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Measurement of brain perfusion in newborns: Pulsed arterial spin labeling (PASL) versus pseudo-continuous arterial spin labeling (pCASL)

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ABSTRACT

Background: Arterial spin labeling (ASL) perfusion-weighted imaging (PWI) by magnetic resonance imaging (MRI) has been shown to be useful for identifying asphyxiated newborns at risk of developing brain injury, whether or not therapeutic hypothermia was administered. However, this technique has been only rarely used in newborns until now, because of the challenges to obtain sufficient signal-to-noise ratio (SNR) and spatial resolution in newborns.

Objective: To compare two methods of ASL-PWI (i.e., single inversion-time pulsed arterial spin labeling [single TI PASL], and pseudo-continuous arterial spin labeling [pCASL]) to assess brain perfusion in asphyxiated newborns treated with therapeutic hypothermia and in healthy newborns.

Design/methods: We conducted a prospective cohort study of term asphyxiated newborns meeting the criteria for therapeutic hypothermia; four additional healthy term newborns were also included as controls. Each of the enrolled newborns was scanned at least once during the first month of life. Each MRI scan included conventional anatomical imaging, as well as PASL and pCASL PWI-MRI. Control and labeled images were registered separately to reduce the effect of motion artifacts. For each scan, the axial slice at the level of the basal ganglia was used for comparisons. Each scan was scored for its image quality. Quantification of whole-slice cerebral blood flow (CBF) was done afterwards using previously described formulas.

Results: A total number of 61 concomitant PASL and pCASL scans were obtained in nineteen asphyxiated newborns treated with therapeutic hypothermia and four healthy newborns. After discarding the scans with very poor image quality, 75% (46/61) remained for comparison between the two ASL methods. pCASL images presented a significantly superior image quality score compared to PASL images ($p < 0.0001$). Strong correlation was found between the CBF measured by PASL and pCASL ($r = 0.61$, $p < 0.0001$).

Conclusion: This study demonstrates that both ASL methods are feasible to assess brain perfusion in healthy and sick newborns. However, pCASL might be a better choice over PASL in newborns, as pCASL perfusion maps had a superior image quality that allowed a more detailed identification of the different brain structures.

1. Introduction

Hypoxic–ischemic encephalopathy is the most common cause of brain injury in term newborns. Therapeutic hypothermia is currently the only existing treatment to minimize brain injury in these newborns, with decreased death and disability rates at 12–18 months and beyond (Azzopardi et al., 2009; Eicher et al., 2005a, b; Gluckman et al., 2005; Jacobs et al., 2007; Shankaran et al., 2005; Shankaran et al., 2012). However, some newborns still develop brain injury despite this treatment (Barks, 2008; Higgins et al., 2006; Higgins and Shankaran, 2009).

Abbreviations: MRI, magnetic resonance imaging; PASL, pulsed arterial spin labeling; pCASL, pseudo-continuous arterial spin labeling; PWI, perfusion-weighted imaging; SNR, signal-to-noise ratio.

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Measuring brain perfusion has been shown to be useful for identifying asphyxiated newborns at risk of developing brain injury, whether or not therapeutic hypothermia was administered (Massaro et al., 2013; Pienaar et al., 2012; Wintermark et al., 2011). In contrast to other approaches previously used in newborns to measure brain perfusion (Levene et al., 1989; Minhas et al., 2003), arterial spin labeling (ASL) perfusion-weighted imaging (PWI) by magnetic resonance imaging (MRI) is the only method allowing direct measurements of brain perfusion in specific brain regions (Biagi et al., 2007; Huisman and Sorensen, 2004; Miranda et al., 2006; Wang and Licht, 2006a; Wang et al., 2006b; Wintermark et al., 2011) and does not require the use of contrast agent, radiotracer or radiation, as it relies upon the magnetic labeling of incoming blood flow to provide a change in contrast that is proportional to the amount of perfusion present. However, this technique has been only rarely used in newborns until now (De Vis et al., 2013; Massaro et al., 2013; Pienaar et al., 2012; Wintermark et al., 2011), because of the challenges to obtain sufficient signal-to-noise ratio (SNR) and spatial resolution to differentiate the cortical gray matter, white matter and basal ganglia in newborns.

Different ASL methods have now been developed in the adult literature, to try to improve this MR technique and obtain better contrast. These methods differ mostly by the extent and the duration of the magnetic labeling of the inflowing blood. Up to now, studies comparing these different ASL methods and determining which one provides the most accurate results are available in the adult literature (Gevers et al., 2011), but not in the newborn literature. However, as previously mentioned, the problem of obtaining sufficient SNR and spatial resolution in this population is more complicated than in adults, as newborns have smaller brain and lower brain perfusion compared to older children and adults. The objective of this study is to compare two methods of ASL-PWI, i.e., single inversion-time pulsed arterial spin labeling (PASL) and pseudo-continuous arterial spin labeling (pCASL) to assess brain perfusion in healthy newborns and in asphyxiated newborns treated with therapeutic hypothermia.

