

# Effect of post-labeling delay on regional cerebral blood flow in arterial spin-labeling MR imaging

Ying Hu<sup>a,\*</sup>, Fajin LV, MD<sup>b</sup>, Qi Li, MD<sup>c</sup>, Rongbo Liu, MD<sup>a</sup>

## Abstract

**Background:** Investigating the effect of post-labeling delay (PLD) on regional cerebral blood flow (CBF) in adults and optimizing the PLD for arterial spin-labeling (ASL) magnetic resonance (MR) imaging are important.

**Methods:** Pseudo-continuous ASL imaging with a three PLDs protocol was performed in 90 healthy adult volunteers from January 2018 to February 2019. Healthy subjects were divided into youth group (mean age, 30.63 years; age range, 20–44 years), middle-aged group (mean age, 52.16 years; age range 45–59 years) and elderly group (mean age, 66.07 years; age range, 60–77 years). After preprocessing, analyses of variance (ANOVA) and volume-of-interest (VOI) were conducted to compare the CBF in each brain region. According to the trends of CBF changing with PLD and the results of ANOVA, we optimized the PLD for ASL imaging in different brain regions and age groups.

**Results:** The CBF values of 87 VOIs [global gray matter (global GM) and other 86 VOIs] for each subject were obtained. Young people had less statistically significant VOIs than middle-aged and elderly people [Numbers of VOIs which had statistical significance ( $P < .05$ ) in the analysis of ANOVA: 42 (youth group), 79 (middle-aged group), and 71 (elderly group)]. In youth group, the deep GM, occipital lobe and temporal lobe were more affected by PLDs than limbic system, frontal lobe and parietal lobe [VOIs with statistical significance ( $P < .05$ )/total VOIs: 8/8 (deep GM) > 8/12 (occipital lobe) > (8/14) (temporal lobe) > 5/12 (limbic system) > 11/28 (frontal lobe) > (2/12) parietal lobe]. In middle-aged group, the limbic system, deep GM and temporal lobe were more affected by PLDs than parietal lobe, frontal lobe and occipital lobe [VOIs with statistical significance ( $P < 0.05$ )/total VOIs: 12/12 (limbic system) = 8/8 (deep GM) > (13/14) (temporal lobe) > (11/12) parietal lobe > 25/28 (frontal lobe) > 9/12 (occipital lobe)]. In elderly group, the temporal lobe, parietal lobe, and frontal lobe were more affected by PLDs than occipital lobe, limbic system, and deep GM [VOIs with statistical significance ( $P < .05$ )/total VOIs: 14/14 (temporal lobe) > 12/12 (parietal lobe) > 22/28 (frontal lobe) > 9/12 (occipital lobe) > 8/12 (limbic system) > 5/8 (deep GM)]. The optimal PLD for most VOIs in youth group was 1525 ms. However, for middle-aged and elderly group, the optimal PLD for most VOIs was 2525 ms.

**Conclusion:** Young people are less affected by PLDs than middle-aged and elderly people. The middle-aged people are most affected by PLDs. In addition, the spatial distributions of PLD effect were different among the three age groups. Optimizing the PLD for ASL imaging according to age and brain regions can obtain more accurate and reliable CBF values.

**Abbreviations:** ANOVA = analysis of variance, ASL = arterial spin labeling, ATT = arterial transit time, CBF = cerebral blood flow, GM = gray matter, MR = magnetic resonance, PLD = post-labeling delay, SNR = signal-to-noise ratio, VOI = volume of interest.

**Keywords:** arterial spin-labeling, cerebral blood flow, post-labeling delay

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<sup>a</sup> Department of Radiology, West China Hospital, Sichuan University, No. 37 Guo Xue Xiang, Chengdu, Sichuan, <sup>b</sup> Department of Radiology, <sup>c</sup> Department of Neurology, The First Affiliated Hospital of Chongqing Medical University, Chongqing, China.

\* Correspondence: Ying Hu, Department of Radiology, West China Hospital, Sichuan University, No. 37 Guo Xue Xiang, Chengdu, Sichuan 610041, China (e-mail: yingh1991@163.com).

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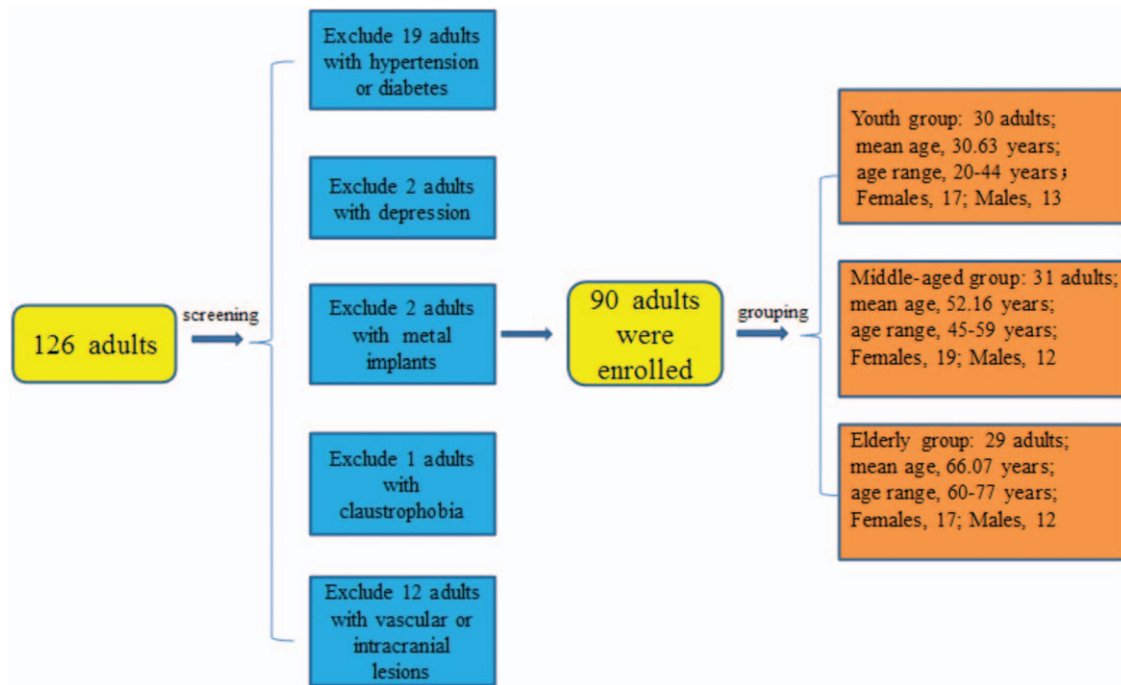
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## 1. Introduction

