ORIGINAL ARTICLE

Reliability of Three Dimensional Pseudo-continuous Arterial Spin Labeling: A Volumetric Cerebral Perfusion Imaging with Different Post-labeling Time and Functional State in Health Adults

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Key words: pseudo-continuous arterial spin labeling; magnetic resonance imaging; intraclass correlation coefficient; reliability

Objective To evaluate the reliability of three dimensional spiral fast spin echo pseudo-continuous arterial spin labeling (3D pc-ASL) in measuring cerebral blood flow (CBF) with different post-labeling delay time (PLD) in the resting state and the right finger taping state.

Methods 3D pc-ASL and three dimensional T1-weighted fast spoiled gradient recalled echo (3D T1-FSPGR) sequence were applied to eight healthy subjects twice at the same time each day for one week interval. ASL data acquisition was performed with post-labeling delay time (PLD) 1.5 seconds and 2.0 seconds in the resting state and the right finger taping state respectively. CBF mapping was calculated and CBF value of both the gray matter (GM) and white matter (WM) was automatically extracted. The reliability was evaluated using the intraclass correlation coefficient (ICC) and Bland and Altman plot.

Results ICC of the GM (0.84) and WM (0.92) was lower at PLD 1.5 seconds than that (GM, 0.88; WM, 0.94) at PLD 2.0 seconds in the resting state, and ICC of GM (0.88) was higher in the right finger taping state than that in the resting state at PLD 1.5 seconds. ICC of the GM and WM was 0.71 and 0.78 for PLD 1.5 seconds and PLD 2.0 seconds in the resting state at the first scan, and ICC of the GM and WM was 0.83 and 0.79 at the second scan, respectively.

Conclusion This work demonstrated that 3D pc-ASL might be a reliable imaging technique to measure CBF over the whole brain at different PLD in the resting state or controlled state.

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ARTERIAL spin labeling (ASL) is a non-invasive cerebral perfusion imaging technique to be used to measure cerebral blood flow (CBF) *in vivo* without exogenous tracers.¹⁻³

In clinical practice, ASL sequence is used to diagnose and monitor stroke,⁴ brain tumor,⁵ and cognitive disorders.⁶ Initially, flow-sensitive alternating inversion recovery (FAIR) technique is applied in the functional imaging to measure CBF.⁷ This approach could label the water molecules of flowing blood as a tracer to obtain the CBF information. However, this technique suffered from low signal intensity, limited spatial coverage of the brain and rapid T1 decay of the labeled spins.⁸

Three dimensional (3D) spiral fast spin echo (FSE) pseudo-continuous ASL (3D pc-ASL) is a novel non-enhancement perfusion sequence on MR750 3.0T. The advantage of this technique includes 3D acquisition, spiral k-space filling, FSE pulse sequence, which would further expand the clinical application of ASL. Therefore, the assessment of the reliability of 3D pc-ASL seems important before the large scale application.

Previous studies⁹⁻¹⁰ demonstrated that continuous and pulsed ASL had a good test-retest reliability of CBF on 1.5T scanners. And compared with ¹⁵O-water positron emission tomography (PET) in Alzheimer's disease, 3D pc-ASL provided a reliable whole brain CBF measurement in young and elderly adult on 3.0T scanner.⁶ A reliability study of pseudo-continuous ASL was also performed at 1.5T and 3.0T, suggesting the fluctuations in perfusion signal seen over the longer term at both 1.5T and 3.0T are likely to reflect genuine fluctuations in resting-state perfusion, and the physiological contributions to the variability of the regional ASL perfusion signal should be furtherly clarified.¹¹

Although some reliability studies focused on the different short term inter-scan interval¹²⁻¹⁴ and different scanners,¹⁵ the reliability of 3D ASL with different post-labeling was not investigated up to now, which would be helpful for the best choice for the different post-labeling time. Besides, it was not assessed that whether the different cerebral functional state may influence the reliability of 3D pc-ASL. In the current study, we hypothesize that a good reliability of 3D pc-ASL could be confirmed with different post-labeling time and functional state. To address this hypothesis, we performed the study as follows: (1) investigate the reliability of 3D pc-ASL with different post-labeling delay time (PLD) at the resting state and right finger taping state over one week interval; (2) clarify the

signal fluctuation by different PLD contributions to the test-retest reliability changes of CBF over the whole brain.

