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Reperfusion beyond six hours reduces infarct probability in moderately ischemic brain tissue

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Abstract

Background and Purpose—We aimed to examine perfusion changes between 3 and 6, and 6 and 24 hours after stroke onset and their impact on tissue outcome.

Methods—Acute ischemic stroke patients underwent perfusion MRI at 3, 6, and 24hr after stroke onset and follow-up FLAIR at 1 month to assess tissue fate. Mean transit time prolongation maps (MTTp = MTT–[median MTT of contralateral hemisphere]) were obtained at 3hr ($MTTp_{3hr}$), 6hr ($MTTp_{6hr}$) and 24hr ($MTTp_{24hr}$). Perfusion changes between 3– 6hrs ($\Delta MTTp_{3\_6}$) and 6– 24hrs ($\Delta MTTp_{3\_6}$) were calculated. A 2-step analysis was performed to evaluate the impact of $\Delta MTTp_{3\_6}$ and $\Delta MTTp_{6\_24}$ on tissue fate. First, a voxel-based multivariable logistic regression was performed for each individual patient with $MTTp_{3hr}$, $\Delta MTTp_{3\_6}$, and ΔMTT_{6_24} as independent variables and tissue fate as outcome. Second, Wilcoxon signed-rank tests on logistic regression coefficients were performed across patients to evaluate whether $\Delta MTTp_{3\_6}$ and ΔMTT_{6_24} had significant impact on tissue fate for varying severities of baseline perfusion.

Results—Perfusion change was common during both time periods: 85% and 81% of patients had perfusion improvement during 3–6hr and 6–24hr time intervals, respectively. ΔMTT_{3_6} significantly influenced 1 month infarct probability across a wide range of baseline perfusion (MTTp 0–15s). ΔMTT_{6_24} also impacted 1 month infarct probability, but its influence was restricted to tissue with milder baseline ischemia (MTTp 0–10s).

Conclusions—Brain tissue with mild to moderate ischemia can be salvaged by reperfusion even after 6 hours. Such tissue could be targeted for intervention beyond current treatment windows.

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Keywords

reperfusion; ischemia; tissue outcome; MR perfusion imaging

Introduction

Pooled data from clinical trials including several thousand patients demonstrate the time-dependence of reperfusion-promoting therapies for enhancing favorable outcomes in acute ischemic stroke.[1, 2] For intravenous tPA, therapeutic benefit is lost 4.5 hours after stroke onset. For endovascular thrombectomy, which may achieve a higher likelihood of recanalization and greater tissue reperfusion, the window is extended up to six hours in patients with large vessel occlusion[3–7].

Tissue fate, however, is influenced not only by the duration of ischemia, but also by the severity of ischemia. Jones et al.[8] performed MCA occlusion in primates varying ischemia times from 15 minutes to permanent. Regional cerebral blood flow (CBF) and neurological deficits were correlated to final tissue outcome. When ischemic duration was < 30 minutes, all tissue survived, even tissue with severe CBF reduction. With permanent occlusion, the majority of tissue died, even tissue with mild ischemia. However, after two to three hours of ischemia, tissue with severely reduced in CBF (<10–12 ml/100g/min) died, while tissue with mild to moderate CBF reduction (12–18ml/100g/min) survived. Heiss and Rosner[9] confirmed the influence of both duration and severity of ischemia on tissue infarction by performing similar MCA occlusions in cats. These data suggested that the therapeutic window for ischemic stroke varied depending on the depth of ischemia: the window was short for tissue with severe ischemia, but prolonged for tissue with milder ischemia. This fundamental concept has not been examined in humans following acute ischemic stroke.

In this study, we performed sequential perfusion imaging at 3, 6, and 24 hours after stroke onset, evaluating the impact of 3–6hr and 6–24hr perfusion changes on tissue outcome and its dependence on baseline ischemic severity. We hypothesized that hyper-acute (between 3 and 6 hr) reperfusion would improve tissue outcome across a wide range of ischemic severity; while reperfusion in the later window (6–24hr) would still influence ischemic tissue fate, and its impact might be limited to mild to moderate ischemia.

Methods

Patients and Inclusion Criteria

This was a prospective, observational MRI study in acute ischemic stroke patients at a large, urban, tertiary care referral center with institutional review board approval. After providing written informed consent, patients were enrolled within 4.5 hours of stroke onset based on pre-specified inclusion criteria: clinically-suspected acute ischemic stroke; age ≥ 18 years; and NIHSS ≥ 5. Exclusion criteria included bilateral strokes, infratentorial stroke, contraindication to MRI or MRI contrast, pregnancy, or any acute endovascular intervention planned. Both IV tPA-treated and untreated patients were included. The study imposed no delay in time-to-tPA treatment and no deviation from standard monitoring practices or

standard inclusion/exclusion criteria for IV tPA administration. Demographic data and past medical history were obtained by the research coordinator at the time of patient enrollment.

