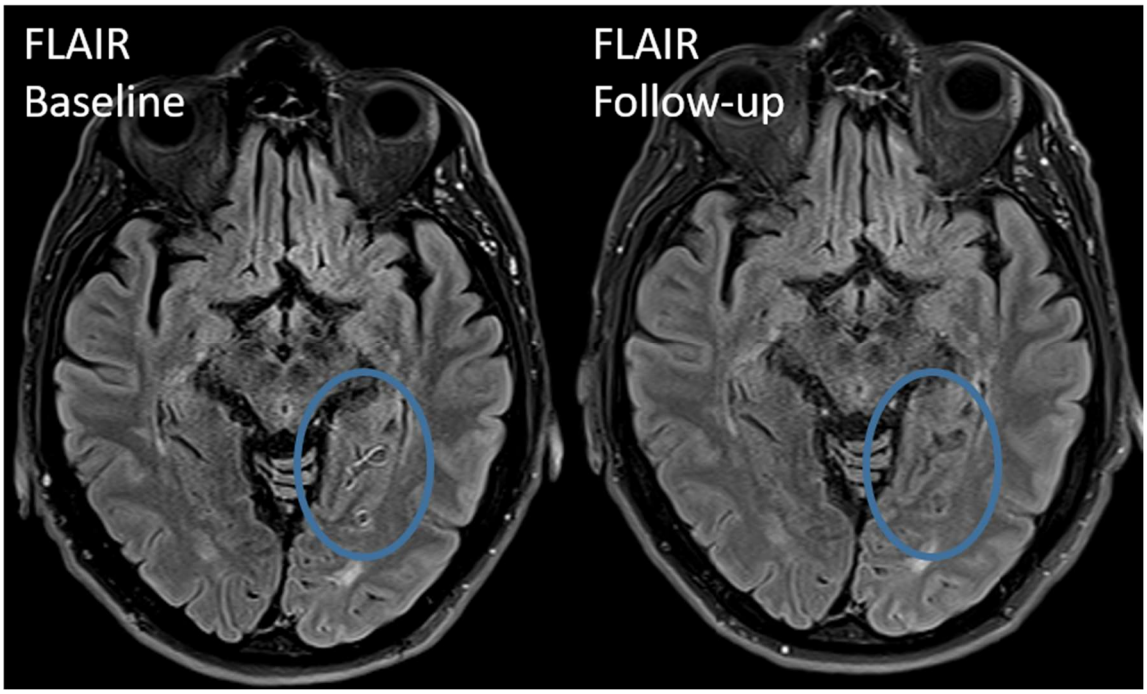

Figure 5. Example of FHV in a patient with an acute ischemic stroke caused by an occlusion of the P3 segment of the left posterior cerebral artery. In the baseline MRI the collaterals are made visible on the FLAIR due to sluggish flow (highlighted in the blue oval). The patient experienced successful recanalization of the occluded vessel segment following tPA and, on follow-up imaging done the next day, the collaterals were no longer visible.

Whether their presence is a good or a bad sign for the acute stroke patient remained an open question due to contradictory results of previous studies[35]. We therefore set out to elucidate the diagnostic and therapeutic value of this imaging marker in the WAKE UP patient cohort. Included were 165 patients with a confirmed unilateral occlusion of a single intracranial vessel[36]. We were unable to find a connection between the extent of FHV and severity of stroke symptoms or stroke size. A clear correlation was also missing between FHV and stroke progression or functional recovery[36]. This was in line with the results of a systematic review of FHV in ischemic stroke published in early 2020, which also found no convincing association between functional outcome and extent of FHV[35]. However, we did find that the extent of baseline FHV modified the treatment effect of thrombolysis, making it more effective in patients with fewer collaterals[36]. This could be explained through a protective influence of FHV, especially in the early hours after stroke onset, making patients who were unable to quickly recruit an extensive collateral network more dependent on tPA[36].

In summary, patient selection for tPA based on “tissue-clocking” using the DWI-FLAIR mismatch was proven safe and effective in a randomized clinical trial. Taking into consideration the results of WAKE UP’s many subsequent sub-analyses, the concept of using an MRI-based imaging biomarker of lesion age appeared safe and at least potentially if not undeniably beneficial to patients, irrespective of the underlying stroke etiology or patient comorbidities.

3.3. “Tissue clocking” using the DWI-FLAIR concept

WAKE UP was also not the only clinical trial using the DWI-FLAIR mismatch criterion to randomize and treat patients with ischemic stroke of unknown onset. Following the end of the WAKE UP trial, the Japanese “mirror” study THAWS[37], which was running in parallel since 2014, was prematurely terminated. It showed no effect on its 131 participants, which could easily be due to the small sample size and the low median size of strokes (2.5ml) in the overall cohort, as a sub-study revealed benefit for tPA treatment in their subpopulation of patients with larger strokes (above 6.4ml)[38]. MR-WITNESS[39] was another trial to employ the DWI-FLAIR mismatch in order to offer thrombolysis in a population of patients with unknown stroke onset. This open-label, phase 2a, prospective study, partially running in parallel to WAKE UP, terminated in 2015 having reached its predetermined sample size of 80 patients and proving the safety of tPA in its cohort.

Considering the mounting body of evidence, it stands to reason that in late 2018 and early 2019, months following the publication of the WAKE UP trial results, both the European[40] and the American guidelines[41] for the early management of patients with acute ischemic stroke were changed to include the recommendation to treat patients with unknown onset stroke based on advanced (MRI) brain imaging and the DWI-FLAIR mismatch. So, “tissue clocking” as a patient selection concept for extending the scope of thrombolytic treatment has stood up to scientific rigor and proven itself in the field. Its foundation was using FLAIR, a T2-weighted sequence with nulled cerebrospinal fluid signal, to approximate the time from stroke onset on the basis of an observed phenomenon of rising T2-signal intensities with elapsed time. This relationship is, however, not necessarily linear nor is its analysis straightforward. The possibly easiest way to evaluate signal intensity on an image is to apply “old school radiology” and simply look at it. This, although quick and technically least demanding, has been shown as having a worryingly low interrater agreement, stemming from the subjectivity of the method. Attempts to make the evaluation more objective through measuring absolute or relative signal intensities, whether in small selected areas of the acute stroke lesion or in its entirety, were surprisingly unable to improve diagnostic accuracy[42] and are therefore typically not in use. Overall, this leaves us with a working, albeit imperfect, method of visual assessment. To make things more complex, the thing that we are visually assessing here does not appear to be only time. It has been shown, time and time again, that just as some patients present with a marked DWI-FLAIR match in a documented early time window others will show a negative or only subtly positive FLAIR even well past the 4.5 hour mark. This observation is further corroborated by the lack of a significant correlation between relative signal intensity on FLAIR and time from symptom onset[42].These are all known limitations to using FLAIR as a surrogate marker of lesion age[42]. They point to the fact that the evolution of signal intensity

