Validation and application of arterial spin labeling MRI for cerebral perfusion
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COMPARISON OF VELOCITY AND ACCELERATION
SELECTIVE ARTERIAL SPIN LABELING WITH
$[^{15}O]H_2O$ POSITRON EMISSION TOMOGRAPHY

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In submission
ABSTRACT

In the last decade spatially non-selective arterial spin labeling (SNS-ASL) methods such as velocity selective ASL (VS-ASL) and acceleration selective ASL (AccASL), have been introduced which label spins based on their flow-velocity or acceleration rather than spatial localization. Since labeling also occurs within the imaging plane, these methods suffer less from transit delay effects than traditional ASL methods. However, there is a need for validation of these techniques. In this study, a comparison was made between these SNS-ASL techniques with \[^{15}O\]H\(_2\)O positron emission tomography (PET), which is regarded as gold standard to measure quantitatively cerebral blood flow (CBF) in humans. Additionally, the question of whether these techniques suffer from sensitivity to arterial cerebral blood volume (aCBV), as opposed to producing pure CBF-contrast, was investigated.

The results show high voxel-wise correlation (0.72–0.89) between the spatial distribution of the perfusion signal from the SNS-ASL methods and the PET CBF-maps. There was a significant underestimation of 16.7% of the GM CBF measured by dual VS-ASL compared with PET (39.2±3.5 versus 47.1±6.5mL/100mL/min, respectively). Finally, only a minor contribution of aCBV-patterns to all SNS-ASL methods was found.

In conclusion, VS-ASL underestimates CBF, but all SNS-ASL methods provide qualitatively similar CBF-maps as \[^{15}O\]H\(_2\)O PET.

**Keywords**

\[^{15}O\]H\(_2\)O positron emission tomography; acceleration selective arterial spin labeling; cerebral blood flow; perfusion imaging; velocity selective arterial spin labeling
INTRODUCTION

Arterial spin labeling (ASL) is an MR technique that uses arterial blood as an endogenous tracer for non-invasive and quantitative local tissue perfusion measurements (Detre et al., 1992). Conventional ASL methods label blood magnetically by inversion or saturation in a slab proximal to the imaging region. Subsequently, the label image is acquired after a post labeling delay (PLD), which is chosen approximately equal to the longitudinal relaxation time of blood ($T_1$) for cerebral perfusion imaging. The PLD represents a compromise between loss of label due to $T_1$ decay and transport time for labeled blood to reach the microvascular bed (Alsop and Detre, 1996). A so-called control image is obtained by repeating the sequence without labeling blood. By subtracting the label image from the control image, contributions from static tissue will be removed. Therefore, solely magnetization of inflowing tagged spins will be measured, resulting in a perfusion-weighted image. To gain sufficient signal-to-noise ratio (SNR) multiple interleaved repetitions of both label and control sequences are performed.

Currently, pseudo-continuous ASL (pCASL) is considered to be the most reliable and robust ASL technique (Alsop et al., 2013). However, when arrival of the labeled blood in the tissue is delayed - for example due to pathology - a longer PLD is required, leading to more relaxation of the label and thereby lower SNR. On the other hand, when the PLD is chosen too short, a severe underestimation of the cerebral blood flow (CBF) will occur. Selecting a proper PLD for accurate CBF values in clinical pathologies represents a delicate balance and would frequently lead to a need for too many signal averages to be clinically practical.

Recently, a new family of ASL techniques has been introduced. These spatially non-selective ASL (SNS-ASL) methods label spins based on their flow velocity (VS-ASL) or acceleration (AccASL) rather than spatial localization (Duhamel et al., 2003; Norris and Schwarzbauer, 1999; Schmid et al., 2014; Wong et al., 2006). As the label is generated globally, i.e. also within the imaging plane, it is labeled much closer to the capillaries. Therefore, the time to reach the tissue, the so-called transit delay, is smaller and more uniform and consequently these SNS-ASL techniques have the potential to be used even under slow and collateral flow conditions (Guo and Wong, 2014; Qiu et al., 2012). Although all these SNS-ASL methods are regarded to reflect perfusion information with one of them thought to be fundamentally weighted to cerebral blood volume (CBV) (single VS-ASL), another purely to CBF (dual VS-ASL) and the third to mixed hemodynamic parameters, both CBF and CBV (AccASL).

Clearly, there is a need for validation of this new family of ASL techniques. Dual VS-ASL has already been compared with traditional ASL techniques (Wong et al., 2006; Wu and Wong, 2007), and Xenon computer tomography (CT) (Qiu et al., 2012), but single VS-ASL and AccASL have only been compared with pCASL (Schmid et al., 2014). None of these SNS-ASL techniques have, however, been compared to the gold standard for quantifying CBF in humans: $[^{15}O]H_2O$ positron emission tomography (PET) (Bokkers et al., 2009). Except for providing parametric CBF-images, $[^{15}O]H_2O$ PET can also be used to generate arterial cerebral blood volume (aCBV) images.

