Original Contribution

Effects of Microvascular Permeability Changes on Contrast-Enhanced T1 and Pharmacokinetic MR Imagings After Ischemia

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Background and Purpose—Brain enhancement on contrast-enhanced T1-weighted imaging (CET1-WI) after ischemic stroke is generally accepted as an indicator of the blood–brain barrier disruption. However, this phenomenon usually starts to become visible at the subacute phase. The purpose of this study was to evaluate the time-course profiles of $K_{\text{trans}}$, cerebral blood volume ($v_p$), and CET1-WI with early detection of blood–brain barrier changes on $K_{\text{trans}}$ maps and their role for prediction of subsequent hemorrhagic transformation in acute middle cerebral arterial infarct.

Methods—Twenty-six patients with acute middle cerebral arterial stroke and early spontaneous reperfusion, whose MR images were obtained at predetermined stroke stages, were included. T2*-based MR perfusion-weighted images were acquired using the first-pass pharmacokinetic model to derive $K_{\text{trans}}$ and $v_p$. Parenchymal enhancement observed on maps of $K_{\text{trans}}$, $v_p$, and CET1-WI at each stage was compared. Association among these measurements and hemorrhagic transformation was analyzed.

Results—$K_{\text{trans}}$ map showed significantly higher parenchymal enhancement in ischemic parenchyma as compared with that of $v_p$ and CET1-WI at early stroke stages ($P<0.05$). The increased $K_{\text{trans}}$ at acute stage was not associated with parenchymal enhancement in CET1-WI at the same stage. Parenchymal enhancement in CET1-WI started to occur at the late subacute stage and tended to be luxury reperfusion–dependent. Patients with hemorrhagic transformation showed higher mean $K_{\text{trans}}$ values as compared with patients without hemorrhagic transformation ($P=0.02$).

Conclusions—Postischemic brain enhancement on routine CET1-WI seems to be closely related to the luxury reperfusion at the late subacute stage and is not dependent on microvascular permeability changes at the acute stage. (Stroke. 2013;44:00-00.)

Key Words: blood-brain barrier ■ $K_{\text{trans}}$ ■ parenchymal enhancement

Reperfusion is known to be essential for functional outcome in acute ischemic stroke. Nonetheless, reperfusion itself also may impose risks of further injury to tissue sustaining ischemia. One of the risks after ischemic reperfusion is the permeability change of blood–brain barrier (BBB), which begins early after ischemic insult and may play an important role in secondary tissue damage and in the risk of hemorrhagic transformation (HT). Therefore, sequential quantifications of BBB changes at earlier stages by means of a noninvasive imaging method potentially can help in elucidating the relationship between the reperfusion and the permeability changes. This may further help in imaging interpretation and in understanding the effects of reperfusion therapy as far as the outcome is concerned.

Contrast-enhanced T1-weighted imaging (CET1-WI) has been routinely used in cerebral stroke study. The presence of parenchymal enhancement (PE) on CET1-WI is generally accepted as an indicator of contrast medium leakage across the disrupted BBB. Previous studies have demonstrated an association between the risk of HT after recanalization therapy and the increased BBB permeability shown as PE on CET1-WI during the acute stage. However, PE in CET1-WI usually does not appear until several days after the ictus. The phenomenon has been explained by BBB breakdown and luxury reperfusion at the subacute stage. Because the BBB disruption is thought to be present as early as 6 hours after cerebral infarction, it is possible that PE in CET1-WI after the subacute stage could result from an insufficient accumulation of contrast agent molecules during acute stroke. An independent measure of the BBB permeability is hence highly desirable, especially in early stage evaluation of stroke.
The endothelial transfer constant, $K_{\text{trans}}$, is an indirect measure of microvascular permeability that has been studied in cerebral diseases.\(^7\) The application of $K_{\text{trans}}$ derived from the pharmacokinetic model to acute stroke studies has not been fully investigated.\(^8\) In this study, we conducted a longitudinal follow-up study of $K_{\text{trans}}$ on the basis of first-pass T2*-weighted (fpT2*-+) perfusion imaging and CET1-WI in patients with acute ischemic stroke and early reperfusion. Because early BBB derangements have been reported linking to reperfusion and a more severe BBB opening would lead to HT,\(^9\) it would be interesting to know if the changes of $K_{\text{trans}}$ are related to the PE on CET1-WI and to the subsequent HT in acute stroke. Therefore, the purposes of this study are to evaluate longitudinal changes of $K_{\text{trans}}$, cerebral blood volume, and CET1-WI in acute middle cerebral arterial infarct, and to correlate $K_{\text{trans}}$ changes of the acute infarcts with subsequent HT.