Magnetic Resonance Imaging Protocol

Patients underwent four serial MRI scans: within 4.5 hours ("3hr"), at 6 hours ("6hr"), at 24 hours ("24hr"), and at 1 month after stroke onset, on a Siemens 3 Tesla whole body Trio scanner. For patients receiving IV tPA, the 3 hr scan was performed during IV tPA infusion. The MRI protocol included diffusion-weighted-imaging (DWI) and apparent diffusion coefficient (ADC) maps, FLAIR [TR/TE=10000/115 ms; inversion time = 2500 ms; matrix=512×416; 20 slices, slice thickness (TH)=5mm], and dynamic susceptibility contrast (DSC) perfusion images with 0.2ml/kg gadolinium contrast injected at 5 ml/sec (a T2*-weighted gradient echo EPI sequence; TR/TE=1500/43ms; 14 slices, TH=5mm, zero interslice gap; matrix=128×128). The DSC method provided the perfusion weighted imaging (PWI) for calculation of cerebral blood flow (CBF) and cerebral blood volume (CBV) maps. Mean transit time (MTT) was calculated as CBV/CBF. Voxels within the proximal middle cerebral artery (MCA) of the contralateral hemisphere were manually chosen and the mean concentration curve of these voxels provided the arterial input function (AIF). A time-shift insensitive block-circulant singular value decomposition method was utilized to minimize effects of time lag of the AIF on perfusion measurements.[10] Measurements were normalized to the contralateral unaffected hemisphere. MR angiography was not performed. Six parameter rigid image registration was performed to align all images across all four scan time-points for each patient using FSL 3.2 (FMRIB, Oxford, UK).[11] Accuracy of image registration was evaluated by a board-certified neuroradiologist (K.D.V.). For delineation of the final infarct, hyperintense lesions were manually outlined on the 1 month FLAIR image by a board-certified vascular neurologist (A.L.F.). Each voxel on the 1 month FLAIR image was assigned a value of "dead" or "alive" as their final fate.

Image and Statistical Analysis

MTT prolongation (MTTp) defined as [MTT]–[median MTT of contralateral hemisphere] was used to represent tissue perfusion. MTTp maps were obtained at 3hr ($MTTp_{3hr}$), 6hr ($MTTp_{6hr}$) and 24hr ($MTTp_{24hr}$) after stroke onset on a voxel-by-voxel basis. MTT was chosen over other time-based perfusion metrics because of a previous study which demonstrated that reperfusion as measured by MTT was most strongly associated with NIHSS improvement compared to Tmax or TTP.[12] Furthermore, MTT is less affected by contrast "delay" from indirect macrovascular pathways of contrast delivery in the setting of large vessel stenosis.[13] Perfusion changes between 3 to 6 hours and 6 to 24 hours were defined as ΔMTT_{3_6}=6hr MTTp –3hr MTTp and ΔMTT_{6_24}=24hr MTTp –6hr MTTp, respectively. To evaluate the impact of ΔMTT_{3_6} and ΔMTT_{6_24} on tissue fate, a two-step analysis was performed. The first step was voxel-based: For each patient, a multivariable logistic regression models was created on a voxel-by-voxel basis, as shown below:

$$Logit(P(tissue\ infarction)) = a0 + a1 \cdot MTTp_{3hr} + a2 \cdot \Delta MTT_{3\_6} + a3 \cdot \Delta MTT_{6\_24}$$

where P(tissue infarction) is the probability of tissue infarction at one month follow-up MRI, baseline $MTTp_{3hr}$ and ΔMTT_{3_6} and ΔMTT_{6_24} are continuous independent variables. The $MTTp_{3hr}$ maps were stratified into four 5-second strata as [0–5], [5–10], [10–15], and [15–

20]. For each patient, a logistic regression model was performed on voxels in each MTTp stratum to obtain a0, a1, a2, and a3. Logistic regression coefficients a2 and a3 characterize the impact of ΔMTT_{3_6} (a2) and ΔMTT_{6_24} (a3) on tissue fate, respectively. The second step (statistical analysis) was performed on a patient-by-patient basis: Wilcoxon signed-rank tests on a2 and a3 (derived from the step 1) were performed across patients to evaluate whether each of these parameters was significantly different from zero. Multiple comparisons were adjusted using a false discovery rate (FDR) of 5%. P values < 0.05 are considered statistically significant.

To directly visualize the impact of perfusion changes during 3 to 6 and 6 to 24 hr epochs on tissue fate, two three-dimensional color plots were generated to demonstrate infarct probability (in-plane color axis) as a function of 3hr MTTp (x-axis) and 6hr MTTp (y-axis) and as a function of 6hr MTTp (x-axis) and 24hr MTTp (y-axis). Infarct probabilities (IP) within each tissue group were defined as: IP= [number of infarcted voxels in a tissue group] / [total number of voxels in a tissue group]. To assess the impact of dynamic perfusion on the evolving ADC lesion, three-dimensional color plots were generated to demonstrate the percentage of voxels with “ADC positivity” (in-plane color axis) at 6hr as a function of 3hr MTTp (x-axis) and 6hr MTTp (y-axis), and “ADC positivity” (in-plane color axis) at 24hr as a function of 6hr MTTp (x-axis) and 24hr MTTp (y-axis). ADC-positivity was defined as a voxel with ADC < 600 mm^2/sec[14]. Isolated regions smaller than 1 ml were removed from analyses to avoid inclusion of voxels with noise-induced variations.