Arterial spin-labeling (ASL) is a non-invasive perfusion imaging technique for quantifying absolute cerebral blood flow (CBF).<sup>[1–3]</sup> CBF is an important quantitative, physiologic indicator associated with brain metabolism and can be used as biomarker for neurodegenerative diseases,<sup>[4]</sup> neurological dysfunction,<sup>[5–7]</sup> and cerebrovascular abnormalities.<sup>[8,9]</sup> Previous studies have demonstrated that the changes of CBF in some specific brain regions can help identify diseases and even serve as predictors for certain diseases.<sup>[10–14]</sup> Therefore, accurate measurements of CBF are very important for guiding clinical diagnosis and acquiring reliable results of scientific research.

Post-labeling delay (PLD) is an important parameter of ASL, which affects the signal-to-noise ratio (SNR) per unit time and CBF values.<sup>[15]</sup> Many studies have used multiple PLDs to reduce error caused by the mismatch between PLD and arterial transit time (ATT).<sup>[16,17]</sup> However, multi-PLD protocol takes longer to image and has lower SNR.<sup>[18,19]</sup> In addition, CBF obtained by single-PLD is robust, reliable, and direct. Therefore, single-PLD is recommended as clinical standard scanning protocol.<sup>[3]</sup>



**Figure 1.** Schematic overview of the screening and grouping of participants.

Currently, except for cerebrovascular disease, many studies still used single PLD. But the PLD protocol these studies adopted was various,<sup>[12,14,20]</sup> even if a standard scanning protocol was issued in 2015.<sup>[3]</sup> The PLDs commonly used in current studies were 1.5, 2, and 2.5 s.<sup>[14,21–24]</sup> Several studies<sup>[25–27]</sup> had reported that the blood velocity in carotid artery decreased with aging and the PLD in ASL imaging was highly dependent on the subjects' physiological state. Thus, optimizing PLD according to the physiological state of subjects is particularly important.

Considering that the physiological status of subjects varied with age, the blood pathways varied with brain regions, this study hypothesized that the effect of PLD on CBF varied with brain regions and age groups. We therefore aimed to investigate the effect of three commonly used PLDs (1.5, 2, and 2.5 s) on regional CBF in adults and optimize the PLD for ASL imaging.

## 2. Materials and methods

### 2.1. Subjects

This prospective study was approved by the institutional review board of Sichuan University. All subjects provided written informed consent. One hundred and twenty-six adult volunteers without brain organic lesions, history of trauma and stroke were screened from January 2018 to February 2019 in our hospital by the following exclusion criteria:

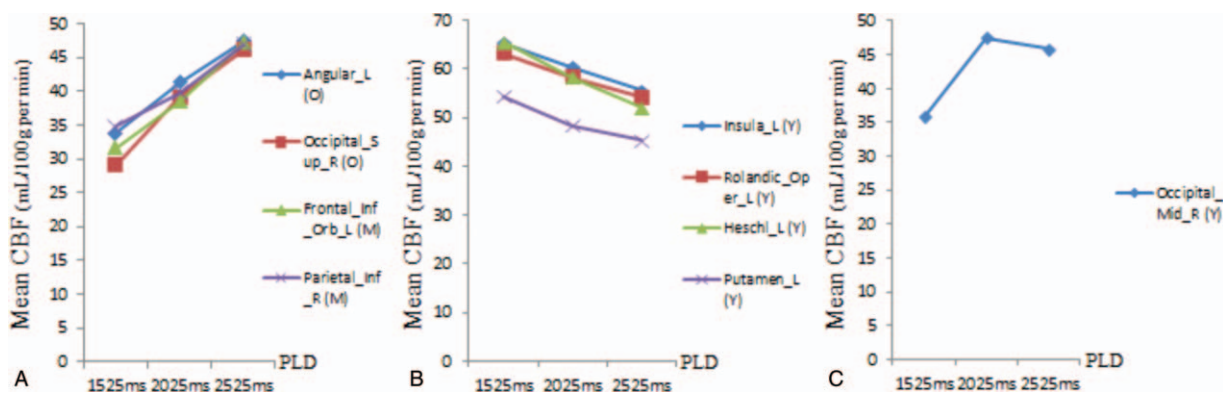
- volunteers with hypertension or diabetes were excluded (19 cases),
- volunteers with mental disorders such as anxiety or depression were excluded (two cases),
- volunteers with metal implants in the body or claustrophobia were excluded (three cases),

- volunteers showed intracranial or vascular lesions on conventional MR imaging or magnetic resonance angiography (MRA) were excluded (12 cases).