Therefore, the aim of this study was to compare SNS-ASL methods with $[^{15}O]H_2O$ PET derived CBF. In addition, it will be investigated whether these SNS-ASL methods suffer from additional sensitivity to aCBV as opposed to pure CBF-contrast, again using $[^{15}O]H_2O$ PET as a standard.
MATERIALS AND METHODS

Subjects and study protocol

This study was performed in compliance with regulations of the Local Institutional Review Boards of the participating centers and written informed consent was obtained from each participant prior to inclusion. This study was part of another study, where the accuracy and precision of pCASL measurements were compared head-to-head with \(^{15}\)O\textsubscript{2}H\textsubscript{2}O PET, by means of a test-retest paradigm as described by Heijtel et al. (Heijtel et al., 2014).

In addition to the previously described results, we studied in this current study three different SNS-ASL scans, which were performed during the second visit. Only the healthy subjects who completed all required scans (three SNS-ASL scans, a pCASL scan, a pCASL scan with vascular crushing as well as the \(^{15}\)O\textsubscript{2}H\textsubscript{2}O PET scan) were included in the present study (n=13, 7 male and 6 female, age 20-24 years).

All MRI scans were performed on a Philips 3T Intera system (Philips Healthcare, Best, the Netherlands) using an 8 channel SENSE head-coil at the Academic Medical Center in Amsterdam. All PET examinations were performed on a Philips Gemini TF-64 PET/CT system (Philips Healthcare, Cleveland, TN, USA) at the VU University Medical Center in Amsterdam. The PET and MRI scans were performed in a random order with a maximum of 7 days between both sessions.

Three types of SNS-ASL approaches were compared with \(^{15}\)O\textsubscript{2}H\textsubscript{2}O PET. The first type, which will be referred to as “single VS-ASL”, uses one velocity-selective labeling module and labels all spins that flow faster than a predefined cut-off velocity (V\textsubscript{C}). This is irrespective of whether these spins are located in arterial or venous blood and therefore this sequence is thought to be fundamentally CBV weighted. The second type, which will be referred to as “dual VS-ASL”, is similar to single VS-ASL, except that a second velocity-selective labeling module is added just before imaging in both the label and control condition. This suppresses all spins accelerating in the PLD between the labelling modules, which is assumed to be the venous component (Duhamel et al., 2003). This is the only quantitative SNS-ASL method and is proposed to be predominantly CBF-weighted (Wong et al., 2006). In literature, this technique is referred to as VS-ASL, but to provide more insight into the labeling process, single VS-ASL was also included into this study, thereby requiring a clear distinguished terminology. The most recently introduced and third type is acceleration selective ASL (AccASL), which contains only a single labeling module and labels all spins that accelerate or decelerate during the labeling module above a certain cut-off acceleration (or deceleration) (A\textsubscript{C}). It has been suggested that the signal is of mixed hemodynamic origin; including both CBF and CBV-weighting. The only difference between the velocity-sensitive and acceleration-sensitive labeling modules is the sign of the second and fourth gradients in the labeling module, inducing an effective zero first-gradient moment, giving no velocity sensitization, but acceleration sensitization due to a second-gradient moment (Priest et al., 2011; Schmid et al., 2014).

pCASL, as employed in the present study for reference purposes, was previously compared with \(^{15}\)O\textsubscript{2}H\textsubscript{2}O PET showing good resemblance (Heijtel et al., 2014; Xu et al., 2009).

MRI Acquisition

Single VS-ASL, dual VS-ASL and AccASL were acquired with interleaved label and control images. The labeling module parameters for VS-ASL, which determine the velocity-sensitivity, were 22
mT/m for the amplitude of the gradients (G), 1 ms for the gradient duration (δ) and 30 ms for the time between the 90° RF-pulses (Δ), corresponding to a V_c of 2 cm/s. The labeling module parameters for AccASL, which determine the acceleration-sensitivity, were G=30mT/m, δ=1ms and Δ=30 ms, corresponding to an AC of 2.3 m/s². Velocity and acceleration encodings were only applied along the slice encoding direction (approximately feet-head direction). For all three techniques background suppression was applied using two adiabatic non-selective inversion pulses at 50 and 1150 ms after labeling to increase the contrast-to-noise ratios (Garcia et al., 2005; St Lawrence et al., 2005; Ye et al., 2000). The post-acquisition delay, the time between the post-acquisition non-selective saturation and subsequent labeling module, was set to 2000 ms. An overview of all imaging parameters can be found in Table 1.

Balanced pCASL was used as a reference representing spatially selective or “conventional” ASL methods (Dai et al., 2008). The labeling pulse duration was 0.5 ms with a 0.5 ms pause between the pulses and a 18° flip angle combined with a 0.6 mT/m average gradient in the direction of the blood flow. pCASL scans were acquired with and without vascular crushing (V_c=5 cm/s) to study the effect of macrovascular crushing. For positioning of the pCASL labeling plane, a time-of-flight (TOF) angiogram was acquired.

