Title:
Infarct Evolution in a Large Animal Model of Middle Cerebral Artery Occlusion

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Running headline: Infarct Evolution in a Canine Stroke Model

Abstract

Mechanical thrombectomy for the treatment of ischemic stroke shows high rates of recanalization; however, some patients still have a poor clinical outcome. A proposed reason for this relates to the fact that the ischemic infarct growth differs significantly between patients. While some patients demonstrate rapid evolution of their infarct core (fast evolvers), others have substantial potentially salvageable penumbral tissue even hours after initial vessel occlusion (slow evolvers). We show that the dog middle cerebral artery occlusion model recapitulates this key aspect of human stroke rendering it a highly desirable model to develop novel multimodal treatments to improve clinical outcomes. Moreover, this model is well suited to develop novel image analysis techniques that allow for improved lesion evolution prediction; we provide proof-of-concept that MRI perfusion-based time-to-peak maps can be utilized to predict the rate of infarct growth as validated by apparent diffusion coefficient derived lesion maps allowing reliable classification of
dogs into fast versus slow evolvers enabling more robust study design for interventional research.

**Key words:** dog, middle cerebral artery occlusion, infarct growth rate, perfusion MRI, time-to-peak, stroke.
**Introduction**

In recent years, randomized-controlled trials have shown that mechanical thrombectomy is a highly effective treatment for selected ischemic stroke patients with emergent large vessel occlusion (ELVO) [1-3]. Although clinical trials and uncontrolled stroke registries report successful recanalization of the primary artery in approximately 85% [4, 5], rates of good clinical outcomes have not yet matched the high rate of technical success. For this reason, novel approaches to multimodal intervention that includes neuroprotection, adjunctive technology to augment brain perfusion, thrombolysis, and recanalization with thrombectomy promise to maximize the probability of a good clinical outcome after ELVO. In particular, methods that can slow infarct progression may expand the availability of mechanical thrombectomy for ELVO patients that present beyond established treatment windows [6, 7]. Approaches under development include transient aortic occlusion [8], stimulation of circle of Willis nerve fibers [9], mild induced hypertension [10], and inhalation of nitric oxide [11]. Perhaps the greatest opportunity is re-exploration of pharmacological neuroprotection combined with mechanical thrombectomy [12-15].

Although multiple animal stroke models are available in a variety of species to study stroke pathophysiology and possible treatment modalities [16], only larger animal species such as rabbits [17], non-human primates [18, 19], and dogs [20-27] are suitable for creating ELVO via an endovascular approach without performing a craniotomy. However, to date only the dog model allows simulation of mechanical thrombectomy for ELVO [28]. Moreover, the gyrencephalic brain of dogs renders it structurally and functionally more similar to the human brain than that of rodents [29]. Unlike sheep [30,
31] and swine [32], dogs do not possess a *rete mirabile*, thus allowing direct endovascular access to the middle cerebral artery (MCA) for creating an occlusion as well as achieving mechanical recanalization [28, 33, 34]. The dog ELVO model has been developed to allow for the assessment of neuroprotectants and other novel therapeutics [35]. Prior studies suggested that the dog model may be limited by its variable ischemic lesion evolution possibly due to irregular collateral circulation [36, 37]. Here we formally explored the possibility that this variability is similar to the human condition and contributes to the different rates of infarct evolution [38]: 1.) fast progressors, who experience rapid infarct growth with a large ischemic core and failing collaterals despite an early diagnosis within 6 h of stroke onset [39, 40]; and 2) slow progressors, who experience slow infarct growth maintaining a small ischemic core and good collaterals with significant salvageable tissue beyond the 6 h time window [41, 42].

Diffusion- (DWI) and perfusion-weighted imaging (PWI) are common magnetic resonance imaging (MRI) techniques used for diagnosing ischemic stroke in the clinical setting [43-49]. The DWI-derived apparent diffusion coefficient (ADC) of cerebral tissue decreases within the region of the irreversibly injured infarct core that can be detected using appropriate thresholds [50-52]. Several PWI parameters derived from dynamic susceptibility contrast (DSC)-MRI including cerebral blood flow (CBF), cerebral blood volume (CBV), mean transit time (MTT), time of maximum value of the tissue residue function (Tmax), bolus arrival time (BAT), and time-to-peak (TTP) concentration become altered as normal cerebral tissue progresses from ischemia to infarction [53]. The DWI/PWI mismatch has been used to predict the extent of infarction and identify patients likely to benefit from reperfusion therapy [54, 55]. However, depending on the PWI
parameter used and the method implemented to compute it, studies have reported an inconsistency in the identification of the final infarct volume especially when using parameters like CBF and MTT [53, 56-68]. On the other hand, PWI parameters like Tmax and TTP have been shown to more accurately estimate the final infarct size [54, 69-77].

The DEFUSE 2 clinical trials reliably showed that patients with target mismatch (based on Tmax thresholds) who underwent early reperfusion after endovascular stroke treatment had more favorable clinical outcomes compared to patients without target mismatch [78]. In a previous dog ELVO model an infarct growth model was developed to predict the infarct growth rate and final infarct volume based on DWI lesion volume and pial collateral quantification from computed tomography (CT) angiograms [37]. However, this model was based on fast evolvers and thus it is uncertain whether results can be translated to slow evolvers. The ability to differentiate fast from slow evolvers early on is an important issue to minimize bias in studies utilizing the dog model to assess novel treatment approaches. To address this issue, we leveraged the presence of slow versus fast evolving ischemic lesions in our established dog ELVO model to predict the infarct evolution using PWI-based TTP maps, derived from DSC-MRI, based only on the first PWI image acquired after the onset of stroke. Correct classification of the infarct evolution from study inception has the advantage to exclude one type of progressor or to prospectively enter animals into appropriate cohorts in therapeutic studies, thereby increasing the scientific rigor.

**Materials and Methods**

*Animal preparation*
All animal research procedures were performed as approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Massachusetts Medical School (Worcester, MA, USA). Fourteen purpose-bred dogs (8 females and 6 males; weight: 8.2–23.9 kg; age: 6.5–36 months) were used in this study. Details of the subject demographics are provided in Table 1. On the day of surgery, the animals were sedated and pre-treated with a single dose of intramuscular acepromazine (0.06 mg/kg), buprenorphine (0.02 mg/kg), and glycopyrrolate (0.01 mg/kg). Anesthesia was induced with an intravenous dose of propofol (3–4 mg/kg), and the animal was intubated. Anesthesia was maintained by mechanical ventilation with 1–3% isoflurane in air. Throughout anesthesia, animals were monitored by electrocardiogram (ECG), peripheral capillary oxygen saturation (spO2), invasive or non-invasive arterial blood pressure, rectal temperature, and end-tidal CO2. All vital measurements were maintained within the physiological range [79]. Using a modified Seldinger technique, an 8F hemostatic introducer was placed in the right femoral artery to allow endovascular access and blood draw for preparing blood clots as previously described in detail [28]. A 6-french Navien-072 catheter (Medtronic Neurovascular, Irvine, CA) was navigated under fluoroscopic guidance to the origin of the right or left internal carotid artery (ICA), at which point an autologous clot [28] was injected and advanced to permanently occlude the MCA (Fig. 1). The side of clot injection was selected on the basis of the larger diameter and length of non-tortuous cervical ICA for delivery of this relatively large catheter.

Imaging protocol

Once correct clot placement at the origin of the MCA was confirmed by fluoroscopy (Allura Xper FD20; Philips Healthcare, Best, the Netherlands), the animal was transferred
to the MRI scanner (Phillips Achieva/Phillips Ingenia 3T, Philips Healthcare), which is physically adjacent to the angiography suite. An 8-channel knee coil (Philips Healthcare) was used to acquire the brain scans. The imaging protocol included time-of-flight (ToF) imaging (TR/TE 20/4 ms, FA = 20°, matrix 332 × 212), DWI (TR/TE 2600/76 ms, FA = 90°, b-value = 0, 1000 s/mm², NSA = 6, matrix 144 × 144), PWI (TR/TE 1500/20.1 ms, FA = 40°, 60 dynamics, matrix 320 × 320), and T2-weighted (T2W)-FLAIR imaging (TR/TE 11000/125 ms, TI 2800 ms, FA = 90°, ETL = 27, matrix = 288 × 288). FLAIR imaging was used to rule out the possibility of subarachnoid hemorrhage. For PWI, 0.2 mmol/kg of gadopentetate dimeglumine (Magnevist, Bayer Healthcare Pharmaceuticals, Leverkusen, Germany) was injected intravenously (IV) after the second of 60 dynamic scans (total scan time = 90 s), followed by 15 ml saline bolus. DWI was obtained approximately every half hour up to 4.5 h post-clot injection. To calculate the infarct core volume, ADC maps were generated from the DWI images. Once the final diffusion image was acquired, the animal was euthanized, and in selected cases the brain was harvested immediately for histological assessment to confirm the final infarct size.