Finally, 90 healthy adult volunteers (mean age, 49.47 years; age range, 20–77 years; 53 females and 37 males) were enrolled and divided into three age groups based on the standard of WHO, including youth group (number of subjects, 30; mean age, 30.63 years; age range, 20–44 years; 17 females and 13 males), middle-aged group (number of subjects, 31; mean age, 52.16 years; age range, 45–59 years; 19 females and 12 males), and elderly group (number of subjects, 29; mean age, 66.07 years; age range, 60–77 years; 17 females and 12 males) (Fig. 1). Subjects were prevented from smoking, drinking coffee or caffeinated stimulating drinks and vigorous exercise 24 hours before MR imaging.

### 2.2. Image acquisition

MR images were acquired with a 3.0-T MR imager (GE Signa HDxt) by using an eight-channel head coil. The following sequences were performed: axial T2-weighted, axial fluid-attenuated inversion-recovery, axial three-dimensional T1-weighted, MR angiography, and ASL perfusion sequences. Pseudo-continuous ASL perfusion images (three-dimensional fast spin-echo acquisition with background suppression) with a three-PLDs protocol were collected by using the following parameters: PLDs, 1525, 2025, and 2525 ms; repetition time, 4521 ms (PLD, 1525 ms), 4912 ms (PLD, 2025 ms), 5216 ms (PLD, 2525 ms); echo time, 9.8 ms; section thickness, 4 mm; number of sections, 30; number of signals acquired, 3; field of view, 24 × 24 cm; matrix, 64 × 64; readout of eight arms × 512 samples; acquisition time, 4 min 22 s (PLD, 1525 ms), 4 min 52 s (PLD, 2025 ms), 5 min 8 s (PLD, 2525 ms).



**Figure 2.** Graphs show three kinds of trends of mean cerebral blood flow (meanCBF) changing with PLDs (1525, 2025, and 2525 ms). Four brain regions (Angular\_L, Frontal\_Inf\_Orb\_L, Parietal\_Inf\_R, Occipital\_Sup\_R) with trend A ( $\text{meanCBF}_{1525\text{ms}} < \text{meanCBF}_{2025\text{ms}} < \text{meanCBF}_{2525\text{ms}}$ ) were displayed in graph (a); Four brain regions (Insula\_L, Rolandic\_Oper\_L, Heschl\_L, Putamen\_L) with trend B ( $\text{meanCBF}_{1525\text{ms}} > \text{meanCBF}_{2025\text{ms}} > \text{meanCBF}_{2525\text{ms}}$ ) were displayed in graph (b); One brain region (Occipital\_Mid\_R) with trend D ( $\text{meanCBF}_{1525\text{ms}} < \text{meanCBF}_{2025\text{ms}}, \text{meanCBF}_{2025\text{ms}} > \text{meanCBF}_{2525\text{ms}}$ ) was displayed in graph (c). M=middle-aged group, O=elderly group, PLD=post-labeling delay, Y=youth group.

### 2.3. Preprocessing and analysis of MR imaging data

Data analyses were carried out by using statistical parametric mapping (SPM8) (<http://www.fil.ion.ucl.ac.uk/spm/software/spm8/>), MATLAB (MathWorks, Natick, MA, R2014a), ADW4.5 (GE workstation), and WFU Pickatlas (<http://fmri.wfubmc.edu/software/PickAtlas>).

Preprocessing of ASL images including generation of CBF maps, removal of nonbrain tissue, motion correction, normalization to Montreal Neurological Institute (MNI) space with a 2-mm isotropic resolution and smoothing with an isotropic kernel of 6 mm were performed by SPM8. Furthermore, quantitative volume of interest (VOI) analysis was performed by WFU Pickatlas. Eighty-seven masks of VOIs (supplementary Figure 1, <http://links.lww.com/MD/E289>) were generated in WFU Pickatlas, including global gray matter (GM) and other 86 VOIs (supplementary Figure 1, <http://links.lww.com/MD/E289>). CBF values of each VOI were extracted using these masks.

We then used the mean CBF values of each VOI to roughly describe the trends of CBF changing with PLDs, there were four kinds of trends as follows:

- (1) Gradually increasing ( $\text{meanCBF}_{1525\text{ms}} < \text{meanCBF}_{2025\text{ms}} < \text{meanCBF}_{2525\text{ms}}$ ), which was expressed as trend A;
- (2) Gradually decreasing ( $\text{meanCBF}_{1525\text{ms}} > \text{meanCBF}_{2025\text{ms}} > \text{meanCBF}_{2525\text{ms}}$ ), which was expressed as trend B;
- (3) Firstly decreasing and then increasing ( $\text{meanCBF}_{1525\text{ms}} > \text{meanCBF}_{2025\text{ms}}, \text{meanCBF}_{2025\text{ms}} < \text{meanCBF}_{2525\text{ms}}$ ), which was expressed as trend C;
- (4) Firstly increasing and then decreasing ( $\text{meanCBF}_{1525\text{ms}} < \text{meanCBF}_{2025\text{ms}}, \text{meanCBF}_{2025\text{ms}} > \text{meanCBF}_{2525\text{ms}}$ ), which was expressed as trend D.