Materials and Methods
For a detailed description of methods and statistical analysis, please see the online-only Data Supplement.

We included 26 patients who had acute stroke undergoing ≥3 MR follow-up perfusion-weighted imaging and CET1-WIs at acute, subacute, and chronic stages. There were 101 MR examinations collected from the 26 patients; 7 were obtained at the hyperacute stage (<6 hours after symptom onset); 15 were obtained at the acute stage (6–48 hours after symptom onset); 19 at the early subacute stage (3–4 days after symptom onset); 19 at the late subacute stage (7–9 days after symptom onset); 18 at the early chronic stage (10–15 days after symptom onset); and 23 at the late chronic stage (30–31 days after symptom onset). $K_{\text{trans}}$ maps were derived according to the method described in detail by Johnson et al.\(^10\) It allows simultaneous mapping of the $K_{\text{trans}}$ and the plasma volume fraction ($v_p$) of the whole brain tissues.\(^7,11\)

We placed the region of interest in the lesion area with maximal abnormality as determined from the $K_{\text{trans}}$ maps at the first examination of the patient. The corresponding $v_p$ also was measured in the same location. A homologous area in the normal-appearing contralateral hemisphere with similar size of region of interest was drawn as the referenced standard. All the regions of interest were applied to the subsequent follow-up images at the corresponding locations for each patient.

PE was defined as the presence of a hypointense area on the $K_{\text{trans}}$ map, $v_p$, or contrast enhancement on CET1-WI within the region of hyperintensity shown on diffusion-weighted images.\(^12\) The presence of HT was evaluated by examining the region of infarct for hyperattenuation indicative of blood products on the noncontrast computed tomography or low signal intensity indicative of magnetic susceptibility from deoxyhemoglobin or methemoglobin on the T2*-weighted or diffusion-weighted image $b_0$ images.\(^3\)

The degree of enhancement on CET1-WI was expressed as $rT1$, a ratio of mean signal intensity of a region of interest on the infarction to that of the contralateral homologous normal brain area.

Statistical Analysis
Pairwise comparisons of $K_{\text{trans}}$ and $v_p$ measurements between lesion and normal-appearing contralateral areas from the perfusion imaging data were performed using Wilcoxon rank sum test. The correlation between parametric variables of $K_{\text{trans}}$ and $v_p$ was assessed using the Pearson correlation coefficient at 3 subdivided periods, including periods of hyperacute to acute stages, early subacute to late chronic stages, and hyperacute to late chronic stages. To evaluate the sensitivity of PE of $K_{\text{trans}}$ maps, Fisher exact test was used to compare the incidence of PE for $K_{\text{trans}}$ versus $v_p$ and $K_{\text{trans}}$ versus CET1-WI at each stage. Patients were then dichotomized according to hemorrhage status at follow-up. The maxima of enhanced $K_{\text{trans}}$ and $v_p$ measurements were compared between the HT and non-HT patient groups using Wilcoxon rank sum test. The occurrence rates of PE on $K_{\text{trans}}$ and $v_p$ were compared between these 2 groups using the Fisher exact test. A statistically significant difference was defined as $P<$0.05.

Results

Time Course of $K_{\text{trans}}$, $v_p$, and $rT1$ measurements
Figure 1 showed the time courses of $K_{\text{trans}}$, $v_p$, and $rT1$. Pairwise comparison demonstrated significantly increased $K_{\text{trans}}$ values in lesion areas as opposed to the normal-appearing contralateral white matter areas at all stages ($P<$0.05; Table I in the online-only Data Supplement). The corresponding $v_p$ values also increased significantly in lesion areas at early subacute, late subacute, and early chronic stages. In general, $K_{\text{trans}}$ values showed higher conspicuity between lesion and normal tissue as compared with those of $v_p$ values. CET1-WI showed no significant enhancement as compared with the contralateral hemisphere at acute and early subacute stages. The enhancement began to increase gradually at late subacute stage and reached its peak at the early chronic stage.

Linear Correlation Analyses Between $K_{\text{trans}}$, $v_p$, and CET1-WI
A significant correlation between $K_{\text{trans}}$ and $v_p$ measurements was found at the subset of regression analysis from period of early subacute to late chronic stage ($R=0.31; P=0.02$), whereas the regression analyses that covered acute period (from hyperacute to acute) or the entire period (from hyperacute to late chronic) showed no significant correlations between $K_{\text{trans}}$ and $v_p$ measurements ($R=0.15$ and $P=0.64$ and $R=0.22$ and $P=0.07$, respectively).

