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CEREBRAL SEQUELAE AND VASCULOPATHY IN SICKLE CELL DISEASE

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CHAPTER 1

GENERAL INTRODUCTION AND OUTLINE OF THE THESIS

V. van der Land

INTRODUCTION

Sickle cell disease is a genetic disorder of hemoglobin synthesis, resulting in severe hemolytic anemia and recurrent vaso-occlusive events. A single point mutation is responsible for the formation of the abnormal hemoglobin S molecule. Hemoglobin S tends to polymerize during deoxygenation, forming long strands, resulting in the typical sickle shaped red blood cells. Sickle red blood cells are rigid and fragile which reduces the lifespan to 15-20 days, with severe hemolytic anemia as a result.[1] Complex interactions between sickled red blood cells and the endothelium will lead to vaso-occlusion with subsequent ischemia and tissue damage. This can occur virtually everywhere in the body, with a predilection for the bone marrow, leading to the hallmark complication of the disease: acute, severe pain in arms, legs, back or abdomen.

Sickle cell disease is associated with a high mortality. Early in life, recurrent vaso-occlusive events in the spleen lead to absent spleen function. Consequently, children have an increased susceptibility for pneumococcal infections, which is an important cause of childhood mortality.[2] Cerebrovascular events, pulmonary complications in the form of acute chest syndrome and splenic sequestration are other important examples of acute, life-threatening complications. Recent advances in care, including prophylactic antibiotics, additional vaccinations, blood transfusion therapy and hydroxycarbamide have improved life expectancy remarkably.[3]

With increased survival, the chronic damage to organ systems becomes a more important cause of morbidity and mortality. Examples of chronic damage include recurrent, asymptomatic vaso-occlusion in the kidneys, leading to impaired urine concentrating ability and renal failure, pulmonary hypertension and chronic lung disease and silent cerebral infarcts, leading to neurocognitive problems. Nowadays, sickle cell disease faces a new challenge: understanding the pathophysiology of these chronic complications and identifying new therapeutic options.

HISTORIC OVERVIEW: FROM SICKLE-SHAPED RED BLOOD CORPUSCLES TO SINGLE POINT MUTATION

In 1910, James B. Herrick published a case report of a patient with severe anemia, in which he described the red blood cells as 'peculiar elongated and sickle-shaped red blood corpuscles'.[4] In the next years, new case reports were published, and in 1922 the term 'Sickle Cell Anemia' was introduced for the first time by V.R. Mason.[5] In July 1949, James Neel published a report in Science suggesting that sickle cell anemia was an autosomal recessive genetic disease.[6]

A couple of months later, Linus Pauling and colleagues published an article in the same journal, describing the abnormalities of the hemoglobin molecule of patients with sickle cell anemia, thereby identifying sickle cell anemia as a molecular disease.[7] Later in 1958, Hunt et al discovered that the substitution of valine for glutamic acid on chromosome 11 was the responsible point mutation, resulting in the formation of hemoglobin S.[8] Besides the homozygous form of sickle cell disease (HbSS, i.e. sickle cell anemia), variant sickle cell syndromes were identified such as hemoglobin SC disease (HbSC), in which the substitution of lysine leads to the formation of hemoglobin crystals. Furthermore, combinations of hemoglobin S disease with beta-thalassemia ($HbS\beta^0$ and $HbS\beta^+$) will also lead to sickle cell disease. Since these initial reports, intensive research has resulted in great number of discoveries into the pathophysiology of sickle cell disease, resulting in the understanding that several different pathways and cells contribute to the disease (Table).[9]

SICKLE CELL DISEASE WORLDWIDE: FROM SUB-SAHARAN AFRICA TO THE NETHERLANDS

The majority of patients with sickle cell disease live in tropical regions of sub-Saharan Africa, in India and in the Middle-east. In Ghana and Nigeria the frequency of sickle cell trait is as high as 15-30% and in Uganda it can reach 45% in certain tribes.[10] It is estimated that sickle cell disease is responsible for 16% of mortality of all children under 5 years, mainly from infections such as malaria and pneumococcal sepsis.[10] Migration has led to an increasing number of patients with sickle cell disease in northern America and Europe, with an estimated 2600 and 1300 annual births of children with sickle cell disease, respectively.[11] With increasing immigration, a rising number of patients with sickle cell disease are expected in the Netherlands. For example, the Ghanaian population living in Amsterdam has increased greatly over the last years.[12] Therefore, it is increasingly important to study mortality and morbidity of patients with sickle cell disease in the Netherlands. Additionally, it is an opportunity to investigate the pathophysiology of sickle cell disease and to make a modest contribution to the understanding of the disease in general.

PATHOPHYSIOLOGY: FROM SINGLE POINT MUTATION TO MULTISYSTEM DISEASE

The solubility of hemoglobin S is low during deoxygenatation, therefore it tends to form long polymers. Multiple polymers will align in strands, and these strands will cause the red blood cell to deform into the typical sickle shape.

Tabel . Evolving paradigm of sickle cell VOC

Year	Scientific observation	Contribution
1910	James Herrick: Description of the first patient with sickle-shaped RBCs on peripheral smear	Original description
1930	Shriver and Waugh: Venous circulation in a patient is enriched in sickle-shaped cells that regain normal shape upon reoxygenation	A disease of the RBC
1949	Linus Pauling demonstrates that the disease originates from a mutated hemoglobin molecule	A molecular disease of hemoglobin
1974	Hofrichter and Eaton: "delay time" for the initiation of rapid phase of deoxy-HbS polymerization	VOC is dependent on deoxy HbS concentration and transit time of the RBCs
1979, 1980	Hebbel and Hoover: Increased propensity of SS-RBCs to adhere to endothelium in vitro and correlation with disease severity	Widened the scope of scientific studies outside the RBC. Adhesive interactions of the RBCs and endothelium leads to VOC
1978-1986	Brugnara and others: increased KCl cotransport and Gardos channel activity in SS-RBCs	SS-RBC dehydration due to cation loss
1989	Kaul and Nagel: SS RBCs adhere in postcapillary venules SS reticulocytes are more adherent than older and denser RBCs	SS reticulocytes initiate VOC by adhering to endothelium, followed by trapping of dense cells, hypoxia, and retrograde extension of obstruction to neighboring vessels
1984-2004	Characterization of multiple discrete mechanisms of sickle RBC adhesion	Role of cell, matrix adhesion molecules and plasma factors in VOC
1994	Platt: Baseline WBC count >15 × 109/L is associated with increase morbidity and mortality	Leukocyte counts are a marker of SCD severity
1997	Pastzy and Narla/Ryan and Townes: Humanized murine models of SCD	Transgenic models expressing only human sickle hemoglobin
2000	Kaul and Hebbel: exaggerated inflammatory response in hypoxia followed by reoxygenation	Contribution of the ischemia-reperfusion injury to inflammation
2002	Turhan and Frenette: SS-RBC interactions with adherent leukocytes initiate VOC. P- and E-selectin deficiency protects against TNF-α induced VOC	First in vivo evidence in a sickle cell murine model for the role of leukocytes in initiating VOC. P and E-selectins are important in mediating this interaction
2002	Reiter and Gladwin: Cell-free hemoglobin limits nitric oxide bioavailability in SCD	Nitric oxide depletion in SCD and its contribution to vasculopathy
2003	Hines and Parise: Role for epinephrine in the regulation of BCAM/Lu dependant SS RBC adhesiveness	Role of physiologic stress as a trigger for VOC
2004-2007	Zennadi and Telen: Epinephrine-induced activation of LW-mediated sickle cell adhesion and VOC in a vivo mouse model is blocked by propranolol	Identifies β-adrenergic receptor antagonism as a potential therapeutic approach
2009	Wallace and Linden: Ischemia reperfusion injury is amplified by the activation of CD1d-restricted iNKT cells	Role of iNKT cells in triggering inflammation
2009	Hidalgo and Frenette: Role of secondary activation signals in neutrophils	Role of E-selectin as an activating signal, Src kinases and αMβ2
2009	Belcher and Vercellotti: Heme oxygenase-1 inhibits vascular stasis in a murine model of SCD	Importance of heme in inflammation and VOC

Table by Manwani et al, Blood 2013; with permission.

Damage to the red cell membrane occurs, as well as dehydration of the red cell and increased expression of adhesion molecules. Polymerization is initially reversible when oxygenation occurs, however repetitive cycles of polymerization will cause extensive damage to the red cell membrane, with hemolysis as a consequence.[13] Polymerization is dependent of intracellular hemoglobin S concentration, factors influencing this concentration are therefore important predictors of polymerization and disease severity. Genotype is the main predictor: anemia and vaso-occlusion are less severe in patients with HbSC disease. The presence of fetal hemoglobin (HbF) lowers the concentration of HbS and limits polymerization, HbF level is a well-recognized predictor of disease severity and mortality.[14]

Endothelial activation

The primary process of HbS polymerization leads to a chronic, systemic vasculopathy through several different, highly intertwined pathways: hemolysis and recurrent episodes of vaso-occlusion result in oxidative stress, ischemia-reperfusion injury, activation of leukocytes and platelets, defects in nitric oxide availability and endothelial and coagulation activation.[15] Endothelial dysfunction is increasingly recognized as an important factor in the pathophysiology of sickle cell disease, being the primary site of adhesion of sickled red blood cells, platelets and neutrophils. During steady disease state, but even more so during vaso-occlusive crisis, adhesion molecules are increased on the surface of the endothelium, circulating endothelial cells are present in increased numbers, and markers of endothelial dysfunction such as von Willebrand factor (vWF) are increased compared to normal controls.[15–17]

COMPLICATIONS: FROM HEAD TO TOE

Besides the occurrence of acute complications, sickle cell disease is also associated with progressive damage to almost all organ systems. Most severely affected are patients with homozygous sickle cell disease (HbSS), anemia is more severe and complications occur more often and are more serious compared to patients with a moderate form (e.g. HbSC).

The most common acute complication is an acute painful episode: pain in arms, legs, back, abdomen, head or chest due to vaso-occlusion and necrosis of bone marrow, often referred to as sickle cell crisis or vaso-occlusive crisis. Acute chest syndrome, characterized by chest pain, fever, wheezing, tachypnea or hypoxemia, and a pulmonary infiltrate on chest x-ray, is the second most common reason for hospital admission and a major cause of mortality in adults.[14,18] In a study in children diagnosed by neonatal screening with a mean follow up of 12 years, almost 60% had had at least one episode of acute chest syndrome.[19]

Acute splenic sequestration mainly occurs in young children, with a median age of 1.4 years at the first episode and is recurrent in up to 67% of patients.[20] It is caused by sickling and subsequent trapping of blood in the spleen, causing hypovolemic shock, and is an important cause of mortality in young children.[21]

More chronic, progressive damage is apparent in almost all organs. The spleen also sustains progressive asymptomatic infarction and fibrosis, resulting in functional asplenia early in life: 91% of children above 12 months will have absent spleen function.[22] This is associated with an increased susceptibility for invasive infection by encapsulated organisms, although prophylactic penicillin and pneumococcal vaccination have greatly reduced the incidence.[2,23] Chronic kidney disease is present in almost 29% at a mean age of 32 years, and end-stage renal disease will develop in 4.2% at a median age of 23 years.[24,25] Chronic lung disease can evolve, even as pulmonary hypertension, which is associated with increased mortality in adults.[26,27] Additionally, the brain sustains progressive damage in patients with a severe genotype.

Cerebral sequelae

Cerebral sequelae are among the most serious complications of sickle cell disease and include overt stroke, transient ischemic attack (TIA), silent cerebral infarction (SCI), seizures and neurocognitive problems, and occur almost exclusively present in patients with a severe genotype. Overt stroke used to occur in 11% by the age of 20.[28] The peak incidence of a first stroke occurs between ages 2-5 years, and affected children are greatly hampered in their development on an academic, social and personal level.[28] Overt stroke is associated with stenosis of the intracerebral arteries due to a process which is not completely understood, but includes the proliferation of smooth muscle cells leading to narrowing of the lumen. This large vessel vasculopathy can be detected by Transcranial Doppler imaging (TCD) by measuring blood flow velocity. The hallmark STOP trial by Adams and colleagues demonstrated that children with a blood flow velocity > 200 cm/second in one of the intracerebral arteries have a very high risk to develop overt stroke, but that chronic transfusion therapy leads to a risk reduction of 92%.[29] Nowadays, with standard TCD screening in patients with HbSS, the incidence of overt stroke has decreased dramatically.

Presently, the focus of research has shifted to the etiology and treatment of SCIs. An SCI is defined as infarct-like lesions on MRI in a patient with no focal neurological deficit.[30] Approximately 40% of children with HbSS will be affected by SCIs by the age of 14.[31] Even though SCIs are not accompanied by neurological deficits, SCIs have detrimental effects on neurocognitive functioning. Most SCIs can be recognized as hyperintensities in the white matter of the brain on MRI, however a detailed and uniform description of the aspect of the lesions is lacking.

Several risk factors for SCIs have been identified, including male sex, a low level of hemoglobin and a relative high systolic blood pressure.[32] However, despite recent studies, the pathophysiology leading to SCIs is still largely unknown. There are two main hypotheses on the etiology of SCIs: local vaso-occlusion elicited by endothelial dysfunction and insufficiency of cerebral blood flow (CBF).

Vaso-occlusion can occur throughout the body and probably also plays an important role in the occurrence of SCIs. The role of cerebral vaso-occlusion and endothelial dysfunction in the etiology of SCIs is supported by the finding of diffuse thickening and sclerosis of intracerebral arterioles as described in one of the few autopsy studies in sickle cell disease.[33] One other small study in patients with SCIs found lower concentrations of tissue plasminogen activator (t-PA) and ADAMTS13 – both markers of endothelial dysfunction - in patients with SCIs compared to patients without SCIs, suggesting that endothelial dysfunction is a risk factor for the development of SCIs.[34]

A second hypothesis for the development of SCIs is related to altered CBF. CBF during steady disease state is known to be increased in children with sickle cell disease, probably as a compensatory mechanism for the chronic anemia.[35] As a consequence, cerebral reactivity, i.e. vasodilatory capacity in reaction to triggers such as hypercapnia, has been demonstrated to be significantly reduced in patients with SCD compared to controls.[36] This may lead to compromised CBF during episodes of increased metabolic demand such as fever or an acute drop in hemoglobin, leading to cerebral ischemia. Normally, it's not possible to measure CBF in the white matter of the brain because of the relatively low blood flow, however due to the higher CBF in patients with sickle cell disease and recent advances in imaging techniques, it is now feasible. As SCIs occur in the white matter, analyzing CBF in this region could give clues on the role of altered CBF in the pathophysiology of SCIs.

CBF can be measured by MRI in a non-invasive way by using arterial spin labeling (ASL), a technique in which blood is magnetically labeled when it passes through the carotid arteries, this signal can be measured when it arrives in the brain tissue. The speed at which this magnetic labeling returns to the ground state, the T1 relaxation time ($T1_{blood}$), is dependent on several factors such as the composition of the labeled blood and surrounding tissue, the amount of macromolecules and also hematocrit.[37] Usually, a standard T1 relaxation time is used to calculate CBF, however as patients with sickle cell disease have a decreased hematocrit, this should possibly be accounted for. Information about the relation between hematocrit and T1 relaxation time in sickle cell disease is not available, but is important to increase the reliability of ASL measurement in sickle cell disease.

Patients with SCIs may develop cognitive impairment, influencing social and academic functioning. Lower full-scale intelligence quotient values have been demonstrated in patients with SCIs compared to patients without SCIs[38,39] however this was not confirmed in other studies.[40–42] The size of SCIs may influence neurocognitive functioning and may vary between patient groups, which may explain some of the inconsistencies in study results. The exact quantitative effect of SCIs on neurocognitive functioning is unclear, however this is relevant in clinical decision making because it can identify the need for neuropsychological evaluation and intervention.

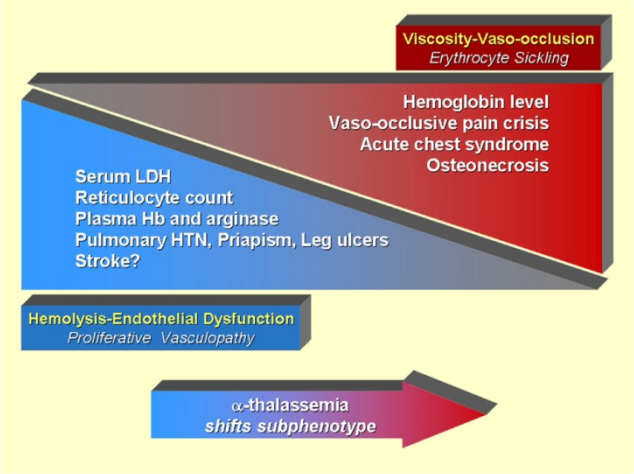
A note on terminology. Classically, cerebral sequelae of sickle cell disease have been classified according to the presence or absence of overt neurological deficits. This has led to the distinction of overt stroke (with neurological deficits) and SCIs (no neurological deficits, the WMHs on the MRI are so-called 'silent'). Although this classification based on the presence or absence of a neurological deficit is useful for patient care, it may not be the most suitable for research purposes. Overt strokes are generally caused by cortical infarctions in the setting of large vessel vasculopathy, and, in contrast, SCIs are recognized as white matter hyperintensities (WMH) on MRI. However, extensive lesions in the white matter, or strategically located infarcts in subcortical grey matter, may also lead to neurological deficits. These would be classified as overt stroke even though the etiology is the same as the WMHs that do not lead to neurological deficits. A careful description of the brain lesions could contribute to a better understanding of the underlying pathophysiology. A classification based on imaging findings may therefore be a more straightforward approach for research purposes because it facilitates the exchange of results between different studies.

Clinical heterogeneity

Sickle cell disease has a remarkable clinical variability, even between patients with the same genotype. Genotype, HbF and other disease modifying factors work together to create a clinical picture which is unique to each patient. Large, observational studies have led to the identification of two clinical subphenotypes: patients with the viscosity-vaso-occlusion subphenotype have a relatively high hemoglobin level and experience frequent vaso-occlusive pain crises, acute chest syndrome and osteonecrosis (Figure). Patients with the hemolysis-endothelial dysfunction subphenotype have high levels of lactate dehydrogenase and a high reticulocyte count and their clinical picture is characterized by stroke, pulmonary hypertension, priapism and leg ulcers.[43,44] The existence of the two subphenotypes suggests that there are at least two separate pathophysiology processes that can lead to specific complications in sickle cell disease.

Therefore, the subphenotypes have been the focus of multiple studies to advance our understanding about the pathophysiology. The two clinical subphenotypes should be considered as two ends of a continuous spectrum, as it is not always possible to identify the subphenotype in all patients. The distinction into the subphenotypes could be useful to predict the risk of specific complications, so that a more targeted screening can be applied. This is of particular interest for children, in which the need to predict outcome is greatest. At the same time, it is particularly difficult to make the distinction into the two subphenotypes at a young age because most of the specific complications do not usually occur in children. Perhaps there is a role for specific biomarkers to identify the subphenotypes in children, however this has not been studied yet.

Figure. Model of overlapping subphenotypes of sickle cell disease. Reproduced with permission by Kato, Blood Reviews 2007.



PREVENTION AND TREATMENT: FROM NEONATAL SCREENING TO HYDROXYCARBAMIDE

Mortality in children with sickle cell disease has decreased significantly in the past decades: in a recent cohort of children diagnosed by neonatal screening, the estimated overall survival of patients with HbSS at the age of 18 years was 93.3%.[3] Early diagnosis can decrease mortality and morbidity in several ways. The prescription of penicillin prophylaxis and pneumococcal vaccination can decrease infections with encapsulated bacteria (e.g. pneumococcal infection).[2,23] Additionally, parental education in the recognition of potentially life-threatening complications such as splenic sequestration or fever can ensure early treatment and intervention, thus increasing survival.

Neonatal screening for sickle cell disease was implemented in 2007 in the Netherlands, and a national cohort study was implemented to evaluate mortality and morbidity of the cohort.

Simple blood transfusions can be necessary to increase circulating blood volume in the case of splenic or hepatic sequestration, but are also used to reduce hemoglobin S percentage and ameliorate vaso-occlusion. Chronic blood transfusion therapy can prevent overt stroke in patients with a stenosis of the intracerebral arteries.[29] Chronic blood transfusion therapy is sometimes used to treat severe recurrent vaso-occlusive events, or for instance therapy resistant leg ulcers in adults.

Hydroxycarbamide is the only disease modifying drug for sickle cell disease with proven efficacy. It increases fetal hemoglobin levels, hereby decreasing hemolysis, increasing hemoglobin level and decreasing the tendency for vaso-occlusion. The first large trial was published in 1995 and demonstrated lower complication rates in patients using hydroxycarbamide compared to placebo.[45] Since then, the indication for hydroxycarbamide has broadened and now includes frequent painful episodes, acute chest syndrome, severe symptomatic anemia, growth retardation, and can also be used to decrease transfusion volume in patients who receive chronic blood transfusion therapy. Most of these indications are based on expert opinion and observational studies, and information is lacking whether hydroxycarbamide can prevent chronic, irreversible complications such as SCIs, avascular bone necrosis, chronic kidney disease and leg ulcers.[46]

OUTLINE AND AIM OF THIS THESIS

In Chapter 2, we investigated whether markers of endothelial dysfunction, including VWF and VWF propeptide, were elevated in young children with sickle cell disease during steady disease state. Additionally, we evaluated whether these markers could be used to identify the clinical subphenotype in young patients. Identifying the clinical subphenotype at a young age can be useful to predict future complications, and install targeted screening and early intervention.

Chapters 3 through 6 focus on the cause and consequences of SCIs, or cerebral white matter hyperintensities. We studied the appearance of WMHs on ultra high-field 7T MRI in Chapter 3, and used existing neuro-imaging standards to describe the lesions. Ultra high-field 7T MRI has a high resolution; to explore the superiority of 7T in identifying imaging abnormalities, we compared our results with 3T MRI.

In Chapters 4 through 6, we present the results of the FIND study, an explorative MRI study in 40 children with sickle cell disease to investigate risk factors for WMHs, and additionally investigate the effect of these lesions on neurocognitive functioning. In Chapter 4, we focused on the two main hypotheses on the pathophysiology of white matter hyperintensities. We investigated whether CBF in the grey or white matter was abnormal in patients with white matter hyperintensities, and whether markers of endothelial dysfunction were associated with the risk for white matter hyperintensities.

In Chapter 5, we will discuss the arterial spin labeling (ASL) technique used to measure CBF. In ASL, blood is magnetically labeled as it flows through the carotids. The $T1_{blood}$ is dependent of the amount of macromolecules in the proximity, i.e. properties of the blood.[37] With a recently developed scan sequence, we were able to measure $T1_{blood}$.

Chapter 6 we will study the consequences of white matter hyperintensities on neurocognitive functioning. Specifically, we investigated whether the severity of neurocognitive dysfunction is associated with the total volume of the lesions.

Early diagnosis and treatment is important in decreasing mortality and organ damage, therefore neonatal screening for sickle cell disease was implemented in 2007 in the Netherlands. In Chapter 7 we will describe the first results of our nationwide cohort study of children diagnosed by neonatal screening. We will describe mortality and the sequelae of sickle cell disease, i.e. sickle cell specific complications and will specifically study weight status.

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Chapter 2

MARKERS OF ENDOTHELIAL DYSFUNCTION DIFFER BETWEEN SUBPHENOTYPES IN CHILDREN WITH SICKLE CELL DISEASE

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ABSTRACT

In adult patients with sickle cell disease two distinct subphenotypes have previously been defined: patients with the viscosity-vaso-occlusion subphenotype (VVO) suffer mainly from vaso-occlusive pain crises and have a relatively high hemoglobin concentration. Patients classified as the hemolysis-endothelial dysfunction subphenotype (HED) suffer from stroke and pulmonary hypertension and have an elevated concentration of lactate dehydrogenase. However, this classification is not possible in children due to low rates of complications. We used laboratory markers to classify children into the two subphenotypes, and measured vWF and vWF propeptide as markers of endothelial dysfunction. We included 106 children with sickle cell disease (mean age 8.7 years), 74 (70%) with HbSS/HbSβ^0 genotype and 32 (30%) with HbSC/HbSβ^+ genotype. vWF and vWF propeptide were significantly elevated in patients with sickle cell disease; this was more pronounced in patients with the HbSS/HbSβ^0 genotype. Patients with the HED subphenotype had higher levels of vWF propeptide, and a trend towards higher levels of vWF compared to those with the VVO subphenotype. We demonstrated that even young children in a stable clinical condition show signs of persistent endothelial dysfunction. A prospective study should demonstrate whether elevated levels of vWF and its propeptide are associated with an increased risk of complications specific for the HED subphenotype.

Abbreviations

CAR: Central African Republic haplotype
ELISA: enzyme-linked immunosorbent assay
F1+2: prothrombin fragment 1 and 2
Hb: hemoglobin
HbF: hemoglobin F percentage
HED: hemolysis-endothelial dysfunction subphenotype
LDH: lactate dehydrogenase
MRI: magnetic resonance imaging
MRA: magnetic resonance angiography
NO: nitric oxide
SCD: sickle cell disease
SCI: silent cerebral infarcts
TAT: thrombin-antithrombin complex
VVO: viscosity-vaso-occlusion subphenotype
vWF: von Willebrand factor

INTRODUCTION

Sickle cell disease (SCD) is a heterogeneous disorder with a highly variable clinical spectrum. To improve our understanding of the diversity in clinical presentation, Kato et al postulated a classification of two clinical subphenotypes in adult patients.[1] According to this classification, patients with the viscosity-vaso-occlusion subphenotype (VVO) have a relatively high hemoglobin level and experience frequent vaso-occlusive pain crises, acute chest syndrome and osteonecrosis. At the other end of the spectrum are patients with the hemolysis-endothelial dysfunction subphenotype (HED); these patients have high levels of lactate dehydrogenase (LDH) and a high reticulocyte count and their clinical picture is characterized by stroke, pulmonary hypertension, priapism and leg ulcers.[2] It would be useful to identify pediatric SCD patients with the HED or VVO subphenotype to be able to screen for these specific complications. However, it is difficult to distinguish the two subphenotypes in young patients because the rate of complications is still low.

The exact pathophysiology of the subphenotypes remains unclear. A reduced bioavailability of nitric oxide (NO) due to intravascular hemolysis has been suggested to play an important role in endothelial dysfunction and in particular in pulmonary hypertension. Whether a decreased level of NO is a true cause of endothelial dysfunction or a secondary phenomenon remains a subject of debate.[3–5]

Von Willebrand factor (vWF) is a sensitive marker of endothelial dysfunction. Several reports have demonstrated elevated levels of vWF in adult patients with SCD.[6–9] The plasma concentration of vWF is higher in patients with a severe genotype (HbSS or HbSβ^0) compared to patients with a moderate genotype (HbSC or HbSβ^+).[8] There is very limited information on vWF plasma concentration in children with SCD. In a small study, 10 children with sickle cell disease and nocturnal hypoxemia were found to have elevated levels of vWF[10] but further studies on vWF in children with SCD are lacking.

VWF is released into the circulation by a constitutive pathway and a regulated pathway. After stimulation of the endothelium, the regulated pathway is activated and vWF that is stored in Weibel-Palade bodies undergoes cleavage of its propeptide. Subsequently, both mature vWF and its propeptide are secreted into the bloodstream. VWF propeptide returns to baseline values within approximately 7 hours after acute activation of the endothelium, whereas the increase of mature vWF is longer due to a four- to fivefold longer half-life.[11] Thus, in chronic endothelial dysfunction, levels of vWF propeptide are not increased. This was illustrated in patients with diabetes who were found to have an elevated level of mature vWF but a normal concentration of vWF propeptide.[12]

Both mature vWF and vWF propeptide may serve as indicators of future complications caused by endothelial dysfunction. This has been demonstrated in a prospective study of 102 children with insulin-dependent diabetes. A subgroup of patients developed elevated plasma concentrations of vWF and vWF propeptide. During a two year follow up, these children developed microalbuminuria, in contrast to the subgroup who did not have elevated plasma concentrations of vWF and vWF propeptide.[13] The results of this study suggest a relation between endothelial dysfunction and nephropathy.

VWF propeptide has never been studied in patients with SCD before. In the present study, we assessed plasma levels of vWF and its propeptide in a large group of young children with sickle cell disease in order to investigate endothelial dysfunction. Furthermore, we investigated whether vWF and vWF propeptide are influenced by genotype, haplotype or subphenotype.

METHODS

Patients

All children between 2-18 years with SCD treated at the Emma Children's Hospital of the AMC were eligible to participate in the study. Collection of blood samples was carried out during regular visits to the outpatient clinic when patients were in a stable clinical condition (i.e. no vaso-occlusive crises, infections or blood transfusions 3 months prior to collection of blood samples). Patients using hydroxyurea and those on chronic blood transfusions were excluded. Patients with HbSS or HbSβ^0 were considered to have a severe genotype and patients with HbSC or HbSβ^+ a mild genotype. The medical ethics committee approved the study protocol and written informed consent was obtained from all participants.

Laboratory parameters

VWF plasma concentration was measured by enzyme-linked immunosorbent assay (ELISA) (Dakopatts).[14] VWF propeptide was assessed using CLB-pro, an antibody against vWF propeptide.[11] To assess coagulation activation, prothrombin fragment 1 and 2 (F1+2) was measured by ELISA using a mouse anti-human antibody.[15] Thrombin-antithrombin complex (TAT) was measured by ELISA using a rabbit anti-human antibody (Enzygnost).[16] Hematological parameters including hemoglobin (Hb), reticulocytes and coagulation factor FVIII were measured. In addition, LDH was assessed. Hemoglobin F percentage (HbF) was only measured in children > 36 months to eliminate the effect of neonatal HbF.

Laboratory parameters were classified as abnormal when they were elevated 2 standard deviations (SD) above the mean of the normal population.

Haplotypes

Haplotype analysis of the β-globin gene cluster was performed by high-resolution melting curve analysis, as described previously[17,18] using the LightScanner instrument and software (LightScanner; Idaho Technologies Inc., USA). Only patients with a severe genotype (HbSS/HbSβ^0) were included in this analysis. Central African Republic (CAR) haplotype is considered to be the most severely affected haplotype and Benin haplotype a more moderate haplotype. Therefore we compared patients with at least one allele of the CAR haplotype (homozygous or heterozygous) with patients homozygous for Benin haplotype.

Subphenotypes

In our pediatric study population the complication rate is low and therefore it was not possible to definitely classify patients into the two subphenotypes based on clinical events. Because a relatively high level of hemoglobin is a characteristic of the VVO subphenotype, we classified patients within the upper quartile of hemoglobin level as the VVO subphenotype. Hemolysis is a hallmark of the HED subphenotype; therefore we classified patients within the upper quartile of LDH as the HED subphenotype. Although in adult patients with SCD, reticulocyte count is the best parameter for hemolysis[3], in children the reticulocyte count is highly dependent on several other factors, such as iron deficiency, bone marrow depression and infection. Therefore we used LDH as a more robust marker of hemolysis in children with SCD. To eliminate the effect of SCD genotype on the results, only patients with a severe genotype (HbSS/HbSβ^0) were included in this analysis.

Definition of clinical events

Data were collected on lifetime complications. We scored admissions for vaso-occlusive pain episodes. Stroke is defined as a focal neurological deficit with evidence on neuroimaging of a cerebral infarct corresponding with the focal deficit. Silent cerebral infarcts (SCI) are areas of increased signal intensity on cerebral imaging (magnetic resonance imaging, MRI) without a history or physical findings of a focal neurological deficit. Cerebral vasculopathy is defined as an abnormal high blood flow velocity as assessed by transcranial Doppler ultrasonography or stenosis of an intracerebral artery of 50% or more as assessed by magnetic resonance angiography (MRA). Acute chest syndrome is defined as a new appearance of an infiltrate on chest radiograph in presence of pulmonary symptoms. Priapism is defined as a persistent and often painful penile erection.

Avascular bone necrosis of hip or shoulder is defined as an abnormality seen on radiography due to bone infarction associated with chronic pain of the affected joint. Leg ulcers are defined as painful skin defects on (lower) legs.

Microalbuminuria

Microalbuminuria was defined as an elevated albumin/creatinine ratio (> 2.5 mg albumin per mmol creatinine in men and > 3.5 mg albumin per mmol creatinine in women). In some patients, only a urine dipstick test was performed. A positive result with urine dipstick was always confirmed by an elevated albumin/creatinine ratio.

Statistical analyses

All presented laboratory values are mean values calculated over 1 to 3 hospital visits per patient. We used a one-sample t-test to evaluate differences between laboratory parameters of our study group and reference values. To test differences between 2 groups, a student's t-test was used in normally distributed data and a Mann-Whitney U test was used when data were not normally distributed. A Fisher's exact test was used to evaluate differences in complication rates between groups. To investigate the association between blood group and levels of vWF and vWF propeptide a one-way ANOVA was performed.

RESULTS

We included 106 children with SCD, mean age 8.7 years (range 2.0 - 17.5 years); the majority (70%) had a severe genotype (HbSS or HbSβ^0). Laboratory parameters are presented in table I. The plasma concentrations of vWF and vWF propeptide were significantly higher in patients with SCD compared to the reference values (Table I, Figure 1). Most patients (53%) had blood group O. vWF plasma concentration was lower in patients with blood group O compared to patients with blood group A, B or AB (p=0.003 by ANOVA). The levels of vWF propeptide were not associated with blood group.

Genetic variability

Patients with a severe genotype (HbSS or HbSβ^0) had significantly higher concentrations of vWF and its propeptide and F1+2 and TAT compared to patients with a moderate genotype (HbSC or HbSβ^+) (Table I, Figure 1). In patients with a severe genotype, vWF and vWF propeptide were above the reference value in 32% and almost 24%, respectively.

The haplotype of the β globin gene cluster is a known modifier of clinical variability in SCD. Haplotype was available in 55 patients (74% of all patients with HbSS or HbSβ^0). Most patients were homozygous for Benin haplotype (32 patients, 58%). Second most prevalent was CAR haplotype, with 7 homozygous patients (13%) and 5 heterozygous patients (9%). The remaining 11 patients (20%) had miscellaneous haplotypes (heterozygous for Benin or Senegal, Cameroon or undetermined haplotypes).

Compared to patients with a homozygous Benin haplotype, patients with at least one CAR haplotype had a lower leukocyte count, LDH and FVIII (Table II). We did not find a difference in markers of endothelial dysfunction or coagulation activation between these two haplotype groups.

