Measurement of microcirculation in clinical research

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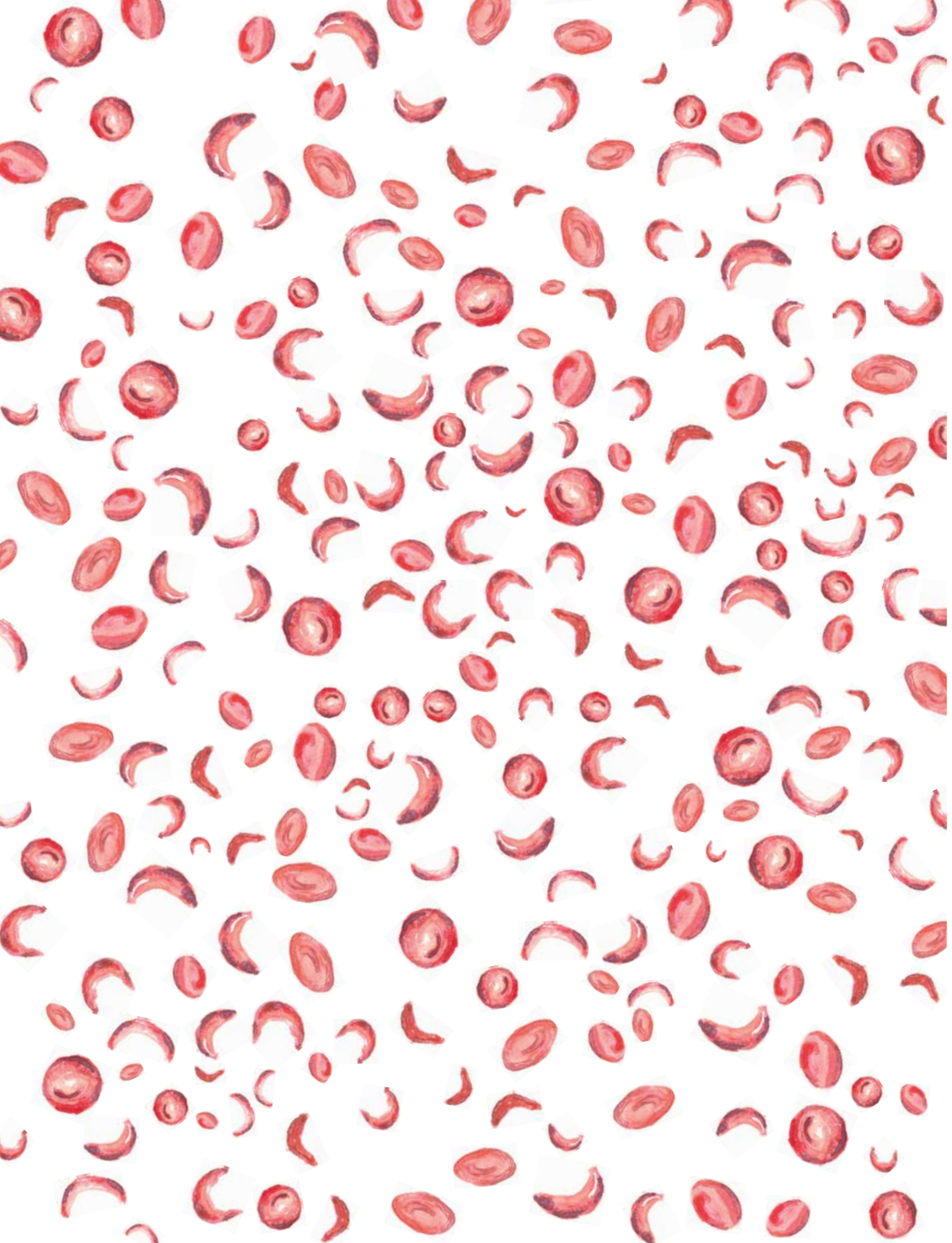

CHAPTER IV

Skin blood flow functions as potential proxy for cerebral blood flow in adults with Sickle cell disease