obviously does not depend solely on time but also on individual patient characteristics. This suggests that FLAIR might indeed be a kind of "tissue clock", reflecting pathophysiology rather than just the passage of time. One piece of evidence for this was the finding that, in patients randomized in the WAKE UP trial, with increasing FLAIR-rSI a smooth continuing trend of decreasing treatment effects in relation to clinical end points was detected[43]. The need for an "individually tailored" approach to stroke treatment has long been postulated; one that would be capable of including all relevant variables into a calculation and returning the particular odds, risks and benefits for every patient. Arguably, various imaging parameters reveal to us the current condition of ischemic brain tissue. Magnetic resonance angiography identifies occlusion of larger brain vessels causal for the stroke. DWI shows us early cytotoxic cell edema leading to cell death. PWI depicts the area of neuronal dysfunction at risk of further demise, caused by insufficient blood supply. FLAIR shows us vasogenic edema as a sign of irrevocable damage stemming from persisting ischemia. Is this information not more relevant and better guiding than mere time? Could we find a way to abandon the time criterion entirely, with treatment decisions based solely on brain imaging findings[39?]

3.4. Advanced imaging in acute ischemic stroke

The DWI and FLAIR juxtaposition is not the only type of advanced imaging aimed at characterizing tissue that has been closely investigated in stroke research. Another frequently used concept is that of penumbra and penumbral imaging, already briefly mentioned on page 56. Penumbra is a term of Latin origin denoting (in the setting of acute ischemic stroke) the tissue which is hypoperfused and therefore non-functional yet still viable and amiable to full recovery, provided that reperfusion takes place. Penumbral imaging done with MRI is a juxtaposition of DWI (depicting the infarct core or irreversibly dying tissue) and PWI (depicting hypoperfused tissue), where the non-overlapping area (showing a PWI deficit but no DWI lesion) represents the penumbra. The same concept has also been applied to computer tomography (CT), using CT perfusion as a way to depict both the infarct core and the penumbra, with a juxtaposition of two different perfusion maps (cerebral blood flow and Tmax, respectively). Many trials have looked into using penumbral imaging (either MRI- or CT-based) to replace or extend the time window. In the EXTEND-IA[44] trial, CT-based penumbral imaging was used as a selection criterion for endovascular treatment with a stent retriever in patients with an occlusion of the internal carotid or middle cerebral artery who could be treated within 6 hours of symptom onset. The DEFUSE-3 trial conducted a similar study with penumbral patient selection in an even more extended time window, 6 to 16 hours post stroke[45]. Both trials were successful in proving the safety and efficacy of their approach. EXTEND[46] and ECASS-4[47] were randomized clinical trials of standard dose alteplase or placebo that used penumbral imaging (either CT perfusion or perfusion-diffusion MRI

in EXTEND or only perfusion-diffusion MRI in ECASS-4) to include patients in a 4.5–9 hour time window of stroke or with WAKE UP stroke. Both prematurely terminated, they nevertheless showed a trend in favor of the benefit of tPA. A meta-analysis done on a large sample of patients with stroke of unknown onset (pooled from WAKE UP, THAWS, EXTEND and ECASS-4 with an N = 843) and selected for randomization based on advanced imaging (either penumbral imaging or MR-based tissue clocking) showed an adjusted odds ratio (OR) for favorable outcome with tPA of 1.48 (95% CI 1.07–2.06)[48]. Furthermore, in this meta-analysis a net benefit was observed for all functional outcomes across the entire range of mRS despite an increased risk of sICH and even a significant treatment benefit in patients with a large vessel occlusion. All of this points to the clinical validity of using imaging-based selection for reperfusion therapy (thrombectomy or intravenous thrombolysis), both in patients with an uncertain time window as well as those with known symptom onset up to 24 hours.

As a general rule, the pooled results of the clinical trials mentioned above show a preference for penumbral selection in patients in the extended time window (4.5h to 24h) and MRI-based tissue clocking in patients in the unknown time window (with the potential addition of PWI in cases with a large vessel occlusion)[49]. Both concepts, MRI-based tissue clocking and penumbral imaging, have their advantages and disadvantages. The former is limited by contraindications to MRI as well as the relative unavailability of MRI as compared to CT. Its strengths are the usage of standard sequences whose assessment requires experience but doesn't necessitate additional post-processing, the ability to avoid using intravenous contrast agent and proven efficacy even for mild and moderate stroke[50]. The latter is technically more challenging and necessitates contrast agent but allows free choice of imaging modality (CT or MRI) and offers up further categories of patients eligible for treatment (for example, through the possibility of combining the DWI-FLAIR mismatch and the PWI-DWI mismatch if using MRI). Ultimately, both CT and MRI can be used to offer advanced imaging-based patient selection. This leaves health care institutions a degree of freedom in determining the imaging strategy best suited to their local environment[50].

Consequently, advanced imaging has certainly won many proponents but is not without its opponents. Efforts are being made to broaden and simplify the screening possibilities for patients with stroke of unknown onset, by either shortening the MRI scanning protocol or allowing CT as the imaging modality. A study showed that, in situations where FLAIR is unavailable or of non-diagnostic quality, DWI alone might suffice to allocate patients within or outside the 4.5h time window[51]. A recent study investigated using ROI-based estimates of water uptake in the ischemic region on non-contrast CT as a way to estimate time from symptom onset[52] , finding a high positive predictive value for a 9.5% increase (relative to corresponding contralateral region), albeit necessitating CT-perfusion to ensure reliable identification of infarct core. These methods seek to bypass or alleviate some of the drawbacks associated with advanced imaging while still acknowledging the need for additional information and

assurance when offering treatment in the extended or unknown time window. However, some believe that thrombolytic therapy may be efficient (and safe) in such patients without resorting to any complex imaging maneuvers. The background of this belief stems from the fact that the imaging selection criteria used in the aforementioned trials were arguably strict and excluded the vast majority of patients they screened[53]. There has been legitimate criticism that some of those patients who do not meet these stringent criteria might also regardless benefit from reperfusion treatment[54-55] arguing that advanced imaging merely separates patients with the greatest odds for favorable outcome but cannot reliably identify patients who will not benefit from therapy. A study published in 2018 showed that a significant percentage of trial-ineligible patients with large vessel occlusion who nevertheless received off-label thrombectomy achieved favorable outcomes[56]. It therefore stands to reason that more inclusive selection paradigms would allow a considerably larger proportion of patients to be (successfully) treated. Such alternative imaging protocols would save time, cut costs and, most importantly, enable the treatment of patients in the extended or unknown time window in centers that lack advanced imaging capabilities. A recently published meta-analysis and systematic review found that, at least for the subpopulation of patients presenting with a large vessel occlusion in the extended time window, a simplified imaging regime (non-contrast CT and CT-angiography) could successfully replace advanced imaging with CT perfusion[57].