Subphenotypes

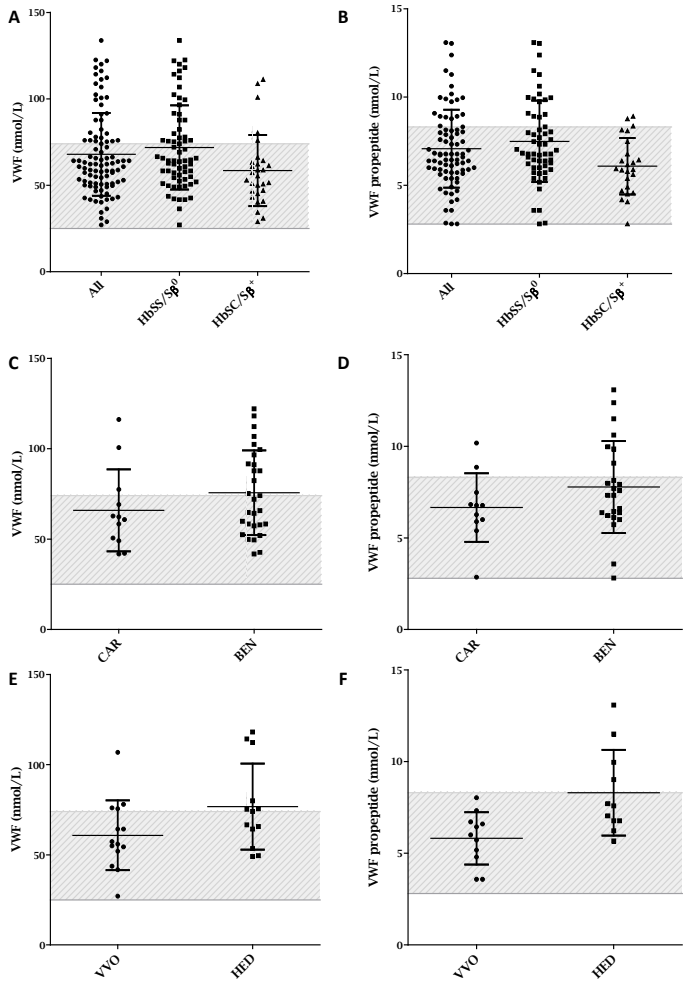
The comparison of the two subphenotypes was restricted to patients with a severe genotype (HbSS or HbSβ^0) (Table III). Age was comparable between VVO and HED subphenotypes (8.5 ± 5.1 years vs 7.5 ± 4.0 years, p=0.569) as was follow up time (13.3 ± 5.1 years vs 15.4 ± 5.8 years, p=0.383) and blood group (53% blood group O vs 62%, p=0.718). Hospitalization rate for vaso-occlusive pain episodes was very low and did not differ between the two subphenotypes. Cerebral vasculopathy was present in four patients with the HED subphenotype but in none of the patients with the VVO subphenotype.

Compared to the VVO subphenotype, patients in the HED group had more pronounced reticulocytosis (11.5% vs 6.8%, p=0.022) and higher leukocyte count (13.7 x10^9/l vs 10.5 x10^9/l, p=0.008). VWF and its propeptide were considerably higher in the HED group: 74 nmol/l vs 57 nmol/l, p=0.104 and 7.6 nmol/l vs 6.0 nmol/l, p=0.008, respectively (Figure 1).

Microalbuminuria

In the total cohort, 17% of patients had micoralbuminuria (19% of patients with HbSS or HbSβ^0 and 13% of patients with HbSC or HbSβ$^+$). In patients with a severe genotype, there was a trend towards a higher vWF propeptide in patients who had microalbuminuria compared to patients who did not have microalbuminuria (8.5 nmol/l vs 7.3 nmol/l, p=0.068). There was also trend towards a higher plasma concentration of vWF in (78 nmol/l vs 70 nmol/l, p=0.130). Also in patients with a mild genotype, there was a trend towards a higher vWF propeptide in patients who had microalbuminuria compared to patients who did not have microalbuminuria (4.4 nmol/l vs 6.5 nmol/l, p=0.062).

Figure 1. Distribution of Von Willebrand factor (VWF) and VWF propeptide.



VWF (A) and VWF propeptide (B) in all patients, patients with a severe genotype (HbSS or HbSβ^0) and patients with a mild genotype (HbSC or HbSβ$^+$). VWF (C) and VWF propeptide (D) in patients homozygous or heterozygous for Central African Republic (CAR) haplotype or homozygous for Benin haplotype (BEN). VWF (E) and VWF propeptide (F) in viscosity-vaso-occlusion subphenotype (VVO) and hemolysis-endothelial dysfunction subphenotype (HED).
Grey area: normal range of VWF or VWF propeptide (2 standard deviations above and below mean of normal population). Error bars represent mean ± standard deviation.

Table I. Laboratory parameters

		Total cohort (*n*=106)			HbSS/HbSβ⁰ (*n*=74)			HbSC/HbSβ⁺ (*n*=32)			
	Reference value			*p**			*p**			*p**	*p*#
Hb (g/l)	***105-161***	90	± 16	<0.001	81	± 10	<0.001	109	± 9	<0.001	< 0.001
HbF (%)	***< 1%***	8.2	(3.9 – 14.0)	<0.001	9.0*	(5.8 – 15.5)	<0.001	2.8*	(1.3 - 5.9)	0.039	< 0.001
Reticulocytes (%)	***0.5-2.5***	7.4	± 4.8	<0.001	8.8*	(6.5 – 12.2)	<0.001	2.7*	(2.0 – 3.4)	<0.001	< 0.001
Leukocytes (x10⁹/l)	***4.6-13.5***	10.3	± 3.8	0.002	12.0*	(9.0 – 13.7)	<0.001	7.1*	(5.1 – 8.9)	0.024	< 0.001
Platelets (x10⁹/l)	***150-350***	366	± 131	<0.001	412	± 120	<0.001	268	± 97	0.300	< 0.001
LDH (U/l)	***0-323***	485	± 183	<0.001	547*	(443 – 634)	<0.001	301*	(267 – 344)	<0.001	< 0.001
VWF (nmol/l)	***25 – 74***	68	± 24	<0.001	72	± 24	<0.001	59	± 21	0.025	0.011
VWF pro (nmol/l)	***2.8 – 8.3***	7.1	± 2.2	<0.001	7.5	± 2.3	<0.001	6.1	± 1.6	0.118	0.008
VWF ratio	***n.a.***	0.108	± 0.026	n.a.	0.105	± 0.025	n.a.	0.114	± 0.030	n.a.	0.191
FVIII (%)	***50-150***	238	± 82	<0.001	263	± 82	<0.001	181	± 49	<0.001	< 0.001
TAT (µg/l)	***0-4.6***	8.8	(5.3 – 13.7)	<0.001	9.3*	(6.4 – 14.7)	<0.001	5.9*	(4.6 – 9.5)	0.002	0.015
F1+2 (nmol/l)	***0.3-1.6***	0.7	(0.5 – 0.9)	0.606	0.8*	(0.5 – 0.9)	0.020	0.5*	(0.4 – 0.8)	0.742	0.006

Data is presented as mean ± standard deviation or median (25th to 75th percentile)
F1+2, prothrombin fragment 1 and 2; FVIII, factor VIII; Hb, hemoglobin; HbF, hemoglobin F; LDH, lactate dehydrogenase; TAT, thrombin-antithrombin complex; VWF, Von Willebrand factor.
* compared to reference values
# HbSS/HbSβ⁰ vs HbSC/HbSβ⁺

Table II. Endothelial dysfunction according to haplotype in patients with a severe genotype (HbSS or HbSβ⁰)

	CAR (*n* = 12)		**BEN** (*n* = 32)		***p***
Hb (g/l)	77	(69 – 82)	79	(74 – 86)	0.128
HbF (%)	11.7	(6.5 – 16.0)	9.8	(5.8 – 15.4)	0.610
Reticulocytes (%)	10.1	(5.9 – 13.8)	9.0	(6.3 – 10.9)	0.282
Leukocytes (x10⁹/l)	10.8	(6.0 – 12.7)	12.4	(9.5 – 14.1)	0.050
Platelets (x10⁹/l)	375	(246 – 456)	433	(359 – 511)	0.074
LDH (U/l)	438	(402 – 542)	553	(469 – 660)	0.030
VWF (nmol/l)	61	(49 – 75)	72	(58 – 94)	0.223
VWF pro (nmol/l)	6.8	(5.9 – 7.5)	7.5	(6.3 – 9.7)	0.183
VWF ratio	0.108	(0.088 – 0.128)	0.102	(0.087 – 0.115)	0.804
FVIII (%)	225	(153 – 276)	278	(242 – 334)	0.014
TAT (µg/l)	8.5	(4.9 – 21.0)	9.5	(7.6 – 14.7)	0.403
F1+2 (nmol/l)	0.64	(0.56 – 0.82)	0.83	(0.54 – 1.07)	0.413

Data is presented as median (25th to 75th percentile)
BEN, homozygous for Benin haplotype; CAR, homozygous or heterozygous for Central African Republic haplotype; F1+2, prothrombin fragment 1 and 2; FVIII, factor VIII; Hb, hemoglobin; HbF, hemoglobin F; LDH, lactate dehydrogenase; TAT, thrombin-antithrombin complex; VWF, Von Willebrand factor.

Table III. Subphenotypes in patients with a severe genotype (HbSS/HbSβ^0)

	VVO (*n* = 15)		**HED (*n* = 13)**		*p*
Hb (g/l)	***94***	*(92–98)*	*73*	*(68–79)*	*<0.001*
LDH (U/l)	*482*	*(293–574)*	***747***	*(669–787)*	*<0.001*
Clinical events					
Vaso-occlusive events (number of events per patient/years of FUP)	0.11	(0.00 – 0.33)	0.16	(0.00 – 0.23)	0.569
Stroke	0		1		0.464
Silent cerebral infarct (affected patients/patients with available imaging)	3/8	(38%)	6/9	(67%)	0.347
Cerebral vasculopathy	0		4		0.035
Acute Chest Syndrome (number of patients with ≥ 1 episode)	4		6		0.433
Priapism (affected boys/total boys)	0/8		1/9		1.000
Avascular bone necrosis (affected patients)	2		0		0.484
Leg ulcers (affected patients)	0		0		1.000
Laboratory parameters					
HbF (%)	11.4	(7.2–19.5)	7.5	(4.6–10.0)	0.143
Reticulocytes (%)	6.8	± 2.5	11.5	± 5.6	0.022
Leukocytes (x10^9/l)	10.5	± 2.9	13.7	± 2.7	0.008
Platelets (x10^9/l)	373	(319–517)	451	(267–482)	0.897
VWF (nmol/l)	57	(50–76)	74	(59–96)	0.104
VWF pro (nmol/l)	6.0	(4.8–6.7)	7.6	(6.8–10.0)	0.008
VWF ratio	0.104	± 0.023	0.110	± 0.020	0.541
FVIII (%)	215	± 64	306	± 77	0.003
TAT (µg/l)	8.2	(4.4–11.3)	12.3	(9.3–26.0)	0.061
F1+2 (nmol/l)	0.9	(0.6–0.9)	0.7	(0.5–1.0)	0.918

Data is presented as mean ± standard deviation or median (25th to 75th percentile).
F1+2, prothrombin fragment 1 and 2; FVIII, factor VIII; FUP, follow up; Hb, hemoglobin; HbF, hemoglobin F; HED, hemolysis-endothelial dysfunction subphenotype; LDH, lactate dehydrogenase; TAT, thrombin-antithrombin complex; VVO, viscosity-vaso-occlusion phenotype; VWF, Von Willebrand factor.

DISCUSSION

Despite the young age and the stable clinical condition at the time of testing, we found an abnormal high plasma concentration of vWF and vWF propeptide in our patients. This was more pronounced in patients with a severe genotype and in patients in the HED subphenotype. This is the first large study to evaluate vWF and vWF propeptide in young children with sickle cell disease. VWF is directly involved in adhesion of sickle red blood cells to the endothelium and is a promising parameter of endothelial dysfunction to study in SCD.[19]

Even in the absence of clinical signs of vaso-occlusion or infection, we found an elevated concentration of vWF propeptide, although not of the same magnitude as previously described in the context of acute illness.[12] This indicates that some form of persistent endothelial activation is present in our patients. VWF propeptide is overwhelmingly elevated in the context of acute severe illness, e.g. malaria, sepsis and thrombotic thrombocytopenic purpura. Previous studies on small groups of adult patients with chronic vascular diseases (i.e. chronic kidney disease and ischemic heart disease) demonstrated an elevated level of vWF propeptide, reflecting continuous acute endothelial activation.[20–24] Our results show that a subgroup of children (24%) with a severe genotype has elevated levels of vWF propeptide (Figure 1B). Whether the elevated concentration of vWF propeptide in this group of patients could potentially predict specific complications in SCD and therefore indentify patients with a HED subphenotype should be studied further. We did find an association between vWF propeptide and micoralbuminuria, but due to the cross sectional design of our study, we could not investigate the time relation between endothelial dysfunction and micoralbuminuria.

We analyzed the association of vWF and its propeptide with genotype, haplotype and subphenotype. The increase in vWF and vWF propeptide was more pronounced in patients with a severe genotype (HbSS or HbSβ^0) compared to patients with a moderate genotype (HbSC or HbSβ^+). The elevated concentration of vWF in patients with a severe genotype was found previously in a study in adults by Mohan et al[8] and a study by Nur et al[9], although this did not reach statistical significance in the latter study.

Our analysis of the association between haplotype and endothelial dysfunction was limited by lack of power, due to the lack of variation in haplotype within the study. There are no previous studies that have analyzed the association between haplotype and endothelial dysfunction. Prior studies on hematological differences between haplotypes are difficult to interpret because of conflicting results.

Patients with the HED subphenotype have a higher concentration vWF propeptide and a trend towards an elevated concentration of mature vWF, compared to patients with the VVO subphenotype.

Chronic hemolysis, the hallmark of the HED subphenotype, is associated with endothelial dysfunction although the exact mechanism remains unclear. Previous observations suggest that intravascular hemolysis leads to elevated plasma levels of cell free hemoglobin which produces reactive oxygen species. This results in scavenging of NO. In addition, hemolysis leads to the release of arginase into the plasma which metabolizes arginine and reduces the availability of substrate for NO production. The reduced bioavailability of NO may result in endothelial activation, enhanced expression of cell adhesion molecules and coagulation activation. However, as stated before, whether a decreased availability of NO is the true cause of endothelial dysfunction or a mere side effect remains unclear.

As vWF propeptide is considerably higher in the HED subphenotype, our results suggest that vWF propeptide is a potential predictor of subphenotype in young children. Our analysis was hampered by low complication rates in our young patient cohort (table III). The admission rate for vaso-occlusive pain episodes was very low, probably because most pain episodes are treated at home. However cerebral vasculopathy, a distinct feature of the HED subphenotype, was present in children with the HED subphenotype and not in patients with the VVO subphenotype ($p=0.035$). A prospective study should determine whether elevated levels of vWF propeptide precede specific complications seen in patients with the HED subphenotype, for example stroke and pulmonary hypertension, and whether it can be used to target specific screening for these complications.

In summary, we demonstrated that even young children during stable clinical condition demonstrate signs of persistent endothelial dysfunction; this is more distinct in patients with a severe genotype and in patients classified as the HED subphenotype. A prospective study should demonstrate whether elevated levels of vWF and vWF propeptide predict an increased risk of complications specific for the HED subphenotype.

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KF and MP designed the study and wrote the study protocol. KF supervised the collection of data. VvdL performed data collection and the statistical analysis and wrote the paper together with KF. CLH is responsible for the haplotyping of the HbS cases. MP, BB, HH and CH contributed to writing the paper and critically reviewed the paper. Competing interests: the authors have no competing interests.

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Chapter 3

CEREBRAL LESIONS ON 7 TESLA MRI IN PATIENTS WITH SICKLE CELL ANEMIA

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ABSTRACT

Background

Patients with sickle cell anemia (SCA) are at high risk to develop cerebral damage. Most common are silent cerebral infarctions (SCIs), visible as white matter hyperintensities (WMHs) on MRI in a patient without neurological deficits. The etiology of SCIs remains largely unclear. In addition, patients are at increased risk for overt stroke which is associated with large vessel disease. This classification based on the presence or absence of neurological deficits may not be the most fitting for research purposes as it does not match the different underlying pathology. A classification based on imaging findings may therefore be a more straightforward approach for research purposes. We explored the feasibility to identify imaging features of SCIs in young, asymptomatic patients with SCA using ultra high-field 7 Tesla (7T) MRI. 7T MRI has a high resolution which offers a unique chance to investigate small subclinical brain lesions in detail. To explore the superiority of 7T in identifying imaging abnormalities, we compared our results with 3T MRI.

Methods

10 young, neurologically asymptomatic patients with SCA underwent 7T and 3T MRI; 10 healthy, age matched controls underwent 7T MRI. We used existing neuroimaging standards to classify the brain lesions. We scored 7T and 3T scans separately, blinded for all other results.

Results

Using 7T MRI, we identified more patients with intracerebral lesions (9/10 versus 5/10), a higher total count of WMHs (203 vs 190, p=0.016) and more lacunes (5 vs 4) compared to 3T MRI. Abnormalities seen on 7T which could not be identified on 3T were cortical hyperintensities (in 3/10) and a different aspect of irregular WMHs, closely associated with cortical hyperintensities in a patient with large vessel stenosis. In 7 controls, a total of 13 WMHs were present.

Conclusion

Using 7T MRI we identified more intracerebral lesions compared to 3T, and found several abnormalities not visible on 3T. 7T MRI in SCA seems of particular interest to study the cortical involvement and the relation between WMHs and the cortex. We found some imaging features that are thought to be representative for small vessel disease, including WMHs, lacunes and prominent perivascular spaces; whether small vessel disease plays a role in SCA requires further research.

INTRODUCTION

Sickle cell anemia (SCA) is characterized by chronic hemolytic anemia and vaso-occlusive events that can lead to complications in almost all organ systems, including the brain.[1] The most common intracerebral lesions are silent cerebral infarctions (SCIs), defined as areas of abnormal hyperintensity on MRI in a patient with no history or physical findings of a focal neurological deficit.[2] SCIs are mainly visible as white matter hyperintensities (WMHs) on MRI; the etiology still remains largely unknown. Although SCIs may not be associated with neurological deficits in the acute phase, patients with multiple SCIs are at risk for cognitive impairment which can influence social and academic functioning.[3,4] Patients with SCA are at increased risk for overt stroke as well, which is associated with large vessel disease. The incidence of stroke has decreased dramatically since the introduction of screening with Transcranial Doppler imaging (TCD) and subsequent treatment of high risk patients with chronic blood transfusion therapy.[5]

The current distinction between the cerebral lesions is based on symptoms, i.e. the presence or absence of a neurological deficit. This classification is useful for patient care, however it may not be the most fitting for research purposes as it does not necessarily match the different underlying pathology. For example, overt strokes are generally cortical infarctions, but extensive WMHs or strategically located infarcts in subcortical grey matter can also lead to neurological deficits. Both would be classified as overt stroke even though the etiology is probably different. A classification based on imaging findings may therefore be a more straightforward approach for research purposes.

In the current study, we explored the feasibility to identify imaging features of SCIs in young, asymptomatic patients with SCA using ultra high-field 7 Tesla (7T) MRI. 7T MRI has a high resolution which offers a unique chance to investigate small subclinical brain lesions in detail. To explore the superiority of 7T in identifying imaging abnormalities, we compared our results with 3T MRI. We used existing neuroimaging standards to describe the brain lesions.[6]

METHODS

Patients

Patients with SCA (HbSS) between 18 and 25 years under treatment at the comprehensive SCA center of the Academic Medical Center, Amsterdam were eligible to participate. Exclusion criteria were a history of stroke, known neurological deficits, hypertension, regular blood transfusions, and exclusion criteria for 7T MRI scanning (metal implants, dental braces etc.).

The study was approved by the institutional ethical review board and written informed consent was obtained from all participants.

Medical history was collected retrospectively using medical charts. Vaso-occlusive crisis was defined as an episode of acute pain in limbs, back, neck or abdomen. Acute chest syndrome was defined as a new pulmonary infiltrate excluding atelectasis, in combination with the presence of chest pain, a temperature >38.5°C, tachypnea, wheezing, or cough.[7] The most recent blood pressure measurement during steady state was recorded. We calculated the mean level of hemoglobin over the last 2 years and collected the last measurement of hemoglobin F percentage. All patients underwent a neurological examination by a trained neurologist in accordance with the National Institutes of Health Stroke Scale (NIHSS).

MRI scanning

A 7T MRI was performed on all 10 patients at the University Medical Center of Utrecht (UMCU) using a volume transmit coil with a 32 channel receive array (Nova Medical, Wilmington, USA). We retrieved 3D MP-FLAIR scans (magnetization prepared fluid attenuated inversion recovery) of 10 age-matched healthy volunteers already available at the UMCU in order to evaluate intracerebral abnormalities in young, healthy volunteers without SCA.[8] 3T scans were performed at the Academic Medical Center of Amsterdam using a SENSE-8-channel head coil (Philips Healthcare, Best, The Netherlands). MRI sequence parameters can be found in the Supplementary Material (for all online suppl. material, see www.karger.com /doi/10.1159/000373917).

MRI analysis

7T and 3T scans were scored separately, blinded for all information including MRI results. Both 7T scans (JH, neuroradiologist and VvdL) and 3T scans (CBLMM; neuroradiologist and VvdL) were scored by two observers in mutual agreement. Cerebral lesions were categorized using the STRIVE (Standards for ReportIng Vascular changes on nEuroimaging) definitions when applicable, see Table 1 of the Supplementary Material.[6] Measurements were performed on transversal MP-FLAIR images using Osirix software version 5.7.1. Perivascular spaces (PVS) were scored as described previously.[9] In short, the number of PVS were scored as 0 = none, 1 = 1–10, 2 = 11–20, 3 = 21–40, and 4 = >40 in one hemisphere, separately in the basal ganglia and centrum semiovale. The hemisphere and slice with the highest number of PVS was chosen, a total score (0-8) was calculated by adding the score of basal ganglia and centrum semiovale. In addition, we described cortical lesions, intracranial hemorrhages and all other incidental findings.

Stenosis of intracerebral arteries was scored on the time-of-flight angiogram by a neuroradiologist (JH), and possible intracranial vessel wall abnormalities were evaluated from the MPIR-TSE (a T1 weighted magnetization prepared inversion recovery turbo spin echo).
A comparison of mean values between groups were performed with a Student's t-test, we tested the differences between categorical variables with a Fisher's exact test. A Wilcoxon signed rank test was used to compare the counts of WMHs and lacunes between 7T and 3T scans.

RESULTS

We included 10 neurologically asymptomatic young SCA patients with a mean age of 23 years (range 19-25 years). All patients had a normal neurological examination (NIHSS score 0). Four patients (A-D) had a relatively mild clinical course without severe complications of SCA, four patients (E-H) had a more severe course with at least one episode of acute chest syndrome; two remaining patients (I-J) had an intermediate course with infrequent admissions for vaso-occlusive pain crises. Hypertension was not present in any of the patients. See Table 2 of the Supplementary Material for a description of clinical and laboratory parameters.

7T MRI findings in SCA patients

Table 1 summarizes the findings at 7T and 3T MRI. Only one patient (patient I) had a completely normal scan. WMHs were observed most frequently: a total of 203 WMHs were present in 9 out of 10 patients, most patients had between 1 and 6 WMHs. Most of the lesions were small (< 5 mm), round and circumscript. WMHs were predominantly located in the frontal lobe, followed by the parietal lobe. Almost all lesions were located in the deep white matter with few periventricular lesions and some juxtacortical lesions.

Patient G was most severely affected with a total of 160 WMHs, mostly small (86% were < 5 mm) and located in the frontal or parietal lobe (86%). About one third of the WMHs were located juxtacortical but no periventricular lesions were found. This patient also had prominent large lesions alongside the optic tract (Figure 1A). Of note, this patient did not experience any large vessel stenosis and the aspect of the perforating arteries was normal. On the vessel wall scan, we observed a minimal irregularity of the carotid bifurcation on the left side, the right side was normal.

The WMHs in patient D displayed a pattern that differed from the other patients (Figure 2A), with larger and more irregular WMHs. This patient had an occluded A1 segment and proximal A2 segment of the left and right anterior cerebral artery, with flow in the distal A2 segment probably due to collaterals.

There were several cortical hyperintensities in the left and right frontal lobe, 4 small cortical hyperintensities (< 5 mm) and 4 somewhat larger cortical hyperintensities (5 – 10 mm). These cortical hyperintensities were not visible on the 3 Tesla MRI scans of this patient (Figure 2B) performed 17 days later.

The appearance of the WMHs in this patient were different compared to the WMHs found in other patients, as they were more irregular in shape and less circumscript. Some lesions were located periventricular which was not seen in other patients. Some of the larger WMHs were positioned close to the cortical hyperintensities (Figure 2A), but they did not appear to be confluent lesions.

We found an isolated cortical hyperintensity in two other patients (patient A and H) of approximately 2x2 mm in the frontal lobe on 7T (Figure 1B), despite the absence of any large vessel vasculopathy. These cortical hyperintensities were not identified on the 3T scans (both performed approximately one month before the 7T scans). Other abnormalities were lacunes of presumed vascular origin and perivascular spaces. Lacunes were present in 2 patients: patient D had one lacune and in patient G we identified 4 lacunes. Perivascular spaces were distinctly visible in high numbers in 7 out of 10 patients (PVS score ≥ 5) (Figure 4). We did not find any microbleeds in any of the patients. With the exception of patient D, there were no occlusions of intracranial vessels. With one exception (patient G), there were no vessel wall abnormalities found on the vessel wall (MPIR-TSE) images.

In patient A, several hypointense areas were visible at the FLAIR scan (Figure 3A) and, to a lesser extent, also on the T2 weighted TSE scan and the slightly T1 weighted vessel wall scan (MPIR-TSE) (Figure 3B). After careful evaluation of the images, these abnormalities were most likely SENSE artifacts originating from wet or gel-containing hair (dreadlock style).

3T MRI findings in SCA patients

Median time between the two MRI scans (3T and 7T) was 13.5 days (interquartile range 6 – 28 days); in 6/10 patients, 3T scanning was performed before 7T. WMHs were present in 6 out of 10 patients at 3T imaging. At 3T we found a total of 190 WMHs compared to 203 using 7T (p=0.016). We identified 4 lacunes at 3T, compared to 5 using 7T (not significant). Importantly, we did not find any cortical lesions at 3T. Even in patient D, cortical lesions were not visible at 3T: all WMHs were limited to the white matter with no visible extension towards the cortex.

7T MRI findings in controls

The mean age of the controls was 28 years (range 18-51) compared to 25 years of patients with SCA (not significant); all controls were Caucasian. In 7 out of 10 controls, a total of 13 WMHs were identified; 6 of the controls had only 1-2 WMHs and one control had 3 lesions.

All WMHs were <5mm except for one. PVS score was ≥ 5 in only one control compared to 7 in the patient group (p=0.01). No other intracerebral lesions were present.

FIGURE 1. Typical white matter hyperintensities and a cortical hyperintensity. Typical white matter hyperintensities in patient G (white arrows) and hyperintensities alongside the optic tract (dotted arrow), 7T MP-FLAIR image (A). A cortical hyperintensity (white arrow) in the frontal lobe of patient A, 7T MP-FLAIR image (B).

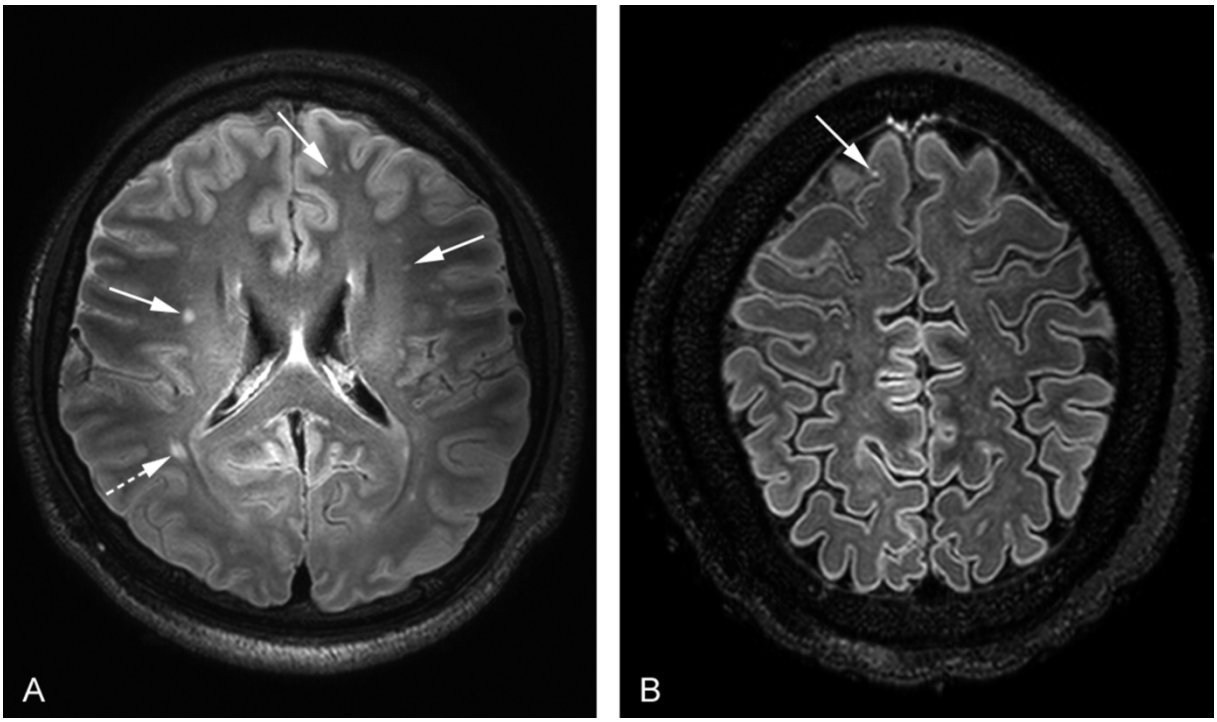

FIGURE 2: White matter hyperintensities and a cortical hyperintensity in a patient with large vessel disease. Cortical hyperintensity (white arrow) and associated white matter hyperintensities in patient D, the cortical hyperintensity is clearly visible on the 7T MP-FLAIR image (A) but not on the 3T FLAIR image (B).

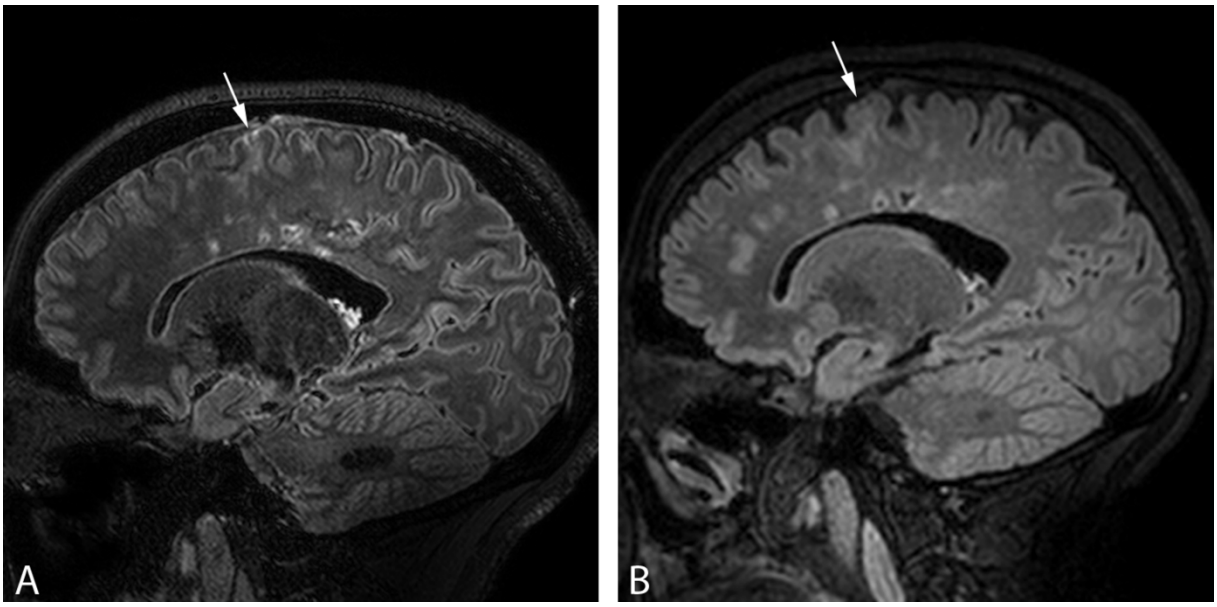

FIGURE 3: Hypointense areas on MP-FLAIR images. Patient A showed hypointense areas (arrow) on 7T MP-FLAIR image (A). Some of these structures were hyperintense on the slightly T1 weighted MPIR-TSE images (B). After careful evaluation, these structures are most probably caused by artifacts from gel/wax or water in the hair of the patient (hair style: dreadlocks), in combination with image acceleration by sensitivity encoding (SENSE).

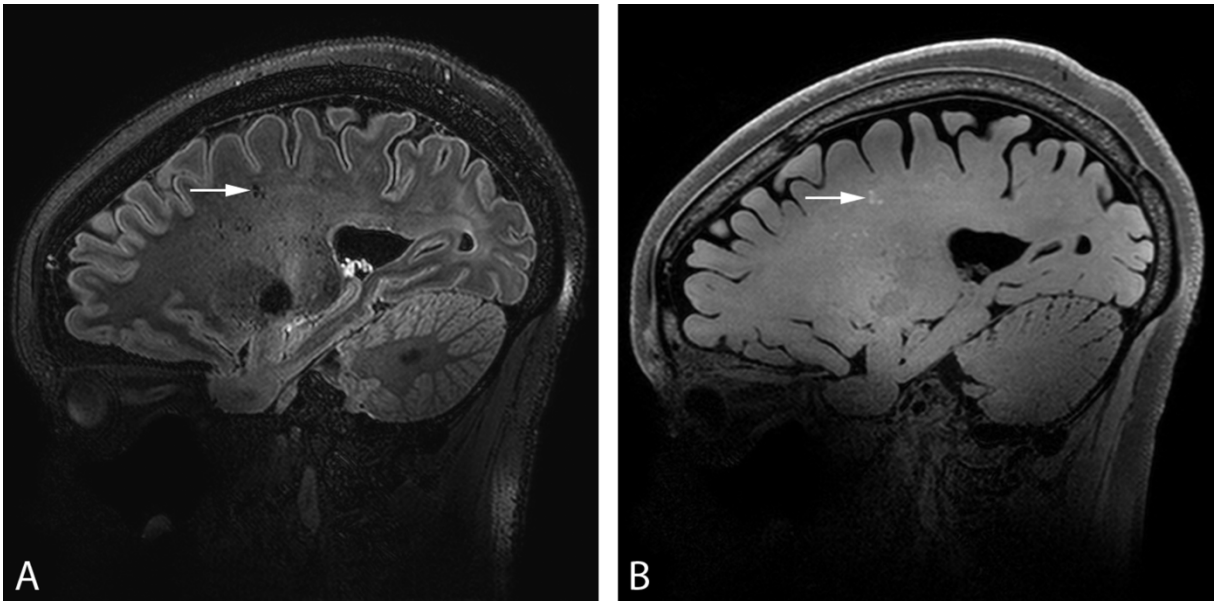

FIGURE 4: Distinctly visible perivascular spaces. Distinctly visible perivascular spaces in patient E; 7T MP-FLAIR image (A) and 7T TSE image (B). The arrow points to an example perivascular space.

