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A review of current imaging methods used in stroke research

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Abstract

Stroke is a serious healthcare problem with high mortality and long-term disability. However, to date, our ability to prevent and cure stroke remains limited. One important goal in stroke research is to identify the extent and location of lesion for treatment. In addition, accurately differentiating salvageable tissue from infarct and evaluating therapeutic efficacies are indispensable. These objectives could potentially be met with the assistance of modern neuroimaging techniques. This paper reviews current imaging methods commonly used in ischemic stroke research. These methods include positron emission tomography, computed tomography, T1 MRI, T2 MRI, diffusion and perfusion MRI, diffusion tensor imaging, blood–brain barrier permeability MRI, pH-weighted MRI, and functional MRI.

Keywords

MRI; PET; Diffusion; Perfusion; Cerebral blood flow; Cerebral ischemia; Blood–brain barrier; Functional MRI

Background

Stroke is the fourth leading cause of mortality and the leading cause of long-term disability in the United States. There are ~795 000 new strokes occurring annually, ~87% of which are ischemic strokes.¹ Ischemic stroke occurs when blood flow to an area of the brain is diminished, leading to death of the compromised tissue. Previously, cerebral infarction was thought to be sudden and irreversible, with episodes of transient ischemic attacks (TIA), where stroke-like symptoms resolve within 24 hours, being attributed to other etiologies such as hypertensive crisis or arterial vasospasm. In the 1950s, the embolic theory for TIA arose, generating the notion that cerebral ischemia may be reversible.²

In the 1970s, Lindsay Symon used a baboon model with middle cerebral artery clipping to demonstrate a mismatch between electrical failure and membrane failure during ischemia.³ His group delineated three basic tissue zones during ischemic episodes: a core zone, the penumbra, and oligemic normal tissue. The core zone suffered electrical and membrane failure and thus was irreversibly injured. The penumbra represented tissue with electrical failure but preserved membrane.⁴ Since then, numerous definitions of the penumbra have arisen, all of which highlight the reversibility of the penumbra's ischemic injury with reperfusion but progression toward infarction without reperfusion.^{5–9}

While salvaging the penumbra has become the focus of stroke treatment, the time window of its existence is highly variable among humans, ranging from as little as 4 hours^{10,11} to as large as 48 hours.^{12–14} This variability stems from numerous factors including the patient's age, gender, comorbidities, collateral flow status, inherent susceptibility of tissue to ischemia (gray versus white matter), location of vessel occlusion, and many others.^{15,16}

As a result of the heterogeneity of patient etiologies that make it difficult to understand the underlying pathophysiology, animal models have been used extensively to gain understanding of stroke. These models have enhanced our knowledge of the microscopic pathways occurring during ischemia, validated different techniques for identifying the penumbra, especially via operationally defined non-invasive methods, and served as arenas for testing neuroprotective agents in experimental stroke.¹⁷

Smaller animal models, such as gerbils and rats, allow monitoring of physiological variables and samples of sufficient size for statistical analysis at low cost;¹⁸ larger ones, such as cats, primates, and pigs, more closely resemble the vascular anatomy and the gray to white matter ratio of humans.¹⁷ While gerbils and small rodents have lissencephalic brains with well-demarcated vascular regions, cats, primates, and pigs have gyrencephalic brains with more heterogeneity in blood flow due to numerous collaterals.¹⁷

Two major forms of ischemia have been studied in animal models: global and focal. Global ischemia mimics injury occurring during cardiopulmonary arrest, while focal ischemia mimics injury occurring during ischemic stroke.¹⁹ Focal ischemia can be generated via two techniques, a mechanically occlusive model or a thromboembolic model. While the latter more closely resembles the slow reperfusion occurring during clot dissolution in resolving strokes, the former is much simpler, more reproducible, and suffers less variability.¹⁹

A major fundamental difference between humans and animal models is the presence of different penumbral and infarct flow thresholds, leading to varying rates and dynamics in infarct evolution. In small rodents, the penumbra at 2 hours post-stroke represents an area of tissue with cerebral blood flow (CBF) between 20–40% of control.²⁰ In humans, at 6 hours, penumbral tissue has CBF between 10–30% of control.²¹ Essentially, infarct evolution occurs over 2–4 hours in rodents, a longer (though unknown exactly how much longer) time period in cats, at least 24 hours in non-human primates (NHP)²² and up to 48 hours in humans (though this time period is highly variable).¹³