We evaluated whether perfusion changes during the 3–6 hr or 6–24 hr epochs correlated with clinical outcome as measured by a change in NIHSS from 3 hours to 1 month follow-up ($\Delta NIHSS_{3hr\_1mo}$) or change in NIHSS from 6 hours to 1 month follow-up ($\Delta NIHSS_{6hr\_1mo}$), respectively. $\Delta NIHSS_{3hr\_1mo}=NIHSS_{3hr}$ -$NIHSS_{1mo}$ and $\Delta NIHSS_{6hr\_1mo}=NIHSS_{6hr}$ −$NIHSS_{1mo}$. Correlation between $\Delta NIHSS_{3hr\_1mo}$ and volume of perfusion improvement with ΔMTT improving more than 2 sec (ΔMTT_{3_6} <=−2 sec) during 3 to 6 hours in mild to severe (3< $MTTp_{3hr}$ ≤ 15 seconds) ischemic tissue was performed. In addition, correlation between $\Delta NIHSS_{6hr\_1mo}$ and volume of perfusion improvement with ΔMTT improving more than 2 sec (ΔMTT_{6_24} <=−2 sec) during 6 to 24 hours in mild to moderate (3< $MTTp_{3hr}$ ≤ 10 seconds) ischemic tissue was performed.

All analyses were performed using Matlab v. 2010b (MathWorks Inc.).

Results

Twenty-seven stroke patients were imaged at a median [Interquartile range (IQR)] of 2.7 hr [2.3, 3.4], 6.2 hr [6.0, 6.5], 24.3 [23.8, 26.0], and 32 days [29, 35] after onset. Eighteen (67%) patients were treated with IV tPA. The remaining nine patients were not treated with IV tPA due to: current oral anticoagulant use (N=4), blood pressure > 185/110 (N=2), history of intracerebral hemorrhage (N=1), recent open heart surgery (N=1), and intracranial meningioma (N=1). Patient characteristics are shown in Table 1.

Highly dynamic perfusion during both 3–6 hr and 6–24 hr time intervals after ischemia was commonly observed. Of the 27 patients, 23 (85%) had perfusion improvement with ΔMTT

improving more than 2 sec (ΔMTT3_6 <=−2 sec) in at least 20% of baseline hypoperfused regions during the hyperacute phase (3–6 hrs after stroke onset); while 81% showed perfusion improvement (ΔMTT6_24 <=−2 sec) in the acute phase (6–24 hrs). Moreover, 16 (59%) and 12 (44%) had at least 20% volume of perfusion deterioration with ΔMTT worse than 2 sec for hyperacute (ΔMTT3_6 >2 sec) and acute (ΔMTT6_24 >2 sec) time intervals, respectively. The absolute and percent volume of tissue showing perfusion change during 3–6 hr and 6–24 hr intervals are shown in Fig. 1. The greatest volumes of perfusion change occurred in a range of ΔMTT of [−6, 6] sec during both 3–6 hr and 6–24 hr phases (Fig. 1). The volumes of perfusion changes were similar during the 3–6 hr and 6–24 hr epochs. Fig. 2 shows examples of spatial and temporal perfusion changes in two acute ischemic stroke patients with ΔMTT overlaid onto 1 month FLAIR images during 3–6 hr (upper row) and 6–24 hr (middle row) after ischemic onset. Patient example (A) shows greatest reperfusion between 3 and 6 hours after stroke onset, with little perfusion change between 6 and 24 hours, while the patient example (B) shows the majority of reperfusion during the acute phase of ischemia with little change during the hyperacute phase.

The impact of ΔMTT_{3_6} and ΔMTT_{6_24} on tissue fate was evaluated using a 2-step analyses in each of the four $MTTp_{3hr}$ strata (0–5, 5–10, 10–15, and 15–20 sec). The median and IQR of logistic regression coefficients a2 and a3 (derived from step 1), representing coefficients for ΔMTT_{3_6} and ΔMTT_{6_24}, respectively, across all patients are summarized in Table 2. After controlling for multiple comparisons using an FDR of 5%, the statistical significance of the Wilcoxon signed-rank tests (step 2) on a2 and a3 was also summarized in Table 2. Coefficients significantly different from zero indicate a significant association between the perfusion parameter and tissue fate at one month. ΔMTT_{3_6} were significantly associated with tissue fate for baseline $MTTp_{3hr}$ ranging from 0–15 sec (mild to severe ischemia); while ΔMTT_{6_24} were significantly associated with tissue fate for baseline $MTTp_{3hr}$ ranging from 0–10 sec (mild to moderate ischemia)—see table 2. Neither ΔMTT_{3_6} nor ΔMTT_{6_24} significantly impact tissue fate in extremely severe ischemia ($MTTp_{3hr}$ 15–20 sec) (Table 2).

In addition to the 5 second MTTp strata, we also performed analyses using MTTp strata of 3 and 4 second. Regardless of the strata size used, we found similar results: perfusion change during the hyperacute phase (3–6hrs) significantly influenced tissue fate in tissue with mild to severe ischemia; while perfusion change during the acute phase (6–24hr) impacted tissue outcome only in tissue with mild to moderate ischemia,.

To visualize the influence of perfusion changes across a wide range of baseline MTTp and their subsequent influence on tissue fate, 3D color plots were created to show infarct probability (in-plane color axis) as a function of 3hr MTTp (x-axis) and 6hr MTTp (y-axis) (Fig. 3A) and as a function of 6hr MTTp (x-axis) and 24hr MTTp (y-axis) (Fig. 3B). The fate of tissue with moderate ischemia is greatly impacted by both 3–6 hr (Fig 3a) and 6–24 hr (Fig 3b) perfusion changes, while the fate of severely ischemic tissue was only affected by 3–6 hr perfusion change. Tissue with extremely severe ischemia was not impacted by either 3–6 hr or 6–24 hr perfusion changes (see Fig 3 legend).

