Multi-delay ASL perfusion imaging: impact of modeling dispersion and interaction with denoising strategies and pathology

Sara Monteiro[1], Joana Pinto[2], Michael A. Chappell[3], Ana Fouto[1], Miguel V. Baptista[4], Pedro Vilela[5], Patricia Figueiredo[1]

[1] Institute for Systems and Robotics - Lisboa and Department of Bioengineering, Instituto Superior Técnico – Universidade de Lisboa, PT; [2] Institute of Biomedical Engineering, Department of Engineering Science, University of Oxford, UK; [3]Sir Peter Mansfield Imaging Centre, School of Medicine, University of Nottingham, UK; [4] Neurology Department, Hospital Egas Moniz, PT; [5] Imaging Department, Hospital da Luz, PT

Corresponding author:

Sara Monteiro

Institute for Systems and Robotics - Lisboa (ISR-Lisboa)
Instituto Superior Técnico, Universidade de Lisboa
Av. Rovisco Pais, 1, 1049-001 Lisboa, Portugal

Email:sarapmonteiro@tecnico.ulisboa.pt

Abstract

Arterial Spin Labeling (ASL) acquisitions at multiple post-labeling delays allow for appropriate kinetic models to be fitted to the data, potentially providing more accurate quantification of cerebral blood flow (CBF) together with other perfusion-related parameters such as the arterial transit times (ATT) or arterial cerebral blood volume (aCBV) if an extended kinetic model is used. Additionally, dispersion of the labelled bolus is a physiological aspect often neglected when modelling ASL signals that might also provide information of interest and improve perfusion quantification. Besides the choice of an appropriate kinetic model, it is also imperative that the data should have sufficient SNR to allow fitting of increasingly complex models.

In this work, we investigated the impact of two denoising strategies, repetition averaging and independent component analysis (ICA), in combination with modeling dispersion effects on multi-delay ASL measurements acquired from a group of small vessel disease (SVD) patients and a group of healthy controls. We found that repetition averaging interacted with modeling dispersion and impacted the estimation of CBF and aCBV, mostly in arterial locations. Moreover, keeping the repetitions without averaging increased the model's free energy, as did ICA denoising and dispersion modeling. These results indicate that including all control-label repetitions rather than averaging provides better noise estimation and hence better model fitting, with special impact on earlier (noisier) time points and hence the estimation of arterial (macrovascular) contributions. I Repetitions averaging and dispersion modelling also interacted with group, such that we found apparently greater CBF in arterial regions of patients but only when averaging and not accounting for dispersion. These findings highlight the importance of modeling dispersion with appropriate noise estimation in pathology.

Keywords: Arterial Spin Labeling, Kinetic Modeling, Independent Component Analysis, Dispersion, Denoising

Introduction

Arterial Spin Labelling (ASL) is a non-invasive technique that uses arterial water as an endogenous tracer, by applying radiofrequency (RF) pulses to selectively invert the magnetization of the spins in the neck region [1]. After a certain post-labelling delay (PLD), an image is acquired comprising signal from the labelled water protons that reached the imaging region and the stationary water protons. Another image is then separately acquired without labelling but with identical RF to control for off-resonance effects [2]. The magnetization difference between these two images (with and without tagged blood) represents the blood conveyed to the brain by perfusion.

One of the main drawbacks of the ASL technique is its inherently low signal to noise ratio (SNR) since the expected difference image is about 1% of the total magnetization in the human brain at typical field strengths. To overcome this limitation, several techniques have been proposed to improve SNR including. acquiring several repetitions of label-control difference images and averaging these. Nevertheless, this approach is associated with increased acquisition time which is not always feasible in clinical settings and may be associated with an increase in motion artefacts [3].

Single PLD acquisitions with delays longer than the time the tracer takes from the labelling region to the brain (Arterial Transit Time, ATT) are commonly used to obtain CBF images [1]. However, this approach does not grant estimation of ATT, neither does it give insight into possible abnormal ATTs in specific physiological and/or pathological conditions ,which in turn may promote inaccuracies in CBF quantification [1]. Several authors have investigated the impact of using multiple PLDs and different labelling durations [4]–[7]. One clear benefit of multi-PLD imaging is that it allows for a kinetic model to be fitted to the ASL signal, being less dependent on temporal assumptions and potentially providing more accurate CBF estimates along with other hemodynamic parameters such as ATT which may also provide relevant information on the arterial blood supply to the brain [8], [9].
Another factor to consider in multi-delay ASL is the dispersion of the labelled bolus. This occurs during the time it takes for the labelled bolus to reach the brain tissue of interest and it is caused by a combination of factors that alter the flow of the tagged water within the blood [10]. Although the effects of flow dispersion in ASL might be reduced when compared to techniques that require the use of contrast agents due to the proximity of the labelling region to the measurement place, it still occurs and should be accounted for. Indeed, it has been shown that the dispersion of the labelled bolus has a significant impact on the ASL signal [11]. This is particularly important when using extended kinetic models to account for macrovascular contributions on data acquired without macroflow suppression, additionally providing estimates of the arterial cerebral blood volume (aCBV) [9]. Although the standard kinetic model assumes no dispersion of the labelled bolus, the combined effects of the flow profile in the feeding arteries and the different arrival times from different paths have been shown to significantly impact ASL signals [12].

