Measuring the Labeling Efficiency of Pseudocontinuous Arterial Spin Labeling

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**Purpose:** Optimization and validation of a sequence for measuring the labeling efficiency of pseudocontinuous arterial spin labeling (pCASL) perfusion MRI.

**Methods:** The proposed sequence consists of a labeling module and a single slice Look-Locker echo planar imaging readout. A model-based algorithm was used to calculate labeling efficiency from the signal acquired from the main brain-feeding arteries. Stability of the labeling efficiency measurement was evaluated with regard to the use of cardiac triggering, flow compensation and vein signal suppression. Accuracy of the measurement was assessed by comparing the measured labeling efficiency to mean brain pCASL signal intensity over a wide range of flip angles as applied in the pCASL labeling.

**Results:** Simulations show that the proposed algorithm can effectively calculate labeling efficiency when correcting for T1 relaxation of the blood spins. Use of cardiac triggering and vein signal suppression improved stability of the labeling efficiency measurement, while flow compensation resulted in little improvement. The measured labeling efficiency was found to be linearly (R = 0.973; P < 0.001) related to brain pCASL signal intensity over a wide range of pCASL flip angles.

**Conclusion:** The optimized labeling efficiency sequence provides robust artery-specific labeling efficiency measurement within a short acquisition time (~30 s), thereby enabling improved accuracy of pCASL CBF quantification. *Magn Reson Med 77:1841–1852, 2017. © 2016 The Authors. Magnetic Resonance in Medicine published by Wiley Periodicals LLC on behalf of International Society for Magnetic Resonance in Medicine. This is an open access article under the terms of the Creative Commons Attribution NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.**

**Key words:** pseudocontinuous; arterial spin labeling (ASL); perfusion imaging; labeling efficiency; MRI

**INTRODUCTION**

Arterial spin labeling (ASL) is a noninvasive MR technique for measuring cerebral blood flow (CBF) (1,2). Labeling efficiency, defined as the difference of blood signal between control and label scan divided by a factor of 2, is one of the most important parameters for accurate ASL CBF quantification (3). Pseudocontinuous ASL (pCASL) is now the most widely accepted ASL approach mainly due to its high signal-noise-ratio (SNR) and easy implementation on clinical MR scanners without the need of extra hardware (3). In principle, the labeling (or inversion) process of pCASL is achieved by applying a series of short radiofrequency (RF) pulses in combination with a gradient in the slice selection direction that manipulates the phase of the flowing spins resulting in a pseudoadiabatic inversion. Thus, its efficiency is vulnerable to factors like flow velocity and field inhomogeneities (4,5), and may vary over arteries, scans, and subjects.

Variation in labeling efficiency is thought to contribute to the intra- and intersubject variability observed in quantitative ASL (6,7). In particular, physiological variation as well as repositioning errors are believed to reduce the long-term reproducibility of pCASL CBF quantification (8). Both factors can cause labeling efficiency variations, for example by means of differences in flow velocity or effective local magnetic field due to another location and/or shim-settings. It is also important to realize that labeling efficiency variation can not only impact global perfusion values, e.g., when all arteries show a lower velocity due to lower cardiac output, but may also lead to perfusion asymmetry between flow territories. Asymmetry of brain perfusion is an important indicator for clinical diagnosis of patients (9) and may presents in specific regions of normal brain (10). Therefore, in order for more accurate and precise ASL perfusion quantification, an artery-specific labeling efficiency measurement accompanying the standard brain pCASL imaging can be considered an essential next step.

However, to date, there is no easy method to address the labeling efficiency of pCASL in a clinically acceptable manner, and usually a constant value (e.g., 0.85) obtained from simulation is adopted (3). Previously, a method based on phase contrast (PC) flow imaging was proposed to estimate labeling efficiency of pCASL by equating the mean CBF as obtained from the PC scan to the mean CBF of the whole
brain pCASL scan (11). Although this method has been applied in some studies (12,13), it has difficulty in obtaining artery-specific labeling efficiency and requires dedicated processing of the PC and pCASL images. Furthermore, its accuracy and precision is not yet fully characterized (14). On the other hand, a more straightforward strategy is to derive the labeling efficiency from the blood signal acquired immediately after the labeling process while the labeled spins are still within the main brain-feeding arteries. Preliminary implementations of this strategy have been used in a few studies in either a phantom or human setting (15,16). However, in these studies, the method for measuring the labeling efficiency was not optimized to enable its use in clinical applications nor was its performance carefully studied, which is important because it may be affected by many factors, such as flow dispersion and cardiac pulsation (17).

