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Incorporating ADC Temporal Profiles to Predict Ischemic Tissue Fate in Acute Stroke

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Abstract

Algorithms to predict ischemic tissue fate based on acute stroke MRI utilized data at a single time point. The goal of this study was to investigate the potential improvement in prediction accuracy when incorporating MRI diffusion data from multiple time points during acute phase to improve prediction accuracy. This study was carried out using data from rats subjected to permanent, 60-min and 30-min of middle cerebral artery occlusion (MCAO). The sensitivity and specificity of prediction accuracy were calculated. In the permanent MCAO group, prediction with multiple time-point diffusion data improved sensitivity and specificity compared with prediction using a single time point. In the 60-min MCAO group, multiple time-point analysis improved specificity but decreased sensitivity compared to the single time-point analysis. In the 30-min MCAO group, multiple time-point analysis showed no statistically significant improvement in specificity and sensitivity compared with the single time point analysis. This is because reperfusion transiently or permanently reversed the decline in ADC values, resulting in increased uncertainty and thus decreased prediction accuracy. These findings suggest that incorporating MRI data from multiple time points could improve prediction accuracy under certain ischemic conditions.

Keywords

Diffusion; Perfusion-diffusion mismatch; MCAO; Focal Ischemia; DWI; PWI; ADC, CBF

INTRODUCTION

Stroke is the fourth leading cause of mortality and the leading cause of long-term disability in the United States (Roger et al., 2012). The only FDA-approved drug to treat ischemic stroke is intravenous administration of recombinant tissue plasminogen activator (rtPA) within 4.5 hours of stroke onset (Hacke et al., 2008). Unfortunately, only 1.8-2.1% of ischemic stroke patients receive treatment with rtPA (Kleindorfer et al., 2008). Imaging modalities have the potential to identify injured but salvageable tissue, known as the ischemic penumbra. In some patients, salvageable tissue exists well beyond the 4.5 hour time window (i.e., up to 24 hours after symptom onset (Darby et al., 1999)). Thus, there is value to accurately predict which group of stroke patients will benefit from therapeutic interventions.

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When cerebral blood flow (CBF) drops below a critical threshold, energetic failure results and the apparent diffusion coefficient (ADC) of water in the tissue starts to decrease (Moseley et al., 1990), although the precise biophysical mechanisms of ADC reduction remains incompletely understood (Duong et al., 1998). Diffusion-weighted magnetic resonance imaging (MRI) in which image contrast is based on water ADC can detect ischemic injury within minutes after onset, whereas computed tomography and other imaging modalities fail to detect stroke injury for at least a few hours (Moseley et al., 1990). The critical ADC threshold below which tissue usually destined to infarct has been reported to be $0.53 \times 10^{-3} \text{ mm}^2/\text{s}$ (Shen et al., 2003). However, the evolution of the initial ADC lesion depends on many conditions (such as duration of ischemia and extent of occlusion or reperfusion). Some tissue with initial ADC reduction is salvageable while other is not (Kidwell et al., 2003; Li et al., 1999). Despite its uncertainty differentiating salvageable from non-salvageable tissue, diffusion-weighted MRI remains commonly used to guide clinical decision making in acute stroke management (Kidwell et al., 2003).

Various sophisticated algorithms have been developed to quantitatively predict ischemic tissue fate, including the generalized linear model (Wu et al., 2001; Wu et al., 2007), probability-of-infarct (Shen et al., 2005b; Shen and Duong, 2008), artificial neural network (Huang et al., 2010) and support vector machine (Huang et al., 2011). These prediction algorithms incorporated imaging data from a single time point. Tissue ADC changes with time after ischemic injury. Incorporating ADC data from multiple time points could improve prediction accuracy. The goal of this study was thus to investigate the potential improvement in prediction accuracy by incorporating ADC measurements at multiple time points during acute stroke phase. We investigated data from rats subjected to permanent, 60-min and 30-min of middle cerebral artery occlusion (MCAO). The sensitivity and specificity of the prediction accuracy were calculated, and comparisons were made with the prediction accuracy when using only a single acute time point for each MCAO group.

