# Identifying the Perfusion Deficit in Acute Stroke with Resting-State Functional Magnetic Resonance Imaging

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**Temporal delay in blood oxygenation level–dependent (BOLD) signals may be sensitive to perfusion deficits in acute stroke. Resting-state functional magnetic resonance imaging (rsfMRI) was added to a standard stroke MRI protocol. We calculated the time delay between the BOLD signal at each voxel and the whole-brain signal using time-lagged correlation and compared the results to mean transit time derived using bolus tracking. In all 11 patients, areas exhibiting significant delay in BOLD signal corresponded to areas of hypoperfusion identified by contrast-based perfusion MRI. Time delay analysis of rsfMRI provides information comparable to that of conventional perfusion MRI without the need for contrast agents.**

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Perfusion and diffusion magnetic resonance imaging (MRI; perfusion-weighted imaging [PWI]; diffusion-weighted imaging [DWI]) are employed in clinical practice and in research to identify pathophysiological patterns in patients with acute stroke.[1–4] DWI is thought to roughly reflect the severely damaged infarct core, whereas PWI reflects the area of hypoperfusion. Their volumetric difference is also termed the PWI/DWI mismatch, and has been suggested as an MRI surrogate of the ischemic penumbra,[5,6] that is, an area of reduced cerebral blood flow (CBF), resulting in impaired neuronal function but preserved brain structure.[7] If normal CBF is restored in time, the ischemic damage in the penumbra can be reversed, and it is therefore regarded as the prime target for any treatment approach.

Although in some centers PWI/DWI information is already used for clinical decision making (eg, for the indication of fibrinolysis[8]), its clinical utility is still controversial, and new approaches are being sought to overcome shortcomings. This includes the application of contrast agent for PWI,[9,10] which has potentially severe side effects (eg, nephrogenic systemic fibrosis), and also precludes repetitive examinations for monitoring purposes. Following recent observations that CBF dynamics may be assessed with resting-state functional MRI (rsfMRI),[11] we hypothesized that rsfMRI should contain information similar to the measures obtained from bolus track PWI, such as mean transit time (MTT) or time to peak. Here, we investigated the time delay in blood oxygenation level–dependent (BOLD) signal of individual voxels using rsfMRI data as a new approach to give pathophysiological information similar to that given by perfusion MRI in the acute phase of stroke without the need for contrast agents.

## Subjects and Methods

### *Participants*

Data were acquired from 17 patients with acute ischemic stroke (age, 35–92years; mean, 68.4years; 9male/8female) at 1 day after stroke onset, and 1 healthy participant. Informed consent was obtained from all participants prior to scanning, and all protocols were approved by the Charité Institutional Review Board EA4/026/08. Due to severe motion confounds in 6 patients, 11 patients were included for final analysis.

### *Magnetic Resonance Data Acquisition*

Magnetic resonance data were acquired on a Siemens (Erlangen, Germany) Tim Trio 3T scanner. The MRI scanning protocol followed the study protocol of a prospective clinical study on the value of the mismatch concept in ischemic stroke (1,000+): (1) DWI: slices = 50, echo time (TE) = 93.1 milliseconds, repetition time (TR) = 7,600 milliseconds, flip = 90°, matrix = 192 × 192, thickness = 2.5 mm, diffusion volumes = 14, time = 2 minutes, 18 seconds; (2) PWI: slices = 21, TE = 29 milliseconds, TR = 1,390, matrix = 128 × 128, slice thickness = 5 mm, gap = 0.5 mm, time = 2 minutes; and (3) rsfMRI: slices = 34, TE = 30, TR = 2,300, flip = 90°, matrix = 64 × 64, voxel dimensions = 3 × 3 × 3 mm + 1 mm gap, volumes = 150, time = 5 minutes, 50 seconds.