The goal of the current study is to optimize and validate a fast labeling efficiency measurement sequence based on the above-described strategy. The arterial blood signal after labeling was monitored by a single slice Look-Locker echo planar imaging (LL-EPI) readout (18) (called LabEff sequence from this point onward). Subsequently, the ASL signal as a function of time was analyzed to enable labeling efficiency calculation. The LabEff sequence was optimized by studying factors that potentially affect stability of the labeling efficiency measurement. Finally, the accuracy of the measurement was investigated by comparing the measured labeling efficiency to mean brain pCASL signal over a wide range of pCASL flip angles (FAs) (i.e., the flip angle of the Hanning-pulses in the labeling module).

METHODS

Theory

The labeling efficiency in the labeling plane can be expressed as weighted average of the labeling efficiency over velocities of the flowing spins:

$$\tilde{\alpha} = \frac{1}{M_0} \sum_{v=0}^{V_{\text{max}}} \alpha(v)M(v)$$

[1]

where $M_0$ is the total equilibrium blood magnetization over the arterial cross-section; $\alpha(v)$ and $M(v)$ are the labeling efficiency and equilibrium longitudinal magnetization of the spins with velocity $v$.

Because the imaging plane is distal to the labeling plane, the labeled spins will undergo velocity-dependent T1 relaxation before being detected. Therefore, the ASL signal as measured in the imaging plane can be expressed as an extension of a previously published model for ASL signal in large arterial vessels (19):

$$\text{ASL}(t) = 2 \sum_{v=0}^{V_{\text{max}}} \alpha(v)M(v)D(v)E(v, t)$$

[2]

where $D(v) = e^{-TT(v)/T_{1b}}$ represents the T1 relaxation of labeled spins with velocity $v$, $TT(v)$ is the spins’ transit time from the labeling to the imaging plane, $T_{1b}$ is the T1 of arterial blood and assumed to be 1650 ms, and $E(v, t)$ is the delivery function of spins at velocity $v$, which is assumed to be a normalized boxcar function with its width equal to the labeling duration and shifted by the spins’ transit time. From the acquired ASL signal in the imaging plane and Eq. [2], $\alpha(v)M(v)$ can be calculated for each velocity component by inverting the matrix $D(v)E(v, t)$. Subsequently, the obtained $\alpha(v)M(v)$ is used to calculate the mean labeling efficiency according to Eq. [1].

Simulations of Labeling Efficiency Calculation

Evolution of the magnetization of the flowing spins within an artery was simulated to verify the proposed algorithm for labeling efficiency calculation. Blood flow hemodynamics were simulated as a simple straight vessel (diameter = 4 mm, 50 mm distance between the labeling and imaging plane) with laminar flow profile (20). Simulations were performed with a resolution of $0.1 \times 0.1 \text{mm}^2$ and subsequently averaged to a lower resolution of $2 \times 2 \text{mm}^2$. Labeling duration of the LabEff sequence was set to 800 ms. Degree of inversion of each labeled spin due to the train of pCASL labeling pulses was simulated using Bloch equations similar to simulations as described by Wu et al (4). The true labeling efficiency in the labeling plane was calculated using Eq. [1] at the original simulation resolution ($0.1 \times 0.1 \text{mm}^2$). Subsequently, from the simulated ASL signal in the imaging plane the labeling efficiency was calculated by solving Eq. [2], as explained above. A series of simulations were performed with the maximum velocities ranging from 10 cm/s to 60 cm/s with an interval of 5 cm/s.

In Vivo Experiments

All MR experiments in this study were performed on a 3 Tesla Philips Achieva scanner (Philips Healthcare, Best, The Netherlands) with a 32-channel head coil. The study protocol was approved by the local Institutional Review Board, and written informed consent was obtained from all participating volunteers. In each scan session, PC angiography scout scans in coronal and sagittal orientation of the carotid arteries were performed for planning purposes, and 2D PC quantitative flow measurements were performed at the labeling and imaging plane of the LabEff scan (single slice, 15 retrospectively triggered cardiac phases, repetition time/echo time/FA (TR/TE/FA) = 13 ms / 8.1 ms / $10^\circ$, field of view (FOV) = $150 \times 103 \text{mm}^2$, voxel size = $1.17 \times 1.17 \times 6 \text{mm}^3$, encoding velocity = 200 cm/s).

