Quantitative signal intensity in Fluid-Attenuated Inversion Recovery (FLAIR) and treatment effect in the WAKE-UP trial

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Abstract

Background and purpose

Relative signal intensity of acute ischemic stroke lesions in fluid-attenuated inversion recovery (FLAIR-rSI) magnetic resonance imaging is associated with time elapsed since stroke onset with higher intensities signifying longer time intervals. In the randomized controlled WAKE-UP trial, intravenous alteplase was effective in patients with unknown onset stroke selected by visual assessment of DWI-FLAIR mismatch, i.e., in those with no marked FLAIR hyperintensity in the region of the acute DWI lesion. In this post-hoc analysis, we investigated if quantitatively measured FLAIR-rSI modifies treatment effect of intravenous alteplase.

Methods

FLAIR-rSI of stroke lesions was measured relative to signal intensity in a mirrored region in the contralesional hemisphere. The relationship between FLAIR-rSI and treatment effect on functional outcome assessed by the modified Rankin Scale (mRS) after 90 days was analysed by binary logistic regression using different endpoints, i.e., favourable outcome defined as mRS 0-1, independent outcome defined as mRS 0-2, ordinal analysis of mRS scores (shift analysis). All models were adjusted for NIHSS at symptom onset and stroke lesion volume.

Results

FLAIR-rSI was successfully quantified in stroke lesions in 433 patients (86% of 503 patients included in WAKE-UP). Mean FLAIR-rSI was 1.06 (SD 0.09). Interaction of FLAIR-rSI and treatment effect was not significant for mRS 0-1 (p=0.169) and shift analysis (p=0.086), but

reached significance for mRS 0-2 (p=0.004). We observed a smooth continuing trend of decreasing treatment effects in relation to clinical endpoints with increasing FLAIR-rSI.

Conclusion

In patients in whom no marked parenchymal FLAIR hyperintensity was detected by visual judgement in the WAKE-UP trial, higher FLAIR-rSI of DWI lesions was associated with decreased treatment effects of intravenous thrombolysis. This parallels the known association of treatment effect and elapsing time of stroke onset.

Introduction

In up to a quarter of stroke patients, time of symptom onset is unknown, precluding thrombolysis.[1] Recent developments in the application of multi-modal magnetic resonance imaging (MRI) and CT perfusion have provided options for effective and safe treatment in patients with an extended or unknown time window.[2] In the WAKE-UP trial, patients with unknown symptom onset times were studied with MRI, including diffusion weighted imaging (DWI) and Fluid-attenuated inversion recovery (FLAIR). Previous studies have shown that in stroke patients with a known time of symptom onset, the presence of a visible ischemic lesion on DWI, combined with the absence of a clearly visible hyperintense signal in the same region on FLAIR was highly predictive of symptom onset within 4.5 hours before imaging.[3–5] In WAKE-UP, visual judgment of a DWI–FLAIR mismatch represented the main imaging criterion for randomization to treatment with alteplase or placebo, which proved successful regarding treatment efficacy and safety.[6]

The DWI-FLAIR mismatch concept is based on the different evolution of signal intensities on DWI and FLAIR over time since onset of ischemia. Specifically, while ischemic lesions become visible on DWI within minutes after ischemic injury, signal intensities in FLAIR increase over several hours after stroke onset, mainly resulting from net water update in ischemic brain tissue. Quantitative analysis of FLAIR signal intensities has demonstrated a linear association between higher FLAIR signal intensities and longer time from symptom onset both in imaging data from stroke patients and experimental animal studies. This association can be explained by the time dependent evolution of T2 relaxation times that contribute to FLAIR signal intensities and are robustly and linearly related to symptom onset time. [5,7–10]

Time after symptom onset is a modifier of treatment effects with intravenous alteplase in ischemic stroke. Pooled data from randomized trials of stroke thrombolysis demonstrated that the magnitude of treatment effects diminishes with increasing delays to treatment with higher

frequency of non-disabling clinical outcomes in the early time window.[11] Given the known continuous relationship between FLAIR signal intensities and stroke onset time, it would therefore be plausible that quantitatively measured FLAIR would relate to treatment efficacy in terms of favorable clinical outcome. In this post-hoc analysis of patients included in WAKE-UP, we therefore investigated if quantitatively measured FLAIR relative signal intensity (FLAIR-rSI) as reported previously[5,7] is a relevant modifier of treatment effect in WAKE-UP.