### *Data Analysis*

*DATA PREPROCESSING.* Preprocessing of fMRI data was performed using both FSL (http://www.fmrib.ox.ac.uk/fsl) and AFNI (http://afni.nimh.nih.gov/afni), including removal of the

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first 4 volumes to allow for signal equilibration, slice-timing correction for interleaved acquisition, and motion correction. Six patients were excluded because of either large head motion (>6mm) or consistent headshaking. Further image preprocessing consisted of spatial smoothing (Gaussian kernel, 6mm full width half maximal) and removing the linear trend. The data were then bandpass filtered (0.01–0.1Hz). DWI and PWI scans were coregistered to the individual's mean functional image.

*TIME-SHIFT ANALYSIS.* We regressed the effect of head motion (using 6 motion parameters), and calculated the average time series across the whole brain. For each voxel, we shifted the time course from −3 TR to 3 TR (−6.9 to +6.9 seconds), and correlated it with the average model time course at each TR. For Patients 3 and 4 (Fig 2), who showed hypoperfusion in the PWI scan across almost half the brain, we modified the analysis and correlated it with average time course of only the healthy hemisphere. Each voxel was then assigned a value based on the time shift required for the maximal correlation coefficient (Fig 1).

*CLINICAL VALIDATION.* Four experts in lesion delineation in the Division of Academic Neuroradiology at Charité Hospital, Berlin traced the hypoperfusion regions (MTT) and time-shift analysis (TSA) for each patient independently. Overlap was calculated using the Dice coefficient (DC), which calculates the ratio of the intersection with respect to the union of each pair of masks. To evaluate the similarity in mask volume, we also calculated the correlation coefficient (CC) of the volume size across the 4 maps across all patients.

## Results

A synopsis of findings in all 11 patients are provided in the Table, Figure 2, and Supplementary Figure 1 (the latter 2 providing DWI, MTT, and TSA maps). In all 11 patients with ischemic stroke, TSA showed areas with a pronounced time delay relative to the respective mean time course (see Fig 2). Overall, these areas corresponded to the areas of hypoperfusion as identified by MTT maps and not to the infarct cores (DWI). The pronounced mismatches in

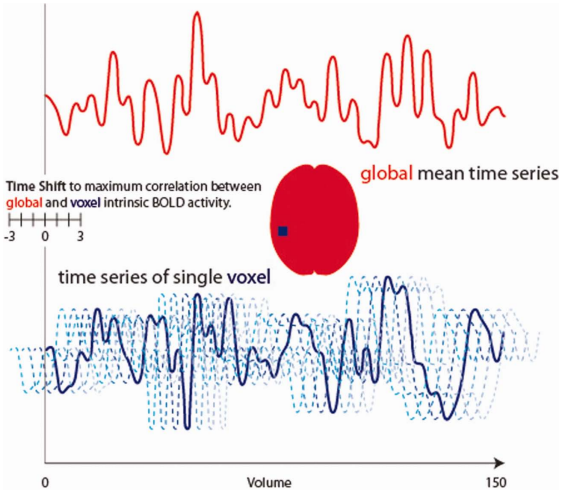


**FIGURE 1: Illustration of the basic methodology for the time-shift analysis. BOLD = blood oxygenation level–dependent. [Color figure can be viewed in the online issue, which is available at www.annalsofneurology.org.]**

Patients 1, 2, 4, and 11 and the smaller mismatch in Patients 5, 7, and 10 are clearly apparent when comparing lesions on TSA maps with DWI. In Patient 1, in addition to the TSA/PWI overlap, the TSA map also showed time delay in medial prefrontal areas. An additional methodological consideration is depicted in Patients 3 and 4, who suffered very large infarctions covering the middle cerebral artery territory. As the whole brain mask would have been too contaminated by signal from hypoperfused tissue, we chose to use the average time course from only the healthy hemisphere as the reference time series. Patient 4 showed a high degree of overlap (DC = 0.67) with areas specific to the perfusion deficit within the affected hemisphere, whereas Patient 3 showed a low degree of overlap (DC = 0.40), likely caused by the large infarct core (DWI). Large head motion in Patient 11 likely caused a low degree of overlap (DC = 0.34). The maximal head motion displacement of this patient was 5.4mm, and there were many spikes in the head motion curve of this patient (Supplementary Fig 4).