Taking it a step further, a small cohort study published in 2009 reported, on patients with stroke of unknown onset, that thrombolytic treatment could be safe even when patients are only selected using non-contrast CT[58]. Encouraged by these findings, the TWIST clinical trial was started in 2017 as an investigator-initiated, multicentre, open-label, randomised controlled trial to test the safety and efficacy of tenecteplase in patients with stroke of unknown onset[59]. These patients were selected based on a non-contrast CT which was used only to exclude intracranial hemorrhage or a large (> 1/3 MCA territory) and already demarcated territorial stroke[59]. The trial concluded in late 2021, having enrolled almost 600 patients, that there was no proven statistical benefit of intravenous thrombolysis for the patients despite it appearing to be safe[60].

Resulting from this, the current European guidelines for the treatment of stroke[40] advocate intravenous thrombolysis in patients in the extended time window (4.5–9 hours after known onset time) only if penumbral imaging, in the form of CT- or MRI perfusion, has been carried out. For patients in the unknown time window, recommendations favor the use of MRI tissue clocking as the screening method of choice, with penumbral imaging within 9 hours from the midpoint of sleep listed as a less preferred but approved alternative. Hence, current recommendations heavily side with the use of advanced imaging for patient selection outside the standard 4.5-hour time window. However, it is clear that the exact scope and indication of advanced imaging remain controversial and warrant clarification through future, well-designed and well-conducted clinical trials.

4. Conclusions

Stroke of unknown onset accounts for up to 20% of all acute ischemic stroke. Prior to the successful completion of the Efficacy and Safety of MRI-Based Thrombolysis in WAKE UP Stroke (WAKE UP) trial, these patients were typically excluded from treatment with IV tPA as this therapy was only approved for cases within 4.5 hours of known symptom onset. WAKE UP utilized a novel imaging biomarker of lesion age, the DWI-FLAIR mismatch (acute stroke visible on DWI but not yet visible on FLAIR), to allocate patients into the early time window for which thrombolysis has been proven safe and efficient; a concept which became known as "tissue clocking". As a multicenter and imaging-heavy trial, WAKE UP relied upon a homogeneous understanding and interpretation of its imaging criteria by all of its many investigators, a process that was safeguarded by dedicated training developed especially for the study's purposes. The study was successful and, upon its completion in 2017, together with two smaller and similar trials that were completed at comparable time points, WAKE UP generated enough high quality evidence to influence a change in official guidelines, now recommending thrombolysis for patients with stroke of unknown onset who satisfy WAKE UP criteria. Various sub-analyses conducted since on the WAKE UP cohort further cemented the credibility of tissue clocking as a patient selection paradigm. But it is not the only such model. In addition to tissue clocking another concept, dubbed penumbral imaging and used as a biomarker of tissue at risk of infarction, has also been investigated in large clinical trials such as EXTEND and ECASS-4, as a way to offer treatment to patients with unknown symptom onset. Both of these methods fall under the umbrella of advanced imaging because they necessitate hardware and/or software as well as expertise in image interpretation that is not routinely available in the majority of the world's hospitals. Tissue clocking (using magnetic resonance imaging and the DWI-FLAIR mismatch) as well as penumbral imaging (using MR or CT based perfusion imaging) offer a lot of additional information, and through it, assurance to the treating physician that potential risks have been minimized and possible benefits of therapy enhanced. In this sense, advanced brain imaging should definitely be considered as part of state of the art, evidence based stroke treatment. Especially in the unknown time window, and due to its ability to perform both tissue clocking and penumbral imaging, MRI as a modality has been proposed as the most inclusive approach to screening ischemic stroke patients in hopes of identifying those still eligible for thrombolytic treatment. However, this approach clearly suffers the drawback of limited availability in everyday clinical practice. Further, well-designed and well-conducted prospective, randomized, controlled trials should be performed to evaluate the exact scope of (advanced) imaging needed for an as-inclusive-as-possible and successful patient selection in the unknown time window.

5. References

1. Katan M, Luft A. Global Burden of Stroke. Semin Neurol. 2018 Apr;38(2):208-211. doi: 10.1055/s-0038-1649503. Epub 2018 May 23. PMID: 29791947.

2. GBD 2015 Neurological Disorders Collaborator Group. Global, regional, and national burden of neurological disorders during 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet Neurol. 2017 Nov;16(11):877-897. doi: 10.1016/S1474-4422(17)30299-5. Epub 2017 Sep 17. PMID: 28931491; PMCID: PMC5641502.

3. The International Stroke Trial (IST): a randomised trial of aspirin, subcutaneous heparin, both, or neither among 19435 patients with acute ischaemic stroke. International Stroke Trial Collaborative Group. Lancet. 1997 May 31;349(9065):1569-81. PMID: 9174558.

4. CAST: randomised placebo-controlled trial of early aspirin use in 20,000 patients with acute ischaemic stroke. CAST (Chinese Acute Stroke Trial) Collaborative Group. Lancet. 1997 Jun 7;349(9066):1641-9. PMID: 9186381.

5. Stroke Unit Trialists' Collaboration. Organised inpatient (stroke unit) care for stroke. Cochrane Database Syst Rev. 2007 Oct 17;(4):CD000197. doi: 10.1002/14651858.CD000197.pub2.

6. National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group. Tissue plasminogen activator for acute ischemic stroke. N Engl J Med. 1995 Dec 14;333(24):1581-7. doi: 10.1056/NEJM199512143332401. PMID: 7477192.

7. Hacke W, Kaste M, Bluhmki E, Brozman M, Dávalos A, Guidetti D, Larrue V, Lees KR, Medeghri Z, Machnig T, Schneider D, von Kummer R, Wahlgren N, Toni D; ECASS Investigators. Thrombolysis with alteplase 3 to 4.5 hours after acute ischemic stroke. N Engl J Med. 2008 Sep 25;359(13):1317-29. doi: 10.1056/NEJMoa0804656. PMID: 18815396.

8. Welch KM, Windham J, Knight RA, Nagesh V, Hugg JW, Jacobs M, Peck D, Booker P, Dereski MO, Levine SR. A model to predict the histopathology of human stroke using diffusion and T2-weighted magnetic resonance imaging. Stroke. 1995 Nov;26(11):1983-9. doi: 10.1161/01.str.26.11.1983. PMID: 7482635.