Besides the choice of an appropriate kinetic model, it is imperative that the data should have sufficient SNR to allow fitting such increasingly complex models. Several denoising strategies can be applied as post-processing steps to compensate for the low SNR of the ASL signal [13]. One common approach consists of averaging multiple repetitions of control-label difference images. Recently, independent component analysis (ICA) has also been proposed to separate the ASL signal of interest from artefacts or other structured noise sources (e.g. head motion or susceptibility artefacts),.increasing SNR of ASL data of up to 50% when compared to data without ICA clean-up [14].

In this work, we aim to study the influence of two different denoising strategies (repetition averaging and ICA clean-up), together with modeling dispersion effects, on multi-delay ASL perfusion imaging in a group of cerebral small vessel disease (SVD) patients as well as in healthy individuals. The impact of different denoising strategies and modeling dispersion will be assessed in terms of CBF, ATT and aCBV

estimates and respective variances, as well as by model free energy (FE) in two different regions of interest (ROI): GM and arterial (aCBV).

2. Methods

2.1 Data acquisition

Data were acquired from 17 SVD patients (SVD, 50+/-9 yrs) with two different SVD specificities: Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopaty (CADASIL) and sporadic SVD (sSVD). The control group consisted of 12 age-matched healthy controls without any medical records of SVD (CTRL, 52+/-8 yrs). To be included in this study, patients had to follow the subsequent criteria: 1) being independent on a daily basis, which was evaluated by the Instrumental Activities of Daily Living (IADL) scale; lack of large vessel disease, as assessed by Doppler ultrasound; 2) for the sSVD group: evidence of SVD in MRI (deep WMH injuries, with no other reasonable justification, with moderate and severe degrees, in accordance to the Fazekas metric [15]; 3) for the CADASIL group: patients with symptoms and with indication of WMH lesions as well as molecular confirmation (mutation on the NOTCH3 gene). The criteria for exclusion were: 1) impossibility to perform MRI; 2) for the patients: existing WMH lesions from other previously identified pathologies; existence of chronic debilitating illnesses; stroke in the past three months; illiteracy; and impaired visual acuity. An experienced neurologist was assigned to evaluate all patients to detect and exclude other neurological disorders and to perform the necessary assessments. This study was approved by the Ethics Committee of Hospital da Luz, and all participants gave written informed consent in compliance with the Declaration of Helsinki.

The participants were scanned on a 3T Siemens Verio scanner (Siemens Healthcare, Erlangen, Germany) using a 12-channel radiofrequency receive head coil. Multi-delay pulsed ASL (PASL) data were acquired using a PICORE-Q2TIPS sequence [16] with 2D-multi-slice Gradient Echo Echo-Planar Imaging (EPI) readout (TR/TE = 2500/11 ms, 28 slices with 3.5x3.5x5.0 mm^3 resolution). Multiple post-labelling delays were sampled using 11 TI_2 values ranging from 400 to 2400 ms, in steps of 200 ms, with 8 control-label repetitions each. The Q2TIPS module allowed limiting the labeling to a maximum of 750 ms by adjusting TI_1 and TI_{1s} for each TI_2: for TI_2 < 1000 ms, $TI_1 = TI_{1s} = TI_2$ - 25 ms; and for TI_2 > 1000 ms, TI_1 = 750 ms and TI_{1s} = 900 ms. A reference image was acquired from each subject for calibration: proton density image with the same readout as the ASL image but with a longer TR (TR = 10 s) [17]. A Magnetization-Prepared Rapid Gradient-Echo (MPRAGE)-T1-weighted (T1WI) structural image (1mm isotropic resolution) was also obtained for registration and segmentation purposes.

2.2 Data analysis

All image analysis was performed using FSL (fsl.fmrib.ox.ac.uk) and MATLAB (2020a, http://mathworks.com).