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Stroke is one of the major causes of morbidity and mortality in patients with Sickle cell disease (SCD). It is caused by vaso-occlusion of major arteries feeding the brain, and experience with life-saving temporal artery ultrasonography in children emphasizes the necessity of knowing the cerebral blood flow (CBF) to assess stroke risk. Less information is available around the CBF in adults with SCD and most of our knowledge is limited to expensive and time consuming magnetic resonance imaging (MRI) analysis. However, based on the pediatric experience it is suspected that it may be highly desirable to ascertain biomarkers of the CBF in adults with SCD. Therefore, in this study, we investigated CBF in adults with SCD using 2 MRI techniques: arterial spin labeling and phase contrast. Additionally we measured skin blood flow using Laser speckle contrast imaging (LSCI) and assessed it as surrogate biomarker of CBF. We enrolled SCD patients (n=17) not undergoing blood transfusion and healthy controls (n=6) in this study. Reliability of blood flow measurements was also studied through test, re-test. In conclusion, we found elevated skin and cerebral blood flow velocity in the adult SCD population compared to matched healthy controls and all measured values exhibited significant longitudinal stability across visits. Furthermore, excellent correlation was found between CBF and skin blood flow in the SCD cohort suggesting skin blood flow measurement using LSCI may be a potential proxy to MRI in routine hemodynamic evaluation in this population.

Sickle cell disease (SCD) is a genetic disorder caused by a point mutation in the β-globin gene of hemoglobin, forming a mutant form known as hemoglobin S. Under deoxygenated conditions, hemoglobin S is abnormally susceptible to polymerization, distorting red blood cells (RBCs) into 'sickled' RBCs (SS-RBCs) and changing their adhesive, rheological, and oxidative properties. SS-RBCs are capable of promoting the formation of heterocellular aggregates that alter blood flow and interrupt normal tissue perfusion. In addition, SCD is associated with aberrant vasomotor responses including impaired vasodilation and vasoconstriction.[1]

Continuous tissue perfusion is crucial for vitality of most organs. Obstructed or inadequate blood flow can lead to ischemic tissue injury, followed by reperfusion injury upon flow restoration. Ischemia and reperfusion injury are major causes of morbidity and mortality in the SCD population. Acute vascular obstruction results clinically in severe painful episodes known as vaso-occlusive crises (VOCs), which often require hospitalization and are a hallmark of SCD. The heterogeneous clinical manifestations of SCD, which in addition to VOC include chronic hemolytic anemia, stroke, and multi-organ dysfunction, likely reflect altered tissue perfusion patterns caused by abnormal SS-RBC associated rheology.[2–5]

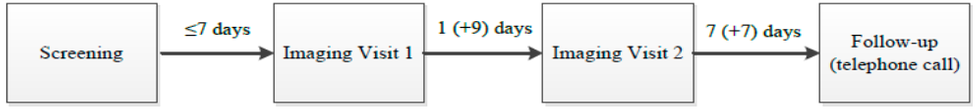
Under stable physiological conditions, most tissues can autoregulate and maintain appropriate blood flow to adequately support tissue perfusion needs. Due to chronic anemia, the cardiac output is significantly elevated, resulting in increased perfusion to certain organs (i.e., brain, kidney, and muscle).[6] Hyper-perfusion is also likely to represent compensatory reactive responses to the slower blood flow rates in occluded microvessels, with increased blood volume diverted around occlusions necessitating increased blood flow through 'detour' vessels. Further effects on blood flow in SCD result from impaired vasomotor responses: flow velocity can increase as a function of abnormally narrowed vessels (as from abnormal vasoconstriction) or if increased flow volume exceeds autoregulatory relaxation (as from impaired vasodilation).

SCD impacts the cerebral hemodynamics and therefore, characterized by high cerebral blood flow (CBF). The brain is particularly susceptible to end organ damage (stroke, silent infarcts) in SCD. Due to the reduction in hematocrit and increase in blood viscosity, adaptive vasodilation tries to maintain the cerebral oxygen supply by increasing the CBF and correspondingly the CBV in SCD.[7] When all the available vessels and collaterals reach their maximal compensatory/vasodilation state, the risk of cerebral stroke is significantly elevated. Most of our knowledge to identify the risk of stroke in SCD patients comes from pediatric population. The prevalence of stroke in adults with SCD is higher compared to children.[8] However, no accepted screening measures are present for identifying adults with SCD at high risk for strokes.[9] Therefore, a robust and accurate assessment of CBF in adults with SCD is highly needed.