SUBJECTS AND METHODS

Subjects

Eight health adults (6 men and 2 women) were recruited from our medical school in April 2016, with a mean age of 23.8 years (ranging from 21 to 33 years). All the subjects were right-handed and highly educated. The exclusion criteria included: cranium trauma, central nervous system inflammatory disease, and use of psychoactive drugs or hormone. All the subjects were scanned twice at the same time each day for one week interval, and none was permitted to do heavy exercise and has caffeinated beverages within one hour of scanning session. Written informed consent was obtained from all subjects and the study was approved by the ethics committee of the local institution.

MR imaging

All the MR data were acquired on a DISCOVERY MR750 3.0T MR system (GE Healthcare, Milwaukee, WI, USA), with a conventional eight-channel phased array head coil. First, fast fluid-attenuated inversion recovery (FLAIR) images with repetition time (TR)/echo time (TE)/inversion time (TI) = 8802 ms/124.3 ms/2200 ms, slice thickness = 4 mm, gap = 1 mm, matrix = 256×256, field of view (FOV) = 24 cm×24 cm, and number of acquisition (NEX) = 1 were obtained for general assessment. The structural imaged data were acquired with a high resolution 3D T1-weighted fast spoiled gradient recalled echo (3D T1-FSPGR) sequence, which was used to generate the 244 contiguous axial slices with parameters as follows: TR/TE = 8.6 ms/3.5 ms, flip angle = 12°, FOV = 22 cm×22 cm, matrix = 256×256, slice thickness = 1.2 mm, and NEX = 1. Volumetric perfusion imaging was obtained using a pc-ASL tagging scheme with a 3D interleaved spiral FSE readout (3D spiral FSE ASL) with parameters of TR/TE = 5128 ms/15.9 ms, flip angle = 111°, FOV = 20 cm×20 cm, x, y matrix = 1024×8 (spiral acquisition), and slice thickness = 3.0 mm. The labeling duration was 1.5 seconds, and PLD was 1.5 seconds and 2 seconds respectively. The first ASL data acquisition was performed with PLD 1.5 seconds, and the second ASL data acquisition with PLD 2 seconds in the uncontrolled resting state for all the subjects, and the third ASL

data acquisition was performed with PLD 1.5 seconds with the right finger tapping for the subjects. All the subjects were advised to take a finger-thumb tapping exercises before MRI scanning, which include repeated, self-paced, rapid (2 Hz or greater), rhythmic tapping of the right thumb with the other fingers respectively.¹⁶ The scan protocols were identical at baseline and follow-up for all subjects.

CBF image processing

All MR structural and ASL data were processed by using Statistical Parametric Mapping 12 (SPM 12) running under MATLAB 7.6 (The Mathworks, Natick, MA, USA) and Advantage Windows workstation (Funtool, General Electric, Milwaukee, USA).

ASL data including perfusion weighted images and proton density-weighted images were processed and 50 axial CBF images were acquired based on the following equation according to the reported literatures:¹⁷⁻¹⁸

$$f = \frac{\lambda}{2\alpha T_{lb}(1 - e^{-\frac{\tau}{T_{lb}}})} \frac{(S_{con} - S_{lbe}) \left(1 - e^{-\frac{t_{sat}}{T_{lg}}}\right)}{S_{ref}} e^{\frac{w}{T_{lb}}}$$

f , flow; $\lambda=0.9$ (brain-blood partition coefficient); $\alpha=0.85$ (labeling efficiency); $T_{lb}=1.6$ seconds (the T1 value of blood); $T_{lg}=1.2$ seconds [the T1 value of gray matter (GM)]; $\tau=1.5$ seconds (labeling duration); S_{con} , S_{lbe} and S_{ref} , the signal of control, label and reference images, respectively; $t_{sat}=2$ seconds (the saturation time for proton density images); w , post-labeling delay.