For ASL quantification purposes, a multi-time point inversion recovery sequence with equal readout properties as the ASL sequence was acquired to estimate the longitudinal magnetization (M₀), followed by a T₁ mapping sequence of the venous blood to estimate the longitudinal relaxation of arterial blood (T₁a) (Varela et al., 2010). In addition, to estimate the pCASL labeling efficiency a phase contrast scan (PC-MRI) was performed immediately after the pCASL scans, using a slice positioned at the center of the pCASL labeling plane, in order to measure the blood flow velocity in the brain feeding arteries.

For anatomical reference a whole brain T₁-weighted MPRAGE image was acquired with a 1 mm isotropic voxel size.

**MRI post processing**

The Oxford Centre for Functional MRI of the Brain (FMRIB)'s Software Library (FSL) was used for realigning the unsubtracted ASL images (Smith et al., 2004; Woolrich et al., 2009) and the time series were motion corrected with Motion Correction FMRIB’s Linear Image Registration Tool (MCFLIRT) with a six-parameter rigid transformation (Jenkinson et al., 2002). ASL-maps were obtained by pairwise subtracting the label from the control images and averaging over time. To enable a valid comparison of the temporal signal-to-noise ratios (tSNR) between all different ASL methods, a fixed total ASL sequence duration of 5 min was chosen. To this end, 35 averages of the SNS-ASL scans and the first 38 averages of the pCASL scans were included in the calculation of the tSNR, using the mean and standard error of the mean (SEM).

Subsequently, CBF was calculated for dual VS-ASL according to the following equation (Wu and Wong, 2007):

\[ \text{CBF_{VSASL}} = \frac{\Delta M \cdot e^{PLD/T_{1a}} \cdot e^{TE/T_{2a}}}{\rho \cdot M_{0a} \cdot PLD \cdot \alpha_{BSup} \cdot (1 - e^{(PLD-TR)/T_{1a}})} \]
Table 1: Scanning parameters the various scans in the MRI protocol.

<table>
<thead>
<tr>
<th>Method</th>
<th>Spatially non-selective ASL</th>
<th>Conventional ASL</th>
<th>Anatomical M_0,CSF</th>
<th>Labeling efficiency</th>
<th>T1s (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FOV (mm²)</td>
<td>240 x 240</td>
<td>240 x 240</td>
<td>240 x 240</td>
<td>240 x 240</td>
<td>240 x 240</td>
</tr>
<tr>
<td>Resolution (mm²)</td>
<td>3 x 3</td>
<td>3 x 3</td>
<td>3 x 3</td>
<td>3 x 3</td>
<td>3 x 3</td>
</tr>
<tr>
<td>Slice thickness (mm)</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>Slices</td>
<td>17</td>
<td>17</td>
<td>17</td>
<td>17</td>
<td>20.0</td>
</tr>
<tr>
<td>TR/TE (ms)</td>
<td>4248/14</td>
<td>4248/14</td>
<td>4248/14</td>
<td>3850/14</td>
<td>3921/17</td>
</tr>
<tr>
<td>Read-out</td>
<td>GE-SSh-EPI</td>
<td>GE-SSh-EPI</td>
<td>GE-SSh-EPI</td>
<td>GE-SSh-EPI</td>
<td>GE-SSh-EPI</td>
</tr>
<tr>
<td>Labeling duration or Δ (ms)</td>
<td>30</td>
<td>30</td>
<td>1650</td>
<td>1650</td>
<td>-</td>
</tr>
<tr>
<td>PLD (ms)</td>
<td>1600</td>
<td>1600</td>
<td>1600</td>
<td>1525</td>
<td>1525</td>
</tr>
<tr>
<td>ΔTI (ms) / nTI</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>200/10</td>
</tr>
<tr>
<td>Bsup (ms)</td>
<td>50/1150</td>
<td>50/1150</td>
<td>1680/2860</td>
<td>1680/2860</td>
<td>-</td>
</tr>
<tr>
<td>G (mT/m)</td>
<td>22</td>
<td>22</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>δ (ms)</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Vc or Ac</td>
<td>2 cm/s</td>
<td>2 cm/s</td>
<td>2.3 m/s²</td>
<td>5 cm/s</td>
<td>80 cm/s</td>
</tr>
<tr>
<td>SENSE</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Fat suppression</td>
<td>SPIR</td>
<td>SPIR</td>
<td>SPIR</td>
<td>SPIR</td>
<td>SPIR</td>
</tr>
<tr>
<td>NSA</td>
<td>35</td>
<td>35</td>
<td>35</td>
<td>38</td>
<td>1</td>
</tr>
<tr>
<td>T_{acq} (s)</td>
<td>297</td>
<td>297</td>
<td>297</td>
<td>419</td>
<td>301</td>
</tr>
</tbody>
</table>