9. Moseley ME, Kucharczyk J, Mintorovitch J, Cohen Y, Kurhanewicz J, Derugin N, Asgari H, Norman D. Diffusion-weighted MR imaging of acute stroke: correlation with T2-weighted and magnetic susceptibility-enhanced MR imaging in cats. AJNR Am J Neuroradiol. 1990 May;11(3):423-9. PMID: 2161612; PMCID: PMC8367476.

10. Go KG. The normal and pathological physiology of brain water. Adv Tech Stand Neurosurg. 1997;23:47-142. doi: 10.1007/978-3-7091-6549-2_2. PMID: 9075471.

11. Ebinger M, Galinovic I, Rozanski M, Brunecker P, Endres M, Fiebach JB. Fluid-attenuated inversion recovery evolution within 12 hours from stroke onset: a reliable tissue clock? Stroke. 2010 Feb;41(2):250-5. doi: 10.1161/STROKEAHA.109.568410. Epub 2009 Dec 24. PMID: 20035068.

12. Thomalla G, Cheng B, Ebinger M, Hao Q, Tourdias T, Wu O, Kim JS, Breuer L, Singer OC, Warach S, Christensen S, Treszl A, Forkert ND, Galinovic I, Rosenkranz M, Engelhorn T, Köhrmann M, Endres M, Kang DW, Dousset V, Sorensen AG, Liebeskind DS, Fiebach JB, Fiehler J, Gerloff C; STIR and VISTA Imaging Investigators. DWI-FLAIR mismatch for the identification of patients with acute ischaemic stroke within 4·5 h of symptom onset (PRE-FLAIR): a multicentre observational study. Lancet Neurol. 2011 Nov;10(11):978-86. doi: 10.1016/S1474-4422(11)70192-2. Epub 2011 Oct 4. PMID: 21978972.

13. Thomalla G, Fiebach JB, Østergaard L, Pedraza S, Thijs V, Nighoghossian N, Roy P, Muir KW, Ebinger M, Cheng B, Galinovic I, Cho TH, Puig J, Boutitie F, Simonsen CZ, Endres M, Fiehler J, Gerloff C; WAKE UP investigators. A multicenter, randomized, double-blind, placebo-controlled trial to test efficacy and safety of magnetic resonance imaging-based thrombolysis in WAKE UP stroke (WAKE UP). Int J Stroke. 2014 Aug;9(6):829-36. doi: 10.1111/ijs.12011. Epub 2013 Mar 12. PMID: 23490032.

14. Ziegler A, Ebinger M, Fiebach JB, Audebert HJ, Leistner S. Judgment of FLAIR signal change in DWI-FLAIR mismatch determination is a challenge to clinicians. J Neurol. 2012 May;259(5):971-3. doi: 10.1007/s00415-011-6284-6. Epub 2011 Oct 27. PMID: 22037953.

15. Galinovic I, Puig J, Neeb L, Guibernau J, Kemmling A, Siemonsen S, Pedraza S, Cheng B, Thomalla G, Fiehler J, Fiebach JB. Visual and region of interest-based inter-rater agreement in the assessment of the diffusion-weighted imaging- fluid-attenuated inversion recovery mismatch. Stroke. 2014 Apr;45(4):1170-2. doi: 10.1161/STROKEAHA.113.002661. Epub 2014 Feb 20. PMID: 24558091.

16. Song SS, Latour LL, Ritter CH, Wu O, Tighiouart M, Hernandez DA, Ku KD, Luby M, Warach S. A pragmatic approach using magnetic resonance imaging to treat ischemic strokes of unknown onset time in a thrombolytic trial. Stroke. 2012 Sep;43(9):2331-5. doi: 10.1161/STROKEAHA.111.630947. Epub 2012 Jun 12. PMID: 22693129; PMCID: PMC3464959.

17. Galinovic I, Dicken V, Heitz J, Klein J, Puig J, Guibernau J, Kemmling A, Gellissen S, Villringer K, Neeb L, Gregori J, Weiler F, Pedraza S, Thomalla G, Fiehler J, Gerloff C, Fiebach JB; WAKE UP Investigators. Homogeneous application of imaging criteria in a multicenter trial supported by investigator training: A report from the WAKE UP study. Eur J Radiol. 2018 Jul;104:115-119. doi: 10.1016/j.ejrad.2018.05.011. Epub 2018 May 15. PMID: 29857856.

18. Scheldeman L, Wouters A, Dupont P, Christensen S, Boutitie F, Cheng B, Ebinger M, Endres M, Fiebach JB, Gerloff C, Muir KW, Nighoghossian N, Pedraza S, Simonsen CZ, Thijs V, Thomalla G, Lemmens R. Diffusion-Weighted Imaging and Fluid-Attenuated Inversion Recovery Quantification to Predict Diffusion-Weighted Imaging-Fluid-Attenuated Inversion Recovery Mismatch Status in Ischemic Stroke With Unknown Onset. Stroke. 2022 May;53(5):1665-1673. doi: 10.1161/STROKEAHA.121.036871. Epub 2022 Feb 2. PMID: 35105179.

19. von Kummer R, Mori E, Truelsen T, Jensen JS, Grønning BA, Fiebach JB, Lovblad KO, Pedraza S, Romero JM, Chabriat H, Chang KC, Dávalos A, Ford GA, Grotta J, Kaste M, Schwamm LH, Shuaib A, Albers GW; DIAS-4 Investigators. Desmoteplase 3 to 9 Hours After Major Artery Occlusion Stroke: The DIAS-4 Trial (Efficacy and Safety Study of Desmoteplase to Treat Acute Ischemic Stroke). Stroke. 2016 Dec;47(12):2880-2887. doi: 10.1161/STROKEAHA.116.013715. Epub 2016 Nov 1. PMID: 27803391.

20. Galinovic I, Dicken V, Heitz J, Klein J, Puig J, Guibernau J, Kemmling A, Gellissen S, Villringer K, Neeb L, Gregori J, Weiler F, Pedraza S, Thomalla G, Fiehler J, Gerloff C, Fiebach JB; WAKE UP Investigators. Homogeneous application of imaging criteria in a multicenter trial supported by investigator training: A report from the WAKE UP study. Eur J Radiol. 2018 Jul;104:115-119. doi: 10.1016/j.ejrad.2018.05.011. Epub 2018 May 15. PMID: 29857856.

21. Thomalla G, Simonsen CZ, Boutitie F, Andersen G, Berthezene Y, Cheng B, Cheripelli B, Cho TH, Fazekas F, Fiehler J, Ford I, Galinovic I, Gellissen S, Golsari A, Gregori J, Günther M, Guibernau J, Häusler KG, Hennerici M, Kemmling A, Marstrand J, Modrau B, Neeb L, Perez de la Ossa N, Puig J, Ringleb P, Roy P, Scheel E, Schonewille W, Serena J, Sunaert S, Villringer K, Wouters A, Thijs V, Ebinger M, Endres M, Fiebach JB, Lemmens R, Muir KW, Nighoghossian N, Pedraza S, Gerloff C; WAKE UP Investigators. MRI-Guided Thrombolysis for Stroke with Unknown Time of Onset. N Engl J Med. 2018 Aug 16;379(7):611-622. doi: 10.1056/NEJMoa1804355. Epub 2018 May 16. PMID: 29766770.