2. Material and methods

2.1. Patients

We conducted a prospective cohort study of 19 term newborns with hypoxic–ischemic encephalopathy admitted at the neonatal intensive care unit within the first 6 h of life, who met the criteria for therapeutic hypothermia (Miller et al., 2005; Rutherford et al., 2004; Wintermark et al., 2008): (1) gestational age $\geq$ 36 weeks and birth weight $\geq$ 1800 g; (2) evidence of fetal distress, e.g., history of acute perinatal event, or cord pH $\leq$ 7.0; (3) evidence of neonatal distress, such as Apgar score $\leq$ 5 at 10 min, postnatal blood gas pH obtained within the first hour of life $\leq$7.0, or need for ventilation initiated at birth and continued for at least 10 min; and (4) evidence of neonatal encephalopathy by physical examination and by abnormal amplitude-integrated electroencephalogram background pattern. Newborns who met the above criteria received whole-body cooling to an esophageal temperature at 33.5 °C, initiated by 6 h of life, and continued for 72 h. Four additional healthy term newborns were also enrolled as controls. This study was approved by the institutional review board, and parental consent was obtained.

2.2. MRI scans

All of the newborns were scanned at least once during the first month of life. If possible, one or two MRI scans were obtained within the first 72 h after birth, i.e., during hypothermia treatment, on day 1 of life (i.e., within 24 h of life) and/or on days 2–3 of life (i.e., 24–72 h after birth). Only asphyxiated newborns, who were hemodynamically stable, underwent the MRI scans during hypothermia treatment. Patients continued to receive hypothermia treatment during the MR imaging without any adverse events (Wintermark et al., 2010). Any ventilation, pressor support, or sedation was maintained during the MRI scanning process; additional sedation was avoided. One or two MRI scans were then obtained after 1 week of life, around day 10 of life and/or around 1 month of life. Excessive movements during imaging were minimized by wrapping the newborns in an MRI-compatible vacuum cushion.

MRI scans were acquired with a 3 T Philips MR Systems Achieva X (Philips Medical Systems, Best, The Netherlands) using a 32-channel head coil (Philips). Conventional anatomic sequences included a 3D T1-weighted gradient-echo (TR/TE, 24/4.6 ms; flip angle, 30°; slice thickness, 1 mm; voxel size, 1 × 1 mm; 110 slices; FOV, 180 × 140 mm) and an axial high resolution T2-weighted turbo spin-echo (TR/TE, 5000/90 ms; TSE factor, 15; flip angle, 90°; slice thickness, 3 mm; voxel size, 0.5 × 0.5 mm; 24 slices; FOV, 150 × 120 mm). In addition, two different arterial spin labeling (ASL) sequences were used. The first was a pulsed arterial spin labeling with a single TI (single TIPASL) sequence, consisting of a multi-slice single-shot echo planar imaging (EPI) sequence with signal targeting and alternating radiofrequency (EPISTAR) for labeling in combination with parallel imaging (SENSE) (Golay et al., 2005) (TR/TE, 2400/15 ms; flip angle, 40°; matrix size, 64 × 64; FOV, 220 × 220 mm; slice thickness, 6 mm; 8–11 axial sections; 69 label/control pairs; total scan time, 5.75 min); the labeling slab had a thickness of 50 mm with a gap of 20 mm; the label delay was 1800 ms. The second perfusion acquisition was a pseudo-continuous arterial spin labeling (pCASL) sequence, consisting of a multi-slice single-shot EPI sequence in combination with parallel imaging (SENSE) (van Osch et al., 2009) (TR/TE, 3749 ms/15 ms; flip angle, 40°; matrix size, 64 × 64; FOV, 220 × 220 mm; slice thickness, 6 mm; 11 axial sections; 45 label/control pairs; total scan time, 5.88 min); the label gap was 20 mm, the label duration was 1650 ms and the post-label delay was 1800 ms. The imaging plane for both ASL sequences was oriented along the corpus callosum and positioned to cover the area from the basal ganglia to the top of the head. The labeling slab for both ASL sequences was oriented parallel to the imaging plane, and had to be above the heart and the aortic arch. To allow quantification with both ASL techniques, a separate equilibrium magnetization map (M0 scan) was also obtained for each technique, with the same parameters for each sequence respectively, except a TR of 10,000 ms was used to allow for complete relaxation of the magnetization. Typically, the PASL sequence was run first followed by the pCASL sequence; if gross motion was too evident at the time of scan, these sequences were repeated if time allowed it.

2.3. Image analysis

Control and tagged images were processed separately. They were registered to the first one of the series using Statistical Parametric Mapping 8 (SPM8). If motion exceeding 1° in angulation or 1 mm in position was detected, the image and its pair were discarded; in general, approximately 0–5 pairs were discarded in each infant. For each scan, the axial slice at the level of the basal ganglia was used for comparisons. An image quality score was developed for this study by one investigator, who reviewed and scored all the images, but was not blinded to the type of ASL images. The image quality score was the sum of two subscores, i.e., a subscore for the difficulty to identify the different brain structures (basal ganglia, white matter, and cortical gray matter) and a subscore for artifacts. In more details, the subscore for the complexity to identify the different brain structures was from 0 to 2, with 0 corresponding to a great difficulty to distinguish the different structures, 1 corresponding to some difficulty to distinguish the different structures, and 2 corresponding to the different structures being easily recognizable. The subscore for artifacts was from 0 to 2, with 0 corresponding to the presence of a major artifact throughout all the images, 1 corresponding to the presence of a minor artifact, and 2 corresponding to the absence of any artifact. The image quality score summed up these two subscores,

and was thus from 0 corresponding to a very poor image quality to 4 corresponding to an excellent image quality. Another investigator, who was blinded to the clinical conditions of the newborns, reviewed all the PASL and pCASL perfusion-weighted images, and scored them for image quality separately and blindly from each other (i.e., at the time of scoring, the investigator did not know if he was scoring PASL or pCASL images). Agreement between the two investigators was good (Kappa 0.79). Only the scoring done by the investigator, who was completely blinded, was presented in the Results section. Scans with an image quality score of 0 were discarded and deemed not appropriate to measure cerebral blood flow.