Abbreviations: Field of view (FOV), repetition time (TR), echo time (TE), time between the 90° RF-pulses of the labeling module (Δ), post labeling delay (PLD), inversion time (TI), background suppression pulses (Bsup), amplitude of the gradients in the labeling module (G), gradient duration of the labeling module (δ), cut-off velocity (Vc), cut-off acceleration (Ac), sensitivity encoding (SENSE), number of signal averages (NSA), acquisition time (Tacq), fast field echo (FFE), gradient echo (GE), single shot (SSh), echo planar imaging (EPI), Spectral Presaturation Inversion Recovery (SPIR).
where CBF is the flow in mL/min/100gr, ΔM the ASL signal intensity, PLD the post-labeling delay (1600 ms for the first slice and increasing with 35 ms for each following slice to correct for the ascending slice time delay), $T_{1a}$ the longitudinal relaxation of arterial blood (as estimated by $T_1$ mapping of venous blood in the sagittal sinus (Varela et al., 2010)), $T_{2a}^*$ the $T_{2}^*$ of arterial blood (50 ms (Teeuwisse et al., 2011)), $T_1t$ the longitudinal relaxation of brain tissue (1200 ms for GM (Lu et al., 2005)), $\alpha_{\text{pCASL}}$ the labeling efficiency derived from the PC-MRI measurement (as simulated by Bloch equations based on the velocities in the labeled arteries (Aslan et al., 2010; Wu et al., 2007)), PLD the post-labeling delay (1525 ms for the first slice and increasing with 35 ms for each following slice), and $\tau$ the labeling duration (1650 ms).

For the vascular crushed pCASL scans it was assumed that only the ASL signal in relatively large arteries with a blood velocity larger than 5 cm/s was affected by crushing (Ye et al., 1997). Therefore, quantification of these scans was performed using the same model as the non-crushed pCASL scans.

An in-plane 5 mm full width at half maximum (FWHM) Gaussian kernel was used to smooth all CBF images in order to obtain an image resolution comparable with that of [15O]H$_2$O PET images and to have a similar smoothing process.

**PET acquisition**

Prior to scanning, all patients received an indwelling radial artery cannula for blood sampling and a venous cannula in the opposite arm for administration of [15O]H$_2$O. Each patient was positioned with the head in the center of the field of view and immobilized with a foam mold to minimize motion. First, a 1 min low-dose CT transmission scan was acquired to enable correction of the subsequent emission scan for photon attenuation and scatter. Next, a dynamic emission scan was performed in 3D acquisition mode, starting at the time of administration of an intravenous bolus of 800 MBq [15O]H$_2$O. This scan consisted of 25 frames with progressively increasing duration over a total scanning period of 10 min. The concentration in arterial blood was monitored continuously using an online blood sampler (Boellaard et al., 2001), which was calibrated using three manual arterial blood samples, taken at 5.5, 8 and 10 min after injection.

**PET post processing**

The [15O]H$_2$O PET data were reconstructed using the row action maximum likelihood algorithm (RAMLA) brain reconstruction protocol as provided by the vendor (128×128 matrix, 2 mm
isotropic voxel size), including all common corrections (random events, dead time, photon decay, attenuation and scatter) required for quantification. Reconstructed images were smoothed with an isotropic 5 mm FWHM Gaussian kernel, resulting in an image resolution of approximately 6.5 mm isotropic FWHM. A single tissue compartment model with arterial blood volume fraction correction was used for CBF quantification:

\[ C(t) = V_a \cdot C_s + (1 - V_a) \cdot f_{\text{PET}} \cdot e^{-(d_{\text{PET}} \cdot V_a)} \otimes C_s + \text{(terms for dispersion, delay, and arterial blood volume)} \]

where \( C_a \) is the tissue concentration of \(^{15}\text{O} \text{H}_2\text{O} \), \( V_a \) the arterial blood volume (aCBV), \( C_s \) the arterial concentration of \(^{15}\text{O} \text{H}_2\text{O} \), \( f_{\text{PET}} \) the blood flow (CBF), \( t \) the time and \( V_t \) the volume of distribution of the water. Subsequently, parametric CBF and aCBV images were generated from the smoothed dynamic images using a basis function method implementation of the single tissue compartment model with corrections for dispersion, delay and arterial blood volume (Boellaard et al., 2005; Bremmer et al., 2010).

**General post-processing**

In FSL, the FMRIB's Automated Segmentation Tool (FAST) was used to segment the anatomical T1-weighted scan of each subject into different tissue types (grey matter (GM), white matter (WM) and cerebrospinal fluid (CSF) probability maps). All ASL and PET CBF-maps were individually co-registered to the segmented GM probability map using FMRIB's Linear Image Registration Tool (FLIRT). A GM mask was generated using a threshold of 55% GM probability. The T1-weighted scan of each subject was registered to the Montreal Neurological Institute (MNI) template with FMRIB's Non-Linear Image Registration Tool (FNIRT). Subsequently, all co-registered perfusion images and GM masks were warped into MNI space using the same transformation parameters.

**Data analysis**

For each subject, the mean GM CBF was calculated for the dual VS-ASL, pCASL and \(^{15}\text{O} \text{H}_2\text{O} \) PET scans using the individual GM-masks. Next, these CBF-values were compared with each other using a paired t-test applying a two-tailed significance level of 0.05. A Bland-Altman analysis (Bland and Altman, 1986) was performed to investigate the spread and measurement agreement between dual VS-ASL and \(^{15}\text{O} \text{H}_2\text{O} \) PET. Bias and 95% limits of agreement were calculated as mean difference and as 1.96 × standard deviation of difference between paired measurements respectively.