During 3–6 hr, ADC lesions grow in a time-dependent manner. To evaluate how early perfusion changes influence the evolving ADC lesion, 3D color plots were created: the

percent of tissue with ADC < 600 mm^2/sec at 6hr (in-plane color axis) was plotted as a function of 3hr MTTp (x-axis) and 6hr MTTp (y-axis) (Supplemental Figure IA) and the percent of tissue with ADC < 600 mm^2/sec at 24hr as a function of 6hr MTTp (x-axis) and 24hr MTTp (y-axis) (Supplemental Figure IB). ΔMTT_{3_6} were associated with the volume of ADC-positive tissue only in tissue with moderate to severe degrees of ischemia. During 6–24 hours, only tissue with moderate ischemia showed an association between the volume of ADC-positive tissue and perfusion changes. These data suggest that as the ADC lesion grows with time, reperfusion-dependent tissue salvage (ischemic penumbra) becomes progressively restricted to tissues with milder ischemia.

The volume of brain tissue with perfusion improvement (defined by ΔMTT ≥2 sec between 3 to 6 hours) was correlated to improvement in neurological deficit between 3 hours and 1 month as measured by serial NIHSS ($\Delta NIHSS_{3hr\_1mo}$). In tissue with mild to severe ischemia, the volume of brain tissue with perfusion improvement during 3–6 hrs inversely correlated with $\Delta NIHSS_{3hr\_1mo}$ (Fig. 4A, R=−0.48, P=0.014). Moreover, a similar inverse correlation was found between volume of perfusion improvement during 6–24 hrs and $\Delta NIHSS_{6hr\_1mo}$ in tissue with mild to moderate ischemia (Fig. 4B, R=−0.41, P=0.038). To verify that these results were not dependent on specific thresholds, we also used a ΔMTT threshold of 3 or 4 sec (ΔMTT≤-3 or -4 sec) to define perfusion improvement. Significant correlations remained, regardless of the ΔMTT threshold used.

Our findings demonstrated that the tissue level perfusion improvement during both 3–6 hr and 6–24 hr epochs was associated with clinical improvement at 1 month.

Discussion

Sequential perfusion MRI was performed at three time-points within 24 hours of stroke onset during which perfusion changes (both improvement and worsening) prior to and beyond six hours after stroke onset occurred in the majority of patients. Our key findings include: (1) perfusion improvement was associated with reduced infarct probability for tissue with mild to moderate ischemia *both* within and beyond six hours; (2) perfusion changes influenced the fate of severe ischemic tissue within but not beyond six hours; while (3) perfusion changes either within or beyond six hours had little impact at extremely severe ischemia. This data provides tissue-level evidence in ischemic stroke patients for a closing therapeutic window that is impacted by both time and depth of ischemia. Moreover, volume of tissue with perfusion improvement during both 3 to 6 hr and 6 to 24 hr epochs was associated with improved NIHSS at 1 month after stroke. Using color-coded duplex sonography to evaluate recanalization, Wunderlich et al have reported that complete recanalization by 30 minutes, between 30minutes to 6 hours, and between 6 to 24 hours after stroke onset were all significantly associated with improved 30 days functional outcome in 99 patients.[15] Our findings are consistent with this study.

Recent randomized clinical trials have demonstrated that endovascular thrombectomy following intravenous tPA improved recanalization of proximal artery occlusions and clinical outcomes compared to intravenous tPA alone[3–6]. In these trials, the mean time from symptom onset to recanalization ranged from 4.0 to 5.5 hours. Our findings suggest that

tissue with mild to moderate ischemia may have a prolonged therapeutic window beyond six hours such that a subgroup of patients may still benefit from late reperfusion-promoting therapies if selected properly.

Our findings in human stroke patients demonstrate that infarct probability is influenced by both ischemic duration and severity. Such findings are consistent with earlier blood flow studies in gyrencephalic animal models of focal ischemia.[8, 9] While animal models of ischemic stroke are controlled with respect to the depth of ischemia and timing of reperfusion, spontaneous perfusion changes in human stroke have been poorly understood until recently with the advent of rapid MR and CT imaging protocols allowing for sequential imaging in the early hours after stroke onset. The current study measured tissue perfusion at three early time-points within 24 hours of stroke onset, evaluating perfusion change within individual voxels over time. We found that perfusion was highly dynamic during both hyperacute (3–6hr) and acute (6–24hr) periods. Not only did tissue demonstrate improvement in perfusion which was associated with lower infarct probability, but also, substantial tissue demonstrated worsening perfusion which was associated with higher infarct probability. We and other groups have previously reported on this observation of "worsening" or "extension" of the perfusion lesion;[16–19] moreover, we previously found this tissue to be associated with prolonged onset-to-IV tPA-treatment, suggesting that IV tPA may exert its beneficial effects not only by promoting reperfusion, but also by preventing worsening perfusion. Our data agree with previous studies[20–26] showing that, regardless of the time interval after stroke onset, tissue with extremely severe baseline ischemia is irreversibly injured.