2.4. Quantification of cerebral blood flow

Quantification of cerebral blood flow was performed using the separate M_{0a} scan and previously described formulas (Gevers et al., 2011).

Quantitative regional CBF requires an estimation of the equilibrium magnetization of the arterial blood as previously described (Gevers et al., 2011):

$$M_{0a} = \frac{S_{csf}\lambda_a}{1-\exp(-\frac{TR}{T_{1csf}})} = 0.93 S_{csf}$$

with M_{0a}, the equilibrium magnetization of the arterial blood; S_{csf}, the signal intensity of cerebrospinal fluid in a manually defined ventricular region; λ_a, the quantity (mL) of water per quantity of blood (mL) (0.87) (Herscovitch and Raichle, 1985); TR, the repetition time of the sequence (10 s); and T_{1csf}, the longitudinal relaxation time of the cerebrospinal fluid (3.7 s) (Clare and Jezzard, 2001; Hopkins et al., 1986).

For quantification of cerebral blood flow using PASL, we used the following model (Gevers et al., 2011):

$$f = CBF = 6000 \frac{\Delta M \exp(\frac{w}{T_{1a}}) \exp(\frac{TE}{T_{2a}{}^*})}{M_{0a}\, 2\, \alpha \tau}$$

with f, the cerebral blood flow (CBF) in mL per 100 g/min; ΔM, the difference between the control and labeled image intensities; w, the post-labeling delay (1.8 s); T_{1a}, the longitudinal relaxation time of arterial blood (1.8 s) (Varela et al., 2011); TE, the echo time of the sequence; $T_{2a}{}^*$, the transverse relaxation time of arterial blood (50 ms) (Gevers et al., 2011); M_{0a}, the equilibrium magnetization of the arterial blood; α, the labeling efficiency (0.95) (Gevers et al., 2011); and τ, the temporal bolus width (assumed to be 1.2 s).

For quantification of cerebral blood flow using pCASL, we used the following model (Gevers et al., 2011):

$$f = CBF = 6000 \frac{\Delta M \exp(\frac{w}{T_{1a}}) \exp(\frac{TE}{T_{2a}{}^*})}{\rho\, M_{0a}\, 2\, \alpha\, T_{1a}}$$

with f, the cerebral blood flow (CBF) in mL per 100 g/min; ΔM, the difference between the control and labeled image intensities; w, the label delay (1.8 s); T_{1a}, the longitudinal relaxation time of arterial blood (1.8 s) (Varela et al., 2011); TE, the echo time of the sequence; $T_{2a}{}^*$, the transverse relaxation time of arterial blood (50 ms) (Gevers et al., 2011; Spees et al., 2001); ρ, the density of brain tissue (1.05 g/mL) (Delpy et al., 1987); M_{0a}, the equilibrium magnetization of the arterial blood; and α, the labeling efficiency (0.85) (Gevers et al., 2011).

One investigator then drew a whole-slice region of interest (ROI) (including only the brain without the skull) on the selected axial slice at the level of the basal ganglia of each PASL or pCASL scan to determine ΔM; the measurement was repeated twice and the mean of the two measurements was used for the abovementioned calculations. S_{csf} was obtained by measuring the signal intensity in a manually drawn ROI in the cerebrospinal fluid, on a slice usually located one or two slices below the axial slice used to measure ΔM, and was used to calculated M_{0a}. Each measurement was repeated twice and the mean was used for the abovementioned calculations.

2.5. Statistical analysis

For each scan, the image quality score and the whole-slice cerebral blood flow was calculated on the axial slice at the level of the basal ganglia for both PASL and pCASL scans. In a first analysis, we compared the image quality score and the cerebral blood flow values measured by PASL or by pCASL using the Wilcoxon signed rank tests. In a second analysis, the association between cerebral blood flow measured by PASL or measured by pCASL for each scan and each patient was explored using Spearman correlations. In a third analysis, we used the different abovementioned tests to explore the relationship between image quality score and CBF measured by PASL or by pCASL, based on the presence or not of brain injury or the day of life at the time of the scan. Analysis was performed using GraphPad Prism version 5.00 (GraphPad Software Inc) and R package (R Core Team, 2013).

3. Results

A total number of 61 concomitant PASL and pCASL scans were obtained; 75% were performed in 19 asphyxiated newborns, and 25% in four healthy newborns. Forty-two percent of the asphyxiated newborns developed brain hypoxic–ischemic (HI) injury despite hypothermia; and 58% did not. The brain MRIs of the healthy term newborns did not reveal any abnormality.