A one-way ANOVA was performed to compare the temporal signal-to-noise ratios (tSNR) for the different ASL techniques. To determine the tSNR in GM, both mean (\( \mu \)) and SEM (\( \sigma / \sqrt{n} \)) were calculated voxel-by-voxel over the pairwise subtracted ASL-maps before averaging:

\[ t\text{SNR} = \frac{\mu \sqrt{n}}{\sigma} \]

Since single VS-ASL and AccASL scans cannot be quantified, the comparison for these scans focused only on the tSNR and the distribution of the signal. In Matlab (R2012b, The MathWorks Inc., Natick,
Comparison of VS-ASL techniques with $[^{15}O]_2$H$_2$O PET

MA) all scans were normalized by dividing each voxel by the average signal intensity in GM. The distribution agreement between the group-averaged normalized scans was visualized in a joint histogram: ASL versus $[^{15}O]_2$H$_2$O derived CBF. The whole brain linear correlation coefficient (Pearson's $r$) was obtained to estimate the degree of correlation between ASL and $[^{15}O]_2$H$_2$O derived CBF and between ASL and $[^{15}O]_2$H$_2$O derived aCBV measurements. In order to assess whether the ASL methods are (also) sensitive to aCBV, the relative distribution of signal in the maps was compared by correlating the normalized, group-averaged ASL maps iteratively with different weighted sums of $[^{15}O]_2$H$_2$O derived CBF and aCBV. The aCBV-fraction at the maximum correlation was interpreted as the aCBV contribution to the ASL scans, where a differentiation was made between the whole brain correlation and the correlation of the whole brain excluding slices containing the Circle of Willis and below.

RESULTS

Normalized $[^{15}O]_2$H$_2$O PET and ASL-images for both the group-average and a single subject example of the are shown in figure 1. For $[^{15}O]_2$H$_2$O PET the lower acquisition resolution is noticeable. On the other hand the ASL scans had a decreased FOV in the z-direction (images not shown), whilst PET covers the entire brain. In AccASL and even more in single VS-ASL an increased signal intensity is visible in the sagittal sinus. Only in the $[^{15}O]_2$H$_2$O derived aCBV maps the Circle of Willis is clearly present.

The $M_0$, measured for CBF quantification, was $3.8 \times 10^5 \pm 0.27 \times 10^5$ and the arterial blood $T_1$ was $1789 \pm 42$ ms for females, which was significantly higher than $1696 \pm 63$ ms for males ($p<0.01$, unpaired student t-test). The mean GM CBF values for dual VS-ASL, pCASL and $[^{15}O]_2$H$_2$O PET are shown in Table 2, together with the GM tSNR of all ASL scans. ANOVA analysis indicated a significant difference in tSNR at the $p<0.05$ level for the different ASL techniques [$F(4, 60) = 37.65$, $p = 9.82 \times 10^{-16}$]. Post hoc comparisons using the Tukey HSD test indicated that the mean tSNR of all ASL techniques were significantly different from each other ($p<0.05$), except for the tSNR of AccASL and non-crushed pCASL ($p=0.80$).

The Bland-Altman plot (figure 2) showed a bias between the mean GM CBF of dual VS-ASL and $[^{15}O]_2$H$_2$O PET. The significant underestimation of GM CBF by dual VS-ASL was on average 16.7% (paired t-test, $p<0.05$). Furthermore, regression analysis showed that the actual bias increased with increasing CBF ($p=0.045$). Moreover, the mean GM CBF-value of dual VS-ASL also was significantly lower than pCASL with or without vascular crushing ($p<0.001$).

In figure 3 the whole brain normalized group-average of the ASL signal versus $[^{15}O]_2$H$_2$O derived CBF is plotted voxel-wise in a joint histogram. In Table 3 the correlation coefficients between ASL and $[^{15}O]_2$H$_2$O PET scans are presented.

Table 2. The average grey matter cerebral blood flow (GM CBF, in mL/100mL/min) of the quantifiable ASL and PET scans and the temporal signal-to-noise ratios (tSNR) of the 5 different ASL scans, both evaluated at subject level [mean ± SD].

<table>
<thead>
<tr>
<th></th>
<th>PET CBF</th>
<th>AccASL</th>
<th>Single VS-ASL</th>
<th>Dual VS-ASL</th>
<th>pCASL no crush</th>
<th>pCASL crush</th>
</tr>
</thead>
<tbody>
<tr>
<td>GM CBF</td>
<td>47.1 ± 6.5</td>
<td></td>
<td></td>
<td>39.2 ± 3.5</td>
<td>60.7 ± 10.9</td>
<td>49.2 ± 9.2</td>
</tr>
<tr>
<td>tSNR</td>
<td>6.96 ± 1.42</td>
<td>5.04 ± 0.85</td>
<td>3.49 ± 0.50</td>
<td>8.40 ± 1.41</td>
<td>7.05 ± 1.19</td>
<td></td>
</tr>
</tbody>
</table>
In Figure 1, an example of 3 transversal slices of the $\textit{[15]O} \text{H}_2\text{O}$ PET and ASL maps of both the group average and a single volunteer is shown. For comparison of the spatial distribution of the signal, all maps were normalized dividing each voxel by the average grey matter value of the corresponding map.