The in vivo labeling efficiency experiments were divided into two separate studies. The first study aimed at studying influence of the acquisition choices on stability of the labeling efficiency measurement. In this study the effect of cardiac triggering, flow compensation (FC) and vein signal suppression (VeinSup) on the acquired signal and labeling efficiencies was studied. The experiments were performed on five healthy volunteers (mean age, 27.8 years; three males). In the second study, accuracy of the labeling efficiency measurement was studied by comparing the measured labeling efficiency to brain pCASL signal intensity over a range of pCASL FAs. By varying the pCASL FA, deliberate changes in labeling efficiency are obtained. For this study, another five healthy volunteers (mean age, 25.4 years; three males) were recruited.

The following parameters were used in all pCASL scans: balanced pCASL, RF duration = 0.5 ms, RF interval = 1.2 ms, FA = $21^\circ$ (unless stated otherwise), mean gradient strength = 0.6 mT/m, maximum gradient strength = 6 mT/m. All LabEff scans were performed with 16 repetitions of control / label pairs. The LL-EPI readout consisted of 45
consecutive 90°-excitation pulses, of which each was followed by a flow-compensated (unless stated otherwise) single-shot gradient echo EPI readout. The labeling plane was placed slightly above the bifurcation of the carotid artery, while the imaging plane was positioned through the beginning of the C2 segment of both two internal carotid arteries (ICAs), as shown in Figure 1. Both labeling and imaging planes were oriented as perpendicular as possible to the left and right ICAs, and then adjusted to ensure perpendicular planning to the vertebral arteries (VAs) when possible. Other parameters of the LabEff sequence were: postlabeling delay (PLD) = 23 ms, no presaturation and background suppression pulses, FOV = 220 × 220 mm^2, voxel size = 2 × 2 × 3 mm^3, TE = 12 ms, sensitivity encoding (SENSE) factor = 2.5, partial Fourier factor = 0.71, resulting in an echo train length of 43, both the start and the end of the labeling module were triggered with minimal delay (unless stated otherwise) to the automatic R-top identification as obtained by pulse oximetry. Unless stated otherwise, a regional saturation (REST) slab (80 mm thickness) was positioned superior to the inferior edge of the imaging plane to suppress signal in the jugular veins (Fig. 1). Each LabEff scan took approximately 1:30 min, depending on the exact settings of the LabEff sequence and the heart rate of the subject. For each scan session, the order of LabEff scans was randomized to mitigate possible modulation due to heart rate variation.

Study 1

Cardiac Triggering Strategy

Cardiac pulsation will modulate the MR signal due to the time-varying flow velocity (21). To investigate the effect of cardiac pulsation on the ASL signal acquired from the main brain-feeding arteries, the LabEff scan was acquired both without triggering (NonTrig) and with two different triggering strategies: (i) only triggering the start of the labeling, named TrigStart, and (ii) triggering both the start and the end of the labeling, named TrigStart&End, resulting in a labeling duration of one cardiac cycle (Fig. 2). Triggering protocols were performed with minimal trigger delay, except when mentioned otherwise. Labeling duration was set to 800 ms for the NonTrig and TrigStart scans; the TrigStart&End scan was repeated with three different trigger-delays (TDs) of 200 ms, 400 ms, and 600 ms.

Flow Compensation

Because velocity of blood spins may vary spatially and temporally within the artery, phase heterogeneities may occur under the influence of gradients and consequently affect the acquired ASL signal (21). To reduce signal variability induced by such velocity-dependent phase dispersion, the LabEff scans were performed with FC, which was realized by replacing the rephasing gradient after slice excitation with a specifically designed bipolar gradient (21). For comparison purpose, one LabEff scan was performed without FC. For all LabEff scans using FC, the interval between two subsequent excitations of the LL-EPI readout was 33.8 ms, while for the non-FC scan this interval was 31.9 ms.

Vein Signal Suppression

In our preliminary experiments, the arterial signal within the ICAs were frequently found to be affected by artifacts originating from the adjacent jugular veins, leading to increased signal variability. To confirm the influence of venous signal, LabEff scans were made with a large (80 mm thickness) and small (5 mm thickness) REST slab, which was applied superior to the inferior edge of the imaging plane, as illustrated in Figure 1. The REST sequence was performed immediately after the labeling module and before the LL-EPI readout. The large REST slab would reduce all signal originating from the jugular vein, whereas the small REST slab will have little influence while keeping the sequence timing of the two scans similar.