25% of scans had to be discarded because of very poor image quality (image quality score = 0) on PASL images (6 scans), pCASL images (7 scans) or both (2 scans). In more details, 22 concomitant scans were obtained in the asphyxiated newborns developing brain HI injury; 18% had to be discarded, including three performed during the first 3 days of life (two because of pCASL images and one because of both PASL and pCASL images) and one performed after the first week of life (because of PASL images). Twenty-four concomitant scans were obtained in the asphyxiated newborns not developing injury; 21% of them had to be discarded, all of the discarded ones were performed after the first week of life (two because of PASL images and three because of pCASL images). Fifteen concomitant scans were obtained in the healthy term newborns; 24% of them had to be discarded, including four performed during the first 3 days of life (one because of PASL images, two because of pCASL images and one because of both) and two performed after the first week of life (two because of PASL images).

After discarding the scans with very poor image quality, 75% of scans (46 scans) remained for comparison between the two ASL methods. Among these scans, pCASL images presented a significantly better image quality score compared to PASL images (i.e., score of 1.87 for PASL images vs score of 2.67 for pCASL images, $p < 0.0001$) (Figs. 1 and 2). The Wilcoxon signed rank test showed that there was no significant difference in whole-slice CBF (mean ± standard-deviation) measured by the two ASL methods, i.e., 29.52 ± 12.44 mL/100 g/min when measured by PASL and 27.57 ± 11.63 mL/100 g/min when measured by pCASL ($p = 0.16$) (Figs. 3 and 4). In addition, strong correlation was found between the CBF measured by PASL and pCASL ($r = 0.61$; $p < 0.0001$) (Fig. 5).

When further comparing according to the presence or not of brain injury, 18 scans were obtained in asphyxiated newborns developing brain HI injury, 19 in asphyxiated newborns not developing brain HI injury, and 9 in healthy newborns. Among the asphyxiated newborns developing brain HI injury, the image quality score was 2.00 for the PASL images and 2.83 for the pCASL images ($p = 0.0005$); the whole-slice CBF was 33.51 ± 16.73 mL/100 g/min when measured by PASL and 32.41 ± 14.43 mL/100 g/min when measured by pCASL ($p = 0.73$) (Fig. 3A); and correlation between both techniques was strong ($r =$

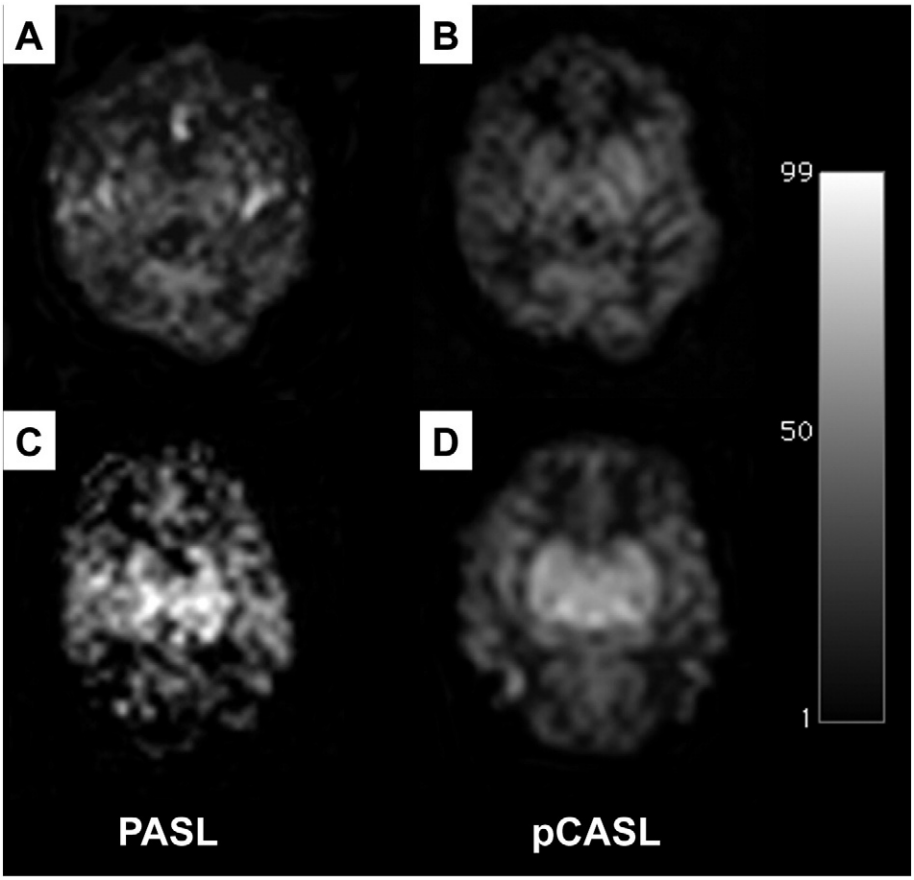

Fig. 1. Example of axial cerebral blood flow (CBF) (mL/100 g/min) maps obtained at the level of the basal ganglia with PASL and pCASL. (A–B) CBF maps in a healthy newborn, (A) obtained by PASL, and (B) obtained by pCASL. (C–D) CBF maps in an asphyxiated newborn developing brain HI injury (in the basal ganglia) despite hypothermia treatment, (C) obtained by PASL, and (D) obtained by pCASL. Perfusion was higher in the gray matter and in the basal ganglia compared to the white matter in the healthy newborns. Perfusion was increased in the basal ganglia of the asphyxiated newborn developing brain HI injury in the basal ganglia. In both patients, the pCASL perfusion maps presented a qualitatively superior image quality, compared to the PASL perfusion maps.