Neuroprotective compounds must be rigorously studied in animal models and have proven beneficial effects before moving to clinical trials, with the end-goal of FDA-approval.²³

While numerous compounds have been efficacious in animals, only recombinant tissue plasminogen activator (rtPA) has shown success in humans. Various hypotheses have been expounded to explain this inability to translate treatments to humans: (1) in animal models, the stroke and therapeutic compound delivery occur at precisely defined time points, whereas this is not possible in the clinical setting; (2) side effects occurring in humans may not occur in animals because those animals that die during the study are often excluded from analysis; (3) animals are sacrificed at 24 hours to perform histology analysis whereas in humans, follow-up studies occur months after stroke;²³ (4) previously, animals used did not have the same comorbid conditions that humans suffering from stroke have, such as hypertension or diabetes – this problem has been addressed with the use of spontaneously hypertensive rats (SHR) and stroke-prone SHR, though more extensive research in these models has yet to be done;¹⁹ (5) previously, therapy would be administered at or soon after ischemic insult in animal models; as this is very difficult in humans, more recent studies have focused on delivering treatment at more reasonable time points;^{24,25} (6) most neuroprotective studies have been performed on mechanical occlusion models rather than thromboembolic, even though the latter more closely resembles human stroke.¹⁹

Nevertheless, animal models have proven utility in the study of stroke. In terms of broadening stroke therapy options, though much genetic homology may exist between humans and other species, the expression of these genes may differ, and thus, studies that are successful across various species are more likely to be successful in humans.²⁶

As noted earlier, rtPA is the only agent with proven beneficial effects across various species as well as humans, and thus, the only FDA approved ischemic stroke treatment is intravenous administration of rtPA within 4.5 hours of stroke onset.²⁷ Despite the fact that a reasonable stroke treatment option exists, many patients are excluded from rtPA administration because of this 4.5-hour time constraint. Only 38.3% of stroke patients arrive to an emergency room within 2 hours with sufficient time for the requisite diagnostic tests prior to rtPA treatment, and of these patients, only 37.5% eventually receive rtPA treatment.²⁸ In the end, only 1.8–2.1% of all ischemic stroke patients receive treatment with rtPA.²⁹ Various imaging modalities have shown the existence of injured but salvageable tissue, known as the ischemic penumbra, well beyond the 4.5-hour time window, up to 48 hours.^{13,14,30} Thus, ideal patient selection for thrombolysis should involve imaging to determine each individual's likelihood of recovery based on the penumbral size, a 'tissue signature', rather than relying on treatment within 4.5 hours of stroke onset.¹⁵ Toward this goal, many techniques for imaging the penumbra have been developed, such as positron emission tomography (PET), computed tomography (CT), and magnetic resonance imaging (MRI). A summary of the strengths and weaknesses pertaining to each major imaging modalities are provided in Table 1.

PET and CT

Of the various techniques for stroke imaging, the first to demonstrate the existence of the penumbra in humans was PET in 1980, when it identified the penumbra as tissue with reduced CBF but normal CMRO₂, secondary to increased OEF.³¹ Given that it was first to image the penumbra and because of the biological plausibility of the parameters it

quantifies, PET is considered the ‘gold standard’ in evaluating early stroke pathophysiology.³² The other major strength of PET is that it offers semi-quantitative or quantitative hemodynamic data.³³ However, its major drawbacks include radiation, high cost, and limited availability, precluding PET application in clinical settings.² Therefore, other more universal imaging techniques, such as MRI, identifying the diffusion-weighted imaging/perfusion-weighted imaging (DWI/PWI) mismatch, are verifying their thresholds against the ‘gold standard’ PET.³⁴