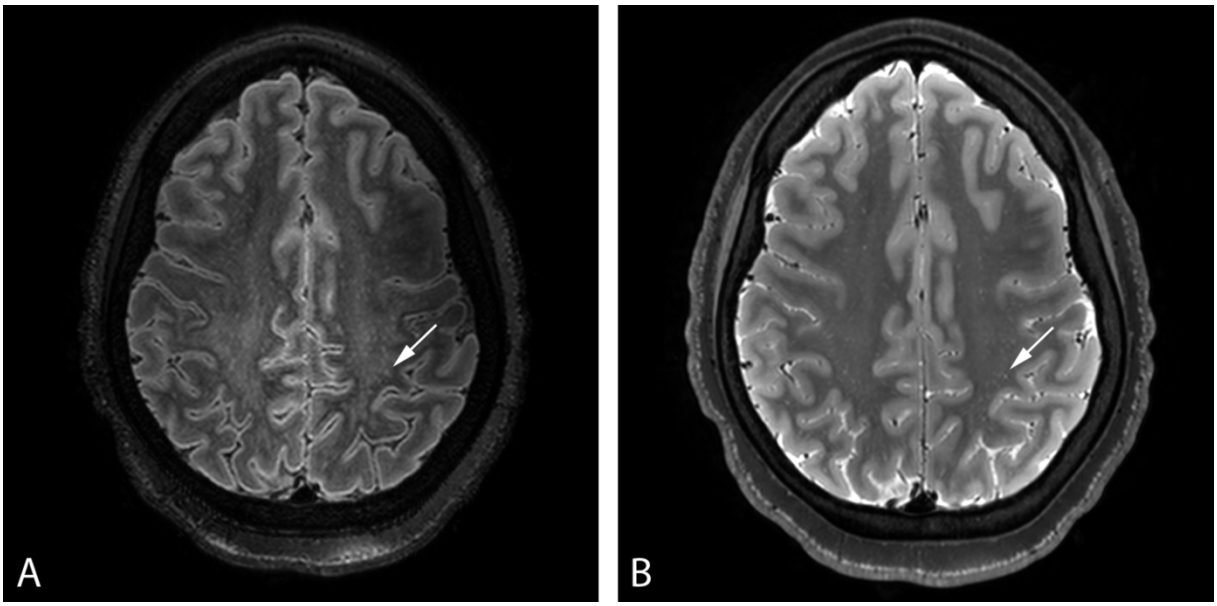

TABLE 1: Overview of findings at 7T and 3T MRI of 10 patients with sickle cell anemia

		WMHs				PVS	Other
		Total	*Size*	*Location*	*Lobe*		
A	7T	2	all < 5 mm	all DWM	all frontal	6	1 cortical hyperintensity (1.7x1.8 mm)‡ Hypointense artifacts on FLAIR#
	3T	-	-	-	-	6	-
B	7T	6	4 x < 5mm 2x ≥ 5 mm	5 x DWM 1 x juxtacort.	4 x frontal 2 x parietal	5	-
	3T	2	all < 5 mm	all DWM	all frontal	4	-
C	7T	1	< 5 mm	DWM	frontal	5	-
	3T	-	-	-	-	5	-
D	7T	24	16 x < 5 mm 9 x ≥ 5 mm	17 x DWM 4 x periventr. 4 x juxtacort.	17 x frontal 7 x parietal	4	Large vessel stenosis[1] 1 lacune (34x4 mm) 8 cortical hyperintensities (19-72 mm)†
	3T	23	15 x < 5 mm 8 x ≥ 5 mm	17 x DWM 4 x periventr. 3 x juxtacort.	20 x frontal 3 x parietal	5	Large vessel stenosis[1] 2 lacunes (7x5 mm and 4x4 mm)
E	7T	1	< 5 mm	DWM	frontal	8§	-
	3T	-	-	-	-	6	-
F	7T	4	2 x < 5 mm 2 x ≥ 5 mm	all DWM	3 x frontal 1 x parietal	6	-
	3T	4	all ≥ 5 mm	all DWM	3 x frontal 1 x parietal	6	-
G	7T	160	137 x < 5 mm 23 x ≥ 5 mm	159 x DWM 1 x juxtacort.	113 x frontal 23 x parietal 16 x temporal 8 x occipital	2	Bilateral large WMHs along optic radiation* 4 lacunes (all < 5 mm)
	3T	158	134 x < 5 mm 24 x ≥ 5 mm	109 x DWM 2 x periventr. 47 x juxtacort.	97 x frontal 26 x parietal 28 x temporal 7 x occipital	2	Bilateral large WMHs along optic radiation 2 lacunes (> 5 mm)
H	7T	2	2 x < 5 mm	2 x DWM	2 x frontal	2	1 cortical hyperintensity (2.2x1.6 mm)
	3T	-	-	-	-	2	-
I	7T	-	-	-	-	6	-
	3T	-	-	-	-	3	-
J	7T	3	3 x < 5 mm	3 x DWM	1 x frontal 2 x parietal	2	Large arachnoidal cyst parietal lobe
	3T	3	3 x < 5 mm	3 x DWM	1 x frontal 2 x parietal	2	Large arachnoidal cyst parietal lobe

DWM: deep white matter; FL, frontal lobe; FLAIR, fluid attenuated inversion recovery; MR: magnetic resonance; periventr., periventricular; PVS: perivascular space; WMH: white matter hyperintensity.
[1] Occluded A1 and proximal A2 segment of left and right anterior cerebral artery, collateral flow in distal A2 segment.
‡ see Figure 1B, # see Figure 3; † see Figure 2A; § see Figure 4;* see Figure 1A.

DISCUSSION

Using 7T MRI, we identified more patients with intracerebral lesions (9/10 versus 5/10), a higher total count of WMHs (203 vs 190, p=0.016) and more lacunes (5 vs 4) compared to 3T MRI. In addition, several abnormalities were seen on 7T which could not be identified on 3T: cortical hyperintensities (in 3/10) and a different aspect of irregular WMHs closely associated with cortical hyperintensities in a patient with large vessel stenosis (patient D).

The superiority of 7T in detecting WMHs has been demonstrated before in patients with multiple sclerosis.[10] Especially cortical lesions are far more easily detected by 7T compared to lower field strengths.[11–13] We detected a total of 10 cortical hyperintensities in 3 patients, which were not visible on 3T. In patient D, WMHs were found to be closely associated with cortical hyperintensities visible at 7T MRI, however this was not visible at 3T MRI. In patients with SCA, 7T MRI seems therefore of particular interest to study the involvement of the cortex and the relation between WMHs and the cortex.

We identified cortical hyperintensities in 3 patients, consistent with possible cortical microinfarcts. Cortical microinfarcts have been identified using 7T MRI in patients with Alzheimer's disease and elderly controls[14,15], but are very unusual at the age of our patients, who did not have any risk factors for cerebrovascular disease. A study comparing histology results with cortical hyperintensities seen at 7T MRI, has confirmed that these hyperintensities are indeed microinfarcts.[16] Cortical infarcts in SCA are usually associated with large vessel disease. The detection of possible cortical microinfarcts in 2 patients who did not have evidence of large vessel disease suggests that the involvement of the cortex is more extensive than previously known.

The WMHs in our patient with large vessel disease (patient D) displayed a very different pattern compared to the other patients, with larger and more irregular WMHs which were located periventricular and juxtacortical, a pattern not seen in the other patients. This may suggest that large vessel disease can lead to the formation of WMHs, but that the underlying mechanism may be different from the other patients without large vessel disease.

WMHs in other diseases such as vascular dementia are commonly classified as small vessel disease, even though histopathological confirmation is not always present.[6] In our study, we found some imaging features that are thought to be representative for small vessel disease, including WMHs, lacunes and prominent perivascular spaces. Recent studies suggest that endothelial dysfunction plays an important role in cerebral small vessel disease.[17–19] Endothelial dysfunction also plays an important role in other, more rare, conditions with WMHs such as CADASIL (Cerebral Autosomal Dominant Arteriopathy with Subcortical infarcts and Leucoencephalopathy) or Fabry disease.[20,21]

It is thought to derive from increased permeability of the endothelium, inflammation and impaired auto-regulation, finally leading to arteriolar wall thickening, luminal narrowing and occlusion. In one of the few autopsy studies in patients with SCA, diffuse thickening and sclerosis of intracerebral arterioles have also been described.[22] In addition, patients with SCA have a high level of endothelial dysfunction even at a young age.[23] We did not investigate markers of endothelial dysfunction in our patients, but endothelial dysfunction has been demonstrated consistently in all patients with SCA, even in those with a relative mild clinical course.[24,25] Endothelial dysfunction is thought to play a key role in the process of vaso-occlusion and vasculopathy, elicited by increased expression of adhesion molecules on red blood cells, hemolysis and impaired vasodilatation due to a decreased bioavailability of nitric oxide.[25,26] The absence of microbleeds in our patients may also indirectly suggest that vaso-occlusion and ischemic damage play a main role. For these similarities, we hypothesize that small vessel disease could potentially play a role in the occurrence of WMHs in SCA; however, this concept requires further research.

PVS scores were ≥ 5 in 7/10 patients compared to 1/10 controls (p=0.01). PVS are a normal finding in controls, but particularly high numbers have been associated with small vessel disease.[9,27] PVS are correlated with a decrease in cognitive functioning in healthy elderly and are more prevalent in vascular dementia compared to controls.[28,29] Although the exact significance of high PVS scores is not yet clear, this finding suggests, too, that SCA is associated with imaging markers similar to those found in small vessel disease.

We should note that WMHs were also present in healthy controls at 7T imaging, but to a much lesser extent compared to patients with SCA. No other abnormalities were present in these controls besides a limited number of WMHs. The incidence of WMHs at 7T imaging in healthy young adults is unknown, and accurate data in lower field strengths is scarce. In a large study in more than 2500 healthy military applicants with a mean age of 20.5 years WMHs were present in only 2.6%; however, this study only used 1T MRI.[30]

The hypointense areas on the 7T FLAIR scan in patient A (Figure 3) are most probably an artifact caused by the dreadlocks of the patient. Transverse reformats of the images showed signal spots outside the skull, suggestive for signal from gel or water in the hair (data not shown), which can lead to artifacts in combination with sensitivity encoding if not enough signal is present in the area to estimate the local coil sensitivity reliably. Recently, this type of artifact has been described in its general form as a 'bright structure reconstruction error', but it can be treacherously difficult to recognize particular examples.[31] For brain imaging, this artifact may particularly be present in hairstyles that use wax and yield signal distant from the skin (i.e. in regions where the coil sensitivity is not known).

As we evaluated a small number of patients, future studies should address the prevalence of WMHs and cortical hyperintensities at 7T in larger patient groups as well as the development over time. For research purposes, we would recommend a classification based on imaging findings instead of using the term SCI. As demonstrated in this study, several different types of lesions can be identified in patients with SCA using high field MRI. Even though STRIVE criteria were developed for a different pathology and may not fully fit the observations in SCA, the advantage of choosing standard criteria is avoiding the term silent cerebral infarct. This term actually encompasses several different imaging features with potentially different underlying pathologies and risk factors. Other imaging techniques such as diffusion-weighted imaging or diffusion tensor imaging may have additional value to further clarify the etiology of WMHs in SCA.

Limitations

We included only ten patients in our explorative study, therefore it is difficult to generalize our findings to the total SCA population. We had a comprehensive scan protocol, but other sequences may be of interest as well. For example, we did not perform diffusion-weighted imaging because the chance of encountering an acute ischemic lesion in asymptomatic patients was negligible, but this may be interesting when studying acute SCIs. Additionally, diffusion tensor imaging could further elucidate the damage to the white matter. We did not assess interobserver variability or reproducibility in our small study, future studies may address this. Regarding the comparison between 3T MRI and 7T MRI: the difference in receive coils (32 channels at 7T vs. 8 channels at 3T) may have contributed to the increased image quality at 7T while, on the other hand, image inhomogeneity due to inhomogeneous excitation at 7T may have reduced the conspicuity of lesions in the temporal lobes and cerebellum at 7T.

Conclusion

Using 7T MRI we identified more WMHs and lacunes compared to 3T MRI and found several cortical hyperintensities not visible on 3T MRI in young patients with SCA. 7T MRI in SCA seems of particular interest to study the cortical involvement and the relation between WMHs and the cortex. We found some imaging features that are thought to be representative for small vessel disease, including WMHs, lacunes and prominent perivascular spaces. Recent insights suggest that endothelial dysfunction plays an important role in cerebral small vessel disease, and this is also apparent in SCA. For these similarities, we hypothesize a shared pathological background between lesions in patients with SCA and cerebral small vessel disease, although this requires further research.

AUTHORSHIP

Design of the study: PJN, KF, JH, VvdL. MRI scanning protocol: PRL, JH, JJZ, AJN, HJMMM, FV. Image analysis: JH, VvdL, CBLMM, JJZ. Drafting /revising manuscript: PJN, KF, JJZ, VvdL. Revising manuscript: all authors.

Conflict of Interest

The authors report no conflict of interest.

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SUPPLEMENTARY MATERIAL

MRI scan protocol

Ultra high field MRI scanning (Philips Healthcare, Cleveland, USA) was performed at the University Medical Center of Utrecht using a volume transmit coil with a 32 channel receive array (Nova Medical, Wilmington, USA). The 7T scan protocol consisted of the following scans, including a brief summary of parameters. A 3D magnetization prepared fluid attenuated inversion recovery (MP-FLAIR) as previously described[1] (0.8 mm isotropic resolution, repetition time (TR) / inversion time (TI) 8000/2250, nominal echo time (TE) 300 ms, field of view (FOV) 250x250x190 mm^3), a fast T1 weighted gradient echo (1.0 mm isotropic resolution, TR between inversion pulses 3000 ms, TI 1163 ms, TR/TE 4.5/2.0 ms, flip angle 5°, FOV 250x250x200 mm^3), a T1 weighted magnetization prepared inversion recovery turbo spin echo (TSE) for vessel wall imaging as previously described[2] (MPIR-TSE, 0.8 mm isotropic resolution, TR/TI 3952/1375 ms, nominal TE 36 ms, FOV 250x250x190 mm^3), a dual echo gradient echo for combined time-of-flight and T2*-weighted imaging[3] (0.4x0.45x0.6 mm^3 resolution, TR/TE1/TE2 19/2.5/15 ms, excitation with tilted non-saturated excitation (TONE) pulses with nominal flip angle variation of 16°-24° in the feet–head direction over the slab, FOV 200x200x100 mm^3), and a 3D T2 weighted TSE[4] (T2w TSE, 0.7 mm isotropic resolution, TR/nominal TE 3158/301 ms, FOV 250x250x190 mm^3).

3T scans were performed at the Academic Medical Center of Amsterdam using a SENSE-8-channel head coil (Philips Healthcare, Best, The Netherlands). The 3T MRI scan protocol consisted of the following scans. A TSE 3DT2 (resolution 0.8 mm isotropic, TE/TR 323/2000 ms, FOV 250x250x180 mm^3). A TSE 3D FLAIR (resolution 1.1 mm isotropic, TE/TR 355/4800 ms, FOV 250x250x180 mm^3). A Fast Field Echo (FFE) 3D T1 (resolution 1.0 mm isotropic, TE/TR 3.5/9 ms, FOV 250x250x180 mm^3). A FFE 3D time-of-flight (TOF) magnetic resonance angiography (MRA) (resolution 0.4x0.6x0.5 mm, TE/TR 4/21 ms, FOV 200x200x90 mm^3).

Table 1. Definitions for neuro-imaging features of small vessel disease according to STRIVE[5]

Recent small subcortical infarct	Neuroimaging evidence of recent infarction in the territory of one perforating arteriole, with imaging features or clinical symptoms consistent with a lesion occurring in the previous few weeks.
Lacune of presumed vascular origin	A round or ovoid, subcortical, fluid-filled cavity (signal similar to CSF) of between 3 mm and about 15 mm in diameter, consistent with a previous acute small subcortical infarct or hemorrhage in the territory of one perforating arteriole.
White matter hyperintensity of presumed vascular origin	Signal abnormality of variable size in the white matter that shows the following characteristics: hyperintensity on T2-weighted images such as fluid-attenuated inversion recovery, without cavitation (signal different from CSF). Lesions in the subcortical grey matter or brainstem are not included in this category unless explicitly stated. If deep grey matter and brainstem hyperintensities are also included, the collective term should be subcortical hyperintensities.
Perivascular space	Fluid-filled spaces that follow the typical course of a vessel as it goes through grey or white matter. The spaces have signal intensity similar to CSF on all sequences. Because they follow the course of penetrating vessels, they appear linear when imaged parallel to the course of the vessel, and round or ovoid, with a diameter generally smaller than 3 mm, when imaged perpendicular to the course of the vessel.
Cerebral microbleed	Small (generally 2–5 mm in diameter, but sometimes up to 10 mm) areas of signal void with associated blooming seen on T2*-weighted MRI or other sequences that are sensitive to susceptibility effects.
Brain atrophy	A lower brain volume that is not related to a specific macroscopic focal injury such as trauma or infarction. Thus, infarction is not included in this measure unless explicitly stated.

CSF: cerebrospinal fluid. MRI: magnetic resonance imaging. STRIVE: standards for reporting vascular changes on neuro-imaging.

Table 2. Overview of clinical outcome and laboratory findings of 10 patients (A-J) with sickle cell disease

	Clinical outcome						Laboratory findings	
	Age	RR	VOC	ACS	Hydrea	Other	Hb	HbF
A	24	135/70	0.2/y	-	-	-	6.0	19.1
B	19	118/63	-	-	-	2005: aplastic crisis due to parvo B19 infection	6.8	3.2
C	25	127/70	0.1/y	-	-	-	6.1	3.3
D	20	*	-	-	5y6m	-	4.8	3.4
E	24	110/65	0.5/y	1	-	-	6.1	3.5
F	24	126/70	0.3/y	2	-	-	5.4	3.2
G	25	130/65	0.7/y	1	8y8m	2003: avascular necrosis femoral head; 2003: white matter hyperintensities on cerebral 3T MRI; 2009: sickle cell retinopathy	6.4	14.9
H	23	100/70	0.2/y	1	7y9m	2002 - 2004: recurrent episodes of priapism	5.9	11.8
I	21	117/69	0.7/y	-	3y2m	-	5.6	16.4
J	25	120/54	1.4/y	-	-	-	4.9	7.2

ACS: acute chest syndrome, total number of episodes during follow-up. Hb: mean level of hemoglobin during last 2 years of follow-up. HbF: most recent HbF percentage. Hydrea: use of hydroxyurea in years and months during follow-up. MRI: magnetic resonance imaging. RR: steady state blood pressure (mmHg). VOC: vaso-occlusive crises, mean number of hospitalizations per years of follow-up.
* No recent data available.

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Chapter 4

RISK FACTOR ANALYSIS OF CEREBRAL WHITE MATTER HYPERINTENSITIES IN CHILDREN WITH SICKLE CELL DISEASE

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SUMMARY

Sickle cell disease (SCD) is complicated by silent cerebral infarcts, visible as white matter hyperintensities (WMHs) on magnetic resonance imaging (MRI). Both local vaso-occlusion, elicited by endothelial dysfunction and insufficiency of cerebral blood flow (CBF) have been proposed to be involved in the etiology. We performed an explorative study to investigate the associations between WMHs and markers of endothelial dysfunction and CBF by quantifying WMH volume on 3.0 Tesla MRI. We included 40 children with HbSS or HbSβ^0thalassemia, mean age was 12.1±2.6 years. Boys demonstrated an increased risk for WMHs (odds ratio 4.5, 95% CI 1.2 - 17.4), unrelated to G6PD deficiency. In patients with WMHs, lower fetal hemoglobin (HbF) was associated with a larger WMH volume (regression coefficient=-0.62, R^2=0.25, p=0.04). Lower ADAMTS13 levels were associated with lower CBF in the white matter (regression coefficient=0.07, R^2 0.15, p=0.03), suggesting that endothelial dysfunction could potentially hamper CBF. The findings of our explorative study suggest that a high level of fetal hemoglobin may be protective for WMHs and that endothelial dysfunction may contribute to the development of WMHs by reducing CBF.

INTRODUCTION

Neurological complications in children with sickle cell disease (SCD) are common and include overt stroke, silent cerebral infarcts (SCIs) and diminished neurocognitive functioning. Overt stroke is usually associated with stenosis of the large intracerebral arteries; the incidence in children has decreased remarkably after the introduction of screening with Transcranial Doppler imaging (TCD) and subsequent treatment of high risk patients with chronic blood transfusion therapy.[1] Nowadays, SCIs are more common compared to overt stroke: approximately 40% of children with a severe genotype will be affected by the age of 14.[2] An SCI is defined as an area of abnormal hyperintensity on magnetic resonance imaging (MRI) of the brain in a patient without a history or physical findings of a focal neurological deficit.[3] Most SCIs are visible as white matter hyperintensities (WMHs) on MRI. SCIs are associated with diminished neurocognitive functioning, potentially hampering social and academic achievement.[4–6] Affected patients are at risk for progression of SCIs and overt stroke.[7] Risk factors include a low level of hemoglobin, male sex, a relatively high systolic blood pressure, intra- and extracranial cerebral vessel stenosis and acute anemic events.[8–10] Despite recent advances in the identification of risk factors, the exact pathophysiology leading to SCIs is still largely unknown.

Two possible disease mechanisms for the development of SCIs have been proposed. First, vaso-occlusion can take place throughout the body in SCD, and may also occur in the small blood vessels of the brain, leading to infarcts in the white matter. Vaso-occlusion occurs in the setting of endothelial and coagulation activation, increased expression of adhesion molecules and impaired vasodilatation due to a decreased bioavailability of nitric oxide.[11–13] These are well recognized phenomena even in young patients, leading to systemic vascular endothelial dysfunction.[14,15] Cerebral involvement is supported by the finding of diffuse thickening and sclerosis of intracerebral arterioles as described in one of the few autopsy studies in sickle cell disease.[16] Another indication for the role of endothelial dysfunction in the etiology of SCIs was demonstrated in a small study which found lower concentrations of tissue plasminogen activator (t-PA) and ADAMTS13 in 9 patients with SCIs compared to 38 patients without SCIs.[17] Although these findings are suggestive, definite evidence for a vascular etiology of SCIs in sickle cell disease is lacking.[18]

A second hypothesis for the development of SCIs is related to altered cerebral blood flow (CBF). Previous studies using an arterial spin labelling (ASL) technique on MRI have demonstrated an increased CBF in the grey matter of children with SCD compared to controls, probably as compensatory mechanism for the chronic anaemia.[19,20]

Cerebral reactivity, i.e. vasodilatory capacity in reaction to triggers such as hypercapnia, has been demonstrated to be significantly reduced in patients with SCD compared to controls.[21] This may lead to compromised CBF during episodes of increased metabolic demand such as fever or an acute drop in hemoglobin, leading to cerebral ischemia. Indeed, acute SCIs have been identified by diffusion weighted imaging in children experiencing an acute decrease in haemoglobin.[22] The exact association between baseline CBF, i.e. during steady disease state, and the risk of SCIs is unclear. Patients with a high baseline CBF could be at risk for the development of SCIs due to insufficient reserve capacity of CBF. On the other hand, SCIs could potentially lead to a decrease in CBF in the white matter because blood flow to necrotic tissue is decreased and subsequently CBF would become lower in the white matter. Therefore, it is of particular interest to study CBF in grey matter and white matter separately.

Probably both mechanisms – endothelial dysfunction leading to small vessel disease and insufficient CBF – are important factors in the etiology of WMHs in sickle cell disease. Additionally, CBF could also be influenced by endothelial and coagulation activation due to altered haemorheology and blood viscosity, besides being influenced directly by hemoglobin and haematocrit.[21,23–25] We performed an explorative study to investigate the association between endothelial and coagulation activation, CBF, and WMHs, and included known risk factors. To study these associations in detail, we calculated the total volume of the WMHs and used this as an outcome measure. Moreover, we analyzed CBF in the grey matter and white matter separately.

PATIENTS AND METHODS

Patients

We prospectively approached all eligible children in two Sickle Cell Comprehensive Care Centers (center 1: Emma Children's Hospital, Amsterdam and center 2: Sophia Children's Hospital, Rotterdam). Inclusion criteria were severe SCD, i.e. HbSS or HbSß^0 thalassaemia and age 8-16 years. Exclusion criteria were prior stroke, stenosis of intracranial arteries (as demonstrated by MRI/MRA prior to participation in this study), abnormal or intermediate velocity on transcranial Doppler imaging (according to [1], chronic blood transfusion therapy, bone marrow transplantation, contra-indications for MRI and concomitant major health problems. As the present study was part of a larger study that included a neuropsychological evaluation, we excluded patients with an inability to undergo neurocognitive testing, e.g. insufficient knowledge of the Dutch language.[6] Patients had to be in a steady disease state, i.e. the absence of infection or crisis for >4 weeks prior to study visit.

All patients underwent a neurological examination performed by a pediatric neurologist (ME) blinded for imaging results. Results were scored according to the Pediatric Stroke Outcome Measure (PSOM). The PSOM yields a score ranging from 0, no deficits, to 10, severe deficits on all 5 subscales (Supplemental Table I).[26] Focal neurological deficits were described separately. Because we specifically studied WMHs regardless of neurological status, patients with a previously unknown neurological deficit that became apparent during the neurological examination were *not* excluded.

Genotype was confirmed by high performance liquid chromatography (HPLC) and DNA analysis when necessary. Alpha-thalassemia was tested by DNA analysis. The use of hydroxycarbamide was recorded and results of the most recent TCD measurement during a regular hospital visit were collected. Blood pressure was measured and converted into percentiles adjusted for age and sex.[27] The Institutional Review Board of the Academic Medical Center in Amsterdam approved the study, written informed consent was obtained from all parents or legal guardians and from children aged twelve years and older.

Laboratory parameters

Blood sampling was performed on the day of study visit. Basic hematologic and biochemical parameters were assessed using standard in-house procedures. Percentage of fetal hemoglobin (HbF) was measured using high-performance liquid chromatography (HPLC). Free hemoglobin (free Hb) was measured using spectrophotometry on a Shimadzu UV-2401 PC. Von Willebrand factor (VWF) plasma concentration was measured by enzyme-linked immunosorbent assay (ELISA) (Dakopatts).[28] VWF activity was determined on an automated coagulation analyzer (Behring Coagulation System, BCS) with reagents and protocol from the manufacturer (Siemens Healthcare Diagnostics, Marburg, Germany). VWF propeptide was assessed by ELISA using two different monoclonal CLB-pro antibodies against VWF propeptide.[29] The activated conformation of VWF was measured by semi-automated ELISA on a TECAN Freedom EVO robot (Tecan, Männedorf, Switzerland) using an antibody against active VWF as described previously.[30] Prothrombin fragment 1 and 2 (F1+2) was measured by ELISA using a mouse anti-human antibody.[31] Thrombin-antithrombin complex (TAT) was measured by ELISA using a rabbit anti-human antibody (Enzygnost).[32] ADAMTS-13 was determined as described previously using a semi-automated assay.[33] Glucose-6-phosphate dehydrogenase activity (G6PD) was measured in all boys using spectrophotometry.

Magnetic Resonance Imaging

Acquisition. Patients were imaged on a 3.0 Tesla (3T) Intera with a SENSE-8-channel head coil (n=32) or on a 3.0 T Ingenia with a SENSE-15-channel head coil (n=8), both Philips Healthcare, Best, the Netherlands. The MRI scan protocol included both a 2 dimensional (2D) T2-weighted and 2D fluid-attenuated inversion recovery (FLAIR) scan (both 5 mm slice thickness) for the visualization of WMHs, and a time of flight (TOF) MR angiography (MRA) of the intracranial arteries for the visualization of arterial stenosis. Added to this protocol was an ASL sequence with pseudo-continuous ASL labelling strategy (PCASL) and a gradient echo single shot echo-planar imaging (EPI) readout, with the following imaging parameters: resolution = 3 × 3 × 7 mm^3; field of view (FOV) = 240 × 240 mm^2; 17 continuous axial slices; TE/TR = 17/4000 ms; flip angle = 90°; SENSE = 2.5; labelling duration = 1650 ms; post-labelling delay for the 17 sequentially acquired slices in ascending order = 1525-2014 ms. Seventy-five label and control pairs were acquired, resulting in a scan duration of ten minutes. Background suppression was implemented with two inversion pulses, 1680 ms and 2830 ms after a pre-labelling saturation pulse. The labelling plane was positioned approximately 9 cm caudal to the anterior commissure-posterior commissure line and perpendicular to the carotid and vertebral arteries, based on 2D coronal and sagittal TOF angiograms.[34]

ASL post-processing: quantification. Matlab 7.12.0 (TheMathWorks, Inc., Natick, MA USA) and the SPM8 toolbox (Statistical Parametric Mapping, Wellcome Trust Centre for Neuroimaging, London, UK) were used for offline data processing with custom-built software. After 3D rigid-body motion correction, the control-label pairs were pair-wise subtracted and a robust perfusion-weighted map was created using linear robust regression with Huber's M-estimator.[35] These perfusion-weighted maps were converted into CBF maps using a single compartment quantification model, assuming that the label decays with the T1 of blood.[36,37] A single M0 value - obtained in a previous study - was used for all participants.[38]

ASL post-processing: spatial normalization. The 2D T2-weighted anatomical scan was segmented into grey matter and white matter tissue probability maps. All CBF maps were transformed into anatomical space by a rigid-body registration of the averaged control image on the grey matter tissue probability maps. A two-stage approach was used to spatially normalize anatomical differences and residual EPI geometric distortion differences between subjects. First, the T1 tissue probability maps were spatially normalized using the Diffeomorphic Anatomical Registration analysis using Exponentiated Lie algebra (DARTEL) algorithm, with the resulting normalization fields applied to the average control images.[39] Secondly, the average control images were segmented into probability maps that were also normalized using DARTEL.

Finally, EPI geometric distortion was corrected by warping the average control images to the average T2. All estimated transformations were applied to the corresponding CBF maps. CBF was calculated for grey matter and white matter separately, for the whole brain and per hemisphere. In addition, we calculated CBF for the total normal appearing white matter by excluding the WMHs itself.

White matter hyperintensities. All scans were evaluated by two independent observers (CBLMM and VvdL or HJMMM); discrepancies were resolved by mutual consent. The observers were blinded for all clinical information and study results except for the diagnosis of SCD. We defined WMHs using the Standards for Reporting Vascular changes on Neuroimaging (STRIVE) definition: a white matter hyperintensity of presumed vascular origin being a hyperintensity of variable size in the white matter on the FLAIR scan, without cavitation.[40] Incidental findings were described separately.

WMH volume was obtained semi-automatically by the procedure as illustrated in Figure 1. On all FLAIR slices WMHs were visually identified and manually selected as regions of interest (ROIs) (Figure 1a) with a wide margin using ITK-Snap.[41] These ROIs were used for an intensity-based segmentation. Grey matter was segmented on all FLAIR scans (Figure 1b), and the mean grey matter intensity of each patient was calculated. All voxels with a signal intensity >1.02 times of the average grey matter intensity were selected (Figure 1c). All selected voxels within the manually delineated ROIs were labelled as WMH (Figure 1d). Stenoses of intracerebral arteries were rated on the TOF MRA by an experienced neuro-radiologist (CBLMM) as follows: <25%; 25-50%; 50-75%; 75-99% or occlusion.

Statistical analysis

For comparison of continuous variables between groups, we used a Student's t-test or a Mann-Whitney U test when data was not normally distributed. For categorical variables, we performed a Fisher's exact test. In patients with WMHs, we investigated the association between potential laboratory risk factors and CBF on one side, and WMH volume on the other side using linear regression analysis. Because the distribution of WMH volume was skewed we used a rank score of WMH volume as the outcome in the linear regression analyses. In addition, we investigated the association between potential laboratory risk factors and CBF using linear regression.

RESULTS

Patient population

We included 40 patients (for inclusion and exclusion, see Supplemental Figure 1). Mean age was 12.1 ± 2.6 years, 58% was male and most patients had homozygous SCD (95%); the remaining patients had HbSß^0 thalassemia. A total of fifteen patients used hydroxycarbamide with a mean duration of 3.5 years: 39% of boys and 35% of girls. G6PD deficiency was not present. TCD was normal in all 40 patients. Endothelial and coagulation activation was present in a large proportion of the patients: VWF antigen, VWF activity and VWF active conformation were above the normal limit in 68%, 51% and 46%, respectively, and TAT in 89% and F1+2 in 29% of the patients.

Description of neurological damage

WMHs were present in half of the patients (Table I); the WMH volume per patient displayed a wide variability. None of the patients had a history of overt stroke. Neurological examination was normal in 34 patients (85%). Three patients had a focal neurological deficit: one boy had a pyramidal tract syndrome, one girl had a partial visual field deficit and another girl experienced saccadic eye movements. These patients all had a high WMH volume (863 mm^3, 15.097 mm^3 and 16.343 mm^3, respectively). The above mentioned boy also had an abnormal MRA: a 50-75% stenosis of the A1 branch of the left anterior cerebral artery despite normal TCD. One other boy had several intracranial stenoses, despite normal TCD: a 75-99% stenosis of the left internal carotid artery, a 50-75% stenosis of the M1 branch of the left middle cerebral artery, a 25-50% stenosis of the M1 branch of the right middle cerebral artery and a 25-50% stenosis of the A1 branch of the right anterior cerebral artery. This patient had extensive WMHs with a total volume of 1420 mm^3; neurological examination was normal. Grey matter involvement was seen in 2 other patients. In one boy we discovered a small (<15 mm) cortical infarct in the occipital lobe with a normal neurological evaluation; this patient had a WMH volume of 697 mm^3, stenosis of intracranial arteries could not be evaluated because of motion artefacts on the TOF scan but TCD was normal. One girl had two small (<5 mm) cortical infarcts in the frontal and temporal lobe, she did not have any WMHs and neurological examination was normal.

Potential risk factors for WMHs including endothelial and coagulation activation

WMHs occurred more often in boys (odds ratio 4.5, 95% CI 1.2 - 17.4) (Table II). This effect was not mediated by age (12.6 years for boys vs 11.5 years for girls, p=0.22), G6PD deficiency (not present) or HbF (11.3% in boys vs 9.7% in girls, p=0.41).

We could not demonstrate significant differences in other potential risk factors between patients with WMHs and patients without WMHs. The use of hydroxycarbamide was not different in patients with (7 out of 20) or without WMHs (8 out of 20 patients).

In patients with WMHs, we studied the association between potential risk factors and WMH volume using linear regression analysis (Table III). A lower HbF was associated with larger WMH volume (Figure 2) (regression coefficient -0.62, R^2=0.25, p=0.04). When we repeated this analysis in the patients who did not use hydroxycarbamide (n=13), the association was still significant (regression coefficient -0.60, R^2=0.36, p=0.04).

Cerebral blood flow and its association with WMHs

Total grey matter and white matter CBF did not differ between patients with WMHs or patients without WMHs. In patients with WMHs, there was no association between WMH volume and CBF in any brain region (p>0.10 for all associations). CBF did not differ between boys and girls.

In patients with WMHs, we calculated CBF in normal appearing white matter by excluding the WMHs itself. Mean CBF in normal appearing white matter was 35.9 mL/100g/min, this was similar to mean CBF in white matter of 37.3 mL/100g/min of patients without WMHs (p=0.41).

Association between CBF and laboratory parameters including endothelial and coagulation activation

Higher CBF values in grey matter and white matter were both significantly associated with lower Hb (regression coefficient -0.47, R^2 0.23, p<0.01 and regression coefficient -0.17, R^2 0.22, p<0.01, respectively) and lower HbF (regression coefficient -0.74, R^2 0.12, p=0.03 and regression coefficient -0.24, R^2 0.11, p=0.04, respectively). Lower CBF in white matter was associated with lower ADAMTS13 (regression coefficient 0.07, R^2 0.15, p=0.03) and the VWF antigen to ADAMTS13 ratio (regression coefficient -2.33, R^2 0.13 , p=0.04) (Table IV).

Table I. Distribution of white matter hyperintensities

Volume category	median	*n*	%
WMH volume (mm^3)			
0 mm^3	0 mm^3	20	50%
1-100 mm^3	46 mm^3	7	17%
101-1000 mm^3	503 mm^3	10	25%
>1000 mm^3	15.097 mm^3	3	8%

WMHs, white matter hyperintensities.

