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VESPA ASL: VELOCITY and SPATIALLY Selective Arterial Spin Labeling

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Abstract:

Purpose: Spatially-selective arterial spin labeling perfusion MRI is sensitive to arterial transit times (ATT) that can result in inaccurate perfusion quantification when ATTs are long. Velocity-selective ASL is robust to this effect because blood is labeled within the imaging region, allowing immediate label delivery. However, velocity-selective ASL cannot characterize ATTs, which can provide important clinical information. Here, we introduce a novel pulse sequence, called VESPA ASL, that combines velocity-selective and pseudo-continuous ASL to simultaneously label different pools of arterial blood for robust cerebral blood flow (CBF) and ATT measurement.

Methods: The VESPA ASL sequence is similar to velocity-selective ASL, but the velocity-selective labeling is made spatially-selective and pseudo-continuous ASL is added to fill the inflow time. The choice of inflow time and other sequence settings were explored. VESPA ASL was compared to multi-delay pseudo-continuous ASL and velocity-selective ASL through simulations and test-retest experiments in healthy volunteers.

Results: VESPA ASL is shown to accurately measure CBF in the presence of long ATTs, while $ATTs < TI$ can also be measured. Measurements were similar to established ASL techniques when ATT was short. When ATT was long, VESPA ASL measured CBF more accurately than multi-delay pseudo-continuous ASL, which tended to underestimate CBF.

Conclusion: VESPA ASL is a novel and robust approach to simultaneously measure CBF and ATT and offers important advantages over existing methods. It fills an important clinical need for non-invasive perfusion and transit time imaging in vascular diseases with delayed arterial transit.

Keywords: VESPA, arterial spin labeling, cerebral blood flow, arterial transit time

1. Introduction

Arterial spin labeling (ASL) techniques magnetically label arterial blood water to create an endogenous tracer for measuring cerebral blood flow (CBF)^{1,2}. Spatially-selective ASL techniques, such as pulsed ASL (PASL)³⁻⁵ and pseudo-continuous ASL (PCASL),⁶ label blood in the large arteries proximal to the brain, making them sensitive to arterial transit times (ATTs) which can compromise CBF measurement.⁵ This sensitivity can be reduced with a post-labeling delay (PLD)⁷ or with multiple PLDs to simultaneously measure CBF and ATT.⁸⁻¹² Nonetheless, in diseases that have pathologically lengthened transit times, such as acute stroke, moyamoya, or carotid stenoses, it can be difficult or even impossible to measure perfusion with spatially-selective ASL.¹³⁻¹⁵

Velocity-selective ASL (VSASL)¹⁶⁻¹⁸ instead labels blood based on its velocity and was developed as an ATT-insensitive technique. With an appropriate velocity cutoff (V_{cut}), labeled blood is delivered to the microvasculature immediately after labeling, permitting robust perfusion measurements in the presence of long ATTs.^{13,19} However, this can also be viewed as a source of weakness because VSASL cannot provide information about ATT, which can be clinically useful.²⁰⁻²⁹

Recently, Bolar et al.¹³ proposed acquiring both spatially-selective ASL and VSASL to robustly and comprehensively evaluate cerebral hemodynamics in pediatric moyamoya, where transit times can be extremely long in affected brain regions. The authors found that VSASL better reflected underlying tissue perfusion, and when acquired together, VSASL and spatially-selective ASL could highlight regions with preserved CBF but delayed transit through collateral pathways. Acquiring both datasets, however, would double the scan time, which is not feasible in high throughput clinical workflows.

In this work, we report the implementation and initial testing of a novel ASL sequence called VESPA ASL,³⁰ which efficiently combines VSASL and PCASL labeling into a single scan to measure both ATT-insensitive CBF as well as ATT. We tested several VESPA sequence settings in vivo to establish their effect on these measurements and determine an effective protocol. Using simulations and in vivo experiments, we then compared CBF and ATT measured by VESPA with CBF and ATT from multi-PLD PCASL and CBF from VSASL.

2. Theory

An outline of the VESPA sequence is shown in Figure 1(A), along with diagrams for the PCASL and VSASL sequences.

In PCASL, each TR starts with a spatially-selective presaturation that only saturates spins within the imaging volume. The labeling plane is positioned inferior to the imaging volume and labeling typically starts immediately after presaturation. After labeling, a PLD provides time for the labeled blood to travel downstream and accumulate within the microvasculature and tissue.⁷ An image is subsequently acquired, where vascular crushing (VC) can be optionally used to saturate labeled blood still present in the large arteries.^{10,31}

For VSASL, a global presaturation is applied, followed by a saturation recovery time to allow the longitudinal magnetization of arterial blood to partially recover. VS labeling is then applied globally followed by an inflow time (TI). An image is then acquired with VC to remove venous signal and define the bolus duration.¹⁸

VESPA ASL is similar to the VSASL sequence but with PCASL labeling inserted between the VS labeling and VC. The presaturation remains global, but the VS labeling is made spatially selective and only labels blood superior to the PCASL plane (Figure 1(B)). PCASL labeling starts immediately after VS labeling so that the leading edge of the PCASL bolus directly follows the trailing edge of the VSASL bolus, an important concept in the VESPA sequence. VC follows immediately after PCASL and suppresses VSASL-related venous signal and macrovascular PCASL signal (Figure 1(C)). Due to the short duration of the VC module (~14-42 ms), the VSASL TI and PCASL label duration (LD) are almost equal and are used interchangeably in this manuscript.

To disambiguate the signal contributions from both ASL contrasts in VESPA, the label/control conditions are varied in an orthogonal pattern over four TRs (Figure 1). This encoding pattern enables the VSASL and PCASL signals to be linearly decoded in post-processing using simple image addition and subtraction similar to Hadamard encoding in vessel-encoded^{32,33} and time-encoded³⁴ ASL. This encoding/decoding process results in a $\sqrt{2}$ gain in SNR efficiency compared to separately acquired VSASL and PCASL scans with identical settings (though this gain may be smaller when compared to optimal separate acquisitions, see discussion).