CBF quantification

CBF GM and white matter (WM) were automatically quantified as follows: (1) All the T1-weighted images (raw T1) were checked visually for artifacts and re-aligned manually according to the anterior commissural-posterior commissural (AC-PC) line to generate the co-registered T1 images (co T1); (2) The individual co T1 images were segmented by Dartel method¹⁹ to generate the normalized GM and WM images; (3) The individual normalized matrix (subject_id_seg8.mat) generated by segment was applied to the raw CBF map and then generated normalized CBF map; (4) The individual normalized GM and WM were overlapped on the normalized CBF map to obtain the CBF value by the rest tool.²⁰ (Fig. 1)

Statistical analysis

The reliability were evaluated using intraclass correlation coefficient (ICC) and Bland-Altman plot for the CBF variance of the GM and WM at the different PLD over one week interval and at different PLD during the same scanning session. ICC was commonly used to assess the reliability of quantitative measurement with different observer or methods measuring the same quantity, which operated on grouped observation while not paired observations. The analysis of variance (ANOVA) method was used to calculate the ICC value. The common quoted guidelines for interpretation for ICC agreement measures were listed as following: < 0.40, poor; 0.40-0.59, fair; 0.60-0.74, good; 0.75-1.00, excellent.²¹ Bland-Altman plot was used to an-

Figure 1. Schematic of measurement of CBF value of GM and WM. CBF: cerebral blood flow; GM: gray matter; WM: white matter; raw T1: the raw T1 weighted images (structural images); co T1: co-registered T1 weighted images.

alyze the consistency between two measurements of the same parameter in the form of plot. The combination of ICC and Bland-Altman would provide a good assessment for the reliability. The statistical analysis was performed by using SPSS 19.0 and MedCalc (V11.4.2.0, <https://www.medcalc.org/index.php>).

RESULTS

Reliability of 3D pc-ASL in CBF measurement at the different PLD, control state and scanning session

Table 1 showed that in the resting state, ICC of GM at PLD 1.5 seconds (0.84) was lower than that of WM (0.92), and ICC of GM (0.88) at PLD 2.0 seconds was also lower than that of WM (0.94), in addition it could be noticed that ICC of both GM and WM at PLD 1.5 seconds was lower than that at PLD 2.0 seconds in the resting state. After being exerted the right finger tapping, ICC of GM (0.88) showed a slightly increase compared with that (0.84) in the resting state at PLD 1.5 seconds, and ICC of WM showed no changes.

The Fig. 2A showed a positive correlation between the CBF value difference and average CBF value of GM at PLD 1.5 seconds, while there was no correlation between the CBF value difference and average CBF value of WM at PLD 1.5 seconds (Fig. 2B). The CBF value difference had rarely correlation with average

CBF value of GM and WM at PLD 2.0 seconds (Fig. 2C and 2D). Fig. 2E indicated that the CBF difference of GM was negatively correlated with average CBF value, and there was no evident correlation between the CBF difference and average CBF value of WM at PLD 1.5 seconds with right finger tapping (Fig. 2F).

Reliability of 3D pc-ASL in CBF measurement at different PLD in the same scanning session

Table 2 demonstrated that in the resting state ICC of GM and WM were 0.71 and 0.78 for PLD 1.5 seconds and PLD 2.0 seconds in the first scan, and ICC of GM and WM were 0.83 and 0.79 in the second scan. Fig. 3 indicated that a positive correlation between the CBF difference and average CBF of GM or WM.

DISCUSSION

Our results demonstrated that 3D pc-ASL had a reliable CBF measurement for different brain tissues with different PLD over one week interval at 3.0T scanner, which suggested that this sequence could reflect the intrinsic CBF state. ICC of GM was lower than that of WM at the same PLD, which indicated that CBF of WM was relatively reliable and stable at the same PLD, and could be considered as a reference value in the brain perfusion study.

The CBF variance could be influenced by random noise and physiological noise. Although higher field strength could increase the perfusion signal intensity and reduce the variability arising from random noise, it would increase the physiological noise. Table 1 demonstrated that ICC of GM and WM was higher at PLD 2.0 seconds than that at PLD 1.5 seconds, which may be associated with the labeling efficiency at different PLD. It was reasonable to speculate that short PLD might increase the physiological noise, which would decrease the reliability of the CBF value for GM and WM at high field strength. Therefore, the detailed mechanisms should be investigated further.