Table II. Potential risk factors for white matter hyperintensities in children with sickle cell disease

	Reference	No WMHs (*n*=20)	WMHs (*n*=20)	*p*
Age (years)		12.2 ± 2.8	12.1 ± 2.5	0.98
Sex, male		8 (40%)	15 (75%)	0.05
Systolic blood pressure (percentile)		54 ± 26	55 ± 27	0.90
Hydroxycarbamide use		8 (40%)	7 (35%)	1.00
Intracranial stenosis		-	2 (10%)	0.49
α thalassemia αα/αα		8 (40%)	10 (50%)	0.75
αα/α-		11 (55%)	8 (40%)	0.75
α-/α-		-	2 (10%)	0.49
missing		1 (5%)	-	1.00
Hemoglobin (g/L)	105-161	86 ± 11	83 ± 12	0.41
Reticulocytes (%)	0.5-2.5	9.4 ± 4.4	10.1 ± 5.3	0.67
Leukocytes (10^9/L)	4.6-13.5	9.9 ± 2.2	10.6 ± 4.1	0.51
HbF (%)	< 1.0%	11.8 ± 6.0	9.3 ± 4.8	0.18
Free Hb (g/L)	0.16-0.48	0.19± 0.15	0.24 ± 0.9	0.41
LDH (U/L 37C)	0-323	491 ± 85	531 ± 128	0.26
VWF Antigen (%)	50-150	178 ± 45	176 ± 41	0.84
VWF Activity (%)	50-150	155 ± 47	144 ± 43	0.45
VWF Propeptide (%)	50-150	120 ± 37	112 ± 23	0.43
VWF Active conformation (%)	50-150	154 ± 36	163 ± 66	0.64
TAT Complex (ug/L)	0.0-4.6	7.0 (5.3 – 12.8)	8.0 (6.5 – 14.7)	0.47
F1+F2 (pmol/L)	53-271	201 (122 – 371)	178 (98 – 303)	0.65
ADAMTS-13 (%)	58-139	99 ± 11	100 ± 32	0.89
Ratio VWF Pro./VWF Ag.		0.64 (0.57 – 0.79)	0.62 (0.54 – 0.73)	0.57
Ratio VWF Antigen/ADAMTS-13		1.75 (1.52 – 2.15)	1.81 (1.28 – 2.45)	0.80

Data are presented as count (percentage) for categorical data, mean ± standard deviation (SD) for continuous data, or median (interquartile range) for continuous data not normally distributed. Age and gender adjusted percentiles were calculated for systolic blood pressure. F1+2, prothrombin fragment 1+2; Ag., Antigen; LDH, lactate dehydrogenase; Pro., Propeptide; TAT, thrombin-antithrombin; VWF, von Willebrand Factor; WMHs, white matter hyperintensities.

Table III. Association between potential risk factors and white matter hyperintensity volume

	Patients with WMHs (*n*=20)		
	β	R^2	*p*
Age (yrs)	-0.11	<0.01	0.85
Systolic blood pressure (percentile)	-0.05	0.04	0.38
Hemoglobin (g/L)	-0.10	0.04	0.40
Reticulocytes (%)	-0.09	0.01	0.72
Leukocytes (10^9/L)	-0.14	0.01	0.69
HbF (%)	**-0.62**	**0.25**	**0.04**
Free Hb (g/L)	1.9	<0.01	0.82
LDH (U/L 37C)	<0.01	<0.01	0.94
VWF Antigen (%)	<0.01	<0.01	0.91
VWF Activity (%)	<-0.01	<0.01	0.85
VWF Propeptide (%)	-0.11	0.17	0.08
VWF Active conformation (%)	<0.01	<0.01	0.72
TAT Complex (ug/L)	0.01	<0.01	0.92
F1+F2 (pmol/L)	<0.01	<0.01	0.78
ADAMTS-13 (%)	-0.05	0.09	0.25
Ratio VWF Pro./VWF Ag.	-12.45	0.14	0.10
Ratio VWF Antigen/ADAMTS-13	1.37	0.05	0.43

Linear regression analysis with WMH volume as outcome, R^2 is explained variance and *β* is unstandardized regression coefficient. Bold denotes statistical significance (p<0.05). Age and gender adjusted percentiles were calculated for systolic blood pressure. F1+2, prothrombin fragment 1+2; Ag., Antigen; LDH, lactate dehydrogenase; Pro., Propeptide; TAT, thrombin-antithrombin; VWF, von Willebrand Factor; WMHs, white matter hyperintensities.

Table IV. Association between laboratory markers and Cerebral Blood Flow

	CBF in grey matter			CBF in white matter		
	β	R^2	*p*	*β*	R^2	*p*
Hemoglobin (g/L)	**-0.47**	**0.23**	**<0.01**	**-0.17**	**0.22**	**<0.01**
Reticulocytes (%)	0.65	0.07	0.09	0.20	0.05	0.16
Leukocytes (10^9/L)	-0.70	0.04	0.22	-0.20	0.03	0.33
HbF (%)	**-0.74**	**0.12**	**0.03**	**-0.24**	**0.11**	**0.04**
Free Hb (g/L)	-12.16	0.03	0.32	-7.49	0.09	0.09
LDH (U/L 37C)	0.01	0.01	0.63	<0.01	<0.01	0.79
VWF Antigen (%)	-0.05	0.03	0.31	-0.01	0.02	0.37
VWF Activity (%)	-0.02	0.01	0.67	-0.01	0.01	0.62
VWF Propeptide (%)	0.05	0.01	0.50	0.02	0.01	0.53
VWF Active conformation (%)	0.07	0.08	0.11	0.02	0.04	0.26
TAT Complex (ug/L)	<0.01	<0.01	1.00	0.09	0.08	0.08
F1+F2 (pmol/L)	<0.01	<0.01	0.71	0.01	0.09	0.08
ADAMTS-13 (%)	0.09	0.03	0.36	**0.07**	**0.15**	**0.03**
Ratio VWF Pro./VWF Ag.	15.60	0.07	0.12	4.50	0.05	0.21
Ratio VWF Antigen/ADAMTS-13	-4.30	0.06	0.18	**-2.33**	**0.13**	**0.04**

Linear regression analysis with CBF in grey matter or in white matter as outcome, R^2 is explained variance and *β* is unstandardized regression coefficient. Bold denotes statistical significance ($p<0.05$). F1+2, prothrombin fragment 1+2; Ag., Antigen; CBF, cerebral blood flow; LDH, lactate dehydrogenase; Pro., Propeptide; TAT, thrombin-antithrombin; VWF, von Willebrand Factor; WMHs, white matter hyperintensities.

Figure 1. Semi-automatic delineation of white matter hyperintensities. A. Fluid attenuated inversion recovery (FLAIR) scan. B: Manual delineation of region of interest containing a white matter hyperintensity. C: Automatic segmentation of grey matter. D: Segmentation of voxels with an intensity of >1.02 times the intensity of grey matter within the region of interest.[6]

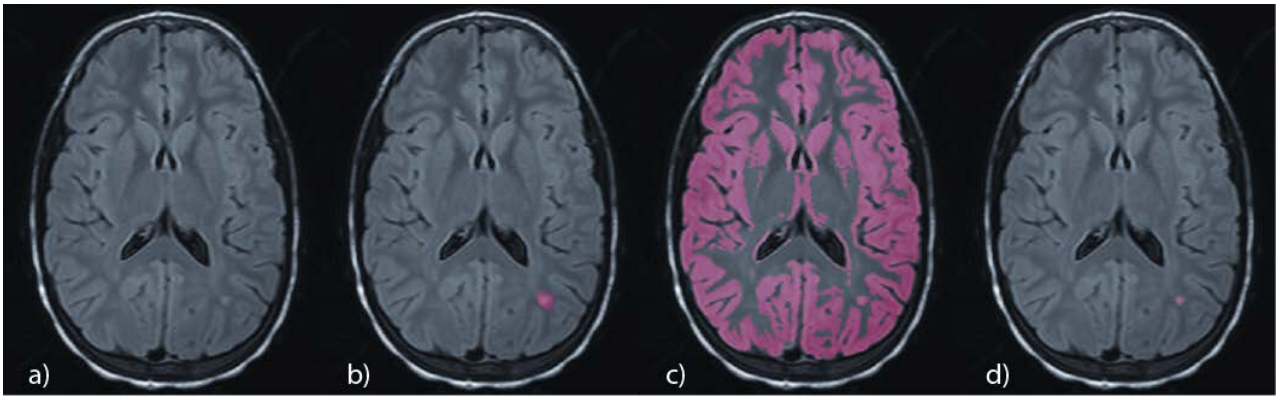


Figure 2. Association between HbF and white matter hyperintensity volume. Patients with white matter hyperintensities were selected. X-axis denotes HbF (%), Y-axis denotes rank score of white matter hyperintensity volume. HbF, fetal hemoglobin; WMHs, white matter hyperintensities.
● patients not using hydroxycarbamide ▲ patients using hydroxycarbamide.

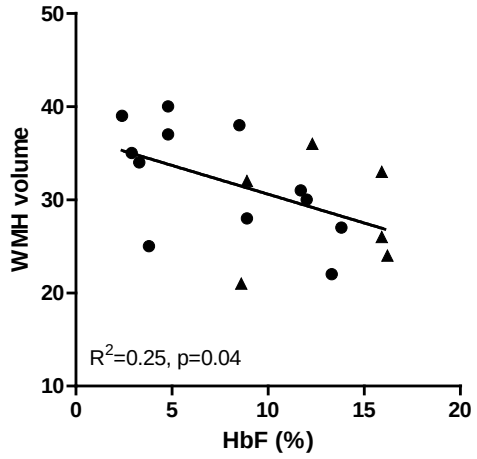
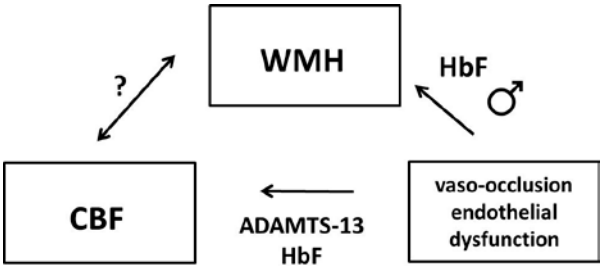


Figure 3. Proposed interaction between endothelial dysfunction, cerebral blood flow and white matter hyperintensities in sickle cell disease



DISCUSSION

We performed an explorative study to investigate risk factors for WMHs, including markers of endothelial and coagulation activation, and to investigate whether CBF was associated with WMHs. We could not demonstrate an association between endothelial or coagulation activation and WMHs, but found that a lower level of HbF was associated with a larger WMH volume, and that boys had an increased risk of WMHs. A low plasma concentration of ADAMTS13 was associated with lower CBF in the white matter; this preliminary finding suggests that endothelial dysfunction is potentially associated with decreased CBF. CBF in grey and white matter was similar in patients with and without WMHs.

We demonstrated that a lower level of HbF is associated with a larger WMH volume. This has not been reported previously, as earlier studies have not taken the WMH volume into account, and therefore this observation awaits confirmation in larger studies. Patients with SCIs in the Créteil newborn cohort had higher HbF values compared to patients without SCIs (13.1% vs 16.5%, p=0.02), however HbF was not a predictor of SCIs in Cox regression analysis.[9] In the Silent Cerebral Infarct Multi-Center Clinical Trial (SITT), there was a trend towards a lower HbF in patients with SCIs compared to patients without SCIs (11.1% vs 12.5%, p=0.06).[8] It would be very interesting to know whether the level of HbF was associated with lesion volume in this very large screenings cohort (n=814). HbF is one of the most important known modifiers of disease severity in SCD, predicting mortality, pain rates, dactylitis and acute chest syndrome.[42–46] Because HbF does not polymerize and reduces the concentration of HbS, high levels can inhibit polymerization and the subsequent detrimental effects such as hemolysis and vaso-occlusion.[47] Increasing HbF by using hydroxycarbamide has been shown to decrease several complications and decrease hemolysis, but it is unclear whether it can protect against the development of WMHs.[48,49] The association between HbF and WMH volume was still significant when we excluded patients who used hydroxycarbamide.

Hemoglobin was not associated with WMHs in our study, this is in contrast to both the SITT (odds ratio of 1.25 for every g/dL decrease in Hb) and a longitudinal study by Bernaudin et al (hazard ratio of 1.42 for every g/dL decrease in Hb).[8,9] A third, retrospective study in 65 young children (mean age 3.7 ± 1.1 years) found lower hemoglobin levels in children with SCIs compared to children without SCIs (77 ± 8 g/L versus 83 ± 11 g/L).[10] However, these relatively small odds and hazard ratio, and the small difference in hemoglobin level between patients with and without SCIs, demonstrate a moderate effect size of hemoglobin on SCIs. In our quantitative analysis, we could not demonstrate that lower hemoglobin levels were associated with a higher WMH volume.

This may be due to our small sample size in combination with a small range of hemoglobin levels. Alternatively, it suggests that when a patient has developed WMHs, the hemoglobin level is not a strong predictor of the total extent of the lesions.

We could also not demonstrate an association between systolic blood pressure and WMHs, in contrast to the SITT study. We used age and gender adjusted z-scores, however, also a post hoc analysis using unadjusted blood pressure could not demonstrate an association between blood pressure and WMHs. Results of the SITT demonstrated that the combination of a low level of hemoglobin and a relatively high systolic blood pressure was associated with the highest odds on SCIs, indicating that these factors have a synergistic effect on the risk for SCIs.

In our study, boys displayed an increased risk for WMHs independent of age, anemia, HbF or G6PD deficiency. This is in agreement with results of the SITT, although their odds ratio of 1.37 is lower compared to our odds ratio of 4.5.[8] Bernaudin et al found a trend towards an increased risk for SCIs in boys (p=0.07) in univariate analysis in their longitudinal cohort study of 217 children.[2] Despite these observations, little is known about the cause of the increased risk in boys.

Cerebral Blood Flow

We observed that CBF in grey and white matter were both associated with hemoglobin level and HbF. The association between hemoglobin or hematocrit and CBF has been demonstrated in an animal model and in neonates undergoing cardiopulmonary bypass[50,51] and in sickle cell disease using Xenon inhalation.[21] However, using ASL in patients with sickle cell disease, a study by Oguz et al could not find an association between CBF and hemoglobin, and Helton et al only found an association between some but not all vascular territories.[19,25]

These differences can presumably be explained by differences in imaging techniques, such as the use of a 1.5T scanner, or challenges with labelling efficiency, leading to asymmetries in CBF. We used state of the art ASL technique yielding high quality CBF results, and found robust associations with CBF in both grey and white matter.

Besides being associated with hemoglobin and HbF, we observed that lower levels of ADAMTS13 were associated with lower CBF in the white matter. ADAMTS13 is released from endothelial cells and cleaves large VWF multimers into smaller units. A (relative) insufficiency of ADAMTS13 indicates endothelial dysfunction. Some evidence for the role of ADAMTS13 in the pathogenesis of WMHs was described by Colombatti et al, who found lower ADAMTS13 levels in patients with WMHs compared to patients without WMHs.[17] Interestingly, recent insights suggest that endothelial dysfunction plays a pivotal role in the occurrence of SCIs in other patient groups such as vascular dementia and hypertension associated WMHs.[52–54]

The role of endothelial dysfunction is also important in other, more rare conditions with WMHs such as CADASIL (Cerebral Autosomal Dominant Arteriopathy with Subcortical infarcts and Leucoencephalopathy) or Fabry disease.[55,56]

We did not find any difference in CBF in grey or white matter between patients with and without WMHs. Nor did CBF in normal appearing white matter differ from CBF in the white matter of patients without WMHs. This may be due to the fact that we performed a cross-sectional study and only investigated patients during steady disease state. Perhaps CBF could become insufficient during episodes of increased metabolic demand. Future studies focusing on the dynamics of CBF could help to elucidate the role of impaired CBF in the etiology of WMHs.

Taken together, our results suggest that endothelial dysfunction, and low hemoglobin and HbF are associated with impaired CBF, which could lead to insufficiency during episodes of increased metabolic demand. The model as suggested in Figure 3 illustrates the suggested interactions between the different factors. This requires further research, but may help us to understand the etiology of WMHs in sickle cell disease.

Strengths and Limitations

MRI scanning was performed using a 3T scanner which yields high resolution images, and additionally we used a semi-automatic method to delineate WMHs, leading to a good estimation of the total volume of the WMHs. We used a state of the art ASL method and were able to quantify CBF in the white matter and grey matter separately. As a new strategy, we used WMH volume as an outcome measure to better understand the quantitative effect of hematological parameters and endothelial dysfunction on WMHs. The main limitation is our small sample size: our results should be interpreted with care and require confirmation in larger studies.

As we performed a cross sectional study, we were not able to investigate the longitudinal association between risk factors and the development of WMHs. As this was an explorative study, we did not perform a correction for multiple testing; this should be taken into account when interpreting the results.

We specifically studied WMHs and used the definition of a recently published international standard on neuro-imaging, and excluded patients with a history of overt stroke. However, we did not exclude patients when a focal neurological deficit was discovered during neurological examination in the context of this study. We realize this is not in line with most previous studies on SCIs, mostly defined as areas of abnormal hyperintensity on MRI of the brain in a patient with no history or physical findings of a focal neurological deficit. Extensive WMHs could result in focal neurological deficits and would lead to the exclusion of these patients when using the definition of SCIs, while the etiology of the lesions is similar.

In an explorative study, excluding patients with severe disease will make it more difficult to investigate risk factors. The term SCI is very useful in clinical practice, but may not be the most fitting for research purposes. Since the exact etiology of the lesions is not completely understood, a descriptive term based on imaging findings seems reasonable to use. Therefore, we chose to use the term WMHs and did not exclude patients with neurological deficits due to WMHs.

Conclusion

Our explorative study demonstrated an increased risk of WMHs in boys, and our results suggest that a low fetal hemoglobin is a risk factor for the development of a large total WMH volume. Lower hemoglobin, HbF and ADAMTS13 levels were associated with lower CBF in the white matter, suggesting that hematological factors and endothelial activation could potentially hamper CBF. Our findings suggest that HbF and endothelial dysfunction may be involved in the pathogenesis of WMHs in patients with SCD, possibly mediated through altered CBF.

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Authorship contributions

VvdL, HJMMM, AJN and KF designed the study, VvdL, HJMMM, ME, CBLMM, AJN and KF analyzed the results. VvdL drafted the article and all authors revised the article and gave final approval.

Conflict-of-interest disclosure

The authors declare no competing financial interests.

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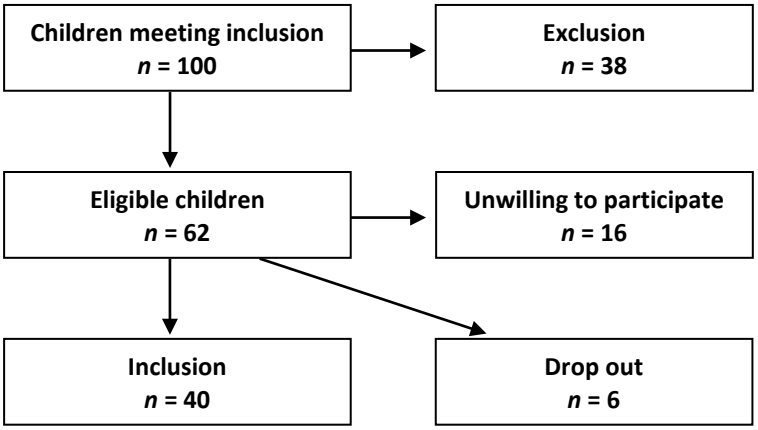
SUPPLEMENTAL MATERIAL

Supplemental Table I. Pediatric Stroke Outcome Measure

Subscales	*Outcome*
Right sensimotor	0 = no deficit
Left sensimotor	0.5 = mild deficit, normal function
Language production	1 = moderate deficit, decreased function
Language comprehension	2 = severe deficit, missing function
Cognitive/behavioral	*Total score: 0-10*

Supplemental Figure 1. Flow chart illustrating inclusion. Reasons for exclusion: exclusion criteria for MRI. i.e. dental braces (n = 10); patients judged not likely to be compliant by treating hematologist and/or frequent missed appointments (n=9); abnormal or intermediate transcranial doppler (n=4); previous overt stroke and/or chronic blood transfusion therapy (n=10); mental retardation or severe depression (n=3); other major health problem (n=2). Reasons for drop out: non-compliant with appointments (n=3); unable to make appointment due to frequent crisis (n=2); child refused MRI (n=1).



Chapter 5

T1 OF BLOOD IN SICKLE CELL DISEASE FOR QUANTIFICATION OF CEREBRAL BLOOD FLOW FROM ARTERIAL SPIN LABELLING MRI

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ABSTRACT

Background and Purpose

Cerebral blood flow (CBF) measurements using arterial spin labelling (ASL) provide insight into the cerebrovascular pathology in children with sickle cell disease (SCD). In ASL, blood is magnetically labeled in the carotid arteries, this signal is measured when it arrives in the brain tissue. The speed at which the magnetic labeling returns to the ground state, $T1_{blood}$, influences CBF measurements and is dependent on factors such as the composition of the labeled blood. Because hematocrit (Hct) and blood composition are abnormal in SCD, we investigated the importance of accounting for $T1_{blood}$, using a recently developed MRI sequence to measure $T1_{blood}$ *in vivo*.

Materials and Methods

$T1_{blood}$ was measured *in vivo* in 39 children with SCD (60%, male, mean age 12.2 ± 2.7 years) with 3.0T MRI. A global inversion pulse preceded a single slice Look-Locker EPI readout in the posterior sagittal sinus to obtain T1 inversion recovery curves from blood for each patient. CBF was measured with pseudo-continuous ASL and calculated in each patient using a) a standard, fixed $T1_{blood}$ of 1650 ms, b) a Hct-derived modelled $T1_{blood}$ value and c) the *in vivo* measured $T1_{blood}$ value. These values were in turn compared to a reference CBF value derived from phase contrast in the labelling plane arteries.

Results

No association between measured $T1_{blood}$ and Hct was observed. The average measured $T1_{blood}$ was significantly lower than Hct-modelled $T1_{blood}$ values (1814 ± 106 ms versus 2058 ± 123 ms, $p<0.001$). Compared to phase contrast CBF (mean 103 ± 15 mL/100g/min), whole brain CBF was a) overestimated when the fixed $T1_{blood}$ was utilized (114 ± 13 mL/100g/min), b) under-estimated when the Hct-modelled $T1_{blood}$ was used (95 ± 10 mL/100g/min), and c) agreed most when the *in vivo* measured $T1_{blood}$ was incorporated in the CBF model(106 ± 14 mL/100g/min).

Conclusion

The incorporation of *in vivo* measured $T1_{blood}$ values in the quantification of CBF provided CBF values that showed the best agreement with reference values. Blood viscosity and hemorheology could provide additional determinants of $T1_{blood}$ that are relevant for quantitative perfusion measurements in SCD. Therefore, *in vivo* measured $T1_{blood}$ values should be incorporated in perfusion quantification with ASL in SCD.

INTRODUCTION

Sickle Cell Disease (SCD) is a systemic disease characterized by severe chronic anemia and hemolysis leading to organ damage and cerebral injury in the form of silent infarct and overt stroke.[1,2] The risk of overt stroke can be identified by Transcranial Doppler ultrasound, and reduced by means of blood transfusions.[3] Aside from overt stroke, as many as 40% of children with SCD (particularly males) are likely to have experienced silent infarct by the age of 14.[4] Maintenance of adequate tissue perfusion may provide unique insights into the cerebrovascular pathology in SCD.

Cerebral perfusion measurements from arterial spin labelling (ASL) have demonstrated perfusion deficits in children with SCD.[5–9] The increased cerebral blood flow (CBF) in SCD is thought to be a compensatory mechanism to maintaining adequate tissue oxygenation, which may be impaired due to low hematocrit (Hct). ASL is an MRI technique that permits quantitative evaluation of CBF in a non-invasive manner, giving it an advantage over methods such as PET or CT.[10] In ASL, blood is magnetically labeled when it passes through the carotid arteries. This signal can be measured when it arrives in the brain tissue. The speed at which the magnetic labeling returns to the ground state, the T1 relaxation time ($T1_{blood}$), is dependent on several factors such as the composition of the labeled blood and surrounding tissue, the amount of macromolecules and also hematocrit.[11] An error of 15% in $T1_{blood}$ can result in a 5% error in the CBF quantification.[12] Usually, a standard $T1_{blood}$ value of 1650 ms is recommended for quantification of CBF in healthy adults at 3.0 T.[10,13]

$T1_{blood}$ is inversely dependent on Hct of blood.[12–20] Healthy children aged 6 to 19 have a Hct range of 38-45%, however in SCD Hct is as low as 18 – 30%.[21,22] Consequently, due to low Hct in SCD, the $T1_{blood}$ is likely to be higher than the standard literature value of 1650 ms would suggest. The inverse linear relationship between $T1_{blood}$ and Hct allows the modelling of $T1_{blood}$ from measured Hct values.[13,15] Hence, if measured Hct values are available, $T1_{blood}$ can be calculated accordingly. Furthermore, since we expect increased blood viscosity in SCD as well[23], we hypothesize that $T1_{blood}$ may not only be dependent on Hct alone, but also on other haemorheology characteristics. We therefore suspect that Hct based $T1_{blood}$ modelling might not suffice and *in vivo* measured $T1_{blood}$ estimates are required to accurately evaluate CBF quantification in SCD.[24]

Inversion recovery of $T1_{blood}$ can be measured rapidly with a recently developed technique that combines a global saturation pulse and a subsequent slice-selective Look-Locker read-out placed perpendicular to the posterior sagittal sinus.[15,16]

The aim of this study was thus twofold. Firstly, we sought to acquire $T1_{blood}$ measurements in children with SCD, and our second goal was to ascertain whether patient-specific $T1_{blood}$ values would be more appropriate than Hct-modelled $T1_{blood}$ or fixed $T1_{blood}$ of 1650 ms as a parameter in the CBF quantification model. He hypothesized that $T1_{blood}$ - Hct relationship may be disturbed due to aberrant blood rheology in SCD, and that incorporating measured $T1_{blood}$ would improve the perfusion calculation with pCASL.

MATERIALS AND METHODS

Subject recruitment

Forty children diagnosed with SCD (genotype HbSS or HbSβ^0) were recruited as described previously[25], and informed consent from parents or legal guardians as well as the from the children themselves was given. Patients were recruited from two centers (Emma Children's Hospital, Amsterdam, and Sophia Children's Hospital, Rotterdam) in the Netherlands, and all experiments were carried out in the Radiology department of the Academic Medical Center (Amsterdam, The Netherlands), where the local institutional review board approved the study. Inclusion criteria were homozygous sickle cell disease (HbSS) or ß-thalassaemia (HbSß^0), 8-17 years old. Exclusion criteria were history of stroke, stenosis of intracranial arteries, abnormal or intermediate velocity on Transcranial Doppler imaging, current chronic blood transfusion therapy, bone marrow transplantation, contra-indications for MRI, major concomitant health problems, or inability to participate in neurocognitive tests as these were also part of the study.

Laboratory measurements

Blood was drawn from an antecubital vein on the day of the MRI examination, and processed by the hospital laboratory using standardized procedures. The following parameters were included: hemoglobin (Hb), normal variant hemoglobin (HbA2), fetal hemoglobin (HbF), sickle hemoglobin (HbS), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and hematocrit (Hct).

MR Acquisition

Thirty-two children were scanned with a 3.0 Tesla Intera clinical scanner with an 8-channel head-coil, and due to a scanner upgrade, the remaining 8 children were scanned with a 3.0 Tesla Ingenia (both Philips Healthcare, Best, The Netherlands), equipped with a 15-channel head coil.

The MRI protocol included a 2D T2-weighted scan for radiological assessment, estimation of brain volume, for tissue segmentation, and for registration purposes.

The longitudinal relaxation of venous blood ($T1_{blood}$) was measured in the posterior sagittal sinus, according to the technique previously described by Varela et al.[15] This technique assumes that the moving blood entering the imaging plane is refreshed constantly and therefore provides a contrast difference from the saturated tissue surrounding the sagittal sinus region of interest (ROI). A non-selective adiabatic 180° inversion pulse (hyperbolic secant pulse, B1 value /duration of the pulse = 13.5 mT/13 ms) preceded a single slice Look-Locker echo-planar imaging (EPI) read-out with the following parameters: flip angle 95°; voxel size = 1.5 x 1.5 mm^2; matrix = 240x240; slice thickness = 2 mm; TE/TR = 15/150 ms; TI_1 = 200 ms; ΔTI = 150 ms; *n*TI = 60, total scan duration 1:20mins.

A gradient-echo single shot EPI pCASL sequence was used to acquire perfusion-weighted images (subtraction of 75 label-control pairs), with the following parameters: resolution 3 × 3 × 7 mm; FOV 240 × 240 mm; 17 continuous axial slices; TE/TR 17/4000 ms; flip angle 90°; SENSE 2.5; labelling duration 1650 ms; post-labelling delay in ascending order 1525-2005 ms. Background suppression with two inversion pulses of 1680 and 2830 ms after a pre-labelling saturation pulse. The labelling plane was adjusted per patient, at approximately 90 mm caudal to the anterior commissure-posterior commissure line and perpendicular to the carotid and vertebral arteries, based on a coronal and sagittal time-of-flight angiogram. Total scan time was 10:07 min.

Velocity was measured with a 2D single slice phase contrast (PC-MRI) fast field echo acquisition in the internal carotid (ICA) and vertebral arteries (VA) to obtain a reference value for flow to the brain. Imaging parameters were: FOV 230 x 230mm^2, voxel size 0.45 x 0.45 mm^2, TR/TE 15/5 ms, flip angle 15°, maximum velocity encoding (V_{ENC}) 140cm/s, slice thickness 4mm, no cardiac synchronization was put in place, total scan time 1:00 min.

Data post-processing

$T1_{blood}$. A square (20 x 20 pixels) ROI was manually placed over the sagittal sinus for each patient and was further restricted to only include the top 5 voxels with the highest signal intensity.

These remaining 5 voxels were averaged and the $T1_{blood}$ was thereafter fitted by solving a 3-parameter model (Nealder-Mead method, MATLAB, MathWorks), with the parameters M_0, offset, and $T1_{blood}$[16]:

Hct-modelled $T1_{blood}$ was calculated per patient using the linear relationship proposed by Varela and colleagues, derived from neonates:[15]

Measured $T1_{blood}$

Mean Hct was 24 ± 3% for 39 children. The mean of the $T1_{blood}$ value per patient (data was normally distributed) was 1814 ± 106 ms (CV 5.9%). There was no significant difference between values obtained on one scanner compared to the second scanner (t-test, $t(37)= -0.14$, $p=0.89$). Error values (sum of least squares) were acceptable and the amount of error was not associated with the $T1_{blood}$ value, which can be visually appreciated in the Supplementary figure S1. Fig. 1a shows a representative inversion recovery curve from one patient. All datasets were found to be of good quality. Measured $T1_{blood}$ values did not correlate with measured Hct values (Pearson's r = 0.08, $p=0.6$; Fig. 1b) or with age or sex. Mean $T1_{blood}$ calculated from measured Hct values was 2058 ± 123 ms. Measured $T1_{blood}$ values were significantly lower than Hct-derived $T1_{blood}$ values (Paired t-test, $p<0.001$)(Fig. 2a). Correlations between clinical parameters and measured $T1_{blood}$ were not significant (data not shown).

Total cerebral blood flow

Mean total flow from PC-MRI (ICAs & VAs) was 1322 ± 242 mL/min, and mean brain volume was 1206 ± 106 mL. Total CBF calculated from PC-MRI was 103 ± 15 mL/100g/min, while mean whole-brain CBF with a fixed $T1_{blood}$ of 1650ms was 114 ± 13 mL/100g/min, mean CBF with Hct-derived $T1_{blood}$ was 95 ± 10 mL/100g/min, and mean CBF with measured $T1_{blood}$ was 106 ± 14 mL/100g/min (Fig. 2a). There were no significant left-right differences between ICA and VA velocities (Table 2).

($T1_{blood}$) CBF quantification comparison

Whole brain CBF values were 114 ± 13 mL/100g/min for fixed $T1_{blood}$, 95 ± 10 mL/100g/min for Hct-modelled $T1_{blood}$, and 104 ± 21 mL/100g/min for measured $T1_{blood}$. Repeated measures ANOVA revealed the following results: Mauchly's test indicated that the assumption of sphericity had been violated, therefore degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity ($\varepsilon = 0.6$). There was a significant effect of $T1_{blood}$ correction on CBF quantification $F(1.8, 56.4) = 12.3$, $p < 0.001$. Post–hoc paired t-tests revealed significant differences between PC-MRI CBF and CBF with fixed $T1_{blood}$ $t(31) = -4.3$, $p < 0.001$, and also between PC-MRI CBF and CBF with Hct-modelled $T1_{blood}$ $t(31) = 2.4$, $p < 0.05$, but not between PC-MRI CBF and CBF with measured $T1_{blood}$; $t(31) = -4.3$, $p = 0.7$. Thus, a fixed $T1_{blood}$ of 1650 ms overestimated CBF, while the Hct-modelled $T1_{blood}$ underestimated CBF, and the best agreement was between PC-MRI CBF and CBF with measured $T1_{blood}$ (Fig. 2b).

Table 1. Demographic and laboratory parameters

Parameter	Value
Sample population (n)	39
Male, n (%)	23 (59)
Age, mean ± SD (range)	12.2 ± 2.7 (8 - 17)
Hemoglobin (mmol/L)	5.2 ± 0.7
Hematocrit (L/L)	24.07 ± 3.4

Table 2. Internal carotid and vertebral artery area and flow

	LICA	(SD)	RICA	(SD)	LVA	(SD)	RVA	(SD)
Area cm^2	0.2348	0.0409	0.2592	0.0597	0.1424	0.1428	0.1455	0.1455
Flow mL/min	468.40	59.80	487.49	103.53	183.19	44.89	179.04	50.06

Total Cerebral Blood Flow ml/min derived from 2D phase contrast (PC) MR imaging.
LICA, left arterial carotid artery; LVA, left vertebral artery; RICA, right internal carotid artery; RVA, right vertebral artery; SD, standard deviation.

Figure 1a. Representative inversion recovery of the $T1_{blood}$ signal in the posterior sagittal sinus in a child with sickle cell disease (mean of 5 voxels).

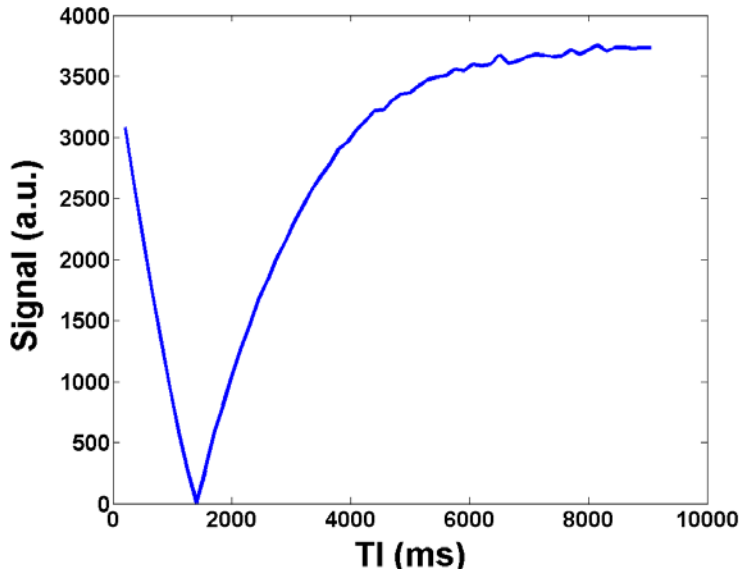


Figure 1b. T1-Hct correlation. Measured $T1_{blood}$ values fell below Hct modelled $T1_{blood}$ values. Measured $T1_{blood}$ did not correlate with measured Hct (mean 23.8 ± 3.4 %). Pearson's r = 0.084, p = 0.614.

