Cerebral Blood Flow Assessment in Children with Sickle Cell Disease

by

Amir Mahmood Behpour

A thesis submitted in conformity with the requirements for the degree of Master of Science

Institute of Medical Science
University of Toronto

© Copyright by Amir Mahmood Behpour 2012
Cerebral Blood Flow Assessment in Children with Sickle Cell Disease

Amir Mahmood Behpour
Master of Science
Institute of Medical Science
University of Toronto
2012

Abstract

This thesis investigated the role of CBF assessment in the management of stroke in children with sickle cell disease (SCD). It is divided into two parts. In the first part, a systematic review of CBF assessment using different imaging modalities in SCD children was designed. The prevalence of CBF abnormalities was found to be equal to or higher than those of structural MRI and transcranial Doppler (TCD) in SCD children who have not experienced stroke. Studies reviewed suggested CBF assessment in SCD could aid in addressing brain abnormalities at the tissue level. In the second part, the arterial spin labeling (ASL) technique was used to depict CBF abnormalities in SCD children. ASL demonstrated perfusion abnormalities that seem to remain invisible in TCD measurements; CBF interhemispheric asymmetries were associated with clinically silent infarctions with no corresponding flow velocity interhemispheric asymmetries assessed with TCD.
Acknowledgments

I would like to thank all the people who have helped and inspired me during this MSc project. I especially want to thank my advisor, Dr. Andrea Kassner, for the guidance she provided in regard to my research and studies at the University of Toronto. Even though I was relatively adventurous at the beginning of my research and I wanted to try every idea I had, she patiently gave me the chance to explore my ideas and to learn how to distinguish between what is practical and what is an ideal.

I would like to thank my thesis committee members, Drs. David J Mikulis and Prakash S. Shah, for their kind support and helpful discussions throughout my MSc study. I also want to express my gratitude to Mrs. Elizabeth Uleryk for coordinating the database search, and to Drs. Suzan Williams and Manohar Shroff for providing helpful comments on the systematic review included in this manuscript. Many thanks also to my colleagues at the Hospital for Sick Children, Mr. Jackie Leung and Dr. Jing Yu, for their enormous inspiration and assistance.

The work in this thesis would not have been possible without the help of all the MRI and ultrasound technicians who prepared and scanned all the subjects; special thanks to Annette Weekes-Holder, Garry Detzler, Ruth Weiss, Tammy Rayner and Arun Mohanta.

On a personal note, my deepest gratitude and appreciation goes to my wife for her love and support throughout my life; this degree would simply not have been impossible without her.

This work was supported by a grant funded by the Canadian Institute of Health Research (CIHR).
Table of contents

Abstract ........................................................................................................................................ ii
Acknowledgments ......................................................................................................................... iii
Table of contents ........................................................................................................................... iv
List of Abbreviations ....................................................................................................................... viii
List of Tables .................................................................................................................................... x
List of Figures ............................................................................................................................... xi
List of Publications and Abstracts ................................................................................................... xvi

Chapter 1 Overview ......................................................................................................................... 1
1.1. Motivation ............................................................................................................................... 2
1.2. Thesis outline ........................................................................................................................... 4

Chapter 2 Background review of literature ....................................................................................... 5
2.1. Sickle cell disease (SCD) ......................................................................................................... 6
  2.1.1 Pathophysiology .................................................................................................................. 6
  2.1.2 Epidemiology ...................................................................................................................... 9
  2.1.3 Treatment ............................................................................................................................ 10
  2.1.4 Cerebrovascular disease (CVD) in SCD ........................................................................... 11
2.2. Clinical imaging techniques of CVD in SCD ........................................................................... 14
  2.2.1 Transcranial Doppler Ultrasonography (TCD) ................................................................. 15
  2.2.2 Structural Magnetic Resonance Imaging (MRI) ............................................................... 17
2.3. Cerebral blood flow (CBF) imaging techniques in SCD ......................................................... 17
2.3.1 Xenon133 Computed Tomography (Xe-CT) ..............................................................18
2.3.2 Positron Emission Tomography (PET) .................................................................19
2.3.3 Single Photon Emission Computed Tomography (SPECT) .................................20
2.3.4 Perfusion MRI ...........................................................................................................20

2.4. CBF measurement ......................................................................................................23

Chapter 3 Hypothesis and aims ......................................................................................26

3.1. Hypotheses .................................................................................................................27
3.2. Aims .............................................................................................................................27

Chapter 4 Cerebral blood flow abnormalities in children with sickle cell disease: a systematic review ..................................................................................................................29

4.1. Introduction ..................................................................................................................30
4.2. Materials and methods ...............................................................................................32
  4.2.1 Criteria used for including studies in this review ..................................................32
  4.2.2 Search strategy .......................................................................................................32
  4.2.3 Study Selection ......................................................................................................33
  4.2.4 Risk of bias assessment .........................................................................................33
  4.2.5 Outcomes ..............................................................................................................34
  4.2.6 Statistical analysis .................................................................................................34

4.3. Results ..........................................................................................................................35
  4.3.1 Search strategy and study selection .................................................................35
  4.3.2 Study analysis .......................................................................................................38
  4.3.3 Outcomes ..............................................................................................................38

4.4. Discussion ....................................................................................................................45
Chapter 5 Absolute values and interhemispheric ratios of CBF assessed by ASL and TCD in children with SCD .................................................................51

5.1. Introduction ..................................................................................52

5.2. Materials and methods ................................................................54
  5.2.1 Study population .......................................................................54
  5.2.2 Magnetic resonance imaging .....................................................54
  5.2.3 Transcranial Doppler ultrasonography (TCD) ............................55
  5.2.4 Data analysis ...........................................................................55
  5.2.5 Statistical Analyses ...................................................................58

5.3. Results ..........................................................................................59
  5.3.1 Patients’ characteristics .............................................................59
  5.3.2 Structural MRI and MRA ..........................................................60
  5.3.3 Cerebral blood flow measured by ASL .....................................61
  5.3.4 Flow velocity measured by TCD .................................................64
  5.3.5 Cerebral blood flow measured by TCD .....................................65
  5.3.6 Correlation of absolute values between ASL, TAPV and TCD-CBF ..67
  5.3.7 Correlation of interhemispheric ratios between ASL, TAPV and TCD-CBF ..69

5.4. Discussion ....................................................................................74

Chapter 6 General discussion .................................................................79

Chapter 7 Conclusions .........................................................................88

Chapter 8 Future Directions ..................................................................91

Chapter 9 References ...........................................................................94
Appendix A  Bias list.........................................................................................................................117

Appendix B  ASL and TCD values obtained in healthy control group: definition of abnormal values and asymmetries ..................................................................................................................119
List of Abbreviations

The abbreviations used in this thesis are as follow:

99mTC 99mTechnetium
ASL Arterial spin labeling
CASL Continuous arterial spin labeling
CBF Cerebral blood flow
CBV Cerebral blood volume
CVR Cerebrovascular reactivity
CNS Central nervous system
CT Computed tomography
CVA Cerebrovascular accident
CVD Cerebrovascular disease
CSF Cerebrospinal fluid
DSC Dynamic susceptibility contrast
ECD Ethyl cysteinate dimer
ECMO Extra corporeal membrane oxygenation
FDG Fluorodeoxyglucose
FLAIR Fluid attenuated inversion recovery
GM Gray matter
HbS Sickle hemoglobin
HbSS Homozygous sickle hemoglobin
HbSC Sickle hemoglobin C disease
HbS/β+ Sickle beta plus thalassemia
HbS/β0 Sickle beta zero thalassemia
Hct Hematocrit
HMPAO Hexamethyl propylenamine oxime
HU Hydroxyurea
ICA Internal carotid artery
MCA Middle cerebral artery
MRI          Magnetic resonance imaging
MRA          Magnetic resonance angiography
MTT          Mean transit time
NO           Nitric oxide
NHLBI        National Heart, Lung and Blood Institute
OEF          Oxygen extraction fraction
PASL         Pulsed arterial spin labeling
PCASL        Pseudo-continuous arterial spin labeling
PET          Positron emission tomography
PLD          Post labeling delay
QUADAS       Quality assessment of diagnostic accuracy studies
RBC          Red blood cell
RF           Radiofrequency
RIND         Reversible ischemic neurological deficit
SCA          Sickle cell anemia
SCD          Sickle cell disease
SD           Standard deviation
SNR          Signal to noise ratio
SPECT        Single photon emission tomography
STOP         Stroke Prevention Trial in Sickle Cell Anemia
TAMV         Time averaged mean velocity
TAPV         Time averaged peak velocity
TCD          Transcranial Doppler ultrasonography
TCD-CBF      Cerebral blood flow derived from TCD
TI           Inversion time
TIA          Transient ischemic attack
TOF          Time of flight
Vav          Cross sectional average blood velocity
WM           White matter
Xe-CT        Xenon enhanced computed tomography
List of Tables

Table 1. Baseline characteristics of included studies .......................................................... 37
Table 2. Risk of bias assessment .......................................................................................... 41
Table 3. Results .................................................................................................................. 43
Table 4. Results (continue) .................................................................................................. 44
Table 5. Patients' characteristics ....................................................................................... 59
Table 6. The mean values of CBF obtained with ASL in GM and WM of MCA territory calculated for the patient group without and with Hct correction ................................. 62
Table 7. Results of correlation between ASL, TAPV and TCD-CBF values .................... 68
Table 8. Association between silent infarctions and interhemispheric asymmetries obtained with ASL, TAPV and TCD-CBF ........................................................................... 74
Table 9. Appendix A, bias list ............................................................................................ 117
Table 10. Appendix B, ASL, TAPV and TCD-CBF values in healthy controls ................ 119
Table 11. Appendix B, definition of abnormal absolute values ....................................... 120
Table 12. Appendix B. definition of asymmetries ............................................................... 121
List of Figures

Figure 1. Pathophysiology of Sickleing.................................................................7

Figure 2. Silent infarctions. A 11 years old SCA male patient with no previous history of stroke. (A) Fluid attenuated inversion recovery (FLAIR) shows bilateral multiple hyperintense lesions in the white matter. (B) MRA shows no major vessel abnormalities. ........................................................................................................12

Figure 3. Transcranial Doppler in a patient with SCD; severe stenosis of left internal carotid artery and left middle cerebral artery consistent with correspondingly very low flow velocity, normal vasculature and normal flow velocity on right side ....16

Figure 4. A schematic description of PASL experiment, perfusion weighted image (ΔM) is obtained by subtraction of the labelled images from the control images (Petersen et al. 2006). ........................................................................................................22

Figure 5. Flow diagram for study selection .........................................................36

Figure 6. Risk of bias assessment in each category ............................................42

Figure 7. Bar graph demonstrates sex ratio in 8 SCD patients without silent infarction (2 males and 6 females) and 7 SCD patients with silent infarction, including inside and outside the MCA territory (one male and 6 females). No significant difference (p value = 0.6) was found in sex ratio between two groups. SI – = patients without silent infarction, SI + = patients with silent infarction, including inside and outside the MCA territory. ........................................................................................................60

Figure 8. Bar graph demonstrates no significant difference (p value = 0.1) in mean age between SCD patients without silent infarction and SCD patients with silent infarction, including inside and outside the MCA territory (13.88 years old and
13.86 years old, respectively). SI − = patients without silent infarction, SI + = patients with silent infarction, including inside and outside the MCA territory. ..........................................................61

**Figure 9.** Correlation of CBF values obtained in GM of MCA territories with ASL between left and right hemispheres. CBF values in GM of MCA territory were significantly correlated between left and right hemispheres in 11 patients with no silent infarction inside the MCA territory (r = 0.78, p = 0.003) and in 8 healthy controls (r = 0.79, p = 0.007). The correlation value in 4 patients with silent infarction inside the MCA territory was 0.68 but was not statistically significant (p = 0.3). MCA = middle cerebral artery, −SI = patients with no silent infarction inside the MCA territory, +SI = patients with silent infarction inside the MCA territory. ..........................................................63

**Figure 10.** Correlation of CBF values obtained in WM of MCA territories with ASL between left and right hemispheres. CBF values in WM of MCA territories were significantly correlated between left and right hemispheres in 11 patients with no silent infarction inside the MCA territory (r = 0.87, p < 0.001) and in 8 healthy controls (r = 0.93, p < 0.001). The correlation value in 4 patients with silent infarction inside the MCA territory was 0.89 but was not statistically significant (p = 0.1). MCA = middle cerebral artery, −SI = patients with no silent infarction inside the MCA territory, +SI = patients with silent infarction inside the MCA territory. ..........................................................64

**Figure 11.** Correlation of TAPV values between left and right MCA. TAPV values obtained from left and right MCA were significantly correlated in 10 patients with
no silent infarction inside the MCA territory \((r = 0.84, p = 0.002)\) and in 7 healthy
controls \((r = 0.89, p = 0.002)\). The correlation value in 4 patients with silent
infarction inside the MCA territory was 0.87 but was not statistically significant
\((p = 0.13)\). MCA = middle cerebral artery. −SI = patients with no silent infarction
inside the MCA territory, +SI = patients with silent infarction inside the MCA
territory.

**Figure 12.** Correlation of TCD-CBF values between left and right MCA territory. TCD-
CBF values obtained in the MCA territory were highly correlated between left
and right hemispheres in 10 patients with no silent infarction inside the MCA
territory \((r = 0.98, p < 0.001)\) and in 5 healthy controls \((r = 0.95, p = 0.01)\). The
correlation value in 4 patients with silent infarction inside the MCA territory was
0.88 but was not statistically significant \((p = 0.12)\). MCA = middle cerebral artery,
−SI patients = patients with no silent infarction inside the MCA territory, +SI
patients = patients with silent infarction inside the MCA territory.

**Figure 13.** Correlation of absolute values between ASL and TAPV. No significant
correlation was found between ASL and TAPV values in MCA territories of 10
patients with no silent infarction inside the MCA territory \((r = 0.01, p = 0.96)\), in
MCA territories of 4 patients with silent infarction inside the MCA territory \((r =
0.08, p = 0.84)\) and in MCA territories of 5 healthy controls \((r = 0.2, p =0.5)\). −SI
= patients with no silent infarction inside the MCA territory, +SI = patients with
silent infarction inside the MCA territory.

**Figure 14.** Correlation of absolute values between ASL and TCD-CBF. A significant
correlation was found between ASL and TAPV values in MCA territories of 4
patients with silent infarction inside the MCA territory ($r = 0.92, p = 0.001$) and in MCA territories of 5 healthy controls ($r = 0.72, p = 0.02$). The correlation value in MCA territories of 10 patients with no silent infarction inside the MCA territory was $0.13$ ($p = 0.59$). $-SI$ = patients with no silent infarction inside the MCA territory, $+SI$ = patients with silent infarction inside the MCA territory.

**Figure 15.** Correlation between interhemispheric ratios of ASL and TAPV. No significant correlation was found between interhemispheric ratios of ASL and TAPV obtained in MCA territories of 10 patients with no silent infarction inside the MCA territory ($r = -0.43, p = 0.2$), in MCA territories of 4 patients with silent infarction inside the MCA territory ($r = 0.53, p = 0.47$) and in MCA territories of 5 healthy controls ($r = 0.1, p = 0.88$). $-SI$ = patients with no silent infarction inside the MCA territory, $+SI$ = patients with silent infarction inside the MCA territory.

**Figure 16.** Correlation between interhemispheric ratios of ASL and TCD-CBF. A significant correlation was found between interhemispheric ratios of ASL and TCD-CBF obtained in MCA territories of 10 patients with no silent infarction inside the MCA territory ($r = 0.75, p = 0.01$) and in MCA territories of 5 healthy controls ($r = 0.94, p = 0.014$). The correlation value in MCA territories of 4 patients with silent infarction inside the MCA territory was 0.81 but was not statistically significant ($p = 0.2$). $-SI$ = patients with no silent infarction inside the MCA territory, $+SI$ = patients with silent infarction inside the MCA territory.

**Figure 17.** Blond-Altman plot shows degree of agreement between interhemispheric ratios measured by ASL and TAPV. The difference between interhemispheric...
ASL and TAPV ratios was plotted against the mean of both ratios. The broken line indicates average of differences and the solid line shows ± 1.96 SD of difference. .................................................................71

**Figure 18.** Blond-Altman plot shows degree of agreement between interhemispheric ratios measured by ASL and TCD-CBF. The difference between interhemispheric ASL and TAPV ratios was plotted against the mean of both ratios. The broken line indicates average of differences and the solid line shows ± 1.96 SD of difference. .................................................................72

**Figure 19.** Three axial slices showing ASL asymmetry in patients with SCD. A. A patient with ASL asymmetry but no TAPV asymmetry B. A patient with both ASL and TAPV asymmetries ............................................................................................................73
**List of Publications and Abstracts**

**Publications:**


**Abstracts:**


Behpour AM, Shah PS, Mikulis DJ, and Kassner A. Cerebral blood flow abnormalities in children with sickle cell disease. 2012 Annual meeting of the Society of Pediatric Research, Boston, USA.

Chapter 1

Outline of thesis and motivations
Chapter 1 Overview

1.1. Motivation

Cerebrovascular disease (CVD) is one of the major causes of morbidity and mortality in children with sickle cell disease (SCD), and as many as 12% of patients will experience a clinically overt stroke before the age of 20 years old (Ohene-Frempong et al. 1998). Furthermore, silent brain infarctions are present in 17%-22% of children with SCD who have not been identified as having a clinically evident stroke (overt stroke) by 14 years of age (Pegelow et al. 2002; Miller et al. 2001); these children are characterized by an increased risk of further overt strokes as well as poor academic attainment, and/or attaining a lower IQ when compared to SCD patients with normal magnetic resonance imaging (MRI) examinations or siblings without SCD (Pegelow et al. 2002; Miller et al. 2001; Moser et al. 1996).

Despite evidence that silent infarctions are prominent in SCD, are associated with significant morbidity and are a significant risk factor related to progressive neurologic disease, the optimal strategy for evaluating and treating such infarctions remains vague. The introduction of Transcranial Doppler (TCD) screening has substantially reduced overt stroke morbidity in children with SCD, but there seems to be no advantage in using TCD to evaluate hemodynamic consequences of silent infarctions (Wang et al. 1998). Thus, this thesis is motivated by a need to provide a viable alternative to the techniques that are currently used in the clinic to evaluate hemodynamic consequences of cerebral infarction, and in particular to evaluate hemodynamic consequences of silent infarction in children with SCD.