The imaging theory of ASL demonstrates when PLD is closer to ATT, the CBF values are more accurate. When PLD is shorter than ATT, the CBF values may be underestimated or overestimated (the labeled blood stay in blood vessels and result in high signals). When PLD is longer than ATT, the SNR will be reduced and the CBF values will be underestimated due to T1 decay.<sup>[9]</sup> Therefore, trend A ( $\text{meanCBF}_{1525\text{ms}} < \text{meanCBF}_{2025\text{ms}} < \text{meanCBF}_{2525\text{ms}}$ ) demonstrates that the optimal PLD among these three PLDs is 2525 ms; trend B ( $\text{meanCBF}_{1525\text{ms}} > \text{meanCBF}_{2025\text{ms}} >$

$\text{meanCBF}_{2525\text{ms}}$ ) demonstrates that the optimal PLD among these three PLDs is 1525 ms; trend D ( $\text{meanCBF}_{1525\text{ms}} < \text{meanCBF}_{2025\text{ms}}, \text{meanCBF}_{2025\text{ms}} > \text{meanCBF}_{2525\text{ms}}$ ) demonstrates that the optimal PLD among these three PLDs is 2025 ms (Fig. 2).

### 2.4. Statistical analysis

Statistical analyses were performed using SPSS 20.0 software (Chicago, IL). Differences among CBF values of three PLDs were assessed by using one-way analysis of variance (ANOVA), with Scheffe post hoc analysis. The tests for normality and variance homogeneity were completed before one-way ANOVA. The extreme values were removed before one-way ANOVA. A  $P$ -value  $< .05$  was considered to indicate a statistically significant difference.

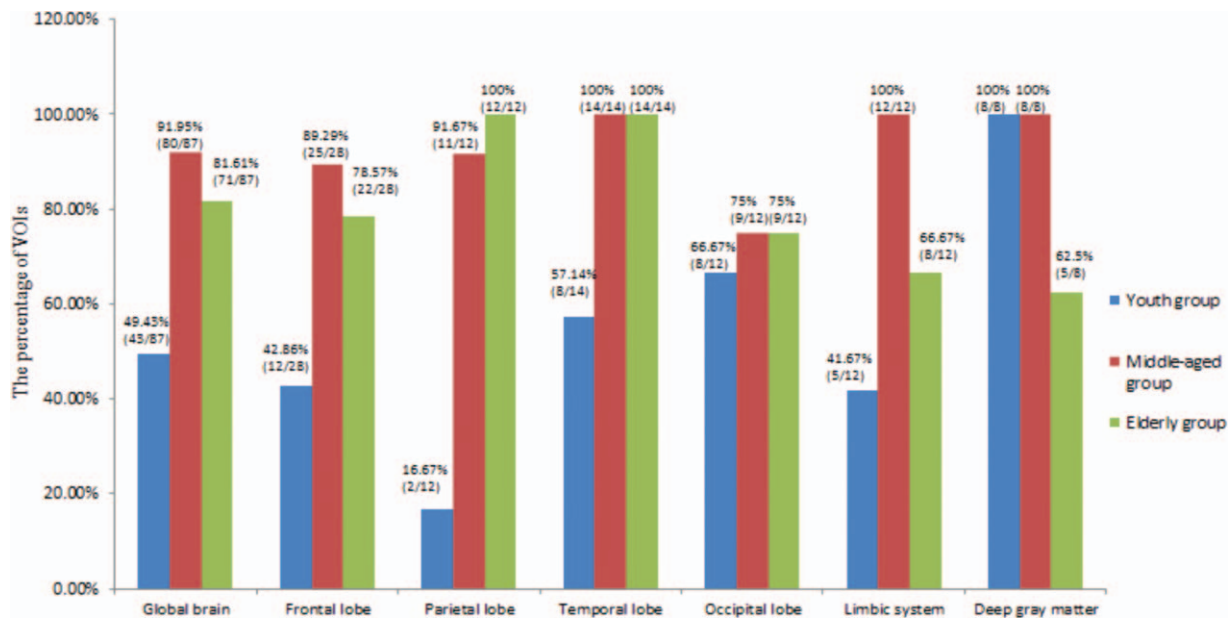
## 3. Results

### 3.1. The effect of PLD on regional CBF

Eighty seven VOIs (global GM and other 86 VOIs) were obtained using WFU Pickatlas. The total numbers of VOIs in frontal lobe, parietal lobe, temporal lobe, occipital lobe, limbic system, and deep GM were 28, 12, 14, 12, 12, and 8, respectively. The mean value and standard deviation of CBF for each VOI was illustrated in supplementary Table 1, <http://links.lww.com/MD/E290>. The results of ANOVA and post hoc analysis were shown in supplementary Table 2, <http://links.lww.com/MD/E291>.

Young people had less statistically significant VOIs than middle-aged and elderly people (Fig. 3). The analysis of ANOVA showed that the young group, middle-aged group and elderly group had 42 VOIs [48.28% (42/87)], 79 VOIs [90.8% (79/87)], and 71 VOIs [81.61% (71/87)] with statistical significance ( $P < .05$ ), respectively (supplementary Table 2, <http://links.lww.com/MD/E291>). Therefore, young people were less affected by PLDs than middle-aged and elderly people. The middle-aged group was most affected by PLDs.