Magnetic resonance imaging (MRI) is the optimal imaging modality to assess the cerebral hemodynamics due to high soft-tissue contrast, high resolution, no use of ionizing radiations and less operator dependence. MRI techniques namely, arterial spin labeling (ASL) and phase-contrast MRI (PC-MRI), are commonly used to measure CBF. These techniques have been applied previously in adults and children with SCD and showed an elevated level of CBF in the SCD population compared to controls.[9–12]

In line with the increased CBF, the microvascular blood flow in the skin was also found higher in the SCD patients compared to healthy group.[13–15] Vasodilation of micro-vessels as a result of sickling of the RBCs or impaired endothelial layer, might

FIGURE 1 Schematic study design followed in the work is shown. Following the screening, eligible subjects underwent two imagingvisits which over the course of 10 days. Individuals were followed-up on phone within a week window.



be the reason of elevated blood flow in the skin in SCD. While MRI is suitable for estimating CBF, Laser speckle contrast imaging (LSCI) offers the possibility to measure the entire physiologic range of blood flow velocities in real time at microvascular level to a depth of approximately 300 μm. These measurements reflect blood flow in capillaries, arterioles, venoles and dermal vascular plexuses.[16] Recent LSCI studies have successfully demonstrated the elevated baseline level of blood flow in the skin of adults with SCD compared to healthy individuals.[13–15]

Despite of being an optimal imaging technique, high scan cost of MRI makes it uneconomical for the screening of high risk SCD patients for stroke. Therefore, we explore the possibility of using LSCI as an economical alternative and to examine the skin blood flow as a peripheral surrogate biomarker of the CBF. In this study, we measure blood flow in the skin and brain of adults with SCD and healthy subjects. For each subject and each technique, the test-retest variability in blood flow was also assessed in the brain and skin.

MATERIALS AND METHODS

Study Design

This was a single-center, non-randomized study of subjects with SCD and healthy subjects. A written informed consent was obtained from each subject prior to any measurement was performed Following a screening visit, patients underwent two imaging visits over the course of 10 days, to establish test-retest reliability. Imaging visit 2 was followed by a telephone follow-up over a week period (*Figure 1*). No drug treatment was administered as part of this study.

Study Population

Clinical hematology and blood chemistry laboratory samples were collected at Screening in SCD patients (n=17) and healthy controls (n=7). Diagnosis of SCD (homozygous for HbS or heterozygous for HbS and β0 thalassemia [HbS-β0]) was confirmed by hemoglobin analysis. SCD patients who had a history of clinical strokes or of other major illness such as diabetes mellitus, renal dysfunction were excluded. Also SCD patients who had an acute pain crisis requiring hospitalization, within ≤4 weeks prior to the first imaging or recent (≤3 months) treatment with hydroxyurea, were excluded.

Imaging

Magnetic resonance imaging

All MRI scans were performed at 3.0 Tesla (T) Siemens Verio (Erlangen, Germany) and a 32-channel head coil was used for acquiring brain and neck images. CBF was estimated using ASL and PC-MRI. No contrast was administered in the scanning.

Arterial spin labeling

Images are acquired with and without labeling and their difference provides the perfusion maps. A pseudo-continuous arterial spin labeling (PCASL) sequence was used to analyze the direct perfusion. A reference series along with thirty measurements from both control and tagged ASL images were acquired separately in order to increase the signal-to-noise (SNR) and make the averaging more efficient. The imaging parameters used are as follows: repetition time (TR) = 3500 ms, echo time (TE) = 22.76 ms, flip angle (FA) =180°, turbo spin-echo (TSE) factor = 17, echo-planar imaging (EPI) factor = 31, pixel bandwidth (BW) = 2003 Hz/pixel, in-plane resolution = 3.5 x 3.5 mm^2, slice thickness (TH) = 3.5 mm and 32 slices per measurement were acquired. Total scan duration was about 4 mins. The CBF maps of individual subjects were generated using the technique from Wang et al., [17] in the units of ml/100g/min. With the purpose of extracting the average CBF information, a region-of-interest (ROI) encompassing the whole brain was manually drawn on each slice of the CBF maps. The mean CBF map for individual subjects was then calculated by taking the average of all the voxels within the ROI and all the slices. Finally, the mean CBF and associated standard error across all the subjects was estimated.

Phase-contrast MRI

PC-MRI estimates the blood flow using the phase shift property of the moving water molecules in the blood to encode the blood flow information. PC-MRI sequence was run with the slice positioned perpendicular to the major vessels at two neck levels: C2-C3 and C5-C6. The data were collected with the following parameters: TE=5.17 ms, TR=66.85 ms, FA = 20°, FOV= 256×256, Matrix size= 448×448, TH=

2.5 mm, velocity encoding (VENC) = 100 cm/sec. All the data was reviewed for quality and any cases that had artifacts or inadequate SNR were removed from the analysis.