Methods

Data from this analysis is available to researchers upon reasonable request. WAKE-UP was a multicenter-randomized, double blind, placebo-controlled trial of MRI-based intravenous thrombolysis in patients with unknown onset stroke. The detailed trial protocol and the results of the original paper were described previously.[6] The mandatory imaging criterion for randomization to treatment with alteplase or placebo was a mismatch between an acute ischemic lesion visible on diffusion weighted imaging (DWI) and absence of a corresponding marked parenchymal hyperintensity on FLAIR (DWI-FLAIR mismatch). Patients or their legal representatives provided written informed consent according to national and local regulations. There was an exception from explicit informed consent in emergency circumstances in some countries. For this secondary analysis, we selected only patients with unilateral ischemic lesions to robustly calculate signal intensities in relation to the hemisphere not affected by stroke. This was done to prevent erroneous measurement of FLAIR-rSI in cases of bilateral stroke lesions. In addition, patients with extensive contralesional white matter hyperintensities in regions selected for reference measurement were excluded. DWI and FLAIR data were collected and data of insufficient quality for quantitative measurement for FLAIR-rSI excluded. Specifically, imaging artefacts (for example due to patient motion) in DWI and FLAIR sequences leading to erroneous registration of DWI and FLAIR and signal measurements were evaluated visually

and data excluded if correct measurement of FLAIR-rSI was impeded. Clinical outcome was assessed using the modified Rankin scale (mRS) 90 days after stroke.

Image analysis

Image data was analyzed using a dedicated software developed for the WAKE-UP trial (Stroke Quantification Tool, SONIA) based on previous methods and functionalities of a stroke imaging toolbox developed in-house.[12] FLAIR-rSI was quantified in relation to contralesional regions of interest mirroring the stroke lesion as described previously[7]. In summary, individual DWI and FLAIR datasets were registered using a non-linear, rigid transformation. Maps of apparent diffusion coefficient (ADC) were calculated based on DWI datasets. Therefore, two datasets were chosen automatically with b-values of 0 s/mm² and b-values of 500 s/mm² ranging to 1500 s/mm² according to the imaging protocol of the individual study site. Stroke lesions were segmented on ADC-maps using a semi-automated procedure with initial manual delineation drawing a generous margin and secondary automated refinement based on an ADC-threshold of 620 mm²/s. A three-dimensional plane was manually defined at the interhemispheric fissure to mirror stroke lesions onto the contralesional hemisphere. Lastly, FLAIR signal intensities were measured at the segmented stroke lesion and contralesional, mirrored region of interest. FLAIR-rSI was calculated as the FLAIR signal intensity within the stroke lesion (as defined above) relative to the unaffected, contralesional regions of interest. In addition, lesion volumes were calculated. All images were visually checked for quality. Patients with extensive white matter lesions preventing accurate measurements of contralesional FLAIR signal intensities were excluded from analysis.

Statistical analysis

Median and mean values of clinical parameters and lesion volumes including 95% Confidence intervals (CI) and interquartile ranges (IQR) were calculated. Values of FLAIR-rSI were dived

into quartiles. Clinical data and lesion volumes were compared between groups of quartiles of FLAIR-rSI using the Cochran Mantel-Haenszel chi-square test for categorical variables, and the Kruskal-Wallis test for continuous variables. Relationship between FLAIR-rSI and treatment effect was analyzed using the primary efficacy criterion of the WAKE-UP trial (favorable outcome 90 days after stroke as defined by mRS 0-1) and secondary efficacy outcome of the score on the modified Rankin scale. The latter was analyzed by fitting a proportional-odds logistic-regression model to calculate the common odds ratio as a measure of the likelihood that alteplase would lead to lower scores on the mRS than would placebo (shift analysis). Proportionality of the odds ratio for the treatment effect was tested on a simpler model with linear treatment effect modification (on the logit scale), using the likelihood ratio test of two models assuming or not a non-proportionality of the treatment effect and its interaction with FLAIR signal intensity. In addition, we investigated the effect applying mRS 0-2 for dichotomization to further explore clinically relevant signal-dependent treatment effects. For each endpoint, separate binary logistic regression models were applied based on a continuous smooth trend (cubic spines) of FLAIR-rSI. We tested for a significant interaction between FLAIR-rSI and treatment (alteplase or placebo). All models were adjusted for NIHSS and patient age at symptom onset and stroke lesion volume. Odds ratios (OR) and 95% CI for pre-defined clinical outcome were calculated. As all analyses were considered exploratory, tests were carried out with an alpha level of 5% without correction for multiple comparisons.