2.2.1 Pre-processing

For each dataset, motion correction was performed using MCFLIRT [18] to align ASL control and label images. Control-label difference image time series were computed for each TI_2. MPRAGE structural images were segmented using FAST [19] to obtain brain tissue masks for gray matter (GM), white matter (WM) and cerebrospinal fluid (CSF) based on the respective partial volume estimate (PVE) maps. The FLIRT tool [18] was used to perform registration between perfusion (ASL) and structural (MPRAGE) spaces using a linear transformation. Additionally, the FNIRT tool [18] was used to perform registration between structural (MPRAGE) and standard (MNI152-2mm standard brain) spaces.

2.2.1 ICA denoising

For each ASL dataset, the control and label magnetization difference images (ΔM) underwent single-subject spatial-ICA using the Multivariate Exploratory Linear Optimised Decomposition of Independent Components (MELODIC) tool [20]. Noise-related Independent Components (ICs) were visually identified by one blinded rater following two criteria, one more conservative than the other:

i) *ICA level 1 (ICA1)*: only ICs related to motion, susceptibility and multiband artefacts, mismatching the expected spatial and temporal properties of the ASL perfusion signal;
ii) ICA *level 2* (*ICA2*): all ICs not consistent with the expected spatial and temporal properties of the ASL perfusion signal.

A representative sample of the ICs selected in *ICA level 2* and *ICA level 1* is presented in supplementary Figure 1. The selected noise ICs were then regressed out from the ΔM time series data using FIX (ref). The two ICA denoising approaches were compared to not performing any ICA denoising (*noICA*, default), in terms of voxelwise percent change in the temporal standard deviation (%ΔSD) of the time series [14][21], defined as:

$$\%\Delta SD = \frac{SD(\Delta M_{NoICA}) - SD(\Delta M_{ICAi})}{SD(\Delta M_{NoICA})} \times 100 \qquad \text{Eq.1}$$

where SD represents the voxelwise standard deviation of the ΔM image time series, and ICAi represents *ICA1* or *ICA2* Each map was then registered to standard space and averaged across subjects in each group.

2.2.2 Repetition averaging

After denoising, two repetition averaging options were considered for the ΔM image time series of each ASL dataset: i) averaging the 8 repetitions for each TI_2 (*averaging*: *11-volumes, default)*); and ii) not performing averaging (*no averaging*: 8x11=*88-volumes*).

2.2.3 Kinetic modeling

An extended kinetic model including a macrovascular component, was then fit to the data. Two different options were considered regarding dispersion effects of the arterial bolus: i) without dispersion (*no disp, default*) - default; and ii) with dispersion described by a gamma kernel (*disp*) [12].

For each denoising strategy (ICA denoising and repetition averaging) and kinetic model dispersion option, the model was fitted to the data using BASIL and the following parameters were estimated in each voxel: CBF, ATT, ATTb (blood arterial transit time), and aCBV [22]. This Bayesian inference method incorporates prior information, which has been demonstrated to facilitate more accurate estimates of multiple model parameters [22]. Maps of estimation variance were obtained for each parameter, as well as maps of model free energy (FE). This parameter reflects the quality of the model fit, taking into consideration model complexity (i.e., the number of degrees of freedom). Specifically, the nearer to 0, the greater is the model's ability to explain the data [22].

To obtain the estimated CBF and aCBV parameters in absolute units, voxelwise calibration was performed. This was achieved by estimating the magnetization of the arterial blood (M_{0b}) based on the tissue magnetization (M_{0t}). The M_{0t} map was generated through the acquisition of a proton density image with the same readout as the ASL image but with a longer TR [17]. Correction for voxelwise T1 relaxation at the acquisition TR was also performed . The M_{0b} is then obtained by normalization, using the tissue's specific partition coefficient (λ) as well as the differences in transverse relaxation between tissue and blood (T2*). In theory, there is a single value of λ_t for each voxel due to the variety of tissues, resulting in partial volume estimates (PVEs). Therefore, λ_t can be considered a PVE-corrected average of λ.

2.3 Statistical analysis

Statistical analysis was performed using MATLAB and IBM SPSS Statistics (version 26.0.0, https://www.ibm.com) to assess the effects of the ICA denoising (ICA), repetition averaging (Averaging) and dispersion modeling (Dispersion), as well as their interactions with each other and with subject group (Group), on perfusion estimation. Firstly, a region-of-interest (ROI) analysis was performed taking all factors into account. Based on significant effects identified in this ROI analysis, voxelwise analyses were then performed to further assess their spatial distribution.

2.3.1. ROI analysis

An analysis based on two different ROIs was performed: i) gray matter (GM), created for each subject based on the respective PVE map using a threshold of >70%; and ii) arterial blood (Arterial), created for each subject and each combination of analysis options (ICA, Averaging and Dispersion), based on the estimated aCBV map using a threshold of 0.1%.

