MRI assessment of cerebral perfusion in clinical trials

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Keywords: arterial spin labeling-MRI; cerebral perfusion; clinical trial; multicenter trials.

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Neurodegenerative mechanisms affect the brain through a variety of processes that are reflected as changes in brain structure and physiology. Although some biomarkers for these changes are well established, others are at different stages of development for use in clinical trials. One of the most challenging biomarkers to harmonize for clinical trials is cerebral blood flow (CBF). There are several magnetic resonance imaging (MRI) methods for quantifying CBF without the use of contrast agents, in particular arterial spin labeling (ASL) perfusion MRI, which has been increasingly applied in clinical trials. In this review, we present ASL MRI techniques, including strategies for implementation across multiple imaging centers, levels of confidence in assessing disease progression and treatment effects, and details of image analysis.

Introduction

Adequate CBF has a crucial role in maintaining the structural and functional integrity of the brain, because it provides a constant supply of oxygenated blood and nutrients.[1] In several neurological disease conditions, reduced brain perfusion and metabolism are commonly observed. There is an association between brain atrophy and reduced CBF, and studies indicate that reduced flow can result in brain atrophy.[2] For example, in Alzheimer's disease (AD), patients display hypoperfusion/hypometabolism most markedly in the temporoparietal lobe and posterior cingulate cortex, precuneus and frontal cortex,[3] and in Parkinson's disease (PD), patients present hypoperfusion in posterior parieto-occipital structures.[4] Furthermore, cardiovascular disease is linked to reduced brain perfusion and subsequent neurodegeneration.[5]

The re-establishment of perfusion appears to aid in alleviation of the symptoms in the case of neurodegeneration associated with cardiovascular dysfunction and could contribute to slowing cognitive impairment.[5] In acute stroke,

perfusion measurement is crucial for assessing compromised tissue, aiding treatment decisions for interval therapy to re-establish circulation, determining collateral perfusion, and predicting clinical outcome.[6,7] Therefore, evaluation of brain perfusion is relevant for the assessment of disease states as well as disease-modifying treatments.

ASL is a non-invasive MRI technique that is an ideal tool to study brain perfusion over time. It does not require radioactive or exogenous contrast agents, and provides a relatively reproducible quantitative CBF measurement.[8] A review of open clinical trials (EU Clinical Trials Register, Clinicaltrials.gov) revealed several studies assessing blood flow in a variety of conditions, including cognitive impairment, multiple sclerosis (MS), oncology, and stroke.

In this review, we provide an understanding of the ASL technique and guidance on the operational aspects of its implementation and image analysis, focusing on neurodegeneration-related clinical trials. For other clinical applications, as well as for perfusion assessment of other organs, such as kidney or heart, the reader is referred to the literature.[9–11]

Arterial spin labeling

ASL is an MRI-based perfusion imaging technique that can quantitatively measure microvascular blood flow or perfusion, as well as other hemodynamic parameters, such as arterial blood volume and arterial transit time.[12–14] In 1992, Williams *et al.* proposed the original ASL method, applied to measure CBF of rat brain,[13] and, in 1994, Detre *et al.* extended the application to measure human brain perfusion.[15] Tissue perfusion measured by ASL assesses the rate of delivery of nutrients, including oxygen, through blood flow in the capillary beds, which is different from macrovascular blood flow in arteries and veins, which can be assessed with other MRI approaches, such as MRI angiography. In particular, using ASL, arterial water spins are labeled and followed until they exchange with the surrounding tissue, where they alter the apparent T1 of the tissue. Various imaging techniques have been developed to measure regional cerebral perfusion in addition to ASL, including positron emission tomography (PET), single photon emission computed tomography (SPECT), Xenon-enhanced computed tomography (CT), dynamic susceptibility contrast MRI (DSC-MR), and Doppler ultrasound (Table 1). ASL is commonly compared with ^{18}F-fluorodeoxyglucose (FDG) PET, although these techniques measure different physiological parameters: blood flow (ASL) versus rate of glucose consumption (FDG PET). However, brain perfusion and metabolism are tightly coupled under normal conditions, and ASL-measured perfusion has shown good correlation with metabolism measured by FDG PET. Additionally, ASL has been used to study brain perfusion/metabolism in place of, or in addition to, FDG PET.[16–18]

Compared with other imaging techniques, ASL MRI has the following advantages: requires no exogenous agent (radioactive or not) or radiation; has whole-brain coverage with a relatively high spatial resolution (2–4 mm) in 5–10 min; and requires no waiting time between two successive exams (reviewed in [19]). The main limitations of ASL are the following: ASL has a relatively low signal-to-noise ratio (SNR) (which can be enhanced by increasing number of repetitions; i.e., scan time); it is susceptible to head motion; and there are a large variety of ASL sequences and analysis methodologies, which makes protocol harmonization and analysis across imaging sites challenging to implement and compare. However, given its wide availability and relative advantages, a community consensus was developed in 2015, and has since had, and will continue to have, an important role for standardized ASL acquisition and analysis across MR platforms and sites. [20] Given that the ASL-MRI requires no exogenous agent and reduces overall cost related to agent purchase, scan preparation, patient waiting time, staffing (nurse for IV injection and patient monitoring), equipment (contrast injector) and reduced scanner time, among others, ASL-MRI could be more cost-efficient compared with PET or DSC-MRI.