Using the tissue similarity mapping algorithm[18] (SPIN, Magnetic Resonance Innovations, Inc, Detroit, MI) the target vessels were demarcated. Two experienced image analysts reviewed the vessel segmentation and adjusted manually if necessary. SPIN software was used for quantifying blood flow rate (ml/sec) from differences in phase-contrast over time through major arteries and veins, including internal carotid arteries (ICA) and vertebral arteries (VA) for in-flow and the internal jugular veins (IJV) for outflow. A robust automatic unwrapping algorithm was implemented where phase aliasing in the vessels occurred due to rapid arterial flow in SCD patients.[18] Non-flow regions were drawn on static tissue to compensate for any phase shifts. Individual vessel flow, as well as bilateral total flow, venous total flow (sum of IJVs) and arterial total flow was calculated (CCA+VA for C5/C6 neck level, and ICA+VA for C2/C3 neck level). C2/C3 arterial total (CBF) was used for PC-MR perfusion calculations.

Laser speckle contrast imaging

Following MRI participants underwent LSCI measurements in a temperature- and light-controlled room. LSCI measurements were performed as previously described,[13] using Pericam PSI (Perimed, PSI-NR). Briefly an area of 3×10 cm² of the ventral forearm was chosen as measurement site. Following 5 mins of baseline perfusion recording, brachial artery blood flow was occluded for 3-5 minutes after occlusion, the reperfusion peak and the return of baseline was recorded for another 5 minutes. LSCI endpoints included basal flow (arbitrary units (AU)), peak flow after occlusion (AU), time to return to basal flow (seconds) (see *Figure 4*) Obtained data were analyzed using PIMSOFT software (Perimed, Järfälla, Sweden).

Statistical analysis

The mean and standard deviation of the CBF and the skin blood flow measurements were calculated. The intraclass correlation coefficient (ICC) were estimated for assessing test-retest reliability. A linear regression analysis was used to investigate the correlation between CBF and skin blood flow measured using MRI and LSCI, respectively.

TABLE 1 Demographic information and basic laboratory report of the healthy subjects and SCD patients are presented.

	Healthy Subjects	SCD Patients
DEMOGRAPHY		
N	6	17
Age (years)	27 (± 5)	34.4 (± 8.7)
Height (cm)	175.1 (± 8.5)	168.8 (± 10.2)
Weight (Kg)	78.9 (± 26.8)	74.2 (± 19.3)
Male (%)	67	47
VITAL SIGNS		
Systolic blood pressure (mmHg)	115.2 (± 10.5)	114.9 (± 12.7)
Diastolic blood pressure (mmHg)	71.7 (± 9.6)	69.4 (± 8)
Pulse (beats/min)	75.3 (± 7.1)	83.8 (± 17.3)

RESULTS

Patient information

A total of 17 adults with SCD and 6 healthy subjects completed the study. Demographics and ethnicity of healthy subjects and SCD patients were similar. Demographics, vital signs and lab measurements are presented in *Table 1*.

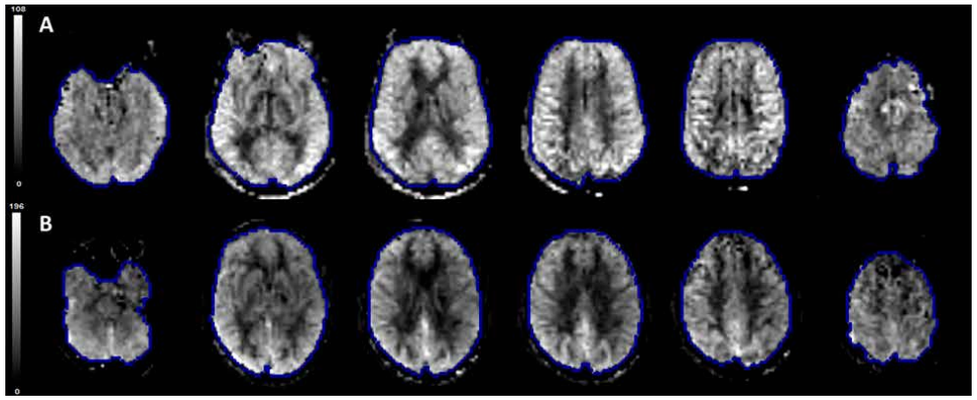
CBF

CBF maps from ASL and PC-MRI images are shown in *Figure 2* and *Figure 3*, respectively. The average CBF measured from ASL was 77.4 21.9) ml/100g/min in SCD population and 48.0 5.3) ml/100g/min in healthy subjects (P = 0.0042). The mean CBF measured using PC-MRI was 68.4 19.9) ml/100g/min (in the SCD population and 43.9 4.3) ml/100g/min in controls (P = 0.0077). The mean CBF was significantly higher in the aduls with SCD compared to the healthy individuals (ASL: *Figure 5*; PC-MRI: *Figure 6*). The test-retest reliability of ASL and PC-MRI measurements of the average CBF showed moderate to high agreement in subjects with SCD (ASL: ICC = 0.66; PC-MRI: ICC = 0.72) and in healthy subjects (ASL: ICC = 0.84; PC-MRI: ICC = 0.75).