$\textit{[15]O} \text{H}_2\text{O}$ derived CBF evaluated at subject level are shown, with an average range between 0.72 and 0.91 when only including slices above the Circle of Willis and between 0.62 and 0.85 for the whole brain. The correlation coefficients for AccASL, both pCASL scans and to a lesser extent dual VS-ASL were comparable. Only single VS-ASL showed a lower correlation and larger standard deviation. In Table 3 the correlation coefficients between the various ASL modalities with $\textit{[15]O} \text{H}_2\text{O}$ derived aCBV are presented as well. For all ASL sequences the correlation coefficients with PET aCBV were lower than PET CBF (p<0.001). The correlation coefficients with $\textit{[15]O} \text{H}_2\text{O}$ derived aCBV of AccASL and both pCASL scans were similar; but both VS-ASL methods showed a lower correlation.
Comparison of VS-ASL techniques with $^{[15O]}$H$_2$O PET

Figure 2. Bland-Altman plot of dual VS-ASL and $^{[15O]}$H$_2$O PET CBF in grey matter. The solid line depicts the mean difference between dual VS-ASL and PET CBF over all volunteers, the dashed lines the corresponding 95% confidence intervals and the dotted line the regression line.

Figure 3. Voxel-wise joint histograms (34 x 30 bins) of ASL versus $^{[15O]}$H$_2$O PET CBF of whole brain intensity normalized group-averaged maps.

Table 3. Whole brain (WB) and above the Circle of Willis (CoW) intensity normalized correlation coefficient between ASL and $^{[15O]}$H$_2$O PET CBF evaluated at subject level [mean ± SD] and the maximum Pearson’s r correlation coefficient between ASL and the weighted sum of PET cerebral blood flow (CBF) and arterial cerebral blood volume (aCBV) evaluated at group level.

<table>
<thead>
<tr>
<th></th>
<th>AccASL</th>
<th>Single VS-ASL</th>
<th>Dual VS-ASL</th>
<th>pCASL no crush</th>
<th>pCASL crush</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correlation with PET CBF</td>
<td>WB</td>
<td>0.85 ± 0.02</td>
<td>0.62 ± 0.08</td>
<td>0.65 ± 0.03</td>
<td>0.84 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>above CoW</td>
<td>0.89 ± 0.02</td>
<td>0.72 ± 0.08</td>
<td>0.84 ± 0.02</td>
<td>0.91 ± 0.01</td>
</tr>
<tr>
<td>Correlation with PET aCBV</td>
<td>WB</td>
<td>0.72 ± 0.06</td>
<td>0.54 ± 0.09</td>
<td>0.58 ± 0.06</td>
<td>0.74 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>above CoW</td>
<td>0.75 ± 0.06</td>
<td>0.60 ± 0.10</td>
<td>0.70 ± 0.06</td>
<td>0.79 ± 0.06</td>
</tr>
<tr>
<td>Max correlation with weighted sum of PET CBF and aCBV</td>
<td>WB</td>
<td>0.90</td>
<td>0.71</td>
<td>0.73</td>
<td>0.88</td>
</tr>
<tr>
<td></td>
<td>above CoW</td>
<td>0.93</td>
<td>0.78</td>
<td>0.90</td>
<td>0.95</td>
</tr>
<tr>
<td>% contribution of PET aCBV</td>
<td>WB</td>
<td>20</td>
<td>30</td>
<td>47</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>above CoW</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>21</td>
</tr>
</tbody>
</table>
Maximizing correlation with respect to the weighted sum of $[15\text{O}]\text{H}_2\text{O}$ derived CBF and aCBV showed only a minor aCBV contribution for all SNS-ASL methods for slices above the Circle of Willis, as can be observed in figure 4 and table 3. The largest influence of aCBV - although still marginal - was detected for pCASL. When looking at the maximum whole brain correlation of ASL with the weighted sum of $[15\text{O}]\text{H}_2\text{O}$ derived CBF and aCBV, AccASL and pCASL showed a similar high correlation with a lower maximum correlation for both VS-ASL methods showed. The contribution of aCBV was increased for all scans when including the slices with the Circle of Willis.

**DISCUSSION**

In the current study we compared three spatially non-selective ASL techniques with the gold standard $[15\text{O}]\text{H}_2\text{O}$ PET and showed pCASL scans, with and without vascular crushing, as a reference for traditional, spatially selective ASL. In addition, the correlation of these ASL methods with aCBV was assessed. To the best of our knowledge this is the first study to extensively compare these SNS-ASL methods with $[15\text{O}]\text{H}_2\text{O}$ PET. The most important findings of the present study are threefold. Firstly, a significant underestimation of the GM CBF by dual VS-ASL compared with $[15\text{O}]\text{H}_2\text{O}$ PET was identified. Secondly, the spatial signal distribution of the SNS-ASL methods showed good agreement with that of $[15\text{O}]\text{H}_2\text{O}$ derived CBF. Finally, for all SNS-ASL methods only a minor presence of aCBV patterns was found.