There are numerous PET techniques to image the penumbra, including multi-tracer PET with ¹⁵O, PET with flumazenil (FMZ) and PET with fluoromisonidazole (FMISO). Each method has its respective strengths and weaknesses. Multi-tracer PET obtains quantitative maps of CBF, cerebral blood volume (CBV), CMRO₂, CMR_{glu}, identifying the penumbra as tissue with ‘misery perfusion’ (reduced CBF but maintained CMRO₂ secondary to increased OEF).³⁵ Studies on cats showed a 60-minute reperfusion group had two outcomes depending on OEF level: if it increased and stayed high throughout the first 24 hours after stroke, the cat survived; if it increased but then decreased at some point during this time, the cat died.³⁵ The problem with multi-tracer PET was the significant variability in penumbral CBF values (from 7 ml/100 g minute to 22 ml/100 g minute in one analysis and 4.8 to 14.1 in another). This portrays how OEF is a poor predictor of tissue viability, and data from a single snapshot in time can be confusing when the pattern over time is unknown. This created the need for a marker able to identify tissue irreversibly injured regardless of time since stroke onset or CBF variations over time.³⁵ Thus, PET with FMZ emerged.³⁶

Radiolabelled ¹¹C-FMZ was used to bind to central benzodiazepine receptors, which are subunits of the GABA receptor complex.³⁷ As inhibitory GABA-ergic synapses are very sensitive to ischemia,³⁸ Sette *et al.* 1993 showed significantly decreased FMZ binding in ischemic cortical tissue. Its binding differentiated intact from infarcted cortex, regardless of reperfusion,³⁹ and its distribution allowed perfusion to be imaged.⁴⁰ The strengths of PET with FMZ are that arterial blood sampling is not required, imaging is independent of patient cooperation, has improved image quality, and can be used with SPECT.³⁵ However, while GABA receptors are abundant in the cortex, they are much less abundant in the basal ganglia and cerebellum and absent in white matter, and consequently, only the cortex can be imaged well. Moreover, imaging first requires the steady-state condition to be reached after injection of FMZ, which takes 30–40 minutes.³⁵

Unlike PET with FMZ, PET with FMISO can reliably image white matter. Yeh *et al.* 1994 first used PET with FMISO, which acutely showed increased uptake around an area of no activity in three of six patients – the penumbra. Chronically, this area showed no uptake, indicating that the tissue either infarcted or was salvaged. These results were confirmed by Read *et al.* 1998. In other words, FMISO identifies the penumbra, labeling hypoxic tissue, as it is reduced in all metabolically active tissue (not the infarcted core), with oxygenated tissue able to re-oxidize the metabolite to its original form while hypoxic tissue is not. Then the metabolite binds to intracellular molecules, labeling the tissue at risk.⁴¹

Hypoxia may be a more reliable indicator of tissue at risk than CBF, since some tissue maintains oxygenation in areas of low CBF by increasing OEF, and since some tissue has a

lower baseline CBF and metabolic rate (such as white matter).⁴¹ Other strengths of FMISO are that it images CBF and cellular metabolism simultaneously, and as tissue binding occurs over time, it provides an approximation of hypoxia over a time interval rather than one snapshot in time. Moreover, it is simpler to perform than other PET methods and can be used in animal studies. Theoretically, one reason for failure of translating neuroprotectants that work in animal models to humans is that animals have a much lower white to gray matter ratio. As FMISO can image hypoxic white matter, this technique would allow testing of white matter neuroprotectants.⁴¹

Weaknesses of FMISO are the time required after injection before the image can be obtained (2 hours),³⁵ and rat studies in Spratt *et al.*'s lab showed binding in irreversibly injured tissue which casts doubt on the utility of FMISO for penumbral identification.⁴¹

Different techniques for using CT for stroke imaging have been developed. While a normal non-contrast CT is typically the first imaging performed during stroke evaluation to exclude hemorrhagic stroke, it has only 31% sensitivity in identifying acutely ischemic tissue and mild correlation with acute NIHSS score.⁴² Another study showed that CT, in normal viewing parameters, has 57% sensitivity and 100% specificity for acute stroke; with soft window and variable settings, this increases to 71% sensitivity with no change in specificity.⁴³ The early changes with infarction include subtle gray matter and/or cortical hypodensity, loss of the insular ribbon, sulcal effacement due to early edema, and the hyperdense MCA sign. Given the poor sensitivity of CT in ischemic stroke, NINDS and ECASS decided normal non-contrast CTs should be used for two reasons: to exclude ICH and to exclude patients with extensive demarcation of ischemic infarctions (> 33% of MCA territory per ECASS, though different, possibly superior definition made by ASPECTS trial [initial results showed higher interobserver reliability with ASPECTS though some other preliminary data contradict these findings]).⁴³