22. Barow E, Boutitie F, Cheng B, Cho TH, Ebinger M, Endres M, Fiebach JB, Fiehler J, Ford I, Galinovic I, Nickel A, Puig J, Roy P, Wouters A, Magnus T, Thijs V, Lemmens R, Muir KW, Nighoghossian N, Pedraza S, Simonsen CZ, Gerloff C, Thomalla G; WAKE UP Investigators. Functional Outcome of Intravenous Thrombolysis in Patients With Lacunar Infarcts in the WAKE UP Trial. JAMA Neurol. 2019 Jun 1;76(6):641-649. doi: 10.1001/jamaneurol.2019.0351. PMID: 30907934; PMCID: PMC6563546.

23. Grosse-Dresselhaus F, Galinovic I, Villringer K, Audebert HJ, Fiebach JB. Difficulty of MRI based identification of lesion age by acute infra-tentorial ischemic stroke. PLoS One. 2014 Mar 20;9(3):e92868. doi: 10.1371/journal.pone.0092868. PMID: 24651570; PMCID: PMC3961416.

24. Galinovic I, Boutitie F, Fiebach JB, Villringer K, Cheng B, Ebinger M, Endres M, Fiehler J, Ford I, Thijs V, Lemmens R, Muir KW, Nighoghossian N, Pedraza S, Simonsen CZ, Roy P, Gerloff C, Thomalla G. Post-

hoc Analysis of Outcome of Intravenous Thrombolysis in Infarcts of Infratentorial Localization in the WAKE UP Trial. Front Neurol. 2019 Sep 11;10:983. doi: 10.3389/fneur.2019.00983. PMID: 31572293; PMCID: PMC6749039.

25. Königsberg A, Sehner S, Arlt S, Cheng B, Simonsen CZ, Boutitie F, Serena J, Thijs V, Ebinger M, Endres M, Fiebach JB, Lemmens R, Muir KW, Nighoghossian N, Pedraza S, Gerloff C, Thomalla G; WAKE UP investigators. Effect of intravenous alteplase on post-stroke depression in the WAKE UP trial. Eur J Neurol. 2021 Jun;28(6):2017-2025. doi: 10.1111/ene.14797. Epub 2021 Mar 22. PMID: 33657675.

26. Jensen M, Sehner S, Cheng B, Schlemm E, Quandt F, Barow E, Wegscheider K, Boutitie F, Ebinger M, Endres M, Fiebach J, Thijs V, Lemmens R, Muir K, Nighoghossian N, Pedraza S, Simonsen CZ, Thomalla G, Gerloff C. Patient-Reported Quality of Life After Intravenous Alteplase for Stroke in the WAKE UP Trial. Neurology. 2022 Oct 27:10.1212/WNL.0000000000201375. doi: 10.1212/WNL.0000000000201375. Epub ahead of print. PMID: 36302662.

27. Miwa K, Koga M, Jensen M, Inoue M, Yoshimura S, Fukuda-Doi M, Boutitie F, Ma H, Ringleb PA, Wu O, Schwamm LH, Warach S, Hacke W, Davis SM, Donnan GA, Gerloff C, Thomalla G, Toyoda K; Evaluation of Unknown Onset Stroke Thrombolysis Trials (EOS) Investigators. Alteplase for Stroke With Unknown Onset Time in Chronic Kidney Disease: A Pooled Analysis of Individual Participant Data. Stroke. 2022 Nov;53(11):3295-3303. doi: 10.1161/STROKEAHA.122.039086. Epub 2022 Aug 23. PMID: 35997023.

28. Schlemm L, Braemswig TB, Boutitie F, Vynckier J, Jensen M, Galinovic I, Simonsen CZ, Cheng B, Cho TH, Fiehler J, Puig J, Thijs V, Fiebach J, Muir K, Nighoghossian N, Ebinger M, Pedraza S, Thomalla G, Gerloff C, Endres M, Lemmens R, Nolte CH; WAKE UP Investigators. Cerebral Microbleeds and Treatment Effect of Intravenous Thrombolysis in Acute Stroke: An Analysis of the WAKE UP Randomized Clinical Trial. Neurology. 2022 Jan 18;98(3):e302-e314. doi: 10.1212/WNL.0000000000013055. Epub 2021 Nov 15. PMID: 34782419; PMCID: PMC8792812.

29. Schlemm L, Kufner A, Boutitie F, Nave AH, Gerloff C, Thomalla G, Simonsen CZ, Ford I, Lemmens R, Muir KW, Nighoghossian N, Pedraza S, Ebinger M, Endres M. Current Smoking Does Not Modify the Treatment Effect of Intravenous Thrombolysis in Acute Ischemic Stroke Patients-A Post-hoc Analysis of the WAKE UP Trial. Front Neurol. 2019 Nov 22;10:1239. doi: 10.3389/fneur.2019.01239. PMID: 31824412; PMCID: PMC6883001.

30. Jensen M, Schlemm E, Cheng B, Lettow I, Quandt F, Boutitie F, Ebinger M, Endres M, Fiebach JB, Fiehler J, Galinovic I, Thijs V, Lemmens R, Muir KW, Nighoghossian N, Pedraza S, Simonsen CZ, Gerloff C, Thomalla G. Clinical Characteristics and Outcome of Patients With Hemorrhagic Transformation

After Intravenous Thrombolysis in the WAKE UP Trial. Front Neurol. 2020 Aug 28;11:957. doi: 10.3389/fneur.2020.00957. PMID: 32982951; PMCID: PMC7483750.

31. Muntendorf LK, Konnopka A, König HH, Boutitie F, Ebinger M, Endres M, Fiebach JB, Thijs V, Lemmens R, Muir KW, Nighoghossian N, Pedraza S, Simonsen CZ, Gerloff C, Thomalla G. Cost-Effectiveness of Magnetic Resonance Imaging-Guided Thrombolysis for Patients With Stroke With Unknown Time of Onset. Value Health. 2021 Nov;24(11):1620-1627. doi: 10.1016/j.jval.2021.05.005. Epub 2021 Jul 31. PMID: 34711362.