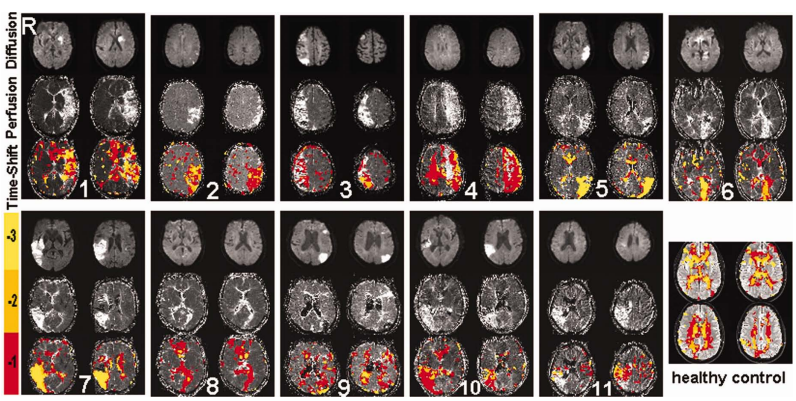


**FIGURE 2: Time-shift analysis results of 11 stroke patients and 1 healthy control with time-shift range from −3 repetition time (TR) to +3 TR. In 11 patients, areas affected by the ischemic stroke (hypoperfused) show a pronounced time delay to the global mean time course. In 1 healthy control, the time-lagged areas were approximately symmetrically distributed within ventricular areas. −1, −2, −3 in color bar indicate −1 TR, −2 TR, −3 TR time shift. [Color figure can be viewed in the online issue, which is available at www.annalsofneurology.org.]**

**TABLE : Patient Information**

| Patient # | Age, yr/Sex | Time, h[a] | NIHSS in Acute Phase | Maximum Motion, mm | Results of Magnetic Resonance Angiography | Brain Mask for TSA | Degree of Overlap, DC[b] |
|---|---|---|---|---|---|---|---|
| 1 | 35/M | 15 | 6 | 2.42 | Occlusion in M2 of MCA on the left side, and dissection of left ACI | Whole | 0.67 |
| 2 | 52/M | 30 | 4 | 1.58 | Stenosis of the left M2 of MCA | Whole | 0.48 |
| 3 | 83/M | 5 | 26 | 0.71 | Occlusion in M2 of MCA on the right side (multimorbid patient) | Healthy hemisphere | 0.40 |
| 4 | 52/M | 10 | 0 | 0.41 | 95% stenosis of the left ACI | Healthy hemisphere | 0.63 |
| 5 | 68/M | 9 | 4 | 2.18 | Posterior MCA territory on the left side with a corresponding hypoperfusion | Whole | 0.66 |
| 6 | 67/M | 2 | 2 | 2.80 | Occlusion in P2 of PCA on the left side | Whole | 0.61 |
| 7 | 78/F | 24 | 12 | 4.69 | Occlusion in M2 of MCA on the right side | Whole | 0.66 |
| 8 | 77/M | 26 | 4 | 0.89 | Occlusion in P3 of PCA on the left side | Whole | 0.31 |
| 9 | 77/M | 22.75 | 11 | 0.97 | No occlusion | Whole | 0.33 |
| 10 | 69/M | 16 | 17 | 2.29 | Occlusion in M1 of MCA on the right side | Whole | 0.70 |
| 11 | 82/F | 9 | 2 | 5.41 | Occlusion in M2 of MCA on the right side | Whole | 0.34 |

[a]Hours after stroke onset.
[b]Overlap between hypoperfusion area and TSA results as measured with DC. ACI = internal carotid artery; DC = Dice coefficient; F = female; M = male; MCA = middle cerebral artery; NIHSS = National Institute of Health Stroke Scale; PCA = posterior cerebral artery; TSA = time-shift analysis.