CT angiography (CTA) and CTA source images (CTA-SI) can provide information about collateral circulation and improve contrast so that early ischemic changes can be identified with increased sensitivity.^{44–46} In addition, CTA and MRA have equal accuracy, and CTA-SI and DWI have nearly equal accuracy of infarct volume.⁴⁷

One of the most clinically applicable techniques for imaging the penumbra is CT perfusion (CTP), for which criteria were made to differentiate reversible from irreversible injury within 6 hours.⁴⁸ In CTP, IV iodinated contrast is administered and two 40-second series of images are acquired 5 minutes apart. Each series acquires one image per second at two adjacent 10 mm sections, leading to a total of 80 images covering four adjacent cross-sections.⁴⁹ CTP measures regional CBV and MTT and then calculates regional CBF (rCBF) via the equation $rCBF = rCBV / MTT$. Healthy parenchyma shows normal MTT, rCBF, and rCBV. Transient ischemic attacks shows increased MTT, normal rCBF, and increased rCBV. Penumbra shows extremely increased MTT, decreased rCBF, and increased rCBV. Infarct shows extremely increased MTT, extremely decreased rCBF and decreased rCBV. Another differentiation is that injured area (penumbra+infarct) is area with $MTT > 145\%$ of that in the healthy contralateral tissue. The penumbra is then tissue with $rCBV > 2 \text{ ml/100 g}$, and infarct is tissue below this rCBV threshold. These areas are measured on each slice

and multiplied by 10 mm to determine the volume of each territory.⁴⁹ CTP has sensitivity > 75% for ischemic stroke, > 90% for infarcts in supratentorial regions, and a high specificity for ischemia.^{48,50–53} CTP-CBV and DWI have significant correlation and CTP-MTT with PWI.^{47,54} In threshold models, there is significant correlation between the CTP core and DWI as well as CTP total ischemia with PWI-MTT.⁵⁵ Because of the linear relationship between contrast concentration and signal intensity, CTP has significant advantage over gadolinium-based perfusion imaging, as CBF can be more quantitatively estimated.⁵⁶ The drawbacks of perfusion CT include the impossibility of serial measurements due to amount of contrast and radiation required and inability to show lacunar or posterior fossa lesions. Another disadvantage is limitation in spatial coverage (number of cross-sections imaged) though this will be overcome with new 64-slice CT scanners.⁴⁹

SPECT is another CT technique for imaging the penumbra, using radiotracers such as xenon, IMP, HMPAO, or ECD to evaluate CBF and cerebrovascular reserve. All of these tracers allow measurement of the CBF thresholds of infarction, while HMPAO can also measure CBV.⁵⁷ SPECT has much higher sensitivity for acute stroke than standard CT (nearly 90% versus 20%).^{58,59} SPECT is also safe, allows for repeated studies for serial measurements, and can provide functional information not given by conventional CT/MRI or PET. However, it cannot measure absolute CBF or metabolism.⁵⁷

Xenon-SPECT measures CBF quantitatively without arterial sampling but has poor spatial resolution due to low energy and rapid clearance from the brain.⁶⁰ It is also costly and difficult to use emergently.⁵⁷ SPECT with IMP, HMPAO, and ECD all have quick brain uptake. IMP has a short brain retention time, leading to poor spatial resolution, but stays trapped in tissue in proportion to CBF and can image high CBF levels better than the other two methods. On the other hand, HMPAO and ECD have slow clearance rates and thus have higher resolution (ECD more so than HMPAO) and allow repeated measurements. ECD also has the advantage of representing cell functional status rather than perfusion as studies have shown that it has low conversion to its trapped hydrophilic form in infarcted areas of subacute stroke lesions. Disadvantages of IMP, HMPAO, and ECD include limited use during emergencies and the high cost of IMP.⁵⁷