A better understanding of the pathophysiology of CVD in SCD is required to identify an adequate strategy to manage cerebral infarctions in children with SCD. Although different mechanisms
characterize the pathophysiology of cerebral infarction in SCD, the inadequate delivery of oxygen and glucose to cerebral tissues, via cerebral blood flow (CBF), seems to be the critical cause of infarction in brain tissue.

The overall purpose of this thesis is to investigate the role of cerebral blood flow (CBF) assessment in the management of cerebral infarction in children with SCD.

To gain a better understanding of CBF assessment in SCD, an overview of currently published studies has been presented. First, a systematic review was designed to summarize the results from research studies that examined CBF imaging modalities in children with SCD. This systematic review explored the prevalence of CBF abnormalities in children with SCD and compared to those abnormalities identified with current clinical imaging modalities, such as structural MRI and TCD.

Given that several studies have suggested that CBF abnormalities could happen in neurologically normal SCD children, we speculated that these abnormalities would be an early indicator of cerebral infarction. Several techniques have been developed to measure CBF. However, a need for radiation and/or the inhalation or injection of contrast agents has necessarily minimized their application in children. Recently, arterial spin labeling (ASL) has been introduced in order to non-invasively assess the CBF in patients. We used ASL to assess CBF in a group of neurologically normal SCD children. CBF abnormalities, in particular CBF asymmetries assessed with ASL were compared with flow velocity as well as CBF abnormalities obtained with TCD; and the association between CBF asymmetries and evidence of silent infarctions on structural MRI was examined.
1.2. Thesis outline

This thesis is presented in nine chapters. Chapter one discusses the motivations for this thesis and introduces the research issues covered in this thesis. In the remaining section of this chapter, outline of this thesis is presented. Chapter two provides an introduction to sickle cell disease, examining the role of “CBF assessment” in SCD children and highlighting the way in which the clinical questions motivating this thesis arise and why the research is important. In chapter three the aims and hypothesis of this thesis are introduced. Chapter four provides a systematic review of the prevalence of CBF abnormalities in SCD children. There, the search methods used in identifying the studies, as well as the method of data collection and analysis, is described. Also, the tools used to assess bias are introduced. Furthermore, the chapter provides a detailed review of included studies and assesses their risk of bias. The studies’ results are then analyzed and discussed with respect to CBF and clinical imaging modalities. Chapter five presents the assessment of CBF using the arterial spin labeling technique in a group of neurologically normal children with SCD. This chapter introduces absolute values and interhemispheric ratios of CBF and TCD velocity, as well as their association with silent infarction. The chapter ends by discussing the CBF abnormalities identified with ASL, as opposed to TCD, as well as the implications and main limitations of ASL-based perfusion measurements. Chapter six summarizes the findings of this thesis and discusses the implication of CBF assessment in patients with SCD. Chapter seven presents a concluding statement and a strategy for the dissemination of the research implications. Future research opportunities are presented in Chapter eight. Finally, a list of references used in this study is provided in chapter nine.
Chapter 2

Background review of literature
Chapter 2 Background review of literature

2.1. Sickle cell disease (SCD)

2.1.1 Pathophysiology

Sickle cell disease is an autosomal recessive hemoglobinopathy caused by the substitution of valine for glutamic acid at position 6 of the beta polypeptide chain of hemoglobin. When two mutant beta chain subunits associate with two alpha chains subunits, Sickle hemoglobin (HbS) is formed. Under the deoxygenated conditions, HbS is polymerized, leading to the formation of long fibers inside the red blood cells (RBC). This aggregation of long fibers distorts the shape of RBCs, causing them to take on a sickle shape. These sickled and rigid RBCs have shortened lifespans and undergo intravascular and extravascular hemolysis. Furthermore, sickled RBCs can adhere to the vascular endothelium, ultimately blocking the normal blood flow through the vasculature (Redding-Lallinger and Knoll 2006).

While the polymerization of HbS is a well known aspect of the disease, the previously used term ‘Sickle cell anemia’ is no longer used, because vascular obliterations, not anemia, are currently believed to play an important role in the pathophysiology of the disease. In particular, nitric oxide (NO) depletion seems to play an important role in the pathophysiology of SCD (Redding-Lallinger and Knoll 2006; Switzer et al. 2006). NO depletion results when large amounts of hemoglobin and red cell arginase are released as a result of the chronic breakdown of red cells that occurs in SCD plasma (Redding-Lallinger and Knoll 2006). The hemolysis and red cell adhesion leads to a pro-inflammatory state characterized by white cell adhesion and platelet aggregation (Switzer et al. 2006). Another result of NO depletion is that the down-regulating effects of NO, on both inflammation and the activation of coagulation, are lost (Redding-
Lallinger and Knoll 2006). NO normally causes vasodilation in smooth muscle. Therefore, unregulated vasoconstriction promotes vaso-occlusion and contributes to tissue hypoxia.

**Figure 1.** Pathophysiology of Sickleing

Vaso-occlusion seems to be derived from an underlying vasculopathy involving both microvascular and large arteries. The narrowing of arteries with sickle shaped RBCs, in addition to impaired endothelial regulation mechanisms, lead to decreased oxygen delivery and is followed by hypoxia, tissue ischemia, painful crises and inflammation. Recurrent episodes of vaso-occlusion and inflammation lead to vasculopathy, which, in turn, results in progressive damage to almost all organs, including the musculoskeletal system, spleen, liver, kidneys, and the brain (Steinberg 2000).
The vaso-occlusive crisis, one of the hallmarks of SCD, may affect many different organs, causing pain (especially in the back, chest, abdomen and extremities), fever and leukocytosis. Vaso-occlusion occurring in the lungs can result in serious lung injury and hypoxia; it is called acute chest syndrome and its occurrence is a medical emergency. The vaso-occlusive events can also occur in bones such as knees and hips. Those are known as avascular necrosis and can eventually necessitate replacement. When vaso-occlusion occurs in the penis, the result is a painful, prolonged erection or priapism. Acute transfusion can be prescribed for acute priapism if the standard therapies fail. Vaso-occlusion in the brain can result in a stroke. This can lead to devastating complications and limitations in cognition, speech and/or movement (see: the cerebrovascular disease section). Vaso-occlusive events can also affect the vascular beds in the eyes, and this can progress significantly before visual problems are revealed. Therefore, comprehensive eye exams are recommended regularly for all SCD patients, regardless of signs or symptoms.

Acute splenic sequestration crisis is a significant cause of death in children with SCD (National Institutes of Health and Resources. 2004). It is a rapid crisis caused by the trapping of red blood cells in vessels leading out of the spleen. The pooling of blood in the spleen results in the massive enlargement of the spleen and acute anemia. Splenic sequestration can be followed by hypotensive shock, with cardiac compromise. If splenic sequestration recurs, a splenectomy is sometimes considered. A vaso-occlusive crisis may also occur in the liver, where it consists of right upper quadrant pain, liver enlargement, fever, jaundice, and elevated liver transaminases. The preferred treatment is exchange transfusion because simple transfusions may be accompanied by the return of sequestered RBCs into the circulation, ultimately leading to a hyperviscosity syndrome (National Institutes of Health and Resources. 2004). Kidneys are found to be very susceptible to injury due to the high tendency of HbS polymerization within renal tissue (Ataga
and Orringer 2000). Some individuals with SCD can even progress to the end stage of renal disease, requiring dialysis and/or renal transplantation.

In general, the above complications start in early life but become more apparent with increasing age. Moreover, various SCD genotypes are characterized by marked and largely unpredictable variability in clinical manifestations and severity (Gill et al. 1995; Platt et al. 1994; Miller et al. 2000). Subjects with homozygous (HbSS) or compound heterozygosity for HbS are symptomatic, whereas heterozygous subjects with only one HbS are asymptomatic genetic carriers. Sickle cell anemia (SCA) is the most sever form of SCD. However, Sickle-hemoglobin disease (HbSC), as well as compound types of sickle cell thalassemia such as Sickle beta plus thalassemia (HbS/β+) and sickle beta zero thalassemia (HbS/β0), also contribute to the clinical spectrum of SCD. Several factors, such as infections, dehydration, fever, cold weather and stress, precipitate the complications. Most of the treatments are directed towards preventing or decreasing sickling, and hence towards reduction in the vasculopathy and clinical complications of SCD.

2.1.2 Epidemiology

Approximately 300,000 infants are born annually with documented SCD. SCD is most common among people whose ancestors originated from Sub-Saharan Africa, India, the Caribbean, the Middle East, the Mediterranean, and South America. SCA occurs in one of every 500 African American births and one of every 1000-1400 Hispanic Americans/Latino births (National institute of health 2007). In the African American community population, the incidence of SCA is 0.2 to 0.3%, the incidence of the SS trait is 9 to 11%, and the incidence of SC disease is 3% (Hamdallah and Bhatia 1995; Petrakis et al. 1970; Nietert, Silverstein, and Abboud 2002). The population at risk of SCD in Canada is unknown. According to a study conducted by the Sickle
Cell Awareness Group of Ontario, 32 out of 40 African-Canadians carry the trait. However, this does not take into account other population groups who are at risk for SCD.

2.1.3 Treatment

The main therapies used to manage SCD consist of blood transfusions, hydroxyurea (HU), and hematopoietic stem cell transplants. Bone marrow transplants are the only cure for SCD; however, that treatment is not available to most people and it is associated with an increased risk of death related to the complication of the transplant itself (Walters 2005). Therefore, the mainstay of therapy is the treatment and prevention of clinical complications.

Single transfusions are recommended for major splenic sequestration, aplastic crises, and acute thoracic syndrome, as well as before major surgery (hemoglobin must be increased to 10 g/dL). The main indication for long-term transfusion programs (designed to maintain low proportions of HbS in the blood long-term) is a central nervous system (CNS) infarction.

Hydroxyurea (HU) is the only pharmacologic agent that is FDA-approved for preventing complications occurring from SCD. HU can reduce the number and severity of pain crises (in 70% to 75% of patients) and can significantly decrease a patient’s number of acute thoracic syndrome episodes, decrease hospitalizations resulting from painful episodes, reduce the prevalence of acute chest syndrome and limit the total number of blood transfusions needed (Platt 2008). HU increases fetal hemoglobin levels, which in turn inhibit the polymerization of HbS. In addition, other mechanisms of action have been described for HU, including an increase in nitric oxide, and a decrease in the expression of red cells, which results in the diminished adhesion of blood cells (Switzer et al. 2006; Steinberg et al. 2003). Nevertheless, HU is only recommended in SCD patients when it is strictly indicated; this is due to its severe side effects, which include
cytopenia, hyperpigmentation, opportunistic infections, azoospermia (in approximately 80% of men), marked hypomagnesemia and teratogenic effects (Kohne).

2.1.4 Cerebrovascular disease (CVD) in SCD

Cerebrovascular disease (CVD) is one of the major complications of SCD and a leading cause of death in both children (Leikin et al. 1989) and adults (Platt et al. 1994) with SCD. Overt stroke, or neurologically symptomatic stroke, can occur in as many as 11% of affected patients by the age of 20 years (Ohene-Frempong et al. 1998). The risk of stroke is highest during the first decade, and it is most significant between the ages of 2 and 5, when it reaches 1.02% per year (Ohene-Frempong et al. 1998). Incidence of CVD in SCD adults is 10 times higher than in the black population without SCD (Pandey and Gorelick 2005). Stroke subtypes vary by age in SCD patients. The incidence of the ischemic variant, which accounts for 54% of all CVD (Ohene-Frempong et al. 1998), is highest during the first decade and before age 20. However, hemorrhagic stroke replaces ischemic stroke as the most likely to occur in patients 20-29 years of age (Ohene-Frempong et al. 1998).

In addition to overt stroke, 17-22% of children with sickle cell anemia show evidence of structural brain defects, without associated neurological symptoms (Moser et al. 1996; Pegelow et al. 2002). Although these lesions have been previously termed silent infarctions they are associated with neuropsychological impairments (Kugler et al. 1993) and an increased risk of future stroke (Miller et al. 2001). The prevalence of silent infarction is also higher in adults than in children, given the effect of age on CVD in SCD (Pegelow et al. 2002; Steen et al. 2003).

Patients with SCD are uniquely vulnerable to critical reductions in the delivery of oxygen to the brain through several complex and interrelated hemodynamic mechanisms. Many children
develop severe narrowing or occlusion of the large arteries of the circle of Willis; these may reduce regional blood flow in the brain. Vasculopathies occurring in the large cerebral arteries, such as the distal internal carotid artery (ICA) and the proximal middle cerebral artery (MCA), are major mechanisms of stroke and are identified in 80% of patients with SCD and stroke (Gerald, Sebes, and Langston 1980; Jeffries, Lipper, and Kishore 1980).

However, large cerebral artery stenosis has not been identified in all SCD patients who have died as a result of neurological problems (Kimmelsteil 1948). In addition, large artery stenosis is usually absent in the context of silent infarction, which mainly happen in the deep white matter of the frontal and parietal lobes. The sickling of red blood cells in the small vessel stenosis is assumed to be a potential cause of a part of overt stroke and most silent infarctions.

**Figure 2.** Silent infarctions. A 11 years old SCA male patient with no previous history of stroke. (A) Fluid attenuated inversion recovery (FLAIR) shows bilateral multiple hyperintense lesions in the white matter. (B) MRA shows no major vessel abnormalities.
Furthermore, increased viscosity, the abnormal adherence of abnormal red blood cells to the endothelium, fat embolisms and moyamoya syndrome (bilateral stenosis and the occlusion of cerebral arteries with collaterals formation) may further impair the flow of blood and hypoxia through narrowed vessels or in normal brain capillaries. The increase in vascular resistance can be overcome through autoregulatory vasodilation mechanisms implemented through the relaxation of arteriolar smooth muscles. When the capacity for autoregulatory vasodilation has been exceeded, autoregulation fails, resulting in reduced blood flow/perfusion. With mild decreases in cerebral perfusion, metabolic function is maintained by increased oxygen extraction. This may be reversible if circulation is restored via a therapeutic intervention such as transfusion therapy or revascularization surgery. Untreated, the persistence of severely reduced perfusion can lead to infarction, which mostly occurs in border zone area located between the ICA and MCA territories (Pavlakis et al. 1989; Adams et al. 1988; Stockman et al. 1972).

Cerebrovascular complications are one of the major causes of morbidity and mortality in patients with SCD. Overt strokes require a rapid and accurate diagnosis due to their narrow treatment time window. Silent infarctions also need to be detected before progression to permanent neuronal loss, with disability, occurs. Imaging methods are designed to provide information about the cause of strokes, the presence of ischemic tissue and vessel abnormalities. More importantly, imaging methods are used to identify the risk of CVD in patients with SCD. This may be followed by preventative therapy in high-risk patients.
2.2. Clinical imaging techniques of CVD in SCD

Non-contrast computed tomography (CT) remains the method of choice in emergency settings due to its cost effectiveness and exquisite sensitivity in detecting hemorrhages and determining the qualification for thrombolysis.

Magnetic resonance imaging (MRI), especially the diffusion-weighted technique, is highly sensitive to the early pathologic changes of ischemic infarction and subtle brain edema. Patients with no evidence of hemorrhagic stroke, according to non-contrast CT, need to undergo MRI to determine the location and extent of ischemic stroke. In addition, MRI is currently used in the detection of silent infarctions (see the MRI section). However, further studies are still required to determine whether MRI can completely replace CT in detecting acute stroke (Fiebach et al. 2004).

Transcranial Doppler ultrasonography (TCD) is a non-invasive ultrasonic technique measuring local blood flow velocity and direction in the proximal portions of large intracranial arteries. This method can be used to screen for intracranial stenosis and occlusion in patients with CVD (Demchuk et al. 2000; Gerriets et al. 2002). The use of TCD in the prevention of ischemic stroke in SCD patients was previously established (see TCD section).

Furthermore, an angiographic assessment may be useful in further characterizing cerebrovascular abnormalities and possibly in aiding decision making in regard to SCD patients with such abnormalities. Digital subtraction angiography (DSA) is the most accurate way to detect cerebrovasculature abnormalities such as stenosis and associated collateral vessels. However, DSA is a highly invasive method and is only indicated when non-invasive methods are inadequate. Magnetic resonance angiography (MRA) and CT angiography are two non-invasive angiography techniques. Although MRI technology has advanced greatly, MRA does not presently provide a high enough resolution to reliably visualize the small arteries that need to be
examined. More recent studies demonstrated that MRA has a 70-86% sensitivity in detecting cerebrovascular stenosis in adults, whereas CT angiography has up to a 98% sensitivity (Katz et al. 1995; Bash et al. 2005). CT angiography currently has a higher sensitivity to small cerebral arteries than does MRA, but exposure to ionizing radiation and the need for contrast injection limit its application, particularly in children.

2.2.1 Transcranial Doppler Ultrasonography (TCD)

A series of studies by Adam et al. showed that time averaged mean flow velocity (TAMV), measured with TCD> 200 cm/s in the distal ICA or proximal MCA, is predictive of a 40% stroke risk in SCA patients (Adams et al. 1990; Adams et al. 1992). Using this information, a stroke prevention in sickle cell disease (STOP) study was designed to examine the role of TCD in the prevention of stroke. The authors demonstrated a 92% reduction in the risk of stroke in patients receiving blood transfusion following abnormal TCD velocity (>200 cm/s), reducing the HbS level to below 30%. TCD was thus introduced as a safe, noninvasive, cost effective and repeatable clinically diagnostic test that can be used to stratify the risk of cerebrovascular diseases in SCD patients.

To date, the National Heart, Lung and Blood Institute (NHLBLI) recommend that TCD screening of children with SCA start at 2 years of age and continue annually, if TCD is normal, and every 4 months if TCD is conditional (170< TAPV<200 cm/s).
Figure 3. Transcranial Doppler in a patient with SCD; severe stenosis of left internal carotid artery and left middle cerebral artery consistent with correspondingly very low flow velocity, normal vasculature and normal flow velocity on right side.