While the present study is not able to elucidate underlying causes of perfusion instability, previous studies have suggested potential mechanisms leading to spontaneous reperfusion or worsening perfusion. Reperfusion may be related to spontaneous or therapy-induced clot lysis (e.g. tPA), or due to recruitment of collateral flow.[27–30] Potential causes of worsening perfusion include: thrombus extending into a nearby branch surrounding the ischemic region that was not previously occluded,[31] previous arterial recanalization prior to baseline imaging followed by re-occlusion with new ischemia on subsequent imaging,[32] and loss of collateral flow that was supplying the territory on baseline imaging but not on subsequent imaging.[30] Peri-infarct depolarizations and cortical spreading depression may also contribute to worsening perfusion surrounding a region of infarction. Such depolarizations are associated with a progressive decline in blood flow and progressive growth in infarct.[33, 3420]

Our study has several strengths. It is a prospective imaging study in a cohort of ischemic stroke patients who were imaged at a median of 2.7 hours after stroke onset. The serial imaging design permitted study of dynamic perfusion changes within the first 24 hours, correlated with 1-month tissue outcome on a voxel-by-voxel basis (via coregistration). Study limitations include: (1) The sample size is small which limits the generalizability of these findings. (2) Discrete imaging time-points limit the knowledge of the exact timing of perfusion change which occurs at some time between two perfusion scans. This limits our knowledge of the exact therapeutic window within the time intervals that were imaged, especially in the 6–24 hours window. (3) Conclusions regarding the impact of dynamic perfusion on tissue fate are valid for perfusion changes at these two time intervals after

stroke onset, but will likely differ for perfusion changes at later time intervals when tissue fate is pre-determined. (4) This cohort included moderate to large strokes with median NIHSS of 16 and thus our results may not apply to patients with smaller, less severe strokes.

We did not perform MR or CT angiograph imaging in this cohort of patients. The lack of vascular imaging does not allow us to assess recanalization. However, it has been demonstrated in several studies that reperfusion is a stronger predictor of tissue fate and clinical outcome than recanalization.[35, 36] Therefore, we think that the tissue-level reperfusion data is more important for our analysis than the patient-level semi-quantitative measures that vascular imaging provides.

These data should not be interpreted as demonstrating threshold MTTp values for baseline ischemia that indicate futility for reperfusion therapies. We used 5 second baseline MTTp strata to investigate the general physiological principle whether the impact of perfusion change on tissue fate during the 3 to 6 and 6 to 24 hours epochs depend on baseline tissue perfusion. Any MTTp thresholds derived from these or similar data would still need to be tested in randomized prospective study with clinical outcomes.

Conclusions

Our findings demonstrate that the fate of regions with mild to moderate ischemia is associated with changes in perfusion even after 6 hours and, thus, such regions may have a longer therapeutic window compared to regions with severe ischemia. Such tissue could be targeted for intervention beyond current treatment windows. Prospective randomized clinical trials of patients with mild to moderately ischemic tissue presenting beyond 6 hours are needed to determine the clinical efficacy of such an approach.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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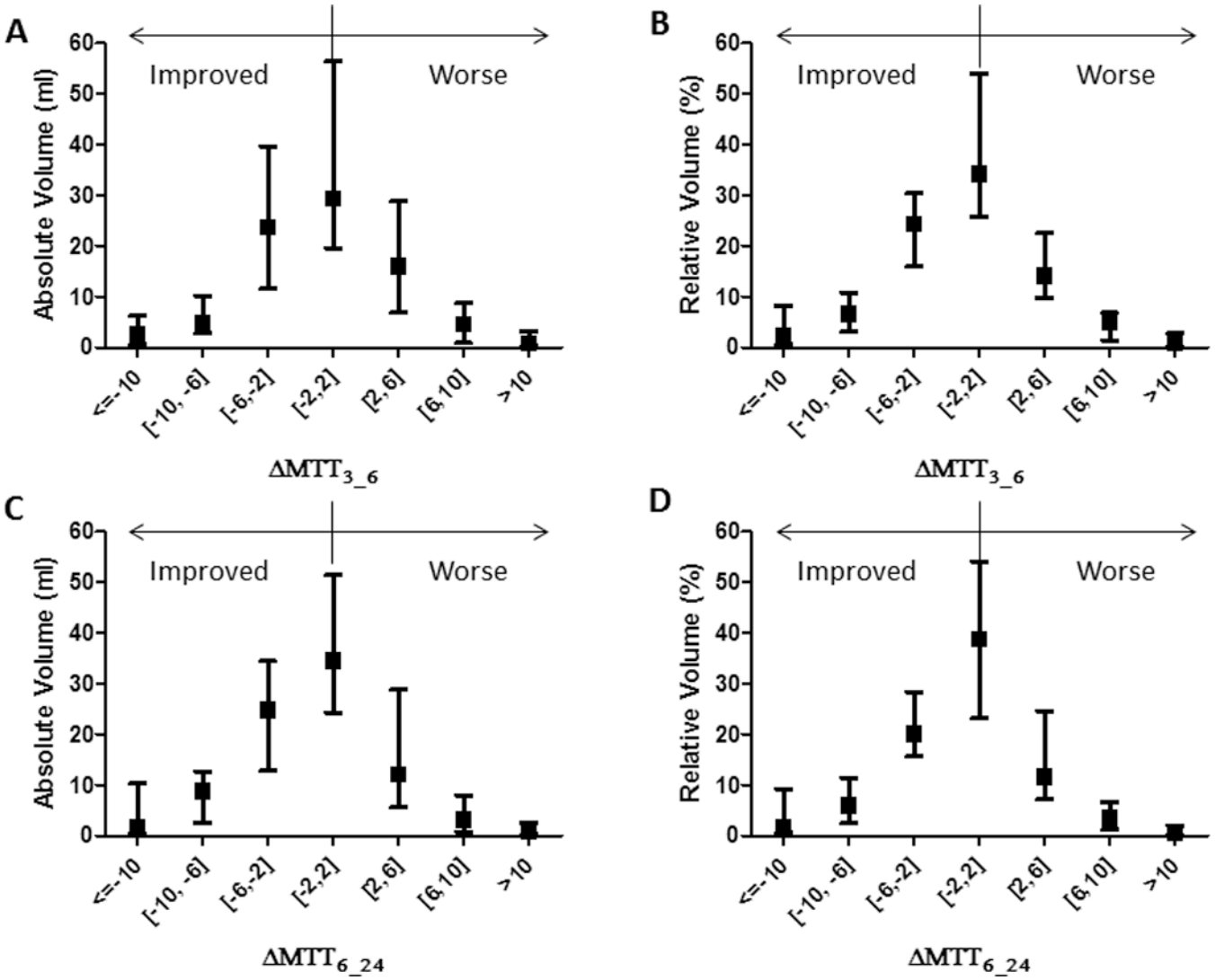