Xenon-CT is another technique for stroke imaging, which works by either IV administration or stable gas inhalation of Xe-133.⁶¹ Two unenhanced CT images are obtained, then gas is inhaled and six xenon-enhanced images are acquired. The unenhanced images are averaged and subtracted from the enhanced images to obtain a large number of voxels representing the brain xenon concentration in each voxel.⁴⁹ Xenon-CT provides quantitative information on CBF, leading to an accurate delineation of penumbral versus core tissue in a fast and cost-efficient manner.⁴⁹

Conventional T1- and T2-MRI

Conventional T1 and T2 MRI (T1WI and T2WI) are part of standard imaging protocol in stroke imaging⁶² and, along with normal non-contrast CT, have been used in acute stroke primarily to rule out hemorrhage.⁶³ While both T1WI and T2WI identify vasogenic edema at later times during stroke,⁶⁴ 90% of infarctions are visible on T2WI at 24 hours, relative to

only 50% on T1WI, and thus T2WI is the ‘gold standard’ in clinical settings for imaging cerebral infarction.⁶⁵ However, both normal non-contrast CT and conventional MRI have shown sensitivities of < 50% in imaging ischemic stroke within 6 hours of onset,^{66,67} despite the fact that T2WI has signal changes as early as 30 minutes post-stroke in cats and primates.⁶⁸ In addition, T1WI and T2WI have shown high false negative rates during the first day after stroke onset.⁶⁵

While T2WI alone is unable to distinguish necrotic tissue from salvageable tissue subacutely, combined with Diffusion-weighting imaging (DWI) it is able to identify salvageable tissue at a much earlier time point.⁶⁹ Also, combined T1WI, T2WI, and DWI data is highly correlated with tissue histology.⁷⁰

Diffusion and Perfusion Weighting Imaging

Multimodal MRI has unique advantages over other imaging modalities for stroke diagnosis by offering extensive information on different tissue contrast. Diffusion-weighting imaging, which detects the movement of water molecules, has been proven to be a powerful tool for early detection (within minutes after stroke onset) of ischemic brain.⁷¹ Under normal conditions, water molecules diffuse randomly in tissue. When ischemia occurs, abnormal water diffusion could be observed as restricted water motion which manifests as hyperintensity on DWI. The degrees of diffusion could be expressed quantitatively using the apparent diffusion coefficient (ADC). Although the origin of these signal changes remains an area of research, it is usually attributed to cytotoxic edema accompanied by dysfunction of ion channels on cell membranes. Diffusion-weighting imaging is considered superior to a non-contrast CT scan and has become routine practice in hospitals.

Disturbed CBF is the first event when ischemic stroke occurs. In addition to CT-perfusion, various perfusion MRI techniques have been developed to visualize CBF reduction. The measurements are made by two major categories of techniques –dynamic susceptibility contrast (DSC) MRI and arterial spin labeling (ASL) MRI. Dynamic susceptibility contrast remains the method of choice in most hospital because of its ease of implementation. It requires a bolus injection of gadolinium-based contrast agent together with dynamic imaging in order to derive hemodynamic parameters. By contrast, ASL, which utilizes radiofrequency pulses to label inflowing blood without exogenous contrast agent, has emerged recently. Although ASL MRI offers absolute quantification of CBF, the optimal imaging parameters for patients with cerebral vascular diseases are sometimes more difficult to determine.

During the acute phase of stroke, the anatomical region defined on the DWI is initially smaller than the area of CBF deficit, but this region expands and eventually coincides with the area defined on perfusion-weighted images (PWI).^{72,73} The difference in the size and extent of the enhancing regions visible in PWI and DWI images is referred to as the ‘perfusion–diffusion mismatch’. This mismatch has been shown to approximate the potentially salvageable ischemic penumbra.⁷⁴ The spatiotemporal evolution of the ischemic lesion during the acute phase is a highly dynamic process as the mismatch gradually decreases over time, and thus represents a rapidly moving target for treatment. The PWI–DWI mismatch was widely observed in humans^{74–77} and animal models^{78–80} and was

suggested as a potential biomarker to guide patient selection for treatments.⁷⁷ However, utilizing PWI–DWI mismatch to help clinical decision-making is not yet a standard practice, primarily because the fate of mismatch tissue is not fully understood. Furthermore, the correlation between the mismatch and the ‘penumbra’, defined by the tissue metabolic status, and the thresholds delineating the mismatch remain somewhat uncertain. These questions are difficult to investigate in patients, and thus, experimental models are needed to address these concerns.