ASL mechanism and approaches

ASL uses blood as an endogenous contrast agent to determine cerebral blood flow (for a review of ASL imaging see Figure 1).[21] In the preparation (or labeling) phase, blood water protons outside of the imaging volume are labeled by magnetic inversion. After a period of time, generally referred to as the 'inflow time' [but more specifically defined as the 'inversion time' (TI) or 'post-labeling delay' (PLD, single or multi-PLDs) depending on the particular ASL technique being used], the image acquisition begins. The inflow time is long enough so that the labeled blood water flows into the imaging tissue and mixes with the (positively magnetized) static tissue water in the parenchyma. The time takes for the labeled blood to reach the vascular or tissue is termed 'arterial transit time'. The long inflow time also allows the more rapid vascular flow to enter and leave the imaged tissue before it is imaged. Once the labeled blood enters, it produces a net reduction of the parenchymal MRI signal in this 'labeled' image through exchange at the capillary level. A relative measure of perfusion can be calculated by acquiring an unlabeled (control) image and calculating the signal difference between the control and labeled images. The difference in intensity can be converted to CBF based on specific model of tracer kinetics. This perfusion-related signal reduction is small in magnitude, ~1–2% of the tissue signal intensity, and is very sensitive to the conditions under which the signal is sampled. It is affected by tissue properties, such as the blood flow rate and T1 relaxation of blood and parenchymal tissue, as well as factors driven by the selection of MRI equipment and pulse sequence used for acquisition. Background suppression techniques are utilized to selectively minimize the static tissue signal from the rest of the brain to boost the SNR in ASL signal.[22]

The two most common labeling approaches for ASL are pseudo-continuous ASL (PCASL) and pulsed ASL (PASL) (Figure 2). For PCASL labeling, a train of radiofrequency (RF) pulses is applied in rapid succession (over milliseconds) and for a relatively long overall duration (1–3 s) to invert and label arterial blood water as it flows through a relatively thin labeling plane. By contrast, PASL typically uses a single RF pulse applied for a much shorter total duration

(e.g., 10–20 ms), to simultaneously invert arterial water in a thick labeling slab. For a comment on the resultant SNR differences between PASL and PCASL please see [20]; it is generally expected for PCASL to have higher SNR and, thus, is becoming the sequence of choice. Note that CASL, in which one single long continuous ASL label is applied, has lower labeling efficiency compared with PCASL and needs an additional labeling coil. Therefore, it is not recommended for clinical imaging.

ASL readout schemes broadly fall in the category of either 2D or 3D, with a segmented 3D readout [such as 3D multi-echo (RARE) stack-of-spirals [22,23] or 3D gradient and spin echo (GRASE)].[24–26] These are the currently recommended default implementations[20] because these readout schemes can be made SNR efficient and are relatively insensitive to off-resonance effects.

The difference between control and labeled ASL scans is a perfusion-weighted image (PWI); to determine absolute perfusion, a separate calibration image (M0) needs to be acquired. This is typically a replication of the ASL 'control' image acquisition but without any background suppression. The process to calculate absolute perfusion from perfusion-weighted images is described below.

ASL test–retest

Crucial to clinical trials, as well as for research and clinical use, an assessment of the degree of reproducibility of CBF measurements utilizing ASL needs to be defined. Many studies have been conducted to evaluate CBF repeatability using different variants of ASL sequences and healthy volunteers, patients, as well as phantoms (summarized in Table 2). Currently, however, there is no systematic review and meta-analysis of all the test-retest studies in the literature. Nevertheless, overall CBF measurements in gray matter (GM) showed moderate to excellent reliability in intraclass correlations (ICC) (0.91>ICC>0.5) in most of these studies; however, there are large variabilities across sites and studies (because of the different ASL techniques used). In the study by Peterson *et al.*,[27] ICC in GM CBF ranged from 0.07 to 0.81 across 28 sites (using QUASAR, a sequence not often used). In addition, the degree of reproducibility varied in different brain regions, from ICC=0.91 in the hippocampus to 0.49 in amygdala[28]; in disease states, the variability could be larger; for instance, ICC ranges from 0.24 to 0.75 for patient with frontotemporal dementia (FTD) in different regions.[29] Whereas most studies focus on CBF, Cohen *et al.*[30] also reported the reliability of arterial transit time (ATT) (ICC range 0.49~0.69), slightly lower than CBF (ICC range 0.66~0.75). The variability across regions and techniques indicates that careful consideration needs to be given when planning a study so that the appropriate statistical power is achieved in the brain regions of interest. In general, a systematic review and meta-analysis of all the test–retest studies should be compiled to establish what the ICC values are in CBF measurements derived from ASL.