Fig. 1. Dynamic perfusion is common during both hyperacute (3–6 hrs) and acute (6–24 hrs) phases of ischemic stroke

Absolute (A, C) and percent (B, D) volume with ΔMTT3_6 (upper row) and ΔMTT6_24 (lower row) in 4 second ΔMTT increments. Data are shown as median [25th, 75th quartiles] across patients.

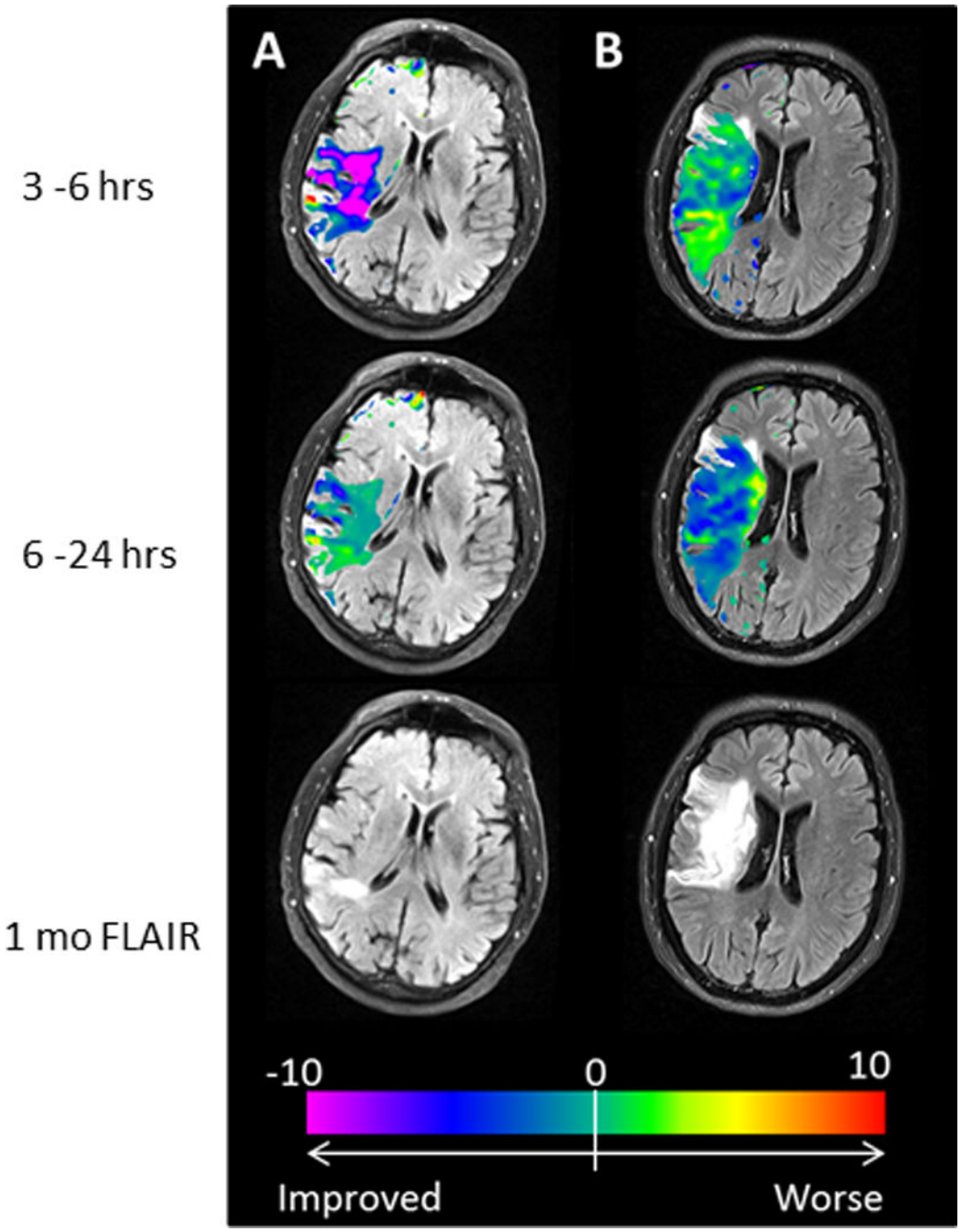

Fig. 2. Two patient examples of spatial and temporal perfusion instability in acute ischemic stroke