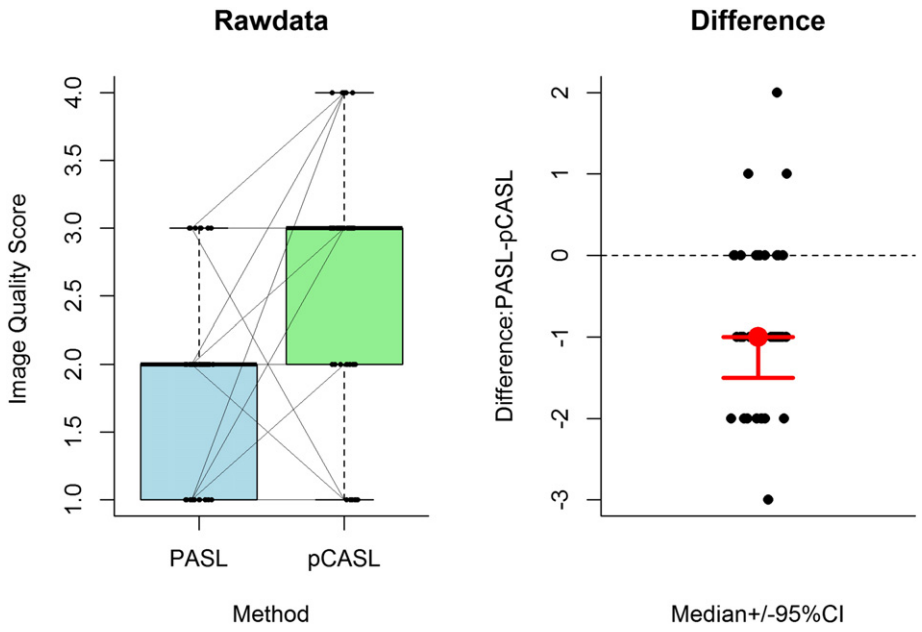

Fig. 2. Comparison between quality score of PASL images and pCASL images in asphyxiated newborns treated with hypothermia and in healthy newborns. Left panel: Box and whisker plot for raw quality scores of PASL and pCASL images; right panel: strip chart for the difference between quality score of PASL image and corresponding pCASL image with median and 95% confidence interval for the mean difference of quality score.

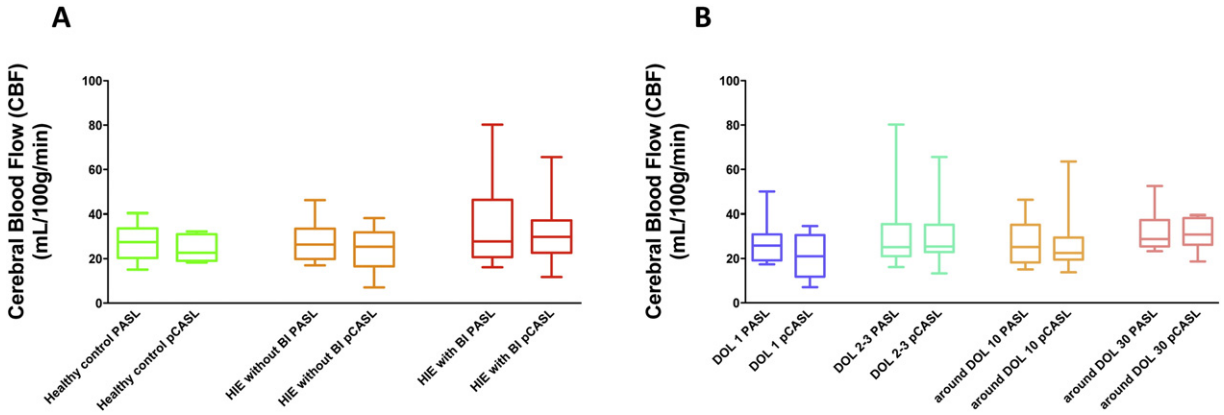

Fig. 3. Cerebral blood flow (CBF) (mL/100 g/min) measured by PASL and pCASL in asphyxiated newborns treated with hypothermia and in healthy newborns. Box and whisker plot (median, minimum, and maximum, in [mL/100 g/min]) representation. All data are first represented, then compared according to the presence or not of brain injury (A) or according to the day of life at the time of the scan (B). Brain perfusion and variations in CBF values between the newborns (i.e., mean and standard-deviations of the different measurements) were the highest on day 2 of life and in the asphyxiated newborns developing brain HI injury.

0.73; $p = 0.0006$). Among the asphyxiated newborns not developing brain HI injury, the image quality score was 1.95 for the PASL images and 2.58 for the pCASL images ($p = 0.03$); the whole-slice CBF was 27.48 ± 8.21 mL/100 g/min when measured by PASL and 24.65 ± 8.82 mL/100 g/min when measured by pCASL ($p = 0.17$) (Fig. 3A); and correlation between both techniques was strong (r = 0.60; $p = 0.007$). Among the healthy newborns, the image quality score was 1.44 for the PASL images and 2.56 for the pCASL images ($p = 0.04$); the whole-slice CBF was 27.22 ± 8.23 mL/100 g/min when measured by PASL and 24.27 ± 5.83 mL/100 g/min when measured by pCASL ($p = 0.30$) (Fig. 3A); and correlation between both techniques was moderate (r = 0.42; $p = 0.27$).