Image acquisition

A major challenge in the use of ASL in clinical trials has been the harmonization of image acquisition protocols in multicenter studies.[20,31] It is likely that sites selected to participate might have MRI systems that differ in manufacturer, field strength, software, and the RF coils used to receive the signal. Although satisfactory ASL imaging can be achieved at 1.5 T, 3 T systems offer greater intrinsic SNR. Furthermore, the lengthening of T1 at higher field strengths provides an additional boost to signal at 3 T because more labeled signal accumulates for a given inflow time. Compensatory reductions in resolution and/or increases in scan time can be made to 1.5 T acquisitions to increase SNR, but introduction of such discrepancies in imaging protocols is undesirable. With regards to RF coil hardware, receiver coils with a greater number of elements will likely offer superior SNR and enable use of parallel imaging methods.

Thankfully, ASL pulse sequences have been implemented by major MRI scanner vendors and are widely available across their platforms. It has been shown that CBF measurements can be reproducibly produced across vendor platforms when the 'identical' pulse sequence is used with parameters as closely matched as possible across platforms.[32] An important recent development is that, for every new scanner now being sold, the ASL pulse sequence provided by the main three manufacturers all follow the White Paper recommendations. As such, including sites with novel equipment and/or latest XXX (SW) level might be one way to ensure comparable pulse sequences across the board. However, such approaches might not be feasible in many clinical trials where the imaging core lab might also be restricted to deploying commercially available sequences offered by the major MRI vendors in legacy systems. At the time of writing, there remains a lack of standardization, both between vendors and within a vendor's portfolio of scanner models and system software versions. Imaging core labs are often confronted with a mixture of sequences across trial imaging sites that vary in spin labeling (PASL, CASL, and PCASL), readout (EPI, Spiral, GRASE, etc.), and 2D and 3D acquisitions. Furthermore, sequence characteristics that commonly vary between systems include choice of inflow times (and whether to acquire single or multidelay data), background suppression, acquisition of M0 image, and achievable resolution. A detailed discussion of ASL implementations is beyond the scope of this paper, but interested readers are directed to publications that provide greater technical detail and insight.[20,33,34]

Finally, ASL readout schemes broadly fall in the category of either 2D or 3D. A segmented 3D readout [such as 3D multi-echo (RARE) stack-of-spirals[22,23] or 3D GRASE[24–26]], currently recommended as the default implementation,[20] because these readout schemes can be made SNR efficient and relatively insensitive to off-resonance effects.

Recommendations

Several studies have indicated that inconsistent scanning equipment and ASL sequences can introduce significant across-site variability.[31,35–37] Recommendations for pulse sequence deployment are highly dependent upon the ASL

endpoints specific to a clinical trial, as well as the number of imaging sites required. When there are a limited number of imaging centers involved, it might be possible to select the sites based on ASL imaging capabilities and limit the sites to those with the same scanning equipment and pulse sequences. When deployment across a variety of scanning equipment and commercial ASL sequences is necessary, it is advised that 3 T scanners be used with RF coils that have an element count greater than or equal to 8. To reduce measurement bias between sites, consistent preparation and acquisition approaches should be used across sites where possible. Pulse sequence parameter guidance for common sequences can be found in the 'Recommended implementation of arterial spin-labeled perfusion MRI for clinical applications' consensus paper published by the ISMRM perfusion study group[20] as well as in Table 3.

In addition, CBF can be affected by many physiological factors, including age, gender, fasted state, caffeine intake, exercise, prescription drug, and disease[35,38–41] (see Clement *et al.*[42] for a systematic review on all these effects, including handy predefined questionnaires to use in clinical trials). To account for these variables, study groups/cohorts should be as closely matched as possible to allow the exploration of the factors of interest, such as drug effects. Physical state of the subjects should be carefully controlled for MRI scans (e.g., no caffeine intake or strenuous activity 3 h before MRI). Prescription drugs that might affect CBF and/or vascular tone should be excluded. Correction for partial volume effects should be considered in studies in which atrophy might be present, or at least interpretation of uncorrected CBF data alongside any volumetric changes.[43,44]

Recently, the Quantitative Imaging Biomarkers Alliance (QIBA) and the European Imaging Biomarkers Alliance (EIBALL) organizations formed a collaborative committee to develop a standardized CBF biomarker calculated from ASL images.[45] Development of this biomarker profile will provide further direction to imaging core labs attempting to deploy ASL in clinical trials.

Quality control of ASL imaging data

When site qualification is complete (see Appendices A and C in the supplemental information online for site setup and qualification recommendations, and for data storage, format, and workflow, respectively) and an approval to scan first subject has been issued to a site, it is important that the imaging core lab continues to monitor the image quality of ASL data using both phantom (every 6 months) and participant (after each data set is collected). For participant data, it is advisable to perform checks for both image quality control (image QC) and protocol adherence (technical QC) as soon as possible after a data set has been submitted by a site. Detecting and resolving issues quickly can limit the number of affected data sets and, therefore, any adverse effects on downstream analysis processes. One approach taken can be to limit a site to imaging a single subject and only allowing it to proceed with imaging of further trial participants once this first data set has passed QC criteria (for subject preparation for scanning, please see Appendix B in the supplemental information online). It is also recommended to repeat the phantom data QC process used in the site qualification process every 6 months to provide information on the readout sequences and potential artifacts. Imaging QC processes can be manual, semi-automated, fully automated, or a mixture thereof in an effort to best detect issues in the data.