An idealized depiction of the labeled blood traveling down the vasculature into the tissue for each sequence is shown in Figure 2. The spatial separation between the labeling plane and tissue results in a non-zero ATT for PCASL, whereas immediate label delivery is seen with VSASL. In VESPA, due to the contiguous spatial placement of VSASL and PCASL, the leading edge of the PCASL bolus immediately follows the trailing edge of the VSASL bolus. The VSASL bolus duration is, therefore, equal to either the TI (if the trailing edge of the VS bolus remains above V_{cut} at TI) or the ATT (if the trailing edge of the VS bolus has passed below the V_{cut} at TI), whichever is shorter. Assuming

plug flow, no outflow of label from the voxel, and the label relaxes with the T_1 of blood, the VESPA ASL tissue signal can be modeled as follows:

$$\begin{aligned} \Delta M_{Z_{VSASL}} &= M_{0B} \cdot f \cdot \alpha_{VS} \cdot \alpha_{BS} \cdot TI \cdot e^{-TI/T_1} \cdot (1 - e^{-T_{sat}/T_1}) & TI < \Delta t \\ \Delta M_{Z_{PCASL}} &= 0 \\ \Delta M_{Z_{VSASL}} &= M_{0B} \cdot f \cdot \alpha_{VS} \cdot \alpha_{BS} \cdot \Delta t \cdot e^{-TI/T_1} \cdot (1 - e^{-T_{sat}/T_1}) & \Delta t < TI \\ \Delta M_{Z_{PCASL}} &= 2 \cdot M_{0B} \cdot f \cdot \alpha_{PC} \cdot \alpha_{BS} \cdot [(\Delta t - TI) \cdot e^{-(T_{sat}+TI)/T_1} + T_1 \cdot (e^{-\Delta t/T_1} - e^{-TI/T_1})] \end{aligned}$$

where $\Delta M_{Z_{VSASL}}$ and $\Delta M_{Z_{PCASL}}$ are the VSASL and PCASL difference signals; M_{0B} is the equilibrium magnetization of blood; α_{VS} and α_{PC} are the labeling efficiencies for VSASL (0.86, simulation with $T_1=1.65$ s, $T_2=0.15$ s) and PCASL (0.85)⁶; α_{BS} is the cumulative efficiency of the background suppression (BS) inversion pulses and VC (0.80, simulation with $T_1=1.65$ s, $T_2=0.15$ s for three direction VC - see Figure 1(C) and below); f is the CBF in units of mL/mL/s, TI is the inflow time (equal to the PCASL LD); Δt is the PCASL ATT; T_1 is the longitudinal relaxation time of blood (1.65 s)³⁵; and T_{sat} is the saturation recovery time. The ATT for VSASL is assumed equal to zero. Simulated delivery curves for VESPA, PCASL, and VSASL are shown in Figure 2 (see Supporting information text 2 for the PCASL and VSASL equations).

After acquiring the VESPA data and decoding the VSASL and PCASL contrasts, the VESPA signal model can be fit to the decoded difference data using a non-linear fitting algorithm to estimate CBF and ATT simultaneously. Both parameters can be estimated from a single TI because each VESPA TI generates two data points with a different dependence on ATT: the decoded VSASL signal has no dependence on ATT while the decoded PCASL signal is delayed by the ATT, thereby giving us two timepoints on the ASL signal curve from a single acquisition. This contrasts with other ASL techniques, such as PCASL and PASL, which require the explicit acquisition of multiple timepoints to measure both CBF and ATT.

3. Methods

All in vivo data were acquired with a 3T system (Discovery MR750; GE Healthcare, Waukesha, WI) using a 32-channel receive-only head coil (Nova Medical, Wilmington, MA). The protocols were approved by the local institutional review board and all volunteers gave informed consent. All reconstructed data, simulation code, post-processing code, and analysis code are available at <https://doi.org/10.5281/zenodo.5817354>.

3.1. VESPA sequence implementation

To make the VS labeling slab selective, the hard 90° pulses typically used in double-refocused hyperbolic secant (DRHS) modules¹⁸ were replaced with symmetric 90° Hamming-windowed sinc pulses (4 ms, TBWP=16) with associated field gradients. The first and zeroth gradient moments were nulled after and before the tip-down and tip-up sinc pulses, respectively, so that they did not contribute to the velocity selectivity, as in Turbo-VSASL.³⁶ A 200 mm nominal slab thickness with $V_{\text{cut}}=2$ cm/s was used, resulting in a 29.4 ms VSASL module duration. The VSASL slab was centered on the middle readout slice. See Supporting information text 1 for more information.

The PCASL LD was set equal to the specified VS TI, with the TI adjusted such that VS labeling ended 1 ms before the start of PCASL. The center of the PCASL plane was automatically placed at the inferior edge of the VSASL slab. PCASL used balanced labeling, 375 μs long Hamming pulses, $\max B_1^+ 10 \mu\text{T}$, max gradient amplitude 16 mT/m, mean gradient 0.9 mT/m, 1 ms inter-pulse spacing.

The VC module was placed 1 ms after PCASL labeling and was a BIR-4 module with monopolar gradients³⁷ (segment duration=1.5 ms, $\omega_{\text{max}}=42.52$ kHz, $\xi=43.58$ and $\tan(\kappa)=69.65$)³⁸ with V_{cut} matched to the VS labeling (2 cm/s). Either a single VC module or three consecutive modules (with flow-weighting gradients played out on orthogonal axes) were used (see Figure 1 and below). The readout started 1 ms after the last VC module.