PE Among $K_{\text{trans}}$, $v_p$, and CET1-WI
Figure 2 illustrates an example of strong PE of the acute ischemic lesion on $K_{\text{trans}}$ map in 1 patient who had right middle cerebral arterial infarction and subsequent HT. The Table shows the comparison results of PE among $K_{\text{trans}}$, $v_p$, and CET1-WI at different stages. $K_{\text{trans}}$ showed a significantly higher occurrence rate of PE than that of $v_p$ at hyperacute ($P=0.01$), early subacute ($P=0.01$), and late subacute stages ($P=0.02$), whereas the rate of occurrence was not significantly different at acute ($P=0.132$), early chronic ($P=0.5$), and late chronic ($P=0.5$) stages. As for the comparisons between $K_{\text{trans}}$ and CET1-WI, $K_{\text{trans}}$ revealed significantly higher occurrence rate of PE when compared with that of CET1-WI at hyperacute ($P=0.01$), acute ($P<0.001$), and early subacute stages ($P<0.001$). In all patients, PE was not observed in any of CET1-WI within 7 days after the onset of ischemic stroke. Both $K_{\text{trans}}$ and CET1-WI revealed PE at late subacute stage, but the enhancement did not show significant difference ($P=0.37$). We found that the abnormal PE areas shown on $K_{\text{trans}}$ maps at acute stage are consistent with the final infarct areas with BBB disruption seen on CET1-WI at chronic stage (Figure II in the online-only Data Supplement).

Comparisons Between HT and Non-HT Patient Groups
A significant group difference in occurrence rate of $K_{\text{trans}}$ PE was found between HT and non-HT patients (100% versus 56.25%; $P=0.02$; Table II in the online-only Data Supplement).
The mean $K_{\text{trans}}$ value for ischemic regions in patients with HT was significantly higher than that of patients without HT ($K_{\text{trans}}$ values of HT versus $K_{\text{trans}}$ values of non-HT group: 0.0071±0.00075 s$^{-1}$ [mean±SD] versus 0.0054±0.00215 s$^{-1}$; $P=0.03$). This trend was not observed in $v_p$ PE between HT and non-HT ($v_p$ PE of HT versus non-HT; $P=0.30$; Table III in the online-only Data Supplement) or in $v_p$ values between HT and non-HT ($v_p$ values of HT versus $v_p$ values of non-HT group: 0.024±0.010 [mean±SD] versus 0.025±0.007; $P=0.48$).

**Discussion**

In this study, we investigated longitudinal evolution of BBB derangements up to 1 month in patients with acute ischemic infarcts and spontaneous reperfusion using fpT2*-based $K_{\text{trans}}$ map, along with $v_p$ and CET1-weighted images. Our results showed high sensitivity of $K_{\text{trans}}$ map in detecting abnormal PE at acute stages of infarct as compared with $v_p$ and CET1-weighted images. These abnormally enhanced areas shown on $K_{\text{trans}}$ maps at acute stages were consistent with the final infarct areas with BBB disruption seen on CET1-WI at chronic stages. Patients with HT had higher occurrence rate of $K_{\text{trans}}$ PE and mean $K_{\text{trans}}$ values as compared with patients without HT. We observed no significant differences in $v_p$ measurements between the HT and the non-HT patient groups.

Theoretically, ischemic tissue can be salvaged by rapid restoration of cerebral flow, the reperfusion. However, controversy remains regarding whether reperfusion is totally beneficial to ischemic brain tissue.$^{13,14}$ Stroke with reperfusion phenomenon could cause endothelial injury by inducing the secretion of strong vasodilatory substances.$^{14}$ Our results

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**Table. Occurrence Rate of PE on $K_{\text{trans}}, v_p$, and CET1-WI at Various Stroke Stages**

<table>
<thead>
<tr>
<th>Stage</th>
<th>$K_{\text{trans}}$</th>
<th>$v_p$</th>
<th>CET1-WI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperacute</td>
<td>5/7 (71)</td>
<td>0/7* (0)</td>
<td>0/7† (0)</td>
</tr>
<tr>
<td>Acute</td>
<td>11/15 (73)</td>
<td>7/15 (47)</td>
<td>0/15† (0)</td>
</tr>
<tr>
<td>Early subacute</td>
<td>16/19 (84)</td>
<td>8/19* (42)</td>
<td>0/19† (0)</td>
</tr>
<tr>
<td>Late subacute</td>
<td>12/19 (63)</td>
<td>5/19* (26)</td>
<td>10/19 (53)</td>
</tr>
<tr>
<td>Early chronic</td>
<td>8/18 (44)</td>
<td>7/18 (39)</td>
<td>16/18† (89)</td>
</tr>
<tr>
<td>Late chronic</td>
<td>1/23 (4)</td>
<td>1/23 (4)</td>
<td>23/23 (100)</td>
</tr>
</tbody>
</table>

*Significant difference between $K_{\text{trans}}$ and $v_p$ ($P<0.05$).
†Significant difference between $K_{\text{trans}}$ and CET1-WI ($P<0.05$).