(A) a 78 year old man with R MCA stroke, NIHSS=5, was treated with IV tPA 2.2 hr after onset. The first two serial perfusion imaging were obtained at 2.7 hr and 6.2 hr after onset. Most ischemic tissue had a perfusion improvement during the hyperacute phase (A, 1st row) and remained mostly stable during the acute phase (A, 2nd row). Most of the ischemic tissue was salvaged as a result of this hyperacute (3–6 hrs) perfusion improvement. (B) a 47 year old woman with R MCA stroke, NIHSS=18, was treated with IV tPA 1.7 hr after onset. The first two serial perfusion imaging were obtained at 2.0 hr and 6.0 hr after onset. There was a

small region of perfusion improvement during the hyperacute phase (B, 1st row); more substantial perfusion improvement occurred during the acute phase (B, 2nd row). Some but not all regions with the acute (6–24 hrs) perfusion improvement survived.

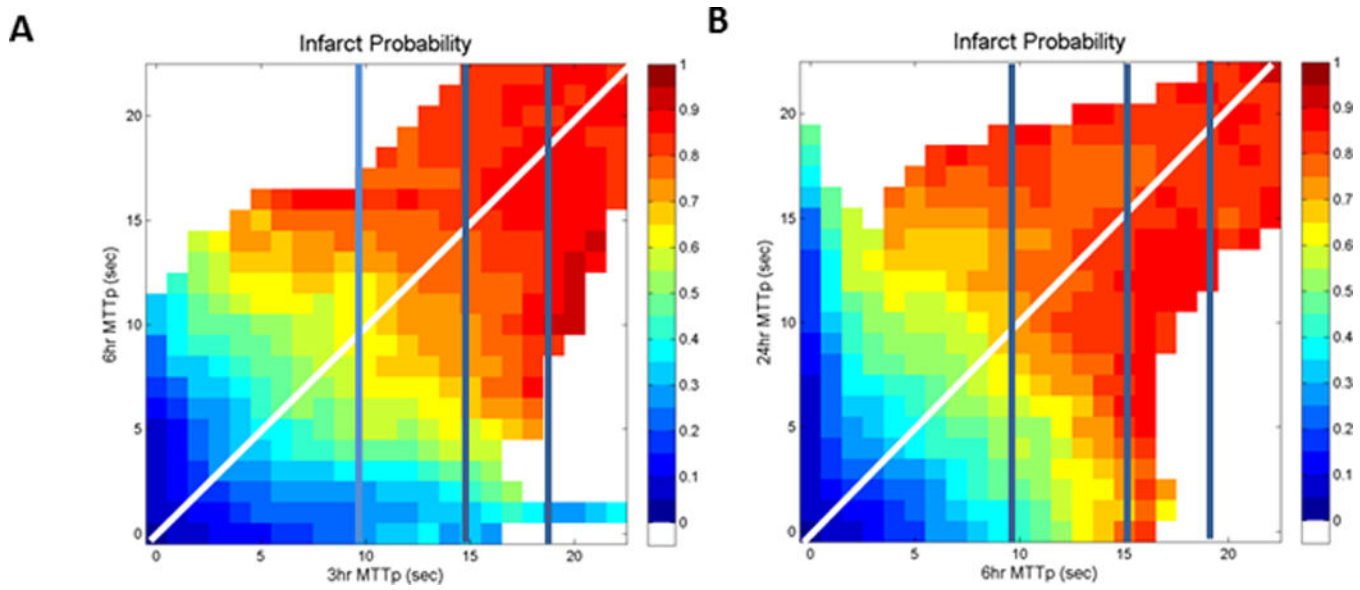

Fig. 3. Dynamic perfusion changes influence tissue fate during 3–6-hour and 6–24-hour time interval, but the degree of tissue salvage depends on baseline severity of ischemia

3D color plots were generated to demonstrate infarct probability (in-plane color axis) as a function of 3hr MTTp (x-axis) and 6hr MTTp (y-axis) (A) and as a function of 6hr MTTp (x-axis) and 24hr MTTp (y-axis) (B). The vertical lines show how subsequent MTTp change may alter infarct probabilities at three given baseline MTTp (9, 15 and 19 seconds) at either 3hrs (A) or 6hrs (B). The diagonal lines (A & B) mark stable perfusion during the 3–6 hrs (A) and 6–24 hrs (B) periods. Area below the diagonal line represent voxels with improved perfusion, while voxels below the line represent voxels with worsened prefusion. For an example of moderate ischemia at 3hr (MTTp of 9s, vertical line in A), if 6hr MTTp improved to 0–2s, infarct probability was low (~25%), however if 6hr MTTp worsened to 17s, infarct probability was high (~85%). Using this same example of tissue with moderate ischemia (MTTp of 9s), infarct probability in the acute phase (6–24 hrs) (B), also varied greatly (from 35% to 85%), suggesting perfusion changes during this acute phase (6–24hrs) also influenced fate in tissue with moderate ischemia. For an example of severe ischemia (vertical lines crossing MTTp of 15s, A&B), infarct probability was still influenced by perfusion change during the hyperacute phase (3–6hrs); however, tissue fate was only minimally affected by perfusion change during the acute phase (6–24hr), suggesting that the therapeutic window for severely ischemic tissue has closed by the later time period. In tissue with extremely severe ischemia (MTTp of 19 seconds) infarct probability has almost no change in relation to perfusion changes during either the hyperacute (3–6hrs) or acute phase (6–24hr).