Presaturation was three consecutive 90° hard RF pulses with orthogonal crusher gradients. BS used two C-shaped FOCI pulses^{39,40} ($\beta=1484$ rad/s, $\mu=1.76$, max modulation=40, $\max B_1^+ 23.5 \mu\text{T}$, duration=10.24 ms), which interleaved the PCASL labeling.⁴¹ BS timings were calculated using the analytical formula given in Günther et al.⁴² to null tissues with two T_1 values (that are constrained to differ by a factor of 2, $T_{1,\text{opt}}$ and $2 \cdot T_{1,\text{opt}}$) 100 ms before the VC module to ensure static tissue was positive at the start of the readout (a necessary condition for magnitude subtraction). By default, we set $T_{1,\text{opt}}=700$ ms, but this value was reduced when necessary to ensure the first BS inversion pulse was after the VSASL module.

The readout was a 2D multi-slice single-shot spiral-out spin-echo readout (Binomial water excitation and sinc refocusing), FOV=220x220 mm², TE=16.1 ms, 5x6 mm thick slices, 8 mm slice spacing. Magnitude data were reconstructed using an iterative off-resonance field correction method^{43,44} using a field map acquired with identical slices to the ASL data. The slices were positioned axially with the middle slice at the top of the thalamus. For the 200 mm VSASL slab used in VESPA, this meant the PCASL plane was just below the cerebellum.⁴⁵

In all scan sessions, six M_0 calibration images were acquired with identical readout but without labeling: TR=5 s, global presaturation, no BS.

3.2. Initial VESPA evaluation

To validate the performance of the contrast decoding, we acquired three VESPA scans on a single subject: TI=1.8 s, TR=5 s, 30 averages (15 repeats of the VESPA encoding matrix), scan duration 5 minutes. The first scan was standard VESPA, while the second and third had VSASL or PCASL RF and gradients turned off, respectively, but with identical timings.

To test the dependence of the VESPA CBF and ATT estimates on the direction of the VS gradients, which are typically only applied along a single direction in VSASL, we acquired three sets of data where the VS and VC gradients were both applied along either the x, y, or z gradient axes (referred to as VSx-VCx, ...). Additionally, to compare the effect of applying VC along either a single direction or all three, another three data sets were acquired where the VC consisted of three consecutive modules applied along each direction (approximating isotropic VC) with the VS gradients applied along either x, y, or z (referred to as VSx-VCxyz, ...). A seventh data set was acquired where both the VS and VC modules were repeated along each direction (referred to as VSxyz-VCxyz). TI=1.8 s, TR=5 s, 20 averages, scan duration 3:20 minutes each, one subject.

To test the dependence of the VESPA CBF and ATT measurements on the TI, we acquired five data sets on a single subject with TI=[1, 1.4, 1.8, 2.2, 2.6] s and TR=[4.2, 4.6, 5, 5.4, 5.8] s, 22 averages (scan durations between 3:05 and 4:15 minutes), slice thickness 5 mm. This choice of TRs kept T_{sat} constant while also being close to the optimal TR for each TI in terms of the VSASL SNR efficiency ($SNR_{\text{eff}} = \Delta M_{\text{ZVSASL}} / \sqrt{TR}$). Monte Carlo simulations with matched timings, but with fixed scan durations of 5 minutes, were run to investigate the effect of the TI on CBF and ATT accuracy in idealized conditions (1000 repeats, CBF=60 mL/100g/min, ATT=0.5-2.6 s sampled every 50 ms, noise SD 0.2% of M_{0B}).

3.3. Comparison with multi-PLD PCASL and VSASL

To compare the VESPA CBF and ATT measurements with established ASL techniques, we acquired VESPA ASL, multi-PLD PCASL, and VSASL data in six volunteers (2 female, age=31.8±7.4 years). The VESPA protocol was TI=1.8 s, TR=5 s, 30 averages, scan duration 5 minutes. The multi-PLD PCASL protocol was LD=1.8 s, PLDs=[0.125, 0.50, 0.875, 1.25, 1.625, 2] s, 8 averages, scan duration 4:57 minutes. The VSASL protocol was TI=1.4 s, TR=4.5 s, 33 averages, scan duration 4:57 minutes. The PCASL PLDs were optimized for both CBF and ATT accuracy while keeping the PLDs equally spaced (optimization ATT range 0.5-1.8 s, taper 0.2 s).⁴⁶ The VSASL TI and TR were optimally SNR efficient. VSASL and PCASL readouts were identical to the VESPA sequence.

To compare the within-session repeatability of each ASL technique, the three ASL scans were acquired twice, with approximately 16 minutes between each test and retest scan. The order of the

three test scans was randomized (with retest scans following this order) for each subject to reduce consistent physiological drifts biasing the results. PCASL used the same BS as VESPA (FOCI pulses that can interleave with the PCASL labeling; optimal timings calculated for each PLD), while VSASL used global HS pulses which were transformed into the form $\cos^{0.2}(t)$ using the VERSE algorithm⁴⁷ to reduce their duration, as in Wong et al.¹⁸ ($\beta=1000$ rad/s, $\mu=3.34$, max B_1^+ 23.5 μ T, duration=3.56 ms). T₁-weighted images (MPRAGE, 1 mm isotropic resolution) were acquired for generating tissue masks.

All three ASL techniques used the previously described three-module VC approach to ensure the ATT and Vcuts were equivalent. Since PCASL does not use a global presaturation, and the time between the VC and the start of labeling in the following TR was short (~ 260 ms), we found that blood destined to be labeled in the next TR was partially crushed. To mitigate this issue, 1 s of dead time was added to each PCASL TR before presaturation to allow fresh unsaturated blood to reach the PCASL plane.

Monte Carlo simulations were used to investigate differences in CBF and ATT accuracy between the protocols in idealized conditions (1000 repeats, CBF=60 mL/100g/min, ATT=0.5-2.6 s sampled every 50 ms, noise SD 0.2% of M_{0B}). As an additional comparison in the simulations, a multi-PLD PCASL protocol with equally spaced PLDs optimized for a longer ATT range (optimization ATT range 0.5-2.6 s, 0.2 s taper)⁴⁶ was included to better evaluate the ability of multi-PLD PCASL to estimate CBF at ATTs longer than would be expected in the GM of young healthy adults (LD=1.8 s, PLDs=[0.3, 0.725, 1.125, 1.55, 1.95, 2.375, 2.8] s, 6 averages, scan duration 5:01 minutes).