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**Figure 1.** Time-course profiles of (A) $K_{\text{trans}}$ (s$^{-1}$) and (B) $v_p$ for the ischemic and normal-appearing contralateral white matter areas. There is less difference between ischemic lesion areas and normal white matter in $v_p$ as compared with $K_{\text{trans}}$ calculations in these patients. C, Time course of rT1. I: hyperacute stage, II: acute stage, III: early subacute stage, IV: late subacute stage, V: early chronic stage, VI: late chronic stage.
are in concert with the notion that reperfusion-induced BBB derangements could lead to subsequent HT, and the degree of BBB derangements adds to the risk of HT.

PE shown on CET1-WI after ischemic stroke is generally accepted as an indicator of BBB disruption with extravasation of contrast material. However, this phenomenon usually starts to be observed at the late ischemic phase (usually ≥7 days after stroke), suggesting a leakage of small molecular contrast medium from the intravascular spaces to the extracellular spaces, which leads to PE occurring at a time when BBB disruption is severe enough to be observed by CET1-WI.6 This hypothesis was tested by a previous study using continuous intravenous infusion of gadolinium in acute infarction. The study demonstrated that PE could occur in the acute stage if the contrast medium was administered continuously >2 hours rather than a clinically-used rapid bolus administration.6 The absence of PE in CET1-WI during acute ischemic stroke, when using rapid bolus injection, should not be regarded as a result of intact BBB. In fact, the disturbance of the BBB is thought to be present as early as 6 hours after cerebral infarction.6 Although the presence of collateral intravascular enhancement on CET1-WI can be detected immediately after the onset of stroke, it is somewhat lacking of specificity. It remains unclear whether the intravascular enhancement is related to the mechanism of BBB derangement or PE.3,6

As shown in our study, the microvascular permeability changes as translated by the $K_{trans}$ constant may serve as an indicator of significant ischemic injury of the brain. The areas delineated by the $K_{trans}$ parenchymal changes at acute stages were consistent with the CET1-WI PE area seen at chronic stages. However, the ischemic microvascular injury is not necessarily correlated with autoregulatory response, such as vessel dilatation and the resultant increase in regional blood volume, the luxury perfusion. Our results showed no correlation between $K_{trans}$ and $v_p$ measurements in acute ischemic stroke with reperfusion. The discrepancy between $K_{trans}$ and $v_p$ suggests different pathophysiologic processes in response to ischemia in terms of microvascular permeability versus cerebral blood volume, which exert different effects on the pharmacokinetic parameters. $K_{trans}$ provides information...
on the underlying pathophysiological changes of vascular permeability,\textsuperscript{10} which potentially can be used to define the ischemic areas with disturbed BBB according to the abnormal kinetic behaviors, even when there is no significant change of cerebral blood volume shown in $v_p$ maps. This is particularly important for patients with stroke with spontaneous reperfusion phenomenon, in whom cerebral blood volume can be normal but significant ischemic injury has already begun.\textsuperscript{13}

In contrast to the inconsistent trend of $v_p$ and $K_{trans}$ at acute stages, we found a significant correlation between the 2 parameters during the period from the early subacute to the late chronic stage. Both $v_p$ and $K_{trans}$ reached their peak values at the late subacute stage and then attenuated progressively at early and late chronic stages. The increased values of $v_p$ during early and late subacute stages coincided with a previous report that hyperperfusion phenomenon typically occurs in the subacute phase (2–72 hours after the onset of symptoms).\textsuperscript{11} It can be associated with the marked opening of the BBB and, therefore, supports the rationale for the significant correlation between $v_p$ and $K_{trans}$ during these subacute stages. The decrease of $v_p$ and $K_{trans}$ values at the chronic stage is in line with the known limitations of pharmokinetic model that $K_{trans}$ may be affected not only by endothelial permeability surface area product but also by blood flow perfusion efficiency,\textsuperscript{10} whereby the reduction of blood flow perfusion at the chronic stage would give rise to the pseudorepaired BBB phenomenon (with $K_{trans}$ recovery) in brain infarction. It is, therefore, clear that CET1-WI still has an important role in detecting BBB leakage in patients with chronic ischemic stroke. Our study demonstrated that the typical PE seen on standard CET1-WI started to occur during the prominent luxury perfusion (high $v_p$) stage when the posts ischemic microvascular changes have already built-up early during the acute stage.