32. Frey BM, Boutitie F, Cheng B, Cho TH, Ebinger M, Endres M, Fiebach JB, Fiehler J, Ford I, Galinovic I, Königsberg A, Puig J, Roy P, Wouters A, Magnus T, Thijs V, Lemmens R, Muir KW, Nighoghossian N, Pedraza S, Simonsen CZ, Gerloff C, Thomalla G; WAKE UP investigators. Safety and efficacy of intravenous thrombolysis in stroke patients on prior antiplatelet therapy in the WAKE UP trial. Neurol Res Pract. 2020 Nov 20;2:40. doi: 10.1186/s42466-020-00087-9. PMID: 33324940; PMCID: PMC7678217.

33. Jensen M, Boutitie F, Cheng B, Cho TH, Ebinger M, Endres M, Fiebach JB, Fiehler J, Ford I, Galinovic I, Königsberg A, Puig J, Roy P, Wouters A, Thijs V, Lemmens R, Muir KW, Nighoghossian N, Pedraza S, Simonsen CZ, Gerloff C, Thomalla G. Polypharmacy, functional outcome and treatment effect of intravenous alteplase for acute ischaemic stroke. Eur J Neurol. 2021 Feb;28(2):532-539. doi: 10.1111/ene.14566. Epub 2020 Oct 28. PMID: 33015924.

34. Scheldeman L, Wouters A, Boutitie F, Dupont P, Christensen S, Cheng B, Ebinger M, Endres M, Fiebach JB, Gerloff C, Muir KW, Nighoghossian N, Pedraza S, Simonsen CZ, Thijs V, Thomalla G, Lemmens R; WAKE UP Investigators. Different Mismatch Concepts for Magnetic Resonance Imaging-Guided Thrombolysis in Unknown Onset Stroke. Ann Neurol. 2020 Jun;87(6):931-938. doi: 10.1002/ana.25730. Epub 2020 Apr 20. PMID: 32227638.

35. Zhou Z, Malavera A, Yoshimura S, Delcourt C, Mair G, Al-Shahi Salman R, Demchuk AM, Wardlaw JM, Lindley RI, Anderson CS. Clinical prognosis of FLAIR hyperintense arteries in ischaemic stroke patients: a systematic review and meta-analysis. J Neurol Neurosurg Psychiatry. 2020 May;91(5):475-482. doi: 10.1136/jnnp-2019-322625. Epub 2020 Mar 26. PMID: 32217786.

36. 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. doi: 10.3389/fneur.2020.623881. PMID: 33613422; PMCID: PMC7890254.

37. Koga M, Yamamoto H, Inoue M, Asakura K, Aoki J, Hamasaki T, Kanzawa T, Kondo R, Ohtaki M, Itabashi R, Kamiyama K, Iwama T, Nakase T, Yakushiji Y, Igarashi S, Nagakane Y, Takizawa S, Okada Y, Doijiri R, Tsujino A, Ito Y, Ohnishi H, Inoue T, Takagi Y, Hasegawa Y, Shiokawa Y, Sakai N, Osaki M, Uesaka Y, Yoshimura S, Urabe T, Ueda T, Ihara M, Kitazono T, Sasaki M, Oita A, Yoshimura S, Fukuda-Doi M, Miwa K, Kimura K, Minematsu K, Toyoda K; THAWS Trial Investigators. Thrombolysis With Alteplase at 0.6 mg/kg for Stroke With Unknown Time of Onset: A Randomized Controlled Trial. Stroke. 2020 May;51(5):1530-1538. doi: 10.1161/STROKEAHA.119.028127. Epub 2020 Apr 6. PMID: 32248771; PMCID: PMC7185058.

38. Toyoda K, Inoue M, Yoshimura S, Yamagami H, Sasaki M, Fukuda-Doi M, Kimura K, Asakura K, Miwa K, Kanzawa T, Ihara M, Kondo R, Shiozawa M, Ohtaki M, Kamiyama K, Itabashi R, Iwama T, Aoki J, Minematsu K, Yamamoto H, Koga M; THAWS trial investigators*. Magnetic Resonance Imaging-Guided Thrombolysis (0.6 mg/kg) Was Beneficial for Unknown Onset Stroke Above a Certain Core Size: THAWS RCT Substudy. Stroke. 2021 Jan;52(1):12-19. doi: 10.1161/STROKEAHA.120.030848. Epub 2020 Dec 10. PMID: 33297866.

39. Schwamm LH, Wu O, Song SS, Latour LL, Ford AL, Hsia AW, Muzikansky A, Betensky RA, Yoo AJ, Lev MH, Boulouis G, Lauer A, Cougo P, Copen WA, Harris GJ, Warach S; MR WITNESS Investigators. Intravenous thrombolysis in unwitnessed stroke onset: MR WITNESS trial results. Ann Neurol. 2018 May;83(5):980-993. doi: 10.1002/ana.25235. Epub 2018 Apr 27. PMID: 29689135; PMCID: PMC6095471.

40. Berge E, Whiteley W, Audebert H, De Marchis GM, Fonseca AC, Padiglioni C, de la Ossa NP, Strbian D, Tsivgoulis G, Turc G. European Stroke Organisation (ESO) guidelines on intravenous thrombolysis for acute ischaemic stroke. Eur Stroke J. 2021 Mar;6(1):I-LXII. doi: 10.1177/2396987321989865. Epub 2021 Feb 19. PMID: 33817340; PMCID: PMC7995316.

41. Powers WJ, Rabinstein AA, Ackerson T, Adeoye OM, Bambakidis NC, Becker K, Biller J, Brown M, Demaerschalk BM, Hoh B, Jauch EC, Kidwell CS, Leslie-Mazwi TM, Ovbiagele B, Scott PA, Sheth KN, Southerland AM, Summers DV, Tirschwell DL. Guidelines for the Early Management of Patients With Acute Ischemic Stroke: 2019 Update to the 2018 Guidelines for the Early Management of Acute Ischemic Stroke: A Guideline for Healthcare Professionals From the American Heart Association/American Stroke Association. Stroke. 2019 Dec;50(12):e344-e418. doi: 10.1161/STR.0000000000000211. Epub 2019 Oct 30. Erratum in: Stroke. 2019 Dec;50(12):e440-e441. PMID: 31662037.

42. Cheng B, Brinkmann M, Forkert ND, Treszl A, Ebinger M, Köhrmann M, Wu O, Kang DW, Liebeskind DS, Tourdias T, Singer OC, Christensen S, Luby M, Warach S, Fiehler J, Fiebach JB, Gerloff C, Thomalla

G; STIR and VISTA Imaging Investigators. Quantitative measurements of relative fluid-attenuated inversion recovery (FLAIR) signal intensities in acute stroke for the prediction of time from symptom onset. J Cereb Blood Flow Metab. 2013 Jan;33(1):76-84. doi: 10.1038/jcbfm.2012.129. Epub 2012 Oct 10. PMID: 23047272; PMCID: PMC3965287.