Study 2

In this study, the accuracy of the labeling efficiency measurement was studied by comparing the measured labeling efficiency to brain perfusion signal over a wide range of labeling efficiencies. The labeling efficiency of pCASL was influenced by changing the pCASL FA (5): the LabEff scan was performed with eight different pCASL FAs (5°, 10°, 15°, 18°, 21°, 25°, 30°, and 35°), whereas standard brain pCASL was performed with four different pCASL FAs (5°, 10°, 21°, and 25°). A planning-free vessel-encoded pCASL (VEASL) scan was performed to identify the flow territories (22). Both the brain pCASL and VEASL sequence had the following parameters:
multislice single-shot EPI, FOV = 240 × 240 × 108 mm³, voxel size = 3 × 3 × 6 mm³, number of slices = 18, TR / TE = 3805 ms / 12 ms, SENSE factor = 3, excitation RF FA = 90°, labeling duration = 1650 ms, PLD = 1575 ms, two background suppression pulses were applied at 1680 ms and 2760 ms after the start of the labeling. The brain pCASL and VEASL scan had the same labeling plane as the LabEff scan, whereas their imaging volume covered the whole brain. The brain pCASL scan had 30 repetitions of control / label pairs, while the VEASL scan had 15 repetitions of 5 different encoding patterns as described in Donahue et al (22). The scan durations of a single brain pCASL and VEASL scan were, respectively, 3:56 and 5:04 min.

Data Processing and Analysis

Figures 3a,b show typical arterial signal as acquired with the LabEff sequence. The ASL signal was calculated as complex difference between the control and the label signal, and was subsequently normalized by two times the modulus of the control signal (Fig. 3c). This normalization was performed individually for each time-point to correct for signal modulation caused by cardiac pulsation. In the normalized ASL signal, which showed a plateau phase followed by an abrupt downslope, the start of the downslope was manually identified and the subsequent 450 ms was analyzed according to Eq. [2]. Note that for the TrigStart&End scans, the labeling duration in Eq. [2] was made equal to the mean cardiac cycle of the scans.

For study 1, stability of the labeling efficiency measurement was evaluated in two ways. First, the voxel with the largest ASL signal on the averaged image over all repetitions was identified for each ICA, and then normalization of ASL signal and calculation of labeling efficiency were performed for each repetition individually. Subsequently, the mean (MEANvox) and standard deviation (STDvox) of the labeling efficiency were calculated over all repetitions. Second, for each ICA the eight voxels with largest ASL signal intensity were identified. For each voxel, mean control and mean label signal were obtained by means of complex averaging over all repetitions, and were subsequently used to calculate labeling efficiency. Then the mean (MEANvox) and standard deviation (STDvox) of the labeling efficiency were calculated over the voxels.

The main brain-feeding arteries (i.e., the two ICAs and two VAs) in PC flow images were manually traced to obtain the velocity profile of the cardiac cycle using a locally developed MATLAB-tool. Because the labeling efficiency is effectively calculated from the last-labeled spins (i.e., the downslope), its relation to flow velocity can be studied with the flow velocity corresponding to the end of the labeling module.

For comparison purpose, the theoretical relationship between labeling efficiency and pCASL FA was simulated with a single spin that had velocity ranging from 10 cm/s to 40 cm/s, using Bloch equations similar to Wu et al (4). The averaged territorial brain pCASL signal intensity was calculated from the standard brain pCASL scan by using territory masks generated from the VEASL data. To compare FA-sweep labeling efficiency and the territorial brain pCASL signal intensity, the labeling efficiencies (i.e. MEANvox and brain pCASL signal intensity acquired with pCASL FAs of 5°, 10°, 21° were normalized by the data acquired with a pCASL FA of 25°.

To determine the necessary number of repetitions and, therefore, the scan duration for the LabEff scan, the dataset of LabEff scans consisting of 16 repetitions was retrospectively divided into 4 subsets of 4 consecutive repetitions each; subsequently, the MEANvox and STDvox were calculated for each subset.