3.4. Alternative VESPA implementations

Several alternative implementations of VESPA ASL were tested. The first was to turn off the VC module (VESPA_{VC-off}). This allows decoding of non-vessel suppressed VSASL and PCASL contrasts, which we hypothesized could be used as a qualitative perfusion-like image and low-resolution angiogram, respectively. The remaining implementations involved combining the VSASL and PCASL contrasts to generate higher SNR images. The most SNR-efficient approach is to alternate the tag and control conditions of both labeling techniques together, referred to as dual VESPA ASL. In this case, the contrasts are no longer separable but can provide either a maximum SNR qualitative perfusion-weighted image (with VC on: dual VESPA_{VC-on}) or an image with a combination of perfusion and angiographic signals (with VC off: dual VESPA_{VC-off}).

These additional VESPA modes were acquired in the same session as the multi-TI VESPA experiment, using the same sequence settings. To preserve the large intravascular signal expected

with the VESPA_{VC-off} scans, flow compensation was used for the readout excitation and refocusing pulses in all scans, increasing the TE to 17.8 ms.

3.5. Post-processing

ASL difference images were decoded from the VESPA data (Figure 1), and pairwise subtractions were used for PCASL and VSASL. Brain masks were generated⁴⁸ from the M_0 images, manually edited using FSLeaves,⁴⁹ and applied to the data.

For the VESPA, PCASL, and VSASL comparisons, the T_1 -weighted images were instead used to generate brain masks,⁴⁸ as well as CSF, GM, and WM partial volume estimates (PVEs).⁵⁰ These were down-sampled to the ASL spatial resolution⁵¹ and thresholds applied as follows: brain 5%, GM 50%, WM 80%. Diffusion attenuation from the VS labeling in VESPA and VSASL was estimated and removed⁵² using the formula:

$$\Delta M_{z,corrected} = \Delta M_z - \sum_{t=1}^3 [M_{0T} \cdot p_t \cdot (1 - e^{-T_{sat}/T_{1t}}) \cdot (1 - e^{-b \cdot D_t}) \cdot \alpha_{VS,t} \cdot \alpha_{BS,t} \cdot e^{-T_1/T_{1t}}],$$

where the index, t , corresponds to the three tissue types (CSF, GM, and WM), M_{0T} is the equilibrium magnetization of tissue, p_t are the partial volume fractions, T_{1t} are the tissue longitudinal relaxation times, D_t are the diffusion coefficients, b is the b -value for each VS module, and $\alpha_{VS,t}$ and $\alpha_{BS,t}$ are the VS labeling efficiencies and combined attenuation from the BS and VC (see Supporting information text 1 for the values used).

The ASL calibration images were averaged then brain masked. The edges were eroded and dilated by two voxels to avoid edge partial volume effects then smoothed in-plane (Gaussian kernel, SD=2.5 mm).⁴⁵ Incomplete signal recovery was corrected, and the images were divided by the brain-blood partition coefficient ($\lambda=0.9$ mL/g).⁴⁵ The ASL difference images were voxel-wise divided by the processed M_0 image to simultaneously correct B_1^- inhomogeneities and T_2 decay during the readout, and to calibrate the data by M_{0B} .⁴⁵

After the T_1 relaxation of the ASL signal between the VC module and each slice was corrected, the unaveraged calibrated ASL difference data were used to voxelwise calculate CBF and ATT for VESPA and PCASL using the signal models described and a coarse least-squares grid-search (grid resolution: 1 mL/100g/min and 10 ms) followed by a non-linear-least-squares fitting routine (MATLAB's `fmincon SQP` function). Bounds on CBF (0, 200) mL/100g/min and ATT (0, 2.6) s were imposed for both fitting steps. CBF was calculated from the VSASL data by inverting the signal model. Parameter estimate SDs (a measure of uncertainty in the fit) were calculated using the CRLB as in Woods et al.⁴⁶ (see Supporting information text 3 for more details).

4. Results

4.1. Initial VESPA evaluation

The in vivo VESPA decoding demonstration is shown in Supporting information Figure S1 and demonstrates that VSASL and PCASL contrasts are accurately decoded.

The results of the in vivo VS and VC gradient direction experiment are shown in Supporting information Figure S2. The VSASL signal was found to vary substantially with the axis of the applied VS gradients, which transfers to the calculated CBF and ATT maps, but is not present in the PCASL images. Due to the spatial dependence of the VSASL signal on the gradient direction (also present in phantom experiments, not shown), we believe these are eddy current effects. The eddy current effects appear most benign with VSz, while VSxyz has the cumulative eddy current effects from all three axes. For these reasons, the VS gradients were only applied along the z-direction in the following experiments.

When the VC gradients were only applied along a single direction, significant amounts of macrovascular PCASL signal remained in arteries whose principal direction of flow was perpendicular to the direction of the applied gradients. Using three consecutive VC modules (applied along different gradient axes) resulted in decoded PCASL images with better suppression of large vessel signal. This translated to longer ATTs calculated in regions with large arteries. Only minor changes to the VSASL data were observed when using the three-direction VC. Therefore, in subsequent experiments, VC along three directions was used to measure CBF and ATT more accurately.