Microvascular skin blood flow

Baseline microvascular blood flow measured by LSCI was significant higher in patients with SCD compared to healthy controls (40.2 7.5) vs. 24.2 4.6) (AU)) (P = 0.0001) (*Figure 7*). However, no differences were observed in peak blood flow, time to peak and in time to return to baseline (see *Table 2*).

FIGURE 2 ASL based perfusion mean CBF maps are shown in A) a 28-year-old healthy control with an average CBF of 57.15 ml/100gin and B) a 44-year-old SCD patient with an average CBF of 116.36 ml/100g/min. The blue line represents the ROIs on six slices from different brain regions.



Correlation between cerebral and skin blood flow

A strong correlation was found between the CBF quantified using ASL or PC-MRI and the baseline blood flow in the skin as measured using LSCI in the population including healthy volunteers and subjects with SCD (ASL vs. LSCI: r = 0.70; PC-MRI vs. LSCI: r = 0.75) (*Figure 8* and *Figure 9*).

DISCUSSION

In this study, we measured CBF and skin blood flow in SCD patients and healthy subjects. In agreement with previous studies, MRI and LSCI measurements of brain and skin blood flow, were higher in SCD patients compared to healthy controls.[9–15] Furthermore CBF measurements showed a high correlation with LSCI measurements.

The increase in blood flow is attributed to the vasodilation of the major vessels and microvascular beds to compensate the oxygen demand of the brain and skin. While high CBF is a well-described phenomenon in children with SCD, less data is available for adults. Elevated CBF in adults with SCD may be a key consideration for stroke risk, as seen in children.[19] Very few studies have been performed with test-retest assessments with ASL and PC-MRI in SCD. Our test-retest reliability showed moderate to high agreement. In addition, ASL and PC-MRI showed no significant difference in the CBF measurements, indicating the comparability of these two MRI techniques for measuring CBF.

Differences in baseline skin blood flow between SCD patients and healthy controls, were in agreement with recent literature.[13] The baseline blood flow in the skin as measured using LSCI was found to be a strong predictor of the average CBF in both SCD patients and controls. Furthermore this correlation was highly reproducible. We hypothesize that the change in rheological properties of RBCs and increasing viscosity might affect the hemodynamic properties of the CNS and peripheral system proportionally, which is captured in the

TABLE 2 LSCI measurements-, for healthy individuals and SCD adult patients with means and SD.

	Healthy Subject	SCD Patients	
N	6	17	
Baseline flow (AU)	24.2 (±4.58)	40.2 (±7.48)	P = 0.0001
Peak flow (AU)	76.7 (±13.06)	80.5 (±14.15)	P = 0.5709
Time to peak (sec)	5.8 (±3.7)	13.1 (±9.9)	P = 0.0962
Time to return to baseline (sec)	97.7 (±44.7)	132.9 (±33.4)	P = 0.054

strong and reproducible correlation between CBF and skin blood flow in SCD patients. Furthermore a high blood flow is also observed in the central retinal vessels which share the same vascular anatomical origin as the CNS vasculature.[13] LSCI might be an inexpensive way to indirectly assess critical hemodynamic changes in the brain. With additional independent validation LSCI may be able to spare SCD subjects from undergoing costly and time intensive MRI scans. Finally, based on these and other available data, MRI and LSCI measurements could be implemented as exploratory endpoints in future sickle cell intervention trials.

There are some limitations of this study. The ASL images are known to be affected by susceptibility and motion artifacts and generally have low SNR, low spatial and temporal resolution.[20] In order to reduce the effect of low SNR, a voxel-wise averaging of 30 individual measurements was implemented using tagged and control ASL data. The CBF measurements using PC-MRI contains error mostly due to partial voluming and noise. Error in the vessel demarcation is controlled largely by the robustness of the segmentation algorithm used. Therefore, manual segmentation of the vessel has been shown to be reliable, particularly where vessels are having faster flow and larger diameters.[21] In addition, VENC value also affects the flow measurements. In this study, we used a larger VENC value of 100 cm/sec, which may have lower overall SNR compared to a lower VENC and might be introducing higher errors in slow venous flow. This was a trade-off in order to mitigate phase aliasing effects and preserve the quality of the rapid arterial flow which is present in SCD.