Although the risk of stroke is highest among those with abnormal TCD velocity, 60% of children with abnormal TCD velocity do not develop a stroke (Adams et al. 1992; Adams, McKie, Hsu, et al. 1998). In addition, stroke events can happen in SCD patients with conditional TCD (Adams et al. 2004). Furthermore, TCD can be limited by operator skill, the need for an ultrasonic window, which is difficult to find in 5-15% of patients, and the difficulty of attaining an accurate measurement of flow velocity, which is dependent on the angle of insonation (Gillams et al. 1998).
2.2.2 Structural Magnetic Resonance Imaging (MRI)

Structural MRI allows for the high-resolution anatomical visualization of cerebral infarction (Verlhac 1999). Such lesions are visualized as hyperintense lesions on T2-weighted images and FLAIR and as hypointense on T1-weighted images. Yet, it has not yet been determined whether structural MRI could replace CT in the emergency setting. In studies in which MRI was used, up to 20% of children SCD have silent infarctions, typically involving watershed areas (Switzer et al. 2006).

2.3. Cerebral blood flow (CBF) imaging techniques in SCD

The development of new technologies, as well as limitations in the current clinical imaging techniques, suggest that, at least in some instances, TCD and MRI may not be sufficient in assessing CVD in patients with SCD.

To identify an adequate strategy in the management of cerebral infarction in children with SCD, a better understanding of the pathophysiology of CVD in SCD is required. Although different mechanisms characterize the pathophysiology of cerebral infarction in SCD, the inadequate supply of cerebral blood flow (CBF) seems to be the critical cause of infarction in brain tissue. It has been shown that the measurement of CBF and blood perfusion can not only predict both the extent and the severity of tissue necrosis in ischemic strokes, but that it also may guide physicians to the appropriate selection of treatment (Albers 1999). Although it is not yet approved, recent research shows that the reversibility or irreversibility of the damage caused to tissues in an acute stroke may be determined by the quantitative measurement of blood perfusion (Hoeffner 2005; Moustafa and Baron 2008). Due to its noninvasive nature and ability to provide regional information, medical imaging has had a profound impact on CBF measurements. Imaging
modalities used to assess CBF can determine the state of perfusion and may indicate tissue that is at risk of cerebral infarction.

Recently, a number of different imaging modalities have been developed to assess CBF at the tissue level. Among these are: Xenon-enhanced computed tomography (Xe-CT), positron emission tomography (PET), single photon emission computed tomography (SPECT), perfusion MRI techniques including dynamic susceptibility contrast (DSC) MRI, arterial spin labeling (ASL) and most recently CT perfusion. The technical aspects of these modalities, alongside some SCD studies using these modalities (except CT perfusion), are briefly described below.

2.3.1 Xenon133 Computed Tomography (Xe-CT)

The inhalation of xenon gas, acting as a contrast agent for CT imaging, can reflect regional variations in blood flow patterns, as well as provide an estimation of CBF (Latchaw et al. 2003). In this method, serial CT scans are performed before, during and after xenon inhalation. CBF can be obtained from the baseline and wash-in studies. Wash-in/wash-out comparisons may be performed to increase the signal-to-noise ratio. In addition, the xenon concentration in blood is indirectly measured by acquiring the xenon concentration in the exhaled air. After scanning, the baseline scans are averaged and subtracted from wash-in scans to obtain a xenon arrival curve for each volume in each scan. The time-dependent xenon concentration within various tissue segments in the brain is used to derive both the local partition coefficient and the CBF in each tissue volume of the CT image.

Xe-CT is not currently widely used. Although some previous studies demonstrated promise in the evaluation of CBF using Xe-CT in adult patients with SCD (Prohovnik et al. 1989; Prohovnik et al. 2009; Huttenlocher et al. 1984), the radiation dose from the CT scanner, a simultaneous
increase in CBF as a result of xenon inhalation (Hartmann, Dettmers, et al. 1991) and the adverse effects of xenon gas, including sensory and cognition symptoms (Yonas, Pindzola, and Johnson 1996), are some of the limitations of the Xe-CT method.

2.3.2 Positron Emission Tomography (PET)

The detection of gamma rays emitted from the radioactive tracer (\(^{15}\text{O}\)) permits an estimate of various physiologic parameters in the brain, such as CBF, cerebral blood volume and oxygen extraction fraction. To measure CBF using PET, an intravenous bolus injection of \(\text{H}_2^{15}\text{O}\) is administered and a sequence of multiple arterial blood samples is then obtained. In another method, the subject inhales \(\text{C}^{15}\text{O}_2\) to reach the steady state concentration in which the delivery and clearance of the tracer, along with tracer decay, are in equilibrium. The tissue concentration of tracers at this steady state represents the CBF. The PET image data is finally modeled using nonlinear, least squared regression analysis on a pixel-by-pixel basis (Ohta et al. 1996).

PET is the current gold standard method for assessing CBF, providing the most complete explanation of how a compromise of CBF leads to cerebral ischemia. PET imaging in SCD patients has demonstrated significantly increased CBF, providing insight into the pathophysiology of stroke in adult and pediatric patients with SCD (Herold et al. 1986). Nevertheless, the need for an arterial catheter to measure the arterial tracer concentration, the application of radioactivity, the cost, and the inaccessibility of PET centers with a cyclotron have limited its use for clinical purposes.
2.3.3 Single Photon Emission Computed Tomography (SPECT)

Single Photon Emission Computed Tomography, also using a radiotracer (a single pass tracer), can provide blood flow information. However, the specific radiopharmaceuticals and implementations of SPECT differ from those used in PET. 99mTc is a widely used SPECT tracer in CBF assessment. 99mTc is attached to a delivery compound, such as hexamethyl propylenamine oxime (HMPAO) or ethyl cysteinate dimer, (ECD) before injection. After being administered intravenously, this single pass radioactive tracer is distributed with cerebral blood flow and passes through the brain blood barrier. The tracer is then taken up proportionally to CBF while collecting decaying photons using tomographic detectors (Matsuda et al. 1992, 1993). The CBF pattern is then mapped and acquired (Dougall, Bruggink, and Ebmeier 2004; Walovitch et al. 1994).

SPECT is a generally less expensive and more widely available technique than PET because it does not depend on a local cyclotron for the production of tracers. To date, a number of SPECT studies in patients with SCD have demonstrated impaired CBF in structurally normal brains (Yalcin et al. 2007; Parsa, Mehregany, and Schulz 1992; Kedar et al. 2006). However, SPECT suffers from low spatial resolution and radiation exposure to patients.

2.3.4 Perfusion MRI

Perfusion MRI is able to measure perfusion parameters including CBF, cerebral blood volume (CBV) and mean transit time (MTT). To assess CBF, perfusion MRI employs either endogenous or exogenous contrast agents. Since perfusion MRI techniques do not rely on X-rays or gamma rays, they are well suited for pediatric applications.
**Dynamic susceptibility contrast (DSC) MRI:** Dynamic susceptibility contrast MRI technique involves the intravenous injection of a paramagnetic contrast agent (gadolinium-DTPA) in combination with rapid dynamic T2 weighted imaging. Signal loss during the first pass of the contrast agent allows CBF to be extracted from signal time intensity curves.

**Arterial spin labeling (ASL):** Arterial spin labeling uses water as an endogenous contrast agent. Blood water is labeled upstream of the slice of interest using a radiofrequency pulse. The magnetically labeled blood water increases the MRI signal when it reaches the tissue. The subtraction of a labeled image from a control image is used to measure the amount of the label that flowed into the tissue. This quantity is closely related to tissue perfusion (Detre et al. 1998).

DSC-MRI and ASL have been applied successfully in SCD (Prengler et al. 2005; Wang et al. 2003; Chalela et al. 2000). ASL is truly non-invasive; it does not even require an injection of a contrast agent. Although ASL is currently restricted to the exploration of a limited region of the brain and has an inherently weak signal-to-noise ratio (Chalela et al. 2000; Detre et al. 1998; Alsop, Detre, and Grossman 2000), future developments are likely to enhance the role of ASL in CBF assessment studies.

There are currently three major categories for ASL: Pulsed ASL (PASL), Continuous ASL (CASL), and Pseudo-Continuous ASL (PCASL). The primary difference between these categories is the technique that magnetically labels inflowing hydrogen protons of arterial blood-water.

In CASL technique, a long and continuous radiofrequency (RF) pulse is used alongside a separate coil continuously inverts the blood’s protons of feeding arteries of the brain (Detre et al. 1992; Detre and Alsop 1999). The longer steady state tagging provides high Signal to Noise Ratio
(SNR). Although CASL has higher SNR than PASL and covers the whole brain, there are several drawbacks that limit application of CASL in the clinical setting. The primary limitation is the need for a continuous RF transmits hardware, which is not always available in commercial clinical scanners. The long RF pulses used in CASL can also exceed the limit allowed for RF deposition. PASL technique uses short RF pulses to selectively invert the protons and images a particular part of the brain due to T1 relaxation of the protons (Edelman et al. 1994). PASL has a higher inversion efficacy and lower RF deposition than CASL.

Figure 4. A schematic description of PASL experiment, perfusion weighted image (ΔM) is obtained by subtraction of the labelled images from the control images (Petersen et al. 2006).

PCASL, as an intermediate method between CASL and PASL, uses a train of discrete RF pulses to mimic continuous tagging seen on CASL without the requirement of the second tagging coil (Garcia, Bazelaire, and Alsop 2005). PCASL has the advantage of high SNR of CASL and high
tagging efficiency of PASL. In all three techniques the difference between two sets of images attribute to flow in the imaging plane.

2.4. CBF measurement

Cerebral blood flow can theoretically be computed by measuring the blood flow that leaves the arterioles to enter the capillary bed of the tissue of interest. However, the very small size of the blood vessels renders this measurement extremely challenging. Instead, most methods measure the concentration of a substance, called a blood tracer, as it travels through the vascular tree and into the capillary network feeding the tissue of interest. In a simplified description, blood tracers, depending on their biochemical properties, can diffuse from the capillaries into the extra-cellular space and from there into tissue cells (diffusible tracers), or they can remain in the capillaries (intra-vascular tracers).

CBF imaging techniques using diffusible tracers such as Xe-CT, PET, SPECT and ASL are dependent on the fact that such tracers are highly diffusible within the brain parenchyma. The measure of CBF in these techniques is achieved by measuring the concentration of the tracer within the arterial and tissue compartments, and with knowledge of the distribution of tracers between the compartments (partition coefficient) at equilibrium (Kety 1956). An alternative approach is to use intravascular tracers, which are retained in the vascular compartment, to measure intravascular flow. CBF is often calculated through the indicator-dilution theory proposed by Meier and Zierler (Meier and Zierler 1954), which describes the passage of a tracer through the microvasculature by means of dynamic functions.

The CBF measured can be absolute or relative. Absolute CBF values have clinical relevance because there are known thresholds of CBF below which reversible (Astrup, Siesjo, and Symon
1981; Hossmann 1983) and irreversible (Heiss, Forsting, and Diener 2001) ischemic injuries can occur. In addition, decrease or increase CBF would be essential for being able to know if the planned interventions achieved their goals (Yonas et al. 2005).

To quantify absolute CBF, different post-processing methods can be employed. Most methods demand user-selected parameters, which may result in variations in the quantitative measurements. For example, using the tracer kinetic model equations to calculate CBF requires the deconvolution of an arterial input function (AIF) from the concentration-time curves (Ostergaard, Weisskoff, et al. 1996; Ostergaard, Sorensen, et al. 1996). However, this function is not known explicitly and currently, the AIF is estimated from the data. In current practice, a trained specialist who examines the data selects a single AIF for the entire brain. While simple, this approach is problematic in case of severe pathologies. For instance, many of the pathological conditions that occur in stroke contradict the assumptions that single AIF selection is based upon (Calamante, Gadian, and Connelly 2002; Wu et al. 2003).

The relative CBF is an alternative approach in practice that is created by comparing data within a region of interest (ROI) to that obtained within a comparable ROI within normal-appearing brain. Relative CBF values do not provide quantitative assessment of brain hemodynamics but instead afford indicators of hemodynamic disturbances that are useful in a clinical setting. A simple example of the relative CBF is CBF asymmetry, which is obtained by comparing the CBF map of two hemispheres against each other. This can be done visually by comparing a CBF map of two hemispheres. CBF asymmetry can also be assessed semiquantitatively by calculating interhemispheric ratio or difference between the absolute CBF measurements in an ROI placed in the abnormal area and a mirror ROI located in the contralateral area regarded as a normal reference. Previous studies using CBF asymmetries have correlated CBF asymmetries with symptom severity in patients suffering stroke (Bonita and Beaglehole 1998; Chalela et al. 2000).
However, it should be noted that currently, there is no standardization in the assessment of CBF asymmetries. An arbitrary threshold of 0.1, equivalent to a 20% difference in hemisphere CBF values can be used to define asymmetry ratio (Detre et al. 1998). Statistical analysis can also be employed to compare interhemispheric ratios between patients and healthy controls; the asymmetry is regarded as the ratios significantly different than controls (Oguz et al. 2003). To date, the selection of one over another method depends on the intrinsic characteristics relating to each imaging technique but also on the settings and on the knowledge and experience of institution staff.
Chapter 3

Hypothesis and aims
Chapter 3 Hypothesis and aims

3.1. Hypotheses

This thesis examines the following hypotheses:

\( H1 \): In neurologically normal SCD children, CBF abnormalities can be identified using ASL in cases with no TCD abnormalities.

\( H2 \): In these cases, CBF abnormalities are associated with evidence of silent infarctions visualized by structural MRI.

\( H3 \): Agreement between ASL and TCD can be improved when TCD velocity measurements are converted into CBF.

3.2. Aims

The aims of this thesis are:

\( A1 \): To systematically review the prevalence of neuroimaging abnormalities in children with SCD, as identified using Xe-CT, PET, SPECT and perfusion MRI techniques (DSC-MRI and ASL), as well as those abnormalities obtained via structural MRI and TCD velocity.

\( A2 \): To obtain absolute values and interhemispheric ratios of CBF in the MCA territory using ASL and compare them to those of flow velocity and CBF obtained with TCD in children with SCD.
A3: To test the association between CBF asymmetries and evidence of silent infarction.
Chapter 4

Cerebral blood flow abnormalities in children with sickle cell disease: a systematic review
4.1. Introduction

Sickle cell disease (SCD) is a spectrum of disorders of the red blood cell, in which the sickle β globin gene is inherited. It is characterized by rigid and sickle shaped red blood cells, which can get destroyed during the passage through the vasculature leading to intravascular hemolysis and anemia. In addition, adhesion of the sickled red blood cells to the vascular endothelium causes occlusion and subsequent tissue ischemia. Cerebral ischemic events, in particular overt stroke, are one of the most devastating complications in children with SCD. Strokes affect 7-11% of children with SCD (Adams 1995; Ohene-Frempong et al. 1998), a prevalence, which is 250 times higher than in the general pediatric population (Earley et al. 1998). Overt stroke is diagnosed as a focal neurological deficit with either motor or sensory deficit that persists for more than 24 hours, and has neuroimaging evidence of a cerebral infarct corresponding to the focal deficit. Interestingly, an even higher number of patients exhibit silent infarcts, defined as increased signal intensity on multiple T2-weighted or fluid attenuated inversion recovery (FLAIR) magnetic resonance (MR) images in the brain with no history or physical findings. Silent strokes occur in approximately 22% of children with SCD (Pegelow et al. 2002), and can be precursors of subsequent overt stroke (Pegelow et al. 2002; Kugler et al. 1993; Miller et al. 2001). Mechanisms responsible for cerebral ischemic events in SCD are complex and seem to be related to impaired blood flow. Blood flow abnormalities can be caused due to narrowing or occlusion of cerebral vessels, increased viscosity, adherence of red blood cells to the vascular endothelium, and exhaustion of autoregulatory vasodilation. Given the high susceptibility to cerebral ischemic events in children with SCD, early screening using advanced neuroimaging tools is important.
Standard Transcranial Doppler (TCD) ultrasonography is a low-cost method to identify SCD patients at increased risk for developing a stroke. The Stroke Prevention Trial in Sickle Cell Anemia (STOP) demonstrated that periodic red blood cell transfusion therapy in patients with TCD velocities above 200 cm/s in the middle cerebral artery or terminal portion of the internal carotid artery can prevent overt strokes (Adams, McKie, Brambilla, et al. 1998), however, a large number of children (60%) with high TCD velocities (> 200 cm/s) do not develop a stroke (Adams et al. 1992; Adams, McKie, Hsu, et al. 1998) whereas others with TCD velocities below 200 cm/s may still be at risk for overt stroke (Adams et al. 2004) or silent infarct (Wang et al. 2000). Furthermore, TCD assessment is dependent on operator skill, the need for an ultrasonic window ability to detect distal branches of intracranial vessel, the dependence of the velocity measurement on the angle and depth of insonation (Gillams et al. 1998).

Advanced modalities for cerebral blood flow measurements include stable Xenon-enhanced computed tomography (Xe-CT), positron emission tomography (PET) and single photon emission tomography (SPECT). Although nuclear medicine techniques such as PET and SPECT can provide accurate regional measures of CBF, they require inhalation, injection or exposure to ionizing radiation. Further disadvantages include low spatial resolution, and in the case of Xe-CT, a concomitant increase in CBF as a result of stable xenon inhalation (Hartmann, Dettmers, et al. 1991). Another disadvantage, specific to positron emission tomography, is limited availability and high cost. More recently, perfusion MRI techniques including dynamic susceptibility contrast (DSC) MRI and arterial spin labeling (ASL) have gained popularity as tools for imaging brain physiology, including CBF. Magnetic resonance imaging is particularly well-suited for pediatric studies, as it is non-invasive and does not require ionizing radiation.

The purpose of this review is to systematically review the prevalence of neuroimaging abnormalities in children with SCD identified on Xe-CT, PET, SPECT and perfusion MRI.
techniques (DSC-MRI and ASL) to those abnormalities obtained with structural MRI and TCD velocity.

4.2. Materials and methods

4.2.1 Criteria used for including studies in this review

*Types of studies and participants:* Cohort studies that assessed children aged 0-18 years with a diagnosis of SCD were included. For completeness of the relevant evidence, we included studies in which median age of population was <18 years recognizing that some patients may be older than 18 years.

*Target condition and reference test:* Studies were included in this review if cerebral ischemic stroke was considered one of the outcomes of interest. Studies reporting only on seizures were excluded. Furthermore, we only included studies that employed either structural MRI or TCD or both as reference test(s).

*Index tests:* Studies that have utilized any test methods of assessment of CBF including Xe-CT, PET, SPECT and perfusion MRI techniques (DSC-MRI and ASL) were included.