RESULTS

With k-means clustering, the ADC data for each rat was separated into four apparent temporal clusters, with each one showing a different pattern across time. We investigated each cluster's proportion of total tissue, infarction rate, variability in infarction rate and ADC trend across time. Sensitivity and specificity calculations formed the prediction analysis. Each of these clusters was mapped onto image space.

Permanent group

Table 1A shows both clustering methods divided the tissue into similar proportions per cluster, with the blue clusters making up about 15% of the total tissue, the green clusters about 30%, and the yellow and red clusters between 24% and 29%. Both methods had similar rates of infarction for each cluster with near 100% infarction for the red clusters, a high percentage of infarction for the yellow clusters, a moderate percentage for the green clusters and a low percentage for the blue clusters. Moreover, the green and blue clusters showed considerable variability in the percent of tissue destined to infarct at the endpoint. In **Figure 1**, while both methods had monotonically decreasing ADC curves for the blue, green and yellow clusters, the single time-point method had a stable red cluster across time whereas the multiple time-point method showed an initial decrease followed by stability in the red cluster.

The prediction analysis (**Table 2**) showed that the multiple time-point method had higher sensitivity (80% versus 73%, $p = 0.009$) and higher specificity (89% versus 85%) $p = 0.045$). Thus, incorporating the temporal behavior of ADC into tissue fate analysis improved prediction accuracy in permanent MCAO.

60-min MCAO group

Table 1B shows both clustering methods had the green cluster making up about 40% of total tissue and the yellow cluster almost 20%. For the remaining tissue, the single time-point method clustered the majority of it into the red cluster while the multiple time-point method clustered the majority of it into the blue cluster. In other words, the single time-point method predicted more tissue destined to infarct at the endpoint than the multiple time-point method. Each cluster's final outcome varied between the two analytical methods. While both methods had similar rates of infarction for the blue clusters, the multiple time-point method had higher percentages of infarction for the green, yellow and red clusters. The green cluster in the multiple time-point analysis had significant variability in the percent of tissue destined to infarct at the endpoint. These findings suggest increased certainty when predicting infarction but decreased certainty when predicting survival with the multiple time-point method. In **Figure 2**, the ADC curves for each method were slightly different. The single time-point method showed all the clusters decreasing before reperfusion. The blue cluster was relatively stable after reperfusion while the remaining clusters showed recovery after reperfusion. The multiple time-point method showed a relatively stable blue cluster both before and after reperfusion, a monotonically decreasing red cluster, and a transiently decreasing with post-reperfusion recovery of the green and yellow clusters.

The prediction analysis (**Table 2**) showed that the multiple time-point method had decreased sensitivity (67% versus 80%, $p = 0.016$) higher specificity (94% versus 86%, $p = 0.002$). Thus, incorporating the temporal behavior of ADC into tissue fate analysis decreased sensitivity but improved specificity in rats subjected to 60-min MCAO.

30-min MCAO group

Table 1C shows both clustering methods divided the tissue into similar proportions per cluster, with the green clusters making up about 36% of the total tissue while the remaining clusters made up between 19% and 25%. The final outcomes of each cluster were similar between the two analytical methods, with a high percentage of infarction for the red clusters, around 50% infarction for the yellow clusters, and a low percentage for the green and blue clusters. In both groups, the yellow and red clusters had considerable variability in the percent destined to infarct at the endpoint. The blue and green clusters sustained relatively stable ADC values across time while the yellow and red clusters showed ADC recovery with reperfusion for both methods (**Figure 3**).

The prediction analysis (**Table 2**) showed that the two methods were not statistically significantly different in (76% versus 84%, $p = 0.09$) and specificity (71% versus 75%, $p = 0.29$).

DISCUSSION

This study reports the use of ADC data at multiple time points to improve prediction accuracy in a rat stroke model of permanent, 60-min and 30-min MCAO. Compared with prediction using a single time point, multiple time-point analysis improved prediction accuracy in the permanent MCAO group. By contrast, multiple time-point analysis improved specificity but decreased sensitivity in the 60-min MCAO group compared to single time point analysis. Multiple time-point analysis showed no statistically significant improvement in specificity and sensitivity compared with single time point analysis in the 30-min MCAO group. This is because reperfusion transiently or permanently reversed the decline in ADC values, resulting in increased uncertainty and thus decreased prediction accuracy.