Results

Of 503 patients randomized in WAKE-UP, we excluded patients with bilateral lesions (n=18), insufficient imaging quality (n=35), and extensive contralesional white matter hyperintensities in regions selected for reference measurement (n=7). In total, data from 443 patients (88% of 503) were included for analysis. Median age of patients was 68 years (IQR 59-74), including 289 (65%) male patients, median NIHSS at admission was 6 (IQR 4-9). Median stroke volume

was 2.3 ml (IQR 1.9-2.7 ml). FLAIR-rSI was successfully quantified in all 443 patients with a median value of 1.06 (IQR 1.02-1.17) and yielded quartiles ranging from 1-1.02, 1.03-1.06, 1.07-1.11 and ≥ 1.11. Mean FLAIR-rSI was 1.06 (95% CI 1.05-1.07). Table 1 describes clinical data and stroke lesion volumes of patients grouped by quartiles of FLAIR-rSI.

Of 443 patients included in this analysis, 215 (48.5%) were treated with alteplase and 218 (51.5%) patients received placebo. Favourable clinical outcome (mRS 0-1) was observed in 120 patients (55.8%) in the alteplase group and in 91 patients (41.7%) in the placebo group.

Regression analysis revealed a continuous association of higher FLAIR-rSI and decreased treatment effect for all three endpoints studied (see figure). The interaction between FLAIR-rSI and treatment effect was not significant for favourable outcome defined as mRS 0-1 (interaction test, p=0.169) and the ordinal analysis across the entire range of the mRS (shift analysis, interaction test: p=0.086, test for non-proportionality of treatment effect modification: p=0.22). For the endpoint independent outcome defined as mRS 0-2, the continuous association was significant with a significant interaction between FLAIR-rSI and treatment effect (interaction test, p=0.004).

Discussion

In the WAKE-UP trial, patients with unknown time of symptom onset were selected based on a visible ischemic lesion on DWI, and the absence of marked parenchymal hyperintensities on FLAIR (DWI-FLAIR-mismatch). In this quantitative analysis, we demonstrate that this approach effectively resulted in a median FLAIR-rSI of 1.06 (i.e. 6% relative increase in relation to a corresponding, contralesional region of interest) of patients considered to meet the criterion of DWI-FLAIR-mismatch. This value is close to the relative FLAIR-SI value of 1.07 which has been previously suggested as an optimal cut-off value for predicting time since

symptom onset ≤ 4.5 hours in stroke patients with known symptom onset.[7] This was based on a comparable methodical voxel-wise approach calculating the mean intensity after three dimensional segmentation of the entire ischemic lesion. Our results therefore support visual assessment of DWI-FLAIR-mismatch for patient selection. Other studies of quantification of FLAIR-rSI have yielded higher thresholds of optimal FLAIR-rSI cut-off values to detect patient with time from symptom onset ≤ 4.5 hours. Here, a value of 1.15 was reported (15% relative increase of signal intensity compared to the opposite hemisphere).[13–15] In these studies, signal intensity was measured in manually selected regions with brightest FLAIR signal intensities inside the DWI lesion which most likely explains the disparity of our findings. For our study, we opted for measurements in the entire lesion to ensure comparability with our previous findings[7] and minimize inter-rater variability arising from manual placement of regions of interests.[16]

FLAIR signal intensities in patients with acute stroke are a surrogate marker of time from onset of ischemia and can be assessed by visual judgement (DWI-FLAIR mismatch) or quantitative analysis.[3] In this study, we did not find a significant interaction between FLAIR-rSI and treatment effect relating to the primary (mRS 0-1) and secondary (mRS score shift) efficacy endpoints of WAKE-UP. However, we observed a negative continuous relationship between increasing FLAIR-rSI and decreasing benefit of thrombolysis (figure 1). As shown in figure 1C, the negative continuous association between FLAIR-rSI and treatment effect was pronounced when applying an alternative frequently used endpoint, i.e. independent outcome defined as mRS 0-2, with a significant interaction. Although this result was not significant given a fixed p-value of < 0.05, we would argue that the observed negative association of FLAIR-rSI and treatment effect resembles the well-known association between time to treatment and treatment response from pooled stroke thrombolysis trials.[11] However, this result has to be considered as an exploratory analysis.