When we calculated the time delay of each voxel's time course in the healthy brain, the areas showing a clear time delay to the global mean were symmetrically distributed and located largely within the ventricles (see Fig 1, Supplementary Fig 1), whereas smaller time delays were identified in adjacent white matter.

To validate the TSA approach for clinical purposes, 4 experts in stroke imaging manually outlined lesions on MTT and TSA maps. We calculated both the CC of the number of voxels included in the lesions between the masks across patients, and the DC for each individual patient, which were then averaged (inter-rater MTT: CC = 0.95, DC = 0.66; inter-rater TSA: CC = 0.93, DC = 0.70). The between modalities results were: CC = 0.79, DC = 0.53.

As the rsfMRI session (6 minutes) was longer than standard PWI scans (2 minutes), we tested the impact of decreasing the scan length on the TSA results. Decreasing the scan length used for the analysis (in increments of 10 volumes), we found that 184 seconds (80 volumes) still showed results similar to those of the full scan session (Supplementary Fig 2). In future data acquisition, such parameters may prove valuable to making practical decisions about acquisition length in clinical settings.

## Discussion

In this study, we successfully used rsfMRI to identify hypoperfused areas in acute stroke. We suggest that an assessment of time delays of the spontaneous low-frequency fluctuations of the BOLD signal may provide information comparable to that provided by parameters of contrast-based perfusion MRI such as MTT,[12] and thus serve as a useful diagnostic tool for stroke MRI without the need for the application of a contrast agent. We show that TSA maps onto the PWI-MTT–defined areas, and not the DWI

lesion. Previous PWI studies have reported delays of MTT in stroke in the range of 0 to 8 seconds,[13] with a difference between the affected and nonaffected hemisphere of 0.4 to 5.6 seconds (1.3 ± 1.9s).[14] In our study, we found an MTT delay of 0.4 to 4.3 seconds (2.3 ± 1.2 seconds; Supplementary Table), suggesting that the time-shift range employed for TSA (± 6.9 seconds) was reasonable.

The primary limiting factor for clinical application of the current approach was the large head motion (>6mm) of 6 patients who had to be excluded from further analysis due to artifacts. Thus, to be clinically viable, it would be valuable to find an effective way to manage head motion, such as prospective motion correction during scanning. Although motion is not a practical issue unique to this imaging modality, real time motion estimation could facilitate decisions regarding repeating or prolonging the measurement—viable clinical options, as no contrast agent application is necessary. Further improvement of the method may be achieved by acquiring BOLD-fMRI at a higher temporal resolution, which is likely to further improve the precision of hypoperfusion assessment.

The current findings suggest TSA as a promising new approach to assessing hemodynamics in stroke. Although it is just a first step, this straightforward measure shows us that there is key information about tissue perfusion that can be extracted from rsfMRI data. The other interesting noninvasive approach to perfusion imaging, arterial spin labeling (ASL), so far has been hampered by poor signal to noise in situations of long transit times,[15] and in a recent study ASL missed 7 of 39 perfusion lesions.[16] Although the present study is essentially a feasibility study, we believe that rsfMRI appears to be sensitive for detecting severe hypoperfusion in acute stroke, and holds promise to provide a valuable alternative to current techniques, opening a new place for rsfMRI in acute stroke diagnostics.

