The goal of perfusion imaging in acute stroke is to identify patients with a large penumbra (and relatively small infarct core) who might have the most to gain from acute reperfusion therapy. Using magnetic resonance imaging (MRI), typically the penumbra is defined as a mismatch between the diffusion lesion (DWI) infarct core and the perfusion lesion, measured by dynamic susceptibility contrast perfusion-weighted imaging (PWI). However, a threshold for PWI measurement of the ischemic penumbra needs to be determined. Additionally, a volumetric ratio of infarct core to penumbra termed mismatch has been used in clinical trials to identify patients who are suitable for reperfusion therapies on the premise that they have a relatively large volume of potentially salvageable tissue.

The rare, but serious, reaction to gadolinium-based contrast agents that occurs in patients with severe renal impairment (nephrogenic systemic fibrosis) now prohibit gadolinium administration unless the renal function is known, or if the glomerular filtration rate is $<30\text{ mL/min.}$ Waiting for renal function results leads to unacceptable delays in (or may even prohibit) MR imaging of patients with hyperacute stroke. Arterial spin labeling (ASL) is a form of perfusion imaging without exogenous contrast administration, using paramagnetic labeling of water in blood flowing to the brain and thus requires no exogenous contrast administration to produce measurements that are proportional to cerebral blood flow (CBF). However, ASL in ischemic stroke may potentially underestimate CBF where there is delay in contrast (labeled blood) reaching the ischemic region because of the short ASL tracer half life (the period the magnetic label lasts).

In a prospective group of patients with hyperacute stroke who underwent CTP followed by MRI (with DWI, PWI, and ASL), we aimed to define the most accurate hyperacute ASL perfusion lesion threshold for tissue at-risk, by comparison with

Background and Purpose—Arterial spin labeling (ASL) is a perfusion magnetic resonance imaging (MRI) technique that does not require contrast administration and thus may be more practical in hyperacute stroke than susceptibility-weighted bolus-tracking perfusion-weighted imaging (PWI). A threshold for PWI measurement of the ischemic penumbra needs to be determined. A total of 58 patients with acute hemispheric ischemic stroke were imaged within 6 hours of symptom onset with MRI including ASL, diffusion-weighted MRI (DWI), and PWI, after perfusion computed tomography (CTP). Patients had repeat MRI at 24 hours. On repeat imaging, 32 patients did not reperfuse and were used to determine the penumbra threshold. A receiver-operating curve and a volumetric analysis were undertaken to identify the ASL-cerebral blood flow (CBF) threshold for the acute penumbra compared with the 24-hour DWI lesion in patients without reperfusion and with the acute PWI and CTP-Tmax thresholds.

Results—An ASL-CBF threshold of 40% showed the highest area under the curve (AUC) for detection of the acute penumbra, defined by 24-hour DWI in nonreperfused patients (AUC 0.76, 95% confidence interval, 0.63–0.85), and was also accurate compared with the acute PWI-Tmax+6 seconds penumbral threshold (AUC 0.79, 95% confidence interval, 0.73–0.84) and acute CTP-Tmax 5.5 seconds penumbral threshold (AUC 0.77, 95% confidence interval, 0.72–0.84). Using a perfusion-to-diffusion mismatch ratio of 1.8:1, an ASL-CBF 40% mismatch compared with PWI-Tmax+ 6 seconds-DWI and CTP mismatch showed good sensitivity (0.81) and specificity (0.71).

Conclusions—ASL-DWI mismatch shows potential to identify salvageable tissue in hyperacute stroke. (Stroke. 2014;45:00-00.)

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the follow-up DWI lesion, in patients without reperfusion. As an additional validation, we compared the acute ASL thresholds for penumbra with PWI and CTP penumbra thresholds in the same patient group from scanning acquired at the same time point.

2. Using the most accurate ASL threshold for at-risk tissue from (1), compare automated quantitative measures of ASL-DWI mismatch with PWI-DWI mismatch and CTP mismatch.