When further comparing according to the day of life at the time of the scan, 8 concomitant scans were obtained on day 1 of life, 13 on days 2–3 of life, 16 around day 10 of life (range: days 9–14 of life) and 9 around 1 month of life (range: days 28–38 of life). On day 1 of life, the image quality score was 1.63 for the PASL images and 2.13 for the pCASL images ($p = 0.50$); the whole-slice CBF was 27.35 ± 10.58 mL/100 g/min when measured by PASL and 20.94 ± 10.74 mL/100 g/min when measured by pCASL ($p = 0.08$) (Fig. 3B); and correlation between both techniques was very strong (r = 0.83; $p = 0.02$). On days 2–3 of life, the image quality score was 1.77 for the PASL images and 2.31 for the pCASL images ($p = 0.20$); the whole-slice CBF was 31.53 ± 17.64 mL/100 g/min when measured by PASL and 30.67 ± 13.36 mL/100 g/min when measured by pCASL ($p = 0.68$) (Fig. 3B); and correlation between both techniques was moderate (r = 0.59; $p = 0.03$). Around day 10 of life, the image quality score was 1.94 for the PASL images and 2.81 for the pCASL images ($p = 0.0005$); the whole-slice CBF was 27.38 ± 10.08 mL/100 g/min when measured by PASL and 26.35 ± 11.95 mL/100 g/min when measured by pCASL ($p = 0.60$) (Fig. 3B); and correlation between both techniques was strong (r = 0.62, $p = 0.01$). Around 1 month of life, the image quality score was 2.11 for the PASL images and 3.44 for the pCASL images ($p = 0.008$); the whole-slice CBF was 32.37 ± 9.25 mL/100 g/min when measured

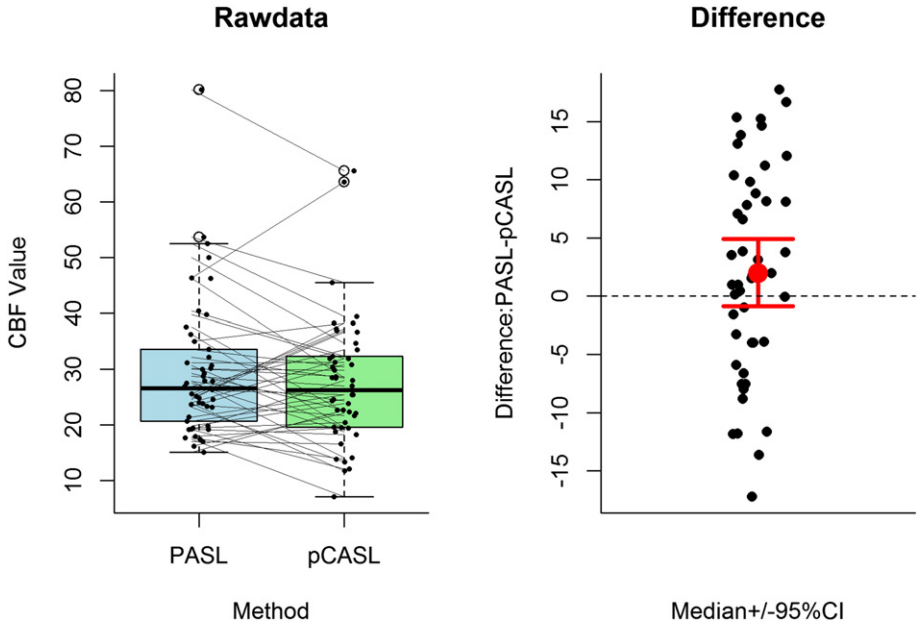

Fig. 4. Comparison between cerebral blood flow (CBF) (mL/100 g/min) measured by PASL and CBF measured by pCASL in asphyxiated newborns treated with hypothermia and in healthy newborns. Left panel: Box and whisker plot for raw CBF values by the two ASL methods; right panel: strip chart for the difference between CBF measured by PASL and that by pCASL with median and 95% confidence interval for the mean difference of CBF values.

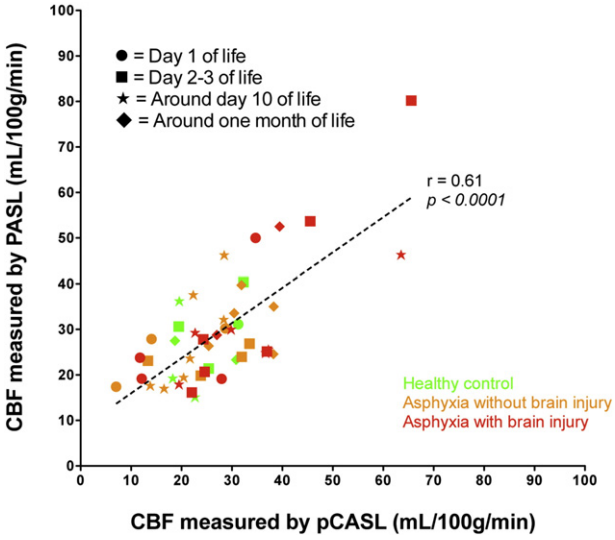

Fig. 5. Correlation between cerebral blood flow (CBF) (mL/100 g/min) measured by PASL and pCASL in asphyxiated newborns treated with hypothermia and in healthy newborns. A strong correlation ($r = 0.61$, $p < 0.0001$) was found between CBF measured by PASL and CBF measured by pCASL, when correlating all the measurements.

by PASL and 31.14 ± 6.86 mL/100 g/min when measured by pCASL ($p = 0.65$) (Fig. 3B); and correlation between both techniques was moderate ($r = 0.47$, $p = 0.21$).