HT is often a natural evolution of ischemic stroke and generally is thought to arise from ischemic damage to BBB followed by reperfusion.\textsuperscript{2,15} HT may not always be symptomatic. Symptomatic HT, usually because of parenchymal hematoma, is potentially devastating. It would be helpful if the noninvasive imaging method can provide additional information on the risk of HT, thus helping the selection of candidates for thrombolytic therapy.\textsuperscript{2,15} In our patients, the occurrence of HT increased with higher $K_{trans}$ PE and values as compared with those without HT, which is in parallel with previous studies.\textsuperscript{2,4,8,9,13–18} It is to be noted that although there is distinction in the occurrence rates of $K_{trans}$ PE for patients without and with HT, the presence of $K_{trans}$ PE should not be used to predict subsequent HT because of the low prediction specificity (Table II in the online-only Data Supplement). In particular, the limited number of HT cases and the fact that all our patients had early spontaneous but not thrombolytic reperfusion precluded recruitment of more patient cases, including those with late reperfusion and severe BBB breakdowns. Spontaneous reperfusion in some patients with stroke has been reported to be nutritional and to have better clinical outcome.\textsuperscript{19} Our results could have been affected by the specific inclusion and exclusion criteria adopted in this study.\textsuperscript{15}

Our study has several possible limitations. First, $K_{trans}$ may be underestimated if the cerebral blood flow is severely attenuated at acute ischemic stages. However, it is obvious that such an approach, which confirms and extends previous observations on the potential of pharmokinetic calculations to provide a better characterization of stroke-related tissue changes at acute stages, only can be applied to patients with reperfusion phenomenon. Second, some investigators have argued whether the first-pass model, as opposed to slow infusion or delayed imaging, is truly measuring capillary endothelia permeability.\textsuperscript{20} Nonetheless, methods that acquire data with prolonged image scanning during the washout of contrast material often require data collection over minutes and more flow effects from the second pass thus would contaminate the $K_{trans}$ measurements. In tumors, it has been reported that typically 12% to 45% of the contrast media leaks into the extravascular–extracellular space during the first pass,\textsuperscript{21} which includes the arrival of contrast medium and lasts for a few cardiac cycles.\textsuperscript{22} The first-pass pharmokinetic model we used in this study has been previously demonstrated to yield a significant correlation with the steady-state T1-weighted method (ssT1).\textsuperscript{7} Still, evidence from previous measurements prove that permeability-related measurements derived from the perfusion-weighted imaging with first-pass analysis can be diagnostically useful in brain tumors and ischemic stroke.\textsuperscript{2,10,11,17,18,23} Nonetheless, limitation may be that the data acquired during the first pass of the bolus could not fully reflect the changes of $K_{trans}$ measurements that connote the BBB permeability changes. Third, considering the paramagnetic effects of MR contrast agents to cause increase in T1 and T2 relaxation rates, the signal drop in T2* dynamic series will be possibly reduced in regions where T1 effects are significant. That is why the postcontrast signal baseline may be above the precontrast baseline.\textsuperscript{10} In our study, however, we did not observe this severe T1 effect even under the relatively higher flip angle used in our perfusion-weighted imaging pulse sequence.\textsuperscript{10} Finally, it is known that $K_{trans}$ inherently has low resistance to noise, which is a common problem for $K_{trans}$ calculations in most pharmokinetic model analyses.\textsuperscript{24} Therefore, image quality threshold is theoretically critical for the clinical use, especially for the case of pixel-by-pixel mapping in routine practice.

In conclusion, our data showed that the T2*-based first-pass pharmokinetic model seems to be more sensitive than conventional CET1-WI in detecting early microvascular permeability changes at the acute stage of ischemic stroke. Patients with HT showed higher $K_{trans}$ PE occurrence rate and values as compared with those without HT in the present study.

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Disclosures
None.