The Monte Carlo simulation and in vivo multi-TI VESPA ASL comparisons are shown in Figure 3 and Figure 4, respectively. The simulations demonstrate that the lowest CBF errors were achieved with TI=1.4 s, similar to standard VSASL, while there was a global increase in the ATT errors with increasing TI. In general, the CBF errors decrease as the ground truth ATT increases. The ATT errors also initially decrease with increasing ATT, but as the ATT approaches the TI, the errors increase before decreasing again when $ATT \approx TI$ due to a ceiling effect. In vivo, the mean CBF was generally stable across TIs except for TI=1 s, where the estimated CBF was moderately elevated. In simulations, there was a mean CBF overestimate of ~2% for $ATTs > TI$ which may have contributed to this overestimate at TI=1 s. From the ATT maps, it is clear the maximum ATT which can be estimated is equal to the TI. There is, therefore, a trade-off between CBF and ATT accuracy and the maximum calculable ATT. The CBF and ATT SD maps demonstrate similar trends to the simulation errors, with the CBF SDs lowest for TIs between 1-1.8 s and ATT SDs increasing with TI. Based on these results, TI=1.8 s was chosen for the VESPA comparison with PCASL and VSASL to maintain

reasonably low CBF and ATT errors while still being able to estimate ATTs up to 1.8 s, which was expected to be adequate for GM in healthy adults.^{45,46}

4.2. Comparison with multi-PLD PCASL and VSASL

The Monte Carlo simulation CBF and ATT errors are shown in Figure 5. For short ATTs, all protocols had minimal CBF bias, but the multi-PLD PCASL protocols had more precise CBF estimates than VSASL and VESPA, in part due to the higher labeling efficiency of PCASL and the short PLDs included in the protocols. However, at long ATTs, PCASL underestimates CBF and ATT and has low precision, even when the PLDs are optimized for the long ATT range (0.5-2.6 s). In contrast, both VSASL and VESPA maintain low CBF bias and high precision at long ATTs because they are not sensitive to these long ATTs. For ATTs between 0.9-1.8 s, VESPA had more precise ATT estimates than both PCASL protocols but cannot estimate ATTs longer than the TI used, 1.8 s. VSASL had slightly more precise CBF estimates than VESPA due to the more SNR-efficient TI used (1.4 s vs 1.8 s), as well as only estimating one parameter.

The CBF and ATT maps from the in vivo comparison are shown in Figure 6. Voxel-wise scatter plots in Figure 7 and quantitative subject-wise comparisons in Figure 8 demonstrate the differences in the CBF and ATT estimates for each ASL technique and test-retest scans. The underlying values in Figure 8 are given in Supporting information Table S1. All three techniques produced spatially similar CBF maps, but with mean CBF in VESPA and VSASL slightly higher than PCASL (GM CBF 5.90 ± 2.68 mL/100g/min [$14.6 \pm 9.2\%$] higher in VESPA and 15.07 ± 3.29 mL/100g/min [$35.7 \pm 7.5\%$] higher in VSASL). PCASL and VESPA produced similar ATT estimates with a negligible difference (GM bias = 0.00 ± 0.09 s).

Subject 4, and to a lesser extent subject 2, showed markedly longer ATTs than the other subjects. Due to the long transit times seen in subject 4, a posterior brain region in the PCASL CBF maps shows apparent hypoperfusion. The CBF in this region is much more similar to the contralateral side in the VESPA and VSASL CBF maps, highlighting their robustness to long transit times. However, a small region of apparent hypoperfusion in the first VESPA CBF map (the "test" scan) remains, suggesting further in vivo validation of VESPA at long ATTs is required. Additionally, the VESPA and VSASL CBF maps for these subjects have qualitatively higher spatial SNR (see Figure 6). This is in line with the Monte Carlo simulations which demonstrate CBF underestimation and low precision at long ATTs for the PCASL protocols.

For the test-retest results, VSASL had lower GM and WM CBF intra-session coefficient of variation⁵³ (CoV) (GM = $11.3 \pm 2.6\%$) than PCASL ($14.3 \pm 6.9\%$) and VESPA ($16.8 \pm 5.4\%$). Although VESPA GM CBF was less reproducible (see correlation, CoV, RMSE) than PCASL for these healthy

subjects, VESPA WM CBF reproducibility was slightly better than PCASL. VESPA had lower GM and WM ATT CoV (GM= $7.1 \pm 1.5\%$ vs $11.1 \pm 3.0\%$) and RMSE (GM= 0.18 ± 0.04 s vs 0.29 ± 0.11 s) than PCASL, which was also the case for ATTs < 1.8 s, so this was not due to an ATT ceiling effect at 1.8 s in VESPA. In general, WM CBF and ATT were less reproducible than GM CBF and ATT for all protocols due to the inherently lower SNR of WM.

4.3. Alternative VESPA implementations

The decoded data for VESPA_{VC-on} (standard VESPA), VESPA_{VC-off}, dual VESPA_{VC-on}, and dual VESPA_{VC-off} are shown in Figure 9. Turning off the VC retains macrovascular signal in the decoded PCASL data (VESPA_{VC-off}), yielding a low-resolution angiogram. The PCASL signal flows further downstream into the parenchyma with increasing TI. However, the absence of VC also leaves venous and arterial macrovascular signal in the decoded VSASL contrast, which means it can no longer be used to quantify CBF. Nonetheless, when comparing the images with VESPA_{VC-on}, at TIs above 1 s the VSASL contrast appears to be mostly parenchymal. Therefore, the decoded VSASL data could still be useful as a qualitative perfusion-weighted image.

With dual VESPA_{VC-on}, the VSASL signal has already reached the parenchyma at short TIs and provides early perfusion-weighting before the PCASL signal has arrived. As the PCASL signal reaches the tissue, the SNR of the data benefits from the accumulation of more ASL label, particularly due to the higher SNR of PCASL labeling. A similar situation occurs for dual VESPA_{VC-off}, but with the addition of the macrovascular signal.

5. Discussion

We have introduced VESPA ASL, a novel ASL sequence capable of robustly estimating CBF in the presence of very long ATTs, while simultaneously measuring ATT. VESPA accomplishes this by combining VSASL and PCASL into the same sequence and labeling different arterial blood water pools. The VSASL label is delivered immediately, whereas the PCASL label is delayed by the ATT. Once the PCASL label arrives in the voxel, two non-zero points on the ASL signal curves are measured (Figure 2) and, because the VSASL and PCASL boluses are contiguous, we can calculate CBF and ATT. By linearly encoding the VSASL and PCASL contrasts, we can efficiently acquire both sets of data within the same scan, yielding a $\sqrt{2}$ SNR efficiency improvement compared to separate acquisitions with identical settings.