The following section describes some of the common issues and artefacts observed in ASL data sets from the participants, how they can be detected, and possible approaches to enhance data by removing errors (e.g., by modifying the acquisition parameters or cleaning/scrubbing the acquired ASL data).

Visual QC

For all neuro ASL acquisitions and scanner-generated outputs, an initial visual check for adequate brain coverage and noticeable artefacts is required. If raw ASL images are available, quick visual assessment of the images can be performed by calculating a temporal variance image. Given that perfused areas display the largest changes in signal intensity because of perfusion, the corresponding temporal variance will also display the largest variance. A variance image resembles a PWI image.

For both PWI (mean or time series) and generated CBF images, the visual image QC process can be divided into two parts: assessment of images for the presence of expected contrast between anatomical regions of the brain (contrast QC) and checks for the presence of artefacts in the images (artefact QC). If other ASL parametric maps (e.g., ATT) are generated by post-processing software, these should also be assessed.

Contrast in PWI (and CBF maps) is driven by the arrival of the labeled blood bolus, and contrast between GM and white matter (WM) tissue regions should be clearly visible. In cases in which ASL image quality is very high, it might also be possible to observe contrast between WM and cerebrospinal fluid (CSF) regions. In some distal brain regions, where the labeled blood must travel further, known as border zone or watershed regions, the ASL signal may be lower.[46] In these regions it is important to identify the cause of the lower PWI and CBF estimates; if it is driven by longer ATT, it might also be necessary to adjust the pulse sequence inflow time to account for this. If the labeling efficiency of the pulse sequence used is too low, or images have been acquired or scaled incorrectly, erroneous PWI and CBF values and poor contrast between WM and GM structures will result. For example, particularly low PWI and CBF in an entire vascular territory might indicate a labeling failure for the artery feeding that territory. The mean CBF value for whole-brain GM should fall within the range of 40–100 ml/min/100 ml,[20] and GM/WM CBF ratios can be of the order of 2–2.5.[47,48] In GM regions, localized regions of spurious PWI and CBF values mgiht indicate an issue, because the signal should be reasonably homogenous and vary smoothly.

Image artefacts need to be detected as part of the ASL image QC process, because resulting deviations in signal intensity can cause significant errors that propagate into the CBF maps. Checks for motion, susceptibility, and vascular artefacts should be assessed at a minimum. ASL pulse sequences are particularly sensitive to head motion,

which causes blurring and a reduction in contrast between WM and GM regions. Motion issues can be identified visually as signal outside of the brain in the PWI and CBF maps that is sometimes described as a 'hyperintense rim'. Magnetic susceptibility effects on ASL data will cause signal to drop out and distort anatomy. An appearance of unnaturally shaped, region-specific areas lacking image contrast might indicate susceptibility-driven artefacts. Regions of spurious signal or 'bright spots' can be indicative of vascular signal, which can be caused by an incorrect ASL inflow time used during imaging or prolonged ATT, which might be seen more often in older subjects or some pathologies.[49]

A scoring method was recently published to remove reader bias and standardize contrast QC and artefact QC activities in ASL clinical trials.[50] This approach might be highly beneficial for increasing sensitivity for the detection of poor-quality imaging data and increasing reproducibility of QC techniques in multisite ASL imaging trials.

Automated QC

In addition to the above visual QC processes, several ASL data-processing strategies have been proposed for the automated detection and exclusion of artifacts in ASL data (referred to herein as 'ASL scrubbing'). We broadly categorize these as either motion-based or signal-based approaches, which can be further subcategorized into threshold-based or threshold-free approaches.

Given that ASL is based on the subtraction of temporally adjacent volumes, any relative displacement will significantly affect CBF estimation; thus, motion is severely detrimental for CBF measurements. Estimating motion in the ASL (control-label) timeseries using motion-based approaches allows identification of individual volumes with excessive motion that can be excluded using either. Miranda *et al.* proposed the rejection of images with more than a threshold 2-mm translation or 1.5° rotation between successive images in the ASL timeseries.[51] More conservatively, Jann *et al.* proposed (implemented in the CereFlow software) a frame-wise displacement (FD) threshold of >0.8 mm for rejecting images from pCASL time series.[52] Signal-based ASL scrubbing can also be used to detect motion artifacts and other imaging artifacts. Tan *et al.* proposed a simple, yet effective PWI signal-filtering approach that removes outliers from the PWI time series if the mean and standard deviation is outside a predefined threshold of the quantities across time.[53] A threshold-free method, named Enhancement of Automated Blood flow Estimates (ENABLE), sorts control-label pairs by motion and cumulatively averages them until the addition of further pairs significantly decreases the temporal voxel-wise signal stability.[54] An implementation of this technique (ExploreASL)[55] uses the median GM voxel-wise temporal SNR (tSNR) as the criterion for signal stability, regularized by an empirically defined minimum tSNR improvement of 5%.[56]