4. Discussion

Two different ASL methods were tested in this study. The first one, a pulsed arterial spin labeling (PASL) sequence, consisted of a multi-slice single-shot echo planar imaging (EPI) sequence with signal targeting and alternating radiofrequency (EPISTAR) (Golay et al., 2005). PASL uses a single short RF pulse (11 ms in our study) to invert a thick slab of arterial water spins (Edelman and Chen, 1998; Kim, 1995; Wong et al., 1998). The second one, a pseudo-continuous arterial spin labeling (pCASL) sequence, consisted of a multi-slice single-shot EPI sequence (van Osch et al., 2009). pCASL uses as many as 1000 or more RF pulses applied in rapid succession (in our study, 1 pulse every millisecond for 1650 ms, i.e., 1650 pulses) to achieve the labeling (Dai et al., 2008). Both techniques were evaluated on a 3-Tesla MRI. Both methods have been previously used in newborns (De Vis et al., 2013; Massaro et al., 2013; Miranda et al., 2006; Pienaar et al., 2012; Wintermark et al., 2011), but never compared to each other in this population.

Cerebral blood flow quantification using ASL methods is based on mathematical models to determine the cerebral blood flow values from the signal intensity. There are known limitations to the quantification formulas used for both the PASL and pCASL methods. In each formula, several parameters are not defined by the design of the sequence and thus may be sources of error in the estimation of the cerebral blood flow. We reviewed the literature about ASL quantification and used the values that were the most appropriate for newborns. Specifically, the relaxation times of arterial blood (T_{1a} and $T_{2a}{}^*$) have not been widely studied in newborns, due to the complexity of these measurements. They both are highly dependent upon field strength (3 Tesla vs 1.5 Tesla), and they both depend on hematocrit (Zhao et al., 2007). The asphyxiated newborns treated with hypothermia often are hemodynamically unstable, so their hematocrit is kept around 35% to keep optimal oxygenation of the body, making it appropriate to assume a constant T_{1a} and $T_{2a}{}^*$ in these infants. For the longitudinal relaxation time of arterial blood (T_{1a}), we initially planned to use 1400–1500 ms like in previous published studies (Cavusoglu et al., 2009; Gevers et al., 2011; Golay et al., 2005; Massaro et al., 2013; van Osch et al., 2009; Wintermark et al., 2011) using estimates derived from adult data rather than actually measured in newborns. However, after further reading, we decided to use 1800 ms, as suggested by Varela et al. (2011) in their study of eighteen newborns, whose mean longitudinal relaxation time of arterial blood was 1799 ± 206 ms. Some authors even suggest 2000 ms for the newborns (Alsop et al., 2014) to give enough time for the labeled blood to reach the tissue. For the transverse relaxation time of arterial blood ($T_{2a}{}^*$), values vary in the literature between 43.6 and 50 ms (Cavusoglu et al., 2009; Gevers et al., 2011), but none were studied in detail in newborns. The quantity of water per quantity of blood (λ_a) depends on the hematocrit and was set at 0.87, as described for a hematocrit at 35% (Herscovitch and Raichle, 1985). For the longitudinal relaxation rate of the CSF (T_{1csf}), values were found only in adults, and not in newborns; described values were either 3.7 s (Clare and Jezzard, 2001; Hopkins et al., 1986) or 4.2 s (Cavusoglu et al., 2009; Chalela et al., 2000). We chose 3.7 s over 4.2 s, due to the difficulty to draw a ROI in a small region at the ventricle level in newborns, without getting any contamination from surrounding tissue; the highest value (4.2 s) is close to the longitudinal relaxation time of pure water. The density of brain tissue (ρ) was set at 1.05 g/mL, as described in the neonatal literature (Delpy et al., 1987); for comparison, the value used for adults is 1.03 g/mL (Lescot et al., 2005). The labeling efficiency (α) was set at 0.95 for PASL and 0.85 for pCASL (Gevers et al., 2011). The temporal bolus width (τ), used for the quantification of cerebral blood flow by PASL, varies from subject to subject. In this study, we arbitrarily assumed it to be 1.2 s to test the hypothesis that a single value of (τ) could be used for this application for all the newborns and all the time-points to get "pseudo-quantitative" values that match the pCASL values. In addition, each sequence had a different number of label/control pairs, which could affect the SNR; however, the total scan time was comparable, allowing a fair comparison between both techniques.