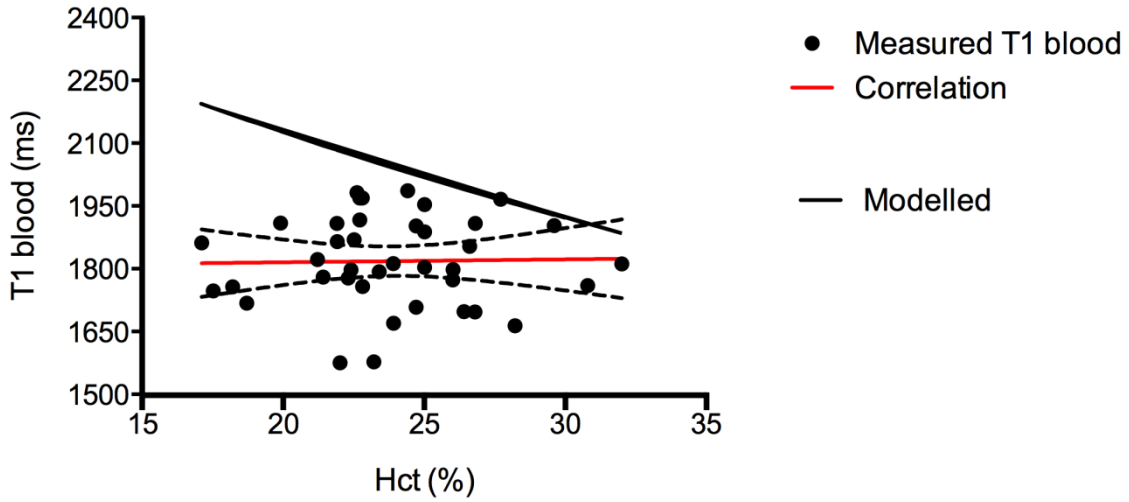


Figure 2a. CBF quantification with various $T1_{blood}$ values. (From left to right): Phase Contrast derived CBF (PC-MRI CBF), CBF with a fixed $T1_{blood}$ of 1650ms (CBF T1 fixed), CBF with a $T1_{blood}$ modelled from measured hematocrit (CBF Hct-$T1_{blood}$; $T1_{blood}$ = a*Hct+b)[15], and finally, CBF calculated with measured $T1_{blood}$ (CBF $T1_{blood}$ measured).

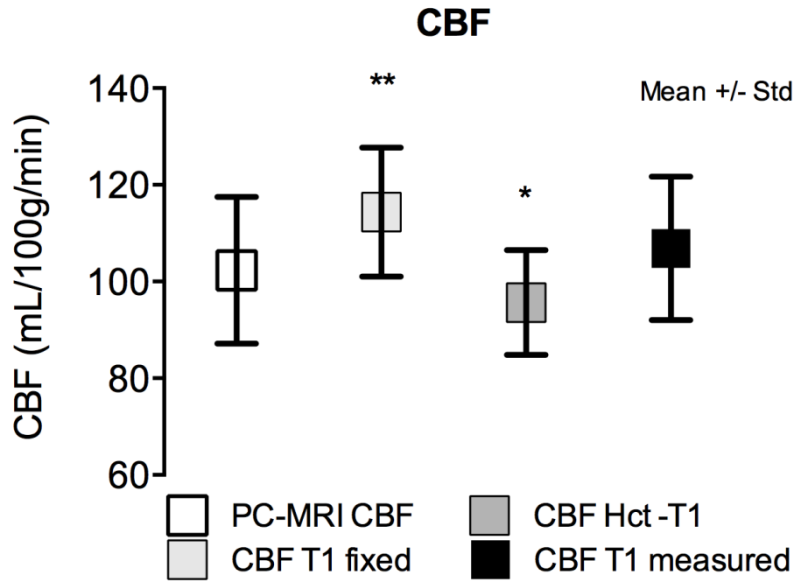
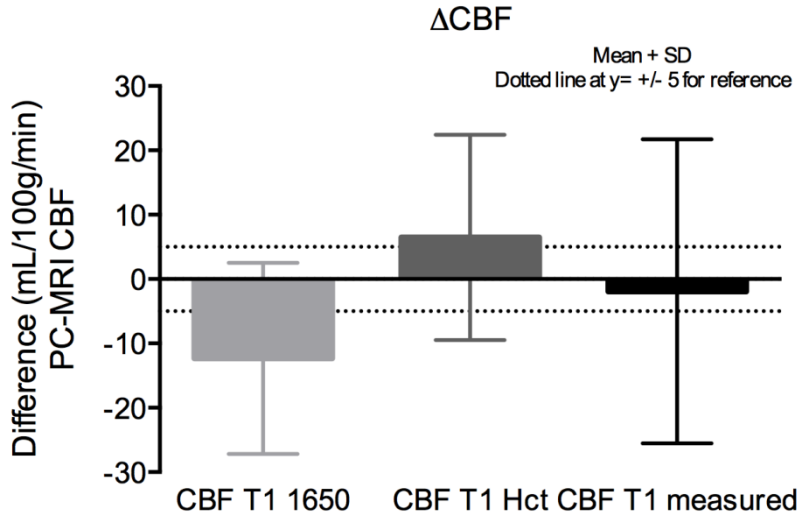


Figure 2b. Delta CBF compared to PC-MRI. **Left:** CBF quantified with fixed $T1_{blood}$ of 1650 ms differed the most from PC-MRI CBF. **Centre:** Whilst mean CBF quantified with Hct-modelled $T1_{blood}$ differed by >5 mL/100g/min. **Right**: CBF quantified with measured $T1_{blood}$ agreed best with PC-MRI CBF.



DISCUSSION

The findings of this study demonstrate that measured $T1_{blood}$ values in children with SCD were lower than what would have been expected if the $T1_{blood}$ – Hct relationship held true in this sample. However, the measured $T1_{blood}$ did not show a correlation with measured Hct. Next to that, varying the $T1_{blood}$ parameter in the quantification model of CBF, allowed us to compare three CBF values from pCASL with those from PC-MRI. This revealed that incorporating the *in vivo* measured $T1_{blood}$ values showed better agreement with CBF measured with PC-MRI, than when incorporating fixed $T1_{blood}$ of 1650ms or Hct-modelled $T1_{blood}$ value. Together these findings support the incorporation of *in vivo* measured $T1_{blood}$ values in the parameters for CBF quantification using ASL.

$T1_{blood}$

The mean longitudinal relaxation time of venous blood ($T1_{blood}$) measured here *in vivo* was 1814 ± 106 ms, which is higher than the recommended value for arterial $T1_{blood}$, which is fixed at 1650ms for adult CBF quantification.[10,13] More importantly, it is lower than the Hct-modelled $T1_{blood}$ (2046.4 ± 70 ms) (Fig. 2), which is calculated from a linear $T1_{blood}$-Hct relationship established previously. Predictions of $T1_{blood}$ based on this relationship have shown reliable results in neonates and adults in either arterial or venous blood.[14,15] We found that when using the formula for Hct, as proposed by Varela - and not dissimilar to Lu and colleagues - Hct-modelled $T1_{blood}$ values were, by and large, overestimated compared to what was measured.

At normal Hct (40%), $T1_{blood}$ has been reported to depend on age[17,24], the magnetic field strength[20], on temperature[33], on oxygenation[11,34], on sex[16,35], as well as on haematocrit.[12–15,17,19,36] The latter is contrary to our finding in children with SCD, in which the association between Hct and $T1_{blood}$ was not apparent (r^2= 0.08). This could be due to our relatively small age and Hct range, or due to other factors influencing $T1_{blood}$, such as the increased viscosity or abnormal haemorheology, which is discussed below.

Pursuant to the linear relationship identified between Hct and $T1_{blood}$ in previous studies, we expected $T1_{blood}$ to be as high as 2000 ms in this cohort of SCD children due to their low Hct. Nevertheless, the $T1_{blood}$ values measured here were ~ 200 ms lower than this. The lower the Hct the higher the $T1_{blood}$ that is calculated. One study by Wu et al showed $T1_{blood}$ values ranging from 1855 - 1887 in girls and 1684-1855 in boys, aged 12- 18 years.[17] According to literature, Hct values in the 12 – 18 year age range are 42 – 45% for males and 39 – 40 % for females[21], which means that the $T1_{blood}$ values reported by Wu and colleagues were much higher than if they had been calculated from literature values of Hct. So, $T1_{blood}$ measured here is unquestionably lower than what would be expected if Hct were in the normal range 38 – 40%.

There were no significant correlations between $T1_{blood}$ and age or sex in this study. We argue that the linear relationship is not apparent in SCD possibly due to the small Hct range, or due to factors that have a higher salience than Hct in influencing $T1_{blood}$ in SCD.

Posture and the location of blood sampling can influence Hct.[37,38] We cannot ignore that there may have been a mismatch between Hct measured from the arm, and Hct in the posterior sagittal sinus. We checked previous Hct values in the patient dossiers, which revealed that Hct did not vary by much more than 2 L/L. This was not concluded to be a substantial amount. Hct values of developing infants and neonates can fluctuate greatly before stabilizing in early adulthood.[15,21] Hence, we can be relatively confident that Hct values were stable in our sample.

It is unlikely that we underestimated $T1_{blood}$ since the Look-Locker T1 technique has previously shown robust results in the same region of interest; the posterior sagittal sinus.[15,16,39] In support of that, visually inspected inversion recovery curves obtained after model fitting were of good quality. Moreover, when the correlation between measured $T1_{blood}$ and Hct was repeated for 10 subjects with the lowest $T1_{blood}$ model error, or best fit, the correlation was still absent. Finally, the $T1_{blood}$ CV was 5.8%, which is an indication of the precision of our measurement. It is therefore improbable that the measurement of $T1_{blood}$ was flawed.

Varela and colleagues determined the linear relationship between $T1_{blood}$ and Hct from both venous and capillary blood, while Lu and colleagues found an almost identical relationship in arterial blood. Therefore, the $T1_{blood}$ values reported here are unlikely to be shorter simply because they were measured in venous blood. On the other hand, venous blood is known to have a shorter T1 than arterial $T1_{blood}$ by ~100 ms at 3.0 Tesla but this is attributed to the differences in oxygenation between the two, not to Hct.[13,14,18] We might expect a lower $T1_{blood}$ in venous blood due to paramagnetic effects of deoxygenated haemoglobin.[11] We measured $T1_{blood}$ on the venous side because until now, it has not been possible to obtain robust arterial $T1_{blood}$ values *in vivo*[17] Thus, although there may be slight differences in $T1_{blood}$ between arterial and venous blood, we can be relatively confident that venous $T1_{blood}$ measured in the sagittal sinus with a Look-Locker technique is a good surrogate for arterial $T1_{blood}$ estimates for CBF quantification from ASL at 3.0 Tesla. Taken together, our findings refute the notion that our lower $T1_{blood}$ values are simply lower because they correspond to venous $T1_{blood}$, since the correlations with Hct in literature were derived from venous, capillary and arterial blood.

$T1_{blood}$ was lower in this study than what would have been expected if it were calculated from Hct values. This, in addition to the lack of correlation between $T1_{blood}$ and Hct suggests that other blood abnormalities drive the decay of $T1_{blood}$ in SCD. However there were no significant correlations between clinical parameters measured here, and $T1_{blood}$. Apart from having a low Hct, several deviations in sickle cell blood have been identified.

These changes include decreased deformability, increased aggregation, and increased viscosity of whole blood and internal RBC viscosity.[22,40] In addition, red blood cells are rigid, less pliable, and more adhesive in SCD.[23,41,42] Additionally, abnormal blood consistency, increased cell aggregation and increased aggregate strength of red blood cells could also have reduced $T1_{blood}$ here.[22,43] Whilst we did not measure blood rheology here, abnormalities have been demonstrated consistently in previous studies, and therefore we speculate that the $T1_{blood}$ values in SCD could be affected by these factors.

Cerebral blood flow quantification

CBF was quantified here using a dual compartment model to account for different relaxation rates of protons in the vascular and tissue compartments respectively.[8,27] Altering the $T1_{blood}$ parameter in three ways allowed us to demonstrate the effect of using $T1_{blood}$ values that were too low (i.e. fixed at 1650 ms), resulting in overestimation of CBF, or too high (i.e. calculated from Hct), resulting in underestimation of CBF in SCD. We found that measured $T1_{blood}$ values resulted in CBF modelling that agreed most with the total CBF derived from PC-MRI in the brain feeding arteries, ICA and VA. Although high, the CBF values found in this study fall within the (large) range of reported values of about 70 to about 150 mL/100g/min reported in children with SCD.[6,8] The high CBF values reported here are not surprising as CBF can be double that of normally developing children.[44] However, the wide range of CBF values reported in literature in SCD emphasizes the need for accurate estimates of perfusion parameters when clinically meaningful interpretations are to be made in the future.

CBF quantification is sensitive to estimates of the additional parameters that are required for quantitative modelling. To the authors' knowledge, CBF parameters specific to SCD have not been investigated until now except for tissue T1 in one child with SCD, which was lower than in healthy children.[44] Our investigation of the $T1_{blood}$ parameter in SCD indicates that $T1_{blood}$ is substantially higher than what is assumed in adults for CBF quantification. Errors in estimating parameters such as $T1_{blood}$, tagging duration and the magnetization equilibrium of blood, can result in large errors in CBF modelling. For example, a 15% error in $T1_{blood}$ can lead to a 5% error in CBF quantification, while a 15% error in arterial transit time can lead to an ~8% error in CBF.[12] We employed an arterial transit time (ATT) of 1500 ms because we expected it to be rather short in SCD patients, given previous reports of increased flow in this population.[45] In support of this, CBF calculated from data acquired with two separate post-labelling delays (PLD; 1525 and 2100 ms), and quantified assuming a fixed ATT of 1500 ms in both cases, left the resulting CBF value unaffected in children with SCD.[8]

Assuming an ATT of 1500 ms was further justified by a recent finding in healthy children aged 7 – 17, in which an ATT of 1538 ± 123 ms was measured *in vivo.*[24] Additionally, a labelling efficiency value of 0.85 was used in this study, which was optimal based on the blood velocity measured in the labelling plane.[8,31] We recognize that CBF modelling relies heavily upon the correct estimation of the parameters additional to the perfusion-weighted measurements themselves. Further studies investigating the assumptions of CBF quantification may provide a more parsimonious model for CBF quantification from pCASL data in general but also in children with SCD.

Noteworthy were the hematological parameters in which correlations between CBF and clinical outcomes were not significant after correction for multiple comparisons, except for a positive association with HbA2, the normal variant hemoglobin, when whole brain CBF was quantified with a fixed $T1_{blood}$ of 1650 m. This shows that although the measured $T1_{blood}$ values were patient-specific, they added an additional source of measurement noise to the CBF quantification.

Limitations

Several limitations concerning this study are noteworthy. First, there was no healthy control group hence CBF measurements cannot be interpreted in a frame of typical values. Next, and importantly, PC-MRI can underestimate flow[24] because it is based on velocity averaged over the entire heart cycle. This raises the question of whether PC-MRI is a good surrogate for total brain CBF measurements. Particularly since the measurement is acquired in the feeding vessels, which also contain blood destined for extracerebral regions, however blood flow to the cerebellum and ocular structures from the ICAs and VAs is negligible as they account for a mere estimated 3.5% of the total flow to the brain.[46] The PC-MRI CBF method relies on proper segmentation of arteries to calculate flow, otherwise partial volume effects could lead to underestimation of velocity.[47] Furthermore, in order to calculate CBF, flow must be divided by the mass of the brain, which may differ between clinical populations. PC-MRI CBF has been used previously as a reference for CBF in adults.[24,31] Hence, although PC-MRI provides an indication of the total CBF in the brain[24,31,32], it may not provide gold standard values for CBF. However, a more reliable reference method is not readily available with MRI.

Future directions could be directed at developing a method for estimating arterial $T1_{blood}$ since the $T1_{blood}$ parameter in CBF modelling refers to the arterial and not the venous T1. In addition, $T1_{blood}$ measurements *in vitro* with variations in viscosity and corresponding Hct would be helpful in determining which $T1_{blood}$ values to include in SCD CBF quantification.

For now we suggest the incorporation of *in vivo* measured $T1_{blood}$ values in the CBF model, particularly since it costs very little time to acquire these measurements.

Conclusion

When an average adult $T1_{blood}$ of 1650 ms was assumed CBF was overestimated in children with SCD because the true $T1_{blood}$ was higher than 1650 ms. Also, when calculating $T1_{blood}$ from Hct (Hct modelled $T1_{blood}$) as proposed by Varela et al[15] we arrived at spuriously high $T1_{bood}$ values that depended only on the low Hct in SCD, while we suggest that other blood characteristics such as viscosity also play a role in T1 decay. Thus, rapid $T1_{blood}$ measurements allow quantification of individual T1 inversion recovery times of blood in the sagittal sinus, per individual patient. Low $T1_{blood}$ values and no apparent association with patient measured hematocrit could perhaps be due to aberrant blood content and rheology, which may also be determinants of $T1_{blood}$ decay. The best agreement on whole brain CBF was shown between PC-MRI CBF and CBF quantified with the measured $T1_{blood}$ parameter. Taken together, these results stress the incorporation of *in vivo* acquired $T1_{blood}$ measurements for CBF modelling from arterial spin labelling experiments, particularly in children with SCD.

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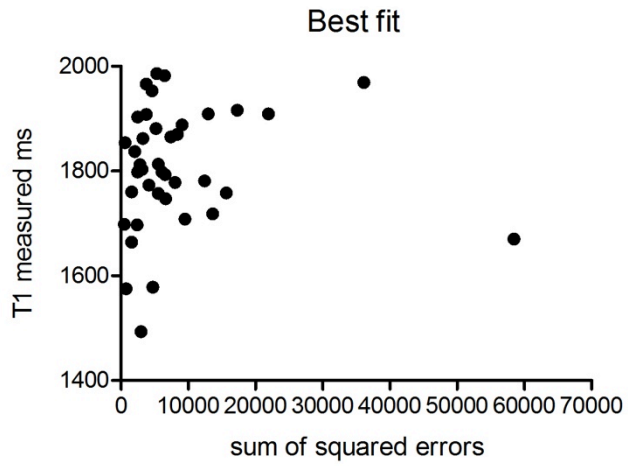
SUPPLEMENTARY MATERIAL

Supplementary Figure 1. Sum of squared errors from fitted $T1_{blood}$ data revealed good overall fitting of the model to the data. There was no association between the error and the T1 value.



Chapter 6

VOLUME OF WHITE MATTER HYPERINTENSITIES IS AN INDEPENDENT PREDICTOR OF INTELLIGENCE QUOTIENT AND PROCESSING SPEED IN CHILDREN WITH SICKLE CELL DISEASE

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ABSTRACT

Introduction

By the age of 14 years nearly 40% of the patients with a severe form (HbSS or HbSß^{0}thalassaemia) of sickle cell disease (SCD) has developed silent cerebral infarctions (SCIs), visible as white matter hyperintensities (WMHs) on magnetic resonance imaging (MRI). Although it has been demonstrated that SCIs are associated with neurocognitive dysfunction, the association between the total volume of WMHs and degree of neurocognitive dysfunction has not yet been characterized. This association could have consequences for the assignment of both neurocognitive interventions and treatment strategies for WMHs.

Methods

Thirty-eight patients with severe SCD underwent MRI and neurocognitive testing. Volume of WMHs was assessed semi-automatically. Data were analysed using multivariate linear regression adjusted for age, sex and haemoglobin level.

Results

WMHs were present in 50%. A higher volume of WMHs was associated with lower full-scale IQ, verbal IQ, Processing Speed Index and more symptoms of fatigue.

Conclusion

Our findings suggest that volume of WMHs is an independent predictor of FSIQ, VIQ, PSI and fatigue. Future studies should consider taking the total volume of WMHs into account as an independent predictor of neurocognitive outcome, instead of only the presence or absence of WMHs. Our results also suggest that the total volume of WMHs is an additional parameter for the evaluation of diagnostic and treatment options; however this should be confirmed in larger studies.

INTRODUCTION

By the age of 14 years nearly 40% of the patients with a severe form (HbSS or HbSß^0-thalassaemia) of sickle cell disease (SCD) has developed silent cerebral infarctions (SCIs).[1] SCIs are defined as an area of abnormal hyperintensity on cerebral magnetic resonance imaging (MRI) in a patient with no history or physical findings of a focal neurological deficit.[2] Affected patients may develop cognitive impairment, influencing social and academic functioning. Lower full-scale intelligence quotient values (FSIQ) have been demonstrated in patients with SCIs compared to patients without SCIs[3,4] however this was not confirmed in other studies.[5–7]

The size of SCIs may influence neurocognitive functioning and may vary between patient groups, which may explain some of the inconsistencies in study results. Only one small study with limited neurocognitive tests accounted for lesion volume and demonstrated that FSIQ was lower in patients with a large lesion volume (n=9) compared to patients with a small lesion volume (n=9).[8] It seems reasonable to assume that patients with extensive SCIs will demonstrate increased neurocognitive impairment compared to patients with limited lesions, but the exact quantitative effect of WMHs on neurocognitive functioning is unclear. For clinical decision making a more differentiated view on WMHs is necessary. Understanding this relation is important for clinical decision making because it can identify the need for neuropsychological evaluation and intervention. In future, it could potentially influence the decision to install treatment strategies for SCIs such as blood transfusion therapy.

SCIs have been defined broadly in some of the previous studies, e.g. all signal abnormalities visible on MRI in a patient with a normal neurological examination.[1] This could result in the selection of mildly affected patients, as those with a focal neurological deficit due to extensive lesions will be excluded. Moreover, besides the most common white matter hyperintensities (WMHs) this definition also includes other lesions, such as lacunes, that can differ in underlying pathogenesis and effect on neurocognitive outcome. Therefore, we focused specifically on WMHs.

We tested the hypothesis that a higher volume of WMHs is associated with lower scores on neurocognitive tests. We measured the total volume of WMHs in a semi-automatic way and adjusted for potential confounders, i.e. age, sex and haemoglobin level.

METHODS

Study Procedure

We prospectively approached all eligible children in two Sickle Cell Comprehensive Care centres (centre 1: Emma Children's Hospital, Amsterdam and centre 2: Sophia Children's Hospital, Rotterdam) during regular hospital visits or by letter. Additional information was provided by telephone calls. Inclusion criteria were age 8-16 years and HbSS or HbSß^{0}thalassaemia. Exclusion criteria were prior stroke, stenosis of intracranial arteries (as demonstrated by MRI/MRA prior to participation in this study), abnormal or intermediate velocity on transcranial Doppler imaging according to Adams et al,[9] chronic blood transfusion therapy, bone marrow transplantation, contra-indications for MRI, concomitant major health problem and inability to undergo neurocognitive testing (i.e. severe mental retardation, insufficient knowledge of the Dutch language). The medical ethics committee of the Academic Medical Center in Amsterdam approved the study. Written informed consent was obtained from all parents or legal guardians and from children aged twelve years and older.

Patients were required to be clinically stable during study visit, i.e. no infection or crisis for >4 weeks prior of the study visit. During the visit, an MRI scan was performed, patients underwent a neurological examination by a trained paediatric neurologist (ME) who was blinded for MRI and neurocognitive testing and blood sampling took place. The caregiver was asked to fill out the parent version of the Behavior Rating Inventory of Executive Function (BRIEF) which takes approximately 20 minutes. Patients were asked to complete the Pediatric Quality of Life Inventory Multidimensional Fatigue Scale (PedsQL Fatigue), this required approximately 10 minutes.

Neurocognitive testing either took place on the same day or during a separate visit. Testing was performed in a fixed order by licensed psychologists using standardized settings. Examiners were blinded for all other results except for diagnosis of SCD. Administration of the entire neurocognitive test battery required a maximum of two hours.

MRI scan protocol

Children from centre 1 were scanned on a 3 Tesla Intera with a SENSE-8-channel head coil. Because of replacement of the scanner, children from centre 2 were scanned on a 3 Tesla Ingenia with a SENSE-15-channel head coil (both Philips Healthcare, Best, the Netherlands). The MRI protocol included a 2-dimensional T2 weighted scan and a 2-dimensional fluid attenuated inversion recovery (FLAIR) scan.

Identical T2 parameters for both scanners: TE/TR = 80/3000 ms, Field of view (FOV) 230x230 mm, matrix 400x320, resolution 0.58 x 0.72 mm, 29 slices with 5 mm thickness. Identical FLAIR parameters for both scanners: TE/TR = 100/11000 ms, FOV 230x230 mm, matrix 224x137, resolution 1.03 x 1.68 mm, 29 slices with 5 mm thickness.

MRI analysis

We defined white matter hyperintensities (WMHs) using the STRIVE definition: a white matter hyperintensity of presumed vascular origin is a signal abnormality of variable size in the white matter that shows the following characteristics, hyperintensity on T2-weighted images such as fluid-attenuated inversion recovery, without cavitation.[10] All other incidental findings were described separately according to STRIVE definitions when applicable in size and location, e.g. incidental cortical infarcts, intracranial haemorrhages, cysts etc. All scans were independently scored by two investigators (CBLMM and VvdL or HJMMM); discrepancies were resolved by mutual consent. Scorers were blinded for all test results except diagnosis of SCD. Volume of WMHs was obtained semi-automatically by the procedure as illustrated in Figure 1.

Neurocognitive testing

Intelligence was assessed using the Wechsler Intelligence Scale for Children-III (WISC-III)[11] or the Wechsler Adult Intelligence Scale-III (WAIS-III)[12] depending on the child's age. Full-scale IQ (FSIQ) was estimated by the subtests Vocabulary, Arithmetic, Block Design and Picture Arrangement. Verbal IQ (VIQ) was estimated using the Vocabulary and Arithmetic subtests and Performance IQ (PIQ) was estimated using the subtests Block Design and Picture Arrangement. Processing speed was assessed using the Processing Speed Index (PSI).

Digit Span Forwards was used to measure verbal short term memory (maximum score is 16) and Digit Span Backwards was used to measure verbal working memory (maximum score is 14); no Dutch normative data exist for the separate measures. The Trail Making Test (TMT) part A was used to assess visual attention and processing speed. It requires children to link numbers from 1 to 25 that are distributed randomly on a page; the time in seconds to complete the task is recorded. TMT part B assesses mental flexibility and requires the child to alternate between numbers and letters, the time in seconds to complete the task is recorded. [13] Dutch normative data is not available.

Visuo-motor functioning was tested using the Beery-Buktenica Developmental Test of Visual-Motor Integration (Beery VMI) in which children have to reproduce geometrical shapes with ascending difficulty, the maximum score is 30. A standardized t-score is calculated.[14]

If neurocognitive testing had already been performed within one year prior of the study visit, in our centre or elsewhere, we did not repeat the assessment to avoid the effect of repeated testing. Instead we requested the previous test results.

Questionnaires

The BRIEF questionnaire was used as a measure for executive functioning.[15] The scores are summarized in a total score and in two indexes, the Behavioral Regulation Index and Metacognition Index. A higher score indicates a worse level of executive dysfunction (minimum score of 75, maximum score of 225). The scores can be transformed into standardized t-scores based on a Dutch normative group.[16]

The PedsQL Fatigue is designed to measure fatigue in children and has been validated in the Dutch population.[17] We used the self-reported version for ages 8-12 and for ages 13-18. Besides a Fatigue Total Score, it consists of three subscales: General Fatigue, Sleep and Cognitive Fatigue. All scores range from 0-100, with a lower score indicating more symptoms of fatigue.

Statistical analysis

Internal consistency of the questionnaires was evaluated by Cronbach's alpha test and was >0.70 for all 8 scales, 2 indexes and total score of the BRIEF questionnaire and for the PedsQL Fatigue Total Score and General Fatigue and Cognitive Fatigue subscales. As Cronbach's alpha was very low in the Sleep Subscale of the PedsQL Fatigue, this subscale was not used in further analysis. We used a one sample t-test to compare results of our patient group with available published reference scores. For all further analyses we used raw scores only.

We used univariate linear regression analysis to identify which variables (age, sex and haemoglobin level) are potential confounders on a neurocognitive outcome measure, i.e. when the p-value was <0.100. Confounders were entered in the first step of the multivariate linear regression model. We present the independent contribution of volume of WMHs on the neurocognitive outcome, adjusted for the confounders. The presented p-values and explained variance (R^2) correspond to the unique contribution of the predictor on the outcome, adjusted for confounders. Because volume of WMHs was not normally distributed, we applied a simple rank score. As this was an exploratory study, we did not correct for multiple testing.[18]

RESULTS

Patient population

We included 38 patients, 30 patients from Amsterdam and 8 patients from Rotterdam; the flow chart of Figure 2 illustrates inclusion of children in the study. Mean age was 12.5 ± 2.7 years (range 8.2 to 17.1 years), 58% was male. Most patients had homozygous SCD (95%), the remaining patients had HbSß^{0}thalassaemia. Three patients had a focal neurological deficit: one patient had a pyramidal tract syndrome, one patient experienced saccadic eye movements and one patient had a partial visual field deficit; these patients all showed extensive WMHs (863 mm^3 to 16.343 mm^3). One patient had two cortical infarcts <5mm, neurological examination was normal. WMHs were present in half of the patients (Table I). Most patients had a limited number of WMHs but there was a large variation.

Neurocognitive functioning

Mean time between MRI and neurocognitive testing was 4.3 ± 3.3 months. Results of neurocognitive testing is presented in Table II. In short, mean FSIQ, VIQ, PIQ and PSI were all between 85 and 90. Visuo-motor functioning was significantly lower in our patient group compared to the norm score (t-score of 46 versus 50, p=0.007). Children with SCD had more symptoms of fatigue compared to the Dutch reference group as represented by a higher PedsQL Fatigue Total Score, General Fatigue Subscale and Cognitive Fatigue Subscale. Patients with WMHs scored significantly lower compared to patients with normal MRI on FSIQ, VIQ, PSI and had more symptoms of fatigue (Table III).

Identification of confounders on neurocognitive outcome

Age was associated with most neurocognitive outcome measures in univariate analysis: Digit Span Forwards and Backwards, TMT A and B, Beery, BRIEF total score and the two indexes and the Cognitive Fatigue Subscale of the PedsQL Fatigue questionnaire. Therefore age was entered as a confounder in the prediction model of these neurocognitive outcomes. Haemoglobin level was only associated with PedsQL Total Score and General Fatigue Subscale and was entered as a confounder in the prediction model of these outcomes. Sex was not associated with any neurocognitive outcomes and therefore not included in the prediction models.

Prediction of neurocognitive functioning by volume of WMHs

Results of the prediction models are presented in Table III, the presented models correspond to the unique contribution of the predictor on the outcome, adjusted for confounders when appropriate. To facilitate comparison between tests and questionnaires we present standardized beta coefficients.

Volume of WMHs could significantly predict the outcomes FSIQ, VIQ and PSI; a larger volume of WMHs is associated with lower scores on these tests. Standardized beta coefficients range from -0.382 to -0.461 indicating a substantial negative effect of an increasing volume of WMHs on IQ scores. The volume of WMHs could explain between 14.6% and 21.2% of the variance on these outcomes. Fatigue Total Score and Cognitive Fatigue Subscale were also predicted independently by the volume of WMHs, adjusted for haemoglobin and age, respectively. Standardized beta coefficients were -0.350 and -0.352, respectively, and explained variance was 12.1% and 12.3%. There was a trend of decreasing executive functioning (BRIEF total score and BRIEF Metacognition Index) with higher volume of WMHs.

Table I. Distribution of White Matter Hyperintensities

Volume category	median	n	(%)
Volume of WMHs			
0 mm^3	0 mm^3	19	(50%)
1-100 mm^3	46 mm^3	7	(18%)
101-1000 mm^3	474 mm^3	9	(24%)
>1000 mm^3	15.097 mm^3	3	(8%)

WMHs, White Matter Hyperintensities.

Table II. Neurocognitive functioning in children with sickle cell disease (n=38)

Neurocognitive tests and questionnaires	Mean score		T-score		Reference	*p**
Intelligence						
Full-scale IQ	85	± 11	-		100	0.000
Verbal IQ	89	± 12	-		100	0.000
Performance IQ	85	± 13	-		100	0.000
Processing speed index	90	± 15	-		100	0.000
Visuo-motor functioning						
Beery VMI	24#	± 3	46	± 8	50	0.007
Executive functioning						
BRIEF, total score	117#	± 25	47	± 10	50	n.s.
BRIEF, Behavioral Regulation Index	42#	± 11	49	± 11	50	n.s.
BRIEF, Metacognition Index	75#	± 17	47	± 9	50	n.s.
Fatigue						
PedsQL Fatigue, Total Score	72#	± 9	-		79†	0.000
PedsQL Fatigue, General Fatigue	76#	± 15	-		83†	0.008
PedsQL Fatigue, Cognitive Fatigue	70#	± 15	-		76†	0.025

Scores are presented as mean ± standard deviation. Results are only presented when standardized scores were available. In three patients, test results were requested from other institutions because testing had been performed within one year prior of the study visit.
Beery VMI, Beery-Buktenica Developmental Test of Visual-Motor Integration; BRIEF, Behavior Rating Inventory of Executive Function Questionnaire; IQ, Intelligence Quotient; n.s. not significant; PedsQL Fatigue, Pediatric Quality of Life Inventory Multidimensional Fatigue Scale.
*One sample t-test between norm score and reference.
Raw score.
†According to Gordijn et al 2011.

Table III. Comparison of neurocognitive outcome between patients with and without white matter hyperintensities

	WMHs +		WMHs -		*p*-value
Full-scale IQ	**81**	± **7**	**89**	± **12**	**0.021**
Verbal IQ	**84**	± **10**	**93**	± **12**	**0.014**
Performance IQ	82	± 11	87	± 15	0.207
Processing speed index	**83**	± **10**	**97**	± **15**	**0.004**
Digit Span Forwards	8.4	± 2.2	8.4	± 2.2	0.926
Digit Span Backwards	4.9	± 1.7	5.4	± 2.6	0.544
TMT, part A	42	± 13	40	± 17	0.652
TMT, part B	100	± 53	91	± 32	0.574
Beery VMI	24	± 2	25	± 4	0.766
BRIEF, total score	125	± 28	113	± 25	0.198
BRIEF, Behavioral Regulation Index	44	± 11	42	± 13	0.513
BRIEF, Metacognition Index	81	± 20	72	± 14	0.123
PedsQL Fatigue, Total Score	**68**	± **8**	**74**	± **9**	**0.042**
PedsQL Fatigue, General Fatigue	72	± 14	81	± 15	0.067
PedsQL Fatigue, Cognitive Fatigue	**65**	± **11**	**76**	± **16**	**0.016**

Scores are presented as mean ± standard deviation. Note that higher scores on BRIEF indicate a decrease in executive functioning, and lower scores on PedsQL Fatigue indicate more symptoms of fatigue.
Beery VMI, Beery-Buktenica Developmental Test of Visual-Motor Integration; BRIEF, Behavior Rating Inventory of Executive Function Questionnaire; IQ, Intelligence Quotient; PedsQL Fatigue, Pediatric Quality of Life Inventory Multidimensional Fatigue Scale; WMHs, white matter hyperintensities.
Bold values: $p<0.05$.