Image analysis

In order to assess the rate of infarct evolution based on DWI images, ADC maps were generated using MATLAB (The Mathworks, Inc., Natick, MA) and the datasets were binned in aggregates of 30 min. Since the goal of the study was to assess the rate of infarct evolution rather than the overall size of the infarct, the infarct size at each time-point was normalized by the final volume. When DWI was unavailable at a time point, linear interpolation was used to estimate the size of the normalized infarct.
Calculation of TTP maps was done offline using ImageJ (Rasband W. ImageJ. Bethesda, MD: http://rsb.info.nih.gov/ij/; 1997–2006) and MATLAB. The dynamic scans from PWI were used to segment the entire brain region in ImageJ. Scripts were written in MATLAB to calculate the TTP maps using the segmented brain slices from the dynamic scans on a voxel-by-voxel basis by locating the dynamic scan that contained the minimum intensity value. To obtain a relative TTP (rTTP) map, TTP maps were normalized by subtracting the mean TTP derived from a contralateral region of interest (ROI; positioned in the periventricular area of the unaffected hemisphere in the mid-brain region) from the absolute TTP in each voxel using the following equation:

\[ r_{\text{TTP}} \text{map} = T_{\text{TTP}} \text{map} - \frac{1}{n} \sum_{i=1}^{n} (ROI_{\text{contralateral}})_i \]

where \( n \) is the total number of voxels in the contralateral ROI.

To allow for exact voxel-wise analysis, all brain slices from the respective ADC and TTP maps were co-registered prior to analysis. This was accomplished by registering the B0 images from the DWI acquisition to the raw PWI images using a Dice similarity coefficient to confirm the best possible registration. This affine transformation was then applied to the ADC maps, which registered them to the rTTP maps. The areas defined as infarct on the final DWI were used to isolate only the voxels within the rTTP maps that ultimately evolved to infarction. The voxels on the rTTP maps were categorized into 4 bins: 4–8 s (slight delay), 9–13 s (moderate delay), 14–18 s (long delay), and 19+ s (extended delay). This categorization was used to predict the rate of infarct evolution using the rTTP maps and comparing them to the true rate of infarct evolution as measured using serial DWI. A summary of the image analysis is shown in Fig. 2. An rTTP infarct
evolution (rTTPIE) index was also calculated using the ratio of the rTTP map voxels in the extended delay and slight delay bins to quantify the rate of infarct evolution in fast and slow evolvers (Table 1). An rTTPIE index greater than 1 would indicate a fast evolver and less than 1 would indicate a slow evolver.

**Final infarct determination**

To minimize confounding of MRI to histology-determined infarct size, we used the ADC-thresholded core assessed on the final image set to determine final infarct volume, which has been previously shown to have a high correlation with TTC-based histology [80, 81]. In addition, we used a subset of animals to assess the final infarct volume histologically. Following extraction, brains were placed in a -80°C freezer for approximately 20 min and then cut into approximately 5-mm thick sections. Each section was stained with 2,3,5-triphenyltetrazolium (TTC) for final infarct volume assessment.

**Statistical analysis**

All statistical analyses were done in R (R Development Core Team (2018). R: A Language and Environment for Statistical Computing (Version 3.5.2) [Software]. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from http://www.R-project.org). For demographic data, analysis of variance (ANOVA) was used to identify any differences between the slow and fast evolvers. The Pearson product–moment correlation coefficient was used to check for significance of the correlations between the infarct volumes of the DWI and PWI images. For the comparison between histograms of the two groups (slow and fast evolvers), a paired t-test was used. Fisher’s exact test was used to examine the significance of association between the rTTP map and DWI categorization of fast and
slow evolvers, i.e. to identify the capability of the rTTP maps to predict the DWI results. A two-sided $p < 0.05$ was considered significant in all analyses. A post-hoc power analysis was also performed using $\alpha = 0.05$ to verify the statistical power of the significant differences between the fast and slow evolvers in this study using the G*Power software package [82].

**Results**

**MCA occlusion**

Embolic MCA occlusion was successfully induced in all animals ($n = 14$) as confirmed by digital subtraction angiography (Fig. 1a, b). The persistence of the MCA occlusion was confirmed by ToF-MRI (Fig. 1c) in each animal. None of the animals displayed any hemorrhagic transformation (data not shown).

**Imaging characteristics of fast evolvers**

Figure 3 depicts a representative animal (Animal #4 from Table 1) that was assigned to the fast evolver group (based on the DWI designation discussed below). A single coronal slice is shown indicating an area of restricted diffusion in the right MCA territory (Fig. 3a). The corresponding ADC map of the same slice shows a hypointense region indicating the extent of infarct with a lesion averaged ADC value of $\sim 0.5 \times 10^{-3}$ mm$^2$/s (Fig. 3b). The corresponding TTC-stained brain section shows concordance of the histology with the ADC-defined region of infarction (Fig. 3c). The rTTP map, corresponding to the ADC map, derived from PWI images is displayed using a pseudo-color map indicating the same infarct region (Fig. 3d). The color intensity of the voxels
indicates that the infarct area with an elevated intensity are at or above ~20 s. Images from DSA in the early (Fig. 3e) and delayed (Fig. 3f) phase indicate a lack of collaterals in the right hemisphere.

*Imaging characteristics of slow evolvers*

Figure 4 shows a representative animal (Animal #1 from Table 1) that was assigned to the slow evolver group (based on the DWI designation discussed below), which revealed a smaller lesion size compared to the fast evolver in Fig. 3. A single coronal slice is shown where the infarct region is visible as an area of restricted diffusion in the left hemisphere (Fig. 4a). The corresponding ADC map of the same slice shows a hypointense region indicating the extent of infarct with an ADC value of ~0.5 \times 10^{-3} \text{mm}^2/\text{s} (Fig. 4b). The corresponding TTC-stained brain section confirms the ADC-defined region of infarct (Fig. 4c). The TTP map, corresponding to the slice from the ADC map, derived from PWI images is displayed using a pseudo-color map indicating the same infarct region (Fig. 4d). The color intensity of the voxels in the infarct area only have a slightly elevated intensity with only a few voxels above ~20 s, which is in contrast to the elevated intensities observed in the fast evolver (Fig. 3d). Images from DSA in the early (Fig. 4e) and delayed (Fig. 4f) phase highlight the impact of the collaterals in the left hemisphere.

*Fast and slow evolver designation using DWI*

Based on the normalized infarct volume as a function of time using DWI (Fig. 5a), animals were categorized to fast versus slow evolvers. After MCA occlusion, fast evolvers showed a constant growth in the ADC-thresholded infarct volume during the first 2 h at which point infarct growth plateaued to reach the final infarct size. Slow evolvers
demonstrated a relatively slow infarct core evolution in the first 2 h with a secondary more rapid constant growth occurring afterwards until the final infarct size was reached at approximately 4 h after MCA occlusion. Thus, animals displaying more than 50% of total infarct volume within 2 h were assigned to the fast evolver group and animals displaying less than 50% of the total infarct volume within 2 h were assigned to the slow evolver group. Based on this definition, each animal in this study was designated with an infarct evolution rate (Table 1). A comparison between the demographics (sex, age, and weight) of the fast and slow evolvers did not show any significant difference (Table 2). The vital measurements between the groups also did not show any significant difference (data not shown).