Despite the approximation of these different parameters, correlation of cerebral blood flow measurements between the two techniques was strong. However, pCASL images presented a significantly superior image quality score compared to PASL images. pCASL images provided thus better identification of the smaller regions of interest for further measurements. pCASL should thus be chosen over PASL in newborns for more detailed assessment of brain perfusion abnormalities. This is mainly due to the fact that temporal duration of the labeled bolus is longer in pCASL than PASL and that the labeling slab is more geometrically defined for pCASL than PASL (Alsop et al., 2014).

Variations in cerebral blood flow between patients were related mostly to the day of life at the time of the MRI scan and the presence or not of brain injury. Brain perfusion and variations in cerebral blood flow values between the newborns were the highest on days 2–3 of life and in the asphyxiated newborns developing brain HI injury. This is in accordance with previously described results obtained with gadolinium-enhanced perfusion MR imaging (Wintermark et al., 2008) or arterial spin labeling (Massaro et al., 2013; Wintermark et al., 2011). Hyperperfusion is one of the mechanisms leading to injury in these newborns despite hypothermia treatment, and hyperperfusion is usually maximal on days 2–3 of life in these patients (Massaro et al., 2013; Wintermark et al., 2011). Cerebral blood flow mean values were the lowest on day 1 of life, probably related to the fact that some asphyxiated newborns have decreased brain perfusion on day 1 of life related to the development of their brain hypoxic–ischemic injury (Massaro et al., 2013; Wintermark et al., 2011). In addition, the whole-slice CBF measured by pCASL on day 1 of life tended to be lower than when measured by PASL; the same trend was not noted for the other time-points. This trend may be explained by the different labeling efficiency values between the two techniques, which may become more evident when the values of brain perfusion are on the lowest side such as on day 1 of life in asphyxiated newborns. However, variations of flow velocity in the first days of life may be another factor influencing the cerebral blood flow (Ilves et al., 2004; Low et al., 1993). The asphyxiated newborns treated with hypothermia had their

heart rate and blood pressure followed carefully and kept cautiously within normal range, especially during MRIs, allowing only minimal variations of heart rate and blood pressure and thus of flow velocity between the newborns.

A main limitation of this study is the lack of gold standard of perfusion measurements in newborns compared to adults, making it difficult to assess accuracy. However, our measurements were in accordance with brain perfusion measurements obtained in newborns by other methods, i.e., positron emission tomography (Altman et al., 1988; Shi et al., 2009) and xenon 133 clearance (Pryds et al., 1990). Another limitation of the study is that the commercially available PASL sequence that was used in this study did not employ QUIPSS2/Q2TIPS type saturation to spoil residual label in the tagging slab. In the PASL sequence from another manufacturer, the addition of QUIPSS2/Q2TIPS minimizes sensitivity of PASL to the variable temporal width of the inversion bolus and allows quantification at a single delay time.

The slices were covering the entire cerebrum of the newborns. However, even though multiple slices were acquired, we decided to only analyze one slice centered at the basal ganglia to make sure to always compare the same part of the brain. Also, since the arterial transit time will generally increase for slices that are further away from the labeling slab and given the expected differences in sensitivity to transit time effects for the PASL and pCASL sequences, this approach was selected to remove additional confounding effects from our analysis. In addition, the whole-slice regions of interest (ROIs) were drawn manually; developing tools to draw these ROIs more automatically in scans of newborns would make future analysis less primitive and permit a wider generalization of those techniques to the newborns.

Motion artifacts and poor image quality remained the other important limiting factors in using ASL methods in non-sedated newborns to measure brain perfusion, as demonstrated by the fact that we had to discard 25% of the scans obtained for this study because of a very poor image quality, despite the use of an MRI-compatible vacuum cushion to wrap around the newborns and a registration to reduce motion artifacts after the scan was acquired. 6 scans were discarded because of PASL images, 7 scans because of pCASL images, and 2 because of both, suggesting that PASL and pCASL sequences had similar success rate. Eighty percent of the scans were discarded in the more active newborns, i.e., healthy newborns (6 scans) and asphyxiated newborns after the first week of life when recovering from their encephalopathy (6 scans). The remaining scans (3 scans) were removed in the first day of life in the asphyxiated newborns developing brain HI injury (i.e., when brain perfusion can be very low). Motion artifacts in the more active newborns also probably explain why the correlation between both sequences was the lowest in healthy newborns ($r = 0.42$) and the asphyxiated newborns after the first week of life ($r = 0.47$ at 1 month of life). More robust prospective and retrospective motion correction processes should thus be developed for newborns to allow the more generalized use of ASL methods in this population of patients. Adding a background suppression should help to reduce the motion sensitivity of the sequence and thus improve image quality (Alsop et al., 2014). However, optimization of background suppression is difficult in newborns as a result of the long T1 relaxation constants for white matter and gray matter, especially in the cases of sick newborns with low brain perfusion.

In conclusion, this study demonstrates that both ASL methods are feasible methods to assess brain perfusion in healthy newborns and in asphyxiated newborns. However, the pCASL sequence was found to be a better choice in this study because pCASL images had superior image quality that allowed a more detailed identification of the different brain structures.

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Conflict of interest

This manuscript has been contributed to, seen and approved by all the authors. Guillaume Gilbert is an employee of Philips Healthcare. All the authors fulfill the authorship credit requirements. No honorarium, grant or other form of payment was received for the preparation of this manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.nicl.2014.08.010.

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