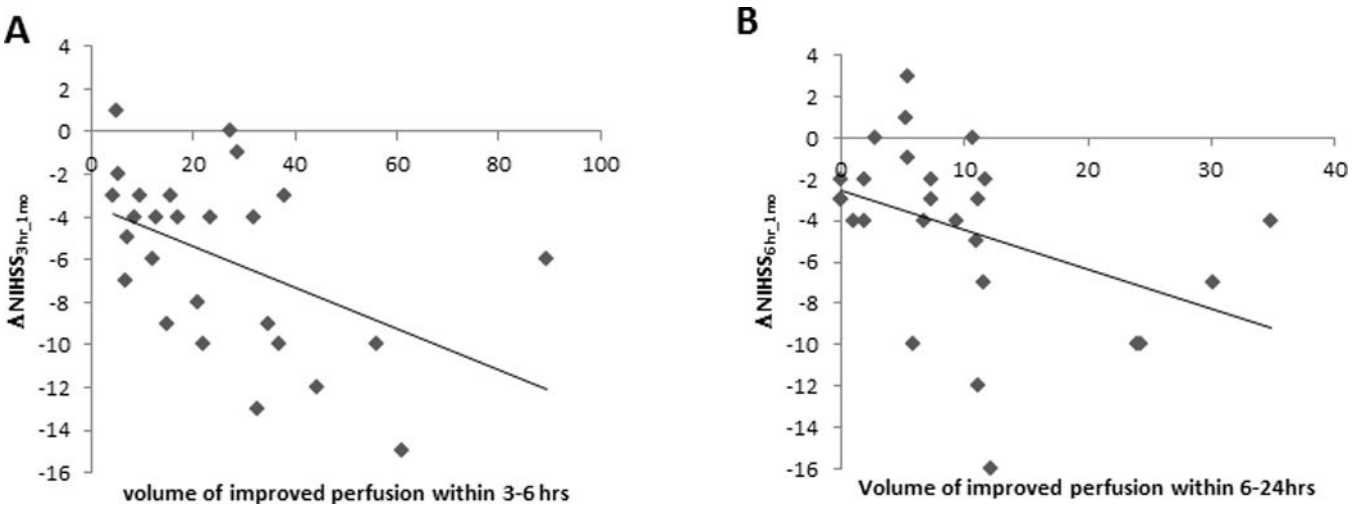

Fig. 4. Correlation between ΔNIHSS and volume of improved perfusion between 3– 6 hours (A) and 6– 24 hours (B)

$\Delta NIHSS_{3hr\_1mo}$ was defined as $NIHSS_{3hr} - NIHSS_{1mo}$ and $\Delta NIHSS_{6hr\_1mo}$ was defined as $NIHSS_{6hr} - NIHSS_{1mo}$. Volumes of improved perfusion were obtained from tissue with mild to severe ischemia ($3<MTTp_{3hr}\leq 15$ seconds) at 3 hours (A) and with mild to moderate ischemia ($3<MTTp_{6hr} \leq 10$) at 6 hours (B).

Table 1

Patient Characteristics (N=27)

Female, n (%)	8 (30%)
Age (years)	65 [55, 74]
Admission NIHSS	16 [8, 20]
African-American, n (%)	10 (37%)
tPA treatment, n (%)	18 (67%)
Admission Mean Arterial Pressure (mmHg)	114 [104, 126]
Onset to tPA treatment time	1.9 [1.7, 2.3]
Onset to 3-hr Scan, hour	2.7 [2.3, 3.4]
Onset to 6-hr Scan, hour	6.2 [6.0, 6.5]
Onset to 24-hr Scan, hour	24.3 [23.8, 26.0]
History of Hypertension, n (%)	23 (85%)
History of Diabetes, n (%)	7 (26%)
History of Congestive Heart Failure, n (%)	4 (15%)
Current Tobacco Use, n (%)	6 (22%)
History of Coronary Artery Disease, n (%)	8 (30%)
History of Stroke or TIA, n (%)	4 (15%)

Continuous data shown as median [interquartile range].
NIHSS = National Institutes of Health Stroke Scale
tp1=time-point 1 MRI; tp2=time-point 2 MRI
TIA=transient ischemic attack

Table 2

Median and [IQR] of the logistic regressions (step 1) coefficients a2 and a3 across all patients. The statistical significance of the Wilcoxon signed-rank tests (step 2) on a2 and a3 were also summarized in Table 2 to evaluate the impact of $\Delta MTT_{3–6hr}$ (a2) and $\Delta MTT_{6–24hr}$ (a3) on tissue fate in each of the four MTTp strata.

MTTp strata (seconds)	a2 (Median [IQR]) $\Delta MTT_{3–6hr}$	a3 (Median [IQR]) $\Delta MTT_{6–24hr}$
0–5	**0.26 [0.11, 0.38]****	**0.10 [0.04, 0.23]***
5–10	**0.19 [0.06, 0.27]****	**0.13 [0.05, 0.17]***
10–15	**0.20 [0.04, 0.25]****	0.00 [−0.05, 0.08] (ns)
15–20	0.07 [−0.06, 0.17] (ns)	−0.02 [−0.07, 0.03] (ns)

**: P<0.001,

*: P<0.05, ns: not significant