4.2.2 Search strategy

We performed a comprehensive search of the Ovid/Medline and Embase databases (1980 to March 2012) to identify eligible studies. We used the following keywords and MeSH terminology: sickle cell, cerebrovascular disorder, hemodynamic, cerebral blood flow, magnetic resonance imaging and transcranial Doppler ultrasonography. Articles were assessed irrespective of language of publication. Bibliographies of acquired articles were then reviewed to complete
the search. We also searched the first 200 hits of Google Scholar™ to identify any articles published in grey literature.

4.2.3 Study Selection

We included peer-reviewed studies that reported CBF measurements along with structural MRI or TCD velocity findings in children with SCD. Case reports and review studies were not included but read to identify additional eligible articles. Two independent reviewers (A.B., A.K.) assessed the title and abstract of each article as the first step. The same reviewers manually reviewed the full text for potentially relevant publications as the next step. Regular consensus meetings were organized between the two reviewers at both steps and any disagreement was discussed and resolved engaging third reviewer (P.S.). Two reviewers (A.B., A.K.) extracted the data independently from eligible articles and discrepancies were resolved by consensus. Data on number and mean age of included patients, number of scanned patients with CBF imaging modalities, inclusion criteria, type of SCD, treatment history, type of reference test, type of CBF imaging modality and different parameters used in the CBF study were collected.

4.2.4 Risk of bias assessment

To analyze the potential for bias, 11 criteria of the quality assessment of diagnostic accuracy studies (QUADAS) scale (Whiting et al. 2003) were used. In addition, to evaluate the effect of treatment as confounding factor in included studies, we added another criteria as “treatment bias”. These 12 criteria (Appendix A) were scored separately in each study either ‘low risk’ (the study is free of evidence of bias or at very minimal risk of bias) or ‘high risk’ (the study assessment reveal evidence of bias) or ‘unclear’ (the assessment indicated uncertainty around potential bias). Two
authors (A.B., A.K.) assessed all articles independently. Discrepancies were checked and consensus achieved by discussion. If needed, a third author reviewed the data to reach a consensus (P.S.).

4.2.5 Outcomes

The outcome of interest in this review was evidence of neuroimaging abnormalities. Patients in each study were categorized into two different groups, the stroke group, which involved patients with SCD and evidence of stroke versus the non-stroke group, which involved either neurologically normal patients with SCD or patients who had a neurological disorder that could not be attributed to ischemic stroke.

4.2.6 Statistical analysis

The prevalence of neuroimaging abnormalities was calculated for each imaging method (test) as a percentage by dividing the number of patients with abnormal scans by the total number of patients scanned in each group. The calculated prevalence of neuroimaging abnormalities was then separately compared to structural MRI and TCD velocity findings (reference), within and between the two groups (stroke versus non-stroke) for each study. With prior knowledge of clinical and methodological heterogeneity between studies, we did not plan any meta-analysis in this review.
4.3. Results

4.3.1 Search strategy and study selection

The database search retrieved 3786 peer-reviewed articles. After reviewing the title and abstract, 26 reports were found potentially relevant for our review. The full texts of potentially relevant articles were then retrieved in the next step with nine papers meeting the criteria for this review. In addition, references of these nine articles were checked and two additional papers were included for a total of 11 eligible papers. A flowchart of the article selection process is summarized in Figure 4. The baseline characteristics of the identified articles are reported in Table 1. All 11 included studies (Numaguchi et al. 1990; Powars et al. 1999; Al-Kandari et al. 2007; Tzika et al. 1993; Kirkham et al. 2001; Grueneich et al. 2004; Helton et al. 2009; Oguz et al. 2003; Strouse et al. 2006; van den Tweel et al. 2009; Schatz et al.) were prospective cohort studies, which utilized different CBF imaging modalities in children with SCD to identify neuroimaging abnormalities. Seven (Numaguchi et al. 1990; Powars et al. 1999; Al-Kandari et al. 2007; Tzika et al. 1993; Strouse et al. 2006; Grueneich et al. 2004; Oguz et al. 2003) of the eleven studies used structural MRI as a reference test and the remaining four used both structural MRI and TCD as a reference test (Kirkham et al. 2001; Helton et al. 2009; van den Tweel et al. 2009; Schatz et al.). The CBF modalities used in these studies included one article using Xe-CT (Numaguchi et al. 1990), one article using PET(Powars et al. 1999), one article using SPECT (Al-Kandari et al. 2007) and eight articles using perfusion MRI techniques, either DSC-MRI (three studies)(Tzika et al. 1993; Kirkham et al. 2001; Grueneich et al. 2004) or ASL (five studies)(Helton et al. 2009; Oguz et al. 2003; Strouse et al. 2006; van den Tweel et al. 2009; Schatz et al.).
Figure 5. Flow diagram for study selection

Articles retrieved for full text review
n=26

Not relevant based on abstracts

Not eligible, n=17
Reasons:
• Review article (n=2)
• Case reports (n=5)
• Animal Study (n=1)
• Evaluation of other cerebrovascular disorders except stroke (n=1)
• Participants were not under 18 year old (n=4)
• Evaluation of only metabolic abnormalities (n=1)
• Methods except MRI used as reference test (n=2)
• No comparison between modalities (n=1)

Included in the review
n=11

Eligible for inclusion
n=9

More articles identified after reviewing references
n=2

Xe-CT (n=1)
PET (n=1)
SPECT (n=1)
DSC-MRI (n=3)
ASL (n=5)

Titles identified as relevant based on Medline and Embase search
n=3786
<table>
<thead>
<tr>
<th>Author, year</th>
<th>Mean age, y (range)</th>
<th>Total no.</th>
<th>Scanned no.</th>
<th>Inclusion criteria (no. in each group)</th>
<th>Type of test</th>
<th>Treatment history</th>
<th>Reference test</th>
<th>Index test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Numaguchi et al</td>
<td>NR (1-16)</td>
<td>25</td>
<td>25 (structural MRI) 25 (Xe-CT)</td>
<td>History of stroke (12) History of CNS symptoms including headache seizures, seizure-like episodes except stroke (13)</td>
<td>NR</td>
<td>Transfusion (12) No treatment (13)</td>
<td>structural MRI Xe-CT</td>
<td>Baseline CBF</td>
</tr>
<tr>
<td>Powars et al</td>
<td>NR (3-19)</td>
<td>49</td>
<td>49 (structural MRI) 49 (PET)</td>
<td>History of stroke (19) Prior hypoxic illness requiring hospitalization or soft neurologic signs (20) Neurological normal (10)</td>
<td>SS (49)</td>
<td>Transfusion (17) No treatment (32)</td>
<td>structural MRI PET (using $^{99m}$Tc HMPAO and FDG)</td>
<td>Baseline CBF and glucose metabolism</td>
</tr>
<tr>
<td>AL- Kandari et al</td>
<td>17 (8-45)</td>
<td>21</td>
<td>19 (structural MRI) 21 (SPECT)</td>
<td>Normal neurological assessment with no history of previous stroke or TIA or abnormal CT or MRI (21)</td>
<td>SS (13), Sj$^\beta$Thal (8) NR$^d$</td>
<td>NR$^d$ structural MRI</td>
<td>SPECT, (using $^{99m}$Tc HMPAO) DSC MRI</td>
<td>Baseline CBF</td>
</tr>
<tr>
<td>Tzika et al</td>
<td>NR (3-20)</td>
<td>33</td>
<td>33 (structural MRI) 33 (DSC MRI)</td>
<td>History of stroke (13) Absence of any clinical indication of stroke with normal MRI (20)</td>
<td>SS (32)$^c$ NR</td>
<td>NR structural MRI</td>
<td>DSC MRI</td>
<td>Baseline CBF</td>
</tr>
<tr>
<td>Kirkham et al</td>
<td>13 (4-34)</td>
<td>48</td>
<td>48 (structural MRI) 44 (TCD) 48 (DSC MRI)</td>
<td>History of stroke (8), TIA (8), RIND (6) seizure (6), severe headache /coma (1) No acute Neurological events (22)</td>
<td>SS (45), SC(1), Sj$^\beta$Thal (2) Transfusion (21) Hydroxyurea (1) No treatment (26)</td>
<td>Structural MRI, TCD DSC MRI</td>
<td>Baseline CBF, CBV, MTT</td>
<td>Baseline CBF</td>
</tr>
<tr>
<td>Grueneich et al</td>
<td>11.9 (9-16)</td>
<td>31</td>
<td>22 (structural MRI) 22 (DSC MRI)</td>
<td>No history of stroke or other neurological disorders (31)</td>
<td>SS (15), SC(9), Sj$^\beta$Thal (6), Sj$^\beta$Thal (1) NR</td>
<td>NR structural MRI</td>
<td>DSC MRI</td>
<td>Baseline CBF, CBV, MTT</td>
</tr>
<tr>
<td>Helton et al</td>
<td>12 (5-17) NR$^b$</td>
<td>21</td>
<td>21 (structural MRI) 21 (TCD) 21 (PASL)</td>
<td>No history of stroke or TIA (30)</td>
<td>SS (30) Hydroxyurea (18) No treatment (3)</td>
<td>Structural MRI, TCD PASL</td>
<td>Hydroxyurea (18)</td>
<td>Base line CBF</td>
</tr>
<tr>
<td>Oguz et al</td>
<td>8.7 (6-12)</td>
<td>18</td>
<td>18 (structural MRI) 16 (CASL)</td>
<td>No history of stroke, TIA, seizure and chronic transfusion or any drugs may affect CBF (18)</td>
<td>SS (18) No treatment (18)</td>
<td>No treatment (18) structural MRI</td>
<td>CASL</td>
<td>Baseline CBF</td>
</tr>
<tr>
<td>Strouse et al</td>
<td>8.5 (6-12)</td>
<td>24</td>
<td>24 (structural MRI) 24 (CASL)</td>
<td>No history of stroke, transfusion, seizure, previous traumatic brain injury and cognitive impairment with TCD less than 200cm/sec (24)</td>
<td>SS (24) Hydroxyurea (22) No treatment (2)</td>
<td>No treatment (2) structural MRI</td>
<td>CASL</td>
<td>Baseline CBF</td>
</tr>
<tr>
<td>Tweel et al</td>
<td>13.4 (12-18)</td>
<td>24</td>
<td>24 (structural MRI) 24 (TCD) 24 (CASL)</td>
<td>No history of neurological events with normal TCD (24)</td>
<td>SS or Sj$^\beta$Thal (24) No treatment (24)</td>
<td>No treatment (24) Structural MRI, TCD CASL</td>
<td>Baseline CBF</td>
<td>Baseline CBF</td>
</tr>
<tr>
<td>Hijmans et al</td>
<td>11.8 (6-18)</td>
<td>37</td>
<td>21 (structural MRI) 34 (TCD) 21 (CASL)</td>
<td>No prior overt stroke or cerebral bleeding receiving treatment for a severe SCD</td>
<td>SS (30), Sj$^\beta$Thal (4) Transfusion (3) No treatment (34)</td>
<td>No treatment (34) structural MRI, TCD CASL</td>
<td>Baseline CBF</td>
<td>Baseline CBF</td>
</tr>
</tbody>
</table>

$^{99m}$Tc HMPAO indicates $99m$ Tc-D, L-hexamethylene-propyleneamine oxime; CASL, continuous arterial spin labeling; CBF, cerebral blood flow; CBV, cerebral blood volume; CNS, central nervous system; DSC, dynamic susceptibility contrast; CT, computed tomography; ECMO, extra corporeal membrane oxygenation; F, female; FDG, fluorodeoxyglucose; M, male; MRI, magnetic resonance imaging; MTT, mean transit time; NR, not reported; PASL, pulsed arterial spin labeling; PET, positron emission tomography; RIND, reversible ischemic neurological deficit; SC, Sickle-Hemoglobin C Disease; Sj$^\beta$Thal, Sickle Beta-Null Thalassemia; Sj$^\beta$Thal, Sickle Beta-Plus Thalassemia; sMRI, structural MRI; SPECT, single photon emission tomography; SS, Sickle Cell Anemia; TCD, transcranial Doppler ultrasonography; TIA, transient ischemic attack; y, years old; Xe-CT, xenon computed tomography.

$^a$Median was under age 18.

$^b$15 out of 21 scanned subjects were male.

$^c$One patient without SCD who had undergone extracorporeal membrane oxygenation after cardiac surgery was also included.

$^d$6 patients had more than 2 transfusions prior to CBF study.
4.3.2 Study analysis

The risk of bias in the included studies is summarized in Figure 2 and Table 2. The overall included studies had moderate risk of bias. Four criteria (differential verification bias, acceptable reference test bias, study examination bias and withdrawal bias) had low risk of bias for all studies. In general, studies scored poorly on diagnostic and test review biases and were susceptible to relevant clinical information bias. Only four publications (Numaguchi et al. 1990; Powars et al. 1999; Tzika et al. 1993; Kirkham et al. 2001) had low risk of spectrum bias, indicating subjects with different clinical history of stroke were included.

4.3.3 Outcomes

To compare the prevalence of CBF abnormalities (index test) to structural MRI and TCD velocity abnormalities (reference tests); hemispheric asymmetry of CBF regardless of the type of CBF imaging modality was identified as the common criteria of CBF abnormality (index test) in ten studies (Numaguchi et al. 1990; Powars et al. 1999; Al-Kandari et al. 2007; Tzika et al. 1993; Kirkham et al. 2001; Grueneich et al. 2004; Helton et al. 2009; Oguz et al. 2003; van den Tweel et al. 2009; Schatz et al.). Hemispheric asymmetry was defined qualitatively based on visual inspection for the majority of the studies or defined as decreased CBF in gray matter relative to the contralateral side. In addition, four studies included a more quantitative approach by comparing absolute CBF measurements in different vascular territories between two hemispheres (van den Tweel et al. 2009; Helton et al. 2009; Oguz et al. 2003; Schatz et al.). One study (Strouse et al. 2006) was excluded from our analysis due to lack of individual CBF measurements and reference test data. For the reference test, tissue with increased T2 signal on structural MRI and a TCD velocity greater than 200 cm/s or lower than 70 cm/s was defined as abnormal. In addition, stroke and non-stroke groups were found in four (Numaguchi et al. 1990; Powars et al.

**CBF abnormalities versus structural MRI abnormalities.** Comparison within non-stroke groups in nine studies showed that the prevalence of CBF abnormalities defined with Xe-CT (Numaguchi et al. 1990), PET (Powars et al. 1999), SPECT (Al-Kandari et al. 2007), DSC-MRI (Grueneich et al. 2004; Kirkham et al. 2001) and ASL (Helton et al. 2009; Oguz et al. 2003; van den Tweel et al. 2009; Schatz et al.) was higher than those of structural MRI abnormalities. One remaining study with DSC-MRI (Tzika et al. 1993) demonstrated the identical prevalence for CBF and structural MRI abnormalities. Comparison within stroke groups showed that two studies with Xe-CT (Numaguchi et al. 1990) and DSC-MRI (Kirkham et al. 2001) identified similar prevalence and in two other studies with PET (Powars et al. 1999) and DSC-MRI (Tzika et al. 1993), the prevalence of structural MRI abnormalities was greater than those of CBF abnormalities. Comparison between groups showed that all four studies (Numaguchi et al. 1990; Powars et al. 1999; Tzika et
al. 1993; Kirkham et al. 2001) demonstrated higher prevalence of CBF and structural MRI abnormalities in stroke groups than non-stroke groups.