All above-described postprocessing was performed in MATLAB (Mathworks, Natick, MA). Paired t-tests were performed to compare the mean and STD of labeling efficiency derived from different LabEff protocols. Repeated-measure analysis of variance (ANOVA) with Bonferroni post hoc test was performed for testing labeling efficiency difference between the different trigger delays. Linear regression of the normalized territorial brain pCASL signal intensity to the normalized artery-specific labeling efficiency was performed. All statistical testing was performed with SPSS software 22.0 (SPSS Inc., Chicago, IL). A P-value of 0.05 was considered statistically significant.
RESULTS

Simulations

Typical simulated arterial signal of the LabEff sequence is shown in Figure 4a. Simulated labeling efficiencies under different flow velocities are shown in Figure 4b. These show that without correction for T1 relaxation the labeling efficiency would be severely underestimated, and by using the proposed algorithm (Eq. [1] and Eq. [2]) the true labeling efficiency in the labeling plane can be well recovered from the ASL signal acquired in the imaging plane.

Cardiac Triggering Strategy

Figure 5a shows that cardiac triggering effectively aligned the control and the label signal, resulting in less pronounced subtraction errors and, therefore, smaller STD<sub>rep</sub> (Fig. 5c). However, the subtraction error was also effectively averaged out when complex averaging over sufficient repetitions (Fig. 5a). Different voxels within an artery showed very different intensity profiles of arterial blood signal, but after normalization, the ASL signal became much more comparable (Fig. 6a). The NonTrig scan showed similar STD<sub>vox</sub> as the cardiac triggered ones (Fig. 6c), but had consistently lower MEAN<sub>vox</sub> (Fig. 6b). No significant differences in MEAN<sub>rep</sub>, MEAN<sub>vox</sub>, STD<sub>rep</sub>, and STD<sub>vox</sub> were found between the TrigStart&End and TrigStart LabEff scans (P values: 0.956 and 0.292 for MEAN<sub>rep</sub> and MEAN<sub>vox</sub>, respectively; 0.248 and 0.475 for STD<sub>rep</sub> and STD<sub>vox</sub>, respectively).

FIG. 3. Modulus (a) and phase (b) signal profiles of a representative signal acquired from a voxel within the ICA. The ASL signal was calculated as the magnitude of the complex difference between the control and label signal. c: Normalized ASL signal obtained by dividing for each time-point the ASL signal with two times the modulus of the control signal. The normalized ASL signal shows typically a plateau phase followed by an abrupt downslope. The beginning of the downslope was manually identified from the normalized ASL signal and the following 450 ms were used to calculate labeling efficiency.

FIG. 4. a: Simulated signal of a pixel at a realistic resolution (2 x 2 mm<sup>2</sup>) in a vessel with laminar flow (maximum velocity of 30 cm/s). b: Labeling efficiencies of a low resolution pixel for different maximum velocities of the laminar flow profile. Mean blood velocities of the pixel are shown on the upper horizontal axis. The uncorrected labeling efficiency corresponds to the maximum intensity of the ASL signal in the imaging plane, i.e., the plateau of the ASL signal shown in (a), divided by 2. The corrected labeling efficiency was calculated from the ASL signal by using Eqs. [1] and [2] for T1 relaxation correction.
However, one should note that a large STD$_{\text{vox}}$ (0.11 for LICA and 0.07 for RICA) was observed for the TrigStart&End scan of subject 4 (see Figure 6c). Checking of the raw control and label images showed that this could be attributed to pulsatility artifacts. As shown in Figures 7a,b and 7e,f, there are significant differences between the labeling efficiencies acquired with different cardiac trigger delays despite the large variance between vessels and subjects. Increasing the trigger delay might lead to larger STD$_{\text{rep}}$ (Figs. 7c,g), but had no influence on STD$_{\text{vox}}$ (Figs. 7d,h).

Flow Compensation

No obvious visible differences in the control and the label signal were observed when comparing the FC and non-FC LabEff scans (Figs. 8a–d). However, the non-FC LabEff scan generally had larger subtraction errors in the ASL signal (Figs. 8g–j). This could be explained by the larger phase deviations between the control and the label measurements when no FC was used (Figs. 8e,f). Nevertheless, the MEAN$_{\text{rep}}$, MEAN$_{\text{vox}}$, STD$_{\text{rep}}$, and STD$_{\text{vox}}$ were not statistically significant different between the two protocols (data not shown).

Vein Signal Suppression

Figure 9a shows the typical location of the jugular vein with respect to the ICA in the imaging plane and suppression of the vein signal. Although the non-VeinSup LabEff scan showed similar MEAN$_{\text{rep}}$ and STD$_{\text{rep}}$ as the VeinSup sequence (data not shown), it had lower MEAN$_{\text{vox}}$, and larger STD$_{\text{vox}}$ (Figs. 9d,e). This is probably caused by ghosts from the jugular vein over-projecting on top of the artery (Figs. 9b,c).