From the three SNS-ASL methods only the CBF of dual VS-ASL can be evaluated quantitatively. The average GM CBF was calculated using the equation proposed by Wu and Wong (Wu and Wong, 2007), with an additional correction factor to account for the two background suppression pulses. A significant difference was found between the GM CBF obtained using dual VS-ASL and $[15\text{O}]\text{H}_2\text{O}$ PET. In addition, a negative relationship was observed between CBF measured by these modalities as shown in the Bland-Altman plot (figure 2), indicating a larger...
underestimation for higher CBF-values. This lower signal in dual VS-ASL could be related to four factors. Firstly, the velocity sensitive gradients of the labeling module were only applied in the z-direction. Most of the blood flowing in the larger vessels is moving in that direction, but there are important exceptions, such as the Circle of Willis and smaller vessels (Wu and Wong, 2007). Ideally, the labeling should be encoded rapidly along all three directions. Secondly, $T_2$-relaxation occurs when spins are in the transversal plane, which is the case during the VS labeling module. Due to the use of two labeling modules significant $T_2$-weighting will be included and, therefore, the ASL signal will decrease leading to an underestimation in CBF. Thirdly, for pCASL and [$^{15}$O]$H_2$O PET a two-compartment model was used, whereas for dual VS-ASL a single compartment model was used to quantify the CBF. Finally, a limitation is that a constant, brain average blood-water partition coefficient was assumed in the ASL quantification, although it is known that this is a function of the hematocrit that may vary between subjects. Furthermore, the partition coefficient also varies throughout the brain, since the water content of the tissue is not constant for the different brain regions (Herscovitch and Raichle, 1985). Since for [$^{15}$O]$H_2$O PET a region specific volume of distribution was calculated, GM-WM discrepancies might occur between both modalities. Nevertheless, the influence on the CBF quantification is hypothesized to be limited, since this was only performed in GM.

In dual VS-ASL only the spins that experience decrease in flow velocity during the PLD will result in ASL signal, thereby removing the contamination of venous signal, which is still present when only a single labeling module is used. However, the use of the second labeling module will eliminate part of the arterial signal as well and thus decrease the amount of detected signal. Despite this, it will not influence the CBF, since it is corrected for in the quantification equation, but it does decrease the tSNR. This decrease in tSNR adds to the already lower tSNR of SNS-ASL due to the use of saturation instead of inversion for labeling.

Although the pCASL data in this study was included for reference purposes only, it was noticed that the calculated mean GM CBF values in this subgroup subjects was $9.8 \pm 4.4 \text{ mL/min/100g}$ higher than reported by Heijtel et. al (Heijtel et al., 2014). The main reason for this difference was that in this present study the GM masks with a different threshold were created, which could lead to differences in the voxels included in the analysis and, moreover, all scans were registered to MNI, whereas in the other study a group-specific atlas was made. Furthermore, some volunteers who were included in the current analysis, were not included in the previous reported analysis and vice versa, resulting in an elevated mean CBF.

The comparison of AccASL and single VS-ASL with [$^{15}$O]$H_2$O derived CBF solely focused on the tSNR and the spatial distribution of the signal by normalized maps, joint histograms, and the calculated correlation coefficients, as these ASL techniques cannot be quantified. When both modalities would have had the same signal distribution, the joint histograms would have shown a straight line as evidence of good agreement. As both the ASL and [$^{15}$O]$H_2$O PET data were normalized to the average signal intensity of the GM, the slope of the line has no specific meaning. In figure 3 it can clearly be seen that the ASL methods showed a good whole brain correlation with PET CBF. WM is represented in the left bottom corner, because the perfusion of WM is lower than that of GM. For [$^{15}$O]$H_2$O PET a region specific volume of distribution was calculated for the blood-water partition coefficient. However, the correlation coefficients were calculated using the ASL data without regional correction for the partition coefficient, which will induce GM-WM
discrepancies between both modalities and might have led to lower correlation values. Some of the other discrepancies can be explained by the EPI read-out, as can be seen in figure 1: it has been shown previously that the EPI read-out with pCASL labeling is prone to signal loss in the prefrontal brain area (Vidorreta et al., 2012). Furthermore, the timing of background suppression pulses was rather aggressive, which could have led to some signal loss in the lowest slices.

Focusing at the correlation coefficients as presented in table 3, it is clear that compared to all other ASL techniques single VS-ASL showed the lowest correlation with $[^{15}\text{O}] \text{H}_2\text{O}$ derived CBF. This weaker correlation could be explained by the presence of venous signal, of which the high signal intensity in the sagittal sinus is a good example, which is less obvious in e.g. AccASL and even absent in the other ASL sequences. Furthermore, it is known that single VS-ASL, and again to a lesser extent for AccASL, has higher signal in CSF regions, due to the combination of a relatively high amount of diffusion and flow in CSF. The diffusion weighting of the labeling module is strong enough to cause diffusion related attenuation in CSF and thereby contamination of the CBF-maps (Wong et al., 2006). To incorporate this contamination into our validation analysis, it was decided to compare signal distribution over the entire brain between ASL and PET (i.e. including ventricles and sagittal sinus), rather than limiting this analysis to GM.