In the permanent and the 30-min groups, the proportions, infarction rates and ADC trends were similar across both single and multiple time-point analyses. In the 60-min group, all of these differed between the two methods. In the single time-point method, the red cluster composed a higher proportion of total tissue, had a lower infarction rate and showed ADC recovery with reperfusion. This suggests the single time-point method predicted a higher rate of infarction than the multiple time-point method by including in the red cluster some pixels with initially low ADC values that recovered with reperfusion, resulting in its increased sensitivity but decreased specificity in predicting infarct tissue.

The single time-point method was not able to differentiate pixels with initially low ADC values that continued to decline from those that recovered with reperfusion. By contrast, the multiple time-point method was able to make this differentiation, and it removed from the red cluster all the pixels that showed ADC recovery with reperfusion, utilizing this temporal information to increase the certainty in its prediction of tissue destined to infarct. Thus, it is evident that ADC recovery with reperfusion did indeed signify potential for survival, though it was not absolute. These findings demonstrate the value of temporal information.

This study has a few shortcomings. First, the clustering method is suboptimal as the k-means clustering is based on the sum of squares of differences in ADC values at each time point. Future studies will improve clustering method to better account the temporal information from multiple time points. Second, the endpoint imaging was 24 hours after MCAO. Although the infarct volume had largely stopped evolving at this time, there could be additional infarct growth, especially for the 30-min and 60-min MCAO groups (Li et al., 1999). Similar analysis of data at a later endpoint (such as 48 or 72 hrs) will need to be investigated to determine how the choice of endpoint MRI affects single and multiple time-point analysis. Third, our analysis used only ADC data. Incorporating additional imaging data (such as CBF) could further improve prediction accuracy. Finally, although performance was evaluated by sensitivity and specificity calculations, future studies will utilize more sophisticated algorithms, such as support vector machine with separate training and experimental groups, to quantitatively predict tissue fate (Huang et al., 2011).

Prediction based on data at multiple time points has the potential to provide quantitative and objective frameworks to extend the treatment window for stroke patients and to aid clinical decision-making in the treatment of acute stroke and drug testing. This approach may also be applied to patients with transient ischemic attack who often return to the emergency room with a large stroke within 48 hours (Rothwell and Warlow, 2005). Objective and accurate prediction models of tissue fate may help drug trials by accelerating the identification of promising potential therapies and help to individualize the treatment window for stroke patients.

METHODS

Animal preparations

The data analyzed in this study were those previously published (Shen and Duong, 2008). Briefly, a total of 35 Sprague-Dawley rats (300-350g) were subjected to 30-min (n = 13), 60-min (n = 12) and permanent (n = 10) MCAO, with reperfusion accomplished remotely without taking the animal out of the scanner.

MR experiments

MRI scanner specifications and settings as well as image acquisition methods were as described elsewhere (Shen and Duong, 2008). Briefly, ADC and CBF data were collected at 30, 60, 90, 120, and 180 min, and again at 24 hrs post-occlusion for all three study groups. For the 30-min and 60-min MCAO groups, the 30-min and 60-min data, respectively, were

acquired before reperfusion. Additionally, for the 30-min and 60-min MCAO groups, an additional imaging time point was performed 10 minutes post-reperfusion, at 40-min and at 70-min respectively. Endpoint T2-weighted MRI was also performed at 24 hrs post-occlusion. Histological infarct volume was determined using TTC (2,3,5-triphenyltetrazolium chloride) staining and with edema correction (Meng et al., 2004).