Motion-based and signal-based strategies can also be combined for artefact detection. Wang *et al.* utilized head motion (both absolute motion and relative motion between each control-label pair) and global signal deviations to exclude outlier timepoints from the ASL series.[57] In further work, the authors proposed an adaptive outlier cleaning approach (AOC) involving using the mean CBF as the reference and iteratively removing timepoints based on the degree to which they vary from the reference [41] Outliers identified based on head motion estimations were removed before calculating the initial mean in the AOC. Similarly, Dolui *et al.* combined both motion-based and signal-based strategies for artefact detection by utilizing a structural correlation-based outlier rejection scheme with pre-processing to remove extreme outliers (SCORE1) to reject outliers in the CBF time series of 2D-PASL data.[38]

Although these approaches help to reduce artifacts introduced into CBF calculation, every effort should be made to minimize them while acquiring the data. For example, head motion can be reduced by properly immobilizing the head for the scan (see Appendix B in the supplemental information online for subject preparation).

Quantitative analysis

The analysis of ASL data begins with pre-processing the raw images to be able to quantify them. Steps include removing nuisance images (scrubbing) and motion correction to generate PWI. According to the acquisition strategy, PWI are then converted to absolute CBF maps. The steps are described in detail below.

Pre-processing steps

Typical pre-processing steps can include scrubbing, motion correction, registration, and, less commonly, distortion correction. Scrubbing has already been discussed in the QC section above.

Motion correction is performed similar to other functional MRI data (i.e., to perform rigid registration between individual volumes). It is important to perform motion correction before the control and label subtraction. ASL data acquired using EPI technique with some scanners can also suffer distortions common to all EPI-based techniques. There are two options for distortion correction: to collect a separate field map[58] or to acquire the calibration image twice with opposite phase-encode directions to the ASL data.[59] In both cases, methods are applied to reduce/correct these distortions.[58,59] For 3D acquisitions, motion correction can be limited because scanners only acquire a handful of tag/control pairs, which not often are saved, and only the resulting PWI or CBF images are saved. In such cases, motion correction provides limited or no help in reducing artifacts.

There is often the need to transform low-resolution ASL data to a template space to be able to report regional statistics on CBF map. It is typically conducted in two steps: (i) registration of the M0 image or mean PWI to the high-resolution structural image (e.g., T1W)and (ii) registration of the structural image to a standard template space (e.g., MNI152). The GM/WM tissue probability maps resulting from these registration steps can also be used for partial volume correction and masking the volumes by GM masks.

After pre-processing, pairwise subtraction between control and label images is performed, and the average of the difference of the images gives the PWI, which is used in clinical practice. The PWI reflects the perfusion in each voxel, but the intensity value does not provide an absolute measure of perfusion.

Quantification of CBF

Although quantification of CBF can be skipped, it can provide quantitative measures for longitudinal comparisons in clinical trials. Under a few assumptions, the PWI can be used to quantify CBF with a simple model. For details of how these equations are derived, the reader is referred to previous publications.[60,61]

Equation 1 is used for PCASL[60]:

$$CBF = \frac{6000*\lambda*(SIcontrol-SIlabel)*e^{\frac{PLD}{T1,blood}}}{2*\alpha*T1,blood*SIPD*(1-e^{\frac{\tau}{T1,blood}})}[ml100g/min] \qquad [1]$$

Equation 2 is used for QUIPSS II PASL[61]:

$$CBF = \frac{6000*\lambda*(SIcontrol-SIlabel)*e^{\frac{TI}{T1,blood}}}{2*\alpha*TI1*SIPD}[ml100g/min] \qquad [2].$$

The parameters for these equations are detailed in Table 3. Issues to be aware of are that SIPD usually is acquired in a different scan, intensity scaling sometimes needs to be applied to accurately calculate CBF, and different scanner manufacturers handle the global scaling factor differently. The reader is referred to Appendix D in the supplemental information online for a description of how to apply it.

Partial volume effects

The spatial resolution achievable with ASL in current scanners is of the order of 2–4 mm in the plane of acquisition and 3–5-mm slice thickness. Accordingly, many voxels will comprise a mixture of gray, white, and CSF components. GM and WM have different perfusion characteristics, and CSF presents no flow determinable by ASL techniques. Compounded with this, disease and age will further alter the relative content of each component. For example, subjects with significant brain atrophy might present reduced cortical GM and enlarged ventricles compared with normal and/or younger subjects. Uncorrected CBF values for partial volume effects can result in artifactual differences if groups present different levels of atrophy.

There has been interest in properly quantifying CBF specifically in regions in which these mixtures are present. Partial volume effect corrections (PVCs) have been approached in a variety of forms, as described below. For more details and implementations, the reader is encouraged to review the cited literature.