In experimental stroke, the spatiotemporal evolution of PWI–DWI has been well characterized from the acute to chronic phase. Meng *et al.* monitored the evolution of the mismatch in rats that underwent middle cerebral artery occlusion (MCAO), and found the mismatch disappears at 60 minutes after occlusion.⁷⁹ The dynamics of PWI–DWI mismatch in NHP, which diminishes about 6 hours post occlusion, was also demonstrated in a recent study.⁷⁸ Both studies showed that early reperfusion could reduce infarct size substantially. Attempts have been made to follow-up the tissue fate of the mismatch. For example, a scatterplot method using quantitative CBF and ADC values was proposed to track the spatiotemporal progression of ischemic tissue fates on a pixel-by-pixel basis.⁸⁰ Shen *et al.* further improved this method by implementing an automatic clustering algorithm to define tissue types.^{81,82} Such objective and automatic approaches could have potential use to predict final infarct. Future studies incorporating more information related to tissue characteristics beyond perfusion and diffusion imaging might help the development of predictive models.

Diffusion Tensor Imaging

Imaging technology has progressed substantially in the last two decades. Diffusion imaging is one example that has greatly benefited from technical advances. Advanced diffusion MRI, such as diffusion tensor imaging (DTI), fully utilizes the information obtained from diffusion anisotropy caused by biological boundaries to infer the exquisite details of tissue microstructure.

After a stroke, the brain remodels and generates new vessels, neurons, and synaptic connections, which in turn improve patient’s outcome. Functional recovery appears in stroke patients variably from weeks to years after the event. However, the underlying mechanisms prompting reorganization remain unclear. Diffusion tensor imaging has been used frequently to visualize the restoration of structural integrity and connectivity. Depending on the different stages post-stroke, different combinations of derived diffusion parameters could be shown. Diffusion parameters are well characterized in rodent stroke models,^{83,84} and as mentioned in the previous section, ADC decreases within minutes after stroke onset, possibly representing acute cell swelling. As ischemia progresses, ADC increases while fractional anisotropy (FA) decreases as cells and myelin breakdown. In the chronic stages, FA increases again to reflect the regeneration of white matter structures.

In contrast to rodents, NHPs have substantially higher white to gray matter ratio, and presumably mimic white matter changes in patients better. Liu *et al.*, measured ADC and FA changes in both permanent and transient MCAO in macaques and compared with T2 and histology to determine the endpoint lesion size.⁸⁵ The authors reported that although the 3-

hour MCAO model produced a permanent stroke lesion, the dynamics of the diffusion indices and T2 are different in permanent and transient stroke groups. Reperfusion accelerates the changes of diffusion parameters when compared with permanent stroke. The temporal dynamics of the diffusion indices and T2 in NHP stroke more closely resemble that in humans as opposed to rodents, suggesting the usefulness of an NHP stroke model and the potential of serial MRIs in stroke research. Chin *et al.* carried out serial DTI in transient MCAO in two macaques and compared diffusion parameters with motor function measurements.⁸⁶ The authors found transient decreases in FA values of affected motor pathways and suggested the recovery of FA correlates with motor recovery in the chronic phase.

Diffusion MRI techniques continue to emerge. Novel variations of diffusion MRI, such as q-space imaging and diffusion spectrum imaging, enable better tractography of white matter bundles. Most recently, it was shown that diffusion MRI is capable of estimating axon diameters *in vivo*.^{87,88} These techniques open up a new avenue for future research to characterize microstructure alterations in stroke.