Methods

Patients

We prospectively studied consecutive patients with acute stroke with hemispheric ischemia presenting within 6 hours of stroke onset with a visible occlusion on imaging with a perfusion lesion present on acute perfusion imaging. All patients underwent baseline multimodal CT examination, MRI within 1 hour of CTP, then follow-up MRI at 24 hours. Eligible patients, based on standard criteria, received intravenous tissue plasminogen activator (tPA), which was administered immediately after CT, and MR was performed during the tPA infusion. The study was approved by the institutional ethics committee, and all patients or relatives gave informed consent.

Imaging Methods

CT imaging was acquired on a 320-slice CT (Toshiba Aquilion, Japan). Whole brain perfusion imaging was acquired simultaneously with 50 mL of contrast agent (ultravist 370; Bayer HealthCare, Berlin, Germany) mechanically injected at 6 mL s⁻¹ followed by 30 mL of saline. Pulsed full rotation scans were acquired, beginning 7 seconds after contrast injection and with 19 time points during 60 seconds.

MR imaging was performed on a 3T MRI (Siemens Verio, Erlangen, Germany) with a 32-channel receive-only head coil. The stroke MRI protocol included an axial isotropic diffusion-weighted imaging (DWI) spin-echo echo-planar imaging (SE-EPI) sequence, time-of-flight MR angiography (TOF-MRA), and whole brain perfusion imaging with dynamic susceptibility contrast PWI. ASL data were collected before PWI contrast injection, as a pulsed sequence using the quantitative imaging of perfusion with a single subtraction, with thin-slice TI1 periodic saturation (Q2TIPS) technique.12 Image parameters were TR 2500 ms; TI 1 500 ms; TI 1s 1500 ms; inversion time (TI) 1700 ms; FOV 240×240 mm; and matrix 64×64. This acquired 9 slices at 8-mm thickness with 28 repetitions for a scan time of 4:02 minutes.

Image Processing

All image analyses were performed on deidentified data. Perfusion maps (ASL-MR, PWI-MR, and CTP) were calculated using commercial software MiStar (Apollo Medical Imaging Technology, Melbourne, Australia).13,14 For PWI and CTP, this required selection of a global arterial input function from an unaffected anterior cerebral artery and a venous outflow function from a large draining vein (sagittal sinus). Deconvolution of the tissue enhancement curve and the arterial input function were performed using model-free singular value decomposition.15 This methodology produced maps of cerebral blood flow (CBF), cerebral blood volume mean transit time (MTT), and Tmax. Given the current lack of consensus around definition of no reperfusion on perfusion imaging, we sort to assess 2 measures (MTT and Tmax) at different thresholds. No reperfusion was defined by either <20% reduction in the acute to 24-hour perfusion lesion (common definition) or <10% reduction (strict definition). We also used the 2 most commonly used thresholds to define the perfusion lesion in the setting of reperfusion assessment, MTT 150% and Tmax 60 seconds.

The raw ASL images were separated and corrected for motion using a 3D rigid registration algorithm and spatially smoothed with a 2D 1.5 voxel Gaussian kernel using MiStar. The raw data images were then separated into label and control pairs and pair-wise subtracted using an odd-even ordering, and CBF was calculated.18

Volumetric ASL Lesion Analysis

An analysis was undertaken to determine the most accurate ASL perfusion lesion threshold for detecting tissue at risk of progressing to infarction. First, the ischemic hemisphere was determined, and the average pixel ASL-CBF value was recorded from the contralateral. ASL thresholds were then determined as percentage changes from the mean baseline contralateral CBF value for each tissue type. Lesion volumes were then automatically calculated from the pixels below the mean contralateral CBF value at decrements of 5%.19