Partial tissue volume content method The initial and most common PVC method for ASL[62,63] assumes a fixed ratio of CBF between GM and WM. It utilizes a high-resolution T1 image to create probability or fractional spatial maps of GM, WM, and CSF in each voxel. The low-resolution CBF map is then co-registered to the high-resolution tissue segmentation and the relative contribution of each one is determined. *However, t*his approach is susceptible to the assumption of fixed GM/WM CBF ratios and to the errors introduced by the segmentation and registration processes.[64]

Local linear regression A local linear regression approach assumes constant CBF for GM and WM voxels within a n^2 kernel around the voxel with partial volume effects using the information obtained from segmentation of anatomical image.[65] This approach produces CBF maps for GM and WM. However, it introduces significant spatial smoothing, reducing the ability to resolve anatomy.[44]

Spatially regularized correction To overcome smoothing introduced by the linear regression approach, a spatially regularized correction approach has been developed. This approach uses a formulation in which GM and WM CBF is subjected to spatial priors within a Bayesian inference scheme, which produces CBF maps that do not display the level of smoothness introduced in the linear regression approach. The method was initially developed for multi-PLD data sets,[44] but it has been shown to also work with single PLD data sets.[43]

Super-resolution reconstruction All the PVC methods described above are applied in the original resolution of the acquired images. *Although* reducing the effect of partial volume composition, they do not allow for an increase in the level of anatomical details in the image. However, super-resolution (SR) reconstruction approaches allow for the correction of partial volume effects and provide higher spatial resolution CBF maps.[66]

The application of deep learning (DL) techniques to increase spatial resolution has been making significant progress across different image modalities,[67] and DL methods for achieving high-resolution, high-SNR ASL have been a focus of recent research. When a large amount of training data is provided, these techniques have been widely used in ASL denoising, with great success.[68–71] Zheng *et al.* suggested a two-stage multi-loss SR network that improves both spatial resolution and SNR of ASL scans.[72] This method, similar to those of other supervised DL approaches, requires a large number of high-quality ASL images for training.

Review of existing software

Several ASL analysis packages (and likely many more in-house scripts and pipelines) exist to aid with the processing of ASL data, such as the aforementioned ASLtbx[57] and ExploreASL,[55] as well as CereFlow (www.transmri.com/cereflowtm) and OxASL (https://oxasl.readthedocs.io/en/latest/). The Open Science Initiative for Perfusion Imaging (OSIPI) inventory is a good resource for ASL pipeline inventory (https://docs.google.com/document/u/1/d/e/2PACX-1vQ-1GF2fmz6Q4IukuKP_-57H-

xi872Xq_uBlX5P0Cwpj4RYd_t73pvZ64UqXegPaVpQJhQQrVRJRPro/pub). Knowing which package to select and use for a particular study can be a daunting task and depends on many factors, such as: (i) *licensing:* is this product licensed to use for my study? Often licensing is free for academic studies, and either not licensed or licensed with a fee for commercial use; (ii) hardware/software: what hardware/software requirements are needed to run the ASL analysis package? Some analysis packages are limited to operating systems (Windows/Mac/Linux) and might require a minimum CPU, hard disk storage, or system memory; (iii) workstations/users: how many workstations and users can access the analysis software at any one time? Depending on the design of the product itself and the licensing agreement, the analysis package might be for a single workstation and single user, single workstation allowing multiple users, or a floating license that can be used on numerous workstations and for multiple users. This is an importance consideration when planning study throughput; (iv) processing capability: can the analysis package process my ASL data and provide the outputs that I require? Some analysis packages are limited to ASL data from certain vendors (e.g., Siemens/GE/Philips), certain 'flavors' of ASL (e.g., pCASL), or single-delay ASL processing; thus, it is important to check for desired processing capability before engaging with a particular analysis package; and (v) regulatory status: what regulatory clearance does the analysis package have and is it suitable for my study? For example, if ASL analysis results are to be submitted to the US Food and Drug Administration (FDA), then a product conforming to 21 CFR Part 11 compliance will be required.

The above checklist is a proposed starting point, which should be coupled with additional consideration of the details for ASL processing itself (i.e., features such as QC, motion correction, outlier detection, modeling, and partial volume correction), as well as the accuracy and reproducibility of the outputs provided by the analysis packages.

Application of machine learning for patient classification and disease definition

Artificial intelligence (AI) and machine learning (ML) approaches could eventually provide automated screening platforms and assist the diagnosis of various types of dementia. Several techniques have been developed for structural MRI scans of the brain that can show tissue loss associated with the disease.[73] However, it is known that the brain undergoes physiological changes before structural changes,[74] such as the regional hypoperfusion in patients with AD compared with healthy controls, as measured by ASL.[62] As a result, functional and ASL scans could be combined for the analysis of patient classification at an earlier disease stage. For example, Collij *et al.* developed a ML approach based on ASL CBF measures to distinguish between patients with AD and two early forms of dementia that can be precursors to AD, mild cognitive impairment (MCI) and subjective cognitive decline (SCD).[3] This approach distinguishes between AD and SCD with 90% accuracy and between AD and MCI with 82% accuracy.[3]

The application of DL approaches has not been investigated for ASL images. One reason why DL has not yet been applied to ASL could be the challenges that researchers face in acquiring the large number of data sets required for such an application. Implementation of the guidance outlined in this review could help to fill this gap by providing robust and reproducible acquisitions in multicenter clinical trials.