**Prediction of fast versus slow evolvers using initial PWI**

The correlation plot (Fig. 5b) shows the agreement of the infarct volumes between the final DWI image and the TTP map generated from the initial PWI images ($R^2 = 0.99$). Based on the relative number of voxels within the “slight delay” and “extended delay” categories, animals were classified into fast and slow evolvers (Fig. 5c): the respective cutoff marks to distinguish between slow and fast evolvers were ~34% for the “slight delay” and ~42% for the “extended delay” bins based on the minimal distance (halfway point) between the boundary points of each category. This classification was more apparent using the rTTPIE index, which demonstrated a significant difference between the fast and slow evolvers (Table 2). For the remaining bins, the fast and slow evolvers did not show a significant difference. This rTTP-based classification correctly predicted the DWI results of all fourteen animals ($p < 0.01$, Fishers exact test for classification). The post-hoc analyses for this study revealed the statistical power to exceed 0.99 for the
detection of differences between the fast and slow evolvers using the “slight delay” and “extended delay” bins, and the rTTPIE index.

Ex vivo infarct assessment and correlation with MRI

In a subset of dogs (n = 6: 4 fast evolvers and 2 slow evolvers) after euthanasia, the brain was removed to histologically quantify the infarct volume using TTC. Overall, there was an excellent correlation between the final ADC-thresholded and the TTC-defined infarct volume ($R^2 = 0.99$) with a slope of 0.96 (data not shown).

Discussion

During human ELVO, the rate of infarct growth is determined by a combination of factors such as genetic background, demographics, physiological parameters, and other conditions that may influence collateral blood flow and ischemic tolerance [5, 38]. A key finding of our study was that the dog ELVO model, similar to monkeys [19], is characterized by variability in the ischemic stroke evolution that closely mimics the clinical scenario [38]. This phenomenon, in conjunction with the ability to study endovascular approaches to recanalization, renders the dog ELVO model as highly relevant to study novel therapeutic approaches in interventional research. Importantly, we show that voxel-wise analyses of PWI images allow to predict whether animals were fast or slow evolvers already on the first PWI scan obtained approximately 30 min after MCA occlusion.

Perfusion is a critical biological mechanism that reflects the delivery of oxygen and essential nutrients to tissue via blood flow. MR-based PWI has the potential to measure brain perfusion in acute stroke subjects and provide treatment options that may
significantly affect the clinical outcome. DSC-MRI, compared to other PWI techniques like dynamic contrast-enhanced (DCE)-MRI and arterial-spin labeling (ASL), offers faster scan times to get a quick measurement of transit time with whole brain coverage, which can be critical in driving treatment decisions with a limited time window [83]. The deconvolved PWI parameters derived from DSC-MRI such as CBF, CBV, and MTT, have been considered to be more reliable in detecting tissue at risk of infarction due to the use of an arterial input function (AIF), which takes into account any variations in physiological and injection conditions [84, 85]. However, the AIF shape is susceptible to errors due to several factors such as motion artifacts and partial volume effects among others.[86, 87] Non-deconvolved PWI parameters derived from DSC-MRI such as TTP and BAT, on the other hand, do not require the measurement of an AIF and are derived directly from the tissue concentration time curve without any deconvolution.

TTP has been suggested to rapidly identify tissue at risk of infarction (penumbral tissue) and predict infarct size and growth in stroke patients [73, 75, 76, 88-91]. Thresholds of TTP delay (TTP > 4 s best identifying penumbral flow) [54, 72-76, 88, 92] were used to determine the volume of tissue that would become viable or that were at risk to progress to infarct. These TTP thresholds have been compared with $^{15}$O-water positron emission tomography (PET) studies and validated using $^{15}$O-water PET-derived CBF threshold of <20 ml/100 g/min, which serves as the “gold standard” for detecting penumbral tissue in ischemic stroke [57, 74-76, 92, 93]. In this study, we expanded the role of TTP maps to assess the extent of critically hypoperfused brain tissue as well as to predict the rate of infarct evolution.
TTP maps have been generated in several different ways in clinical and animal studies over the last couple of decades. TTP map calculations have mainly been reported using three different methods where: 1.) TTP was defined as the time difference in peak intensity and the BAT [85, 94]; 2.) TTP was defined as the time from bolus injection to peak intensity [53, 54, 76, 95]; and 3.) TTP maps were generated using a normalization by subtracting a mean contralateral ROI of the unaffected hemisphere [54, 71-73, 75]. Since the latter method using normalization of the TTP map reduces AIF dependency (by lowering variability due to factors such as injection rate, catheter size, and cardiac output among others) and performs as well as deconvolved parameters in infarct prediction [57, 85, 86, 96], we used rTTP maps in this study to perform the histogram binning.

Histogram binning of rTTP maps successfully classified the animals into fast and slow evolvers based on the infarct growth rate observed in the ADC maps. The “slight delay” and “extended delay” bins in the histogram clearly distinguished between the fast and slow evolvers, which is numerically evident using the rTTPIE index. Utilizing the rTTP map binning scheme promises to be useful in prospectively determining whether an animal will progress as a fast or slow evolver – a major advantage when studying novel therapeutics as it would allow to control for variability in infarct progression as part of the randomization scheme.

**Limitations**

One limitation from our analysis was that only two types of evolvers (fast and slow) were distinguishable using the rTTP analysis. If the rTTPIE index value equaled close to
one, then that would correspond to having approximately equal number of voxels in the
“slight delay” and “extended delay” histogram bins of the rTTP maps, which would make it
difficult to assign the animal as a fast or slow evolver. In that case, an “intermediate
evolver” designation maybe more appropriate. The rTTP analysis to predict fast and slow
evolvers was done retrospectively, which requires prospective confirmation of these
preliminary findings. There was a notable variation in animal age and weight related to
the different strains of dogs included into our retrospective analysis. However, we did not
find any significant age and weight difference between fast and slow evolvers, assuaging
concerns of major confounding and may in fact indicate broad applicability of our results.
During the course of this proof-of-concept study, histology was not performed in all dogs
for confirmation of the ADC thresholded infarct volume. However, in our past experience,
the selected ADC threshold is highly concordant with histology [27, 28].

Conclusion

The canine model of embolic stroke resulting in the occlusion of the MCA results
in both slow and fast infarct evolution. Preliminary evidence demonstrates that infarct
evolution pattern can be determined immediately after clot placement using perfusion-
weighted MRI. These data support more robust preclinical assessment of complex,
multimodal acute stroke therapies.

Compliance with Ethical Standards

Conflict of Interest
Dr. Henninger is supported by K08NS091499 from the National Institute of Neurological Disorders and Stroke of the National Institutes of Health (NIH). The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH. Dr. Henninger serves on the advisory board of Omniox, Inc. and serves as consultant to Astrocyte Pharmaceuticals, Inc. Dr. Gounis has been a consultant on a fee-per-hour basis for Cerenovus, Imperative Care, Mivi Neurosciences, Phenox, Route 92 Medical, Stryker Neurovascular; holds stock in Imperative Care and Neurogami; and has received research support from the NIH, the United States – Israel Binational Science Foundation, Anaconda, Cerenovus, Ceretrieve, Cook Medical, Gentuity, Imperative Care, InNeuroCo, Magneto, Microvention, Medtronic Neurovascular, MIVI Neurosciences, Neuravi, Neurogami, Philips Healthcare, Rapid Medical, Route 92 Medical, Stryker Neurovascular, Syntheon, and the Wyss Institute. All authors declare that they have no potential conflicts of interest in regard to the research, authorship, and publication of this paper.

Ethical Approval

All animal research procedures were performed as approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Massachusetts Medical School (Worcester, MA, USA). This article does not contain any studies with human participants performed by any of the authors.

Author Contribution Statement
The concept of study was developed by Matthew Gounis, Nils Henninger, Johannes Boltze, and Ajit Puri. Robert King and Matthew Gounis performed the animal experiments and collected the imaging data. Olivia Brooks performed data analysis. Mohammed Salman Shazeeb developed the image analysis pipeline, performed image analysis, and wrote the manuscript. Robert King also performed image analysis. Mathew Gounis, Nils Henninger, Johannes Boltze, and Robert King made significant edits to the manuscript for intellectual content. All authors read and approved the final manuscript.

Supplementary Information

None

References


**Figure Legends**

**Fig. 1** Placement of an autologous clot in the middle cerebral artery (MCA) of the dog. (a) Digital Subtraction Angiography (DSA) prior to clot placement is shown. The arrows indicate the location of the MCA (b) Total MCA occlusion is shown in DSA post-clot placement. The dashed arrows depict the original course of the MCA, which is now absent. (c) Time-of-flight (ToF) MRI shows an open internal carotid artery (ICA) and an occluded MCA.