FA-Sweep Experiment

The measured in vivo labeling efficiency as a function of pCASL FA showed similar behavior as our simulations as well as previously published simulations (5): the labeling efficiency increases rapidly for small pCASL FAs, reaching a plateau-phase around 20°, after which it...
shows a gradual decline (Fig. 10a); with larger flow velocity, the labeling efficiency has a slower increasing rate at first, reaches the maxim at a larger FA and finally has a larger value (Figs. 10a–c). However, note that the maximally achieved in vivo labeling efficiency values showed much more variation, which cannot be explained by the velocity differences among the arteries. Regression between the normalized territorial brain pCASL signal intensity and the normalized labeling efficiency yielded a slope of 1.014 with an R of 0.973 ($P < 0.001$) (Fig. 10d).

Although the 4-repetition subsets yielded a significantly higher labeling efficiency as compared with the 16-repetition dataset, the increase was relatively small (for TrigStart&End: 0.892 versus 0.884, $P = 0.001$; for NonTrig: 0.850 versus 0.838, $P = 0.002$). Reduction of repetitions also resulted in a small increase of labeling efficiency variability (i.e., $STD_{vox}$), which is more pronounced for Non-Trig (0.039 versus 0.032; $P < 0.001$) than TrigStart&End (0.049 versus 0.047; $P = 0.084$). Note that the mean of the subjects’ cardiac cycles during the LabEff scans was 855 ms (range from 763 to 1020 ms) for study 1, and 902 ms (range from 724 to 1072 ms) for study 2.

**DISCUSSION**

In this study, the labeling efficiency measurement sequence for pCASL perfusion imaging was implemented, optimized, and validated. Stability of the measurement was tested with regard to cardiac triggering strategy, flow compensation and vein signal suppression. Accuracy of the measured artery-specific labeling efficiency was validated by comparing it to averaged territorial brain pCASL signal intensity over a wide range of labeling efficiencies. Finally, it was proven that with only four averages (approximately 30 s) already reasonable labeling efficiency measurement can be achieved.

Compared with the PC-based method, i.e., equating total brain perfusion to total blood flow in the feeding arteries, the proposed LabEff sequence has several advantages. First, the labeling efficiency measured by the LabEff sequence is artery-specific, thereby assisting the clinical interpretation of, e.g., an asymmetrical perfusion distribution. Second, no complicated postprocessing that may introduce errors into the analysis is required. Although in the current study we relied on manual delineation of rectangular ROIs encompassing the target arteries and the begin of the ASL signal downslope, the
whole data processing could easily be fully automated by using existing artery and signal detection algorithms (23). Furthermore, the current approach involves direct measurement of the labeling efficiency. This would also enable the use of the PC-approach to investigate other sources of error in pCASL-quantification, such as deviations from the single compartment model.

In this study, the labeling efficiency was calculated from the downslope of the normalized ASL signal. The normalized ASL signal is assumed to have a plateau-phase followed by a downslope reflecting the velocity-dependent passage of the bolus of labeled spins (Fig. 4a). According to the simulations with laminar flow (data not shown), this assumption is valid when the minimum velocity of the spins within the voxel is large enough (i.e., at the location of the slowest velocities, the labeled spins did arrive before the fastest spins have already passed, otherwise no plateau phase would be observed) and a relatively long labeling duration is used. We ensured that this assumption was met in our in vivo experiments by choosing only those voxels with high ASL signal intensity and a labeling duration approximately equal to the human cardiac cycle.

In this study, cardiac triggering was found to increase stability of the labeling efficiency measurement (Fig. 5c). The NonTrig LabEff scan yielded consistently lower labeling efficiency than the TrigStart&End scan, which can be explained by two effects: (i) the labeling efficiency of NonTrig scan provides an average measure over the whole cardiac cycle, while the TrigStart&End scan reflects the labeling efficiency of a specific part of the cardiac cycle. Data acquired with additional trigger