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SUPPLEMENTAL MATERIAL

Supplemental Methods

Subjects

This was a retrospective study in a cohort of patients who had acute ischemic stroke admitted to Tri-Service General Hospital between August 2000 and April 2004. Data analysis of this study was approved a waiver of informed consent by a local institutional review board. Twenty-eight out of 127 patients who had acute stroke met the following criteria: 1) had acute ischemic infarcts of the unilateral middle cerebral arterial territory with early reperfusion determined by MR angiography at acute stage, 2) at least three MR follow-up perfusion-weighted imaging (PWI) and CET1-WIs were available respectively at acute, subacute, and chronic stages, 3) no thrombolytic therapy was administered. Two subjects who met the above criteria were excluded from the study due to suboptimal image quality; one was due to severe signal loss in region of infarct on PWI measurements and the other had severe unconscious movements during MR examination. Finally, data from 26 patients were enrolled in this study, these including 13 men and 13 women with ages ranging from 34 to 81 years (mean=56.10 years, STD=17.48 years). There were totally 101 MR examinations collected from the 26 patients; seven were obtained at the hyperacute
stage (< 6 hours after symptom onset); 15 at the acute (6-48 hours after symptom onset); 19 at the early subacute (3-4 days after symptom onset); 19 at the late subacute (7-9 days after symptom onset); 18 at the early chronic stage (10-15 days after symptom onset); and 23 at the late chronic stage (30-31 days after symptom onset) (online-only Data Supplemental Table S1).

**Magnetic Resonance Imaging**

All MR imagings were performed at a 1.5T system (Magnetom Vision Plus system; Siemens, Erlangen, Germany). Unenhanced axial T1-weighed images were first obtained with TR/TE = 700/14 ms, matrix = 256 × 256, gap = 1.5 mm, and 20 slices with 5 mm thickness. Diffusion-weighted imaging (DWI) using a single-shot echo-planar imaging sequence was acquired with diffusion-weighted factors b = 0 and 1000 sec/mm² at three orthogonal gradient axes (TR/TE = 4700/120 ms, matrix = 256 × 256, field of view = 230 mm, slice thickness = 5 mm with gap = 1.5 mm at 20 slice locations).

While the $K_{trans}$ calculation is based on the steady-state T1-weighted method (ssT1), the first-pass T2*-weighted (fpT2*) perfusion imaging may gain more ground in clinical environment due to its wide adoption in acute stroke imaging for perfusion.
study $^{1-5}$. $K^{\text{trans}}$ measurement based on the fpT2* method has been shown to have a potential for prediction of hemorrhagic transformation (HT) during the acute phase of stroke $^{1-4}$. In this study, we conducted a longitudinal follow-up study of $K^{\text{trans}}$ based on the fpT2* method. T2* susceptibility-based PWI (TR/TE = 1000/44 ms, matrix = 128 × 128, flip angle = 60° - 90°, 6 slices with slice thickness = 5 mm) with contrast injection were performed at 1-second intervals with 75 dynamic time points. A manual bolus injection of 0.2 mmol per kilogram of body weight gadopentetate dimeglumine (Magnevist; Schering AG, Berlin, Germany) at about 4-5 mL/sec through a 20-gauge venous catheter inserted in the antecubital vein was given, followed immediately by saline flush (total of 20 mL at the same rate). Finally, we obtained axial CET1-WI after the PWI, by using the same parameters as the precontrast T1-weighted images.

**Image analysis**

$K^{\text{trans}}$ maps were derived according to the method described in detail by Johnson et al $^{5}$. The dynamic concentration-time curve for brain tissue following contrast administration, $C_i(t)$, can be described by the equation:

$$C_i(t) = v_p C_p + K^{\text{trans}} \int_0^t C_p(\tau) e^{-K^{\text{trans}}(t-\tau)/v_e} d\tau,$$

where $C_p$ is the tracer concentration in the plasma, and $v_e$ is the volume fraction of the
extravascular-extracellular space. The plasma concentration, which is associated with the arterial input and recirculation, is estimated by employing the vascular contrast medium concentration obtained from the normal-appearing contralateral white matter. This model allows simultaneous mapping of the endothelial transfer constant, $K_{\text{trans}}$, and the plasma volume fraction, $v_p$, of the whole brain tissues. Although measurement of BBB $K_{\text{trans}}$ requires several assumptions which may not be true in complex ischemic brain environment and thus limit the absolute quantification of $K_{\text{trans}}$, this method was shown to yield good correlation with the ssT1 method in brain tumor.

Because of the different contributions of perfusion baseline values from the gray and white matter areas in $K_{\text{trans}}$ measurements, we focused our measurements on the white matter areas in this study. Previous studies of PWI-derived parametric maps have shown that measurement of maximal abnormality provides the highest intra- and inter-observer reproducibility. We therefore placed the ROI in the lesion area with maximal abnormality as visually determined from the $K_{\text{trans}}$ maps at the first examination of the patient. The corresponding $v_p$ was also measured in the same location. The size of the ROI was kept as constant as possible (typically containing 25–30 pixels) to minimize confounding factors in ROI analyses. A homologous
area in the normal-appearing contralateral white matter with similar size of ROI was drawn as the referenced standard to ensure that our calculated values are in reasonable agreement with the literatures 5, 9. All the ROIs were applied to the subsequent follow-up images at the corresponding locations for each patient.