Table IV. Linear regression prediction model of neurocognitive outcome by volume of white matter hyperintensities adjusted for confounders

	ß	R^2	*p*
Full-scale IQ	**-0.382**	**0.146**	**0.018**
Verbal IQ	**-0.460**	**0.212**	**0.004**
Performance IQ	-0.170	0.029	0.314
Processing speed index	**-0.461**	**0.212**	**0.005**
Digit Span Forwards*	-0.065	0.004	0.680
Digit Span Backwards*	-0.197	0.039	0.234
TMT, part A*	0.127	0.016	0.459
TMT, part B*	0.171	0.029	0.279
Beery VMI*	-0.105	0.011	0.487
BRIEF, total score*	0.257	0.066	0.103
BRIEF, Behavioral Regulation Index*	0.168	0.028	0.282
BRIEF, Metacognition Index*	0.282	0.079	0.080
PedsQL Fatigue, Total Score#	**-0.350**	**0.121**	**0.025**
PedsQL Fatigue, General Fatigue#	-0.289	0.083	0.071
PedsQL Fatigue, Cognitive Fatigue*	**-0.352**	**0.123**	**0.026**

p-values correspond to the unique contribution of volume of WMHs on the outcome, adjusted for confounders when appropriate; explained variance (R^2) is the independent explained variance of volume of WMHs on the neurocognitive outcome, adjusted for confounders when appropriate; standardized ß coefficients are presented.
Beery VMI, Beery-Buktenica Developmental Test of Visual-Motor Integration; BRIEF, Behavior Rating Inventory of Executive Function Questionnaire; IQ, Intelligence Quotient; PedsQL Fatigue, Pediatric Quality of Life Inventory Multidimensional Fatigue Scale; TMT, Trail Making Test; WMHs, white matter hyperintensities.
*Model adjusted for age.
Model adjusted for haemoglobin.
Bold values: $p<0.05$.

Fig 1. Semi-automatic delineation of white matter hyperintensities (WMHs). On all FLAIR slices (A), WMHs were visually identified and manually selected as regions of interest (ROIs) (B) with a wide margin. These ROIs were used for the intensity-based segmentation. Grey matter was segmented on all FLAIRs (C) and the mean grey matter intensity of each patient was calculated. All voxels within the manually delineated ROIs that had an intensity >1.02 times the average grey matter intensity, were labelled as WMH (D). WMH volume was then calculated as the number of segmented voxels times voxel volume (0.45x0.45x5 mm).

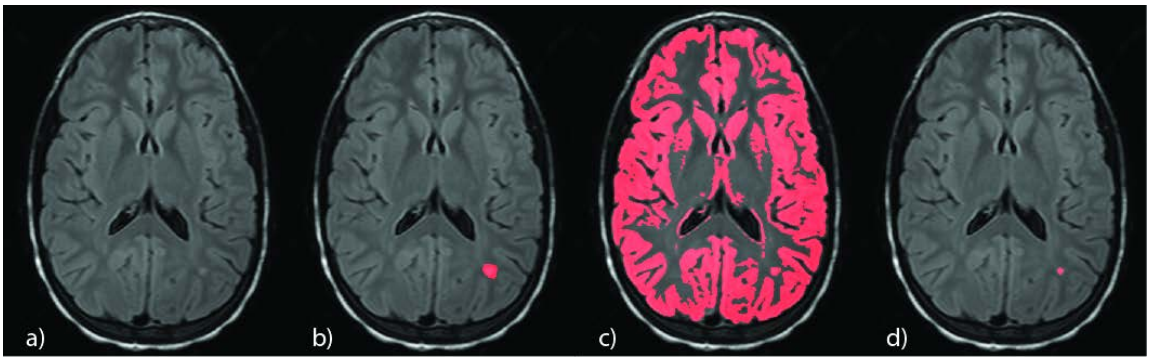
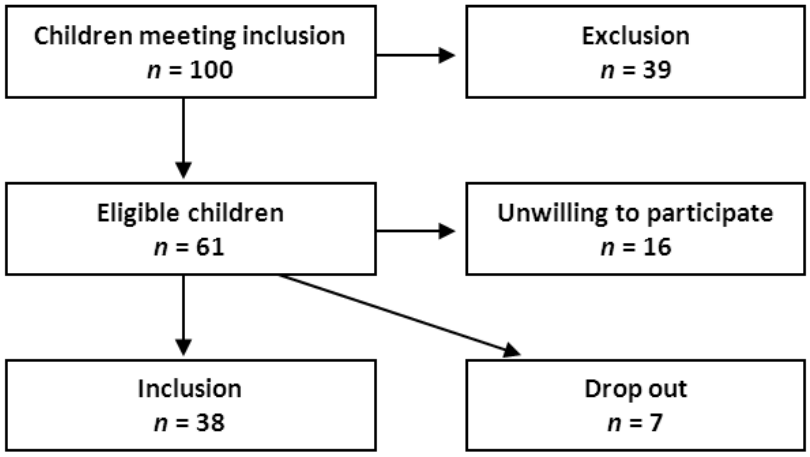


Fig. 2. Flow chart illustrating inclusion. Reasons for exclusion were as follows: exclusion criteria for MRI, i.e. dental braces (*n*=10); patients judged not likely to be compliant by treating haematologist and/or frequent missed appointments (*n*=9); abnormal or intermediate transcranial doppler (*n*=4); previous overt stroke and/or chronic blood transfusion therapy (*n*=10); mental retardation (*n*=3); other major health problem (*n*=3). Reasons for drop out were as follows: non-compliant with appointments (*n*=4); unable to make appointment due to frequent sickle cell crisis (*n*=2); child refused MRI (*n*=1).



DISCUSSION

This study demonstrated that a higher volume of WMHs is associated with a reduction in FSIQ, VIQ and PSI and increased fatigue in children with a severe form of SCD. The most notable association was present for PSI and VIQ with an explained variance of 21% for each. This is quite high considering the many other environmental and genetic factors that contribute to these measurements. Demonstrating this quantitative association between the volume of WMHs and the severity of neurocognitive dysfunction allows for a more quantified approach in neuropsychological diagnostic and treatment interventions. However, larger studies are needed to confirm our results before specific recommendations for clinicians can be made.

As expected, children with WMHs scored worse on IQ tests and fatigue compared to children without WMHs (Table III). FSIQ was 85 for the total group which is quite low; notably, we did not include a control group of the same socio-economic status. Our results on the neurocognitive tests are in line with previous studies that demonstrated reduced neurocognitive outcome in patients with SCIs compared to patients with a normal MRI, but did not address the extent of the lesions. A lower FSIQ in patients with SCIs compared to patients with a normal MRI was demonstrated before in older, retrospective studies[3,4], using broader definitions of SCIs[19] and in a selected group of patients screened for the SIT trial.[20] In contrast to these results, a large study in 144 children by Bernaudin et al could only demonstrate a lower VIQ and verbal comprehension, whereas there was no apparent difference in FSIQ for children with SCIs.[6] Processing Speed index was studied by Steen et al, children with SCIs displayed a lower score when compared to children without SCIs (85 vs. 94) although this difference was not significant.[21] The discrepancies between the studies can possibly be explained by the fact that the extent of the lesions was not taken into account, or due to different patient characteristics.

The association between WMHs and measures of fatigue has not been investigated before. Overall our patients displayed higher scores on the PedsQL Fatigue compared to the norm group, this is in accordance to 2 earlier studies of patients with sickle cell disease.[22,23] The PedsQL Fatigue is easy and quick to administer, has a high validity and could therefore be a promising tool to administer in larger studies. Our results also suggest a trend towards an association between volume of WMHs and diminished executive functioning as measured by the BRIEF questionnaire. In contrast to previous studies, overall scores on the BRIEF questionnaire in our patient group were not significantly different from norm scores; however these previous studies were limited by small patient numbers.[24–26]

Most WMHs in patients with SCD are located in the frontal lobe which was also the case in our study (data not shown).[27,28] Frontal lobe lesions are well-known to be associated with impaired executive functioning and disruption of frontal lobe integrity is associated with impairment of executive functioning and PSI in non-SCD populations.[29–31] We demonstrated that an increased volume of WMHs was associated with a lower score on PSI, but not mental flexibility as assessed with the TMT test. Possibly, this last aspect of executive functioning is less influenced by WMHs as compared to other executive functions such as planning or inhibition.

Haemoglobin level could be a potential confounder for the association between volume of WMHs and neurocognitive outcome. The association between haemoglobin and FSIQ has been established by some studies,[6,21,32] however Mackin et al could not confirm the results of Vichinsky in the same patient cohort.[33] We only found an association with PedsQL Fatigue Total Score and General Fatigue Subscale and adjusted for this association. Haemoglobin level in our patient group showed relatively little variation with a mean value of 8.5 g/dL ± 1.1 g/dL which may be an explanation for the lack of association with other neurocognitive outcomes.

Results of the Silent Cerebral Infarct Multi-Center Clinical Trial (SIT) suggest that in children with SCIs, blood transfusion therapy results in a 58% relative risk reduction for recurrent cerebral infarction (overt and silent) compared to observation.[34] However, IQ measurements (FSIQ, VIQ and PIQ) and BRIEF scores were not different between study arms, indicating no effect on cognition. The short follow up period (median of 3 years) and low number of patients with an endpoint may explain this. Lesion volume was not taken into account, perhaps a subgroup of patients with more extensive WMHs could benefit from transfusion therapy by preventing further cerebral damage and thereby preserving cognition.

For future studies in this field, there is a need for a brief neurocognitive test battery to maximize collection of psychometrically robust neurocognitive data. This has been accomplished in paediatric oncology.[35] In addition, a new upcoming NIH toolbox[36] for standard neurocognitive testing may also facilitate the comparison between studies of which normative data have recently been published still only in the English language.[37]

A possible limitation of our study is the number of patients who were not willing or able to participate in our study, mostly due to lack of time. These patients did not differ according to age, sex or haemoglobin level compared to participants. Not all neurocognitive testing was performed in the context of the present study, in three cases test results were requested from other institutions. As this concerned only three patients, and we only used well-known standardized tests, we assume this has not affected our results. As this was an explorative study, we did not perform a correction for multiple testing; this should be taken into account when interpreting the results.

In conclusion, our findings suggest that volume of WMHs is an independent predictor of FSIQ, VIQ, PSI and fatigue. Future studies should consider taking the total volume of WMHs into account as an independent predictor of neurocognitive outcome, instead of only the presence or absence of WMHs. Our results also suggest that the total volume of WMHs is an additional parameter for the evaluation of diagnostic and treatment options; however this should be confirmed in larger studies.

AUTHOR CONTRIBUTION

VvdL designed the study, analysed the results and wrote the paper. CTH designed the study, performed neurocognitive testing, analysed neurocognitive results and participated in writing the paper. MdR assisted in interpreting the neurocognitive results and participated in writing the paper. HJMMM designed the study, performed MRI analysis and participated in writing the paper. MHC designed the study and participated in writing the paper. ME designed the study, performed neurological examination and participated in writing the paper. AJN designed the study, assisted in MRI analysis and participated in writing the paper. CBLMM designed the study, performed and supervised MRI analysis and participated in writing the paper. MAG designed the study, analysed the results and wrote the paper. KF designed the study, analysed the results and wrote the paper. All authors approved of the final version of the paper. The authors report no potential conflicts of interest. The authors have no competing interests.

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Chapter 7

FIRST REPORT ON SEQUELAE OF SICKLE CELL DISEASE IN CHILDREN DIAGNOSED THROUGH NEONATAL SCREENING IN THE NETHERLANDS

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ABSTRACT

Introduction

Sickle cell disease (SCD) is complicated by high mortality and morbidity, especially in undiagnosed children. Neonatal screening for SCD was introduced in the Netherlands in 2007. We aimed to evaluate sequelae of SCD in this neonatal screening cohort, including mortality, morbidity and weight status.

Methods

All children diagnosed with SCD by neonatal screening in the Netherlands, and seen at least once in one of the two major Comprehensive Sickle Cell Centers, were included. Complications were collected prospectively. Weight was evaluated by calculating body mass index, z-scores were calculated using the WHO growth standard. Underweight was defined as BMI z-score ≤ -1.64. Overweight was defined as BMI z-score ≥ 1.04 to < 1.64 and obesity was defined as BMI z-score ≥ 1.64.

Results

Between January 1st, 2007 and May 1st, 2014 we included 162 patients, yielding information on 471 patient-years. Median age was 2.8 years (range 15 days to 7.2 years) and 57% were classified as having severe genotype (HbSS or HbSβ0). Mortality was 0.21/100 patient-years. There were 8.2 and 20.9 admissions per 100 patient-years for dactylitis and acute painful episodes, and 7.8 episodes of acute chest syndrome per 100 patient-years in children with a severe genotype. Underweight was not apparent. At ages 1 through 5, obesity was present in 12-23% of all patients.

Conclusion

Mortality and morbidity is low in this recent neonatal screening cohort compared to previously published cohorts, suggesting that outcome is continuously improving even in the last decade. The absence of underweight patients also reflects this. The high rate of obesity in children is of particular concern for the long-term outcome because obesity is an additional risk factor for cardiovascular, neurological and renal disease.

INTRODUCTION

Sickle cell disease (SCD) is characterized by chronic hemolytic anemia and vaso-occlusive events, which can lead to irreversible damage in all vital organs, including the spleen, brain, bones and lungs. Undiagnosed SCD has a high mortality and morbidity from potentially treatable or preventable causes such as pneumococcal infection and splenic sequestration.[1,2] Young children are at increased risk for infection with encapsulated bacteria due to functional asplenia early in life; prophylactic penicillin and pneumococcal vaccination can reduce infectious complications.[3–5] A second important cause of childhood mortality is splenic sequestration: without early intervention, severe episodes may lead to hypovolemic shock and death.[6] Parental education and instruction to seek medical attention when a child develops potential life-threatening symptoms such as fever, splenomegaly, pallor or lethargy, are therefore crucial. Neonatal screening facilitates the early implementation of prophylactic penicillin administration and parental education and has been demonstrated to reduce mortality.[1,7,8] A study in the Netherlands in 2003 reported that 61% of children diagnosed with SCD were not regularly seen by a pediatrician, even though national and international guidelines state that patients with SCD should be managed by clinicians with adequate experience.[9,10] To reduce mortality and morbidity, and to ensure that all children with SCD receive the care of specialized pediatricians, neonatal screening for SCD was implemented in the Netherlands in 2007. All newly diagnosed children are seen regularly in one of the Comprehensive Sickle Cell Centers.

Previous studies have shown decreased weight and growth in children with SCD varying from 0.5 to 2 standard deviations (SD) below the population mean.[11,12] Weight impairment in SCD may have various causes, such as the severe chronic hemolytic anemia with subsequent increased hematopoiesis, the presence of a hyperdynamic circulation, and the high infection rate. Most studies on weight in SCD were conducted several decades ago or in developing countries, and may therefore not be applicable to the current situation in the Netherlands. One recent, cross-sectional study in 665 children with SCD in the USA actually demonstrated that overweight and obesity were 3 times more common than underweight.[13] This finding highlights the need for information on weight status in children diagnosed with SCD by neonatal screening, which is currently unavailable.

We aimed to investigate sequelae of SCD of the Dutch neonatal screening cohort. Besides evaluating mortality and morbidity, we specifically investigated weight status and its association with genotype and hemoglobin level.

METHODS

Cohort

Starting on January 1st, 2007, universal neonatal screening for SCD was introduced in the Netherlands. In the present analysis, we included all patients diagnosed with SCD and referred to either the Comprehensive Sickle Cell Center of the Emma Children's Hospital (Academic Medical Center, Amsterdam) or Sophia Children's Hospital (Erasmus Medical Center, Rotterdam), who had had at least one visit. These two centers comprise about 65% of the Dutch SCD population. Patients were considered lost to follow up in case of: emigration or moving to an area outside the reach of the current SCD center or no show for > 2 years despite reminders. Patients with HbSS and HbSβ^0thalassemia were classified as having a severe genotype; patients with HbSC, HbSβ^+thalassemia, HbS$\delta\beta$thalassemia and HbSE were classified as having a moderate genotype. The study was approved by the Institutional Review Board and written informed consent was obtained from all parents and/or legal guardians.

Routine care at the Comprehensive Sickle Cell Centers

Diagnosis was confirmed by high-performance liquid chromatography (HPLC). Antibiotic prophylaxis with penicillin was started at 3-4 months and additional vaccination with 23-valent polysaccharide pneumococcal vaccine was performed at the age of 2 years. Folic acid supplementation started at the age of 4-6 months. Parental education was started at the first visit and repeated at subsequent visits. Follow up consisted of regular checkups starting at the age of 1 month and were repeated every 6 months. During visits, parents were questioned about sickle cell specific complications, infections and other disease episodes. A physical examination was performed including measurement of weight and height. Hydroxycarbamide was prescribed in children with recurrent severe acute painful episodes or one or more episodes of acute chest syndrome (ACS).

Patients with severe genotype were screened for stenosis of intracranial arteries by transcranial Doppler imaging (TCDi) twice a year from the age of 2 years. Chronic transfusion therapy was started in patients with abnormal TCDi results (≥ 180cm/sec).

Data collection

Data was prospectively collected during standard outpatient hospital visits by the treating pediatric hematologist using a case record form and reviewed by a single investigator (VvdL). Data collection took place at the age of: 1 month, 6 months, 12 months, 18 months, 2 years, and hereafter annually.. Gestational age and birth weight were recorded.

Prematurity was defined as a gestational age <37 weeks; weight was corrected for gestational age up to the age of 24 months and length was corrected up to the age of 40 months. Small for gestational age was defined as a birth weight <p10 according to recently published international standards.[14] Sickle cell specific complications were defined as presented in Table 1. As a chest X-ray was not routinely performed in all patients with suspected ACS in our centers, this was not required for the diagnosis of ACS.

We scored admissions separately from complications that were treated at home. Laboratory testing (e.g. full blood count and hemolytic parameters, fetal hemoglobin [HbF]) was performed at age 1 month, 6 months, 1 year and annually. Results of laboratory testing were only collected when patients were in steady disease state, i.e. having suffered a mild complication >1 week ago or a severe complication >4 weeks ago.

Statistical analysis

Morbidity was assessed by calculating complications per 100 patient-years of follow up at the following age groups: 6 months, 12 months, 18 months, 2 years, and hereafter annually, separately for severe and moderate genotypes. For this analysis, only the patient-years without hydroxycarbamide therapy or chronic blood transfusion therapy were used. This was similar to the method of Telfer et al and enabled comparison.[7]

We defined adverse clinical outcome according to a previously described method by Miller et al.[15] Adverse clinical outcome was present if one of more of the following criteria was fulfilled: 1. An average of at least two acute painful episodes per year for the entire follow-up period; 2. An average of at least one episode of acute chest syndrome per year for the entire follow-up period, 3. Death known or believed to be related to SCD and 4. Stroke. We adapted two of the criteria as follows: for the 1st criterion we also included episodes of dactylitis because this is the most common complication at a young age; for the 4th criterion we also included stenosis of intracranial arteries as demonstrated by magnetic resonance imaging (MRI) and/or abnormal TCD results. In accordance with Miller, follow up was ended when therapy with hydroxycarbamide or chronic blood transfusion therapy was started.

For analysis of weight, we collected weight and height and calculated body mass index (BMI). Patient-years on hydroxyurea or chronic blood transfusion therapy were included in this analysis. BMI was transformed into z-scores adjusted for age and gender using Growth analyzer, a program based on the growth standard of the World Health Organization (WHO) (available at www.who.int/childgrowth/software/en/) which is applicable for all ethnicities.[16] Mean z-scores were calculated per age group, we used a one sample t-test to investigate whether this mean z-score was significantly different from zero. Underweight was defined as BMI ≤ 5th percentile, corresponding with a z-score ≤ -1.64.

Overweight was defined as BMI ≥85th to <95th percentile, corresponding with a z-score ≥ 1.04 to < 1.64. Obesity was defined as BMI ≥ 95th percentile, corresponding with a z-score ≥ 1.64.[17] By using the above mentioned definitions, 5% of the total cohort would be expected to be underweight or obese, and 10% would be expected to be overweight. A Fisher's exact test was used to evaluate whether the actual percentage of patients with underweight, overweight or obesity was significantly different compared to this expected value. The association between hemoglobin of HbF and BMI z-scores was evaluated by linear regression analysis for each age group.

RESULTS

Cohort description

Between January 1st, 2007 and May 1st, 2014, 170 children were diagnosed with SCD and seen at least once in one of the two Comprehensive Sickle Cell Centers. Parents of 8 children (4.7%) refused participation, which leaves 162 patients in this study. Total follow-up on May 1st, 2014 was 471 patient-years, median age was 2.8 years (range 15 days to 7.2 years).

The median age at first visit was 1.3 month (range 0–13.5 months) and 93% was seen before the age of 4 months. Sixteen patients (9.9%) were born prematurely (<37 weeks), and fourteen patients (8.6%) were small for gestational age.

Severe genotype was present in 92 patients (57%) and 63 (39%) had a moderate genotype (Table 2). At the time of data analysis, the definitive diagnosis (e.g. the distinction between HbSS and HbSβ^0) was not yet known in 7 patients (4%). Hydroxycarbamide therapy was started in 20 patients (12.3%, 18 patients with HbSS, 1 patient with HbSC and 1 patient with HbSβ^+) at a median age of 3.1 years. The main indication was frequent acute painful episodes (16/20, 80%). Chronic blood transfusion therapy was started in 2 patients, one because of stenosis of intracranial arteries and in one because of severe anemia with frequent crises complicated by auto-immune hemolytic anemia. One other patient received transfusions for a period of 9 months after pneumococcal meningitis complicated by cerebral infarction, as demonstrated by MRI. Excluding chronic blood transfusion therapy and transfusions due to prematurity (in 2 patients), a total of 50 transfusions were administered to 24 patients (14.8% of all patients, 21 with severe genotype and 3 with moderate genotype) with a median of 2 per patient. Stem cell transplantation was not performed.

Mortality

One patient with a severe genotype died (0.6% of the total cohort) at the age of 6 months during a visit abroad, presumably due to pneumococcal sepsis and non-adherence of penicillin prophylaxis. Mean mortality of the total cohort is 0.21/100 patient-years (95% Confidence Interval [CI] 0.00 - 0.63); in patients with severe genotype mortality is 0.38/100 patient-years (95% CI 0.00 – 1.11).

Sickle cell disease specific complications

Complication rates per age group are presented in Table 3. Rates for all complications were higher in patients with a severe genotype compared to a moderate genotype. Dactylitis was the most common complication in children up to 2 years, 31.5% of patients with a severe genotype had had at least one episode (Figure 1). ACS was diagnosed infrequently, the peak incidence was 8.0/100 patient-years in age group 0-2 years in patients with a severe genotype.

Splenic sequestration occurred 4 times in 4 different patients, the peak incidence was 2.0/100 patient-years in age group 0-2 years in patients with severe genotype. One patient developed pneumococcal meningitis at the age of 1 year, and proved to be non-adherent to penicillin prophylaxis. No other pneumococcal infections occurred besides the presumed pneumococcal sepsis in the patient who died.

Adverse clinical outcome

Nineteen patients (12%) met the criteria for adverse clinical outcome using the adapted score of Miller et al: 1 sickle cell related death, 1 stroke and 1 stenosis of intracranial arteries, and 16 patients with recurrent painful episodes. There were no patients with recurrent ACS.

Weight status

Mean BMI z-scores of the total cohort, and for severe and moderate genotype separately, are presented in Table 4. Mean BMI z-scores of the total cohort at ages 1 year through 5 are all significantly above zero, indicating a higher BMI compared to the WHO reference. Mean BMI z-scores of the patients with severe genotype were significantly higher compared to WHO reference at 1.5 and 2 years, and for patients with a moderate genotype, at ages 6 months through 5 years (Figure 2). Only patients with severe genotype at the age of 6 months had a significantly lower mean z-score compared to the reference. At this age, 15.1% of the patients with severe genotype were defined as underweight (<5th percentile), a significantly higher percentage compared to the expected 5% ($p<0.01$).

For the total group at age 1 through 5, obesity was present in 12.2-23.0%, significantly higher compared to the expected 5% of the WHO reference (Table 4). Even in patients with severe genotype, obesity was present in 21.6% and 23.4% at ages 1.5 and 2 years, respectively. The Supplemental Figure represents the frequency distribution of z-scores for BMI in patients with severe genotype; the distribution of z-scores is broad, with a relatively high number of patients with either low or high z-scores. We could not demonstrate an association between BMI z-scores and hemoglobin level or HbF. Due to the low number of patients using hydroxycarbamide or chronic blood transfusions, we were not able to analyze their association with weight status.

Figure 1. Kaplan-Meier curve indicating time to first dactylitis, separate for severe and moderate genotype.

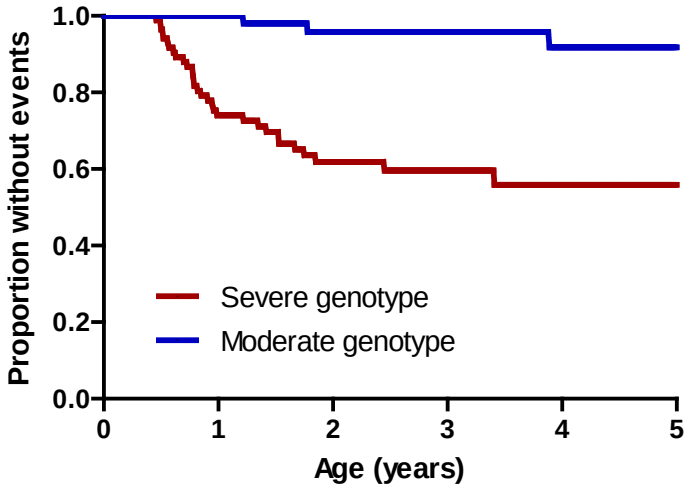

Figure 2. Mean BMI z-score per age group, separate for severe genotype and moderate genotype, with 95% confidence interval (95% CI)

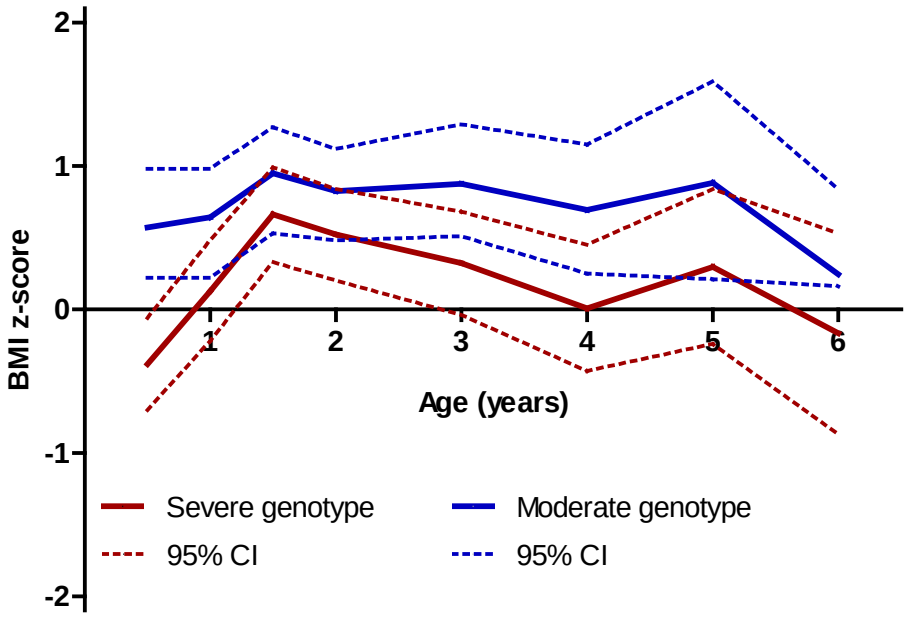

Table 1. Definitions of sickle cell disease complications

Complication	Definition
Dactylitis	Pain and swelling in one or more hands or feet, for which the administration of (oral) analgesia was necessary
Acute painful episodes	Pain in the extremities, back, abdomen, chest or head for which no other explanation can be found judged clinically by the treating physician and for which the administration of (oral) analgesia was necessary
ACS	Acute pulmonary illness in combination with one or more of the following symptoms: fever, hypoxemia or thoracic pain
Stroke	An acute neurologic syndrome secondary to occlusion of an artery or hemorrhage with resultant ischemia and neurologic symptoms and signs, with evidence of infarction and/or hemorrhage on MRI or CT scan
Acute Splenic Sequestration	Acute enlargement of the spleen, accompanied by an acute drop of hemoglobin level compared to steady state, with no or little evidence of increased hemolysis
Aplastic crisis	Acute drop in hemoglobin level accompanied by a drop in reticulocyte count

ACS, acute chest syndrome; CT scan, computerized tomography scan, MRI, magnetic resonance imaging.

Table 2. Genotype

Genotype	*n*	(%)
HbSS	86	(53.1)
HbSC	46	(28.4)
HbSβ$^+$	10	(6.2)
HbSβ^0	6	(3.7)
HbSδβ	5	(3.1)
HbSE	2	(1.2)
Unknown	7	(4.3)
Total	*162*	*(100.0)*

HbSS indicates sickle cell anemia; HbSC, sickle hemoglobin C disease; HbSβ$^+$, sickle β$^+$-thalassemia; HbSβ^0, sickle β^0-thalassemia; HbSδβ, sickle δβ-thalassemia and HbSE, sickle hemoglobin E disease.

Table 3. Sickle cell disease specific complication rates per age group

	Age group 0-2		Age group 3-4		Age group 5-6		Age group 0-8	
	Severe	Moderate	Severe	Moderate	Severe	Moderate	Severe	Moderate
Patient-years of follow up	150.7	104.6	68.5	65.0	25.0	26.0	244.2	196.7
Number of patients	92	63	46	37	22	16	92	63
Rates/100 patient-years								
Dactylitis, total	43.1	1.9*	8.8	1.5	4.0	0	29.5	1.5*
Dactylitis, admissions	11.3	0*	2.9	0	4.0	0	8.2	0*
Acute pain, total	35.8	3.8*	89.1	40.0*	120.0	53.8*	59.4	23.4*
Acute pain, adm.	15.3	2.9*	35.4	9.2*	20.0	15.4	20.9	7.1*
Acute Chest Syndrome	8.0	3.8	7.3	0*	4.0	0	7.8	2.0
Stroke/abnormal TCD	0.7¥	0	0	0	4.0#	0	0.8	0
Acute splenic sequestration	2.0	0	0	0	1	0	1.6	0
Aplastic crisis	3.3	1.0	0	1.5	1	0	2.5	1.0
Pneumococcal sepsis	0	0	1.5†	0	0	0	0.4	0
Death	0.7	0	0	0	0	0	0.4	0

Age group 7-8 years is not shown because of a low patient number (n=5). Severe denotes genotype HbSS and HbSβ^0, moderate denotes genotype HbSC, HbSβ^+ and other genotypes. Because patients were followed several years, multiple measurements of each child appear in this table. All patient-years and complications after the start of therapy (chronic blood transfusion or hydroxycarbamide) are excluded. Acute pain, acute painful episode; adm, admission; pyrs, patient-years; TCD, transcranial Doppler. *Severe versus moderate, $p<0.05$. ¥One case of meningitis with subsequent cerebral infarction. #Abnormal TCD. †No confirmed cases, 1 possible case of pneumococcal sepsis abroad, patient died.

Table 4. Body mass index: underweight, overweight and obesity

Age groups	**0.5**	**1**	**1.5**	**2**	**3**	**4**	**5**	**6**
Total group, *n*	*90*	*87*	*87*	*80*	*69*	*49*	*31*	*24*
Mean z-score	0.0 ± 1.2	0.3 ± 1.2#	0.8 ± 1.1#	0.6 ± 1.0#	0.6 ± 1.1#	0.4 ± 1.1#	0.6 ± 1.1#	0.0 ± 0.9
Underweight, %	8 (8.9)	5 (5.7)	1 (1.1)	0 (0)	1 (1.4)	3 (6.1)	1 (3.2)	1 (4.2)
Overweight, %	11 (12.2)	13 (14.9)	8 (9.2)	5 (6.3)	10 (14.5)	7 (14.3)	4 (12.9)	2 (8.3)
Obesity, %	7 (7.8)	11 (12.6)*	20 (23.0)*	18 (22.5)*	9 (13.0)*	6 (12.2)*	5 (16.1)*	1 (4.2)
Severe, *n*	*53*	*53*	*51*	*47*	*40*	*23*	*17*	*13*
Mean z-score	-0.4 ± 1.2#	0.1 ± 1.3	0.7 ± 1.2#	0.5 ± 1.1#	0.3 ± 1.1	0.0 ± 1.0	0.3 ± 1.1	-0.2 ± 1.2
Underweight,%	8 (15.1)*	5 (9.4)	1 (2.0)	0 (0)	1 (2.5)	2 (8.7)	1 (5.9)	1 (7.7)
Overweight, %	5 (9.4)	8 (15.1)	6 (11.8)	3 (6.4)	5 (12.5)	0 (0)	2 (11.8)	1 (7.7)
Obesity, %	2 (3.8)	6 (11.3)	11 (21.6)*	11 (23.4)*	4 (10.0)	3 (13.0)	2 (11.8)	1 (7.7)
Moderate, *n*	*35*	*34*	*36*	*32*	*28*	*25*	*14*	*11*
Mean z-score	0.6 ± 1.1#	0.6 ± 1.1#	0.9 ± 1.1#	0.8 ± 0.9#	0.9 ± 1.0#	0.7 ± 1.1#	0.9 ± 1.2#	0.2 ± 0.5
Underweight,%	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (4.0)	0 (0)	0 (0)
Overweight, %	7 (20)	5 (14.7)	3 (8.3)	3 (9.4)	5 (17.9)	7 (28.0)†	2 (14.3)	1 (9.0)
Obesity, %	5 (14.3)*	5 (14.7)*	9* (25.0)	7 (21.9)*	5 (17.9)*	3 (12.0)	3 (21.4)*	0 (0)

Mean BMI z-scores were calculated using the WHO growth standard. Age group 7 years is not shown because of a low patient number (n=5). Because patients were followed several years, multiple measurements of each child appear in this table. Severe denotes HbSS and HbSβ^0, moderate denotes HbSC, HbSβ^+ and other genotypes. Underweight is defined as BMI ≤ 5th percentile, corresponding with a z-score ≤ -1.64. Overweight is defined as BMI ≥85th to <95th percentile, corresponding with a z-score ≥ 1.04 to < 1.64. Obesity is defined as BMI ≥ 95th percentile, corresponding with a z-score ≥ 1.64. BMI, body mass index; WHO, World Health Organization.
One sample t-test compared to WHO reference z-score of 0, p< 0.05. * Fisher's exact test compared to the expected value of 10%, p<0.05.
† Fisher's exact test compared to the expected value of 5%, p<0.05.