Averages of the following quantities were obtained in each ROI, for each subject and each combination of analysis options (ICA, Averaging and Dispersion):

- Values of estimated parameters (CBF, aCBV, ATT and ATTb)
- Variances of estimated parameters (Var_CBF, Var_aCBV, Var_ATT and Var_ATTb)
- Model free energy (FE)

For each ROI and quantity, a 3-way repeated measures ANOVA was performed to assess the effects of within-subjects factors ICA (NoICA, ICA level 1, ICA level 2), Averaging (no averaging, averaging) and Dispersion (no disp, disp), and between-subjects factor Group (CTRL, SVD). For significant interactions, post-hoc pairwise t-tests were performed.

2.3.2. Voxelwise analysis

Given that significant interactions were found between several factors for estimated parameters CBF, aCBV and ATT (as shown in Results, section 3.2), voxelwise analysis were performed separately for each factor, using permutation testing as implemented by the FSL tool Randomise [18]. In each case, maps of percent change in each parameter estimate between different analysis options were computed for each subject and averaged across subjects in each group.

3. Results

3.1 ICA denoising

The number of independent components (ICs) estimated by principal component analysis before ICA was 28 ± 5, ranging from 21 to 45. The ICA *level 1* approach resulted in the identification of 43±7% / 36±8% noisy ICs in patients / controls, whilst ICA *level 2* generated 65±9% / 54±10% noisy ICs in patients / controls. The maps of percent change in ASL control-label temporal SD with ICA denoising are presented in Supplementary Figure 1 (top), showing evident effects on the edges and in the central part of the brain. These were larger for ICA level 2 than ICA level 1, as expected, achieving reductions of up to 30%. Interestingly, greater reductions were observed in patients compared with controls, with differences of up to +/-10%, as can be seen in Supplementary Figure 2 (bottom). This is consistent with the fact that a larger number of ICs was classified as noise and removed in patients relative to controls.

When looking at the effect of ICA on the model's FE, the voxelwise maps of No ICA vs. ICA level 2 reveal that ICA denoising has a greater impact on the FE of Patients when compared to Controls and on the data that was not averaged when compared to the averaged data, being apparently independent on the dispersion model implemented (Figure 1). This highlights the positive impact of denoising ASL data on the goodness of the model fit, especially in pathology.

3.2 ROI analysis

The distributions across subjects of the GM and Arterial ROI average estimates of CBF, aCBV, ATT and ATTb, for each condition of ICA denoising, repetition averaging and dispersion modeling, and for each group (Controls and Patients), are presented in Figure 2.

The most consistent finding across all estimated parameters was a significant (or close to significant) interaction between Averaging and Dispersion in both ROIs. A significant (or close to significant) main effect of Averaging was also found for all parameters in both ROIs (except ATT in GM). Effects of Dispersion were found significant for all parameters in GM, but its effects in the Arterial ROI strongly depended on averaging and group.

, modeling dispersion reduced CBF in the arterial region; however, this was only verified when no averaging was performed. This interaction may be explained by the fact that averaging resulted in significantly reduced aCBV estimates in this area, implying that it may have compromised crucial information in the early time points for the estimation of the macrovascular contributions. Indeed,] an increase in aCBV was also only found when not averaging. Consistently, arterial transit times also exhibited effects of dispersion in the Arterial ROI only when not averaging, with reduced ATT and increased ATTb.

Most interestingly, a significant triple interaction between Averaging, Dispersion and Group was found for CBF and aCBV (close to significant for ATT and ATTb) in the Arterial ROI. Indeed, an apparently greater CBF was found in SVD vs. CTRL, but only when not modeling dispersion or when averaging; this difference disappeared when using all 88 volumes without averaging and modeling dispersion. This effect is accompanied, and may be explained by, a greater aCBV in the Arterial ROI for SVD vs. CTRL in the same conditions (with 88 volumes and dispersion). Moreover, consistent trends were also found for the transit times, with the arterial regions of patients showing greater reduction of ATT (consistent with reduced CBF estimate), making them comparable with controls, and greater increase in ATTb (consistent with increased aCBV estimate), suggesting slower blood velocity relative to controls.

In GM, estimates of CBF, aCBV and ATTb significantly decreased with Averaging, in interaction with Dispersion. Remarkably, despite the main effect of Dispersion, aCBV only increased with dispersion without averaging, once more suggesting that aCBV could only be accurately estimated under these circumstances. Main effects of ICA denoising were found for ATTb in GM, with trends also for aCBV and ATT.