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## Potential Conflicts of Interest

A.V.: consultancy, Lundbeck; speaking fees, Boehringer Ingelheim, SANOFI, Bayer, AstraZeneca, Novartis; Patents/patents pending, Max Plank Institute/Virginia Tech (patent pending for described work; no compensation as yet). D.G.: consultancy, Bayer Pharma. M.E.: grants/grants pending, AstraZeneca, Sanofi; consultancy, Bayer, Boehringer Ingelheim, Bristol-Myers Squibb, MSD, Pfizer, Sanofi; grants/grants pending, AstraZeneca, Sanofi, Maquet; speaking fees, AstraZeneca, Bayer, Berlin Chemie, Bristol-Myers Squibb, Boehringer Ingelheim, Desitin, Eisei, Sanofi, Ever, GlaxoSmithKline, MSD, Novartis, Pfizer, Takeda, Trommsdorff. J.F.: board membership, Lundbeck; consultancy, Lundbeck, Sygnis, Synarc; speaking fees, Siemens, Boehringer Ingelheim.

## References

1. Dani KA, Thomas RG, Chappell FM, et al. Computed tomography and magnetic resonance perfusion imaging in ischemic stroke: definitions and thresholds. Ann Neurol 2011;70:384–401.
2. Merino JG, Warach S. Imaging of acute stroke. Nat Rev Neurol 2010;6:560–571.
3. Sorensen AG, Buonanno FS, Gonzalez RG, et al. Hyperacute stroke: evaluation with combined multisection diffusion-weighted and hemodynamically weighted echo-planar MR imaging. Radiology 1996;199:391–401.
4. Wardlaw JM. Neuroimaging in acute ischaemic stroke: insights into unanswered questions of pathophysiology. J Intern Med 2010;267:172–190.
5. Karonen JO, Vanninen RL, Liu Y, et al. Combined diffusion and perfusion MRI with correlation to single-photon emission CT in acute ischemic stroke. Ischemic penumbra predicts infarct growth. Stroke 1999;30:1583–1590.
6. Schlaug G, Benfield A, Baird AE, et al. The ischemic penumbra: operationally defined by diffusion and perfusion MRI. Neurology 1999;53:1528–1537.
7. Astrup J, Siesjo BK, Symon L. Thresholds in cerebral ischemia—the ischemic penumbra. Stroke 1981;12:723–725.
8. Rother J, Schellinger PD, Gass A, et al. Effect of intravenous thrombolysis on MRI parameters and functional outcome in acute stroke <6 hours. Stroke 2002;33:2438–2445.
9. Rosen BR, Belliveau JW, Vevea JM, Brady TJ. Perfusion imaging with NMR contrast agents. Magn Reson Med 1990;14:249–265.
10. Villringer A, Rosen BR, Belliveau JW, et al. Dynamic imaging with lanthanide chelates in normal brain: contrast due to magnetic susceptibility effects. Magn Reson Med 1988;6:164–174.
11. Tong Y, Frederick BD. Time lag dependent multimodal processing of concurrent fMRI and near-infrared spectroscopy (NIRS) data suggests a global circulatory origin for low-frequency oscillation signals in human brain. Neuroimage 2010;53:553–564.
12. Ostergaard L, Weisskoff RM, Chesler DA, et al. High resolution measurement of cerebral blood flow using intravascular tracer bolus passages. Part I: Mathematical approach and statistical analysis. Magn Reson Med 1996;36:715–725.
13. Schellinger PD, Latour LL, Wu CS, et al. The association between neurological deficit in acute ischemic stroke and mean transit time: comparison of four different perfusion MRI algorithms. Neuroradiology 2006;48:69–77.
14. Mihara F, Kuwabara Y, Tanaka A, et al. Reliability of mean transit time obtained using perfusion-weighted MR imaging; comparison with positron emission tomography. Magn Reson Imaging 2003;21:33–39.
15. Petersen ET, Zimine I, Ho YC, Golay X. Non-invasive measurement of perfusion: a critical review of arterial spin labelling techniques. Br J Radiol 2006;79:688–701.
16. Bokkers RP, Hernandez DA, Merino JG, et al. Whole-brain arterial spin labeling perfusion MRI in patients with acute stroke. Stroke 2012;43:1290–1294.