Table 5. Mortality and morbidity of international neonatal cohorts

Cohort	Year	Cohort description				Mortality	Morbidity				
		n	pyrs	Age	Genotype		Stroke /100pyrs	ACS /100pyrs	Dactylitis /100pyrs	Acute pain /100pyrs	ASS /100pyrs
Jamaica, Stevens*[18]	1973-1981	146	n.a.	0-2 yrs	HbSS, 100%	10% at 2yrs	-	-	45% at 2yrs	-	18% at 2yrs
Jamaica, Balkaran#[19]	1973-1981	310	n.a.	9-17 yrs	HbSS, 100%	-	7.8% at 14 yrs	-	-	-	-
USA, California, Vichinsky¶[1]	1975-1985	89	n.a.	7.2 yrs	HbSS, 69%	1.8%	1.8%	-	-	-	18.2%
					HbSC, 28%	0	0	-	-	-	0
USA, CSSCD, Gill†[20]	1978-1988	694	2908	0-10 yrs	HbSS, 62%	1.1/100pyrs	1.2	26.3 at 2 yrs	11.0 at 2 yrs	38.3 at 2 yrs	5.3 at 2 yrs
					HbSC, 38%	0	0.0	10.4 at 2 yrs	1.2 at 2 yrs	15.3 at 2 yrs	0.0 at 2 yrs
UK, East London, Telfer¥[7]	1983-2005	252	2138	7.8 yrs	HbSS, 71%	1.0% at 16yrs 0.13/100 pyrs	0.3	17.1	33% at 2yrs	63.9	2.7
					HbSC, 29%	0% at 16yrs	-	4.1	-	13.9	0.8
USA, Dallas Quinn 2007§[21]	1983-2005	264	n.a.	12.1 yrs	HbSS, 100%	-	-	32.6% at 3yrs	6.1% at 3 yrs	20.5% at 3 yrs	-

USA, Dallas Quinn 2010[22]	1983-2007	940	8857	9.2 yrs	HbSS, 63%	6.1% at 18 yrs 0.5/100 pyrs	-	-	-	-	-
					HbSC, 37%	1.6% at 18 yrs 0.1/100 pyrs	-	-	-	-	-
	2000-2007	n.a.	n.a.	n.a.	HbSS, 100%	0.15/100 pyrs	-	-	-	-	-
Guadeloupe, Foucan**[23]	1984-1998	114	n.a.	n.a.	HbSS, 100%	7.9% 1.1/100 pyrs	0.7	10.9	10.0	25.2	4.6
France, Créteil Bernaudin##[24]	1988-2007	217	1609	6.2 yrs	HbSS, 100%	2.5% at 18 yrs 0.3/100 pyrs	0.2 1.9% at 18 yrs	-	-	-	-
Rio, Da Silva Filho¶¶[25]	2001-2008	96	n.a.	2.5 yrs	HbSS, 82%	1.3%	1.3%	4.1%	38.8%	32.7%	71.4%
					HbSC, 18%	5.9%	0	0	15.4%	15.4%	15.4%
Current study, Netherlands	2007-2014	162	471	0-7	HbSS, 57%	0.21/100 pyrs	0.4	7.8	8.2	20.9	1.6
					HbSC, 39%	0	0	2.0	0	7.1	0

Rates are presented as number of episodes per 100 patient-years, unless stated otherwise. Definition of clinical events, unless stated otherwise: Stroke: an acute neurologic syndrome secondary to occlusion of an artery or hemorrhage with resultant ischemia and neurologic symptoms and signs. ACS: a new appearance of an infiltrate on chest radiograph or, in presence of pulmonary symptoms and negative chest radiograph, abnormalities on an isotopic scan of the lungs. Dactylitis: pain and swelling of the digits and/or dorsum of the of one or more hands or feet. Acute pain/acute painful episode/ vaso-occlusive crisis: pain in the extremities, back, abdomen, chest or head for which no other explanation could be found. ASS: a decrease of hemoglobin or hematocrit ≥20% accompanied by an increase in spleen size of at least 2 cm from baseline.

* Patients were followed for a maximum of 2 years. Exclusion of patients who died <6 months or in whom HbF was not measured <6 months. Dactylitis: definition not given. ASS: a fall in hemoglobin of more than 2 gm/dl associated with acute enlargement of the spleen and evidence of increased marrow activity.

Patients were aged 9-17 yrs. Stroke was defined as a neurologic deficit lasting more than 24 hours, excluding transient ischemic attacks, seizures without neurologic deficit, meningitis, and encephalitis.

¶ Only hospital admissions were scored.

†CSSCD, subset of patients enrolled <6 months of age, observed over a 10-year period. Only events that brought the child to the center for medical care were recorded. Patient-years on regular transfusion are excluded. Data of age category 2 yrs is presented, see the original article for all age groups.

¥ Patient-years on hydroxyurea or regular transfusion are excluded. Dactylitis and acute pain: admission not required.

§ Patients aged 5-20 yrs were included. Only inpatient episodes were recorded. ACS: acute pulmonary illness in a person who has SCD that is characterized by a new radiographic pulmonary infiltrate and some combination of fever, hypoxemia, thoracic pain, and signs and symptoms of respiratory illness. Episodes < 3 yrs of age are presented.

**Only hospital admissions were scored. Acute chest syndrome was defined as a pneumonia like illness, or the presence of a new infiltrate on chest radiography. Cerebrovascular accident was defined as an acute neurological syndrome including ischemic strokes and hemorrhagic strokes.

##Only patients who had had at least 1 TCD were included. Stroke: an acute neurologic deficit with new ischemic lesions on MRI or a neurologic deficit lasting more than 24 hours in the absence of new ischemic lesions on MRI.

¶¶ Retrospective study, unclear selection of patients. One death in a patient with HbSC of unknown cause. Splenic sequestration crisis: the presence of splenomegaly associated with a significant decrease of Hb from baseline levels. Acute chest syndrome: chest radiography with recent pulmonary infiltrate associated with severe respiratory symptoms, low oxygen saturation and requirement of a blood transfusion for clinical and radiological improvement.

ACS, acute chest syndrome, age, mean age of the cohort, or total range; ASS, acute splenic sequestration; CSSCD, Cooperative Study of Sickle Cell Disease; n.a., not assessed; pyrs, patient-years; TCD, Transcranial Doppler imaging.

DISCUSSION

Our cohort study of 162 patients diagnosed with SCD by neonatal screening has demonstrated a low mortality, low complication rates and a low number of patients who were underweight, but in contrast a high percentage of patients who are overweight or obese. Mortality in our total cohort was 0.21/100 patient-years (95% CI 0.00 - 0.63) and 0.38/100 patient-years (95% CI 0.00 – 1.11) in patients with severe genotype. This is comparable to the recent UK, France and Dallas neonatal screening cohorts, and lower compared to the older cohorts of Jamaica and the CSSCD (Table 5).[7,18,20,22,24] Mortality in the Netherlands before the introduction of neonatal screening was 0.27/100 patient-years (95% CI 0.15-0.43), however median follow up in this cohort was 13.0 years. A direct comparison between the historic and neonatal screening cohort is difficult, because mortality in the historic cohort does not account for patients who died without a diagnosis of SCD, thereby incorrectly lowering mortality.

The hospital admission rates of acute complications in our cohort are low, for example the admission rate for acute painful episodes in age group 0-2 years was 15.3/100 patient-years, compared to 38.3/100 patient-years in the CSSCD cohort and 27.8/100 patient-years in the UK cohort (Table 5). This may partly be explained by differences in criteria for hospital admission, health care organization and insurance policies instead of true differences in complication rates. In our cohort, admissions for acute painful episodes or dactylitis represent about 30% of the total episodes (admissions and episodes treated at home), indicating that total morbidity of these complications is in fact much larger. Episodes of ACS were remarkably low, especially when considering we used a more liberal definition by not including a mandatory chest X-ray, in contrast to other studies where a chest X-ray is necessary for the diagnosis of ACS.

Compared with the study by Miller et al[15], we found less children with an adverse clinical outcome (12% vs 18%), even though we used broader criteria, also including episodes of dactylitis. This can probably be explained by the older patient group of Miller (mean follow up of 10 years) and the improvement of care and therapeutic options since the inclusion of their cohort, which was from 1978 to 1988. Even though our cohort is small, our results suggest that with neonatal screening and subsequent early treatment, morbidity is notably lower compared to the older cohort of the CSSCD. This highlights the continuous need for recent, large cohort studies to update the knowledge on complication rates, and to detect current challenges in the treatment of children with SCD.

In addition to low rates of complications, there were almost no underweight patients. Only in patients with severe genotype at the age of 6 months a significant proportion of 15.1% of patients were underweight. This growth impairment was not seen in older age groups and seems therefore to be transient, nor was it present in patients with moderate genotype.

A surprisingly high percentage of patients – even patients with severe genotype – were obese: between 12-23% at ages 1 through 5 years. In comparison, obesity was present in 0.7-3.3% at ages 2 through 5 years in a recent large growth study in the Netherlands, indicating that obesity is even more prevalent in our patients with SCD compared to the normal population.[26] Because we performed a longitudinal study, we have multiple data points for each patient this should be taken into account when interpreting our data. Furthermore, our cohort is small and consisted of young children, our results need to be confirmed in larger cohort studies.

However, our results do not stand alone. A large cross-sectional study in 665 children with SCD with a mean age of 10.8 years in the USA demonstrated that overweight and obesity, taken together, was present in 22.4% of patients.[13] This study demonstrated an association between hemoglobin and overweight and obesity, although this was not adjusted for genotype. We did not find an association between hemoglobin level and BMI, presumably due to a lack of power, but overweight and obesity were more outspoken in patients with moderate genotype compared to severe genotype.

Weight status of very young children (3 months to 3.5 years) was evaluated in the BabyHUG study, with BMI z-scores calculated using the WHO reference, for both the placebo group and the hydroxycarbamide group.[27] Mean BMI z-scores were above the norm score at all ages. After 2 years of follow up, a trend towards higher BMI z-scores in the hydroxycarbamide group was found. By lowering hemolysis and increasing hemoglobin level, it would be expected that hydroxycarbamide has the potential to lower energy expenditure and thereby to increase weight status. Due to a low number of patients using hydroxycarbamide (n=20), and the short duration of hydroxycarbamide therapy in our cohort, we were not able to investigate the association with weight status in our cohort. With the increasing use of hydroxycarbamide at a young age, it will be valuable to investigate the effect on weight status in longitudinal studies.

While significant growth impairment has been documented in previous, older SCD cohorts, the findings of our cohort and other recent studies suggest that the scales have actually tipped towards obesity for an important proportion of the patients. In the general population the prevalence of childhood obesity has increased substantially in the last two decades in both the USA and the Netherlands.[26,28] Our results suggest that this trend towards increased BMI in children is also apparent in children with SCD. A possible explanation for this is the decrease in complication rates over the last decades, especially infectious complications and painful episodes, leading to lower energy expenditure. This low rate of complications was also visible in our cohort, especially in children with a moderate genotype.

The trend towards overweight and obesity is of particular concern for the long term outcome of patients with SCD. Overweight and obesity will lead to additional problems such as hypertension, the increased risk of diabetes, sleep apnea, and has a detrimental effect on bones and joints. With exclusion of the latter, these are all additional risk factors for cardiovascular, neurological and renal disease in SCD, which are already responsible for significant morbidity and mortality later in life.

Careful evaluation of weight status in children and adolescents with SCD is warranted, and clinicians should be aware that nowadays, overweight or obesity are in fact a bigger problem than underweight. Care should be taken to aim for a normal weight status by encouraging parents and patients to follow a healthy diet and to perform moderate exercise. Further studies are needed to investigate which patients are at increased risk to develop obesity and to investigate the effect of hydroxycarbamide on weight status. Although the current study had a small patient numbers and relatively short follow up time, it brings into view a clear and disturbing trend of increased weight in children with sickle cell disease.

CONTRIBUTORS' STATEMENT

Van der Land, Peters, Dors and Fijnvandraat designed the study. Van der Land, Peters, Heijboer, Van Ommen, Cnossen, Dors, Suijker and Fijnvandraat were involved in data collection, supervised and reviewed by Van der Land. Analysis and interpretation of data was performed by Van der Land, Van der Lee and Fijnvandraat. The initial manuscript was drafted by Van der Land and Fijnvandraat. All authors reviewed and accepted the final manuscript.

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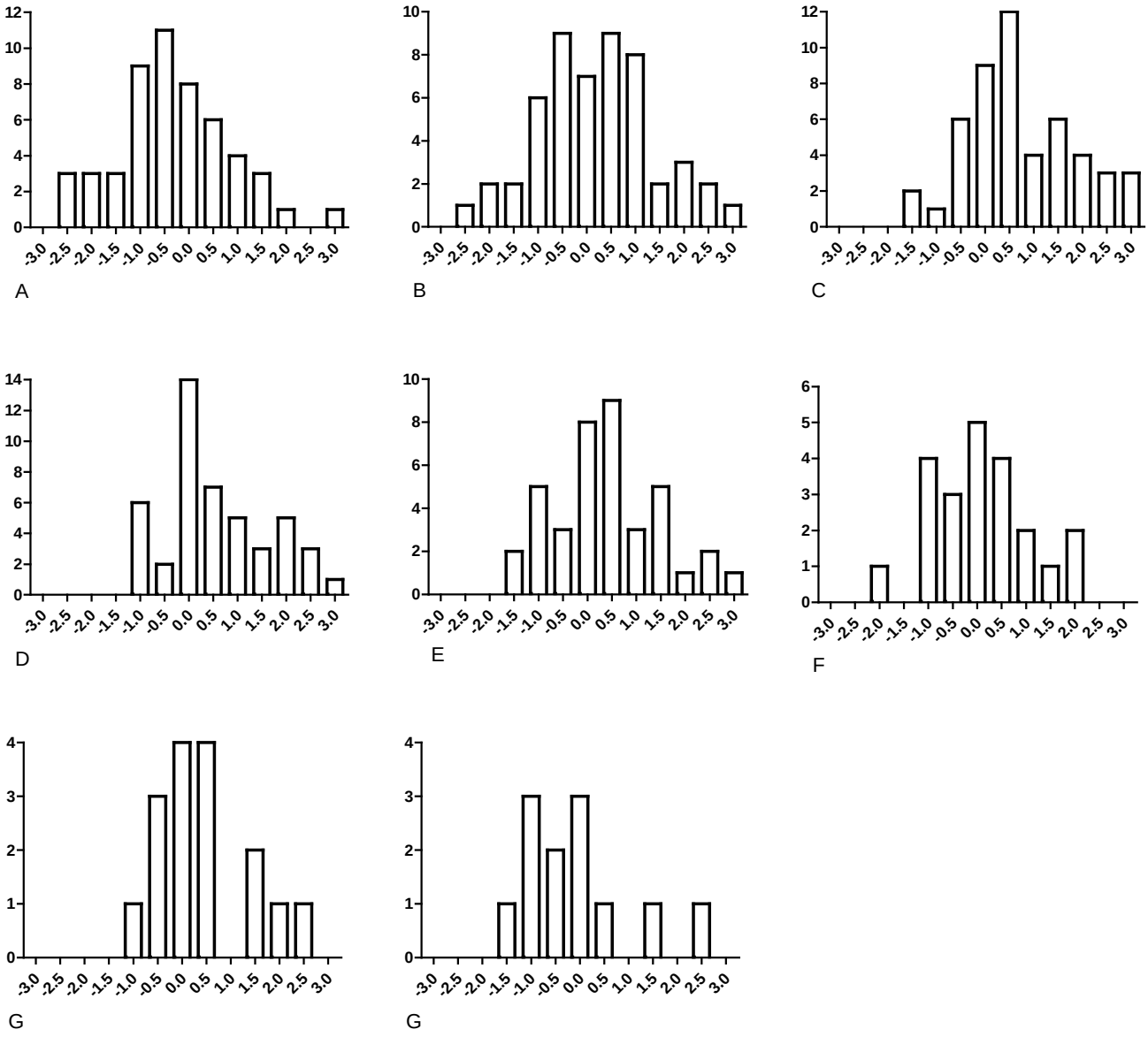
SUPPLEMENTAL MATERIAL

Supplemental Figure

Frequency distribution of z-scores for BMI in patients with severe genotype. A. Age group 6 months (n=53). B. Age group 1 year (n=53). Age group 1.5 years (n=51). D. Age group 2 years (n=47). E. Age group 3 years (n=40). F. Age group 4 years (n=23). G. Age group 5 years (n=17). H. Age group 6 years (n=13).



CHAPTER 8

SUMMARY AND GENERAL DISCUSSION

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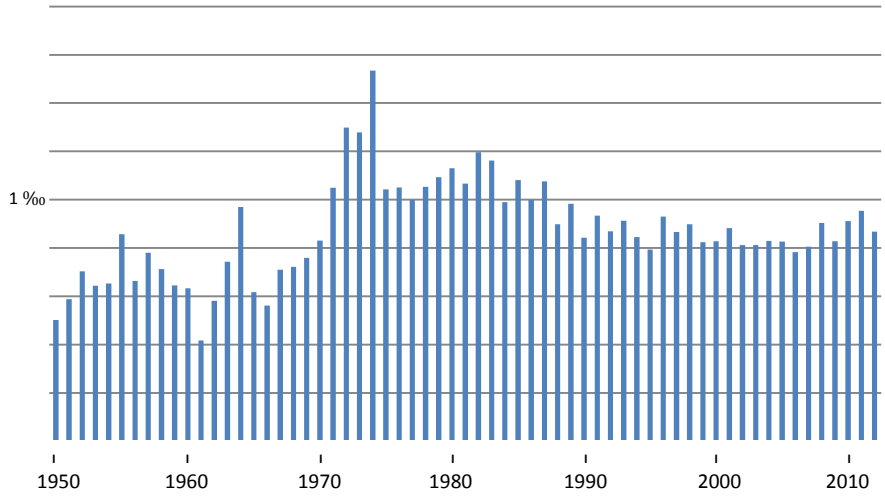
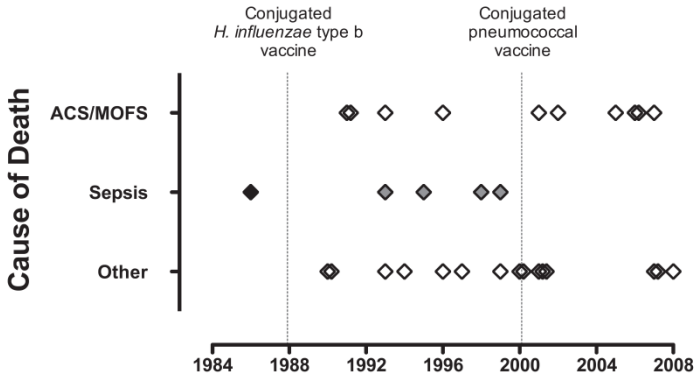
Since the first reports on sickle cell disease in scientific literature, considerable efforts have been made to gain insight into the natural history, pathophysiology and treatment options which have increased our knowledge of sickle cell disease remarkably. In fact, from 1980 onwards, a steady 1 promille of all papers published in Pubmed are on sickle cell disease (Figure 1).[1] This body of evidence has increased our understanding of the pathophysiology of the disease, and has led to improvements in patient care.

Figure 1. Proportion of papers on sickle cell disease on total number of published papers in Pubmed, from 1950 to 2012.



Childhood mortality has decreased substantially in countries where adequate care is available: nowadays nearly all children survive beyond the age of 18 years. In a recent newborn cohort study, mortality decreased from 0.67/100 patient-years for patients born between 1983-1990 to 0.15/100 patient-years for patients born between 2000-2007.[2] In children aged 0-3 years, deaths due to infections decreased by 70% in the time period 1999-2002 compared to 1995-1998; this decrease was primarily attributed to the introduction of the 7-valent pneumococcal conjugate vaccine in 2000.[3] Indeed, the combined effects of the introduction of penicillin prophylaxis, the administration of pneumococcal vaccination, the immunization against *Haemophilus influenza*, and the introduction of newborn screening have led to a considerable decrease in early mortality due to infections (Figure 2).[2,3]

Figure 2. Figure from the Dallas Newborn Cohort by Quinn et al[2]: temporal changes in causes of death in children with sickle cell disease. Three categories of death are shown on the y-axis: deaths due to acute chest syndrome (ACS) and multiorgan failure syndrome (MOFS), deaths due to sepsis, and deaths of all other causes (including non-sickle cell related deaths). Together, deaths due to ACS and MOFS are now more common than fatal sepsis in the most recent years. Notably, no member of the cohort has died from *Haemophilus influenzae* type b sepsis (◆) or *Streptococcus pneumonia* sepsis (◈) since the availability of the protein-conjugate vaccine against either bacterium (dotted lines). Figure reproduced with permission.



With increased survival of children, the challenge of research in pediatric sickle cell disease lies now in the prevention of chronic complications such as neurological and cardiovascular disease, nephropathy and chronic lung disease. These complications are still not very well understood and treatment options are limited, leading to significant morbidity and mortality later in life. Further research is urgently required in order to develop effective treatment strategies for these specific complications and improve their prognosis. A substantial proportion of childhood morbidity concerns the sequelae of cerebral infarction, in particularly the silent cerebral infarcts (SCIs): cerebral infarction in the white matter of the brain, visible as white matter hyperintensities (WMHs) on MRI in a neurologically asymptomatic patient. SCIs are of particular concern because of the associated neurocognitive deficits, which will influence academic achievement, career development, and social skills, not only during childhood but lifelong.

In this thesis, we studied WMHs using new imaging techniques such as 7 Tesla MRI and arterial spin labeling (ASL), in order to gain further insight into the pathophysiology, and investigated the quantitative effect of total lesion volume on neurocognitive outcome. We explored whether endothelial dysfunction was present in children with sickle cell disease, and whether endothelial dysfunction was associated with an increased risk of WMHs. Additionally, we have presented the first follow up study of children with sickle cell disease, born in the Netherlands and diagnosed by neonatal screening. In this final chapter, we will summarize our findings, provide a context for our findings, and discuss future perspectives for clinical practice and research.

ENDOTHELIAL DYSFUNCTION AND VASCULOPATHY

Polymerization of sickle hemoglobin (HbS), leading to sickle shaped red blood cells prone to hemolysis and vaso-occlusion, is the primary pathological process in sickle cell disease. This process leads to the activation of multiple pathways involved in the pathophysiology of sickle cell disease. These include activation of red blood cells, platelets and leukocytes, endothelial and coagulation activation, decreased bioavailability of nitric oxide (NO), abnormal hemorheology and increased blood viscosity. Recent insights have demonstrated an important role for the endothelium in the pathophysiology of acute and chronic complications, being the site of interaction between red blood cells, platelets and neutrophils.[4] Hemolysis and endothelial dysfunction are increasingly recognized to contribute to a cluster of specific complications, including stroke, pulmonary hypertension, priapism and leg ulcers. This has led to the distinction of two clinical subphenotypes of sickle cell disease: the above mentioned hemolysis endothelial dysfunction (HED) subphenotype and the viscosity vaso-occlusion (VVO) subphenotype, complicated by frequent vaso-occlusive episodes, acute chest syndrome and osteonecrosis.[5,6] Interestingly, endothelial dysfunction is also linked to cardiovascular disease, cerebral small vessel disease and (diabetic) nephropathy, suggesting further evidence for the role of endothelial dysfunction in these specific complications in sickle cell disease.[7–13]

In Chapter 2, we demonstrated that endothelial and coagulation activation are clearly present in young children, even during steady disease state. Even in the absence of vaso-occlusive crisis or infection, children with sickle cell disease display signs of chronic endothelial activation, as represented by elevated levels of Von Willebrand Factor (VWF) and VWF propeptide. Genotype was found to be an important determinant of VWF and VWF propeptide levels, patients with a severe genotype (HbSS or HbSβ^0) had higher levels compared to those with a moderate genotype (HbSC or HbSβ^+). Patients classified as belonging to the HED subphenotype had higher levels of VWF propeptide, and a trend towards higher levels of VWF compared to those with the VVO subphenotype.

These findings are a first indication that endothelial and coagulation activation are present in children with sickle cell disease in various degrees. Longitudinal studies are needed to investigate whether an elevated VWF or VWF propeptide at a young age is a predictor for specific complications associated with the HED subphenotype. A better understanding of the role of endothelial dysfunction and other pathways involved in sickle cell disease may eventually lead to strategies to prevent these specific complications.

Several therapies have been tested in phase 1 or 2 trials that intervene at a specific pathway such as anti-adhesive, anti-oxidant and anti-inflammatory agents.[14] However the effect size of these interventions are moderate, and the clinical application of these therapies has so far been limited. Most of these therapies have been tested in the context of acute complications such as vaso-occlusive events or acute chest syndrome in a hospital setting. Perhaps the limited therapeutic effects are explained by the relatively late initiation of therapy; possibly early administration would be more effective, however challenging to bring into practice. Alternatively, maybe a higher benefit can only be achieved by combining agents to block not one, but several pathways. This multimodal approach deserves more attention in future research. Additionally, long-term effects of maintenance therapy of the above mentioned agents on chronic organ damage are promising, but have not yet been studied.

IMAGING OF CEREBRAL SEQUELAE USING ULTRA-HIGH FIELD 7T MRI

In Chapter 3, we performed 7T MRI in 10 patients with sickle cell disease, and carefully described the lesions according to recently published neuro-imaging standards[15] and compared our results with 3T MRI. We identified white matter hyperintensities in 9 out of 10 patients, lacunes in 2 patients and prominent perivascular spaces in 7 patients. As a completely new finding, we also identified cortical hyperintensities in 3 patients, presumably cortical microinfarcts. These cortical hyperintensities were not visible on 3T MRI, and have not been described in sickle cell disease before. One patient had several stenosis of the intracranial arteries. Interestingly, the WMHs in this patient displayed a very different pattern compared to the other patients: WMHs were irregular of shape instead of the normal circumscribed lesions, and were closely associated with cortical hyperintensities. This could suggest that WMHs that are associated with large vessel disease may have a different pathophysiology compared to WMHs that arise in the absence of large vessel disease. However, this is a preliminary finding, because, in previous studies, the exact appearance of WMHs has not been described in detail.

Using 7T MRI, we could identify more lesions in the white matter compared to 3T MRI, and discovered several hyperintensities in the cortex which were not visible on 3T. The additional value of 7T MRI in sickle cell disease seems therefore mainly to investigate the involvement of the cortex. Our study suggests that cortical involvement is much more common than has previously been demonstrated.

The intracerebral lesions we found on 7T MRI, met the imaging criteria for cerebral small vessel disease. Small vessel disease is a descriptive term used to describe (the combination of) WMHs, lacunes, prominent perivascular spaces, and microbleeds. Small vessel disease is found in the context of conditions such as cerebral amyloid angiopathy and hypertension associated small vessel disease. It is thought to derive from increased permeability of the endothelium, inflammation and impaired auto-regulation, finally leading to arteriolar wall thickening, luminal narrowing and occlusion.[16] Interestingly, recent studies have suggested that endothelial dysfunction is involved in the pathophysiology of small vessel disease.[7,8,16] In one of the few autopsy studies in patients with sickle cell disease, diffuse thickening and sclerosis of intracerebral arterioles have also been described[17] and endothelial dysfunction is present in various degrees (Chapter 2). For these similarities, we hypothesize that small vessel disease and WMHs in sickle cell disease share a similar pathogenesis.

Definite evidence that small vessel disease is involved in the pathophysiology of WMHs in sickle cell disease is difficult to obtain, as there is no animal model specifically for WMHs available to study this, and autopsy studies to investigate the histopathology are very challenging to conduct. To study sickle red blood cell adherence to the endothelium, an in vitro model using flow chambers with cultured endothelial cells is available, as well as a mouse model with a dorsal skin fold chamber to study vaso-occlusion *in vivo*. However these models are not specific for the intracerebral situation.[18–20] Further imaging studies using high field MRI should focus on describing their findings using internationally accepted standards to improve comparability between studies. New imaging techniques can bring further insight into the pathological changes of the white matter, for example diffusion tensor imaging can be used to visualize white matter fiber tracts.

PATHOPHYSIOLOGY OF WHITE MATTER HYPERINTENSITIES IN SICKLE CELL DISEASE

Insight into the pathophysiology of WMHs is crucial to identify patients at risk, and to determine treatment options. Two processes have been suggested to play a role: vaso-occlusion elicited by endothelial dysfunction, and insufficiency of cerebral blood flow (CBF). However, despite several studies on risk factors, the exact role of these two processes in the etiology of WMHs remains unclear.

In Chapter 4, we performed an explorative study to investigate the associations between markers of endothelial dysfunction, CBF and WMH volume. We could not establish an association between WMHs and markers of endothelial and coagulation activation.

However, we did demonstrated that a higher fetal hemoglobin percentage was associated with a lower volume of WMHs, suggesting that fetal hemoglobin may protect against large WMHs.

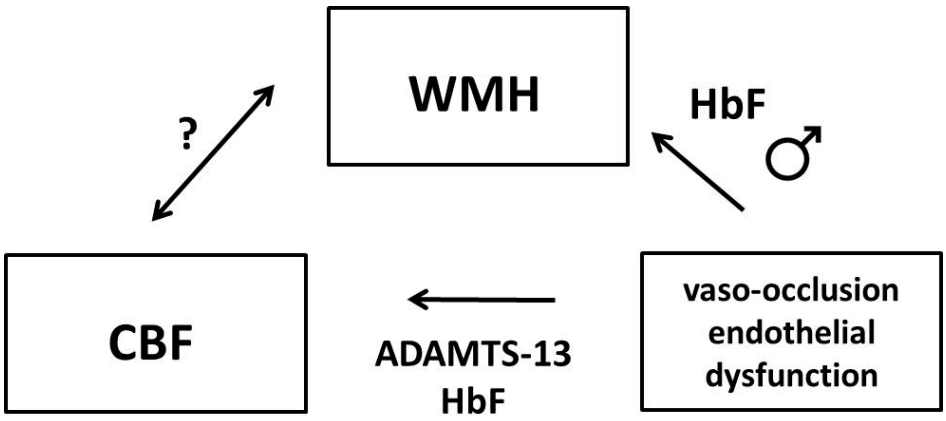
A higher fetal hemoglobin (HbF) concentration is associated with a lower concentration of hemoglobin S, and will therefore reduce the sickling of red blood cells. HbF has been demonstrated to be an important disease modifier, higher HbF is associated with lower rates of complications and mortality.[21–25] There is some evidence that HbF also decreases endothelial dysfunction, however this has not been studied in depth.[26] Unpublished results of our data of Chapter 2 reveal a negative association between HbF and VWF in patients with a severe genotype (Pearson's correlation 0.506, p=0.001). Increasing HbF by using hydroxyurea could be a promising therapy to help prevent white matter hyperintensities, but this has not been investigated before.

We could not demonstrate an association between hemoglobin level and the volume of WMHs, perhaps due to a very narrow range of hemoglobin levels, our small sample size, or due to the small effect size of hemoglobin on WMHs. A low level of hemoglobin has been identified as a risk factor for WMHs in previous studies, although the effect size is small: an odds ratio of 1.25 (95% confidence interval 1.06 - 1.49) for every g/dL decrease in hemoglobin in the Silent Cerebral Infarct Multi-Center Clinical Trial (SITT) and a hazard ratio of 1.42 (95% confidence interval 1.03 – 1.96) for every g/dL decrease in hemoglobin in a longitudinal study by Bernaudin et al.[27,28] It must be noted that hemoglobin level was not a risk factor for SCIs in the Cooperative Study of Sickle Cell Disease (CSSCD), a large observational study which included patients between 1978 and 1988.[29] Differences in these study results may be caused by differences in patient selection, definition of SCIs or neuroimaging standards. Further insight into the role of hemoglobin in SCIs may be obtained by performing a quantitative analysis by taking the total lesions volume into account.

In the same study, we investigated CBF using arterial spin labeling (ASL). Patients with sickle cell disease have an increased CBF, probably to compensate for the severe anemia in order to maintain adequate oxygen delivery.[30] During episodes of increased demand, or when hemoglobin level further decreases, CBF could become insufficient and ischemia and infarction can occur. This is supported by the appearance of acute diffusion defects on diffusion-weighted imaging, so called acute SCIs, directly after acute drops in hemoglobin level.[31,32] Due to high resolution MRI we were able to measure CBF in the white matter and grey matter separately. We did not find any CBF differences between patients with or without WMHs. Possibly, this is due to a lack of power. Alternatively, CBF reserve capacity may be more important than CBF during steady disease state. Therefore, we proposes to investigate CBF reserve capacity and the risk of WMHs in a subsequent study, this will help elucidate the role of impaired CBF in the pathophysiology of WMHs and could potentially identify patients at high risk.

CBF in sickle cell disease is not only dependent on anemia, but could possibly also be influenced by endothelial dysfunction, which is associated with nitric oxide (NO) deficiency and subsequent defective vasodilatation. We found that lower levels of ADAMTS13, a marker of endothelial dysfunction, were associated with impaired CBF in the white matter. This suggests that endothelial dysfunction might influence CBF, and thereby may contribute to WMHs (Figure 3). However, determinants of CBF in general, and in sickle cell disease specifically, have not been studied in great detail. Studying the dynamics of CBF in sickle cell disease, and the specific interaction between endothelial dysfunction and CBF, may lead to new insights on the pathophysiology of WMHs in sickle cell disease.

Figure 3. Proposed interaction between endothelial dysfunction, cerebral blood flow and white matter hyperintensities in sickle cell disease. In Chapter 4, we demonstrated that a higher HbF percentage was associated with a lower volume of WMHs, suggesting that HbF may protect against large WMHs. Male sex was an independent risk factor for WMHs. Lower levels of ADAMTS-13, a sign of endothelial dysfunction, were associated impaired CBF. We could not demonstrate an association between CBF and WMHs in this study, perhaps because we measured CBF during steady state, while CBF reserve capacity may be a more important predictor of WMHs. CBF, cerebral blood flow; HbF, fetal hemoglobin; WMH, white matter hyperintensities.



MEASURING CEREBRAL BLOOD FLOW IN SICKLE CELL DISEASE

As stated above, abnormal cerebral blood flow may play a crucial role in the etiology of WMHs. CBF can be measured in a non-invasive way by arterial spin labeling (ASL), by magnetically labeling the inflowing blood as it passes through the carotids and measuring this signal in the brain. The labeled protons will return to their equilibrium after a certain amount of time: the T1 relaxation time ($T1_{blood}$). $T1_{blood}$ is dependent on several factors including the amount of macromolecules in their environment.[33] Normally, a standard value of 1650 ms is assumed in adults.

$T1_{blood}$ is inversely dependent on hematocrit as demonstrated in studies in neonates, healthy children and adults.[34–36] It is possible to calculate $T1_{blood}$ from a wide range of hematocrit values, to account for this. However, whether the linear relationship between $T1_{blood}$ and hematocrit is also true in patients with sickle cell disease is yet unknown. There are several other factors that may influence $T1_{blood}$ which is abnormal in sickle cell disease, such as hemorheology and blood viscosity.

In Chapter 5, we were able to measure $T1_{blood}$ *in vivo* in children with SCD using a recently developed technique. We measured a mean $T1_{blood}$ of 1846 ms, this is longer compared to the standard value of 1650 ms, but shorter compared to the calculated value based on hematocrit values, which was 2058 ms. Using the calculated $T1_{blood}$ seemed to underestimate CBF and using the standard value of $T1_{blood}$ seemed to overestimate it. CBF calculated with the measured $T1_{blood}$ was comparable to CBF measured by phase-contrast MRI. This would encourage the use of *in vivo* measured $T1_{blood}$ values to improve the quantification of CBF in sickle cell disease. Care must be taken to interpret ASL derived CBF in patients with sickle cell disease, because there are still additional parameters besides $T1_{blood}$ that require accurate estimation, for example labeling efficiency and arterial transit time, i.e. the time it takes for arterial blood to reach the capillary bed of the brain.

NEUROCOGNITIVE CONSEQUENCES OF WMHS

Neurocognitive deficits are a relatively common problem in children with sickle cell disease and are associated with SCIs. Socio-economic status is low in a substantial part of the families, which can hinder treatment and rehabilitation and is a risk factor for neurocognitive deficits in itself. Previous studies on the association between SCIs and neurocognitive outcome have yielded conflicting results. Some studies demonstrated lower full-scale IQ in children with SCIs compared to children without SCIs,[37,38] but this was not confirmed in other studies.[39–41] Possibly, differences in patient characteristics, definition of SCIs, or neurocognitive testing may explain this discrepancy. As these previous studies did not take the total volume of the SCIs into account, heterogeneity in lesion volume could be another explanation for the conflicting results. The quantitative effect of the total volume of the SCIs on neurocognitive outcome is unknown, but would be useful to take into account when treatment options are considered, both for SCIs and for neurocognitive functioning.

In Chapter 6, we measured the total volume of the WMHs and demonstrated the association between volume of WMHs and full-scale IQ and processing speed index. WMH volume could explain approximately 20% of the variance of IQ and processing speed.