ATTb estimates tend to be decreased with dispersion in the aCBV ROI although no main effects were found. However, in the GM ROI both averaging, dispersion and ICA affect the ATTb estimations: higher ATTb is obtained when not averaging and modeling dispersion. Only in this case, significant differences were also found between Groups – suggesting that blood velocity is reduced in patients - once more suggesting that apparent changes in CBF estimates are intrinsically related with ATTb estimates and that physiological changes are more likely to be captured with dispersion. In the arterial ROI, there were significant interactions among these covariates and Group: when averaging, the perfusion estimates were higher in patients than controls.

The distributions across subjects of the GM and Arterial ROI average parameter estimate variances and model FE are displayed in Figure 3. CBF, ATT and ATTb estimation variances were significantly (or close to significantly) reduced with ICA denoising in both ROIs, indicating an improvement in estimation precision in agreement with a previous report [14]. Consistently, better model fitting as evidenced by increased FE was obtained with ICA denoising (in the GM ROI). Moreover, averaging drastically increased FE in both ROIs, probably because of the difference in degrees of freedom between averaging (11 volumes) and not averaging (88 volumes).

Interestingly, we found that modeling dispersion significantly (or close to significantly) reduced the variance of all parameter estimates in both regions (except aCBV in the Arterial ROI), once more suggesting that accounting for bolus dispersion may provide more precise perfusion measurements. The variance reduction was particularly large for ATTb in the Arterial ROI. Accordingly, modeling dispersion increased FE (close to significance), but only in the Arterial ROI, indicating that this is more crucial in regions with larger macrovascular contributions. Interactions between ICA and Averaging and/or Dispersion were systematically observed, indicating that the precision of dispersion model estimates depends on appropriate denoising strategies.

3.3 Voxelwise analysis

Since ICA denoising generally improved model fitting and parameter estimation, by increased FE and decreasing estimation variance, while leaving the values of the parameter estimates largely unaffected, we proceed with the analysis of the datasets that underwent the ICA level 2 denoising pipeline. Given the effects of Averaging and Dispersion, and their interactions with each other and with Group, we present voxelwise analysis of the effects of these two factors for each combination of the other factors (with ICA level 2): effects of Dispersion in Fig.4 and effects of Averaging in Fig.5.

The voxelwise analysis revealed significantly greater aCBV when including dispersion in the model in regions that agree with the expected arterial signal location, but only without averaging (Fig. 4). The area of significant differences was greater for patients compared with controls, with 47138 and 16577 voxels, respectively. These effects are consistent with the Arterial ROI analysis of aCBV; however, they were only close to significant in this case, indicating a high degree of spatial specificity probably not accurately captured by our definition of the ROI. On the other hand, no significant effects were found for the other parameters, in contrast with the GM ROI analysis. This may be explained by the fact that these effects are relatively small but quite widespread, therefore gaining sensitivity through spatial averaging into ROIs.

Regarding the voxelwise comparisons between averaging approaches, significantly greater aCBV estimates were found when not averaging, but only when modeling dispersion, in apparently arterial regions (Fig. 5). As for Dispersion, the area of significant differences was greater for patients compared with controls, with 20714 and 16990 voxels, respectively. These effects are also consistent with the Arterial ROI analysis of aCBV. In contrast, when not modeling dispersion, significantly reduced aCBV values were estimated in a widespread region across the brain, which was not captured by the ROI analysis. This is probably due to the loss of spatial specificity when spatial averaging into the ROIs. Also only partly consistent with the ROI analysis, we found significantly greater ATT values across the brain, both with and without dispersion, but only in patients. This finding again highlights the importance of the spatial specificity of voxelwise analyses, whenever sensitivity is sufficient.

Discussion

We assessed the effects of modeling the label bolus dispersion, together with two denoising strategies (averaging of the control-label repetitions per PLD – Averaging, and Independent Component Analysis), on multi-delay ASL data collected from a group of patients with SVD and their healthy controls. We found that repetition averaging strongly interacted with modeling dispersion and significantly impacted the estimation of perfusion and macrovascular contributions, mostly in brain regions consistent with arterial locations. On the other hand, ICA denoising significantly improved model fitting and reduced the variance of parameter estimation, while not affecting the values of the parameter estimates.

ROI definition

The effects of dispersion modeling, repetition averaging and ICA denoising were analysed across two different ROIs, defined for each subject. The choice of ROI is crucial since it establishes the area of the brain being investigated for changes regarding the different post-processing options and model fitting in pathology. For this reason, we considered a global GM region, representative of the brain tissue from where an ASL signal is typically measured. Alongside, we created an Arterial ROI to specifically analyse regions where the ASL signal is more contaminated by macrovascular contributions, since the analysis options we investigated were expected to impact them mostly.