Our findings suggest that quantification of FLAIR-rSI might be of additional value for estimating the potential benefit of intravenous thrombolysis. The largest treatment effect was observed for patients in the lowest values of FLAIR-rSI, i.e., patients with virtually no measurable water uptake within the ischemic lesion reflecting very early strokes. Given the known pathophysiological association between FLAIR signal intensities and time since stroke onset, it would therefore be plausible that increases of FLAIR-rSI in visually pre-selected patients (based on a DWI-FLAIR-mismatch) is associated with a reduced efficacy of thrombolysis. In other words, in patients with a high probability of time since symptom onset ≤4.5 hours, shorter delays to thrombolysis are associated with larger treatment effect. Beyond time from symptom onset, rates of FLAIR-rSI evolution are influenced by other factors underlying the development of vasogenic oedema such as the integrity of cerebral microvasculature[17] or degree of arterial collateralisation.[18] Specifically, association of FLAIR-rSI and time from symptom onset was stronger in patients with poor collateral status compared to patients with good collateral function in a previous subgroup analysis.[18] Since perfusion imaging data was not systematically collected in our group of patients, we are however unable to report on this potential confounder, which has to be considered as a limitation of our study. In addition, interpretation of our results should be made with caution since the WAKE-UP trial was not powered to demonstrate differences in treatment effect between subgroups. We also like to emphasize that these effects occurred in patients in whom, according to the WAKE-UP inclusion criteria, no marked parenchymal FLAIR lesions were detected by visual judgement, and in whom overall treatment with alteplase had a beneficial effect.[6] We would therefore not propose to exclude patients with unknown time of symptom onset from treatment using a fixed FLAIR-rSI threshold when a DWI-FLAIR mismatch is present judged by visual analysis. Since clinical outcomes of patients with visible FLAIR lesions (and markedly higher FLAIR-rSI) were not randomized for WAKE-UP, we are unable to report on optimal cut-off values for treatment effect with thrombolysis in the entire population of patients with unknown time of

symptom onset screened for the WAKE-UP trial. In this study, we quantified FLARI-rSI in a time-efficient and straightforward approach that is methodically close to visual assessments performed in WAKE-UP. More complex classification algorithms are expected to add predictive value beyond FLAIR signal intensities as a surrogate marker for time since symptom onset. These would include imaging parameters not systematically collected in WAKE-UP such as quantitative measurements of T2-relaxation times[9,10], markers of cerebral perfusion or arterial collateralisation[14].

Summary

In summary, **i**n patients without marked parenchymal FLAIR hyperintensity detected by visual judgement in the WAKE-UP trial, higher FLAIR-rSI of DWI lesions was associated with decreased treatment effects of intravenous thrombolysis in line with the known association of treatment effect and elapsing time of stroke onset, although this did not reach statistical significance. Our results demonstrate that FLAIR-rSI can be considered as a valuable factor in multivariate and imaging-based individualized approaches for treatment of patients with acute stroke.

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Figure legend

Figure 1: Predicted odds ratios for clinical outcome measured by the modified Rankin scale based on the analysis of a continuous smooth trend of FLAIR relative signal intensity (FLAIR-rSI). All models were adjusted for NIHSS, patient age at symptom onset and stroke lesion volume. Odds ratios are shown in relation to favourable outcome defined as mRS 0-1 (A), mRS 0-2 (B) and the mRS ordinal analysis (C; shift analysis). Higher Odds ratios indicate effects in favour of treatment with alteplase. Predicted Odds ratios (blue line) and 95% Confidence intervals (blue ribbons) are shown.

Table

	FLAIR relative signal intensity (quartiles)				**p-value**
	Q1: 0-1.02	**Q2: 1.03-1.06**	**Q3: 1.07-1.11**	**Q4: >1.11**	
N	132	95	121	95	
Age [years] – mean (SD)	66.1 (10)	64.4 (10.3)	65.1 (12.7)	65.3 (10.8)	0.198
Male sex - number (%)	92 (69.7%)	59 (62.1%)	75 (62.0%)	63 (66.3%)	0.234
Time between last seen well and MRI [hours] - median (IQR)	9.8 (7.3-11.6)	9.5 (7.5-11.4)	9.9 (7.7-11.2)	10.4 (8.4-12.1)	0.093
NIHSS score - median (IQR)	5 (3-9)	6 (4-9)	6 (4-9)	6 (4-10)	0.559
DWI lesion volume at baseline [ml] - median (IQR)	1.7 (0.7-5.4)	2.5 (0.9-8.9)	3.1 (1.1-9.7)	2.4 (0.6-13.4)	0.031

Table 1: Demographic data and stroke lesion volumes grouped by increasing categories of relative FLAIR-SI quartiles (Q1 – Q4). Abbreviations: FLAIR, Fluid-attenuated inversion recovery; SD, standard deviation; MRI, magnetic resonance imaging; IQR interquartile range; NIHSS, National Institutes of Health Stroke Scale; DWI, diffusion-weighed imaging.