**Fig. 2** Flowchart of image analysis. Apparent diffusion coefficient (ADC) and time-to-peak (TTP) maps were generated from diffusion-weighted imaging (DWI) and perfusion-weighted imaging (PWI), respectively. ADC maps were used to calculate the final infarct volume. The entire brain in TTP maps was normalized with respect to a representative area on the contralateral side of the infarct region to generate relative TTP (rTTP) maps. rTTP maps were registered with ADC maps to count the voxels within the infarct volume. All voxels within the ADC-defined lesion were binned into one of four categories in the rTTP map. The total size of each bin, across the entire lesion volume, was used to predict the rate of infarct evolution.

**Fig. 3** Representative animal from the fast evolver group (Animal #4 from Table 1). (a) Final diffusion-weighted imaging (DWI) image showing the total extent of the infarct as a hyperintense region. (b) Apparent diffusion coefficient (ADC) map showing a hypointense region indicating the extent of infarct. The red line on the scale bar indicates the intensity
level of the infarct region. (c) Post-mortem triphenyltetrazolium chloride (TTC) staining showing the final histological infarct size. Scale bar: 10 mm. (d) Relative time-to-peak (rTTP) map derived from perfusion-weighted imaging (PWI) images. The color intensity of the infarct area voxels indicate an elevated intensity at or above the 20-s delay. Digital Subtraction Angiography (DSA) is shown in early (e) and delayed phase (f), which denote a lack of collaterals in the right hemisphere. The arrows indicate the location of the clot.

**Fig. 4** Representative animal from the slow evolver group (Animal #1 from Table 1). (a) Final diffusion-weighted imaging (DWI) image showing the total extent of the infarct as a hyperintense region. (b) Apparent diffusion coefficient (ADC) map showing a hypointense region indicating the extent of infarct. The red line on the scale bar indicates the intensity level of the infarct region. (c) Post-mortem triphenyltetrazolium chloride (TTC) staining showing the final histological infarct size. Scale bar: 10 mm. (d) Relative time-to-peak (rTTP) map derived from perfusion-weighted imaging (PWI) images. The area of occlusion is seen only slightly elevated with a few voxels above the 20-s delay. Digital Subtraction Angiography (DSA) is shown in early (e) and delayed phase (f), which highlights the impact of the collaterals in the left hemisphere. The arrows indicate the location of the clot.

**Fig. 5** Quantification of fast and slow evolvers using diffusion-weighted imaging (DWI) apparent diffusion coefficient (ADC) maps and perfusion-weighted imaging (PWI) time-to-peak (TTP) maps. (a) Ischemic core evolution as derived by ADC-thresholded maps and dichotomized to fast (orange) versus slow (blue) evolvers are shown. Based on lesion
growth rate, animals were divided into fast (>50% total volume within 2 h) and slow (<50% total volume within 2 h) evolver groups. The plot shows the progression of slow and fast evolvers with the greatest difference occurring at about 2 h. Individual growth curves are shown for each animal in lighter shade. Error bars indicate SEM. (b) Correlation plot is shown indicating the agreement of infarct volumes between the final DWI image and the TTP map generated from the initial PWI images ($R^2 = 0.99$). The dotted lines indicate 95% confidence level. (c) Histogram binning from rTTP maps for fast and slow evolvers are shown. The voxels in the “slight delay” bin (4–8 s) of fast evolvers are significantly fewer compared to that of slow evolvers (***$p < 0.0001$). The dashed line near the left axis of the plot shows a cutoff at ~34%, which distinguishes between the fast and slow evolvers in the “slight delay” bin. The voxels in the “extended delay” bin (19+ s) of fast evolvers are significantly more compared to that of slow evolvers (*$p = 0.0013$). The dashed line near the right axis of the plot shows a cutoff at ~42%, which distinguishes between the fast and slow evolvers in the “extended delay” bin. The two middle bins of “moderate delay” (9–13 s) and “long delay” (14–18 s) did not show any significant differences between the slow and fast evolvers. Error bars indicate SEM.
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Abstract

Mechanical thrombectomy for the treatment of ischemic stroke shows high rates of recanalization; however, some patients still have a poor clinical outcome. A proposed reason for this relates to the fact that the ischemic infarct growth differs significantly between patients. While some patients demonstrate rapid evolution of their infarct core (fast evolvers), others have substantial potentially salvageable penumbral tissue even hours after initial vessel occlusion (slow evolvers). We show that the dog middle cerebral artery occlusion model recapitulates this key aspect of human stroke rendering it a highly desirable model to develop novel multimodal treatments to improve clinical outcomes. Moreover, this model is well suited to develop novel image analysis techniques that allow for improved lesion evolution prediction; we provide proof-of-concept that MRI perfusion-based time-to-peak maps can be utilized to predict the rate of infarct growth as validated by apparent diffusion coefficient derived lesion maps allowing reliable classification of
dogs into fast versus slow evolvers enabling more robust study design for interventional research.

Key words: dog, middle cerebral artery occlusion, infarct growth rate, perfusion MRI, time-to-peak, stroke.
Introduction

In recent years, randomized-controlled trials have shown that mechanical thrombectomy is a highly effective treatment for selected ischemic stroke patients with emergent large vessel occlusion (ELVO) [1-3]. Although clinical trials and uncontrolled stroke registries report successful recanalization of the primary artery in approximately 85% [4, 5], rates of good clinical outcomes have not yet matched the high rate of technical success. For this reason, novel approaches to multimodal intervention that includes neuroprotection, adjunctive technology to augment brain perfusion, thrombolysis, and recanalization with thrombectomy promise to maximize the probability of a good clinical outcome after ELVO. In particular, methods that can slow infarct progression may expand the availability of mechanical thrombectomy for ELVO patients that present beyond established treatment windows [6, 7]. Approaches under development include transient aortic occlusion [8], stimulation of circle of Willis nerve fibers [9], mild induced hypertension [10], and inhalation of nitric oxide [11]. Perhaps the greatest opportunity is re-exploration of pharmacological neuroprotection combined with mechanical thrombectomy [12-15].

Although multiple animal stroke models are available in a variety of species to study stroke pathophysiology and possible treatment modalities [16], only larger animal species such as rabbits [17], non-human primates [18, 19], and dogs [20-27] are suitable for creating ELVO via an endovascular approach without performing a craniotomy. However, to date only the dog model allows simulation of mechanical thrombectomy for ELVO [28]. Moreover, the gyrencephalic brain of dogs renders it structurally and functionally more similar to the human brain than that of rodents [29]. Unlike sheep [30,
31] and swine [32], dogs do not possess a *rete mirabile*, thus allowing direct endovascular access to the middle cerebral artery (MCA) for creating an occlusion as well as achieving mechanical recanalization [28, 33, 34]. The dog ELVO model has been developed to allow for the assessment of neuroprotectants and other novel therapeutics [35]. Prior studies suggested that the dog model may be limited by its variable ischemic lesion evolution possibly due to irregular collateral circulation [36, 37]. Here we formally explored the possibility that this variability is similar to the human condition and contributes to the different rates of stroke evolution [38]: 1.) fast progressors, who experience rapid infarct growth with a large ischemic core and failing collaterals despite an early diagnosis within 6 h of stroke onset [39, 40]; and 2) slow progressors, who experience slow infarct growth maintaining a small ischemic core and good collaterals with significant salvageable tissue beyond the 6 h time window [41, 42].

Diffusion- (DWI) and perfusion-weighted imaging (PWI) are common magnetic resonance imaging (MRI) techniques used for diagnosing ischemic stroke in the clinical setting [43-49]. The DWI-derived apparent diffusion coefficient (ADC) of cerebral tissue decreases within the region of the irreversibly injured infarct core that can be detected using appropriate thresholds [50-52]. Several PWI parameters derived from dynamic susceptibility contrast (DSC)-MRI including cerebral blood flow (CBF), cerebral blood volume (CBV), mean transit time (MTT), time of maximum value of the tissue residue function (Tmax), bolus arrival time (BAT), and time-to-peak (TTP) concentration become altered as normal cerebral tissue progresses from ischemia to infarction [53]. The DWI/PWI mismatch has been used to predict the extent of infarction and identify patients likely to benefit from reperfusion therapy [54, 55]. However, depending on the PWI
parameter used and the method implemented to compute it, studies have reported an inconsistency in the identification of the final infarct volume especially when using parameters like CBF and MTT [53, 56-68]. On the other hand, PWI parameters like Tmax and TTP have been shown to more accurately estimate the final infarct size [54, 69-77].