ICA denoising

The main disadvantage of ASL is that the image created is only 1% of the total magnetization, resulting in an intrinsically low SNR [23]. The use of ICA denoising in post-processing has been shown to successfully remove artefactual components and, thus increase the precision of the results [13], [17]. Manual IC classification has been considered the appropriate procedure and was used in this study following two selection criteria with different degrees of signal preservation [24]. Although the total number of IC's obtained was similar in both patient and control groups, for both ICA denoising levels (ICA level 2 and ICA level 1) about 10% more components were selected as noise in SVD patients when compared to healthy controls. This difference might be attributed to expected increased head motion in the patients group. Additional evidence that our denoising was effective was obtained by verifying that the location of the change in the ASL difference signal variance corresponded to the expected head motion locations, such as the brain rim and deep white matter (WM) around the ventricles (Supplementary Fig. 2).

ICA denoising did not significantly impact parameter quantification, but it had a positive influence on the quality of the model fitting as evidenced by increased model FE and decreased parameter estimate variances. Since an improvement was found also between ICA level 1 and ICA level 2, the latter was chosen to perform a more detailed voxelwise analysis of the other two factors, Averaging and Dispersion.

Interactions between repetition averaging and dispersion modeling

In the ROI analysis we found that repetition averaging interacted with modeling dispersion and impacted the estimation of perfusion (CBF) and macrovascular (aCBV) contributions, mostly in arterial locations. aCBV values systematically increased in both ROIs when no Averaging was performed and with dispersion modeling, probably due to the inclusion of signal that was wrongly assigned as CBF in other analysis. On the other hand, in the case of the Averaged dataset, the voxelwise analysis evidenced regions of decreased aCBV when including dispersion in the model which seem mostly artifactual (Figure 4). This outlines the increase in noise/artefactual components potentially arising from the averaging of the early time points. This aCBV underestimation might be related to the averaging of the earlier ATTs, which is very variable and may cause the mismatch of CBF for aCBV.

Moreover, keeping the repetitions without averaging increased the model's free energy, as did ICA denoising and dispersion modeling. These results indicate that including all control-label repetitions rather than averaging provides better noise estimation and hence better model fitting, with special impact on earlier time points and hence the estimation of arterial (macrovascular) contributions. In fact, averaging these noisier time points might provoke undesired outliers.

Interactions with pathology

Interestingly, averaging and dispersion also interacted with group, such that we found greater CBF in arterial regions of patients if averaging and not modeling dispersion. These findings may be explained by differences in dispersion between groups that manifests as apparent differences in CBF when not appropriately accounting for dispersion, highlighting the importance of modeling dispersion in pathology. Furthermore, differences observed in ATT between SVD and CTRL when averaging was not performed and dispersion was modelled once more suggest that this strategy is better at modeling the decreased blood velocities in large vessels expected with this pathology, which might be more prone to dispersion [25].

Regarding the impact of these options in pathology, SVD patients normally have severe alterations of their hemodynamic responses and vessel properties. Indeed, this disease is associated with higher vessel wall stiffness, which implies higher pulsatility of the vessels generated by the cardiac flow [26]. We hypothesize that this increased pulsatile energy will also be associated with greater dispersion [10], [27]. For this reason, hemodynamic parameter estimation in patients is more likely altered by the inclusion of dispersion in the model than healthy controls.

Limitations

Although the main goal of our work is to create a systematic and standardized way to analyse ASL data, some limitations should be considered. In this study, the use of PASL labelling rather than pCASL (the recommended approach according to the ASL White Paper [1]) might impact the reproducibility of the results. Furthermore, the 2D EPI readout implemented allows for shorter TR's and hence more time points, which is intrinsically related to better temporal resolution (essential for ICA denoising). However, it is not possible to implement ICA on a single delay 3D GRASE due to the insufficient time points. For this reason, our conclusions regarding the benefits of ICA denoising are not necessarily generalizable to studies using 3D readouts and background suppression. Moreover, alternative denoising methods should be considered such as the Outlier Removal tool of ExploreASL [28] that eliminates outliers and the Enable volume selection tool of Quantiphyse (https://eng.ox.ac.uk/quantiphyse/) that resorts to the removal of data time points. Compared to Quantiphyse, our approach does not exclude data, which can be seen as an advantage.
Furthermore, even though manual IC quantification has been extensively implemented [14], [29], it is dependent on the operator's expertise and very time-consuming. The possibility to automate this process should be contemplated, although it might be difficult due to the wide range of ASL acquisition protocols and scanners.