Receiver-operating characteristic (ROC) curve analysis was then used to test the accuracy of the respective incremental 5% threshold ASL-CBF lesions to predict the reference follow-up 24-hour MRI DWI infarct core in patients without reperfusion.20 Tmax PWI and CTP maps were tested at 0.5-second increments versus the 24-hour DWI lesion in patients without major reperfusion for the ROC analysis to determine the most accurate penumbral threshold. In each of these analyses, the DWI maps were considered to be the true lesion, and the pixels where the DWI lesion and the perfusion lesion on ASL-CBF, or PWI-Tmax, or CTP-Tmax lesion (for each percentage threshold) overlapped were considered true positive (TP). DWI pixels not within the perfusion lesion were considered true negative. Pixels within the respective perfusion lesion but not within the DWI lesion were assigned false-negative (FN). Specificity (TN/ [TN + FP]) and sensitivity (TP/ [TP + FN]) were calculated for each perfusion map. Results presented are area under the curve (AUC) and 95% confidence interval (CI) for the whole ROC curve for the specific perfusion maps (ASL, MR-Tmax, CTP-Tmax).

Mismatch ratios were calculated for each patient using each of the 3 optimized perfusion techniques. Infarct core volumes for these calculations were defined by acute DWI for MRI-defined mismatch (ASL and PWI-Tmax) and CBF 40% for CTP-defined mismatch.21 Mismatch ratios were assessed as a continuous variable and with cutoffs that were set at 1.2 and 1.8 in accordance with those used in current clinical trials.22

Results

Patients

During the period from 2009 to 2012, 67 sub–6-hour anterior circulation ischemic stroke patients were identified because of perfusion lesions in the anterior region and were prospectively
studied with hyperacute multimodal CT, followed by hyperacute MRI. Average age was 62 years (range 28–84). The median acute NIHSS was 17 (range 8–28), with an average time to acute CT of 167 minutes (range 50–390) and MRI of 247 minutes (range 100–450), median time between CT and MR was 80 minutes (range 50–215) and mean time from symptom onset to iv-rtPA of 180 minutes. Of the 67 patients, 28 patients received iv-rtPA according to the institutional guidelines. Two patients were excluded because of excessive motion on acute imaging, and 7 patients were excluded because they showed either partial (5) or complete reperfusion (2) between acute CTP and acute MRI, which would invalidate acute CTP versus MR perfusion comparisons. Of the 58 included patients, 8 had occlusions of internal carotid artery, 41 had middle cerebral artery occlusions, and 9 had anterior cerebral artery occlusions. No reperfusion was seen in 32 patients using the common definition of MTT 150% lesion reduction of <20% (14 of whom received tPA), whereas 27 patients showed no reperfusion if we use a strict <10% definition. The same patients showed no reperfusion when a Tmax threshold was used at either the <20% (32 patients) cutoff or <10% (27 patients). Mean acute DWI infarct volume was 26 mL (95% CI, 15–37 mL) and mean acute penumbra volume (Tmax +6 seconds) was 56 mL (95% CI, 40–72 mL).

**Identifying the Perfusion Lesion With ASL**

**ASL, PWI, and CTP Versus 24-Hour DWI in Nonreperfused Patients (Using the Common Reperfusion of <20% Lesion Reduction)**

An ASL-CBF threshold of <40% was clearly the most accurate predictor of the 24-hour DWI lesion in patients without reperfusion (Figure 1), with high accuracy (AUC 0.76, 95% CI [0.63–0.85]). ASL-CBF <40% tended to overestimate the 24-h DWI lesion (mean 16±12 mL, \( R^2 0.318; P=0.067 \)).

For PWI-Tmax, the most accurate AUC for the 24-hour DWI was >6 seconds (AUC 0.79, 95% CI [0.73–0.84]). PWI-Tmax >6 seconds tended to overestimate the 24-h DWI lesion (mean 7±9 mL, \( R^2 0.416; P=0.018 \)).