BBB permeability imaging

The blood–brain barrier (BBB) is a physical boundary that separates blood from tissue of the central nervous system, while allowing passive diffusion of small molecules and active cellular transportation of metabolite. Immediately following stroke and/or after reperfusion, BBB disrupts which might be related to subsequent hemorrhagic transformation and edema.^{89–91} In the late stages, vascular remodeling and formation post-stroke is a normal and vital step associated with neurogenesis and recovery.^{92,93} Some studies have related angiogenesis, especially in the ischemic core region, to post-stroke hyper-perfusion. Such angiogenesis involves a highly complex physiological cascade of processes and has been extensively reviewed elsewhere. At the early stage of post-stroke angiogenesis, the density of microvessels increases but the BBB of the newly formed vessels is leaky. A decrease in BBB permeability is observed overtime as the vessels mature. Therefore, non-invasive imaging of BBB integrity is a valuable tool for stroke, because it not only offers a way to predict the potential severity of stroke but also can provide spatiotemporal information associated with tissue regeneration.

Blood-brain barrier permeability could be measured using DSC MRI by monitoring the leakage of exogenous contrast agents into brain tissue in order to derive parameters such as the transfer constant. Absolute quantification of BBB permeability also remains challenging due to the complexity of dynamic modeling that involves multiple compartments. Methods that estimate the water exchange rate with or without contrast have also been proposed.^{94–96} Studies in experimental stroke have shown that treatments with albumin,⁹⁷ sildenafil,⁹⁸ matrix metal-loproteinases,⁹⁹ hyperbaric oxygen,¹⁰⁰ and many others reduced BBB leakage. However, the impact of these treatments on reducing the final infarct and improving outcome is controversial. Future studies are needed to better understand how BBB integrity affects subsequent hemorrhage or recovery.

pH MRI

Defining PWI abnormality highly depends on the threshold chosen and often could include tissue with benign oligemia. Diffusion-weighting imaging is considered a marker for cell membrane failure, but studies have shown some tissue with abnormal diffusion could potentially be reversible. Tissue acidosis is one of the early events involved in the ischemic cascade and might be a better biomarker for membrane failure and ion depolarization. MR spectroscopy (MRS) can be used to assess lactate concentration and pH as indicators of impaired energy metabolism. However, MRS techniques suffer from relatively low sensitivity compared to MRI. Thus, it often has low spatial resolution that may not be sufficient to delineate salvageable tissue in acute stroke.

A variation of the chemical shift saturation transfer (CEST) MRI, called amide proton transfer (APT), was found to be sensitive to tissue pH status, and has been shown the potential usage of sub-dividing the perfusion–diffusion mismatch.^{101,102} Although APT (or pH-imaging) is still early in its development, the technique highlights a new direction for future imaging.

Functional MRI

Functional MRI (fMRI) using blood oxygen-level depend (BOLD), CBF, or CBV contrast allows us to visualize brain activity non-invasively in the living human brain, and therefore offers great potential for the study of functional recovery and plasticity in clinical populations.^{35,103,104} BOLD-fMRI is the most widely used approach due to its high sensitivity and ease of implementation.

Experimental stroke models produced by occluding the MCA territory will induce ischemic lesions in the sensorimotor area specifically. Reliable models, with precisely controlled passive sensory stimuli, provide excellent tools for studies of functional recovery and for evaluating rehabilitation strategies. In rodent stroke models, electrical forepaw and/or hindpaw stimulation is one popular paradigm. Restoration of sensory-motor function was accompanied by contralesional activation early (1–3 days) after stroke, and recruitment of perilesional cortical areas was observed in later stages (weeks to months).^{105,106} The degree of shift and the time of restoration of the ipsilesional activation may be associated with functional outcome.¹⁰⁶ A study of MCAO in rats reported that after unilateral stroke, bilateral activation was shown when stimulating the unaffected limb. This suggested that the impact of focal ischemia on structurally and functionally connected brain regions should also be considered.^{107,108}

From a network perspective, any physiological and pathological processes could affect the underlying intra- or inter-network interactions. Resting-state fMRI provides a means to investigate the functional connectivity between brain regions.^{109,110} Using resting-state functional connectivity MRI to characterize cortical plasticity might offer unique insights regarding within-network (intra-hemispherical and inter-hemispherical) and between-networks interactions and their relationship with behavioral outcomes.¹¹¹ An improvement in sensory-motor function in rodent stroke is related to the recovery of inter-hemispherical (within-network) signal synchronization.^{107,112} In NHP stroke, Liu *et al.* have carried out a