In addition, the spatial distributions of PLD effect were different among the three age groups. The percentages [VOIs with statistical significance ( $P < .05$ )/total VOIs] of deep GM



**Figure 3.** The numbers and percentages of VOIs which had statistically significant results in the one-way analysis of variance (ANOVA) in global brain, frontal lobe, parietal lobe, temporal lobe, occipital lobe, limbic system, and deep gray matter were displayed in this figure. In global brain, the total number of VOIs was 87; In frontal lobe, the total number of VOIs was 28; In parietal lobe, the total number of VOIs was 12; In temporal lobe, the total number of VOIs was 14; In occipital lobe, the total number of VOIs was 12; In limbic system, the total number of VOIs was 12; In deep gray matter, the total number of VOIs was 8. For example, the number of VOIs which had statistical significance for youth group in global brain was 43; the percentage of VOIs which had statistical significance for youth group in global brain was 49.43% (43/87). VOI=volume of interest.

[100% (8/8)], occipital lobe [66.67% (8/12)], and temporal lobe [57.14% (8/14)] were higher than limbic system [41.67% (5/12)], frontal lobe [39.29% (11/28)], and parietal lobe [16.67% (2/12)] (Table 1) in youth group. Thus, the deep GM, occipital lobe, and temporal lobe were more affected by PLDs than limbic system, frontal lobe and parietal lobe in youth group. However, the limbic system [100% (12/12)], deep GM [100% (8/8)] and temporal lobe [92.86% (13/14)] were more affected by PLDs than parietal lobe [91.67% (11/12)], frontal lobe [89.29% (25/28)], and occipital lobe [75% (9/12)] in middle-aged group. In elderly group, the temporal lobe [100% (14/14)], parietal lobe [100% (12/12)], and frontal lobe [78.57% (22/28)] were more affected by PLDs than occipital lobe [75% (9/12)], limbic system [66.67% (8/12)], and deep GM [62.5% (5/8)] (Fig. 3, supplementary Table 2, <http://links.lww.com/MD/E291>).

### 3.2. Optimization of PLD for ASL imaging in different age and brain regions

We had demonstrated when the mean CBF showed as trend A ( $\text{meanCBF}_{1525\text{ms}} < \text{meanCBF}_{2025\text{ms}} < \text{meanCBF}_{2525\text{ms}}$ ), the optimal PLD among these three PLDs was 2525 ms; when the mean CBF showed as trend B ( $\text{meanCBF}_{1525\text{ms}} > \text{meanCBF}_{2025\text{ms}} > \text{meanCBF}_{2525\text{ms}}$ ), the optimal PLD among these three PLDs was 1525 ms; when the mean CBF showed as trend D ( $\text{meanCBF}_{1525\text{ms}} < \text{meanCBF}_{2025\text{ms}}, \text{meanCBF}_{2025\text{ms}} > \text{meanCBF}_{2525\text{ms}}$ ), the optimal PLD among these three PLDs was 2025 ms (Fig. 2).

The optimal PLD in different brain regions of the same age group was not the same. In youth group, among the VOIs which had statistical significance ( $P < .05$ ) in the analysis of ANOVA, there were 19 VOIs [five in frontal lobe, two in parietal lobe, five in temporal lobe, seven in occipital lobe] in which the mean CBF

showed as trend A (Table 1, Fig. 2). Thus, the optimal PLD for these VOIs was 2525 ms. However, the optimal PLD for 20 VOIs (four in frontal lobe, three in temporal lobe, five in limbic system, eight in deep GM) in which the mean CBF showed as trend B was 1525 ms; the optimal PLD for one VOIs (in occipital lobe) in which the mean CBF showed as trend D was 2025 ms. Therefore, the optimal PLD for most VOIs in youth group was 1525 ms.

However, for middle-aged and elderly group, the optimal PLD for most VOIs was 2525 ms. Because, there were 54 VOIs (22 in frontal lobe, 10 in parietal lobe, 10 in temporal lobe, eight in occipital lobe, three in limbic system, one in global GM) showed as trend A (Table 2, Fig. 2) in middle-aged group and there were 60 VOIs (22 in frontal lobe, 12 in parietal lobe, 12 in temporal lobe, nine in occipital lobe, four in limbic system, one in global GM) showed as trend A (Table 3, Fig. 2) in elderly group. In addition, the optimal PLD for one VOIs (Rolandic\_Oper\_L) in which the mean CBF showed as trend B (Table 2, Fig. 2) was 1525 ms in middle-aged group.

## 4. Discussion

In this study, 3D-pCASL imaging technique and VOI analysis were used to detect the effect of PLDs on regional CBF values and optimize the PLD for arterial spin-labeling (ASL) magnetic resonance (MR) imaging in different age group. Our study demonstrated that young people were less affected by PLDs than middle-aged and elderly people and the middle-aged group was most affected by the PLDs. In addition, the spatial distributions of PLD effect were different among the three age groups. At the same time, our study investigated the optimal PLD among the three commonly used PLDs (1525, 2025, and 2525 ms) for those brain regions which had statistical significance ( $P < .05$ ) in the