LSCI is highly sensitive to motion, which was overcome by averaging the measurements over a longer period of time.[22] Although, we found a strong correlation between the CSF and skin blood flow, our sample size is small in this study. Further validation of these findings with larger groups is therefore needed.

In conclusion, this study showed that CNS macrovascular blood flow measured by ASL and PC-MRI was elevated in SCD patients compared to controls. Additionally, microvascular skin blood flow, measured using LSCI, was also elevated and could potentially be used as surrogate biomarker for blood flow measurements in the brain. This could potentially provide an affordable solution to screen critical CNS hemodynamics and possible stroke risk in adult sickle cell patients and will open new avenues to find more sensitive biomarkers in this disease.

FIGURE 3 **PC-MRI based flow estimation is shown. Major vessels are demarcated at C2/C3 neck level in magnitude (left) and complex-divided phase image (right).**
R: right, L: left, IJV: internal jugular vein, ICA: internal carotid artery, VA: vertebral artery.

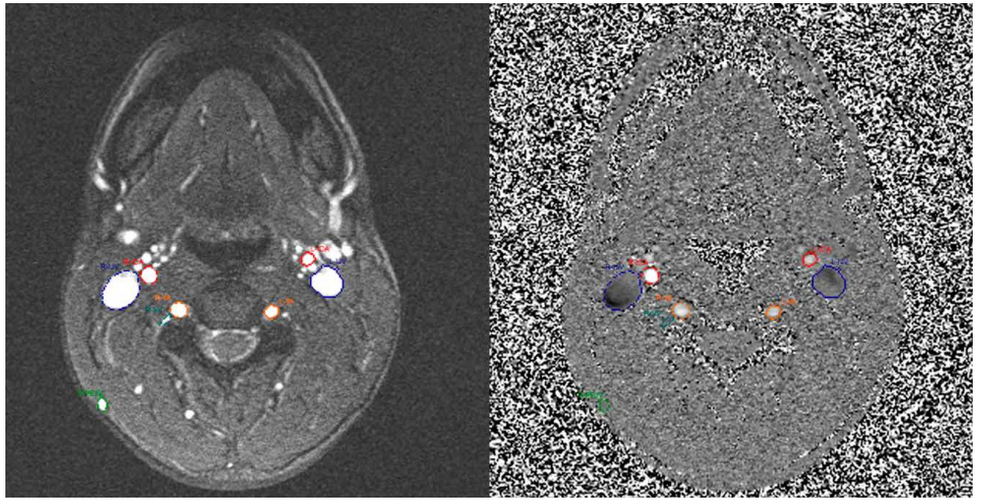


FIGURE 4 **Graphical rendering of LSCI blood flux measurements and the different parameters included in this research. The flux measurements were measured in AU and the time measurements in seconds.**

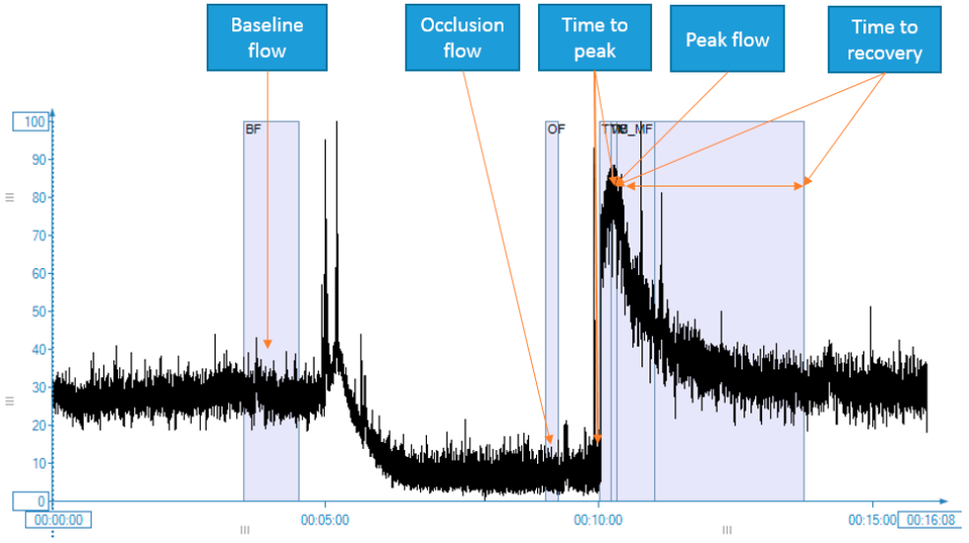


FIGURE 5 **Cerebral blood flow measured using ASL-MRI was found to be significantly higher in the sickle cell patients compared to the healthy group. The test-retest showed high reliability.**