The DEFUSE 2 clinical trials reliably showed that patients with target mismatch (based on Tmax thresholds) who underwent early reperfusion after endovascular stroke treatment had more favorable clinical outcomes compared to patients without target mismatch [78]. In a previous dog ELVO model an infarct growth model was developed to predict the infarct growth rate and final infarct volume based on DWI lesion volume and pial collateral quantification from computed tomography (CT) angiograms [37]. However, this model was based on fast evolvers and thus it is uncertain whether results can be translated to slow evolvers. The ability to differentiate fast from slow evolvers early on is an important issue to minimize bias in studies utilizing the dog model to assess novel treatment approaches. To address this issue, we leveraged the presence of slow versus fast evolving ischemic lesions in our established dog ELVO model to predict the infarct evolution using PWI-based TTP maps, derived from DSC-MRI, based only on the first PWI image acquired after the onset of stroke. Correct classification of the infarct evolution from study inception has the advantage to exclude one type of progressor or to prospectively enter animals into appropriate cohorts in therapeutic studies, thereby increasing the scientific rigor.

**Materials and Methods**

*Animal preparation*
All animal research procedures were performed as approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Massachusetts Medical School (Worcester, MA, USA). Fourteen purpose-bred dogs (8 females and 6 males; weight: 8.2–23.9 kg; age: 6.5–36 months) were used in this study. Details of the subject demographics are provided in Table 1. On the day of surgery, the animals were sedated and pre-treated with a single dose of intramuscular acepromazine (0.06 mg/kg), buprenorphine (0.02 mg/kg), and glycopyrrolate (0.01 mg/kg). Anesthesia was induced with an intravenous dose of propofol (3–4 mg/kg), and the animal was intubated. Anesthesia was maintained by mechanical ventilation with 1–3% isoflurane in air. Throughout anesthesia, animals were monitored by electrocardiogram (ECG), peripheral capillary oxygen saturation (spO$_2$), invasive or non-invasive arterial blood pressure, rectal temperature, and end-tidal CO$_2$. All vital measurements were maintained within the physiological range [79]. Using a modified Seldinger technique, an 8F hemostatic introducer was placed in the right femoral artery to allow endovascular access and blood draw for preparing blood clots as previously described in detail [28]. A 6-french Navien-072 catheter (Medtronic Neurovascular, Irvine, CA) was navigated under fluoroscopic guidance to the origin of the right or left internal carotid artery (ICA), at which point an autologous clot [28] was injected and advanced to permanently occlude the MCA (Fig. 1). The side of clot injection was selected on the basis of the larger diameter and length of non-tortuous cervical ICA for delivery of this relatively large catheter.

*Imaging protocol*

Once correct clot placement at the origin of the MCA was confirmed by fluoroscopy (Allura Xper FD20; Philips Healthcare, Best, the Netherlands), the animal was transferred
to the MRI scanner (Phillips Achieva/Phillips Ingenia 3T, Philips Healthcare), which is physically adjacent to the angiography suite. An 8-channel knee coil (Philips Healthcare) was used to acquire the brain scans. The imaging protocol included time-of-flight (ToF) imaging (TR/TE 20/4 ms, FA = 20°, matrix 332 × 212), DWI (TR/TE 2600/76 ms, FA = 90°, b-value = 0, 1000 s/mm², NSA = 6, matrix 144 × 144), PWI (TR/TE 1500/20.1 ms, FA = 40°, 60 dynamics, matrix 320 × 320), and T2-weighted (T2W)-FLAIR imaging (TR/TE 11000/125 ms, TI 2800 ms, FA = 90°, ETL = 27, matrix = 288 × 288). FLAIR imaging was used to rule out the possibility of subarachnoid hemorrhage. For PWI, 0.2 mmol/kg of gadopentetate dimeglumine (Magnevist, Bayer Healthcare Pharmaceuticals, Leverkusen, Germany) was injected intravenously (IV) after the second of 60 dynamic scans (total scan time = 90 s), followed by 15 ml saline bolus. DWI was obtained approximately every half hour up to 4.5 h post-clot injection. To calculate the infarct core volume, ADC maps were generated from the DWI images. Once the final diffusion image was acquired, the animal was euthanized, and in selected cases the brain was harvested immediately for histological assessment to confirm the final infarct size.

Image analysis

In order to assess the rate of stroke infarct evolution based on DWI images, ADC maps were generated using MATLAB (The Mathworks, Inc., Natick, MA) and the datasets were binned in aggregates of 30 min. Since the goal of the study was to assess the rate of infarct evolution rather than the overall size of the infarct, the infarct size at each time-point was normalized by the final volume. When DWI was unavailable at a time point, linear interpolation was used to estimate the size of the normalized infarct.
Calculation of TTP maps was done offline using ImageJ (Rasband W. ImageJ. Bethesda, MD: http://rsb.info.nih.gov/ij/; 1997–2006) and MATLAB. The dynamic scans from PWI were used to segment the entire brain region in ImageJ. Scripts were written in MATLAB to calculate the TTP maps using the segmented brain slices from the dynamic scans on a pixel-by-pixel basis by locating the dynamic scan that contained the minimum intensity value. To obtain a relative TTP (rTTP) map, TTP maps were then normalized by subtracting the mean TTP derived from a contralateral region of interest (ROI); positioned in the periventricular area of the unaffected hemisphere in the mid-brain region) from the absolute TTP in each pixel, yielding a relative TTP (rTTP) map voxel using the following equation:

\[ r_{TTP}^{\text{map}} = TTP_{\text{map}} - \frac{1}{n} \sum_{i=1}^{n} (ROI_{\text{contralateral}})_{i} \]

where \( n \) is the total number of voxels in the contralateral ROI.

To allow for exact voxel-wise analysis, all brain slices from the respective ADC and TTP maps were co-registered prior to analysis. This was accomplished by registering the B0 images from the DWI acquisition to the raw PWI images using a Dice similarity coefficient to confirm the best possible registration. This affine transformation was then applied to the ADC maps, which registered them to the rTTP maps. The areas defined as infarct on the final DWI were used to isolate only the voxels within the rTTP maps that ultimately evolved to infarction. The voxels on the rTTP maps were categorized into 4 bins: 4–8 s (slight delay), 9–13 s (moderate delay), 14–18 s (long delay), and 19+ s (extended delay). This categorization was used to predict the rate of stroke infarct evolution using the rTTP maps and comparing them to the true rate of stroke infarct.
evolution as measured using serial DWI. A summary of the image analysis is shown in Fig. 2. An rTTP stroke rate infarct evolution (rTTPIE) index (rTTP-SRI) was also calculated using the ratio of the rTTP map voxels in the extended delay and slight delay bins to quantify the rate of stroke infarct evolution in fast and slow evolvers (Table 1). An rTTP-SRI\textsubscript{rTTPIE} index greater than 1 would indicate a fast evolver and less than 1 would indicate a slow evolver.

**Final infarct determination**

To minimize confounding of MRI to histology-determined infarct size, we used the ADC-thresholded core assessed on the final image set to determine final infarct volume, which has been previously shown to have a high correlation with TTC-based histology [80, 81]. In addition, we used a subset of animals to assess the final infarct volume histologically. Following extraction, brains were placed in a -80°C freezer for approximately 20 min and then cut into approximately 5-mm thick sections. Each section was stained with 2,3,5-triphenyltetrazolium (TTC) for final infarct volume assessment.

**Statistical analysis**

All statistical analyses were done in R (R Development Core Team (2018). R: A Language and Environment for Statistical Computing (Version 3.5.2) [Software]. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from http://www.R-project.org). For demographic data, analysis of variance (ANOVA) was used to identify any differences between the slow and fast evolvers. The Pearson product–moment correlation coefficient was used to check for significance of the correlations between the infarct volumes of the DWI and PWI images. For the comparison between histograms of the two groups (slow
and fast evolvers), a paired t-test was used. Fisher’s exact test was used to examine the significance of association between the rTTP map and DWI categorization of fast and slow evolvers, i.e. to identify the capability of the rTTP maps to predict the DWI results. A two-sided \( p < 0.05 \) was considered significant in all analyses. A post-hoc power analysis was also performed using \( \alpha = 0.05 \) to verify the statistical power of the significant differences between the fast and slow evolvers in this study using the G*Power software package [82].