43. Cheng B, Boutitie F, Nickel A, Wouters A, Cho TH, Ebinger M, Endres M, Fiebach JB, Fiehler J, Galinovic I, Puig J, Thijs V, Lemmens R, Muir KW, Nighoghossian N, Pedraza S, Simonsen CZ, Gerloff C, Thomalla G; WAKE UP (Efficacy and Safety of MRI-Based Thrombolysis inWAKE UP Stroke Trial) investigators and administrative staff are as follows:. Quantitative Signal Intensity in Fluid-Attenuated Inversion Recovery and Treatment Effect in the WAKE UP Trial. Stroke. 2020 Jan;51(1):209-215. doi: 10.1161/STROKEAHA.119.027390. Epub 2019 Oct 30. PMID: 31662118.

44. Campbell BC, Mitchell PJ, Kleinig TJ, Dewey HM, Churilov L, Yassi N, Yan B, Dowling RJ, Parsons MW, Oxley TJ, Wu TY, Brooks M, Simpson MA, Miteff F, Levi CR, Krause M, Harrington TJ, Faulder KC, Steinfort BS, Priglinger M, Ang T, Scroop R, Barber PA, McGuinness B, Wijeratne T, Phan TG, Chong W, Chandra RV, Bladin CF, Badve M, Rice H, de Villiers L, Ma H, Desmond PM, Donnan GA, Davis SM; EXTEND-IA Investigators. Endovascular therapy for ischemic stroke with perfusion-imaging selection. N Engl J Med. 2015 Mar 12;372(11):1009-18. doi: 10.1056/NEJMoa1414792. Epub 2015 Feb 11. PMID: 25671797.

45. Albers GW, Marks MP, Kemp S, Christensen S, Tsai JP, Ortega-Gutierrez S, McTaggart RA, Torbey MT, Kim-Tenser M, Leslie-Mazwi T, Sarraj A, Kasner SE, Ansari SA, Yeatts SD, Hamilton S, Mlynash M, Heit JJ, Zaharchuk G, Kim S, Carrozzella J, Palesch YY, Demchuk AM, Bammer R, Lavori PW, Broderick JP, Lansberg MG; DEFUSE 3 Investigators. Thrombectomy for Stroke at 6 to 16 Hours with Selection by Perfusion Imaging. N Engl J Med. 2018 Feb 22;378(8):708-718. doi: 10.1056/NEJMoa1713973. Epub 2018 Jan 24. PMID: 29364767; PMCID: PMC6590673.

46. Ma H, Campbell BCV, Parsons MW, Churilov L, Levi CR, Hsu C, Kleinig TJ, Wijeratne T, Curtze S, Dewey HM, Miteff F, Tsai CH, Lee JT, Phan TG, Mahant N, Sun MC, Krause M, Sturm J, Grimley R, Chen CH, Hu CJ, Wong AA, Field D, Sun Y, Barber PA, Sabet A, Jannes J, Jeng JS, Clissold B, Markus R, Lin CH, Lien LM, Bladin CF, Christensen S, Yassi N, Sharma G, Bivard A, Desmond PM, Yan B, Mitchell PJ, Thijs V, Carey L, Meretoja A, Davis SM, Donnan GA; EXTEND Investigators. Thrombolysis Guided by Perfusion Imaging up to 9 Hours after Onset of Stroke. N Engl J Med. 2019 May 9;380(19):1795-1803. doi: 10.1056/NEJMoa1813046. Erratum in: N Engl J Med. 2021 Apr 1;384(13):1278. PMID: 31067369.

47. Ringleb P, Bendszus M, Bluhmki E, Donnan G, Eschenfelder C, Fatar M, Kessler C, Molina C, Leys D, Muddegowda G, Poli S, Schellinger P, Schwab S, Serena J, Toni D, Wahlgren N, Hacke W; ECASS-4 study group. Extending the time window for intravenous thrombolysis in acute ischemic stroke using

magnetic resonance imaging-based patient selection. Int J Stroke. 2019 Jul;14(5):483-490. doi: 10.1177/1747493019840938. Epub 2019 Apr 4. PMID: 30947642.

48. Thomalla G, Boutitie F, Ma H, Koga M, Ringleb P, Schwamm LH, Wu O, Bendszus M, Bladin CF, Campbell BCV, Cheng B, Churilov L, Ebinger M, Endres M, Fiebach JB, Fukuda-Doi M, Inoue M, Kleinig TJ, Latour LL, Lemmens R, Levi CR, Leys D, Miwa K, Molina CA, Muir KW, Nighoghossian N, Parsons MW, Pedraza S, Schellinger PD, Schwab S, Simonsen CZ, Song SS, Thijs V, Toni D, Hsu CY, Wahlgren N, Yamamoto H, Yassi N, Yoshimura S, Warach S, Hacke W, Toyoda K, Donnan GA, Davis SM, Gerloff C; Evaluation of unknown Onset Stroke thrombolysis trials (EOS) investigators. Intravenous alteplase for stroke with unknown time of onset guided by advanced imaging: systematic review and meta-analysis of individual patient data. Lancet. 2020 Nov 14;396(10262):1574-1584. doi: 10.1016/S0140-6736(20)32163-2. Epub 2020 Nov 8. PMID: 33176180; PMCID: PMC7734592.

49. Thomalla G, Gerloff C. Acute imaging for evidence-based treatment of ischemic stroke. Curr Opin Neurol. 2019 Aug;32(4):521-529. doi: 10.1097/WCO.0000000000000716. PMID: 31116116.

50. Simonsen CZ, Leslie-Mazwi TM, Thomalla G. Which Imaging Approach Should Be Used for Stroke of Unknown Time of Onset? Stroke. 2021 Jan;52(1):373-380. doi: 10.1161/STROKEAHA.120.032020. Epub 2020 Dec 11. PMID: 33302796.

51. Madai VI, Galinovic I, Grittner U, Zaro-Weber O, Schneider A, Martin SZ, von Samson-Himmelstjerna FC, Stengl KL, Mutke MA, Moeller-Hartmann W, Ebinger M, Fiebach JB, Sobesky J. DWI intensity values predict FLAIR lesions in acute ischemic stroke. PLoS One. 2014 Mar 21;9(3):e92295. doi: 10.1371/journal.pone.0092295. PMID: 24658092; PMCID: PMC3962388.