PCASL and PASL are sensitive to long ATTs due to the physical separation of the labeling region and imaging volume. When the ATT is long, shorter PLDs miss the ASL label resulting in low CBF sensitivity and over/underestimation (Figure 5). Even when long ATTs are expected and appropriately long PLDs are used, T_1 relaxation of the label inherently limits the accuracy of these

approaches. By labeling within the imaging volume, VSASL eliminates the ATT and yields accurate CBF measurements even in the setting of extremely long transit delays; however, VSASL cannot characterize ATTs, which also have clinical importance. VESPA ASL, therefore, fills an important methodological gap by addressing both limitations.

In vivo experiments demonstrated that VESPA ASL generates similar CBF and ATT measurements compared to multi-PLD PCASL and CBF compared to VSASL. However, VESPA and VSASL gave higher CBF measurements than PCASL. This effect has previously been seen in the VSASL literature^{38,52} and could have several sources, including lower than expected PCASL labeling efficiency, VS labeling eddy current effects, incomplete correction of VS diffusion attenuation, artificial VS labeling of static tissue and CSF due to pulsation, and differing times spent in the tissue relaxing with a shorter T_1 . These varying effects can make it difficult to compare ASL techniques without measuring and accounting for each one (which we cannot do with these data).

In this study, multi-PLD PCASL yielded more reproducible test-retest CBF measurements than VESPA, while VESPA had more reproducible ATT. These CBF results were consistent with our simulations, given the short ATTs seen in our healthy subjects. In this ATT range, the higher labeling efficiency of PCASL, modest T_1 decay, and short PLDs result in high SNR for CBF and ATT measurements with PCASL.⁵² Additionally, VSASL had more reproducible CBF measurements than VESPA, as predicted by the simulations in Figure 5. This is due to the more CBF optimal TI used in VSASL (1.4 s) compared to VESPA (1.8 s) and only CBF being estimated from the VSASL data, compared to both CBF and ATT with VESPA.

Monte Carlo simulations demonstrate that VESPA can robustly estimate CBF at long ATTs. The long ATTs witnessed in subject 4 (Figure 6) allowed for an in vivo demonstration of this CBF robustness: the PCASL CBF maps were noisier and underestimated CBF in the posterior cerebral artery territories (presumably due to the longer ATTs in these territories⁵⁴), whereas VESPA and VSASL both had more homogenous CBF throughout all vascular territories, though with the "test" VESPA scan still showed a small residual region of hypoperfusion. Although VESPA cannot measure $ATTs > TI$, we still know that the ATT is longer than the TI, which is clinically useful information. Future work will investigate the CBF robustness of VESPA, and how it compares to VSASL and PCASL, in subjects with pathologically delayed ATTs.

For subject 1, there was a substantial change in mean CBF between test and retest scans for VESPA (GM change=-9.58 mL/100g/min) and VSASL (-7.23 mL/100g/min), but not PCASL (0.71 mL/100g/min). Since the test-retest mean CBF values for the other subjects were relatively much more constant for all protocols (Figure 6) and the order of the scans for this subject were VSASL_{test}-

VESPA_{test}-PCASL_{test}-VSASL_{retest}..., the apparent global decrease in CBF for subject 1 could be a result of falling asleep⁵⁵ (a possibility, as reported by the subject) or habituation, with CBF higher at the start of the scan then reducing to a relatively constant level by the third scan. However, we did not monitor subject consciousness or collect information about other CBF modifiers, such as caffeine consumption, so we cannot rule out other causes.

In vivo and simulated data demonstrated that VESPA CBF measurements were reasonably robust to the choice of TI, with the TI only affecting the measurement precision and maximum ATT that could be estimated. Although long TIs permit the measurement of long ATTs, this comes at the cost of decreased SNR. Despite it being possible to measure both CBF and ATT from a single TI with VESPA, multi-TI VESPA protocols could be used to enable the measurement of long ATTs with less pronounced reductions in SNR by spreading the TIs across short and long times. Such protocols could be objectively optimized using a Cramér-Rao Lower Bound optimization framework.⁴⁶

When the TI is sufficiently long, removing the VC in VESPA yields a qualitative perfusion map from VSASL and a low-resolution angiogram from PCASL, which may be useful to identify arteriovenous shunt lesions and arterial transit artifacts.^{22,56} Dual VESPA instead efficiently combines VSASL and PCASL contrasts into a single image. Dual VESPA_{VC-on} may be useful in clinical situations where absolute quantification is unnecessary and the priority is to acquire a high SNR perfusion-weighted image robust to long transit times (for example, to detect subtle relative CBF changes in acute stroke).

Experiments varying the VC direction showed that using VC along three orthogonal directions achieved more robust macrovascular signal suppression. When VC is only applied along a single direction, macrovascular PCASL signal flowing perpendicular to this direction is left unsuppressed (Supporting information Figure S2),⁵⁷ which can shorten the measured ATT and erroneously increase CBF in both VESPA and multi-PLD PCASL. However, the Vcut profile will no longer be matched between VS labeling and VC¹⁸ because VS labeling was only applied along a single direction to reduce eddy current and diffusion errors. While it might seem this would negatively affect quantification, this is not entirely the case. When the Vcut profile is matched, VSASL produces accurate whole-brain CBF, but some label can be displaced upstream, causing local CBF overestimation and downstream underestimation. In contrast, the three-direction VC reduces the problem of upstream CBF overestimation but leaves distal voxels with potentially underestimated CBF.

In VESPA, we assume the trailing edge of the VSASL bolus and leading edge of the PCASL bolus are contiguous. Ignoring dispersion, which affects all ASL techniques, this is a reasonable assumption given the use of z-direction VS gradients, the high TBWP of the DRHS excitation pulses, and the well-defined shape of the PCASL bolus. The validity of this assumption was supported by the excellent agreement in ATTs between VESPA and multi-PLD PCASL.