Conclusion

Our results suggest that the use of ICA denoising of multi-PLD ASL data to improve the quality of CBF and ATT estimates, and further suggest that it may differentially affect data collected from patients and controls, significantly influencing the statistical analysis of group differences in perfusion related parameters. ICA denoising appears to have more impact in the patient group, most likely because these are more prone to artifacts such as head motion. The analysis and comparison of various ASL post-processing strategies, along with the different kinetic modeling options, offers understanding

into their usability to studies related to ASL and haemodynamic parameter estimation. This study highlightsthe great discrepancy in ASL results when following different pre-processing and modeling approaches, which will be of great relevance in the analysis of future ASL datasets.

Acknowledgements

Refs

[1] D. C. Alsop *et al.*, "Recommended Implementation of ASL Perfusion MRI for Clinical Applications," *Magn. Reson. Med.*, vol. 73, no. 1, pp. 102–116, 2015.

[2] R. B. Buxton, *Introduction to functional magnetic resonance imaging - principles and techniques*, 2nd ed. New York: Cambridge University Press, 2009.

[3] M. Grade, J. A. Hernandez Tamames, F. B. Pizzini, E. Achten, X. Golay, and M. Smits, "A neuroradiologist's guide to arterial spin labeling MRI in clinical practice," *Neuroradiology*, vol. 57, no. 12, pp. 1181–1202, 2015, doi: 10.1007/s00234-015-1571-z.

[4] W. Dai, P. M. Robson, A. Shankaranarayanan, and D. C. Alsop, "Reduced resolution transit delay prescan for quantitative continuous arterial spin labeling perfusion imaging," *Magn. Reson. Med.*, vol. 67, no. 5, pp. 1252–1265, May 2012, doi: 10.1002/mrm.23103.

[5] D. J. J. Wang *et al.*, "Multi-delay multi-parametric arterial spin-labeled perfusion MRI in acute ischemic stroke - Comparison with dynamic susceptibility contrast enhanced perfusion imaging," *NeuroImage Clin.*, vol. 3, pp. 1–7, 2013, doi: 10.1016/j.nicl.2013.06.017.

[6] M. Mezue, A. R. Segerdahl, T. W. Okell, M. A. Chappell, M. E. Kelly, and I. Tracey, "Optimization and reliability of multiple postlabeling delay pseudo-continuous arterial spin labeling during rest and stimulus-induced functional task activation," *J. Cereb. Blood Flow Metab.*, vol. 34, no. 12, pp. 1919–1927, 2014, doi: 10.1038/jcbfm.2014.163.

[7] A. Tominaga, M. Sasaki, and R. Shirane, "Clinical Benefits of Multiple Post-Labeling Delay Pseudo-Continuous Arterial Spin Labeling (Multi-PLD PCASL) in Pediatric Patients with Moyamoya Disease," no. 73, pp. 29–40, 2019.

[8] P. M. Figueiredo, S. Clare, and P. Jezzard, "Quantitative perfusion measurements using pulsed arterial spin labeling: Effects of large region-of-interest analysis," *J. Magn. Reson. Imaging*, vol. 21, no. 6, pp. 676–682, 2005, doi: 10.1002/jmri.20329.

[9] M. A. Chappell, A. R. Groves, B. J. MacIntosh, M. J. Donahue, P. Jezzard, and M. W. Woolrich, "Partial volume correction of multiple inversion time arterial spin labeling MRI data," *Magn. Reson. Med.*, vol. 65, no. 4, pp. 1173–1183, 2011, doi: 10.1002/mrm.22641.

[10] D. Gallichan and P. Jezzard, "Modeling the effects of dispersion and pulsatility of

blood flow in pulsed arterial spin labeling," *Magn. Reson. Med.*, vol. 60, no. 1, pp. 53–63, 2008, doi: 10.1002/mrm.21654.

[11] W. C. Wu, Y. Mazaheri, and E. C. Wong, "The effects of flow dispersion and cardiac pulsation in arterial spin labeling," *IEEE Trans. Med. Imaging*, vol. 26, no. 1, pp. 84–92, 2007, doi: 10.1109/TMI.2006.886807.

[12] M. A. Chappell, M. W. Woolrich, S. Kazan, P. Jezzard, S. J. Payne, and B. J. MacIntosh, "Modeling dispersion in arterial spin labeling: Validation using dynamic angiographic measurements," *Magn. Reson. Med.*, vol. 69, no. 2, pp. 563–570, 2013, doi: 10.1002/mrm.24260.

[13] S. Moia *et al.*, "ICA-based denoising strategies in breath-hold induced cerebrovascular reactivity mapping with multi echo BOLD fMRI," *Neuroimage*, vol. 233, 2021, doi: 10.1016/j.neuroimage.2021.117914.