**Table 1**  
**Results of the VOI analysis in youth group.**

Lobes	Trend	Statistical significance ( $P < .05$ )*	
		Yes	No
Frontal lobe <sup>[28]</sup>	A	5 (Frontal_Mid_Orb_L,Frontal_Mid_Orb_R,Frontal_Sup_L,Frontal_Sup_R,Frontal_Sup_Orb_L)	3 (Frontal_Mid_L,Paracentral_Lobule_R,Precentral_R)
	B	4 (Insula_L,Insula_R,Rolandic_Oper_L,Rolandic_Oper_R)	4 (Frontal_Inf_Oper_R,Frontal_Med_Orb_L,Frontal_Sup_Medial_R,,Rectus_R)
	C	2 (Frontal_Inf_Orb_R,Frontal_Sup_Medial_L)	7 (Frontal_Inf_Oper_L,Frontal_Inf_Orb_L,Frontal_Inf_Tri_L,Frontal_Inf_Tri_R,Frontal_Med_Orb_R,Frontal_Sup_Orb_R,Rectus_L)
	D	0	3 (Frontal_Mid_R,Paracentral_Lobule_L,Precentral_L)
Parietal lobe <sup>[12]</sup>	A	2 (Parietal_Sup_L,Parietal_Sup_R)	5 (Angular_L,Parietal_Inf_L,Parietal_Inf_R,Postcentral_L,Postcentral_R,)
	B	0	0
	C	0	2 (SupraMarginal_L,SupraMarginal_R)
	D	0	3 (Angular_R,Precuneus_L,Precuneus_R)
Temporal lobe <sup>[14]</sup>	A	5 (Temporal_Inf_L,Temporal_Inf_R,Temporal_Pole_Mid_L,Temporal_Pole_Mid_R,Temporal_Pole_Sup_R,)	3 (Temporal_Mid_L,Temporal_Mid_R,Temporal_Pole_Sup_L,)
	B	3 (Heschl_L,Heschl_R,Temporal_Sup_L)	1 (Temporal_Sup_R)
	C	0	0
	D	0	2 (Fusiform_L,Fusiform_R)
Occipital lobe <sup>[12]</sup>	A	7 (Cuneus_L,Cuneus_R,Occipital_Inf_L,Occipital_Inf_R,Occipital_Mid_L,Occipital_Sup_L,Occipital_Sup_R)	0
	B	0	0
	C	0	0
	D	1 (Occipital_Mid_R)	4 (Calcarine_L,Calcarine_R,Lingual_L,Lingual_R)
Limbic system <sup>[12]</sup>	A	0	0
	B	5 (Cingulum_Mid_L,Cingulum_Mid_R,Cingulum_Post_L,Cingulum_Post_R,Hippocampus_L)	5 (Amygdala_L, Cingulum_Ant_L, Cingulum_Ant_R, Hippocampus_R, ParaHippocampal_L)
	C	0	1 (Amygdala_R)
	D	0	1 (ParaHippocampal_R)
Deep GM <sup>[8]</sup>	A	0	0
	B	8 (Caudate_L, Caudate_R, Pallidum_L, Pallidum_R, Putamen_L, Putamen_R, Thalamus_L, Thalamus_R)	0
	C	0	0
	D	0	0
Global GM <sup>[1]</sup>	A	0	0
	B	0	0
	C	0	1
	D	0	0

VOI = volume of interest; GM = gray matter; A: meanCBF<sub>1525ms</sub> < meanCBF<sub>2025ms</sub> < meanCBF<sub>2525ms</sub>; B: meanCBF<sub>1525ms</sub> > meanCBF<sub>2025ms</sub> > meanCBF<sub>2525ms</sub>; C: meanCBF<sub>1525ms</sub> > meanCBF<sub>2025ms</sub>; meanCBF<sub>2025ms</sub> < meanCBF<sub>2525ms</sub>; D: meanCBF<sub>1525ms</sub> < meanCBF<sub>2025ms</sub>, meanCBF<sub>2025ms</sub> > meanCBF<sub>2525ms</sub>.

\*Data are the numbers of VOIs.

analysis of ANOVA and showed that 1525 ms was optimal for most brain regions in youth group and 2525 ms was optimal for most brain regions in middle-aged and elderly group.

PLD is an important parameter, which determines the accuracy of CBF values.<sup>[28]</sup> Accurate measurements of CBF in specific brain regions, which serve as predictors for certain diseases<sup>[10–14]</sup> are critical. For example, patients with Alzheimer disease (AD) exhibited lower CBF in parietal lobe, posterior cingulate cortex and precuneus.<sup>[4,10,11]</sup> Parkinson disease (PDD) showed lower perfusion in right occipital cortex and precuneus.<sup>[14,20]</sup> According to our results, the optimal PLD for middle-aged and elderly people in most brain regions of parietal lobe and occipital lobe is 2525 ms. While the PLD of 1800 ms (healthy subjects < 70 years) and 2000 ms (healthy subjects > 70 years) were recommended by the standard scanning protocol which was issued in 2015. These two recommended PLDs may underestimate the CBF of parietal lobe and occipital lobe and result in false positive.

Many studies have used multiple PLDs to estimate ATT and optimize PLD. Melvin Mezue studied the reliability of a multi-PLD protocol and investigated optimal PLD for ASL imaging in different brain regions. In their study, the PLDs (0.25, 0.5, 0.75, 1, 1.25, and 1.5 s) were not commonly used in recent studies.<sup>[17]</sup> Our study found that the commonly used PLDs in current studies were 1.5, 2, and 2.5 s.<sup>[14,21–24]</sup> In Melvin Mezue study, the 10 VOIs (Grey Matter, White Matter, Frontal lobe, Temporal lobe, Parietal lobe, Occipital lobe, Insular, Thalamus, Caudate, and Putamen) may be a bit big to ignore the differences among smaller VOIs. Therefore, our study subdivided the brain lobes into 87 VOIs, including global GM and other 86 VOIs. Melvin Mezue also believed that choosing appropriate PLD according to brain regions was important and they reported the optimal PLD for most brain regions was <1 s. However, in our study, the optimal PLD in most brain regions of youth group was 1525 ms. This contradiction may be due to the subjects in Melvin Mezue study was relatively younger (mean age, 28.3). Neville D. Gai