Discussion

In this review, we have detailed the flexibility and utility of ASL MRI for measuring CBF. However, ASL has limitations in its application. Knowledge of these limitations and how to identify, limit, and control them, is key to providing high-quality, interpretable, and meaningful results for a study. For example, the inherently low SNR of ASL MRI is a limitation, which can be improved by careful selection of ASL labeling and readout schemes. The lack of widely used ASL phantoms is also a limitation, because, without phantoms, the precise measurement error for a given combination of ASL sequence and system is unknown. Advances have been made in the ASL phantom; for example, a phantom called Quantitative Arterial Spin Labeling Perfusion Reference (QASPER) has been available for purchase since 2018 (www.goldstandardphantoms.com/products/qasper/). Our recommendation, if possible, is to establish in-house data on baseline CBF variability (test–retest) for a particular set-up on a given study. In research studies that include a control group and an experimental group that will receive an intervention or treatment that is expected to affect the measured outcome (e.g., CBF), the aforementioned test–retest data enable *a priori* calculation of the required magnitude of change to observe a target effect size and, in turn, to calculate required sample sizes.[29]

When exploring the central (i.e., cerebral) effects of interventions and treatments on CBF, any undesirable peripheral cardiovascular effects also need to be considered and potentially controlled. For example, compounds that alter heart rate, blood pressure, and arterial transit time might impact the measured CBF,[75] which is particularly important in single-delay ASL, because the measurement of CBF is established from a single snapshot in time. Capturing supplementary clinical data (e.g., heart rate or blood pressure), or performing simulation work, can assist in evaluating these changes and interpreting CBF results.[76] Additionally, a second compound could be co-administered to block the undesirable peripheral cardiovascular effects (e.g., co-administration of a low dose of the beta-blocker nadolol).[76–79] In this case, assessment of any central effects of each compound individually, as well as when co-administered, is essential.

Concluding remarks

ASL allows for the quantification of CBF and its utility in clinical trials is expanding, although remains hampered by the diversity of acquisition modalities and analysis approaches present within the clinical market. The QIBA is leading in developing a profile for ASL, which will help researchers and clinicians adopt standards in the process of acquiring, measuring, and interpreting CBF values through ASL. In addition, the commercial availability of the prescribed sequence based on the ASL White Paper as part of the latest software packages from each of the main

vendors will further reduce this heterogeneity. The lack of use of exogeneous contrast agents (e.g., gadolinium-based) and low risk associated with MRI renders ASL a promising tool for CBF measurement. As more PET radiotracers are developed to assess different mechanisms of neurodegeneration, ASL could provide a useful alternative to standard metabolic measures of blood flow obtained with FDG PET and $H_2[^{15}O]$ PET,[80] because use of ASL would reduce the radiation exposure for the subject. With improvement in MRI equipment, standardization of acquisition protocols, and development of analysis methodologies, ASL could become an important tool for assessing cerebral physiology in neurodegenerative conditions and treatments.

Acknowledgments

The authors would like to thank Kristen E. Murfin and Elizabeth Best for providing medical writing support. D.W. is a shareholder of Translational MRI LLC, which developed Cereflow software.

Declaration of interests

X.W., C.B., J.O'C., A.G., J.A., W.T., and L.B. are employees of Invicro, and this publication was supported by Invicro.

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Figure 1. Arterial spin labeling (ASL) mechanism. The difference (right) between the control (left) and labeled (middle) image determines the ASL. In the labeled images, blood water protons (red arrows) outside of the imaging volume (blue box) are labeled by magnetic inversion (negative magnetization). After a period of time, generally referred to as the 'inflow time', the image acquisition begins. The inflow time is long enough so that the labeled blood water flows into the imaging volume and mixes with the (positively magnetized) static tissue water in the parenchyma (blue arrows within the blue box). This results in a net reduction of the parenchymal magnetic resonance imaging (MRI) signal in this 'labeled' image. A relative measure of perfusion can be calculated by acquiring an unlabeled (control) image (note that in control image the blood water protons are not inverted) and calculating the signal difference compared with the labeled image. The static tissue signal from control and labeled image will be cancelled in the difference image, and the net difference is related to cerebral blood flow.

Figure 2. The two most common labeling approaches for arterial spin labeling (ASL): pseudo-continuous ASL (PCASL) and pulsed ASL (PASL). For PCASL, many radiofrequency (RF) pulses are applied in rapid succession (over the order of milliseconds) and for a relatively long overall duration (1–3 s), to invert and label arterial blood water as it flows through a relatively thin labeling plane **(a)**. By contrast, PASL typically uses a single RF pulse applied for a shorter total duration (e.g., 10–20 ms) to simultaneously invert arterial water in a thick labeling slab **(b)**.