Data analysis

Five anterior slices were analyzed to avoid susceptibility distortion around the ear canals. Images were co-registered using custom-designed semi-automatic co-registration software between acute phase and 24-hour data within the same animals and between animals as described previously (Liu et al., 2004; Schmidt et al., 2006; Shen et al., 2005a). ADC maps with intensity in unit of mm^2/s (Meng et al., 2004; Shen et al., 2005a) and CBF maps with intensity in units of mL/g/min were calculated (Duong et al., 2000). Image displays and overlays were performed on the STIMULATE software (University of Minnesota). All data were reported as mean $\pm$ SEM.

K-means clustering

Codes written in Matlab (MathWorks, Natick, MA) were used to perform k-means clustering analysis. First, data were analyzed via a single time point – the ADC values at the 30-min time point for each rat were used to form four clusters. This was done by first clustering the tissue into two groups, then clustering each of these groups further into two sub-groups. For each cluster, the ADC values of the pixels were averaged together to form the curves. The endpoint tissue outcome for each group was determined by comparison with ISODATA at 24 hours. The percentage of tissue made up by each cluster was calculated as well as the percent of each cluster to infarct at the endpoint.

The 30-min time point data were chosen for single time-point analysis because in our rat stroke model, there was substantial mismatch at 30-min after stroke but it disappeared within 2-3 hours after stroke onset (Shen et al., 2003), whereas in humans the mismatch exists considerably longer (Hacke et al., 2008). The 30-min time point was also chosen because only a pre-reperfusion time point can be reasonably used for single time-point analysis, as prediction using a post-reperfusion time point, when ADC decline has reversed, was unreliable. To be consistent, the 30-min time point data were used for all three groups.

Data were analyzed via multiple time points – all the time points for the reperfusion groups and the first four time points for the permanent group – forming four ADC clusters. Again, the tissue was clustered into two groups which were each split further to form a total of four clusters. Only the first four time points were used for the permanent group as the fifth and last time point was used to determine endpoint tissue outcome. And the percentage of tissue made up by each cluster was calculated as well as the percent of each cluster to infarct at the endpoint.

Prediction analysis

The single time-point analysis was compared with the multiple time-point analysis via sensitivity and specificity calculations. The four curves obtained via k-means were combined into two groups – one which included the lower two clusters, red and yellow, and the other which included the higher two clusters, green and blue. The grouping was performed in this manner as the lower ADC group included the clusters having any ADC value near or below the threshold value of $0.53 \times 10^{-3} \text{ mm}^2/\text{s}$. This threshold value was determined as the value at which the ADC-defined lesion volume best approximated the TTC-identified final infarct volume (Shen et al., 2003). The higher ADC group, consisting of ADC values well above threshold, was assumed to predict 100% survival, while the lower

group, consisting of any ADC value below or near threshold, was assumed to predict 100% infarction. The sensitivity and specificity for each group were then determined by comparing the predictions with the actual outcome determined by ISODATA. These were calculated for each rat, and subsequently, the means and standard deviations were calculated.

Statistical analysis

For each group, the sensitivity and specificity values of the single time-point analysis were compared to that of the multiple time-point analysis via paired one-tailed t-test, and $p < 0.05$ was taken as significant.

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Highlights

Multiple ADC time-point analysis improved prediction accuracy in the permanent stroke group compared with single time-point prediction.

Multiple ADC time-point analysis improved specificity but decreased sensitivity in the 60-min stroke group.

Multiple time-point analysis showed no statistically significant improvement in specificity and sensitivity in the 30-min MCAO group.

Figure 1. Permanent MCAO group

(A) Time-series ADC maps from a representative animal, chosen based on its superior depiction of lesion evolution. The image labeled “final infarct” indicates the endpoint tissue outcome – survival or infarct – determined via ISODATA analysis. The image labeled “cluster map” depicts an example from the same rat of the tissue clustered into four groups, with the blue pixels representing the cluster with the highest ADC values, the green pixels representing the next highest and so forth. As this map represents the clusters from only one rat, it may not exactly correlate with the numbers shown in Table 1, which are group averages. (B) Averaged ADC values of different clusters versus time for the single time-point group, and (C) averaged ADC values of different clusters versus time for the multiple time-point group. The color of the pixels in the cluster maps correlates with the color of the ADC curves shown in the graphs.