**Table 2**  
**Results of the VOI analysis in middle-aged group.**

Lobes	Trend	Statistical significance ( $P < .05$ )*	
		Yes	No
Frontal lobe <sup>[28]</sup>	A	22 (Frontal_Inf_Oper_L, Frontal_Inf_Oper_R, Frontal_Inf_Orb_L, Frontal_Inf_Orb_R, Frontal_Inf_Tri_L, Frontal_Inf_Tri_R, Frontal_Med_Orb_L, Frontal_Med_Orb_R, Frontal_Mid_L, Frontal_Mid_R, Frontal_Mid_Orb_L, Frontal_Mid_Orb_R, Frontal_Sup_L, Frontal_Sup_R, Frontal_Sup_Medial_L, Frontal_Sup_Medial_R, Frontal_Sup_Orb_L, Frontal_Sup_Orb_R, Precentral_L, Precentral_R, Rectus_L, Rectus_R),	1 (Paracentral_Lobule_R)
	B	1 (Rolandic_Oper_L)	0
	C	2 (Insula_R, Rolandic_Oper_R)	2 (Insula_L, Paracentral_Lobule_L)
	D	0	0
Parietal lobe <sup>[12]</sup>	A	10 (Angular_L, Angular_R, Parietal_Inf_L, Parietal_Inf_R, Parietal_Sup_L, Parietal_Sup_R, Postcentral_L, Postcentral_R, SupraMarginal_L, SupraMarginal_R)	1 (Precuneus_L)
	B	0	0
	C	1 (Precuneus_R)	0
	D	0	0
Temporal lobe <sup>[14]</sup>	A	10 (Temporal_Inf_R, Temporal_Mid_L, Temporal_Mid_R, Temporal_Pole_Mid_L, Temporal_Pole_Mid_R, Temporal_Pole_Sup_L, Temporal_Pole_Sup_R, Temporal_Sup_L, Temporal_Sup_R, Fusiform_R)	1 (Temporal_Inf_L)
	B	0	0
	C	3 (Fusiform_L, Heschl_L, Heschl_R)	0
	D	0	0
Occipital lobe <sup>[12]</sup>	A	8 (Cuneus_L, Cuneus_R, Occipital_Inf_L, Occipital_Inf_R, Occipital_Mid_L, Occipital_Mid_R, Occipital_Sup_L, Occipital_Sup_R)	0
	B	0	1 (Calcarine_L)
	C	1 (Lingual_R)	2 (Calcarine_R, Lingual_L)
	D	0	0
Limbic system <sup>[12]</sup>	A	3 (Amygdala_L, Amygdala_R, ParaHippocampal_L)	0
	B	0	0
	C	9 (Cingulum_Ant_L, Cingulum_Ant_R, Cingulum_Mid_L, Cingulum_Mid_R, Cingulum_Post_L, Cingulum_Post_R, Hippocampus_L, Hippocampus_R, ParaHippocampal_R)	0
	D	0	0
Deep GM <sup>[8]</sup>	A	0	0
	B	0	0
	C	8 (Caudate_L, Caudate_R, Pallidum_L, Pallidum_R, Putamen_L, Putamen_R, Thalamus_L, Thalamus_R)	0
	D	0	0
Global GM <sup>[11]</sup>	A	1	0
	B	0	0
	C	0	0
	D	0	0

VOI = volume of interest; GM = gray matter; A:  $\text{meanCBF}_{1525\text{ms}} < \text{meanCBF}_{2025\text{ms}} < \text{meanCBF}_{2525\text{ms}}$ ; B:  $\text{meanCBF}_{1525\text{ms}} > \text{meanCBF}_{2025\text{ms}} > \text{meanCBF}_{2525\text{ms}}$ ; C:  $\text{meanCBF}_{1525\text{ms}} > \text{meanCBF}_{2025\text{ms}} < \text{meanCBF}_{2525\text{ms}}$ ; D:  $\text{meanCBF}_{1525\text{ms}} < \text{meanCBF}_{2025\text{ms}} > \text{meanCBF}_{2525\text{ms}}$ .

\* Data are the numbers of VOIs.

investigated the optimal PLD based on specific estimation of blood velocity in the carotid artery.<sup>[15]</sup> Eleven normal volunteers (age:  $42 \pm 12.9$  years) were enrolled and eight PLDs which were equally spaced between 600 and 2000 ms were adopted in their study. Phase contrast (PC) imaging was performed to measure the blood velocity in carotid artery and a formula was calculated to obtain the optimal PLD. In their study, PLD with the best SNR in global GM was optimal and the optimal PLD of the volunteers was between 1102 and 1787 ms. Neville D. Gai sample size was relatively small, so the reliability of their formula needed further confirmation. In addition, Neville D. Gai did not refer to the CBF

in white matter for believing that the signal in white matter was poor, the variation of ATT in white matter was larger and it took longer to image white matter. However, the VOIs in our research consisted of gray and white matter, which may be one of the reasons that the optimal PLD in our study was longer. There were many studies which involved white matter had obtained relatively accurate CBF values.<sup>[21]</sup> Moreover, the perfusion in some white matter was associated with many diseases.<sup>[12,13,29,30]</sup> Therefore, accurate measurements of perfusion in brain regions which composed of GM and white matter are particularly important.