Previous study demonstrated that regional CBF was more reliable when measured on separate days with pseudo-continuous ASL,²² and the high reliability was also confirmed in Alzheimer's disease.⁶ The current study demonstrated that the high reliability of CBF measurement with 3D pc-ASL in young healthy adults based on the measurements of whole brain. Besides, we also observed that controlled state, such as motion, could influence the reliability of CBF measurements

Table 1. Test-retest reliability of CBF values at different PLD in the resting state and right finger tapping state for the brain over one week interval in 8 healthy volunteers[§]

Acquisition parameters	CBF value [ml/(min·100 ml)]		ICC (95%CI)
	First scan	Second scan	
rs1.5			
GM	47.03±7.46	50.25±5.94	0.84 (0.05-0.97)
WM	43.09±5.53	44.19±5.83	0.92 (0.67-0.98)
rs2.0			
GM	50.41±5.36	51.13±3.97	0.88 (0.54-0.97)
WM	45.30±4.60	46.25±4.50	0.94 (0.69-0.99)
ft1.5			
GM	48.78±6.79	47.55±8.20	0.88 (0.54-0.97)
WM	42.18±4.93	42.66±5.22	0.92 (0.66-0.98)

§Plus-minus values are means±SD. rs1.5: PLD 1.5 seconds in the resting state; rs2.0: PLD 2.0 seconds in the resting state; ft1.5: PLD 1.5 seconds in the right finger tapping state; PLD: post-labeling delay time; ICC: intraclass correlation coefficient.

Figure 2. Bland and Altman plot of CBF difference of GM (A, C, E) and WM (B, D, F) for the normal subjects at different PLD and control state for the brain over one week interval. A and B. PLD 1.5 seconds in the resting state; C and D. PLD 2.0 seconds in the resting state; E and F. PLD 1.5 seconds in right finger taping state. circle: the subject; purple dotted line: regression line of difference; light yellow dotted line: 95% confidence interval.

Table 2. Reliability of CBF values of GM and WM acquired at the different PLD during the same scanning session in 8 healthy volunteers^s

Region	CBF value (ml/min·100 ml)		ICC (95%CI)
	rs1.5	rs2.0	
First scan			
GM	47.03±7.46	50.41±5.36	0.71 (0.08-0.94)
WM	43.09±5.53	45.30±4.60	0.78 (0.20-0.95)
Second scan			
GM	50.25±5.94	51.13±3.97	0.83 (0.39-0.96)
WM	44.19±5.83	46.25±4.50	0.79 (0.26-0.96)

^sPlus-minus values are means±SD.

with different PLD for different brain tissues. It has been demonstrated that 3D PC-ASL had a higher reliability for CBF measurements of WM with the different PLD under rest state, while it had a higher reliability to detect CBF of GM with the different PLD at control state (such as motion), which may be associated with the contribution of physiological noise to reliability of CBF measurements. Therefore, it should be cautious as explaining CBF value with different control state.

Bland and Altman plot analysis showed a positive correlation of CBF value difference with average CBF value of GM, while there was no correlation of CBF value difference with average CBF value of WM at PLD

Figure 3. Bland and Altman plot of CBF difference of GM (A) and WM (B) for the normal subjects in the resting state at different PLD during the same scanning session. circle: the subject; purple dotted line: regression line of difference; light yellow dotted line: 95% confidence interval.

1.5 seconds, which suggested that the reliability of CBF measurement for GM was more easily affected by the perfusions state. It should be careful to explain the results when the lesions in the GM had a higher CBF value, since it might be related to the hypervascularity of GM.²³ Fig. 2 also presented that there was no significant correlation between CBF value difference and average CBF value of GM and WM at PLD 2.0 seconds in the resting state, which also suggested that the CBF measurements for GM and WM were reliable when PLD was set at 2.0 seconds. However, the variance of CBF value of GM presented negative correlation with the average CBF of GM in right finger tapping state, which indicated that the CBF value of GM was influenced by the control state (motion state). This study demonstrated that the CBF value of WM measured with 3D pc-ASL was reliable in finger tapping state. The detailed mechanisms of difference between the CBF value of GM and WM in control state should be investigated further.

There were some limitations in our study. First, the sample size was relatively small. Second, more controlled state (cognitive state, visual stimulation, etc) should be applied. Third, the subjects were all young adults, and elderly adults should be included in the future study. Last, the reliability should also be evaluated in different disease entities because the CBF could be affected by different disease state.

In conclusion, 3D pc-ASL offered a good reliability for CBF measurement over the whole brain at different PLD in the resting state or controlled state.

Conflicts of interest statement

The authors have no conflicts of interest to disclose.

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