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**FIG. 7.** In vivo labeling efficiencies acquired by the LabEff sequence using different cardiac trigger delays. **a,c:** Mean and standard deviation of labeling efficiency over all 16 repetitions. **b,d:** Mean and standard deviation of labeling efficiency over eight voxels within a single artery. For clarity, data in **a–d** were averaged over all vessels for each trigger delay as shown in **e–h.** Repeated-measures ANOVA was performed to test for differences between trigger delays, and Bonferroni post hoc comparison was performed for identifications of the differences between the individual delays. Data in **a–c** and **e–g** all had significant ($P < 0.05$) overall differences between trigger delays. Note that the TrigStart scan with 800 ms labeling duration is also included here, because it is almost equivalent to a Trig-Start&End scan with 800 ms trigger delay.
delays show that indeed the measured labeling efficiency varies within the cardiac cycle (Fig. 7b). (ii) The misalignment of the control and the label signal in NonTrig scans results in imperfect subtraction and, therefore, underestimated labeling efficiency. In addition, signal fluctuations of the control signal may also add to errors in estimating labeling efficiency due to the use of it in the normalization procedure.

Use of flow compensation in the LabEff sequence was shown to reduce subtraction errors for a single ASL subtraction, but averaging the signal over multiple repetitions quickly averaged out such subtraction errors, resulting in a nonsignificant difference when using 16 averages as in the current study. Due to the high sensitivity of the EPI readout to blood flow, ghosts of the nearby-located jugular veins may contaminate the signal within the ICAs. The contamination increases the variation of labeling efficiency over voxels (Figs. 9c,e), as well as decreases the labeling efficiency (Fig. 9d). Note that venous signal contamination happens only in the ICAs, not in the VAs. In the proposed LabEff sequence, a large saturation slab was performed immediately after the labeling to saturate venous signal. Due to the large flow velocity in the ICAs and short distance between the labeling and imaging plane, the labeled arterial spins can be imaged without venous artifacts before unsaturated venous signal reaches the imaging location.

The pCASL FA-sweep LabEff experiment of study 2 proved the capability of the LabEff sequence to robustly measure the labeling efficiency as can be concluded from the similarity to simulation results as well as the high regression coefficient (Figs. 10a–d). This suggests that the LabEff sequence is able to detect relatively large labeling efficiency changes as achieved by changing the pCASL FA in this study. However, because the exact labeling efficiency is unknown, it is not yet determined whether this approach also allows capturing more subtle labeling efficiency changes. From simulation (Fig. 10a), flow velocity was found to modulate both maximally achievable labeling efficiency as well as the shape of the pCASL FA-labeling efficiency profile. Probably due to influence of other factors (e.g., off-resonance effect) that also differs between arteries, the measured maximally achievable labeling efficiencies

FIG. 8. a–j: Representative signal of individual repetitions acquired by the LabEff sequence with and without flow compensation (FC). The control and the label signal is plotted as complex vectors in (e) and (f). For clarity, only 8 of 16 repetitions are shown in all charts. The signal segments with large subtraction errors are highlighted with green frames.
were not related to the flow velocity as expected from simulations (Figs. 10a–c). However, the shape modulation was observed in the in vivo data, especially for the ICAs (Fig. 10b). This suggests that the LabEff sequence can address the labeling efficiency in terms of flow velocity. Therefore, the variability of pCASL CBF quantification caused by flow velocity-induced labeling efficiency change may be reduced by using the LabEff sequence for labeling efficiency correction.

Regression results of the pCASL FA-sweep LabEff and brain pCASL data further indicate usefulness of the LabEff sequence for calibrating pCASL CBF measurement. Because the brain pCASL perfusion scan and the LabEff scan have exactly the same labeling condition, the labeling efficiency change evoked by manipulating the pCASL FAs is expected to be the same for the two scans. The fact that the regression slope is close to one indicates that the measured artery-specific labeling efficiency can explain most of the variability of the brain pCASL signal and, therefore, proofs its potential for calibration of the (territorial) brain pCASL perfusion.

The retrospective analysis of four-repetition sub-data-sets shows that the labeling efficiency measured by the proposed method has high temporal SNR, especially when cardiac triggering is used. This suggests that the LabEff sequence can be performed with few repetitions and within 30 s (current LabEff scan with 16 repetitions takes less than 2 min) without sacrificing accuracy of the labeling efficiency measurement.