For CTP-Tmax, the most accurate AUC for 24-hour DWI was >5.5 seconds (AUC 0.77, 95% CI [0.72–0.84]). CTP-Tmax

![Figure 2](http://stroke.ahajournals.org/)

**Figure 2.** Area under the curve measures of arterial spin labeling (ASL)-cerebral blood flow (CBF), perfusion-weighted imaging (PWI)-Tmax and perfusion computed tomography (CTP)-Tmax at defining the acute penumbra compared with 24-h diffusion-weighted imaging (DWI) in nonreperfused patients. This figure shows the difference in accuracy between ASL-CBF, PWI-Tmax, and CTP-Tmax at determining the acute perfusion lesion when compared with 24-hour DWI in patients without major reperfusion.
ASL Versus PWI Penumbral Lesions
The optimal ASL-threshold of <CBF 40% tended to overestimate the perfusion lesion compared with the PWI-Tmax 6 volume by a mean of 17mL (±8 mL; R² 0.392; P=0.056).

ASL Versus CTP Penumbral Lesions
The CTP-Tmax >5.5 seconds and ASL <CBF 40% perfusion lesions were also similar (R²=0.477; P<0.001).

Definition of Reperfusion
Using the stricter definition of no reperfusion (<10% reduction in acute–24-hour MTT 150% lesion) did not show significantly different results from the common definition (<20% reduction in acute–24 hour MTT 150% lesion) for ASL (strict <10% definition AUC 0.77, 95% CI [0.65–0.83]; P=0.432), PWI (strict <10% definition AUC 0.81, 95% CI [0.67–0.85]; P=0.121) or CTP (strict <10% definition AUC 0.78, 95% CI [0.67–0.85]; P=0.347). Additionally, the results were similar when using a Tmax threshold (Tmax+6 seconds) for ASL (<20% lesion reduction AUC 0.75, 95% CI [0.62–0.85], <10% lesion reduction AUC 0.76, 95% CI [0.64–0.84]; P=0.432), PWI (<20% lesion reduction AUC 0.79, 95% CI [73–84], <10% lesion reduction AUC 0.81, 95% CI [0.67–0.85]; P=0.121) and CTP (<20% lesion reduction 0.78, 95% CI [72–86], <10% lesion reduction AUC 0.78, 95% CI [0.67–0.85]; P=0.347). There was no significant differences in AUC result between using MTT and Tmax to determine no reperfusion (P=0.671).

Assessment of MRA data identified that all of the patients defined as no reperfusion by the <20% reduction in acute–24-hour MTT 150% lesion had persistent occlusion on MRA, but another 7 patients with persistent occlusion had much greater reductions in the acute to 24-hour perfusion lesion (range 24%–59%, presumably reflecting improved collateral flow, or partial antegrade flow not seen on MRA). These 7 patients had much less infarct growth compared with the patients with persistent MRA occlusion and no tissue reperfusion on perfusion imaging (23 versus 41 mL; P=0.009).

Although our <20% reduction in perfusion lesion (no reperfusion) group all had no change (or worsening) in acute to 24 NIHSS, there were another 7 patients with no change in acute to 24-hour NIHSS who had reperfusion >20% and no infarct growth (this included 4 with persistent MRA occlusion). This likely reflects reperfusion into already infarcted tissue. Thus, using lack of NIHSS to define lack of tissue reperfusion is problematic because we would be including patients with minimal infarct growth.

Automated Volumetric Mismatch Assessment for Potential Reperfusion Treatment Suitability
Lesion volumes derived from mismatch of ASL-CBF 40%-DWI and PWI-DWI mismatch ratio of 1.8:1 (Tmax>6 seconds for PWI) showed a strong correlation (r²=0.73; P<0.01), but ASL determined mismatch volume was larger (mean volume difference 12 mL SD 8 mL; P=0.066; Figure 2; Table). Lesion volumes derived from a mismatch ratio of 1.8:1 of ASL-CBF 40%-DWI and CTP-Tmax mismatch showed a strong correlation (r²=0.59; P=0.03) but again, ASL determined mismatch volume was larger (mean volume difference 11 mL SD 6 mL; Table).