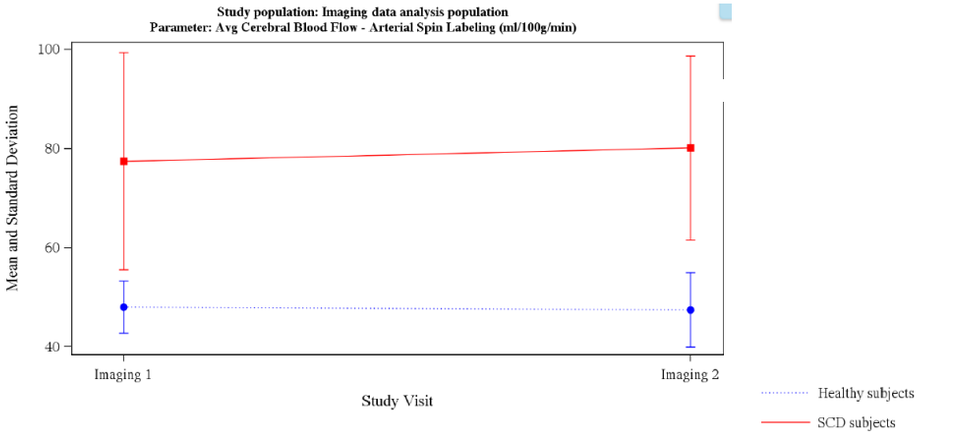


FIGURE 6 Cerebral blood flow measured using PC-MRI was found to be significantly higher in the sickle cell patients compared to the healthy group. The test-retest was also reliable in both the groups.

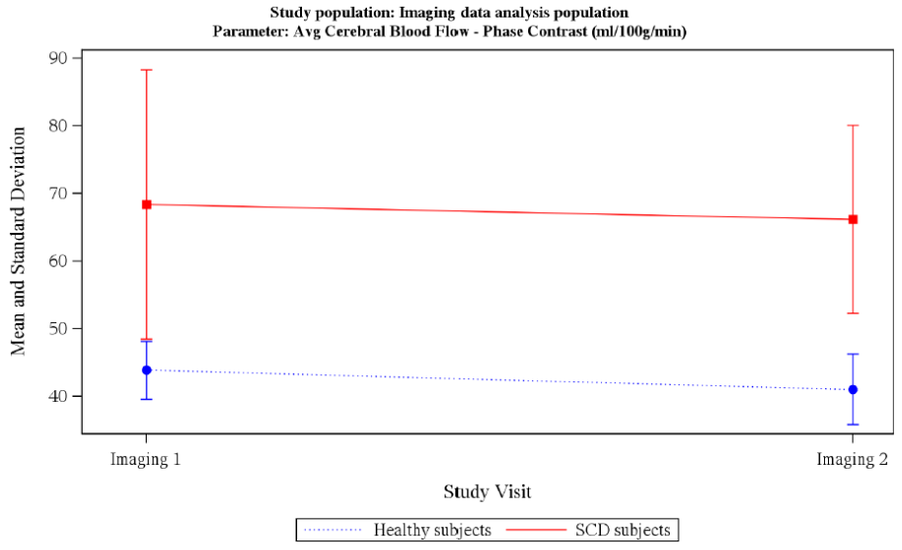


FIGURE 7 The baseline skin blood flow measured using LSCI was found to be significantly higher in the sickle cell patients compared to the healthy group. Higher test retest reliability was found in both the groups.