*CBF abnormalities versus TCD velocity abnormalities.* CBF abnormalities were more prevalent than TCD velocity abnormalities in non-stroke groups of the three ASL studies (Helton et al. 2009; van den Tweel et al. 2009; Schatz et al.) and one DSC-MRI study (Kirkham et al. 2001). Comparison within the stroke groups in the same DSC-MRI (Kirkham et al. 2001) study also showed a higher prevalence of CBF abnormalities than those of TCD velocity abnormalities.
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Representative of spectrum</td>
<td>Low risk</td>
<td>Low risk</td>
<td>High risk</td>
<td>Low risk</td>
<td>Low risk</td>
<td>High risk</td>
<td>High risk</td>
<td>High risk</td>
<td>High risk</td>
<td>High risk</td>
<td>High risk</td>
</tr>
<tr>
<td>3. Acceptable delay between tests</td>
<td>High risk</td>
<td>Unclear</td>
<td>High risk</td>
<td>Low risk</td>
<td>Low risk</td>
<td>Low risk</td>
<td>Low risk</td>
<td>Low risk</td>
<td>Low risk</td>
<td>Low risk</td>
<td>High risk</td>
</tr>
<tr>
<td>4. Partial verification avoided</td>
<td>Low risk</td>
<td>Low risk</td>
<td>Low risk</td>
<td>High risk</td>
<td>Low risk</td>
<td>Low risk</td>
<td>Low risk</td>
<td>Low risk</td>
<td>Low risk</td>
<td>Low risk</td>
<td>Low risk</td>
</tr>
<tr>
<td>5. Differential verification avoided</td>
<td>Low risk</td>
<td>Low risk</td>
<td>Low risk</td>
<td>Low risk</td>
<td>Low risk</td>
<td>Low risk</td>
<td>Low risk</td>
<td>Low risk</td>
<td>Low risk</td>
<td>Low risk</td>
<td>Low risk</td>
</tr>
<tr>
<td>6. Incorporation avoided</td>
<td>Low risk</td>
<td>Low risk</td>
<td>Low risk</td>
<td>High risk</td>
<td>High risk</td>
<td>High risk</td>
<td>High risk</td>
<td>High risk</td>
<td>High risk</td>
<td>High risk</td>
<td>High risk</td>
</tr>
<tr>
<td>7. Reference standard results blinded</td>
<td>Unclear</td>
<td>Low risk</td>
<td>Unclear</td>
<td>Unclear</td>
<td>Unclear</td>
<td>Unclear</td>
<td>Unclear</td>
<td>Unclear</td>
<td>Unclear</td>
<td>Unclear</td>
<td>Unclear</td>
</tr>
<tr>
<td>8. Index test results blinded</td>
<td>Low risk</td>
<td>Unclear</td>
<td>Unclear</td>
<td>Unclear</td>
<td>Unclear</td>
<td>Low risk</td>
<td>Unclear</td>
<td>Unclear</td>
<td>Unclear</td>
<td>Unclear</td>
<td>Unclear</td>
</tr>
<tr>
<td>9. Relevant clinical information</td>
<td>Unclear</td>
<td>Unclear</td>
<td>Unclear</td>
<td>Unclear</td>
<td>Unclear</td>
<td>Unclear</td>
<td>Unclear</td>
<td>Unclear</td>
<td>Unclear</td>
<td>Unclear</td>
<td>Unclear</td>
</tr>
<tr>
<td>10. Uninterpretable results reported</td>
<td>Low risk</td>
<td>Low risk</td>
<td>Low risk</td>
<td>Low risk</td>
<td>Low risk</td>
<td>Low risk</td>
<td>Low risk</td>
<td>Low risk</td>
<td>Low risk</td>
<td>Low risk</td>
<td>Low risk</td>
</tr>
<tr>
<td>11. Withdrawals explained</td>
<td>Low risk</td>
<td>Low risk</td>
<td>Low risk</td>
<td>Low risk</td>
<td>Low risk</td>
<td>Low risk</td>
<td>Low risk</td>
<td>Low risk</td>
<td>Low risk</td>
<td>Low risk</td>
<td>Low risk</td>
</tr>
<tr>
<td>12. Treatment history reported</td>
<td>Low risk</td>
<td>Low risk</td>
<td>High risk</td>
<td>Low risk</td>
<td>Low risk</td>
<td>High risk</td>
<td>Low risk</td>
<td>Low risk</td>
<td>Low risk</td>
<td>Low risk</td>
<td>Low risk</td>
</tr>
</tbody>
</table>
Figure 6. Risk of bias assessment in each category
<table>
<thead>
<tr>
<th>Study, year</th>
<th>Type of CBF imaging modality</th>
<th>Criteria of abnormality</th>
<th>Prevalence of abnormalities %, (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CBF measurement</td>
<td>structural MRI</td>
</tr>
<tr>
<td>Numaguchi et al (1990)</td>
<td>Xe-CT</td>
<td>(1) Definite: obvious decreased flow areas in more than two contiguous slices relative to the normally perfused cortex of the comparable opposite hemisphere. (2) Possible: extensive areas of decreased flow in a single slice area or multiple small areas of decreased flow in a single slice area, or in multiple noncontiguous slices areas</td>
<td>High signal intensity on T2-w images</td>
</tr>
<tr>
<td>Powars et al (1999)</td>
<td>PET</td>
<td>Decrease of blood perfusion in the gray matter regions</td>
<td>High signal intensity on T2-w images</td>
</tr>
<tr>
<td>AI-Kandari et al (2007)</td>
<td>SPECT</td>
<td>The perfusion deficit: mild (10%–25%), moderate (25%–50%), severe (more than 50%): The contralateral side of each slice was used as the control for each patient with hypoperfusion.</td>
<td>Evidence of infarction on T1-w, T2-w and PD images</td>
</tr>
<tr>
<td>Tzika et al (1993)</td>
<td>DSC-MRI</td>
<td>Asymmetry in reduction of signal intensity between right and left</td>
<td>High signal intensity on T2-w images</td>
</tr>
<tr>
<td>Kirkham et al (2001)</td>
<td>DSC-MRI</td>
<td>Focal reduction in CBF, increase in MTT, reduction or increase in CBV, or increase in TTP</td>
<td>Changes in signal intensity on T2-w High ADC on DWI MCA velocity: &lt;70 or &gt;200 cm/s</td>
</tr>
</tbody>
</table>
### Table 4. Results (continue)

<table>
<thead>
<tr>
<th>Study, year</th>
<th>Type of CBF imaging modalities</th>
<th>Criteria of abnormality</th>
<th>Prevalence of abnormalities %, (95% CI, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>stroke group</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>structural MRI</td>
</tr>
<tr>
<td>Helton <em>et al</em> (2008)</td>
<td>PASL</td>
<td>rCBF asymmetry of CBF within ACA, ACAP, MCA, MCPP, PCA, PCAP defined as: if a statistically significant (P values&lt;0.0002) difference existed between the left and right brain.</td>
<td>High signal intensity areas on T2-w and FLAIR</td>
</tr>
<tr>
<td>Oguz <em>et al</em> (2003)</td>
<td>CASL</td>
<td>rCBF asymmetry within ACA, MCA, PCA defined as: $R = \frac{f_L - f_R}{\text{max}(f_L, f_R)}$, Then ratio $R$ with a central reduced normal variable $K(R)$ were associated, defined as: $K(R) = \frac{R - \bar{R}}{\sigma_R}$</td>
<td>High signal intensity areas on T2-w and FLAIR</td>
</tr>
<tr>
<td>Strouse <em>et al</em> (2006)</td>
<td>CASL</td>
<td>NR</td>
<td>High signal intensity area ≥ 3 mm in diameter on T2-w or FLAIR consistent with ischemia and seen in two planes</td>
</tr>
<tr>
<td>Tweel <em>et al</em> (2009)</td>
<td>CASL</td>
<td>rCBF asymmetry within ACA, MCA, PCA defined as: difference in rCBF lower than 11.7 mL/100 g/min between right and left.</td>
<td>High signal intensity on T2-w images</td>
</tr>
<tr>
<td>Hijmans <em>et al</em> (2011)</td>
<td>CASL</td>
<td>A difference in blood flow between both hemispheres&gt;11.7 mL/100 g/min</td>
<td>Evidence of silent infarct on MRI</td>
</tr>
</tbody>
</table>

- indicates no entry; $f_L$, $f_R$ indicate CBF in the left and right territories; $\bar{R}$ and $\sigma_R$, nonbiased estimators of the mean and SD of $R$ over a normal population calculated with data from the group of volunteers; ACA, anterior cerebral artery; ACAP, anterior cerebral artery perforator; ADC, apparent diffusion coefficient; CASL, continuous arterial spin labeling; CBF, cerebral blood flow; CBV, cerebral blood volume; CI, confidence interval; dICA, distal internal carotid artery; DSC, dynamic susceptibility contrast; DWI, diffusion weighted imaging; FLAIR, fluid attenuated inversion recovery; MCA, middle cerebral artery; MCPP, middle cerebral artery perforator; MTT, mean transit time; MRI, magnetic resonance imaging; NR, data is not reported; PASL, pulsed arterial spin labeling; PCA, posterior cerebral artery; PCAP, posterior cerebral artery perforator; PD, proton density; PET, positron emission tomography; pMCA, proximal middle cerebral artery; rCBF, regional cerebral blood flow; SPECT, single photon emission tomography; T1-w, T1 weighted; T2-w, T2 weighted; TCD, transcranial Doppler ultrasonography; Xe-CT, xenon computed tomography.
4.4. Discussion

In this review, we included 11 studies, which assessed CBF abnormalities in pediatric patients with SCD and compared them to TCD velocity and structural MRI abnormalities. We identified that CBF and structural MRI abnormalities were more prevalent in patients who previously had a stroke compared to those who did not. In the stroke groups, the prevalence of CBF abnormalities was equal to or lower than those of MRI abnormalities. In the non-stroke groups, the prevalence of CBF abnormalities was equal to or greater than those of structural MRI abnormalities. In addition, CBF abnormalities were more prevalent than TCD velocity abnormalities in four studies of non-stroke patients as well as in one study of stroke patients.

To our knowledge, this review is the first to systematically compare a range of CBF imaging modalities with structural MRI and TCD velocity measurements in children with SCD. In previous reviews the role of CBF imaging modalities in children with SCD was only partly discussed (Powars 2000; Winrow and Melhem 2003; Arkuszewski, Melhem, and Krejza). Furthermore, additional CBF studies, in particular ASL studies were not included in these reviews. In 2000, a review by Powars et al (Powars 2000) discussed the management of cerebral vasculopathies in children with SCD and briefly reviewed Xe-CT, PET and SPECT CBF studies. However, perfusion MRI methods examining CBF were not considered. In 2003, Winrow and Melhem (Winrow and Melhem 2003) evaluated utility and accuracy of clinical as well as neuroimaging examinations in children with SCD. The authors did review CBF measurements using Xe-CT, PET, DSC-MRI studies as well as one preliminary ASL study but omitted a detailed assessment of bias. In 2010, Arkuszewski et al (Arkuszewski, Melhem, and Krejza) reviewed neuroimaging in assessment of risk of stroke in children with SCD. The authors discussed a comprehensive array of neuroimaging modalities such as CT, MRI, TCD as well as nuclear medicine techniques. However, the discussion on CBF measurements was very minimal,
except for two PET studies with only one being performed in children with SCD. Furthermore, the assessment of bias was lacking and advanced CBF methods based on MRI such as DSC and ASL were not included. However, the authors provided a good overview of the use TCD and structural MRI in the assessment of children with SCD. Overall, the listed reviews lacked a comprehensive analysis in order to draw a reliable conclusion with regard to the utility of CBF assessment in children with SCD. Although the CBF assessment was recommended in children with SCD, the recommendations were drawn solely from individual results of the included studies and had no comparison to standard reference tests.

Cerebral blood flow is an important indicator of cerebral function and impaired CBF seems to be the pathway through which cerebral injury occurs in SCD. In vivo, CBF can be assessed in several ways, for example by measuring absolute or relative CBF or by comparing the CBF map of two hemispheres against each other to evaluate CBF asymmetry. In general, measurements of CBF require a tracer whose tissue distribution can be modeled. Different types of tracers (diffusible, intravascular, endogenous or exogenous) are used depending on the type of CBF imaging modality. Almost all CBF imaging modalities employ modeling of tracer kinetics (the contrast agent transport in tissue), using either compartment models developed by Kety and Schmidt (Verlhac 1999) for example in PET, SPECT, Xe-CT and ASL or some version of the indicator dilution theory developed by Meier and Zierler (Meier and Zierler 1954) in case of DSC MRI. However, a series of assumptions using these models as well as complex technical requirements can lead to variation in absolute CBF values. A simpler alternative is the qualitative assessment of CBF asymmetry. This can be done visually by comparing a CBF map of two hemispheres (Adams et al. 1988). CBF asymmetry can also be assessed more quantitatively by comparing absolute CBF measurements of the different vascular territories between hemispheres. In our review, there was a lack of individual absolute CBF in the evaluated studies, we could
therefore only systematically assess CBF asymmetries compared to the reference tests (structural MRI or TCD).

The comparison of CBF asymmetries with structural MRI within the included studies identified a considerable percentage of children with no previous history of overt stroke who had CBF asymmetries but a normal structural MRI exam. Stenosis of large cerebral arteries is probably the main reason for this CBF asymmetry, and in fact progression of stenosis is more often associated with clinically overt stroke (Stockman et al. 1972; Gerald, Sebes, and Langston 1980; Russell et al. 1984). However, a number of CBF asymmetries without corresponding stenosis of large cerebral arteries were also reported in four of the included studies (Kirkham et al. 2001; Oguz et al. 2003; Helton et al. 2009; van den Tweel et al. 2009). Occlusion of small cerebral arteries caused by abnormal adherence of sickle shaped blood cells as well as impaired vasodilatory response are thought to be responsible for perfusion deficiencies in the absence of large artery stenosis. Asymmetry of CBF in patients with normal structural MRI presumably represents some brain tissue at risk for ischemia (Levine, Rozental, and Nickles 1992).

Currently, risk of ischemic stroke in SCD children is determined with a threshold TCD velocity $>200$ cm/s and correlates with either a focal stenosis $\geq 50\%$ in the large cerebral arteries (Wintermark et al. 2005), hyperemia or both. However, abnormal TCD velocity in this review was less common compared to CBF asymmetries identified by perfusion MRI techniques (DSC and ASL). TCD velocity seems to be less sensitive to perfusion disturbances at tissue level than CBF. Furthermore, concerns about the accuracy of this TCD velocity threshold as well as operator dependence of TCD measurements may invalidate the interpretation of TCD measurements.
It is also important to note that TCD cannot distinguish whether high blood flow velocities in SCD are due to stenosis or hyperemia. TCD does not provide CBF values, as TCD cannot easily measure the diameter of the large cerebral arteries accurately. Furthermore, previous studies demonstrated poor correlation between TCD velocity and perfusion measurements at the tissue level (Hartmann, Ries, et al. 1991; Bishop et al. 1986). However, it should be noted that TCD velocity can be used in children with SCD to reveal perfusion asymmetry when compared to the contralateral hemisphere (Arkuszewski et al.), but this measure has not been used as a comparison in any of the studies we evaluated. Further studies are needed to investigate whether perfusion asymmetries obtained by TCD correlate with other neuroimaging modalities.

This review has several strengths including a comprehensive search strategy, no language restriction, inclusion of studies limited to pediatric population, inclusion of various CBF imaging modalities such as Xe-CT, PET, SPECT and perfusion MRI techniques, comparison to current clinical standards (structural MRI and TCD) and risk of bias assessment. However, our review has some limitations that warrant consideration. Heterogeneity among the CBF imaging modalities prevented meta-analysis. We also were not able to compare the specificity of CBF abnormalities with those obtained with structural MRI and TCD velocity. Patient selection can be biased in individual studies; however, bias at article selection was avoided by strict inclusion and exclusion criteria.

Based on the findings of this review, we are not in the position to provide clear guidelines for clinical practice. To date, TCD and structural MRI have remained the first choice in clinical decision making in SCD, at least in part due to the lack of research with regard to hemodynamic risk factors associated with stroke in SCD such as CBF abnormalities.
However, the usefulness of CBF measurements in children with SCD should be further investigated. Perfusion MRI techniques including DSC and ASL, due to the lack of radiation exposure are usually preferred to nuclear medicine techniques in children. Compared to ASL, DSC provides higher spatial resolution, requires shorter scanning time and can measure other hemodynamic parameters such as cerebral blood volume (CBV), mean transit time (MTT) simultaneously. However, DSC requires the use of gadolinum based contrast agents, which can potentially increase the risk of vaso-occlusive crisis. Safety of these contrast agents has not been yet fully established in SCD patients (OptiMark 2009). Therefore, ASL is probably a better choice for CBF assessment in children, as it only uses blood water as an endogenous contrast agent. Although, ASL is currently a bit more restricted to the exploration of a limited region of the brain and has an inherently weak signal-to-noise ratio, future developments such as availability of high-field MRI can enhance the role of ASL in CBF assessment studies.

Application of other neuroimaging methods may further characterize CBF abnormalities and possibly assist in managing SCD patients with such abnormalities. In particular, the need for a high resolution angiographic assessment is necessary to exclude stenosis of intracranial arteries. Conventional digital subtraction angiography is the most reliable way to infer cerebrovascular complications such as stenosis and associated collateral vessels, but is usually risky compared to other techniques such as Magnetic resonance imaging (MRA). MRA is frequently the angiography of choice in children, which can readily be combined with MRI perfusion techniques.

Other physiological parameters should be considered to thoroughly understand the complex pathophysiology of SCD. For instance, autoregulatory cerebral vasodilation can be assessed to reflect the reserve capacity of the cerebral circulation to increase CBF. In addition, the possible role of blood viscosity in regulation of CBF should be examined in SCD patients.
In summary, our results suggest that the assessment of CBF in children with SCD merits further investigation, as CBF abnormalities are potentially associated with risk for ischemia. Longitudinal studies are required to determine the clinical value of CBF.
Chapter 5

Absolute values and interhemispheric ratios of
CBF assessed by ASL and TCD in children
with SCD
Chapter 5 Absolute values and interhemispheric ratios of CBF assessed by ASL and TCD in children with SCD

5.1. Introduction

Sickle cell disease (SCD) is among the most common causes of ischemic stroke in children, which leads to increased morbidity and mortality rate (Platt et al. 1994; Prengler et al. 2002). The mechanisms responsible for cerebral ischemic events in sickle cell disease (SCD) are very complex and seem to be related to abnormal blood flow (Adams 2007; Cheung et al. 2002). Blood flow abnormalities can be due to the narrowing or occlusion of cerebral vessels, as well as hyperemia associated with chronic anemia. Currently, the blood flow velocity of the middle cerebral artery (MCA), or the terminal portion of the internal carotid artery as measured by transcranial Doppler (TCD) ultrasonography, is used to stratify the risk of overt stroke in children with SCD. However, a significant percentage of low risk patients with normal TCD velocity still suffer silent infarction, which in turn increases the risk of overt stroke and is also associated with neurocognitive deficits.

Silent infarctions reveal a different pathology of cerebral infarction in SCD. They are usually seen in the deep WM, where deep penetrating arterioles have limited anastomoses, thereby reducing cerebrovascular reserve capacity and hence are predisposed to ischemia under circumstances in which CBF is insufficient to meet metabolic demand (Ovbiagele and Saver 2006). This pattern of infarction suggests a more complex inherited vasculoopathology in SCD, affecting not only large arteries of the Circle of Willis, but also smaller arteries at the tissue level (Cheung et al. 2001). Thus, advanced neuroimaging techniques may be required to identify related mechanisms, which are likely to remain unseen when relying solely on TCD.
Cerebral blood flow (CBF) imaging techniques can provide information on the effects of small vessel disease on cerebral perfusion, by demonstrating the delivery of oxygen and nutrients at the tissue level, which is quantitated in mL blood/minute/100 gram brain tissue. Various imaging techniques—such as Xenon computed tomography (Xe-CT), Positron emission tomography (PET), Single photon emission tomography (SPECT) and dynamic susceptibility contrast MRI—have been previously used to assess CBF at tissue level. However, the need for radiation and/or inhalation or injection of a contrast agent limits their application in children. Recently, arterial spin labeling (ASL) has been developed to non-invasively assess the cerebral vasculature. ASL does not require a contrast agent injection; but instead employs the magnetization of flowing blood as an endogenous tracer. ASL has been successfully employed in previous studies of SCD. Researchers have demonstrated that CBF abnormalities exist in otherwise neurologically normal children with SCD (Oguz et al. 2003; van den Tweel et al. 2009; Helton et al. 2009) and have raised the question as to whether these CBF abnormalities could be an indicator of cerebral infarction. In the present study, we tested the hypothesis that CBF abnormalities assessed with ASL, that exist in the absence of established TCD velocity abnormalities, can be identified in neurologically normal SCD children and if they are associated with silent infarctions. The purpose of our study was to obtain absolute values and interhemispheric ratios of blood flow in the MCA territory using ASL and TCD in children with SCD, and to examine the association between CBF abnormalities and the evidence of silent infarction imaged on structural MRI.
5.2. Materials and methods

5.2.1 Study population

Children with SCD were recruited from the Sickle cell clinic in the department of Hematology, at the Hospital for Sick Children in Toronto, from January 2011 to March 2012. Children between 10 and 18 years old—with different variants of SCD including HbSS and HbSβ^+/0, as confirmed by high performance liquid chromatography—were eligible for this study. Patients with a history of focal neurologic events lasting more than 24 hours and with medical documentation of, or a history of, prior overt stroke or chronic blood transfusion therapy in the preceding 3 months were excluded.