**Results**

*MCA occlusion*

Embolic MCA occlusion was successfully induced in all animals \( (n = 14) \) as confirmed by digital subtraction angiography (Fig. 1a, b). The persistence of the MCA occlusion was confirmed by ToF-MRI (Fig. 1c) in each animal. None of the animals displayed any hemorrhagic transformation (data not shown).

*Imaging characteristics of fast evolvers*

Figure 3 depicts a representative animal (Animal #4 from Table 1) that was assigned to the fast evolver group (based on the DWI designation discussed below). A single coronal slice is shown indicating an area of restricted diffusion in the right MCA territory (Fig. 3a). The corresponding ADC map of the same slice shows a hypointense region indicating the extent of infarct with a lesion averaged ADC value of \( \sim 0.5 \times 10^{-3} \) mm\(^2\)/s (Fig. 3b). The corresponding TTC-stained brain section shows concordance of the histology with the ADC-defined region of infarction (Fig. 3c). The rTTP map,
corresponding to the ADC map, derived from PWI images is displayed using a pseudo-color map indicating the same infarct region (Fig. 3d). The color intensity of the voxels indicates that the infarct area with an elevated intensity are at or above ~20 s. Images from DSA in the early (Fig. 3e) and delayed (Fig. 3f) phase indicate a lack of collaterals in the right hemisphere.

**Imaging characteristics of slow evolvers**

Figure 4 shows a representative animal (Animal #1 from Table 1) that was assigned to the slow evolver group (based on the DWI designation discussed below), which revealed a smaller lesion size compared to the fast evolver in Fig. 3. A single coronal slice is shown where the infarct region is visible as an area of restricted diffusion in the left hemisphere (Fig. 4a). The corresponding ADC map of the same slice shows a hypointense region indicating the extent of infarct with an ADC value of ~0.5 × 10^{-3} \text{mm}^2/\text{s} (Fig. 4b). The corresponding TTC-stained brain section confirms the ADC-defined region of infarct (Fig. 4c). The TTP map, corresponding to the slice from the ADC map, derived from PWI images is displayed using a pseudo-color map indicating the same infarct region (Fig. 4d). The color intensity of the voxels in the infarct area only have a slightly elevated intensity with only a few voxels above ~20 s, which is in contrast to the elevated intensities observed in the fast evolver (Fig. 3d). Images from DSA in the early (Fig. 4e) and delayed (Fig. 4f) phase highlight the impact of the collaterals in the left hemisphere.

**Fast and slow evolver designation using DWI**

Based on the normalized stroke infarct volume as a function of time using DWI (Fig. 5a), animals were categorized to fast versus slow evolvers. After MCA occlusion, fast
Evolvers showed a constant growth in the ADC-thresholded infarct volume during the first 2 h at which point infarct growth plateaued to reach the final infarct size. Slow evolvers demonstrated a relatively slow infarct core evolution in the first 2 h with a secondary more rapid constant growth occurring afterwards until the final infarct size was reached at approximately 4 h after MCA occlusion. Thus, animals displaying more than 50% of total infarct volume within 2 h were assigned to the fast evolver group and animals displaying less than 50% of the total infarct volume within 2 h were assigned to the slow evolver group. Based on this definition, each animal in this study was designated with an infarct evolution rate (Table 1). A comparison between the demographics (sex, age, and weight) of the fast and slow evolvers did not show any significant difference (Table 2). The vital measurements between the groups also did not show any significant difference (data not shown).

*Prediction of fast versus slow evolvers using initial PWI*

The correlation plot (Fig. 5b) shows the agreement of the infarct volumes between the final DWI image and the TTP map generated from the initial PWI images ($R^2 = 0.99$). Based on the relative number of voxels within the “slight delay” and “extended delay” categories, animals were classified into fast and slow evolvers (Fig. 5c): the respective cutoff marks to distinguish between slow and fast evolvers were ~34% for the “slight delay” and ~42% for the “extended delay” bins. Based on the minimal distance (halfway point) between the boundary points of each category, this classification was more apparent using the $rTTP-SRI_{rTTPIE}$ index, which demonstrated a significant difference between the fast and slow evolvers (Table 2). For the remaining bins, the fast and slow evolvers did not show a significant difference. This $rTTP$-based classification correctly
predicted the DWI results of all fourteen animals \( (p < 0.01, \text{Fishers exact test for classification}) \). The post-hoc analyses for this study revealed the statistical power to exceed 0.99 for the detection of differences between the fast and slow evolvers using the “slight delay” and “extended delay” bins, and the \( r\text{TTP-SRIrTTPIE} \) index.

**Ex vivo infarct assessment and correlation with MRI**

In a subset of dogs \( (n=6: 4 \text{ fast evolvers and 2 slow evolvers}) \) after euthanasia, the brain was removed to histologically quantify the infarct volume using TTC. Overall, there was an excellent correlation between the final ADC-thresholded and the TTC-defined infarct volume \( (R^2 = 0.99) \) with a slope of 0.96 (data not shown).

**Discussion**

During human ELVO, the rate of infarct growth is determined by a combination of factors such as genetic background, demographics, physiological parameters, and other conditions that may influence collateral blood flow and ischemic tolerance [5, 38]. A key finding of our study was that the dog ELVO model, similar to monkeys [19], is characterized by variability in the ischemic stroke evolution that closely mimics the clinical scenario [38]. This phenomenon, in conjunction with the ability to study endovascular approaches to recanalization, renders the dog ELVO model as highly relevant to study novel therapeutic approaches in interventional research. Importantly, we show that voxel-wise analyses of PWI images allow to predict whether animals were fast or slow evolvers already on the first PWI scan obtained approximately 30 min after MCA occlusion.
Perfusion is a critical biological mechanism that reflects the delivery of oxygen and essential nutrients to tissue via blood flow. MR-based PWI has the potential to measure brain perfusion in acute stroke subjects and provide treatment options that may significantly affect the clinical outcome. DSC-MRI, compared to other PWI techniques like dynamic contrast-enhanced (DCE)-MRI and arterial-spin labeling (ASL), offers faster scan times to get a quick measurement of transit time with whole brain coverage, which can be critical in driving treatment decisions with a limited time window [83]. The deconvolved PWI parameters derived from DSC-MRI such as CBF, CBV, and MTT, have been considered to be more reliable in detecting tissue at risk of infarction due to the use of an arterial input function (AIF), which takes into account any variations in physiological and injection conditions [84, 85]. However, the AIF shape is susceptible to errors due to several factors such as motion artifacts and partial volume effects among others.[86, 87] Non-deconvolved PWI parameters derived from DSC-MRI such as TTP and BAT, on the other hand, do not require the measurement of an AIF and are derived directly from the tissue concentration time curve without any deconvolution.

TTP has been suggested to rapidly identify tissue at risk of infarction (penumbral tissue) and predict infarct size and growth in stroke patients [73, 75, 76, 88-91]. Thresholds of TTP delay (TTP > 4 s best identifying penumbral flow) [54, 72-76, 88, 92] were used to determine the volume of tissue that would become viable or that were at risk to progress to infarct. These TTP thresholds have been compared with $^{15}$O-water positron emission tomography (PET) studies and validated using $^{15}$O-water PET-derived CBF threshold of <20 ml/100 g/min, which serves as the “gold standard” for detecting penumbral tissue in ischemic stroke [57, 74-76, 92, 93]. In this study, we expanded the
role of TTP maps to assess the extent of critically hypoperfused brain tissue as well as to predict the rate of stroke evolution.

TTP maps have been generated in several different ways in clinical and animal studies over the last couple of decades. TTP map calculations have mainly been reported using three different methods where: 1.) TTP was defined as the time difference in peak intensity and the BAT [85, 94]; 2.) TTP was defined as the time from bolus injection to peak intensity [53, 54, 76, 95]; and 3.) TTP maps were generated using a normalization by subtracting a mean contralateral ROI of the unaffected hemisphere [54, 71-73, 75]. Since the latter method using normalization of the TTP map reduces AIF dependency (by lowering variability due to factors such as injection rate, catheter size, and cardiac output among others) and performs as well as deconvolved parameters in infarct prediction [57, 85, 86, 96], we used rTTP maps in this study to perform the histogram binning.