This study mainly focused on labeling efficiency measurement for ICAs, because the imaging plane was positioned optimally for the ICAs, whereas this was not the case for the VAs. Therefore, the LabEff signal of VAs suffered more from partial volume effects, which are already more pronounced in the VAs due to the smaller diameter as compared to ICAs. Although the partial volume problem was mitigated to some extent due to saturation of static tissue signal by the consecutive execution of 90°-excitation pulses in the LL-EPI readout, the measured pCASL FA-sweep labeling efficiency for VAs showed more fluctuations as compared to measurements in the ICAs (Figs. 10b,c). Moreover, the VA segments (V2–V4) between the used labeling and imaging planes are tortuous, which may result in complicated flow hemodynamics and affect the labeling efficiency measurement. Therefore, further optimizations are needed.

FIG. 9. Signal and labeling efficiencies acquired by the LabEff sequence with and without vein signal suppression. a: Phase contrast complex difference image and LabEff control images acquired at the same location in subject 5, showing the jugular vein (green arrow) located close to the right ICA (red arrow) and the vein signal suppressed in the VeinSup scan. b,c: Normalized ASL signal of the voxels within the right ICA of subject 5. d,e: Mean and standard deviation of labeling efficiency over eight voxels within a single artery. Statistical comparisons of the labeling efficiency were performed between the two protocols using a paired t-test. P values for statistical comparisons are stated in the charts when <0.05.
for labeling efficiency measurement in the VAs. It should also be noted that the VAs were not included for the regression analysis in Figure 10d, because blood flow in the two VAs may or may not mix within the basilar artery, resulting in a complicated relation of the labeling efficiency in the VAs with the measured perfusion signals in the posterior circulation territory (24).

Note that for both the ICAs and VAs, some of the measured labeling efficiencies were larger than one, which is, of course, unrealistic. In this study, the ASL signal (i.e., the difference signal) of LabEff scan was normalized by the control signal, which was assumed equal to the equilibrium blood signal. However, due to the use of an imperfect control condition, the control signal is usually smaller than the true equilibrium blood signal. This may explain the overestimation of the labeling efficiency. Therefore, it should be considered to introduce a third condition, besides label and control, during which the pCASL RF pulses are completely switched off to acquire the true equilibrium blood signal.

There are several limitations of the current implementation of the LabEff sequence. First, the specific layout of the LabEff sequence imposes certain restrictions on the location of the labeling slab. Because the distance between the labeling and imaging plane of the LabEff scan cannot be too small, the labeling plane needs to be positioned slightly lower than commonly done (Fig. 1). This position of the labeling plane might not always be optimal for brain pCASL perfusion imaging (11), depending on the extent of the RF transmit coil and the effectiveness of shimming of the magnetic field at this labeling location. Furthermore, a slightly longer transit time might be expected for the perfusion pCASL scan when the labeling is positioned at the same location as for the labeling efficiency measurement. Finally, labeling should not be performed too close to the carotid bifurcation, because complex flow patterns might result in lower labeling efficiency. Other planning-strategies for the LabEff sequence can be considered, but these need to be validated in the future. Second, reproducibility of the

FIG. 10. PCASL flip angle (FA)-sweep labeling efficiency and brain pCASL signal intensity. a: Simulated dependency of the labeling efficiency on pCASL FA for different flow velocities as encoded by the color of the curves. b,c: In vivo labeling efficiencies of ICAs and vertebral arteries (VAs) acquired with different pCASL FAs on five subjects. When calculating the labeling efficiency, 8 voxels within ICA and 2 within VA were used, respectively. All curves in (a–c) are color-coded according to the mean velocity as measured in that specific artery at the labeling plane; color scale according to the colorbar show on top of the figure. d: Relation between the normalized territory brain pCASL signal intensity and the normalized labeling efficiency. Normalization refers to the fact that the brain pCASL signal intensity and labeling efficiencies acquired with pCASL FAs of 5°, 10°, and 21° were divided by their respective values acquired with pCASL FA of 25°. Only ICAs data are included in (d).
labeling efficiency measurement was not investigated in the current study, although short-term reproducibility was proven by splitting the dataset into four subsets. A further study investigating this topic is warranted, which should also try to differentiate between true variations in labeling efficiency and measurement errors. Third, due to the lack of a gold standard for labeling efficiency measurement, the measured labeling efficiency was validated in an indirect manner. Further validation, for example by comparing labeling efficiency-corrected brain perfusion pCASL with other perfusion techniques like PET, is warranted.

CONCLUSIONS
In this study, a pCASL-based sequence was optimized to measure artery-specific labeling efficiency. The proposed approach was shown suitable for calibration of brain pCASL perfusion measurement, which should improve the accuracy of pCASL CBF quantification.

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