Healthy children matched for age and gender with no previous history of cerebrovascular disease, were recruited as control subjects. All experiments were performed under informed consent and the guidelines approved by the Hospital for Sick Children Research Ethics Board. All participants received verbal and written explanations of the objectives and procedures of the study.

5.2.2 Magnetic resonance imaging

Each subject was scanned in Department of Diagnostic Imaging at the Hospital for Sick Children. MRI was performed on a 3.0T MRI scanner (Magnetom TrimTrio, Siemens Medical Solutions, Erlangen, Germany) using a 32-channel head coil. Aside from time of flight (TOF) angiography, high resolution FLAIR (fluid attenuation inversion recovery) and T_2-weighted fast spin echo images used for clinical purposes, pulsed ASL (PASL) was obtained using a PICORE Q2T sequence (Wong, Buxton, and Frank 1998). The imaging parameters for the PASL sequence were: TR/TE 2500/13 ms; FOV 220×220; matrix size: 64×64; slices: 13; slice thickness: 4.5 mm;
slice gap: 0.5 mm; single shot EPI; post-labeling delay time: 1800 ms; Saturation stop time: 1600 ms; background suppression; number of dynamics: 45.

5.2.3 Transcranial Doppler ultrasonography (TCD)

In addition, blood flow velocities of the right and left MCA of all subjects were assessed by a TCD system (iU-22 xMatrix; Philips Electronics, Best, the Netherlands) equipped with a S5-1 transducer. With the subject in a supine position and breathing normally, an ultrasound probe was placed on the acoustic temporal window to locate the MCA. The time average peak velocity (TAPV) of the M1 segment of each MCA was then recorded three consecutive times.

5.2.4 Data analysis

**ASL**

The conventional MRI series, including T2, FLAIR and MRA were reviewed by an experienced neuroradiologist (M.S.), who was blinded to the clinical and ASL data; special attention was paid to the presence of cerebral infarction and vasculopathy. Cerebral infarctions were identified as an area of hyperintensity on T2 or FLAIR and lesions were classified according to their size and location. Cerebral vasculopathy was scored based on focal or diffuse changes in the caliber of intracranial arteries.

The ASL data were transferred to an independent workstation and imported into MATLAB (Mathworks Inc.). An SPM5-based ASL data processing toolbox (ASLtbx), provided by Wang et al. 2007 (http://cfn.upenn.edu/perfusion/software.htm), was used for ASL data analyses. The raw images were preprocessed using the following steps before quantitative CBF was calculated. Each
ASL dynamic was realigned to the first dynamic of the series using a 6 parameter motion correction algorithm. The realigned ASL images were coregistered to each subject’s structural image by applying a transformation; this was achieved by modifying the header file of each functional image directly. The SPM Gaussian smoothing kernel was then used to spatially smooth the realigned and co-registered images to reduce noise. A mask based on the mean of the functional images was then created; it was used to exclude outliers during ASL perfusion subtraction.

Anatomic images were automatically segmented into gray matter (GM), white matter (WM) and cerebrospinal flow (CSF) using the FAST tool (FMRIB's Automated Segmentation Tool). Because vascular territories vary among individuals, we arbitrarily determined the MCA territories according to the atlas prepared by Kretchman and Wolfgang (Kretschmann HJ 2003). The cortical and subcortical MCA territory included the middle and inferior frontal gyri, precentral gyrus; superior and inferior parietal gyri, angular gyrus, postcentral gyrus; supramarginal gyrus; superior, middle, inferior and transverse temporal gyri, and superior and middle occipital gyri. The basal ganglia, internal capsule, and thalamus as well as the anatomic watershed areas between the MCA and anterior cerebral artery and between the MCA and posterior cerebral artery were excluded in the analysis to examine MCA territory independently. Areas of hyperintensities on the FLAIR MR images, depicting areas of infarction, were excluded from the respective MCA territory. Subsequently, these territories were applied to registered ASL images. T1 arterial blood was corrected for the individual hematocrit (Hct) level according to the following equation (Lu et al. 2004):

$$T1a = \frac{1}{0.52 \text{ Hct} + 0.38}$$
Hematocrit values obtained from the last clinic visit within one month of the experiment were used for patients. A Hct level of 0.4 was also assumed for healthy subjects. The CBF was then calculated in each subject for each MCA territory that was thresholded for both GM and WM before and after Hct correction.

**TCD**

The highest TAPV among the three measurements of each MCA was selected for analysis. For all TCD measurements, CBF in the MCA territory (T) was obtained from velocity with the following equation (Schatlo and Pluta 2007):

\[
\text{TCD-CBF} = \frac{(V_{av} \times A)}{T}
\]

Where \( V_{av} \) and \( A \) are the area and the cross sectional average blood velocity (\( V_{av} \)) of the MCA, respectively. Therefore, to calculate CBF from the blood velocity of the MCA, one must know the cross sectional average blood velocity as well as the luminal area and perfusion territory of the MCA.

In this study, \( V_{av} \) was calculated based on the following formula for TAPV used by Seidel et al (Seidel et al. 1999):

\[
V_{av} = \frac{(\text{TAPV}/2)}
\]

MRA images (TOF) were acquired for evaluation of MCA diameter (\( d \)) in each hemisphere and luminal area (\( A \)) at the same levels as TCD measurements and was calculated as follows:

\[
A = (d/2)^2 \times \pi
\]
T1-weighted images with a voxel size of 1×1×1 mm were used to determine the volumes of different brain tissue types including grey matter (GM), WM and CSF by summing all voxels designated as such within the MCA territory. Subsequently, an estimation of weight of MCA territory can be acquired by converting the MRI-derived volumes of parenchyma (GM and WM) and CSF into grams per milliliter and then by summing these values as follows:

MCA territory in grams = (GM volume + WM volume in milliliters x 1.0365 g/mL) + (CSF volume in milliliters x 1.00 g/mL) (Frontera 1958; Stephan 1960).

**Interhemispheric ratio**

For ASL, TAPV and TCD-CBF values, a left to right (L/R) interhemispheric ratio was also calculated. If the interhemispheric ratio exceeded two standard deviations from the mean established by the control group, it was regarded as asymmetric (Appendix B).

### 5.2.5 Statistical Analyses

Statistical analysis was performed using the STATA software (Stata Corporation, USA). All data are expressed as mean ± SD. An unpaired Student’s *t* test was used to compare the average age, Hct, ASL, TAPV and TCD-CBF values between the patients and controls. Interhemispheric correlation of ASL and TCD measurements as well as correlation between ASL, TAPV and TCD-CBF absolute values and interhemispheric ratios were assessed using a Pearson correlation (*r*). In addition to correlation analysis, Bland–Altman analyses were performed to display the spread and the limits of agreement between ASL and TCD for inter-hemispheric ratio when TAPV and CBF were obtained with TCD. Bland Altman presents a range in which 95% of the differences lie. Absolute ASL, TAPV and TCD-CBF values were defined as abnormal if they exceeded two
standard deviations from the mean established by the control group (Appendix B). For all statistical tests used, \( P \) values < 0.05 were considered significant.

## 5.3. Results

### 5.3.1 Patients’ characteristics

From January 2011 to March 2012, we enrolled 15 patients with SCD (12 girls, 3 boys; average age at time of imaging: 13.86 years; age range: 11-18 years) and 10 control subjects (5 girls, 5 boys; average age at time of imaging: 13.8 years; age range: 10-18 years). Thirteen patients had homozygous sickle cell (HbSS), two had sickle-\( \beta \) thalassemia (HbS/\( \beta \)+) and one was taking Hydroxyurea. None of the SCD patients had any neurologic abnormalities based on the standardized examination. Hct values checked during the patient’s last clinic visit were compared to reference value (0.4 g/L) in healthy children (Table 5).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients</th>
<th>Controls</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>13.86</td>
<td>13.8</td>
<td>0.72</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>3/12</td>
<td>5/5</td>
<td>0.12</td>
</tr>
<tr>
<td>Hct (g/L)</td>
<td>0.265</td>
<td>0.4(^a)</td>
<td>&lt; 0.001(^c)</td>
</tr>
<tr>
<td>Stenosis on MRA</td>
<td>0/15</td>
<td>0/10</td>
<td>-</td>
</tr>
<tr>
<td>Silent infarction</td>
<td>7(^b)/15</td>
<td>0/10</td>
<td>-</td>
</tr>
</tbody>
</table>

\(^a\) Hct 0.4 was assumed for control group.

\(^b\) Four were in MCA territory and three were out of MCA territory.

\(^c\) statistically significant
5.3.2 Structural MRI and MRA

Seven patients (one male and six females) had silent infarctions on FLAIR images. Four of seven patients had silent infarctions inside the MCA territories (unilaterally in two patients and bilaterally in other two patients) and the remaining three patients had silent infarctions outside the MCA territory (unilaterally in two patients and bilaterally in another patient). Eight patients (two males and six females) had normal appearing studies (Figure 7). The average ages of patients with and without silent infarction were 13.86 and 13.88, respectively (Figure 8). There was no significant difference between two groups with respect to age and gender ($p = 0.1$ and $p = 0.6$, respectively). No vessel stenoses or tortuosity was identified in any of the included patients.

![Figure 7](image_url)

**Figure 7.** Bar graph demonstrates sex ratio in 8 SCD patients without silent infarction (2 males and 6 females) and 7 SCD patients with silent infarction, including inside and outside the MCA territory (one male and 6 females). No significant difference ($p$ value = 0.6) was found in sex ratio between two groups. $SI - =$ patients without silent infarction, $SI + =$ patients with silent infarction, including inside and outside the MCA territory.
Figure 8. Bar graph demonstrates no significant difference ($p$ value = 0.1) in mean age between SCD patients without silent infarction and SCD patients with silent infarction, including inside and outside the MCA territory (13.88 years old and 13.86 years old, respectively). $SI−$ = patients without silent infarction, $SI+$ = patients with silent infarction, including inside and outside the MCA territory.

5.3.3 Cerebral blood flow measured by ASL

ASL data were acquired from 15 patients and 8 healthy subjects. Two controls were excluded from ASL analysis due to severe motion artifact. The average CBF obtained with ASL for the MCA territory was $55.25 \pm 8.98$ and $47.33 \pm 8.1$ ml/min/100g without and with Hct correction in patients, respectively and $32.49 \pm 5.99$ ml/min/100g in controls. Mean CBF in patients was significantly higher compared to control subjects ($p < 0.001$ without and with Hct correction). The mean CBF values of GM and the WM of MCA territory in patients without and with Hct correction are presented in Table 6.
Table 6. The mean values of CBF obtained with ASL in GM and WM of MCA territory calculated for the patient group without and with Hct correction

<table>
<thead>
<tr>
<th>CBF measurements</th>
<th>- Hct correction</th>
<th>+ Hct correction</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GM (ml/min/100g)</td>
<td>67.15 ± 13.1</td>
<td>54.48 ± 8.5</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>WM (ml/min/100g)</td>
<td>40.12 ± 9.76</td>
<td>36.31 ± 10.77</td>
<td>0.158</td>
</tr>
</tbody>
</table>

CBF values presented with and without Hct correction; Results presented as Mean (±SD).

Abnormal absolute ASL values (>2SD of control mean) were found bilaterally in the MCA territories of nine patients and unilaterally in other two patients. The CBF values between the two hemispheres, in both the GM and the WM, were significantly correlated (p values, 0.001 and 0.001 in patients, 0.007 and < 0.001 in controls, respectively). The correlation values in the patient group were less than those in the control group (r values, 0.75, 0.79 and 0.89, 0.93 in GM and WM of patients and controls, respectively (Figures 7 and 8).
Figure 9. Correlation of CBF values obtained in GM of MCA territories with ASL between left and right hemispheres. CBF values in GM of MCA territory were significantly correlated between left and right hemispheres in 11 patients with no silent infarction inside the MCA territory \((r = 0.78, p = 0.003)\) and in 8 healthy controls \((r = 0.79, p = 0.007)\). The correlation value in 4 patients with silent infarction inside the MCA territory was 0.68 but was not statistically significant \((p = 0.3)\). MCA = middle cerebral artery, \(-SI\) = patients with no silent infarction inside the MCA territory, \(+SI\) = patients with silent infarction inside the MCA territory.
Figure 10. Correlation of CBF values obtained in WM of MCA territories with ASL between left and right hemispheres. CBF values in WM of MCA territories were significantly correlated between left and right hemispheres in 11 patients with no silent infarction inside the MCA territory ($r = 0.87$, $p < 0.001$) and in 8 healthy controls ($r = 0.93$, $p < 0.001$). The correlation value in 4 patients with silent infarction inside the MCA territory was 0.89 but was not statistically significant ($p = 0.1$). $MCA =$ middle cerebral artery, $-SI =$ patients with no silent infarction inside the MCA territory, $+SI =$ patients with silent infarction inside the MCA territory.

5.3.4 Flow velocity measured by TCD

TCD data was acquired from 14 patients and 7 healthy controls. In one patient, the acoustic window was not found. Three healthy subjects did not agree to participate in TCD experiment. The average TAPV was $94.02 \pm 17.54$ cm/s and $65.89 \pm 16.13$ cm/s in patients and controls, respectively. Averaged TAPV value was, as expected, significantly higher in patients compared to the control group ($p < 0.001$). In addition, abnormal absolute TAPV values (>2SD of control
mean) were only identified bilaterally in six and unilaterally in two of those patients with abnormal ASL values \((p = 0.04\)). The correlation between left and right TAPV values was 0.81 \((p < 0.001\)) in patients and 0.89 \((p = 0.002\)) in controls (Figure 9).

![Figure 11. Correlation of TAPV values between left and right MCA. TAPV values obtained from left and right MCA were significantly correlated in 10 patients with no silent infarction inside the MCA territory \((r = 0.84, p = 0.002\)) and in 7 healthy controls \((r = 0.89, p = 0.002\)). The correlation value in 4 patients with silent infarction inside the MCA territory was 0.87 but was not statistically significant \((p = 0.13\)). MCA = middle cerebral artery. −SI = patients with no silent infarction inside the MCA territory, +SI = patients with silent infarction inside the MCA territory.](image)

### 5.3.5 Cerebral blood flow measured by TCD

To obtain CBF from TCD (TCD-CBF), both MRI and TCD data was required. TCD-CBF was acquired from 14 patients and 5 healthy controls. One patient and five healthy subjects were excluded due to lack of either MRI or TCD data. TCD-CBF was \(83.29 \pm 31.89\) ml/min/100g and
50.86 ± 6.98 ml/min/100g in patients and controls, respectively. Mean TCD-CBF in patients was significantly higher when compared to control subjects ($p = 0.0006$). Abnormal TCD-CBF values were found bilaterally in eight and unilaterally in two of those patients with abnormal ASL value. The TCD-CBF values between the two hemispheres were significantly correlated ($r = 0.93$ and $p < 0.001$ in patients, $r = 0.95$ and $p = 0.01$ in controls) (Figure 10).

![Figure 12](image_url)

**Figure 12.** Correlation of TCD-CBF values between left and right MCA territory. TCD-CBF values obtained in the MCA territory were highly correlated between left and right hemispheres in 10 patients with no silent infarction inside the MCA territory ($r = 0.98, p < 0.001$) and in 5 healthy controls ($r = 0.95, p = 0.01$). The correlation value in 4 patients with silent infarction inside the MCA territory was 0.88 but was not statistically significant ($p = 0.12$). MCA = middle cerebral artery, −SI patients = patients with no silent infarction inside the MCA territory, +SI patients = patients with silent infarction inside the MCA territory.
5.3.6 Correlation of absolute values between ASL, TAPV and TCD-CBF

The individual ASL data were plotted separately against TAPV and TCD-CBF values (Figures 11 and 12). These plots and the results of the statistical analysis shown in Table 7 suggest that a close correlation is observed between ASL and TCD measurements in both patients and controls only when CBF values derived from TCD were used.

Figure 13. Correlation of absolute values between ASL and TAPV. No significant correlation was found between ASL and TAPV values in MCA territories of 10 patients with no silent infarction inside the MCA territory ($r = 0.01, p = 0.96$), in MCA territories of 4 patients with silent infarction inside the MCA territory ($r = 0.08, p = 0.84$) and in MCA territories of 5 healthy controls ($r = 0.2, p = 0.5$). $-$SI = patients with no silent infarction inside the MCA territory, +SI = patients with silent infarction inside the MCA territory.
Figure 14. Correlation of absolute values between ASL and TCD-CBF. A significant correlation was found between ASL and TAPV values in MCA territories of 4 patients with silent infarction inside the MCA territory ($r = 0.92$, $p = 0.001$) and in MCA territories of 5 healthy controls ($r = 0.72$, $p = 0.02$). The correlation value in MCA territories of 10 patients with no silent infarction inside the MCA territory was 0.13 ($p = 0.59$). $-SI$ = patients with no silent infarction inside the MCA territory, $+SI$ = patients with silent infarction inside the MCA territory.

Table 7. Results of correlation between ASL, TAPV and TCD-CBF values

<table>
<thead>
<tr>
<th>Group</th>
<th>ASL vs. TAPV</th>
<th>ASL vs. TCD-CBF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$r$</td>
<td>$P$ value</td>
</tr>
<tr>
<td>Patients</td>
<td>0.05</td>
<td>0.8</td>
</tr>
<tr>
<td>Healthy controls</td>
<td>0.2</td>
<td>0.5</td>
</tr>
</tbody>
</table>
5.3.7 Correlation of interhemispheric ratios between ASL, TAPV and TCD-CBF

The interhemispheric ratios obtained with ASL and TAPV in patients ranged between 0.84-1.21 and 0.73-1.13, respectively. No significant correlation was found between ASL and TAPV ratios within either patient or control group ($r = 0.1, p = 0.75$ and $r = 0.1, p = 0.88$, respectively) (Figure 13).