Based on the best thresholds for penumbra from the above analyses (CBF <40%, PWI-Tmax 6 seconds and CTP-Tmax >5.5 seconds), automated penumbra and core maps were generated (Figure 3). Because of ASL overestimation of the perfusion lesion volume, using an automated mismatch ratio of >1:1.2 core to penumbra ratio,22 26 out of 58 patients had >1.2 ASL-DWI mismatch, but 6 of these patients were below this cutpoint using PWI-DWI (P=0.044) and CTP mismatch (P=0.041). Using an automated ASL-DWI mismatch ratio of >1.8, 23 out of 58 patients had a mismatch ratio >1.8, but 5 of these were below this cutpoint using PWI-DWI (P=0.026) or CTP (P=0.019) mismatch. Therefore, with the most accurate respective penumbral thresholds for each modality, ASL mismatch of 1.8 had a good sensitivity (0.81) and specificity (0.71) when compared with corresponding PWI/DWI mismatch (Table), whereas a mismatch ratio of 1.2 had a slightly lower sensitivity (0.72) and specificity (0.66). A mismatch ratio of 1:2.5 showed the highest sensitivity (0.92) and specificity (0.85), with only one misclassification, comparing ASL-DWI mismatch with CTP mismatch (P<0.001) and PWI-DWI mismatch (P=0.002).

Discussion
In this study, we determined the accuracy of ASL to predict final infarction, and compared it with both CT and MR bolustacking perfusion modalities, in the same patients. We found that ASL identifies the acute perfusion lesion and tissue at-risk of infarction with similar accuracy to PWI and CTP-Tmax thresholds. It is notable that the ASL-CBF <40% threshold performed similarly to PWI-Tmax >6 seconds and CTP-Tmax >5.5 seconds thresholds, which are known to be accurate measures of penumbra.19 However, using the ASL-CBF

Table. Comparison of ASL Mismatch With PWI and CTP Mismatch With DWI at Ratios of 1:1.2, 1:1.8, and 1:2.5 in Selecting Patients for Treatment

<table>
<thead>
<tr>
<th>Mismatch</th>
<th>ASL-DWI Mismatch</th>
<th>PWI-DWI Mismatch</th>
<th>CTP-DWI Mismatch</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:1.2</td>
<td>26/58</td>
<td>20/58</td>
<td>22/58</td>
</tr>
<tr>
<td>1:1.8</td>
<td>23/58</td>
<td>18/58</td>
<td>18/58</td>
</tr>
<tr>
<td>1:2.5</td>
<td>16/58</td>
<td>15/58</td>
<td>15/58</td>
</tr>
<tr>
<td>Mean mismatch volume</td>
<td>94 mL (32 mL)</td>
<td>71 mL (29 mL)</td>
<td>75 mL (23 mL)</td>
</tr>
</tbody>
</table>

ASL <40% threshold is derived from contralateral mixed gray/white matter CBF value. ASL indicates arterial spin labeling; CBF, cerebral blood flow; CTP, perfusion computed tomography; DWI, diffusion-weighted imaging; and PWI, perfusion-weighted imaging.
<40% threshold, lesion volumes tended to be overestimated compared with PWI-Tmax–DWI mismatch and CTP-Tmax lesions volumes; however, this result did not reach significance. Our study focused on the potential for clinical use of ASL, particularly in defining mismatch tissue and whether it might have potential to replace PWI-DWI mismatch. Previous assessments of visual mismatch have been shown an agreement in 77% or 34 out of 44 patients, whereas this study saw a higher agreement when using an ASL-threshold (89% or 52 out of 58 patients). However, it is important to note that the previous work was performed on a 1.5T, whereas this study was undertaken on a 3T MRI that would substantially increase the signal-to-noise ratio and produce clearer images.