52. Sporns PB, Höhne M, Meyer L, Krogias C, Puetz V, Thierfelder KM, Duering M, Kaiser D, Langner S, Brehm A, Rotkopf LT, Kunz WG, Fiehler J, Heindel W, Schramm P, Wiendl H, Minnerup H, Psychogios MN, Kemmling A, Minnerup J. Simplified Assessment of Lesion Water Uptake for Identification of Patients within 4.5 Hours of Stroke Onset: An Analysis of the MissPerfeCT Study. J Stroke. 2022 Sep;24(3):390-395. doi: 10.5853/jos.2022.00220. Epub 2022 Sep 30. PMID: 36221942; PMCID: PMC9561216 AND Broocks G, Leischner H, Hanning U, Flottmann F, Faizy TD, Schön G, Sporns P, Thomalla G, Kamalian S, Lev MH, Fiehler J, Kemmling A. Lesion Age Imaging in Acute Stroke: Water Uptake in CT Versus DWI-FLAIR Mismatch. Ann Neurol. 2020 Dec;88(6):1144-1152. doi: 10.1002/ana.25903. Epub 2020 Oct 2. PMID: 32939824.

53. Lee KJ, Kim BJ, Kim DE, Ryu WS, Han MK, Kim JT, Choi KH, Cho KH, Cha JK, Kim DH, Nah HW, Park JM, Kang K, Lee SJ, Kim JG, Oh MS, Yu KH, Lee BC, Hong KS, Cho YJ, Park TH, Lee KB, Lee J, Lee JS, Lee J, Bae HJ; Clinical Research Collaboration for Stroke in Korea (CRCS-K) Investigators. Nationwide Estimation of Eligibility for Endovascular Thrombectomy Based on the DAWN Trial. J Stroke. 2018

May;20(2):277-279. doi: 10.5853/jos.2018.00836. Epub 2018 May 31. PMID: 29886718; PMCID: PMC6007293.

54. Borst J, Berkhemer OA, Roos YB, van Bavel E, van Zwam WH, van Oostenbrugge RJ, van Walderveen MA, Lingsma HF, van der Lugt A, Dippel DW, Yoo AJ, Marquering HA, Majoie CB; MR CLEAN investigators. Value of Computed Tomographic Perfusion-Based Patient Selection for Intra-Arterial Acute Ischemic Stroke Treatment. Stroke. 2015 Dec;46(12):3375-82. doi: 10.1161/STROKEAHA.115.010564. Epub 2015 Nov 5. PMID: 26542698.

55. Ducroux C, Khoury N, Lecler A, Blanc R, Chetrit A, Redjem H, Ciccio G, Smajda S, Escalard S, Desilles JP, Mazighi M, Ben Maacha M, Piotin M, Fahed R. Application of the DAWN clinical imaging mismatch and DEFUSE 3 selection criteria: benefit seems similar but restrictive volume cut-offs might omit potential responders. Eur J Neurol. 2018 Aug;25(8):1093-1099. doi: 10.1111/ene.13660. Epub 2018 May 10. PMID: 29667266.

56. Desai SM, Rocha M, Molyneaux BJ, Starr M, Kenmuir CL, Gross BA, Jankowitz BT, Jovin TG, Jadhav AP. Thrombectomy 6-24 hours after stroke in trial ineligible patients. J Neurointerv Surg. 2018 Nov;10(11):1033-1037. doi: 10.1136/neurintsurg-2018-013915. Epub 2018 May 17. PMID: 29773716.

57. Dong Z, Deng S, Zhang J, Chen S, Ye Z, Zhang L, Hu R, Zhong C, Liu X, Qin C. Simplified stroke imaging selection modality for endovascular thrombectomy in the extended time window: systematic review and meta-analysis. J Neurointerv Surg. 2022 Dec 7:jnis-2022-019556. doi: 10.1136/jnis-2022-019556. Epub ahead of print. PMID: 36597953.

58. Barreto AD, Martin-Schild S, Hallevi H, Morales MM, Abraham AT, Gonzales NR, Illoh K, Grotta JC, Savitz SI. Thrombolytic therapy for patients who WAKE UP with stroke. Stroke. 2009 Mar;40(3):827-32. doi: 10.1161/STROKEAHA.108.528034. Epub 2009 Jan 8. PMID: 19131657; PMCID: PMC2676861.

59. Roaldsen MB, Lindekleiv H, Eltoft A, Jusufovic M, Søyland MH, Petersson J, Indredavik B, Tveiten A, Putaala J, Christensen H, Kõrv J, Jatužis D, Engelter ST, Marco De Marchis G, Wilsgaard T, Werring DJ, Robinson T, Mathiesen EB, Berge E. Tenecteplase in WAKE UP ischemic stroke trial: Protocol for a randomized-controlled trial. Int J Stroke. 2021 Oct;16(8):990-994. doi: 10.1177/1747493020984073. Epub 2021 Jan 14. Erratum in: Int J Stroke. 2021 Feb 10;:1747493021995410. PMID: 33446083; PMCID: PMC8554491.

60. Roaldsen MB, Eltoft A, Wilsgaard T, Christensen H, Engelter ST, Indredavik B, Jatužis D, Karelis G, Kõrv J, Lundström E, Petersson J, Putaala J, Søyland MH, Tveiten A, Bivard A, Johnsen SH, Mazya MV, Werring DJ, Wu TY, De Marchis GM, Robinson TG, Mathiesen EB; TWIST Investigators. Safety and efficacy of tenecteplase in patients with WAKE UP stroke assessed by non-contrast CT (TWIST): a

multicentre, open-label, randomised controlled trial. Lancet Neurol. 2022 Dec 19:S1474-4422(22)00484-7. doi: 10.1016/S1474-4422(22)00484-7. Epub ahead of print. PMID: 36549308.

6. Acknowledgments

First and foremost, I must thank the members of the WAKE UP consortium for giving me the opportunity to work side-by-side with such incredible individuals and hence take part in the amazing adventure of planning, conducting and ultimately successfully completing a multicenter clinical trial. This has certainly been among the most exciting and fulfilling experiences of my professional career.

I would unquestionably like to show gratitude to my supervisor, Prof. Fiebach, for his continuing faith in my abilities and support through the challenging and frustrating moments of my career at the Charite. A very special thanks goes to my closest colleagues: Ahmed Khalil, who is always here to brainstorm a project or troubleshoot a solution to a problem with me and Kersten Villringer, who has taught me much of what I know about clinical neuroradiology. I must also express my gratitude to my wider team, whose kindness and collegial spirit have created an exceptional working atmosphere. It's been a privilege and a joy being a part of the group all these years. A great thank you also to the many students I have mentored over the years for their hard work and contribution to the team, especially Anne Sophie Grosch for her involvement in my last WAKE UP associated manuscript and Asmaa Foda for her incredible dedication and unexpected friendship. A final thank you goes to my physician colleagues and nursing staff at the neurology department of the Charite, Campus Benjamin Franklin, for the many years of smooth and collegial cooperation.

A last but never least mention goes to my family and friends, without whose love and support even a successful professional career would lose all luster and meaning.

Erklärung

§ 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, 02.02.2023

Dr. Ivana Galinovic