**Figure 15.** Correlation between interhemispheric ratios of ASL and TAPV. No significant correlation was found between interhemispheric ratios of ASL and TAPV obtained in MCA territories of 10 patients with no silent infarction inside the MCA territory ($r = -0.43, p = 0.2$), in MCA territories of 4 patients with silent infarction inside the MCA territory ($r = 0.53, p = 0.47$) and in MCA territories of 5 healthy controls ($r = 0.1, p = 0.88$). -SI = patients with no silent infarction inside the MCA territory, +SI = patients with silent infarction inside the MCA territory.
However, as shown in Figure 14, CBF interhemispheric ratio obtained with ASL correlated significantly with those from TCD for both patients and controls ($r = 0.79$, $p = 0.001$ and $r = 0.94$, $p = 0.014$, respectively). This was consistent during the measurements for patients without silent infarction in the MCA territory as well as for patients with silent infarction in the MCA territory, where the correlation varied from 0.75 to 0.81.

**Figure 16.** Correlation between interhemispheric ratios of ASL and TCD-CBF. A significant correlation was found between interhemispheric ratios of ASL and TCD-CBF obtained in MCA territories of 10 patients with no silent infarction inside the MCA territory ($r = 0.75$, $p = 0.01$) and in MCA territories of 5 healthy controls ($r = 0.94$, $p = 0.014$). The correlation value in MCA territories of 4 patients with silent infarction inside the MCA territory was 0.81 but was not statistically significant ($p = 0.2$). $-SI$ = patients with no silent infarction inside the MCA territory, $+SI$ = patients with silent infarction inside the MCA territory.
The results of the Bland-Altman plots are shown in Figure 15 A and B. Interhemispheric ratios obtained with ASL plotted against the corresponding interhemispheric TAPV and TCD-CBF ratios. Mean difference (±95% limits of agreement) was 0.093 (±0.27) between ASL and TAPV, −0.03 (±0.17) between ASL and TCD-CBF. There did appear to be an improvement in association between ASL and TCD measurements when the later were being normalized for vessel diameter as well as perfusion territories.

Figure 17. Bland-Altman plot shows degree of agreement between interhemispheric ratios measured by ASL and TAPV. The difference between interhemispheric ASL and TAPV ratios was plotted against the mean of both ratios. The broken line indicates average of differences and the solid line shows ± 1.96 SD of difference.
Figure 18. Blond-Altman plot shows degree of agreement between interhemispheric ratios measured by ASL and TCD-CBF. The difference between interhemispheric ASL and TAPV ratios was plotted against the mean of both ratios. The broken line indicates average of differences and the solid line shows ± 1.96 SD of difference.

Interhemispheric asymmetries assessed with ASL were observed in 4 patients (29%), whereas only one of them (7%) had interhemispheric TAPV asymmetry (Figure 16). All patients with ASL asymmetry had silent infarction in the MCA territory. Three other patients, with evidence of silent infarction located outside of the MCA territory, had no asymmetry in either ASL or TAPV.

However, a higher association was observed between ASL and TCD asymmetries when interhemispheric TCD-CBF ratios were used. Moreover, TCD-CBF asymmetry was identified in three (21 %) out of four patients with silent infarction in the MCA territory who also had ASL.
asymmetry. Therefore, a higher association between silent infarction, ASL asymmetry and TCD asymmetry can be achieved when the same parameter (i.e. CBF) being taken into account by both ASL and TCD techniques (Table 8).

**Figure 19.** Three axial slices showing ASL asymmetry in patients with SCD. A. A patient with ASL asymmetry but no TAPV asymmetry B. A patient with both ASL and TAPV asymmetries
Table 8. Association between silent infarctions and interhemispheric asymmetries obtained with ASL, TAPV and TCD-CBF

<table>
<thead>
<tr>
<th>Patient</th>
<th>Location of Silent Infarction</th>
<th>ASL interhemispheric ratio</th>
<th>TCD-TAPV interhemispheric ratio</th>
<th>TCD-CBF interhemispheric ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 2</td>
<td>R F</td>
<td>Symmetric</td>
<td>Symmetric</td>
<td>Symmetric</td>
</tr>
<tr>
<td>Patient 3</td>
<td>R L</td>
<td>Symmetric</td>
<td>Symmetric</td>
<td>Symmetric</td>
</tr>
<tr>
<td>Patient 5</td>
<td>L P</td>
<td>Asymmetric (L&lt;R)</td>
<td>Asymmetric (L&lt;R)</td>
<td>Asymmetric (L&lt;R)</td>
</tr>
<tr>
<td>Patient 7</td>
<td>R L</td>
<td>Asymmetric (L&gt;R)</td>
<td>Symmetric</td>
<td>Asymmetric (L&gt;R)</td>
</tr>
<tr>
<td>Patient 9</td>
<td>L FP</td>
<td>Asymmetric (L&lt;R)</td>
<td>Symmetric</td>
<td>Symmetric</td>
</tr>
<tr>
<td>Patient 11</td>
<td>R L</td>
<td>Asymmetric (L&gt;R)</td>
<td>Symmetric</td>
<td>Asymmetric (L&gt;R)</td>
</tr>
<tr>
<td>Patient 14</td>
<td>R F</td>
<td>Symmetric</td>
<td>Symmetric</td>
<td>Symmetric</td>
</tr>
</tbody>
</table>

Four patients (5, 7, 9, 11) had silent infarction inside the MCA territory; three patients (2, 3, 14) had silent infarction outside the MCA territories. F indicates Frontal; FP, Frontoparietal; L, Left; O, Occipital; P, Parietal; PT, Parietotemporal; R, Right; T, Temporal.

5.4. Discussion

We have demonstrated that absolute CBF and TCD velocity values are significantly higher in children with SCD compared to those obtained in controls. CBF abnormalities, including absolute values and interhemispheric asymmetries can occur in children with SCD who have normal TCD velocity measurements. In addition, CBF asymmetries were associated with evidence of silent infarction as seen on structural MRI.

In the present study, the ASL technique was used to assess CBF at the tissue level. This technique
has advantages over Xe-CT and nuclear medicine techniques such as PET and SPECT, in terms of increased spatial resolution and the absence of ionizing radiation exposure. ASL also utilizes endogenous spin-labeled arterial blood protons, as opposed to injected contrast (gadolinium), to quantify CBF. The ASL technique has been used in adults to determine the effects of acute cerebral ischemia and endarterectomy on CBF (Chalela et al. 2000; Ances et al. 2004). Additional studies (Oguz et al. 2003; Wang et al. 2003; Strouse et al. 2006; van den Tweel et al. 2009; Helton et al. 2009) have demonstrated the usefulness of ASL in the evaluation of CBF in children with SCD.

We demonstrated in our study that absolute CBF, in both GM and WM, was higher in SCD patients than in controls, both before and after Hct correction, supporting hyperemia in children with SCD, which is due to chronic anemia. These findings are consistent with previously published studies in children with SCD that assessed CBF by ASL as well as by other imaging techniques such as Xe-CT or PET (Prohovnik et al. 2009; Prohovnik et al. 1989; Herold et al. 1986; Oguz et al. 2003; van den Tweel et al. 2009; Helton et al. 2009; Strouse et al. 2006). Furthermore, the CBF obtained in GM was higher than that obtained in WM, in both patient and control groups. Oguz et al. demonstrated abnormally high resting CBF perfusion in all of the GM vascular territories of children with SCD compared to CBF values obtained in normal controls; this likely reflects microcirculatory adaptation to chronic anemia (Oguz et al. 2003). GM has a higher metabolic rate than WM and is easier to characterize using perfusion techniques.

The mean CBF in our non-treated cohort was relatively low in comparison to previous studies that measured cerebral blood flow in patients with SCD; however, mean CBF in our control population (31.9±4.5 mL/100g/min) was in agreement with previous studies, but was within the lower range of the spectrum of CBF values that have been reported in the ASL literature (Petersen, Lim, and Golay 2006; Chen, Wang, and Detre). The variation in CBF values obtained
in prior studies may be due to differences in perfusion imaging techniques. Because the evaluation of CBF is not yet standardized to an absolute measure, values obtained via different techniques cannot be compared. The relative change of CBF, expressed in terms of unbiased ratios such as the asymmetry ratio, is therefore very important in any assessment of perfusion abnormalities.

We identified ASL asymmetry between the left and right hemisphere in four patients (31%), whereas TAPV asymmetry was present in only one of those four patients. Interestingly, ASL asymmetries were associated with the presence of silent infarctions in all four patients. In older children with SCD, silent infarctions have been shown to progress in both number and size over time (Pegelow et al. 2002), and the associated neurocognitive abnormalities may also be progressive. This supports the need for early interventions to halt such progression. Prior studies found no correlation between TCD velocity measurements and silent infarctions (Wang et al. 2000), suggesting that other mechanisms, other than narrowing or occlusion of large cerebral arteries, may be contributing to the pathologies of cerebral infarction in SCD. Impaired hemodynamic reserve capacity, as well as the narrowing of microvasculature, can be the cause of CBF asymmetries in the absence of large vessel vasculopathy. The lack of symmetry in CBF may thus be an early indicator of subclinical pathological changes in the cerebral microvasculature or hemodynamics. However, a longitudinal study would be required to investigate this possibility, and to establish a relationship between CBF asymmetries and subsequent infarctions.

The present study demonstrated a close correlation between interhemispheric ratios of ASL and TCD-CBF. Interhemispheric TCD-CBF asymmetries were also associated with evidence of silent infarction. Therefore, the sensitivity of TCD for silent infarctions seems to be increased when velocity measurements are converted to CBF values. TCD primarily measures blood flow velocity, which has been compared to standard methods of CBF assessment with varying results.
In theory, TCD can measure CBF if the sectional area of cerebral artery and the corresponding perfusion territory are known. It is, however, not yet possible to measure intracranial vessel diameters reliably using TCD and hence, application of angiography techniques is warranted. In addition, information about perfusion territory volume requires a structural neuroimaging method such as MRI. Nonetheless, with this knowledge, a more reliable method in evaluation of brain perfusion with TCD would be employed, enabling consideration of CBF than flow velocity measurements.

Our study is limited by its relatively small sample size, the large size of arterial territories examined and technical issues such as the time delay used in this ASL study. We were not able to localize regional CBF changes related to the presence of cerebral pathology. This could be due to the limited spatial resolution of ASL, or to the large size vascular territories in which CBF was measured. In a study such as ours, decreased CBF in smaller territories, which might be an early indicator of cerebral ischemia, may have been overlooked due to the averaging of CBF over a larger volume. The transit time of blood will influence the labeling efficiency and the time between initial labeling and imaging (post-labeling delay) will influence the observed perfusion signal. Currently, the choice of an appropriate post-labeling for ASL study is controversial. The optimum post-labeling delay will depend on the age of patient and pathophysiology of disease. A recent study by Liu et al (Liu, Uh, and Lu 2011) studied compartment localization of labeled spins in ASL and concluded that a post-labeling delay of 2000 ms is sufficient to allow the tagged blood to enter the gray matter. However, children with SCD due to autoregulatory response to chronic anemia have increased blood flow velocities and likely require a shorter post-labeling delay. On the other hand, a choice of very short post-labeling delay such as 1200 and 1400 ms used in previous SCD studies was found to overestimate the CBF asymmetries (Gevers et al.). Performing ASL at multi post-delay times can provide the unique opportunity to calculate arterial timing parameters such as the arterial transit time (Golay, Hendrikse, and Lim 2004). However,
such a technique is time consuming and is thus hard to perform in children. In our study, a post-labeling delay time between 1500-2000 ms was chosen as a reasonable compromise.

In conclusion, we have demonstrated higher CBF in both the GM and WM of children with SCD compared to those in healthy subjects. We associated CBF abnormalities with silent infarctions in the corresponding arterial regions, while TCD velocity values were neither related to CBF nor sensitive enough to detect silent infarctions. The combination of a CBF imaging modality, such as ASL, and current clinical standard methods is likely to allow a more comprehensive assessment of cerebrovasuclar disease in children with SCD. ASL seems to be very attractive tool for measuring cerebral blood flow in children and it has a great potential in clinical settings for the diagnosis of neurological disorders that occur before anatomical changes are observed. However, the hematocrit correction, as well as the effect of transit time, should be taken into consideration when interpreting perfusion data measured by ASL. Prospective studies are needed to follow CBF abnormalities over time and to determine the predictive value of CBF abnormalities for cerebral infarctions.
Chapter 6

General discussion
Chapter 6 General discussion

This thesis work investigates the importance of CBF evaluation in children with SCD. We performed a systematic review (see chapter four) to assess the prevalence of CBF abnormalities compared to current clinical imaging methods (structural MRI and TCD) used in the management of children with SCD. Perfusion abnormalities, in particular CBF asymmetries, were identified to be more prevalent than structural MRI and TCD abnormalities in SCD children who had never experienced overt stroke.

We also successfully applied ASL to neurologically intact SCD children in chapter five, allowing us to investigate the correlation between CBF and TCD velocity abnormalities in addition to their association with silent infarction on structural MRI. Absolute CBF, as well as TCD velocity measurements, were significantly higher in patients compared to the control group. No significant correlation was found between ASL and TCD velocity measurements, whether abnormal absolute values or asymmetry ratios were examined. While ASL asymmetries were shown to appear in all patients with silent infarction in the corresponding arterial regions, TCD velocity asymmetries were absent in most patients. However, a close correlation was found between TCD and ASL absolute values as well as interhemispheric ratios, when CBF was derived from TCD velocity measurements. In addition, the TCD-CBF asymmetries appeared to have a stronger association with silent infarctions than velocity asymmetries.

Ischemic stroke is one of the serious complications of SCD, occurring in approximately 11% of patients in the first two decades of life (Ohene-Frempong et al. 1998; Pegelow et al. 2001; Schatz et al. 2001). Furthermore, silent cerebral infarctions occur in up to 22% of pediatric patients and are associated with poor educational and cognitive functioning. Elevated cerebral blood flow velocity, measured by TCD, has been identified as a risk factor for overt stroke in SCD. However,
while the prevalence of silent infarction is twice that of overt stroke, there is no tool available to determine the risk of silent infarctions.

The mechanisms responsible for cerebral infarctions in SCD are poorly understood. However, they seem to be the result of inadequate CBF to particular regions of brain. The occlusion of large cerebral arteries, in the case of overt stroke, as well as cerebral microvascular disease and the exhaustion of cerebrovascular reserve, which are prominent in the pathophysiology of silent infarction, are believed to be the causes of CBF disruption in SCD.

Several studies of patients with carotid artery stenosis have demonstrated that impaired CBF is an indicator of subsequent stroke and can be used to predict the cerebral infarctions earlier than they would be identified through other imaging methods (Kuroda et al. 2001; Klijn et al. 1997). Therefore, the question has been raised as to whether CBF assessment in SCD can also be used to identify cerebral abnormalities prior to them becoming visible on TCD and structural MRI and, importantly, whether CBF assessment can help in identifying SCD patients who are at risk not only of overt stroke but also silent infarctions.

In this thesis, a systematic review was designed first in order to summarize the available evidence of CBF assessment in SCD children, particularly in those who were neurologically normal. The review was also designed to obtain a precise comparison between abnormalities identified with CBF imaging modalities and those obtained through clinically used imaging methods, such as structural MRI and TCD; this allowed us to explore potential guidelines for future studies.

To our knowledge, no systematic review had yet been performed to evaluate the role of CBF assessment in children with SCD, although some narrative reviews have briefly discussed the role of CBF imaging modalities in children with SCD. The term ‘systematic review’ entails a number
of key stages that distinguish it from the more traditional narrative review. Systematic reviews start from a well defined research question, employ systemic and explicit methods to identify potentially relevant studies, use explicit selection criteria to include eligible studies, critically appraise relevant research studies, explore heterogeneity, collect and assess data from the studies that are included in the review and, if possible, synthesize study results (meta-analysis).

Systematic reviews can also help practitioners keep abreast of the medical literature by summarizing large bodies of evidence and helping to explain differences among studies focused on the same question. Systematic reviews can help in identifying gaps in the evidence and may provide a quantitative basis for new research initiatives (Cooper, Jones, and Sutton 2005).

In our analysis, covering 32 years of published literature, we found eleven studies focused on various CBF imaging modalities, such as Xe-CT, PET, SPECT, DSC MRI and ASL, that compared those modalities to structural MRI and/or TCD. Overall, CBF abnormalities were identified to be more prevalent than structural MRI and TCD abnormalities in neurologically normal SCD children, suggesting that the assessment of CBF in children with SCD warrants further investigation, as CBF abnormalities are associated with an increased risk for ischemia.

Although this study could support both our primary hypothesis and the value of CBF assessment in children with SCD, the heterogeneity existing among studies prevented us from performing meta-analysis. We were also not able to compare the specificity of CBF abnormalities to those obtained with structural MRI and TCD. Patient selection can be biased in individual studies; however, bias in article selection was avoided by the use of strict inclusion and exclusion criteria.

The multiple imaging techniques dedicated to CBF all address the same types of pathological conditions, and each has its own advantages and pitfalls. Most CBF imaging modalities, such as Xe-CT, PET, SPECT and DSC MRI, require the injection or inhalation of tracers and/or radiation
exposure, but recent advances in MRI techniques have led to the development of ASL perfusion MRI. ASL is a non-invasive CBF imaging technique that employs magnetically labeled arterial water as a contrast agent. It visualizes perfusion abnormalities and measures CBF in a quantitative way. In typical ASL experiments, two images are collected. One is collected following a tagging period and after adding a delay, which is called an inversion delay (TI) in pulsed ASL and a post-labeling delay (PLD) in continuous ASL (MacIntosh et al. 2010); this delay is generally introduced so that the tagged blood will travel from the tagging slabs to the volume of interest. The other image is collected after a control period, during which the tag clears and the system is allowed to return to equilibrium. The subtraction of these two images and accounting for magnetic decay of the labeled water (T1 relaxation time), yields a signal that is proportional to the amount of tag that is in the tissue, and can be used for absolute quantification of CBF in units of ml/100g/min.

ASL-based measures of CBF are reproducible (Jahng et al. 2005) and have been validated against established techniques such as PET (Ye et al. 2000). Because radioactive tracers or ionizing radiation are not required, ASL is well suited for children. In addition, there is typically a 70 % greater SNR in children tested using CBF measurements, as compared to adults (Wang et al. 2003). Despite its advantages, ASL has not yet replaced the more invasive perfusion imaging methods that are currently used in clinical practice. ASL suffers from intrinsic limitations such as its relative complexity of the method, low SNR and relatively high sensitivity to motion artifacts. Nevertheless, the technical improvements to ASL sequences during the past decade have increased the SNR and shortened scanning times. Also, whole brain coverage can be achieved using ASL, and at a resolution that is often comparable with the resolution of other perfusion imaging techniques.