[14] D. Carone *et al.*, "ICA-based denoising for ASL perfusion imaging," *Neuroimage*, vol. 200, no. April, pp. 363–372, 2019, doi: 10.1016/j.neuroimage.2019.07.002.

[15] F. Fazekas, J. Chawluk, A. Alavi, H. Hurtig, and R. Zimmerman, "MR signal abnormalities at 1.5 T in Alzheimer's dementia and normal aging," *Am. J. Roentgenol.*, vol. 149, no. 2, pp. 351–356, Aug. 1987, doi: 10.2214/ajr.149.2.351.

[16] W. M. Luh, E. C. Wong, P. A. Bandettini, and J. S. Hyde, "QUIPSS II with thin-slice TI1 periodic saturation: A method for improving accuracy of quantitative perfusion imaging using pulsed arterial spin labeling," *Magn. Reson. Med.*, vol. 41, no. 6, pp. 1246–1254, 1999, doi: 10.1002/(SICI)1522-2594(199906)41:6<1246::AID-MRM22>3.0.CO;2-N.

[17] J. Pinto *et al.*, "Calibration of arterial spin labeling data—potential pitfalls in post-processing," *Magn. Reson. Med.*, vol. 83, no. 4, pp. 1222–1234, 2020, doi: 10.1002/mrm.28000.

[18] M. Jenkinson, C. F. Beckmann, T. E. J. Behrens, M. W. Woolrich, and S. M. Smith, "FSL," vol. 62, pp. 782–790, 2012, doi: 10.1016/j.neuroimage.2011.09.015.

[19] Y. Zhang, M. Brady, and S. Smith, "Segmentation of brain MR images through a hidden Markov random field model and the expectation-maximization algorithm," *IEEE Trans. Med. Imaging*, vol. 20, no. 1, pp. 45–57, 2001, doi: 10.1109/42.906424.

[20] C. F. Beckmann and S. M. Smith, "Probabilistic Independent Component Analysis for Functional Magnetic Resonance Imaging," *IEEE Trans. Med. Imaging*, vol. 23, no. 2, pp. 137–152, Feb. 2004, doi: 10.1109/TMI.2003.822821.

[21] D. Carone, R. Licenik, S. Suri, L. Griffanti, N. Filippini, and J. Kennedy, "Impact of automated ICA-based denoising of fMRI data in acute stroke patients," *NeuroImage Clin.*, vol. 16, no. January, pp. 23–31, 2017, doi: 10.1016/j.nicl.2017.06.033.

[22] M. A. Chappell, A. R. Groves, B. Whitcher, and M. W. Woolrich, "Variational Bayesian inference for a nonlinear forward model," *IEEE Trans. Signal Process.*, vol. 57, no. 1, pp. 223–236, 2009, doi: 10.1109/TSP.2008.2005752.

[23] T. T. LIU and G. G. BROWN, "Measurement of cerebral perfusion with arterial spin

labeling: Part 1. Methods," *J. Int. Neuropsychol. Soc.*, vol. 13, no. 03, May 2007, doi: 10.1017/S1355617707070646.

[24] F. De Martino *et al.*, "Classification of fMRI independent components using IC-fingerprints and support vector machine classifiers," *Neuroimage*, vol. 34, no. 1, pp. 177–194, Jan. 2007, doi: 10.1016/j.neuroimage.2006.08.041.

[25] W. H. Lin, Q. Hao, B. Rosengarten, W. H. Leung, and K. S. Wong, "Impaired neurovascular coupling in ischaemic stroke patients with large or small vessel disease," *Eur. J. Neurol.*, vol. 18, no. 5, pp. 731–736, May 2011, doi: 10.1111/j.1468-1331.2010.03262.x.

[26] I. Makedonov, S. E. Black, and B. J. MacIntosh, "BOLD fMRI in the White Matter as a Marker of Aging and Small Vessel Disease," *PLoS One*, vol. 8, no. 7, pp. 1–9, 2013, doi: 10.1371/journal.pone.0067652.

[27] J. J. M. Zwanenburg and M. J. P. Van Osch, "Targeting cerebral small vessel disease with MRI," *Stroke*, vol. 48, no. 11, pp. 3175–3182, 2017, doi: 10.1161/STROKEAHA.117.016996.

[28] H. J. M. M. Mutsaerts *et al.*, "ExploreASL: An image processing pipeline for multi-center ASL perfusion MRI studies," *Neuroimage*, vol. 219, p. 117031, Oct. 2020, doi: 10.1016/j.neuroimage.2020.117031.

[29] C. Rummel *et al.*, "Time Course Based Artifact Identification for Independent Components of Resting-State fMRI," *Front. Hum. Neurosci.*, vol. 7, 2013, doi: 10.3389/fnhum.2013.00214.