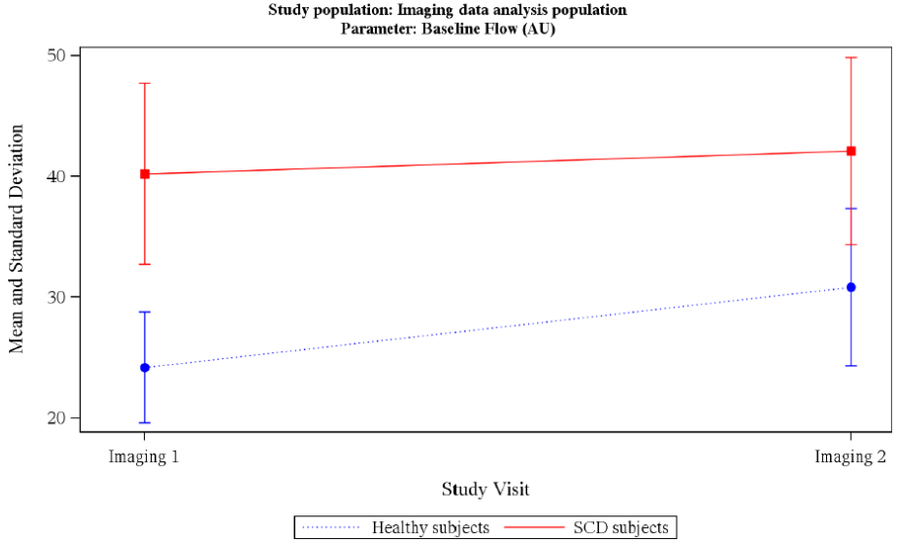


FIGURE 8 Correlation between average CBF using ASL-MRI and baseline flow using LSCI was shown in healthy and sickle cell patients for in imaging visits. A significant correlation was observed in the measurements of two imaging modalities and it remained consistent in the test-retest.

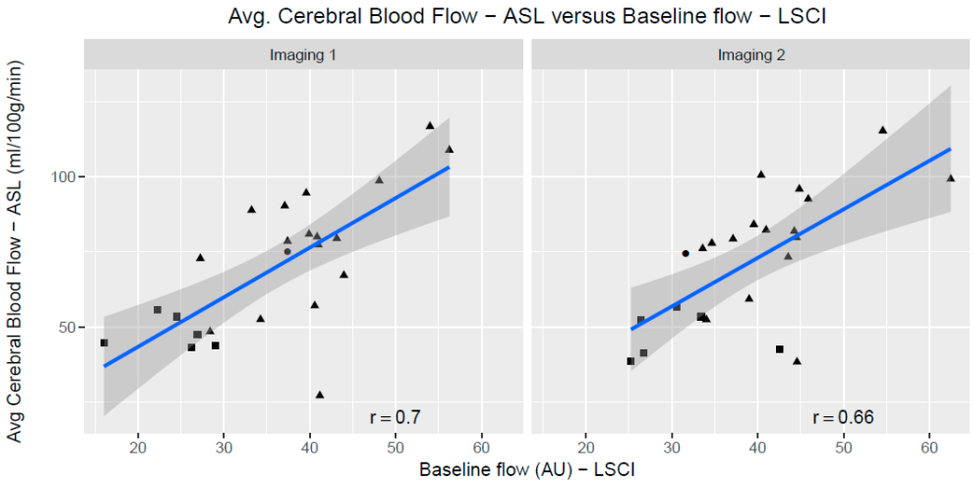
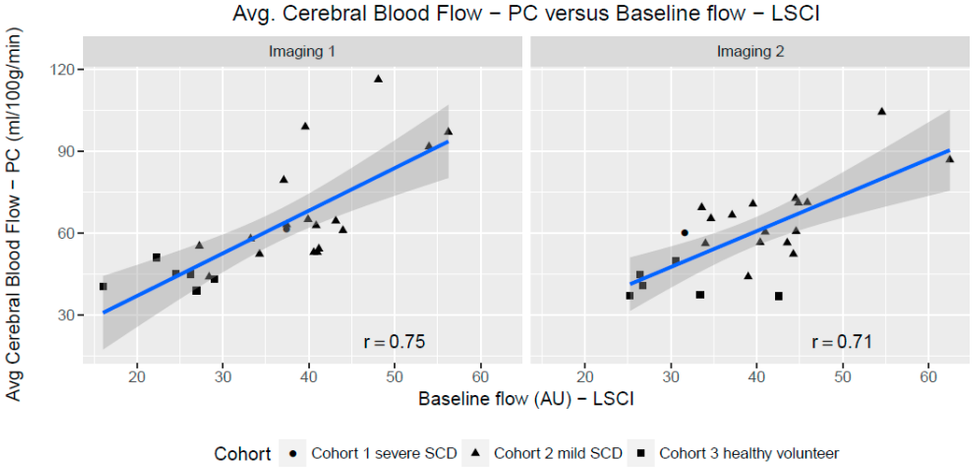


FIGURE 9 Correlation between average CBF using PC-MRI and baseline flow using LSCI was shown in healthy and sickle cell patients for in imaging visits. A significant correlation was observed in the measurements of two imaging modalities which was consistent in both visits.



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