The presence of parenchymal enhancement (PE) and HT in lesion areas was assessed by an experienced neuroradiologist (C.Y.C, with more than 20 years experience in MR brain image interpretation) who was blinded to the clinical data and follow-up images. PE was defined as the presence of a hyperintense area on the Ktrans map or contrast enhancement on CET1-weighted image, in the region of hyperintensity seen on DW images 10. The presence of HT was evaluated by examining the region of infarct for hyperattenuation indicative of blood products on the non-contrast computed tomography and/or low signal intensity indicative of magnetic susceptibility from deoxyhemoglobin or methemoglobin on the axial T2*-weighted or DWI b0 images 11. Although HT can be categorized as hemorrhagic infarction or parenchymal hematoma, these subtypes of HT were not individually analyzed owing to the limited number of hemorrhages in the present study.

The degree of enhancement on CET1-WI was expressed as rT1, a ratio of mean
signal intensity of an ROI on the infarction to that of the contralateral homologous normal brain area. Time course of rT1 was obtained from the follow-up images at different stroke stages using the same-size ROI as for the acute image.

**Statistical Analysis**

Pairwise comparisons of $K^{\text{trans}}$ and $v_p$ measurements between lesion and normal-appearing contralateral white matter areas from the longitudinal perfusion imaging data were carried out by using Wilcoxon rank sum test. The correlation between parametric variables of $K^{\text{trans}}$ and $v_p$ was assessed using the Pearson correlation coefficient at three subdivided periods, including periods of hyperacute to acute stages, early subacute to late chronic stages and hyperacute to late chronic stages. To evaluate the sensitivity of PE of $K^{\text{trans}}$ maps, Fisher’s exact test was used to compare the incidence of PE for $K^{\text{trans}}$ vs. $v_p$, and $K^{\text{trans}}$ vs. CET1-WI at each stage.

Patients were then dichotomized according to hemorrhage status at follow-up, and differences between the HT and non-HT patient groups were assessed. Because the main clinical application of measuring $K^{\text{trans}}$ is to predict HT by assessing BBB integrity, and the reduction of blood flow perfusion efficiency at the chronic stage would give rise to the pseudo-repaired BBB phenomenon in brain infarction, therefore,
the strategy we adopted to evaluate the difference of HT vs. non-HT patient groups in the present study is to extract the maxima of enhanced $K^{\text{trans}}$ measurement for each patient in their longitudinal follow-up study, and do the group comparison by using Wilcoxon rank sum test. Similar comparison between HT and non-HT patient groups was also conducted for $v_p$ measurements. The occurrence rates of PE on $K^{\text{trans}}$ and $v_p$ were further compared between these two groups by using the Fisher exact test.

Binary logistic regression models were used to predict HT outcomes (coded as 1 for HT and 0 for non-HT) using $K^{\text{trans}}$ PE (coded as 1 for PE and 0 for non-PE) and $K^{\text{trans}}$ values (from the ROI measurements). Regression analyses showed that there was no significant effect of $K^{\text{trans}}$ PE occurrence rate to predict HT outcome ($P = 0.99$) but a trend of correlation between $K^{\text{trans}}$ values and subsequent HT ($P = 0.06$). Similar regression analyses were also performed to test the association between HT occurrence and both $v_p$ PE and $v_p$ values. There was no significant effect of $v_p$ in the regression analyses ($P=0.65$ and 0.73 for $v_p$ PE and $v_p$ values, respectively). The sensitivities, specificities, and positive and negative predictive values of $K^{\text{trans}}$ and $v_p$ PE were calculated by using the $2 \times 2$ contingency table (see Supplementary Tables S2 and S3). A statistically significant difference was defined as $P < 0.05$. 
Fisher’s exact test and Wilcoxon rank sum test were employed for the comparisons of difference of gender and the means of the age between the HT and non-HT groups, respectively. We found no statistically significant difference in patient age (P = 0.91) or gender (P = 0.23) between HT (mean age, 55.80 ± 18.06 years; age range, 34-81 years; 4 men, 6 women) and non-HT (mean age, 55.40 ± 17.82 years; age range, 35-81 years; 9 men, 7 women) groups.