**Table 3**  
**Results of the VOI analysis in elderly group.**

Lobes	Trend	Statistical significance ( $P < .05$ )*	
		Yes	No
Frontal lobe <sup>[28]</sup>	A	22 (Frontal_Inf_Oper_L,Frontal_Inf_Oper_R,Frontal_Inf_Orb_L, Frontal_Inf_Orb_R, Frontal_Inf_Tri_L,Frontal_Inf_Tri_R, Frontal_Med_Orb_L, Frontal_Med_Orb_R, Frontal_Mid_L, Frontal_Mid_R, Frontal_Mid_Orb_L, Frontal_Mid_Orb_R, Frontal_Sup_L, Frontal_Sup_R, Frontal_Sup_Medial_L, Frontal_Sup_Medial_R, Frontal_Sup_Orb_L, Frontal_Sup_Orb_R, Precentral_L, Precentral_R, Rectus_L, Rectus_R),	2 (Paracentral_Lobule_L, Paracentral_Lobule_R)
	B	0	1 (Rolandic_Oper_R)
	C	0	3 (Insula_L, Insula_R, Rolandic_Oper_L)
	D	0	0
Parietal lobe <sup>[12]</sup>	A	12 (Angular_L, Angular_R,Parietal_Inf_L,Parietal_Inf_R, Parietal_Sup_L,Parietal_Sup_R, Postcentral_L,Postcentral_R, Precuneus_L,Precuneus_R, SupraMarginal_L,SupraMarginal_R)	0
	B	0	0
	C	0	0
Temporal lobe <sup>[14]</sup>	D	0	0
	A	12 (Fusiform_L, Fusiform_R,,Temporal_Inf_L,Temporal_Inf_R, Temporal_Mid_L, Temporal_Mid_R,Temporal_Pole_Mid_L, Temporal_Pole_Mid_R,Temporal_Pole_Sup_L, Temporal_Pole_Sup_R, Temporal_Sup_L, Temporal_Sup_R)	0
	B	0	0
	C	2 (Heschl_L,Heschl_R)	0
Occipital lobe <sup>[12]</sup>	D	0	0
	A	9 (Calcarine_L,Cuneus_L,Cuneus_R,Occipital_Inf_L, Occipital_Inf_R,Occipital_Mid_L, Occipital_Mid_R, Occipital_Sup_L,Occipital_Sup_R)	3 (Calcarine_R, Lingual_L, Lingual_R)
	B	0	0
	C	0	0
Limbic system <sup>[12]</sup>	D	0	0
	A	4 (Amygdala_R, Hippocampus_L, ParaHippocampal_L, ParaHippocampal_R)	0
	B	0	2 (Cingulum_Post_L, Cingulum_Post_R)
	C	4 (Amygdala_L, Cingulum_Ant_L, Cingulum_Ant_R, Hippocampus_R)	2 (Cingulum_Mid_L, Cingulum_Mid_R)
Deep GM <sup>[8]</sup>	D	0	0
	A	0	0
	B	0	0
	C	5 (Caudate_L, Caudate_R, Putamen_R, Thalamus_L, Thalamus_R)	3 (Pallidum_L, Pallidum_R, Putamen_L)
Global GM <sup>[1]</sup>	D	0	0
	A	1	0
	B	0	0
	C	0	0
	D	0	0

VOI = volume of interest; GM = gray matter; A: meanCBF<sub>1525 ms</sub> < meanCBF<sub>2025 ms</sub> < meanCBF<sub>2525 ms</sub>; B: meanCBF<sub>1525 ms</sub> > meanCBF<sub>2025 ms</sub> > meanCBF<sub>2525 ms</sub>; C: meanCBF<sub>1525 ms</sub> > meanCBF<sub>2025 ms</sub>; meanCBF<sub>2025 ms</sub> < meanCBF<sub>2525 ms</sub>; D: meanCBF<sub>1525 ms</sub> < meanCBF<sub>2025 ms</sub>, meanCBF<sub>2025 ms</sub> > meanCBF<sub>2525 ms</sub>.  
\* Data are the numbers of VOIs.

One limitation of our study was that only adults were recruited. The consideration is that many diseases, especially those related to brain function, mainly occur in middle-aged and elderly people (age > 46 years). Another limitation was that we only performed three PLDs due to our total acquisition time was nearly half an hour. Many subjects said they felt uncomfortable when the acquisition time was more than half an hour. Hence, our study used three PLDs, which were commonly used in clinical settings and scientific research.

To conclude, our study suggests that young people are less affected by PLDs than middle-aged and elderly people. The middle-aged group is most affected by PLDs. In addition, the deep GM, occipital lobe and temporal lobe were more affected by PLDs than frontal lobe, limbic system, and parietal lobe in youth group. In middle-aged group, the limbic system, deep GM, and temporal lobe were more affected by PLDs than parietal lobe, frontal lobe, and occipital lobe. In elderly group, the temporal lobe, parietal lobe, and frontal lobe were more affected by PLDs

than occipital lobe, limbic system, and deep GM. In youth group, the optimal PLD for most brain regions is 1525 ms. In middle-aged and elderly group, the optimal PLD for most brain regions is 2525 ms.

### Author contributions

Guarantors of integrity of entire study, all authors; study concepts and study design, all authors; literature research, Y.H., F.L.; clinical studies, Y.H., Q.L.; experimental studies and data analysis, Y.H.; statistical analysis, Y.H., R.L; and manuscript editing, Y.H., R.L.

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