In VSASL, it is typically assumed that the supply of labeled blood is longer than the TI. This is often true due to the size of body transmit coils and the use of $TIs < 2$ s,⁵² but is not always guaranteed. By design, the VSASL bolus duration in VESPA is either equal to the TI or the PCASL ATT, whichever is shorter, thus reducing this source of uncertainty and facilitating the simultaneous measurement of CBF and ATT.

The use of a global presaturation in VESPA reduces the SNR of the PCASL labeling because upstream blood that will be labeled by PCASL is also saturated at the start of each TR. This introduces a dependence on T_{sat} in the PCASL part of the VESPA signal model, as inflowing blood relaxes during the T_{sat} and TI. The use of a spatially-selective presaturation could improve the SNR of the PCASL contrast but could also introduce an ATT dependence to the VSASL signal. For example, if the inferior edge of the presaturation slab is placed at the PCASL labeling plane and $ATT > T_{sat}$, unsaturated blood will not have fully refilled the arterial vasculature by the start of VS labeling and the first $ATT - T_{sat}$ section of the VSASL bolus will comprise of partially relaxed blood (when $ATT - T_{sat} > TI$, the entire bolus will be partially relaxed). This effect could be included in the VESPA signal model, but it would only be possible to characterize when $TI > ATT$, i.e. when ATT can be estimated. As such, VESPA would lose its ability to accurately quantify CBF in these situations, which may particularly arise in disease. In contrast, the use of a global saturation assumes complete saturation of upstream blood, which may not occur due to the limited size of the body transmit coil used.⁵² Incomplete up-stream saturation could lead to higher-than-expected PCASL signal, leading to ATT underestimation.

A spatially-selective DRHS module was used for VS labeling to only label blood superior to the PCASL plane because BIR-8 modules³⁸ cannot easily be made spatially selective. However, DRHS modules are more sensitive to B_1^+ variation and eddy current effects. In the future, the DRHS excitation pulses could be made more B_1^+ insensitive by using spatially-selective composite⁵⁸ or adiabatic⁵⁹ pulses. The eddy current sensitivity could be improved using a recently developed VSASL eddy current minimization framework.⁶⁰ The post-processing approach used in VESPA could also be used to combine standard separate scans of single-PLD PCASL and non-spatially selective VSASL data to estimate both CBF and ATT by assuming the VSASL bolus duration is equal to the TI. This could reduce the SNR efficiency by up to $\sqrt{2}$ compared to the presented VESPA sequence, but the

use of optimal settings for the separate VSASL and PCASL scans (such as TI, LD, global VS labeling, spatially-selective presaturation for PCASL) could potentially mitigate some of this reduction.

A natural approach to improve the SNR of the VSASL data in VESPA would be to design a spatially-selective Fourier Transform VS inversion (FT-VSI) module,¹⁷ potentially yielding 30-40% SNR improvement for the VSASL contrast.⁶¹ However, to mitigate the effect of the longer spatially-selective pulse durations, it may be necessary to reduce the number of velocity encoding steps. Additionally, FT-VSI is similarly sensitive to B_1^+ as DRHS.

A weakness of the equally-spaced multi-PLD PCASL protocols used for comparison is that they do not represent the most SNR-efficient techniques within the literature. For example, longer LDs^{9,14,62,63} and time-encoding^{9,34} can improve the accuracy of CBF and ATT measurements. However, spatially-selective ASL techniques are still fundamentally limited at long ATTs by T_1 relaxation. Future work could compare these more advanced PCASL techniques with VESPA to establish the ATT boundaries where each is most useful.

6. Conclusions

We have introduced VESPA ASL, a novel ASL sequence that can yield robust ATT-insensitive CBF measurements while simultaneously measuring ATT. We validated this method against conventional multi-PLD PCASL and VSASL in healthy volunteers and found an excellent agreement. Due to the promising robustness of VESPA at both short and very long ATTs, we believe it offers advantages over multi-PLD PCASL and could replace single-PLD PCASL as the standard clinical ASL technique.

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Figure 1: (A) Sequence timing diagrams and labeling and readout regions (left); encoding patterns (middle), and decoding formulas (right) for PCASL, VSASL, and VESPA. (B) The RF amplitude and gradient waveforms for the VSASL spatially-selective DRHS module. (C) The RF amplitude and gradient waveforms for the three-direction BIR-4 VC module. Sat = presaturation, VC = vascular crushing, RO = readout, LD = label duration, PLD = post-labeling delay, T_{sat} = saturation recovery time, TI = inflow time.

Figure 2: Left: Schematic of labeled blood traveling down the arterial tree to the tissue. Right: corresponding tissue signal models with simplified sequence diagrams to highlight the relevant timings. The signal difference between control and label is shown for PCASL (orange) and VSASL (blue). The non-zero ATT in PCASL is due to the physical distance between the labeling plane and tissue. VSASL is insensitive to ATT because the leading edge of the labeled blood is created in the microvasculature close to the target tissue. In VESPA, VS labeling delivers signal to the tissue immediately, ensuring CBF can be measured no matter how long the PCASL ATT is. The arrival of PCASL signal allows for measurement of the ATT. Parameter settings: $f=0.01$ mL/mL/s, $\Delta t=1$ s, $\alpha_{PC}=0.85$, $T_1=1.65$ s. PCASL settings: LD=1.8 s and PLD=1.8 s. VSASL settings: TI=1.4s, $T_{sat}=2.85$ s, $\alpha_{VS}=0.92$. VESPA settings: TI=1.8 s, $T_{sat}=2.89$ s, $\alpha_{VS}=0.86$.

Figure 3: Monte Carlo simulations for VESPA ASL at different TIs to evaluate CBF and ATT accuracy. A minimum CBF RMSE is achieved with TI=1.4 s, which is also the optimally SNR efficient TI for VSASL (see methods). A longer TI allows longer ATTs to be estimated, but the ATT RMSEs also increase with TI.