Supplemental References


Supplemental Tables

TABLE S1. Measurements of $K^{trans}$ (sec$^{-1}$) and $v_p$ as compared to the contralateral normal white matter (WM) at various stroke stages

<table>
<thead>
<tr>
<th>Stage</th>
<th>$K^{trans}$</th>
<th>$P$</th>
<th>$v_p$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lesion</td>
<td>WM</td>
<td>Lesion</td>
<td>WM</td>
</tr>
<tr>
<td>Hyperacute</td>
<td>Mean</td>
<td>0.0061</td>
<td>0.0025</td>
<td>0.0043*</td>
</tr>
<tr>
<td>n = 7</td>
<td>std</td>
<td>0.0012</td>
<td>0.0004</td>
<td></td>
</tr>
<tr>
<td>Acute</td>
<td>Mean</td>
<td>0.0066</td>
<td>0.0023</td>
<td>0.0043*</td>
</tr>
<tr>
<td>n = 15</td>
<td>std</td>
<td>0.0007</td>
<td>0.0002</td>
<td></td>
</tr>
<tr>
<td>Early subacute</td>
<td>Mean</td>
<td>0.0062</td>
<td>0.0027</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>n = 19</td>
<td>std</td>
<td>0.0013</td>
<td>0.0012</td>
<td></td>
</tr>
<tr>
<td>Late subacute</td>
<td>Mean</td>
<td>0.0068</td>
<td>0.0028</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>n = 19</td>
<td>std</td>
<td>0.0010</td>
<td>0.0007</td>
<td></td>
</tr>
<tr>
<td>Early chronic</td>
<td>Mean</td>
<td>0.0053</td>
<td>0.0026</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>n = 18</td>
<td>std</td>
<td>0.0013</td>
<td>0.0006</td>
<td></td>
</tr>
<tr>
<td>Late chronic</td>
<td>Mean</td>
<td>0.0042</td>
<td>0.0025</td>
<td>0.006*</td>
</tr>
<tr>
<td>n = 23</td>
<td>std</td>
<td>0.0014</td>
<td>0.0010</td>
<td></td>
</tr>
</tbody>
</table>

Note. - $P$: comparison between lesion area and contralateral normal white matter (WM).
- *$P$ < 0.05
TABLE S2 Parenchymal enhancement in $K_{trans}$ maps ($K_{trans}$ PE) v.s. hemorrhagic transformation (HT)  In all HT cases, parenchymal enhancement in $K_{trans}$ maps was identified at acute stroke stages in our study but not all cases with $K_{trans}$ PE developed HT at later stages.

<table>
<thead>
<tr>
<th></th>
<th>HT</th>
<th>Non-HT</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_{trans}$ PE</td>
<td>10</td>
<td>9</td>
<td>53%</td>
<td></td>
</tr>
<tr>
<td>No- $K_{trans}$ PE</td>
<td>0</td>
<td>7</td>
<td>100%</td>
<td>44%</td>
</tr>
</tbody>
</table>

Note. - PPV, positive predictive value; NPV, negative predictive value.
TABLE S3. Parenchymal enhancement in $v_p$ maps ($v_p$ PE) v.s. hemorrhagic transformation (HT)

<table>
<thead>
<tr>
<th></th>
<th>HT</th>
<th>Non-HT</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>$v_p$ PE</td>
<td>4</td>
<td>5</td>
<td>PPV = 44%</td>
</tr>
<tr>
<td>No-$v_p$ PE</td>
<td>6</td>
<td>11</td>
<td>NPV = 65%</td>
</tr>
</tbody>
</table>

Sensitivity 40%  Specificity 69%

Note. - PPV, positive predictive value; NPV, negative predictive value
Supplemental Figure Legends

Figure S1

MR images in a 54-year-old male with left middle-cerebral artery infarction at 3 (first column), 14 (second column) and 30 days (third column) after ischemic symptom onset. From top to bottom: $K_{\text{trans}}$ maps, $v_p$ maps, contrast-enhanced T1-weighted, diffusion-weighted and T2 FLAIR images. No hemorrhagic transformation was found in the follow-up imaging for this patient.

Figure S2

Representative cases with parenchymal enhanced areas shown on $K_{\text{trans}}$ maps demonstrated that those abnormal enhanced areas at acute stage are consistent with the final infarct areas with BBB disruption seen on CET1-WI at chronic stage.
Figure S1
MR images in a 54-year-old male with left middle-cerebral artery infarction at 3 (first column), 14 (second column) and 30 days (third column) after ischemic symptom onset. From top to bottom: $K_{\text{trans}}$ maps, $v_p$ maps, contrast-enhanced T1-weighted, diffusion-weighted and T2 FLAIR images. No hemorrhagic transformation was found in the follow-up imaging for this patient.
Figure S2
Figure S2, continued
Figure S2, continued