The above mentioned technical improvements have significantly advanced the clinical
applicability of ASL. The clinical application of ASL perfusion MRI has already been demonstrated for several neuroimaging applications, such as those related cerebrovascular disease, dementia, neoplasms and functional MRI (Aguirre et al. 2002; Alsop et al.; Bokkers et al. 2008; Chalela et al. 2000; Bokkers et al. 2009; Chen et al. 2009; Du et al. 2006; Hendrikse et al. 2005; Kimura et al. 2005; Lim et al. 2008; Wolf et al. 2001). However, the advantages and disadvantages of different labeling strategies should be identified for optimal use in the clinical setting; in choosing specific sequences, hemodynamic changes resulting from pathology or drug use should be taken into consideration. In particular, one of the major sources of error in perfusion quantification using ASL (Golay, Hendrikse, and Lim 2004) is arterial transit time, which is the time blood takes to travel from the tagging region to the imaging slices. As arterial transit time varies depending on the subject’s condition, the amount of PLD chosen for a given ASL experiment can differ accordingly. For example, in patients with cerebrovascular stenosis, the use of a long PLD would allow for all the labeled spins to reach the brain parenchyma via collaterals, while a short PLD would result in the underestimation of CBF. In elderly patients with a relatively lower CBF, the choice of a long post-labeling delay would be inappropriate because the relaxation of the relatively smaller amount of labeled spins would result in insufficient SNR. One approach to meeting this transit time challenge is to perform ASL at various inversion times between labeling and image acquisition (multi TI). A multi TI sequence offers the unique opportunity to calculate arterial timing parameters, such as the arterial transit time (Golay, Hendrikse, and Lim 2004).

Another error that can interfere with the accuracy of CBF measurements occurs when patients with chronic anemia are scanned. The longitudinal relaxation time of arterial blood increases when hematocrit is decreased, causing a higher perfusion signal to be measured by ASL. Thus, the longitudinal relaxation time of arterial blood should be defined according to the individual’s hematocrit level using a separate sequence; or, if the scanning time is limited and hematocrit
values are available, it can be corrected using models such as the one introduced by Lu et al (Lu et al. 2004).

Until recently, different study groups (Oguz et al. 2003; Helton et al. 2009; van den Tweel et al. 2009) used different ASL sequences to measure perfusion in children with SCD. They reported increased CBF abnormalities, which could be an early indicator of brain tissue being at risk of ischemia. In this thesis, we described the evaluation of CBF using ASL in children with SCD. The purpose of our study was to evaluate the relationship between CBF and TCD abnormalities, and to determine whether CBF abnormalities are associated with cerebral infarction and if those abnormalities can also be identified using TCD (see chapter five).

The results of this study confirmed the existence of cerebral hyperemia in SCD patients. Furthermore, the significant differences in ASL perfusion measurements between patients and controls still remained after Hct correction. Although Hct correction slightly improved the agreement between ASL and phase contrast flow measurements in a recent study (Gevers et al.), the value of adding Hct correction should be further evaluated in future studies. We also found perfusion asymmetries and showed their association with silent infarction. Unlike previous ASL studies (Oguz et al. 2003; Helton et al. 2009; van den Tweel et al. 2009), in which CBF asymmetries in SCD children were not correlated with the evidence of cerebral infarction on structural MRI, all patients with CBF asymmetries in this study were found to have silent infarction in one or both hemispheres. That illustrates that the perfusion asymmetries in children with SCD, as has been described before, can at least partly be attributed to silent infarction. However, we were not able to localize regional CBF changes related to the presence of cerebral pathology. This could be due to the limited spatial resolution of ASL, to the fact that we did not measure CBF at the time ischemic lesions occurred, or to the use of relatively large region of interests for the calculation of perfusion asymmetries. Moreover, most pathology occurs in WM,
whereas perfusion signals in ASL mainly originate from GM and adequate SNR in WM requires longer scanning times to collect accurate perfusion measurements.

Currently, it is unclear whether CBF asymmetries result in or are subsequent to cerebral infarcts. Thus, the ability of ASL to predictively determine patients at risk for silent infarctions should be assessed in a prospective longitudinal study design, which should employ longer scanning times to allow for the measurement of WM perfusion. In addition, the interpretation of ASL perfusion imaging in children with SCD should consider the physiological characteristics of this patient population, as well as the technical pitfalls affecting perfusion maps. To avoid the underestimation of perfusion in patients who have normal or increased CBF, a relatively long PLD should be employed. However, a long PLD can result in the overestimation of CBF asymmetries in patients with vascular stenosis or occlusion. In this situation, a multi-TI sequence can be used to measure the temporal characteristics of the inflowing arterial blood.

Moreover, single assessment of CBF alone may not adequately assess cerebral hemodynamic status. CBF may be normal despite some pre-existing autoregulatory vasodilation, which in turn reduces oxygen carrying capacity or perfusion pressure due to the disease. However, with further reductions in oxygen carrying capacity or perfusion pressure, the capacity of autoregulatory vasodilation to maintain normal blood flow is overcome and blood flow begins to decrease. The autoregulatory capacity of the cerebrovasculature can be assessed by measuring cerebrovascular reactivity (CVR) index. CVR relies on paired CBF measurements with the initial measurement obtained at rest and the second measurement obtained following a cerebral vasodilatory stimulus such as hypercapnia or acetazolamide. A variety of quantitative or qualitative imaging techniques can be used to assess CVR changes in response to a stimulus, including Xe-CT, SPECT, PET, and perfusion MRI techniques (Powers 1991; Ostergaard et al. 1998). Some study groups have reported chronic hyperemia caused by the exhaustion of cerebrovascular reserve capacity, leading
to infarction in the cases of slight increases in the metabolic demand (Prohovnik et al. 1989; Prohovnik et al. 2009).

Although exhausted autoregulation fails to further increase CBF and delivery of oxygen, the brain can increase the amount of oxygen it extracts from the blood (oxygen extraction fraction (OEF)) to maintain normal cerebral oxygen metabolism and function (Kety et al. 1950). This phenomenon of reduced blood flow and increased oxygen extraction has been termed misery perfusion (Baron et al. 1981). Once oxygen extraction becomes maximal, further decreases in oxygen carrying capacity or perfusion pressure (and consequently blood flow) will lead to disruption in normal oxygen metabolism and ultimately to infarction. Assessment of OEF can be used to identify patients with increased oxygen extraction. Currently, regional measurements of OEF can be performed using O-15 labeled PET (Herscovitch, Mintun, and Raichle 1985). Both absolute and relative OEF have been used for the determination of abnormal from normal. MRI measurements using pulse sequences sensitive to deoxyhemoglobin show increased signal in regions with increased oxygen extraction and are being developed to provide similar information (Lin et al. 1998).
Chapter 7

Conclusions
Chapter 7 Conclusions

We investigated the role of CBF assessment in children with SCD. A systematic review was conducted to gather evidence from a number of SCD studies into one report, which pools and analyses all available data comparing CBF assessment, TCD and structural MRI. The prevalence of CBF abnormalities was equal to or higher than those obtained with structural MRI and TCD. The studies suggest that the assessment of CBF in SCD can be of potential value in addressing brain abnormalities at the tissue level. Further investigations are required to assess CBF in children with SCD, as CBF abnormalities are potentially associated with risk of ischemia.

We also explored the correlation between CBF and TCD abnormalities. We successfully performed ASL, a non-invasive perfusion imaging method, to depict, specifically, CBF abnormalities in children with SCD; thereby, we confirmed ASL’s ability to demonstrate perfusion abnormalities at tissue level, which seem to remain invisible on TCD velocity measurements. Abnormal absolute CBF was found in a group of SCD patients whose TCD velocity was normal. In addition, CBF asymmetries were associated with silent infarction in SCD children with no corresponding TCD velocity asymmetry. Nevertheless, converting TCD velocity to CBF can significantly increase the sensitivity of TCD for silent infarction. However, the need for accurate measurements of vessel diameter and perfusion territory currently limits direct measurement of CBF from TCD measurements. ASL, a non-invasive perfusion technique, is suitable for perfusion imaging in patients with sickle cell disease; however, the Hct correction, as well as the effect of transit time, should be taken into consideration when interpreting perfusion data measured by ASL.
The combination of a CBF imaging modality such as ASL and current clinical standard methods likely allows for the full assessment of cerebrovascular disease in children with SCD. Arterial spin labeling or for that matter, any perfusion imaging technique at the tissue level may provide information about the effects of small vessel disease and hemoglobin on brain tissue perfusion that is usually unattainable with conventional MR imaging, MR angiography, or TCD (Oguz et al. 2003). While much work still needs to be done, we speculate that CBF assessment has potential to help with decision making in three types of patients with SCD: (1) a screening technique to select patients, who are at a high risk for stroke, silent or overt, so that intervention (hydroxyurea, blood transfusions) can be initiated; (2) an evaluation method in patients who have been on a chronic transfusion regimen for a few years to decide what the probability of recurrent stroke is, shall they come off transfusions; (3) another factor in deciding which patients with severe cerebrovascular disease are the optimal candidates for vascular surgery. However, validation of these issues will require more work and perhaps a multicenter controlled population.

Furthermore, the use of other physiological parameters, such as cerebrovascular reactivity, as well as other neuroimaging methods, such as high resolution angiographic assessment, may further characterize CBF abnormalities and could possibly assist in managing SCD patients with such abnormalities.
Chapter 8

*Future Directions*
Chapter 8 Future Directions

To date, little is known about the mechanisms that cause perfusion abnormalities in patients with SCD, and it has not yet been elucidated whether CBF abnormalities play a causative role in stroke, or if they are a result of SCD-related hemodynamic disturbances (Nur et al. 2009). Longitudinal studies are required to follow CBF abnormalities over time and to determine the predictive value of CBF abnormalities for cerebral infarction. On the other hand, whether CBF assessment will help improve therapeutic decisions based on TCD findings and surpass TCD for prediction of cerebral infarction and cognitive deficits requires further evaluation.

There is insufficient evidence to support the use of one CBF imaging modality over another, or to change the current practices by adopting CBF assessment over current clinical standard methods (TCD and structural MRI). ASL is a very attractive tool for non-invasive perfusion imaging that can be used in every patient suitable for MR imaging. However, the physiological characteristics of the individual patient, as well as the technical pitfalls of the chosen ASL sequence and quantification methods, should be addressed. Work is currently under way in a number of centers to address the problems underlying the quantification of CBF, and with appropriate modeling these problems may be overcome.

To date, differences in perfusion imaging techniques, as well as in kinetic models, have led to variations in absolute CBF values; therefore, values obtained by different techniques cannot be compared. The relative changes of CBF, such as the asymmetry ratio, can be used in part to assess perfusion abnormalities in the absence of absolute values; however, there is also no standard definition for the asymmetry ratio, which varies arbitrarily depending on the method used. Hence, the evaluation of CBF needs to be standardized to an absolute measure, and
prospective studies on large healthy populations are also required to identify a precise asymmetry ratio.

There remain opportunities for future research related to the management of cerebral infarction in SCD patients, as well as achieving an optimal diagnostic and screening tool for children with SCD.
Chapter 9

References
Chapter 9 References


frontotemporal dementia and Alzheimer disease by arterial spin labeling MRI. 
*Neurology* 67 (7):1215-20.


MacIntosh, B. J., N. Filippini, M. A. Chappell, M. W. Woolrich, C. E. Mackay, and P. Jezzard. 2010. Assessment of arterial arrival times derived from multiple


108


children with sickle cell disease using continuous arterial spin labeling at 3.0-Tesla MRI. *Stroke* 40 (3):795-800.


Appendix A

Bias list
### Table 9. Appendix A, bias list

<table>
<thead>
<tr>
<th>Item</th>
<th>Bias</th>
<th>Potential answers to meet the bias</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Spectrum bias</td>
<td>SCD Patients with different clinical evidence of stroke (including patients with and without clinical history of stroke)</td>
</tr>
<tr>
<td>2</td>
<td>Acceptable reference standard</td>
<td>Structural MRI and/or TCD used as reference test</td>
</tr>
<tr>
<td>3</td>
<td>Disease progression bias</td>
<td>No delay occurs between Results of Index test (hemodynamic measurements) and reference (MRI, TCD)</td>
</tr>
<tr>
<td>4</td>
<td>Selection bias</td>
<td>Consecutive selection of patients</td>
</tr>
<tr>
<td>5</td>
<td>Differential verification bias</td>
<td>Patients received verification of their disease status using the same reference standard (MRI, TCD)</td>
</tr>
<tr>
<td>6</td>
<td>Incorporation bias</td>
<td>The index tests (hemodynamic measurements) do not form part of the reference standard test (MRI, TCD)</td>
</tr>
<tr>
<td>7</td>
<td>Diagnostic review bias</td>
<td>Blind interpretation of index test without knowledge of reference test</td>
</tr>
<tr>
<td>8</td>
<td>Test review bias</td>
<td>Blind interpretation of reference test without knowledge of index test</td>
</tr>
<tr>
<td>9</td>
<td>Observer variability bias</td>
<td>Similar clinical data are available when interpreting MRI, TCD and other hemodynamic measurements</td>
</tr>
<tr>
<td>10</td>
<td>Study examination bias</td>
<td>&lt;10% of indeterminate or uninterpretable result</td>
</tr>
<tr>
<td>11</td>
<td>Withdrawal bias</td>
<td>&lt;10% of patients withdraw after the index test</td>
</tr>
<tr>
<td>12</td>
<td>Treatment bias</td>
<td>Treatment history of included patients was reported</td>
</tr>
</tbody>
</table>
Appendix B

ASL and TCD values obtained in healthy control group: definition of abnormal values and asymmetries
Appendix B  ASL and TCD values obtained in healthy control group: definition of abnormal values and asymmetries

<table>
<thead>
<tr>
<th>Subject no.</th>
<th>ASL</th>
<th>TCD</th>
<th>TAPV</th>
<th>TCD-CBF</th>
</tr>
</thead>
</table>

**Table 10.** Appendix B, ASL, TAPV and TCD-CBF values in healthy controls
<table>
<thead>
<tr>
<th></th>
<th>Absolute values (mm/100gr/min)</th>
<th>L/R</th>
<th>Absolute values (cm/s)</th>
<th>L/R</th>
<th>Absolute values (ml/min/100gr)</th>
<th>L/R</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>R</td>
<td>N/A</td>
<td>71.7</td>
<td>1.08</td>
<td>52.66</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td></td>
<td>77.5</td>
<td></td>
<td>52.65</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>R</td>
<td>36.01</td>
<td>78.8</td>
<td>0.87</td>
<td>57.73</td>
<td>1.02</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>38.60</td>
<td>68.8</td>
<td></td>
<td>58.91</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>R</td>
<td>N/A</td>
<td>48.8</td>
<td>0.97</td>
<td>42.53</td>
<td>1.07</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td></td>
<td>47.1</td>
<td></td>
<td>45.59</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>R</td>
<td>22.70</td>
<td>78.2</td>
<td>0.86</td>
<td>39.50</td>
<td>1.02</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>23.00</td>
<td>67</td>
<td></td>
<td>40.22</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>R</td>
<td>30.18</td>
<td>93.5</td>
<td>0.94</td>
<td>60.42</td>
<td>0.96</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>29.29</td>
<td>88.3</td>
<td></td>
<td>58.01</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>R</td>
<td>37.44</td>
<td></td>
<td>0.96</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>36.00</td>
<td></td>
<td></td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>R</td>
<td>24.36</td>
<td></td>
<td>1.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>24.53</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>R</td>
<td>35.12</td>
<td></td>
<td>0.91</td>
<td>54.40</td>
<td>0.97</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>31.82</td>
<td></td>
<td></td>
<td>52.77</td>
<td>0.97</td>
</tr>
<tr>
<td>9</td>
<td>R</td>
<td>35.80</td>
<td></td>
<td>1.06</td>
<td>44.7</td>
<td>1.14</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>37.93</td>
<td></td>
<td></td>
<td>50.9</td>
<td>1.14</td>
</tr>
<tr>
<td>10</td>
<td>R</td>
<td>39.07</td>
<td></td>
<td>0.97</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>38.02</td>
<td></td>
<td></td>
<td>N/A</td>
<td></td>
</tr>
</tbody>
</table>

**Table 11. Appendix B, definition of abnormal absolute values**

<table>
<thead>
<tr>
<th>Modality</th>
<th>Mean</th>
<th>SD</th>
<th>Mean±2SD</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>ASL (ml/min/100gr)</em></td>
<td>32.49</td>
<td>5.99</td>
<td>44.48</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>20.50</td>
</tr>
<tr>
<td><em>TAPV (cm/s)</em></td>
<td>65.89</td>
<td>16.13</td>
<td>98.16</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>33.62</td>
</tr>
<tr>
<td><em>TCD-CBF (ml/min/100gr)</em></td>
<td>50.86</td>
<td>6.98</td>
<td>64.82</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>36.89</td>
</tr>
</tbody>
</table>

➢ Abnormal absolute ASL value was defined as ASL >44.48 or <20.50
- Abnormal absolute TAPV value was defined as TAPV >98.16 or <33.62
- Abnormal absolute TCD-CBF value was defined as TCD-CBF >64.82 or <36.89

Table 12. Appendix B. definition of asymmetries

<table>
<thead>
<tr>
<th>L/R ratio</th>
<th>Mean</th>
<th>SD</th>
<th>Mean±2SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASL</td>
<td>1</td>
<td>0.05</td>
<td>1.10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.90</td>
</tr>
<tr>
<td>TAPV</td>
<td>0.98</td>
<td>0.10</td>
<td>1.18</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.78</td>
</tr>
<tr>
<td>TCD-CBF</td>
<td>1</td>
<td>0.05</td>
<td>1.10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.90</td>
</tr>
</tbody>
</table>

- ASL asymmetry was defined as ASL L/R ratio >1.10 or <0.90
- TAPV asymmetry was defined as TAPV L/R ratio >1.18 or <0.78
- TCD-CBF asymmetry was defined as TCD-CBF ratio >1.10 or <0.90