For both single VS-ASL and AccASL, it has been postulated that they would be more weighted towards CBV than CBF, since all blood above a certain velocity or acceleration is labeled (Schmid et al., 2014). To investigate this hypothesis, weighting towards aCBV was studied in two ways. Firstly, the whole brain correlation coefficients of ASL with $[^{15}\text{O}] \text{H}_2\text{O}$ derived aCBV were examined. The highest correlations with PET aCBV were observed for pCASL and AccASL, closely followed by dual VS-ASL. Only the correlation coefficient of single VS-ASL with PET aCBV differed from the other sequences, being approximately 20% lower than the traditional ASL methods. However, all ASL methods showed lower whole brain correlation coefficients with PET CBF than with PET aCBV. Therefore, in the second analysis, the whole brain correlation coefficient of the various ASL methods for a range of different weighted sums of PET CBF and aCBV was calculated. The highest whole brain correlation coefficient between the SNS-ASL sequences and the weighted sum of PET CBF and aCBV was found when no additional aCBV-weighting was included. aCBV-maps mainly show besides a normal GM/WM contrast, large vessels and the signal is only arterial, whereas the single VS-ASL and AccASL are thought to create label closer to the tissue due to the relatively long PLD and the images also include a venous component (Guo and Wong, 2014; Schmid et al., 2014). In summary, the present results show that single VS-ASL and AccASL have much lower weighting towards aCBV than towards CBF. Unfortunately, these findings cannot answer the question whether those techniques are more weighted towards total CBV and this remains to be answered.

For dual VS-ASL the maximum correlation coefficient with the weighted sum of $[^{15}\text{O}] \text{H}_2\text{O}$ derived CBF above the Circle of Willis was observed for full $[^{15}\text{O}] \text{H}_2\text{O}$ derived CBF-weighting without additional aCBV-weighting included. The maximum whole brain correlation was detected at an almost 50% aCBV-weighting. This difference due to including of slices with the Circle of Willis into the analysis could be explained by the directionality of labeling in the velocity selective module, which is in the feet-head direction, while the vessels of the Circle of Willis are in different directions.
Remarkably, no significant difference was identified in the maximum whole brain correlation coefficient of the weighted sum of $^{15}$O H$_2$O derived CBF and aCBV with pCASL with and without vascular crushing. This is remarkable, because vascular crushing is known to be able to resolve areas with overestimated CBF in pCASL to a large extent (Ye et al., 1997). Also no significant difference was observed in the percentile contribution of $^{15}$O H$_2$O derived aCBV. However, it should be noted that this was evaluated at group-level and that for both pCASL methods only a minor aCBV pattern contribution was established.

In the present study, the $^{15}$O H$_2$O derived aCBV were used only for comparison of the relative signal distributions and not for quantitative purposes. This approach was followed because of uncertainties in quantitative accuracy of aCBV as measured by $^{15}$O H$_2$O PET. One problem is the potential confounding effect of dispersion in the arterial line. When the dispersion is not estimated correctly due to artefacts in the image reconstruction, aCBV will become less accurate and could especially show a global bias (Lammertsma and Jones, 1983). Visual inspection of the individual aCBV-maps was performed to identify any clear errors, which were not observed. PET would be able to provide more accurate CBV-measurements by means of $^{15}$O-labeled carbon monoxide (C$^{15}$O) (Bremmer et al., 2010).

The study protocol could have been improved by acquiring the PET and ASL scans on the same day or even at the same time, thereby minimizing the effects of the physiological fluctuations in the perfusion (Zhang et al., 2014). Unfortunately, this was logistically impossible with the imaging centers at two different locations, since at the time of the study no combined PET/MRI system was available in either if the two centers at the start of this study. Nevertheless, it should be noted that it was verified that both pCASL and VS-ASL demonstrate similar variations over time, with changes on the order of 10% or less over a 6 month period (Zun et al., 2013).

In future work, a comparison of the results from spatially non-selective ASL methods with images that are (totally) CBV-weighted should be made, to better understand in the weighting of the different ASL methods. Furthermore, the current recommended clinical application for SNS-ASL is pathology with slow or collateral flow. Therefore, a comparison between conventional and spatially non-selective ASL methods and $^{15}$O H$_2$O or $^{15}$O CO PET in patients with large vessel disease could give more insight into the preferred perfusion measurement technique for such pathologies with delayed arrival times.

In conclusion, dual VS-ASL underestimates CBF, but qualitatively it provides similar CBF-maps as compared to $^{15}$O H$_2$O PET. From the spatially non-selective ASL methods, AccASL was most similar to PET CBF, showing only a 2% lower correlation coefficient compared with pCASL. This opens up the possibility of exploring the clinical applications and validations for especially dual VS-ASL as a quantitative technique and AccASL for qualitative purposes.

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