Figure 2. 60-min MCAO group

(A) Time-series ADC maps from a representative animal, chosen based on its superior depiction of lesion evolution with occlusion and resolution with reperfusion. The image labeled “final infarct” shows the endpoint tissue outcome – survival or infarct – determined via ISODATA analysis. The image labeled “cluster map” depicts an example from the same rat of the tissue clustered into four color-coded groups. As this map represents the clusters from only one rat, it may not exactly correlate with the numbers in Table 1, which are group averages. (B) Averaged ADC values of different clusters versus time for the single time-point group, and (C) averaged ADC values of different clusters versus time for the multiple time-point group. The color of the pixels in the cluster maps correlates with the color of the ADC curves shown in the graphs. “REP” and arrow on x-axis indicate the time of reperfusion.

Figure 3. 30-min MCAO group

(A) Time-series ADC maps from a representative animal, chosen based on its superior depiction of lesion evolution with occlusion and resolution with reperfusion. The image labeled “final infarct” shows the endpoint tissue outcome – survival or infarct – determined via ISODATA analysis. The image labeled “cluster map” depicts an example from the same rat of the tissue clustered into four color-coded groups. As this map represents the clusters from only one rat, it may not exactly correlate with the numbers in Table 1, which are group averages. (B) Averaged ADC values of different clusters versus time for the single time-point group, and (C) averaged ADC values of different clusters versus time for the multiple time-point group. The color of the pixels in the cluster maps correlates with the color of the ADC curves shown in the graphs. “REP” and arrow on x-axis indicate the time of reperfusion.

Table 1

Single Time-Point and Multiple Time-Point Analyses For each cluster, “% infarcting” represents the percentage of tissue comprising that cluster which is destined to infarct at the end point. “% of tissue” represents the percentage of total tissue made up by each cluster. This is shown for **(A)** the permanent, **(B)** the 60-min, and **(C)** the 30-min MCAO groups.

(A)				
Permanent MCAO				
	Single Time-Point		Multiple Time-Point	
Cluster	% Infarcting	% of tissue	% Infarcting	% of tissue
Blue	25 ± 18	15 ± 7	17 ± 13	15 ± 10
Green	45 ± 21	32 ± 13	38 ± 25	28 ± 11
Yellow	86 ± 9	29 ± 9	89 ± 10	28 ± 13
Red	96 ± 7	24 ± 8	98 ± 2	29 ± 8

(B)				
60-min MCAO				
	Single Time-Point		Multiple Time-Point	
Cluster	% Infarcting	% of tissue	% Infarcting	% of tissue
Blue	15 ± 9	12 ± 4	17 ± 7	25 ± 14
Green	21 ± 10	40 ± 11	31 ± 28	39 ± 15
Yellow	77 ± 9	19 ± 5	86 ± 8	18 ± 8
Red	91 ± 5	29 ± 7	98 ± 2	19 ± 7

(C)				
30-min MCAO				
	Single Time-Point		Multiple Time-Point	
Cluster	% Infarcting	% of tissue	% Infarcting	% of tissue
Blue	7 ± 8	19 ± 7	8 ± 10	19 ± 8
Green	11 ± 16	36 ± 9	18 ± 22	36 ± 5
Yellow	52 ± 29	20 ± 5	47 ± 38	24 ± 10
Red	73 ± 25	25 ± 6	69 ± 27	21 ± 6

Table 2

Prediction analysis For each group, the sensitivity and specificity calculations are shown.

	Permanent MCAO		60-min MCAO		30-min MCAO	
	Single Time-Point	Multiple Time-Point	Single Time-Point	Multiple Time-Point	Single Time-Point	Multiple Time-Point
Sensitivity	73 $\pm$ 16 %	80 $\pm$ 19 %	80 $\pm$ 8 %	67 $\pm$ 15 %	84 $\pm$ 12 %	76 $\pm$ 14 %
Specificity	85 $\pm$ 10 %	89 $\pm$ 5 %	86 $\pm$ 9 %	94 $\pm$ 3 %	75 $\pm$ 11 %	71 $\pm$ 18 %