Figure 4: In vivo VESPA (A) CBF, (B) ATT, (C) CBF SD, and (D) ATT SD maps at five TIs for two slices, with the combined data at all TIs fit in the righthand column ('All'). The mean brain CBF and ATT estimates, with errors relative to the 'All' estimates, are inset. In the SD maps, voxels with SDs > 10¹⁰ are set to zero to better see the underlying trends (these voxels are typically either very noisy or where ATT > TI). The CBF estimates are relatively stable across TIs despite many of the ATTs being longer than the shortest TIs. The ATT maps remain visually relatively stable after TI=1.8 s since most of the ATTs are < 1.8 s. The CBF and ATT SD maps show similar trends to those seen in simulation: the CBF SDs are lowest for TI=1.4 s and the ATT SDs are lowest for TI=1 s.

Figure 5: Monte Carlo simulation CBF and ATT mean bias (A, B) and SD (C, D) for the VESPA, PCASL, and VSASL comparison. The PCASL Opt_short protocol was optimized for a short ATT range (0.5-1.8 s), while the PCASL Opt_long protocol was optimized for a longer ATT range (0.5-2.6 s). The TIs of VESPA and VSASL were 1.8 s and 1.4 s, respectively. At long ATTs, the PCASL protocols can no longer accurately estimate CBF and ATT due to a loss in precision (higher SDs) and over/underestimation of both parameters. VESPA and VSASL maintain low CBF bias and SDs at long ATTs due to their insensitivity to ATT. The VESPA measured ATTs are more precise than both PCASL protocols for the range $0.9 < \text{ATT} < 1.8$ s, but ATTs $> \text{TI}$ (1.8 s) cannot be measured with VESPA.

Figure 6: A single slice of the CBF (left) and ATT (right) maps for each subject for the VESPA, PCASL, and VSASL comparison. The mean whole brain GM (gray text) and WM (white text) CBF and ATT estimates are inset below each image. The green arrows highlight a region in subject 4's PCASL CBF map where CBF is underestimated due to long ATTs, while VESPA and VSASL better reflect the underlying CBF, due to their ATT insensitivity.

Figure 7: Scatter plots of the in vivo voxel-wise CBF (A) and ATT (B) measurements in the VESPA, multi-PLD PCASL, and VSASL comparison. The graphs show the test-retest comparisons and the comparisons between each ASL technique (for which the test-retest data were concatenated). GM voxels are shown in blue and WM voxels are shown in red. Pearson correlation coefficients are inset for each comparison. All correlations were significant ($p < 0.001$).

Figure 8: Quantitative subject-wise test-retest errors and comparisons between VESPA, multi-PLD PCASL, and VSASL CBF and ATT measurements. The top-left quadrant shows GM CBF, the top-right quadrant shows GM ATT, the bottom-left quadrant shows WM CBF, and the bottom-right quadrant shows WM ATT results. The intra-session coefficient of variation (CoV) (A,C,E,G) is a normalized error, while the RMSE, SD, and bias (B,D,F,H) are in physiological units. The SD and bias of the differences aid understanding of the RMSE. Each metric was calculated on a subject-wise basis, with the mean and SD taken across subjects (error bars are shown for CoV and RMSE only). Two-sided paired Wilcoxon signed-rank tests were performed only on the test-retest CoV and RMSE results with significant differences displayed in the figure ($p < 0.05$). The bias was calculated as $\text{mean}(A-B)$ e.g., $\text{mean}(\text{VESPA}-\text{PCASL})$ for the 'VESPA vs PCASL' comparison.

Figure 9: A single slice at each TI for each contrast in the alternative implementations of the VESPA sequence. The top two rows show VESPA_{VC-on}, which is the standard VESPA sequence. The third and fourth rows are the decoded VSASL and PCASL contrasts when the VC is turned off (VESPA_{VC-off}), leaving the macrovascular signal unsuppressed. The last two rows are dual VESPA (where the VSASL and PCASL tag/control conditions are alternated together) with either VC on (dual VESPA_{VC-on}) or VC off (dual VESPA_{VC-off}). The data has been divided by the M_{0B} image to correct B_1^- inhomogeneity. Several of the contrasts have been scaled up by x5 to better visualize them next to the large arterial signal in the VC-off images.

Supporting Information Figure S1: By selectively turning off either the PCASL or VSASL modules (RF and gradients set to zero), while keeping all other sequence parameters fixed, the accuracy of the VSASL and PCASL contrast decoding with VESPA is demonstrated. When VSASL is turned off, no VSASL perfusion signal is decoded but the PCASL contrast remains relatively unchanged. When PCASL is turned off, no PCASL perfusion signal is decoded but the VSASL contrast remains relatively unchanged.

Supporting Information Figure S2: Two slices showing VESPA generated (A) VSASL data, (B) PCASL data, (C) CBF maps, and (D) ATT maps, using different velocity-selective (VS) and vascular crushing (VC) directions. The data has been calibrated by the M_{0B} images. The arrows in (A) and (D) highlight the direction of the applied VS gradients as well as the axis of the largest eddy current errors. The arrows in (B) highlight regions of unsuppressed macrovascular PCASL signal when only using VC along one direction (anterior cerebral artery [top row] and middle cerebral artery [bottom row]). The eddy current errors and unsuppressed macrovascular PCASL signal both modify the estimated CBF and ATT values. Using VS gradients along only z results in the smallest eddy current errors for the scanner used (supported by phantom experiments, not shown), while applying the VC along x, y, and z results in better suppressed macrovascular PCASL signal.

Supporting Information Table S1: The intra-session Coefficient of Variation (CoV), RMSE, bias [mean(A-B)], and SD [std(A-B)] of the test-retest scans and comparisons between ASL techniques. Each metric was calculated on a subject-wise basis, with the mean and SD taken across subjects. CBF values are in units of mL/100g/min and ATT values are units of seconds.