resting-state fMRI study. In this preliminary study, they reported that the spontaneous fMRI signal fluctuation altered in the peri-infarct area and suggested that resting-state fMRI might provide additional information to diffusion and perfusion MRI.¹¹³ A recent study combined resting-state fMRI with DTI and behavioral assessments in patients with relatively large stroke, showing that the degree of functional recovery after stroke is closely related to the extent of preservation or restoration of corticospinal tracts and to the reinforcement of inter-hemispherical neuronal signal synchrony.¹¹² Hypercapnia or hypoxia in fMRI resulting from global physiological challenges might provide interesting information regarding the vascular reactivity and the oxygen metabolic status respectively.^{114,115,116} The so-called calibrated fMRI technique and other methods such as applied biophysical modeling that quantify tissue metabolism have shown promising results in experiment stroke and have the potential for translation into clinics but remain active areas of research.^{117–120}

Prediction of ischemic tissue fate: An important goal of acute stroke imaging is to predict tissue fate based on acute data. Generalized linear model,^{121,122} probability-of-infarct,^{123,124} and artificial neural network (ANN)¹²⁵ and Support vector machines (SVM)¹²⁶ have been used to provide statistical or probabilistic maps of infarct likelihood on a pixel-by-pixel basis utilizing only the acute MRI data. Performance analysis showed accurate prediction when compared with endpoint T2 MRI and/or histology. Other potential a priori information can be incorporated in these predictive models.^{127,128} Prediction accuracy were quantified using receiver-operating characteristic (ROC) analysis.

Conclusions and Future Perspectives

The current consensus is that no imaging method alone could provide sufficient information to unambiguously identify potentially salvageable tissue or monitor tissue reorganization. Combining multimodality imaging approaches has the potential to give complementary information that might help clinical diagnosis and/or resolve pathophysiological events following ischemia. Positron emission tomography of rCBF and cerebral energy metabolism was the first imaging technique employed for penumbra detection. The regions with mismatch between blood flow and metabolism, i.e. the region of ‘miser perfusion’,³¹ was the first imaging-based definition of the ischemic penumbra, and it remains the gold standard for identifying penumbral tissue. However, due to technical demands and the short half-life of ¹⁵O radiotracers, the utility of PET is limited in clinics. The PWI–DWI mismatch is the most reliable MRI method currently available for detecting the ischemic penumbra albeit controversial. The development of innovative MRI methodologies and the improvement of existing techniques will surely benefit the field of stroke imaging. For example, ¹⁷O-MRI, as a close analog of ¹⁵O-PET, could provide the opportunity to better delineate the ischemic penumbra base on tissue metabolic status. Although this technique might be difficult to implement in clinics on a daily basis (potentially limited by the cost and other technical challenges), the development of ¹⁷O-MRI might be able to help establish more reliable perfusion and diffusion thresholds of the penumbra in patients and animal models.

Other novel imaging techniques might also be useful. Besides inventing novel imaging techniques, optimizing current available imaging methods is also very important for

immediate clinical use. For example, perfusion imaging using ASL has recently been implemented in hospitals. However, because of the diversity of ASL methods, having a standardized imaging protocol for optimal performance is also very important.

Recently, multimodality imaging methods, which offer complementary information to MRI, started finding their place in the research and clinical environments. For example, simultaneous MR-PET has the potential to provide information about tissue metabolic status with sufficient spatial resolution to minimize scan time. At least it offers a great platform to cross-validate novel MRI methodologies that are developed. Simultaneous EEG-MRI could also advance our understanding of stroke pathological phenomena, such as peri-infarct depolarization (PID), that often occur in acute stroke.

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Table 1

Comparison of the strengths and weaknesses of different imaging modalities for stroke

Modality	Strengths	Weaknesses
PET	Standard for ischemic penumbra delineation Quantitative measurements	Expensive Limited availability Time-consuming when using certain radiotracers (such as ^{18}F -labeled compounds) Radiation exposure Poor spatial and temporal resolution
CT Perfusion	Widely accessible Cheap Fast	Radiation exposure Contrast agent required Semi-quantitative, quantitation issues
SPECT	Serial measurements possible	Relatively expensive Difficult to use in emergency settings
MRI	Good spatial resolution High sensitivity (especially diffusion imaging) Non-invasive/no radiation Great versatility and image contrasts	Time-consuming Expensive