Table 1. Main imaging techniques for measuring brain perfusion for clinical research[a]

Feature	Imaging technique		
	ASL	**FDG PET**	**DSC**
Contrast	None (endogenous contrast)	Radioisotope	Gadolinium-based contrast
Spatial resolution	2–4 mm (in plane) and 4 mm (slice thickness)	4–6 mm	2 mm
Radiation	None	Yes	None
Acquisition time	5–10 min	5–10 min (plus uptake time)	2 min
Parameters assessed	CBF, ATT	Glucose metabolism	CBV, CBF, MTT
Limitations	Can require separate license/agreement for certain ASL sequences More efforts on standardization needed (variety of ASL sequences available)	Radiation dose limit per year Tracer dose logistics (tracer needs to be used with certain time to avoid decay in radioactivity)	Patients can have acute adverse reactions to contrast agent and potential long-term gadolinium retention in brain Can affect accuracy of other scans following gadolinium injection
Subject selection		Not for pediatric patients	Not for subjects with history of previous contrast reaction, asthma, or renal problems
Patient preparation	Avoid caffeine and drugs that affect perfusion	Fasting 4–6 h before scan to reduce glucose level	

[a]Abbreviations: CBV, cerebral blood volume; MTT, mean transit time.

Table 2. Summary of ASL test–retest studies

Author (year)	Study population	Interval	site/sequences	ICC for CBF	Refs
Petersen *et al.* 2010	284 healthy volunteers	2 weeks on average (13±10 days)	28 sites, QUASAR	GM (range: 0.07~0.81 in all sites)	29
Chen *et al.* 2011	12 young healthy subjects	Up to 1 week	Single site, 3T, PCASL, PASL	GM/WM is 0.911/0.877 (PCASL); 0.835/0.825 (PASL)	27
Kilroy *et al.* 2014	13 older MCI:6, mild AD:1	~4 weeks	3D GRASE pCASL, 2D EPI pCASL	0.707 (GRASE pCASL), 0.362 (EPI pCASL) across 24 ROIs	30

Hodkinson *et al.* 2013	16 healthy male volunteers (age range: 18–50 years)	5 sessions, separated by <1 week or 2–4 weeks	Single site, 3T, PCASL	Inter- and intrasession reliability of postsurgical pCASL CBF measurements ICC >0.6, between pre- and postsurgical states ICC >0.4	[31]
Lin *et al.* 2020	20 adult volunteers (age 56.6±17.2 years)	1 h	3D pCASL with standard (1500 ms) and long (3500 ms) delay	Precentral cortex (0.84 in left and 0.81 in right)	[32]
Jann *et al.* 2021	45 older Latinx subjects	~6 weeks	3D pCASL	Whole brain 0.84, WM 0.77,	[33]
Cohen *et al.* 2020	52 healthy, male subjects	4 sessions over 45 days	3D pCASL with Hadamard-Encoded multiple PLD, (seven PLDs from 1.0–3.7 s)	ICC CBF (0.75, 0.66,0.72, 0.72 for Day 7, 14, 30, 45, respectively) ICC ATT (0.69, 0.57, 0.55, 0.49 for Day 7, 14, 30, 45, respectively)	[34]
Ssali *et al.* 2021	13 healthy controls and eight patients with FTD or progressive supranuclear palsy	1 month	pCASL	0.5~0.8 for controls, 0.24~0.75 for patients, <0.4 for amygdala and temporal pole	[28]
Binnie *et al.* 2022	54 older adults with small vessel disease (mean age: 66.9 years)	>7 days	Multisite pCASL PASTIS (trial)	GM: 0.771, deep GM: 0.872	[35,36]
Wang *et al.* 2021	3D-printed perfusion phantom	One session per week for 5 weeks	2D pCASL with 20 PLDs	ICC=0.96 (95% CI 0.83–1.00)	[37]

Table 3. Parameters for calculation of CBF

Parameter	Value	Notes
λ (blood–brain partition coefficient)	0.9 ml/g	
T1, blood	3 T/1.5 T: 1650/1350 ms	
SI control–SI label	Average signal intensity in control–label subtraction (i.e., PWI)	If order of control–label is wrong, perfusion value will be negative
SI PD	Intensity in PD image	When acquired separately from control/label scan, rescaling is needed for accurate quantification
TI (Inversion time)		For 2D multi-slice acquisition, TI value needs to be adjusted for each slice to take into account time delay between slices; no need for 3D acquisition
α (labeling efficiency)	PCASL: 0.85; PASL: 0.98	
Background suppression efficiency	GE 3D spiral: 0.75 (from [81]) Philips 2D EPI or Siemens 3D GRASE: 0.83 Philips 3D GRASE: 0.81	When background suppression is applied, overall efficiency is combination of both inversion efficiency and background suppression efficiency
PCASL, PLD (post-labeling delay)		Extract from DICOM header/protocol, for details please refer to the supplemental information online For multiple PLD sequences there will be multiple PLD values
PASL TI1, TI		
τ (tau)	Label duration	