Histogram binning of rTTP maps successfully classified the animals into fast and slow evolvers based on the infarct growth rate observed in the ADC maps. The “slight delay” and “extended delay” bins in the histogram clearly distinguished between the fast and slow evolvers, which is numerically evident using the rTTP-SRI_{rTTP}E index. Utilizing the rTTP map binning scheme promises to be useful in prospectively determining whether an animal will progress as a fast or slow evolver – a major advantage when studying novel therapeutics as it would allow to control for variability in infarct progression as part of the randomization scheme.

Limitations
One limitation from our analysis was that only two types of evolvers (fast and slow) were distinguishable using the rTTP analysis. If the \textit{rTTP-SR/rTTPrIE index} value equaled close to one, then that would correspond to having approximately equal number of voxels in the “slight delay” and “extended delay” histogram bins of the rTTP maps, which would make it difficult to assign the animal as a fast or slow evolver. In that case, an “intermediate evolver” designation maybe more appropriate. The rTTP analysis to predict fast and slow evolvers was done retrospectively, which requires prospective confirmation of these preliminary findings. \textit{There was a notable variation in animal age and weight related to the different strains of dogs included into our retrospective analysis. However, we did not find any significant age and weight difference between fast and slow evolvers, assuaging concerns of major confounding and may in fact indicate broad applicability of our results.} During the course of this proof-of-concept study, histology was not performed in all dogs for confirmation of the ADC thresholded infarct volume. However, in our past experience, the selected ADC threshold is highly concordant with histology [27, 28].

\textbf{Conclusion}

The canine model of embolic stroke resulting in the occlusion of the MCA results in both slow and fast infarct evolution. Preliminary evidence demonstrates that infarct evolution pattern can be determined immediately after clot placement using perfusion-weighted MRI. These data support more robust preclinical assessment of complex, multimodal acute stroke therapies.
Compliance with Ethical Standards

Conflict of Interest

Dr. Henninger is supported by K08NS091499 from the National Institute of Neurological Disorders and Stroke of the National Institutes of Health. (NIH). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health (NIH). Dr. Henninger serves on the advisory board of Omniox, Inc. and serves as consultant to Astrocyte Pharmaceuticals, Inc. Dr. Gounis has been a consultant on a fee-per-hour basis for Cerenovus, Imperative Care, Mivi Neurosciences, Phenox, Route 92 Medical, Stryker Neurovascular; holds stock in Imperative Care and Neurogami; and has received research support from the NIH, the United States – Israel Binational Science Foundation, Anaconda, Cerenovus, Ceretrieve, Cook Medical, Gentuity, Imperative Care, InNeuroCo, Magneto, Microvention, Medtronic Neurovascular, MIVI Neurosciences, Neuravi, Neurogami, Philips Healthcare, Rapid Medical, Route 92 Medical, Stryker Neurovascular, Syntheon, and the Wyss Institute. All authors declare that they have no potential conflicts of interest in regard to the research, authorship, and publication of this paper.

Ethical Approval

All animal research procedures were performed as approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Massachusetts Medical School (Worcester, MA, USA). This article does not contain any studies with human participants performed by any of the authors.
Author Contribution Statement

The concept of study was developed by Matthew Gounis, Nils Henninger, Johannes Boltze, and Ajit Puri. Robert King and Matthew Gounis performed the animal experiments and collected the imaging data. Olivia Brooks performed data analysis. Mohammed Salman Shazeeb developed the image analysis pipeline, performed image analysis, and wrote the manuscript. Robert King also performed image analysis. Mathew Gounis, Nils Henninger, Johannes Boltze, and Robert King made significant edits to the manuscript for intellectual content. All authors read and approved the final manuscript.

Supplementary Information

None

References


Figure Legends

**Fig. 1** Placement of an autologous clot in the middle cerebral artery (MCA) of the dog. (a) Digital Subtraction Angiography (DSA) prior to clot placement is shown. The arrows indicate the location of the MCA (b) Total MCA occlusion is shown in DSA post-clot placement. The dashed arrows depict the original course of the MCA, which is now absent. (c) Time-of-flight (ToF) MRI shows an open internal carotid artery (ICA) and an occluded MCA.

**Fig. 2** Flowchart of image analysis. Apparent diffusion coefficient (ADC) and time-to-peak (TTP) maps were generated from diffusion-weighted imaging (DWI) and perfusion-weighted imaging (PWI), respectively. ADC maps were used to calculate the final strokeinfarct volume. The entire brain in TTP maps was normalized with respect to a representative area on the contralateral side of the strokeinfarct region to generate relative TTP (rTTP) maps. rTTP maps were registered with ADC maps to count the voxels within the strokeinfarct volume. All voxels within the ADC-defined lesion were binned into one of four categories in the rTTP map. The total size of each bin, across the entire lesion volume, was used to predict the rate of strokeinfarct evolution.

**Fig. 3** Representative animal from the fast evolver group (Animal #4 from Table 1). (a) Final diffusion-weighted imaging (DWI) image showing the total extent of the infarct as a hyperintense region. (b) Apparent diffusion coefficient (ADC) map showing a hypointense region indicating the extent of infarct. The red line on the scale bar indicates the intensity
level of the infarct region. (c) Post-mortem triphenyltetrazolium chloride (TTC) staining showing the final histological stroke-infarct size. **Scale bar: 10 mm.** (d) Relative time-to-peak (rTTP) map derived from perfusion-weighted imaging (PWI) images. The color intensity of the infarct area voxels indicate an elevated intensity at or above the 20-s delay. Digital Subtraction Angiography (DSA) is shown in early (e) and delayed phase (f), which denote a lack of collaterals in the right hemisphere. The arrows indicate the location of the clot.

**Fig. 4** Representative animal from the slow evolver group (Animal #1 from Table 1). (a) Final diffusion-weighted imaging (DWI) image showing the total extent of the infarct as a hyperintense region. (b) Apparent diffusion coefficient (ADC) map showing a hypointense region indicating the extent of infarct. The red line on the scale bar indicates the intensity level of the infarct region. (c) Post-mortem triphenyltetrazolium chloride (TTC) staining showing the final histological stroke-infarct size. **Scale bar: 10 mm.** (d) Relative time-to-peak (rTTP) map derived from perfusion-weighted imaging (PWI) images. The area of occlusion is seen only slightly elevated with a few voxels above the 20-s delay. Digital Subtraction Angiography (DSA) is shown in early (e) and delayed phase (f), which highlights the impact of the collaterals in the left hemisphere. The arrows indicate the location of the clot.

**Fig. 5** Quantification of fast and slow evolvers using diffusion-weighted imaging (DWI) apparent diffusion coefficient (ADC) maps and perfusion-weighted imaging (PWI) time-to-peak (TTP) maps. (a) Ischemic core evolution as derived by ADC-thresholded maps
and dichotomized to fast (orange) versus slow (blue) evolvers are shown. Based on lesion growth rate, animals were divided into fast (>50% total volume within 2 h) and slow (<50% total volume within 2 h) evolver groups. The plot shows the progression of slow and fast evolvers with the greatest difference occurring at about 2 h. Individual growth curves are shown for each animal in lighter shade. Error bars indicate SEM. (b) Correlation plot is shown indicating the agreement of infarct volumes between the final DWI image and the TTP map generated from the initial PWI images ($R^2 = 0.99$). The dotted lines indicate 95% confidence level. (c) Histogram binning from rTTP maps for fast and slow evolvers are shown. The voxels in the “slight delay” bin (4–8 s) of fast evolvers are significantly fewer compared to that of slow evolvers (**$p < 0.0001$). The dashed line near the left axis of the plot shows a cutoff at ~34%, which distinguishes between the fast and slow evolvers in the “slight delay” bin. The voxels in the “extended delay” bin (19+ s) of fast evolvers are significantly more compared to that of slow evolvers (*$p = 0.0013$). The dashed line near the right axis of the plot shows a cutoff at ~42%, which distinguishes between the fast and slow evolvers in the “extended delay” bin. The two middle bins of “moderate delay” (9–13 s) and “long delay” (14–18 s) did not show any significant differences between the slow and fast evolvers. Error bars indicate SEM.
We thank the reviewers for their comments and have included the responses to their queries below in red. In addition, we rephrased terms like stroke volume and stroke rate with infarct volume and infarct evolution rate throughout the manuscript. We also updated the funding/conflict of interest information.