The use of mismatch imaging selection for acute reperfusion therapy is not yet proven in the clinical setting; however, there are several current trials underway using PWI-DWI mismatch and CTP mismatch to select patients for acute reperfusion treatment. This study showed that ASL-DWI mismatch at the currently used ratios potentially misclassifies patients; however, if a higher cutpoint is used, there is virtually no difference in mismatch classification compared with PWI-DWI or CTP classification because the specificity becomes similar to that of PWI and CTP-DWI mismatch.
The classification of reperfusion was a critical element of this study because partial reperfusion can result in extremely variable infarct core growth and thereby invalidate penumbral threshold assessments. Using either the <20% or <10% reduction in acute-24 hour perfusion lesion definition did not change the results of this study nor did the use of MTT or Tmax to define the perfusion lesion. Other options for defining lack of reperfusion we considered were to assess lack of MRA recanalization or lack of NIHSS improvement. The problem with both these measures is that they are indirect assessments of perfusion at the tissue level. The weakness of MRA is that this is not as sensitive at measuring perfusion at the tissue level, meaning that changes on MRA may be because of improved collateral flow (or partial antegrade flow not seen on MRA). Therefore, using MRA to define reperfusion is problematic because we would be including patients with minimal infarct growth because of improved distal tissue perfusion, which invalidates the determination of an accurate penumbral threshold. Similarly, using lack of NIHSS change to define lack of tissue reperfusion is problematic because we would be including patients with minimal infarct growth. Additionally, we have reported patients who had no change (or worsening) in acute to 24 NIHSS, with reperfusion >20% and no infarct growth. This likely reflects reperfusion into already infarcted tissue. Therefore, assessing change in perfusion lesion volume is the most accurate way to assess lack of reperfusion into already infarcted tissue. Other options for defining lack of reperfusion we considered were to assess lack of reperfusion >20% and no infarct growth. This likely reflects reperfusion into already infarcted tissue. Therefore, assessing change in perfusion lesion volume is the most accurate method to assess tissue reperfusion because it directly reflects the level of blood flow to the tissue and correlates much better with infarct growth than do indirect measures such as vessel or clinical status. This is particularly important when determining penumbral perfusion thresholds.

The standard pulsed ASL acquisition used in this study tended to overestimate hypoperfused tissue truly at risk of progression to infarction, particularly in white matter. This may relate to delayed contrast arrival to ischemic tissue. Notably, this problem is not unique to ASL and can also reduce the accuracy of bolus-tracking perfusion. However, this is particularly an issue for ASL where there is a lower signal-to-noise ratio compared with PWI in the white matter where CBF is inherently lower and leads to underestimation of CBF in hypoperfused white matter. This underestimation of CBF is especially problematic in the infarct core, where CBF is already low; however, this is not as clinically relevant to ASL as it is to CTP because we have the advantage of combining ASL with DWI to produce mismatch maps. The current issue with ASL does not specifically relate to the labeling of exogenous water, rather the short labeling time compared with continuous techniques and the short postlabeling delay that is required for stroke imaging to capture gradations in lower than normal CBF. Future studies that use variations of this study’s ASL parameters or ASL technique may draw different conclusions attributable to the effect of changes to the ASL acquisition parameters that have a significant effect on ASL signal and map quality.

There are variants of the ASL technique proposed that may improve issues of low signal-to-noise ratio in white matter such as pseudocontinuous, multi-TI acquisitions, or techniques that use background suppression to decrease noise that may be suitable in the future. With these sequence-enhanced abilities to distinguish low flow from noise, particularly in white matter, our ability to measure delayed perfusion will greatly improve. Finally, ASL has substantial potential for clinical use as a broadly used imaging technique especially ASL-DWI mismatch in acute ischemic stroke but requires further independent validation.

ASL shows promise in the clinical setting where contrast administration is contraindicated or where waiting for renal function results is unacceptable (ie, acute stroke). Although further refinement of the acquisition is required, it is foreseeable that ASL will become the preferred MR perfusion imaging option for acute ischemic stroke. Furthermore, in centers where one has ready access to both CT and MRI, rapid, automated ASL-DWI mismatch may be preferred to CT because ASL measures the at-risk perfusion lesion with similar accuracy to CTP and has the advantage of DWI to more accurately measure the infarct core.

Disclosures

None.

References

Arterial Spin Labeling Versus Bolus-Tracking Perfusion in Hyperacute Stroke
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