Reviewer #1: In this study, the authors used DWI and PWI technology to divide ELVO dogs into fast evolvers and slow evolvers increasing the scientific rigor, which enabling more robust study design for follow-up interventional research.

Some points need to be pay attention to:

1. In the figure 3 and figure 4, why were the infarcts located in the different side? Generally we select the same side of infarts in animal model for the rigor of the study.

   Thank you for pointing this out. The choice of left versus right side for infarct modeling was based on the angiographic features of the cervical ICA. We selected the larger ICA with sufficient non-tortuous origin for placement of the catheter. We revised our methods section to include this information as follows:
   "The side of clot injection was selected on the basis of the larger diameter and length of non-tortuous cervical ICA for delivery of this relatively large catheter."

2. The authors should add a scale to figure 3C and figure 4C.

   A scale has been added to the figures and the figure caption has been edited to include the scale information. We apologize for the oversight.

3. How is it defined in terms of distinguishing between slow and fast evolvers were ~34% for the "slight delay" and ~42% for the "extended delay" bins

   The definition was based on the spread of the data points in the fast and slow evolver categories in Fig 5C for the "slight delay" and "extended delay" bins. ~34% and ~42% were approximately the halfway values between the boundary data points of the fast and slow evolvers. Hence, the rTTPIE (rTTP infarct evolution) index was used as the more quantitative measure to designate fast and slow evolvers. The Results section has been revised to now read as follows: "the respective cutoff marks to distinguish between slow and fast evolvers were ~34% for the “slight delay” and ~42% for the “extended delay” bins based on the minimal distance (halfway point) between the boundary points of each category.

4. The authors should give a calculation formula or specific method of rTTP and compare the affected area with the mirror image area.

   Thank you for the suggestion. As requested, we added this information to the revised methods section as follows: "To obtain a relative TTP (rTTP) map, TTP maps were normalized by subtracting the mean TTP derived from a contralateral region of interest (ROI; positioned in the periventricular area of the unaffected hemisphere in the mid-brain region) from the absolute TTP in each voxel using the following equation:
\[ r_{TTP_{map}} = TTP_{map} - \frac{1}{n} \sum_{i=1}^{n} (ROI_{contralateral})_i \]

where \( n \) is the total number of voxels in the contralateral ROI. 

5. Why is there such a big difference in the selection of the age of the animals in the group, and the weight of the older animals in the aging state is very low, which may be an unstable factor in this study. The variability in animal age and weight relates to the different strains of dogs used in this study. However, size and age of the animal were not statistically different between the slow and fast evolver groups (see Table 2) assuaging concerns that these factors biased our results. Nevertheless, your point is well taken and we revised our limitation to briefly address this issue as follows: “There was a notable variation in animal age and weight related to the different strains of dogs included into our retrospective analysis. However, we did not find any significant age and weight difference between fast and slow evolvers, assuaging concerns of major confounding and may in fact indicate broad applicability of our results.”

Reviewer #2: An excellent experimental model to hopefully get closer to answering the topical question of reasons for the gap between recanalized patients and those with good clinical outcome. The canine model is a close replica of the human circle of willis and hence can be a good experimental model to assess novel therapeutic targets in the future. Well designed study with MCA occlusion model. Good demonstration of the hypothesis of selecting out animals with slow progression of infarct volume vs fast progression. The utilization of MRI every 30 minutes to closely follow infarct evolution is a robust mechanism to study the phenomenon in question. This model can be applied to further prospective evaluation to validate further and then can be utilized to study interventions with novel therapeutic targets.

We appreciate the enthusiasm of the reviewer and agree that this model will be applicable to prospective studies to validate therapeutic interventions.
Figure 1

(a) MCA

(b) MCA occlusion

(c) MCA occlusion
Figure 2

- DWI
  - ADC map
  - Define total infarct volume
- PWI
  - TTP map
  - Normalize w.r.t. contralateral side
  - Register TTP map with infarct volume voxels
  - Bin infarct voxels in rTTP map

- Slight delay: 4–8 s
- Moderate delay: 9–13 s
- Long delay: 14–18 s
- Extended delay: 19+ s

- Calculate true infarct evolution rate from serial images
- Predict infarct evolution rate
### Table 1. Demographic data and infarct evolution rate of subjects

<table>
<thead>
<tr>
<th>Animal ID</th>
<th>Age (months)</th>
<th>Sex</th>
<th>Weight (kg)</th>
<th>Infarct evolution rate</th>
<th>rTTPIE(^a) index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal 1</td>
<td>7.0</td>
<td>Female</td>
<td>21.2</td>
<td>Slow</td>
<td>0.003</td>
</tr>
<tr>
<td>Animal 2</td>
<td>6.5</td>
<td>Male</td>
<td>14.7</td>
<td>Fast</td>
<td>2.615</td>
</tr>
<tr>
<td>Animal 3</td>
<td>17.0</td>
<td>Male</td>
<td>8.9</td>
<td>Slow</td>
<td>0.125</td>
</tr>
<tr>
<td>Animal 4</td>
<td>8.0</td>
<td>Female</td>
<td>21.3</td>
<td>Fast</td>
<td>5.043</td>
</tr>
<tr>
<td>Animal 5</td>
<td>16.5</td>
<td>Male</td>
<td>9.3</td>
<td>Fast</td>
<td>2.676</td>
</tr>
<tr>
<td>Animal 6</td>
<td>6.75</td>
<td>Female</td>
<td>22.8</td>
<td>Slow</td>
<td>0.074</td>
</tr>
<tr>
<td>Animal 7</td>
<td>15.5</td>
<td>Male</td>
<td>13.0</td>
<td>Slow</td>
<td>0.882</td>
</tr>
<tr>
<td>Animal 8</td>
<td>16.0</td>
<td>Male</td>
<td>12.5</td>
<td>Slow</td>
<td>0.099</td>
</tr>
<tr>
<td>Animal 9</td>
<td>10.5</td>
<td>Female</td>
<td>23.9</td>
<td>Fast</td>
<td>2.634</td>
</tr>
<tr>
<td>Animal 10</td>
<td>10.0</td>
<td>Female</td>
<td>23.4</td>
<td>Fast</td>
<td>1.721</td>
</tr>
<tr>
<td>Animal 11</td>
<td>26.0</td>
<td>Male</td>
<td>8.2</td>
<td>Slow</td>
<td>0.286</td>
</tr>
<tr>
<td>Animal 12</td>
<td>36.0</td>
<td>Female</td>
<td>10.1</td>
<td>Fast</td>
<td>1.908</td>
</tr>
<tr>
<td>Animal 13</td>
<td>30.5</td>
<td>Female</td>
<td>9.8</td>
<td>Slow</td>
<td>0.767</td>
</tr>
<tr>
<td>Animal 14</td>
<td>32.5</td>
<td>Female</td>
<td>9.4</td>
<td>Fast</td>
<td>3.896</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>17.1 ± 10.2</td>
<td>14.9 ± 6.2</td>
<td>1.62 ± 1.59</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) rTTPIE – relative time-to-peak infarct evolution
Table 2. Statistical summary of fast and slow evolvers

<table>
<thead>
<tr>
<th></th>
<th>Fast Evolvers</th>
<th>Slow Evolvers</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex (% female)</strong></td>
<td>71</td>
<td>43</td>
<td>0.32</td>
</tr>
<tr>
<td><strong>Age (months)</strong></td>
<td>17.1 ± 12.1</td>
<td>17.0 ± 8.9</td>
<td>0.98</td>
</tr>
<tr>
<td><strong>Weight (kg)</strong></td>
<td>16.0 ± 6.7</td>
<td>13.8 ± 5.9</td>
<td>0.52</td>
</tr>
<tr>
<td><strong>rTTPIE&lt;sup&gt;a&lt;/sup&gt; index</strong></td>
<td>2.93 ± 1.17</td>
<td>0.32 ± 0.36</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

<sup>a</sup> rTTPIE – relative time-to-peak infarct evolution