Considering that neurocognitive outcomes are influenced by many other factors, including socio-economic status, this is quite high. Patients severely affected by WMHs should therefore be monitored carefully and adequate counseling and psychological support should be offered. Severely affected patients could qualify for more aggressive therapies to prevent further neurological damage. Results of the SITT trial, mentioned above, suggest that chronic blood transfusion therapy could prevent the recurrence of overt and silent stroke in patients with SCIs.[42] However, there was an unexplained high incidence of overt stroke in the control group of this trial, much higher than would normally be expected. This high stroke rate in the control group was mainly responsible for the positive outcome of the SITT trial. If only SCIs were taken into account in the SITT trial, the effect of chronic blood transfusion therapy is not convincing, considering there were 7 SCIs in the control group compared to 5 in the blood transfusion group. In addition, chronic blood transfusion therapy has a high impact on social life, school attendance, and can be complicated by allo-immunization and iron overload. Therefore, care should be taken to select patients for this treatment who benefit most. Measuring WMH volume allows for a more quantified approach in neuropsychological diagnostic and treatment interventions.

NEONATAL COHORT STUDY

Early diagnosis of sickle cell disease with subsequent follow up and education of parents is important to reduce mortality and morbidity early in life.[43] Many of the acute complications can be greatly reduced when adequate preventive measures are installed, especially the infectious complications and painful episodes. Whether early diagnosis and treatment will also have a positive effect on the more chronic organ damage is unknown. To prevent early mortality and morbidity, neonatal screening for sickle cell disease has been implemented in 2007 in the Netherlands.

In Chapter 7, we discuss the first results of a nationwide cohort study of children diagnosed through neonatal screening, initiated to evaluate sequelae of sickle cell disease, including mortality, morbidity and weight status. Mortality was low, only 1 patient with a severe genotype died, leading to a mortality of 0.21/100 patient-years. This is comparable to recent neonatal screening cohorts such as the Dallas newborn cohort and cohorts in France and the UK.[2,44,45] Other sequelae of sickle cell disease were markedly lower compared to these cohorts, with low admission rates for dactylitis, painful episodes and acute chest syndrome.
This can partly be explained by differences in admission criteria, in the Netherlands most complications (dactylitis and painful episodes) are treated at home.

Compared to the largest longitudinal cohort study, the CSSCD which included patients between 1978 and 1988, our mortality and morbidity is considerably lower, reflecting improvements in patient care.[46] This stresses the need for more recent follow up studies, so current issues in diagnosis, prognosis and treatment can be detected. To achieve this, national and international collaboration is needed.

So far, we have not been able to investigate cerebral sequelae in this patient cohort due to the young age of the patients (0-7 years), with the exception of screening for intracranial stenosis with transcranial Doppler imaging. Screening for WMHs will start at the age of 8 years, as well as neurocognitive testing. Whether the low morbidity of our cohort will also lead to a low incidence of WMHs and subsequent good neurocognitive outcome, is therefore yet unknown. Acute anemic events are a risk factor for SCIs, therefore a low incidence of this complication could potentially lead to less SCIs.[31,32]

Besides evaluating mortality and morbidity, we specifically evaluated weight status. Previous studies have demonstrated severe growth retardation in children with sickle cell disease, ranging from 0.5 to 2 standard deviations below the population mean.[47,48] However, these studies were performed several decades ago, before the introduction of neonatal screening and with a different standard of care compared to the current situation. In our neonatal screening cohort, growth retardation was not present. Instead, a significant part of the young children were obese, ranging from 12-23% between the age of 1 and 5 years. Obesity was more prevalent in patients with a mild genotype, but even in the patient group with a severe genotype, mean body mass index (BMI) was above average. The high rate of obesity in this young patient group is surprising, and may be associated with the trend towards increased weight in the general population. Recently, a large cross-sectional study demonstrated that 22.4% of the 665 included children was overweight or obese, however they did not present results of obesity alone.[49] Our results need confirmation in a larger patient cohort with a longer follow up. However the trend towards overweight and obesity is of particular concern for the long-term outcome of patients with sickle cell disease. Obesity and ensuing complications such as hypertension and diabetes, are additional risk factors for neurological, cardiovascular and renal disease. Patients with sickle cell disease are already at increased risk for these complications, which can be responsible for significant morbidity and mortality later in life. Further research should focus on the determinants over overweight and obesity in children with sickle cell disease, and treatment options should be explored. As a start, awareness of the risk of obesity should arise in treating physicians, parents and patients.

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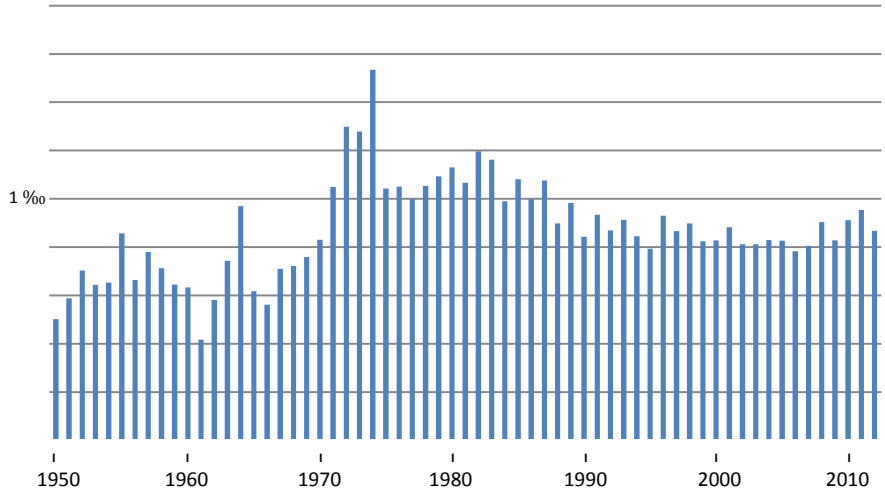
APPENDICES

NEDERLANDSE SAMENVATTING EN DISCUSSIE

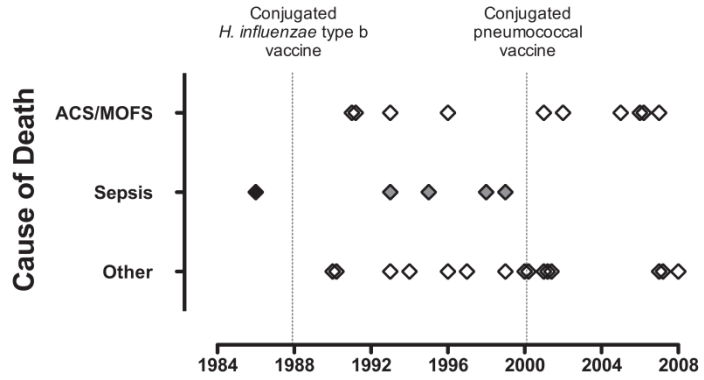
Sinds de eerste publicaties over sikkelcelziekte in de wetenschappelijke literatuur heeft veel onderzoek plaatsgevonden naar het natuurlijk beloop, de pathofysiologie en behandelopties, resulterend in een enorme toename van kennis van het ziektebeeld. Vanaf 1980 is het aantal wetenschappelijke publicaties op het gebied van sikkelcelziekte zelfs stabiel rond de 1 promille van alle artikelen die in Pubmed gepubliceerd worden (Figuur 1).[1] Deze kennis heeft geleid tot een beter begrip van de pathofysiologie van de ziekte en daardoor een betere zorg voor patiënten met sikkelcelziekte.

Figuur 1. Aantal artikelen over sikkelcelziekte als deel van het totale aantal artikelen, gepubliceerd in Pubmed van 1950 tot 2012.



In landen waar adequate zorg beschikbaar is, is de mortaliteit op kinderleeftijd substantieel gedaald, tegenwoordig bereiken vrijwel alle kinderen de 18-jarige leeftijd. In een recente studie naar kinderen gediagnosticeerd door middel van neonatale screening daalde de mortaliteit van 0.67/100 persoonsjaren voor kinderen geboren tussen 1983-1990 naar 0.15/100 persoonsjaren voor kinderen geboren tussen 2000-2007.[2] Bij kinderen van 0-3 jaar was de mortaliteit ten gevolge van infecties gedaald met 70% in de tijdsperiode 1999-2002 in vergelijking tot de periode 1995-1998. Deze daling wordt met name toegeschreven aan de introductie van het 7-valente pneumokokken vaccin in 2000.[3] Het effect van de introductie van penicilline profylaxe, de toediening van pneumokokken vaccinaties en immunisatie tegen *Haemophilus influenza*, én de invoering van neonatale screening voor sikkelcelziekte, hebben allen bijgedragen aan de substantiële daling van mortaliteit bij jonge kinderen ten gevolge van infecties (Figuur 2).[2,3]

Figuur 2. Figuur uit het Dallas Neonatale Screenings Cohort door Quinn et al.[2]: verandering in de doodsoorzaken bij kinderen met sikkelcelziekte. Drie categorieën worden getoond op de y-as: mortaliteit als gevolg van acuut chest syndroom (ACS) en multi-orgaanfalen syndroom (MOF) , mortaliteit als gevolg van sepsis, en mortaliteit ten gevolge van alle andere oorzaken (inclusief mortaliteit niet geassocieerd met sikkelcelziekte). In de meest recente jaren, mortaliteit ten gevolge van ACS en MOF is hoger dan mortaliteit ten gevolge van sepsis. Sinds de introductie van de vaccins tegen *Haemophilus influenzae* type b en *Streptococcus pneumonia* (stippellijnen) zijn er geen sterfgevallen meer geweest ten gevolge van deze infecties. Figuur overgenomen met toestemming.



Door de toegenomen overleving van kinderen ligt de uitdaging van het wetenschappelijk onderzoek nu met name op het gebied van preventie van chronische complicaties zoals de neurologische en cardiovasculaire aandoeningen, nierziekte en chronische longziekte. Er is nog weinig kennis over deze complicaties en de behandelopties zijn beperkt. Dit zorgt voor aanzienlijke morbiditeit en mortaliteit op latere leeftijd. Verder onderzoek is dan ook cruciaal om effectieve behandelstrategieën te ontwikkelen voor deze specifieke complicaties, om zo de prognose op lange termijn te verbeteren.

Een substantieel deel van de morbiditeit op kinderleeftijd vloeit voort uit de gevolgen van hersenschade, en dan met name de zogenoemde 'silent cerebral infarcts' (SCIs). Dit zijn infarcten die zichtbaar zijn als hyperintensiteiten in de witte stof (wittestofafwijkingen) op een MRI bij een patiënt zonder neurologische afwijkingen. Deze SCIs zorgen voor neurocognitieve problemen, die op hun beurt de academische prestaties, carrièremogelijkheden en sociale vaardigheden beïnvloeden, niet alleen op de kinderleeftijd maar levenslang.

In dit proefschrift onderzochten we wittestofafwijkingen met behulp van nieuwe beeldvormende technieken zoals 7 Tesla MRI en arteriële spin labeling (ASL), om meer inzicht te krijgen in de pathofysiologie. Tevens onderzochten we het kwantitatieve effect van het totale laesie volume van de wittestofafwijkingen op verschillende neurocognitieve uitkomsten. We onderzochten of er sprake was van endotheel dysfunctie bij kinderen met sikkelcelziekte en of endotheel dysfunctie geassocieerd was met een verhoogd risico op wittestofafwijkingen.

Daarnaast hebben we de eerste follow-up studie gepresenteerd van kinderen, geboren in Nederland die werden gediagnosticeerd door middel van neonatale screening. In dit laatste hoofdstuk vatten we onze bevindingen samen, plaatsen deze in de context van eerder onderzoek en bespreken we toekomstperspectieven voor de klinische praktijk en het onderzoek.

ENDOTHEEL DYSFUNCTIE EN VASCULOPATIE

Polymerisatie van sikkel hemoglobine (HbS) is het primaire pathologische proces dat leidt tot sikkelvormige rode bloedcellen die gevoelig zijn voor hemolyse en vaso-occlusie. Dit proces leidt tot de activering van meerdere routes die betrokken zijn bij de pathofysiologie van sikkelcelziekte. Deze omvatten onder andere activatie van rode bloedcellen, bloedplaatjes en leukocyten, endotheel en coagulatie activatie, verminderde biologische beschikbaarheid van stikstofoxide (NO), abnormale hemorheologie en verhoogde bloedviscositeit. Recente inzichten wijzen een belangrijke rol toe aan het endotheel in de pathofysiologie van acute en chronische complicaties in sikkelcelziekte, aangezien hier de interactie tussen rode bloedcellen, bloedplaatjes en neutrofielen plaatsvindt.[4]

Recente observationele onderzoeken hebben geleid tot het onderscheid van twee klinische subfenotypes van sikkelcelziekte: het hemolyse endotheel dysfunctie (HED) subfenotype, gecompliceerd door herseninfarcten, pulmonale hypertensie, priapisme en ulcus cruris, en het viscositeit vaso-occlusie (VVO) subfenotype, gecompliceerd door frequente vaso-occlusieve crisen, acuut chest syndroom en osteonecrose.[5,6] Ook in andere ziektebeelden is aangetoond dat endotheel dysfunctie een rol speelt, zoals cardiovasculaire ziekten, cerebrale small vessel disease en (diabetische) nefropathie.[7–13] Dit suggereert dat endotheel dysfunctie een belangrijke rol speelt kan spelen bepaalde, specifieke complicaties van sikkelcelziekte.

In hoofdstuk 2 hebben we aangetoond dat endotheel en stollingsactivatie duidelijk aanwezig zijn bij jonge kinderen, zelfs tijdens stabiele ziekte toestand. Zelfs tijdens de afwezigheid van vaso-occlusieve crisis of infectie is er bij kinderen met sikkelcelziekte dus sprake van chronische endotheel activatie, zoals bijvoorbeeld blijkt uit een verhoogde concentratie van Von Willebrand factor (VWF) en VWF propeptide. Het genotype bleek een belangrijke determinant te zijn van de concentratie van VWF en VWF propeptide, patiënten met een ernstige genotype (HbSS of HbSβ^0) hadden hogere concentraties in vergelijking met patiënten met een mild genotype (HbSC of HbSβ^+). Ook de patiënten die ingedeeld waren in het HED subfenotype hadden hogere concentraties van VWF propeptide en een trend naar hogere VWF concentraties in vergelijking met patiënten met het VVO subphenotype.

Deze bevindingen zijn een eerste aanwijzing dat endotheel en stollingsactivatie aanwezig zijn bij kinderen met sikkelcelziekte in verschillende mate. Longitudinale studies zullen moeten uitwijzen of een verhoogd VWF of VWF propeptide op jonge leeftijd een voorspeller is voor de specifieke complicaties die kunnen optreden bij het HED subphenotype. Een beter begrip van de rol van endotheel dysfunctie en de andere routes die betrokken zijn bij de pathofysiologie kunnen uiteindelijk leiden tot strategieën om deze specifieke complicaties te voorkomen.

Er zijn verschillende fase 1 en 2 onderzoeken geweest naar middelen die ingrijpen op een specifiek punt van de boven beschreven routes, zoals anti-oxidanten, anti-inflammatoire middelen en middelen die specifiek bepaalde adhesiemoleculen blokkeren.[14] Echter deze middelen lijken afzonderlijk niet erg effectief te zijn, wat de klinische toepasbaarheid dusver heeft beperkt. De meeste van deze middelen zijn getest tijdens acute complicaties zoals vaso-occlusieve crisen of acuut chest syndroom in een ziekenhuissetting. Wellicht kan het beperkte therapeutische effect worden verklaard door de relatief late start van de therapie; mogelijk dat vroege toediening vele malen effectiever is, echter dit is moeilijk uit te voeren. Een ander alternatief is mogelijkheid dat het combineren van verschillende middelen, die elk aangrijpen op andere routes, een groter therapeutisch effect geeft. Deze multimodale benadering verdient meer aandacht in toekomstig onderzoek. Daarnaast kunnen deze middelen op de lange termijn mogelijk wel chronische orgaanschade beperken. Dit is veelbelovend, maar ook nog niet onderzocht.

BEELDVORMING VAN CEREBRALE COMPLICATIES MET BEHULP VAN 7T MRI MET ULTRA-HOGE VELDSTERKTE

In hoofdstuk 3 hebben we 7T MRI scans gemaakt bij 10 patiënten met sikkelcelziekte en de resultaten vergeleken met 3T MRI scans. De gevonden afwijkingen zijn beschreven volgens een recent gepubliceerde neuro-imaging standaard.[15] We identificeerden wittestofafwijkingen in 9 van de 10 patiënten, lacunes in 2 patiënten en prominente perivasculaire ruimten in 7 patiënten. Als een geheel nieuwe bevinding hebben we hiernaast ook corticale hyperintensiteiten gevonden in 3 patiënten, waarschijnlijk zijn dit corticale microinfarcten. Deze corticale hyperintensiteiten waren niet zichtbaar op 3T MRI, en zijn niet eerder beschreven in sikkelcelziekte. Eén patiënt had verschillende stenoses van de intracraniële arteriën, en opmerkelijk genoeg vertoonde de wittestofafwijkingen bij deze patiënt een heel ander patroon dan bij de andere patiënten: de wittestofafwijkingen waren zeer onregelmatig van vorm en grenzend aan de corticale afwijkingen.

Dit zou kunnen suggereren dat wittestofafwijkingen die zijn geassocieerd met grote vaatafwijkingen een andere pathofysiologie hebben dan wittestofafwijkingen die optreden in afwezigheid van grote vaatafwijkingen. Aangezien eerdere studies geen precieze omschrijving van de gevonden laesies bevatten, kan dit nog niet met zekerheid worden vastgesteld.

Met behulp van 7T MRI werden meer laesies in de witte stof geïdentificeerd in vergelijking met 3T MRI, en werden verscheidene hyperintensiteiten in de cortex ontdekt die niet zichtbaar waren op 3T. De toegevoegde waarde van 7T MRI bij sikkelcelziekte lijkt voornamelijk te liggen in het onderzoeken van de cortex. Onze studie suggereert ook dat de cortex veel vaker aangedaan is dan voorheen werd gedacht.

De afwijkingen die werden gevonden op 7T MRI voldoen aan de criteria voor small vessel disease op MRI. Small vessel disease is een beschrijvende term die gebruikt wordt voor (de combinatie van) wittestofafwijkingen, lacunes, prominente perivasculaire ruimten, en microbloedingen. Onder small vessel disease valt onder andere cerebrale amyloïde angiopathie maar ook hypertensie geassocieerde wittestofafwijkingen. Waarschijnlijk ontstaat het small vessel disease ten gevolge van een verhoogde permeabiliteit van het endotheel, vaatwandontsteking en een verminderde autoregulatie van de kleine hersenvaten, wat uiteindelijk leidt tot verdikking van de wand van de arteriolen, leidend tot stenosen en uiteindelijk occlusie van het lumen.[16] Recente studies suggereren dat endotheel dysfunctie betrokken is bij de pathofysiologie van small vessel disease.[7,8,16] In één van de weinige autopsie studies bij patiënten met sikkelcelziekte werd ook diffuse verdikking en sclerose van de intracerebrale arteriolen beschreven.[17] Endotheel dysfunctie is aanwezig in verschillende gradaties in patiënten met sikkelcelziekte (hoofdstuk 2). Vanwege deze gelijkenissen verwachten wij dat small vessel disease en de wittestofafwijkingen die voorkomen bij sikkelcelziekte een soortgelijke pathogenese hebben.

Definitief bewijs dat small vessel disease betrokken is bij de pathofysiologie van wittestofafwijkingen in sikkelcelziekte is moeilijk te verkrijgen omdat er geen diermodel bestaat waarin dit specifiek bestudeerd kan worden. Histologisch onderzoek van de hersenafwijkingen zou erg inzichtelijk zijn, maar kan alleen maar uitgevoerd worden in het kader van autopsie studies. Er bestaat een *in vitro* model om de hechting van sikkelcellen aan het endotheel te onderzoeken met behulp van gekweekte endotheelcellen. En verder bestaat er een muismodel waarbij vaso-occlusie *in vivo* onderzocht kan worden in de dorsale huidplooi van de muis, echter deze modellen zijn zeker niet specifiek voor de intracerebrale situatie.[18–20]

Het dient de aanbeveling dat nieuwe MRI studies gebruik maken van de internationaal gepubliceerde neuro-imaging standaarden om de vergelijkbaarheid tussen studies te verbeteren.

Nieuwe beeldvormende technieken kunnen tot nieuwe inzichten leiden in de pathologische veranderingen van de witte stof in sikkelcelziekte, diffusion tensor imaging kan bijvoorbeeld gebruikt worden om de zenuwbanen in de witte stof te visualiseren.

PATHOFYSIOLOGIE VAN WITTESTOFAFWIJKINGEN BIJ SIKKELCELZIEKTE

Inzicht in de pathofysiologie van wittestofafwijkingen bij sikkelcelziekte is belangrijk om nieuwe behandelingen te kunnen ontwikkelen en om hoog-risico patiënten te kunnen identificeren. Twee verschillende processen dragen waarschijnlijk bij aan de pathofysiologie: vaso-occlusie veroorzaakt door endotheel dysfunctie, en insufficiëntie van de cerebrale bloedvoorziening (CBV). Ondanks meerdere studies naar risicofactoren voor wittestofafwijkingen blijft de exacte rol van deze twee processen in de pathofysiologie ervan nog onduidelijk.

In hoofdstuk 4 beschrijven we de resultaten van een exploratief onderzoek naar de associaties tussen markers van endotheel dysfunctie, CBV en wittestofafwijkingen. We konden geen associatie aantonen tussen wittestofafwijkingen en markers van endotheel- en stollingsactivatie. Onze resultaten toonden wel aan dat een hoger percentage foetaal hemoglobine (HbF) was geassocieerd met een lager volume van wittestofafwijkingen. Dit suggereert dat een hoog HbF mogelijk beschermend werkt tegen het optreden van uitgebreide wittestofafwijkingen. Een hoger HbF gaat gepaard met een lagere concentratie hemoglobine S, waardoor sikkeling van rode bloedcellen wordt geremd. Het HbF percentage is een bekende voorspeller van het klinisch beeld, waarbij een hoger HbF gepaard gaat met minder complicaties en een lagere mortaliteit.[21–25] Daarnaast is er enig bewijs dat een hoger HbF percentage ook endotheel dysfunctie kan tegengaan, maar dit is nog onvoldoende onderzocht.[26] Een post-hoc analyse op basis van gegevens uit Hoofdstuk 2 laat zien dat een hoger HbF gepaard gaat met een lagere concentratie van VWF (Pearson's correlatie 0.506, p=0.001). Het verhogen van HbF door middel van hydroxycarbamide is een veelbelovende therapie ter voorkoming wittestof-afwijkingen, echter de effectiviteit hiervan is nog niet onderzocht.

Verder hebben we geen associatie gevonden tussen het hemoglobinegehalte en het volume van de wittestofafwijkingen. Dit is misschien het gevolg van de smalle spreiding van het hemoglobine gehalte in onze studie, het kleine aantal patiënten, of de kleine effectgrootte van hemoglobine op wittestofafwijkingen.

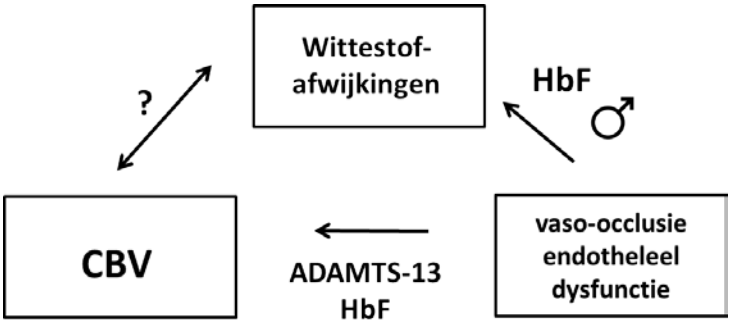
Uit eerder onderzoek is wel gebleken dat een laag hemoglobinegehalte een risicofactor is voor het optreden van SCIs, hoewel het effect klein is: een odds ratio van 1.25 (95% betrouwbaarheidsinterval 1.06-1.49) voor elke g/dl daling van hemoglobine in de Silent Infarct Multicenter Clinical Trial (SITT) en een hazard ratio van 1.42 (95% betrouwbaarheidsinterval 1.03-1.96) voor elke g/dl lager hemoglobine in een longitudinale studie van Bernaudin et al.[27,28] Overigens was hemoglobine geen risicofactor voor SCIs in de Cooperative Study of Sickle Cell Disease (CSSCD), een grote observationele studie uit de Verenigde Staten waar patiënten werden geïncludeerd tussen 1978 en 1988.[29] Deze verschillen kunnen mogelijk worden verklaard door verschillen in patiënten selectie, de definitie van SCIs, of verschillen in scansequenties. Om verder inzicht te krijgen in het belang van het hemoglobine gehalte bij het ontstaan van SCIs en wittestofafwijkingen is het belangrijk om een kwantitatieve analyse uit te voeren door het totale laesies volume van de wittestofafwijkingen mee te nemen.

In dezelfde studie onderzochten we CBV met behulp van arteriële spin labeling (ASL). Patiënten met sikkelcelziekte hebben een verhoogde CBV, waarschijnlijk ter compensatie van de ernstige anemie om de zuurstoftoevoer te garanderen.[30] Op momenten van toegenomen metabole behoefte, of wanneer er sprake is van een verdere daling van het hemoglobinegehalte, is de reserve capaciteit van CBV onvoldoende waardoor er ischemie optreed en infarcten kunnen ontstaan. Een tweetal onderzoeken heeft inderdaad laten zien dat er ischemie in de hersenen kan ontstaan bij kinderen met sikkelcelziekte, zogenaamde acute SCIs, die optraden na acute dalingen in het hemoglobinegehalte.[31,32] Aangezien we gebruik maakte van hoge resolutie MRI konden we CBV apart in de witte stof en de grijze stof meten. In onze studie vonden we geen verschillen in CBV tussen patiënten met of zonder wittestofafwijkingen. Mogelijk was dit ook een power probleem van de studie. Echter, misschien is het belangrijker om de reserve capaciteit van CBV te meten in plaats van CBV terwijl de patiënt niet ziek is en er dus geen sprake is van een verhoogde metabole behoefte. In een toekomstige studie zullen we daarom de reservecapaciteit van CBV onderzoeken en het risico op wittestofafwijkingen om beter inzicht te krijgen in de rol van CBV in de pathofysiologie van wittestofafwijkingen.

CBV in sikkelcelziekte is niet alleen afhankelijk van de mate van anemie, maar kan mogelijk ook worden beïnvloed door endotheel dysfunctie, aangezien dit geassocieerd is met stikstofoxide (NO) deficiëntie en verminderde vasodilatatie. In onze studie was een lagere concentratie van ADAMTS13, een marker van endotheel activatie, geassocieerd met verminderde CBV in de witte stof. Dit suggereert dat endotheel dysfunctie inderdaad CBV kan beïnvloeden, en daardoor kan bijdragen tot de vorming van wittestofafwijkingen (figuur 3). Determinanten van CBV in het algemeen, en in sikkelcelziekte in het bijzonder, zijn echter nog weinig onderzocht.

Het bestuderen van de dynamiek van CBV in sikkelcelziekte, en de specifieke interactie tussen endotheel dysfunctie en CBV, zal kunnen bijdragen aan nieuwe inzichten in de pathofysiologie van wittestofafwijkingen in sikkelcelziekte.

Figuur 3. Voorgestelde interactie tussen endotheeldysfunctie, cerebrale bloedvoorziening en wittestofafwijkingen in sikkelcelziekte. In hoofdstuk 4 hebben we aangetoond dat een hoger HbF percentage was geassocieerd met een lager volume van wittestofafwijkingen, wat suggereert dat HbF beschermend werkt tegen het optreden van uitgebreide wittestofafwijkingen. Mannelijke sekse was een onafhankelijke risicofactor voor wittestofafwijkingen. Een lagere concentratie van ADAMTS-13, een teken van endotheeldysfunctie, was geassocieerd met verminderde CBV. We konden geen associatie tussen CBV en wittestofafwijkingen aantonen in deze studie, wellicht omdat CBV gemeten terwijl de patiënt niet ziek was en er dus geen sprake was van verhoogde metabole behoefte. CBV, cerebrale bloedvoorziening; HbF, foetaal hemoglobine.



HET METEN VAN DE CEREBRALE BLOEDVOORZIENING IN SIKKELCELZIEKTE

Abnormale CBV speelt een cruciale rol spelen in de etiologie van wittestofafwijkingen in sikkelcelziekte. CBV kan niet-invasief worden gemeten met behulp van arteriële spin labeling (ASL) door het bloed magnetisch te labelen als het door de carotiden stroomt. Dit signaal kan worden gemeten als het aankomt in de hersenen. De gelabelde protonen keren na een bepaalde tijd weer terug naar de grondstatus, dit is de T1 relaxatietijd ($T1_{bloed}$). $T1_{bloed}$ is afhankelijk van verschillende factoren waaronder de hoeveelheid macromoleculen in de nabije omgeving van de protonen.[33] Normaliter wordt een standaardwaarde van 1650 ms gebruikt in volwassenen. Echter, in studies bij pasgeborenen, gezonde kinderen en volwassenen is aangetoond dat $T1_{bloed}$ afhankelijk is van hematocriet.[34–36] Door de lineaire associatie tussen hematocriet en $T1_{bloed}$ is het mogelijk om $T1_{bloed}$ te berekenen uit een breed scala van hematocrietwaarden. Of de lineaire relatie tussen $T1_{bloed}$ en hematocriet ook van toepassing is voor patiënten met sikkelcelziekte is echter nog niet bekend. Er zijn verschillende andere factoren die $T1_{bloed}$ kunnen beïnvloeden en die afwijkend zijn in sikkelcelziekte, zoals hemorheology en bloedviscositeit.

In hoofdstuk 5, hebben we $T1_{bloed}$ *in vivo* gemeten bij kinderen met sikkelcelziekte met behulp van een recent ontwikkelde scantechniek. Gemeten $T1_{bloed}$ was gemiddeld 1846 ms, dat is hoger dan de standaardwaarde van 1650 ms, maar korter vergeleken met de berekende waarde op basis van hematocrietwaarden, die 2058 ms was. CBV gemeten met behulp van de berekende $T1_{bloed}$ leek een onderschatting te zijn, terwijl het gebruik van de standaard waarde van $T1_{bloed}$ de CBV juist leek te overschatten. CBV berekend met de gemeten $T1_{bloed}$ waarde benaderde het beste de CBV gemeten met fasecontrast MRI. Het gebruik van de *in vivo* gemeten $T1_{bloed}$ verbetert de CBV meting in patiënten met sikkelcelziekte. Dit onderschrijft het belang van voorzichtige interpretatie van CBV metingen met behulp van ASL bij patiënten met sikkelcelziekte. Er kunnen naast $T1_{bloed}$ nog andere factoren zijn waar rekening mee gehouden moet worden, bijvoorbeeld de labelingsefficiëntie en de arteriële transit tijd, dat wil zeggen de tijd die nodig is voor het arteriële bloed om in het capillaire bed van de hersenen te arriveren.

NEUROCOGNITIEVE GEVOLGEN VAN WITTESTOFAFWIJKINGEN

Neurocognitieve problemen komen relatief veel voor bij kinderen met sikkelcelziekte en zijn geassocieerd met SCIs. Bij een substantieel deel van de patiënten is de socio-economische status laag, dit bemoeilijkt de behandeling en revalidatie en is op zichzelf ook een risicofactor voor neurocognitieve problemen. Eerdere studies naar de associatie tussen SCIs en neurocognitieve uitkomsten hebben tegenstrijdige resultaten opgeleverd. Sommige studies toonden een lager totaal IQ bij kinderen met SCIs in vergelijking met kinderen zonder SCIs,[37,38] maar dit werd niet bevestigd in andere studies.[39–41] Deze verschillen kunnen mogelijk verklaard worden door verschillen in patiëntgroepen, de definitie van SCIs, of de gebruikte neurocognitieve testen. Aangezien deze eerdere studies het totale volume van de SCIs niet hebben meegenomen , kan ook heterogeniteit in het volume van de laesies een verklaring zijn voor de tegenstrijdige resultaten. Het kwantitatieve effect van het totale volume van de SCIs op neurocognitieve uitkomsten is namelijk nog onbekend. Kennis van deze relatie zou nuttig zijn om mee te nemen bij de behandelingen van zowel de SCIs en het neurocognitief functioneren.

In hoofdstuk 6 hebben we het totale volume van de wittestofafwijkingen gemeten, en een verband aangetoond tussen het totale volume van de wittestofafwijkingen en het totale IQ en de verwerkingssnelheid. Het volume van de wittestofafwijkingen kon ongeveer 20% van de variantie van IQ en verwerkingssnelheid verklaren. Dit is vrij hoog aangezien neurocognitieve uitkomsten worden beïnvloed door vele andere factoren zoals o.a. socio-economische status.

Patiënten met uitgebreide wittestofafwijkingen moeten daarom zorgvuldig worden gecontroleerd op neurocognitief functioneren, deze patiënten hebben recht op adequate begeleiding en psychologische ondersteuning. Patiënten met uitgebreide wittestofafwijkingen kunnen ook in aanmerking komen voor meer agressieve behandelingen om progressie van de wittestofafwijkingen te voorkomen. De resultaten van de reeds genoemde SITT trial suggereren dat een chronische bloedtransfusie schema bij patiënten met SCIs de verdere progressie van SCIs en herseninfarcten kunnen voorkomen.[42] Echter, in de controlegroep van deze studie trad een onverklaarbaar hoog aantal herseninfarcten op, veel hoger dan wat normaal te verwachten valt. Dit hoge aantal herseninfarcten in de controlegroep was voornamelijk verantwoordelijk voor het positieve resultaat van de SITT trial. Als alleen naar de SCIs wordt gekeken, dan zijn de resultaten van de SITT trial niet overtuigend, aangezien er 7 SCIs in de controlegroep voorkwamen tegenover 5 SCIs in de bloedtransfusie groep. Bovendien zijn er veel nadelen te noemen van een chronische bloedtransfusie schema, zoals de grote impact op het sociale leven, schoolbezoek, en kunnen er complicaties optreden zoals allo-immunisatie en ijzer-stapeling. Daarom is het nodig om juist die patiënten te selecteren die het meeste baat hebben bij de behandeling. Door het totale volume te meten van de wittestofafwijkingen kan er een meer gekwantificeerde aanpak plaatsvinden, dit geldt voor de neuropsychologische diagnostiek en interventie maar ook voor de behandelingsopties zoals het bloedtransfusie schema.

NEONATALE COHORTSTUDIE

Vroege diagnose van sikkelcelziekte met adequate follow-up zorg én de educatie van ouders zijn allen belangrijk om de mortaliteit en morbiditeit op jonge leeftijd te verminderen.[43] Veel van de acute complicaties kunnen worden voorkomen indien preventieve maatregelen worden genomen, dit geldt met name voor de infectieuze complicaties en pijnlijke crisen. Of vroegtijdige diagnose en behandeling ook een positief effect zal hebben op de meer chronische orgaanschade is nog niet duidelijk. Om vroege mortaliteit en morbiditeit te voorkomen is neonatale screening op sikkelcelziekte in 2007 ingevoerd in Nederland.

In hoofdstuk 7 presenteerden we de eerste resultaten van een landelijke cohort studie van de kinderen gediagnosticeerd door middel van neonatale screening. Hierin werden mortaliteit, morbiditeit en ook het gewichtsbeloop geëvalueerd. De mortaliteit in ons cohort was laag, slechts 1 patiënt met een ernstige genotype was overleden. De mortaliteit was hiermee 0.21/100 persoonsjaren.

Dit is vergelijkbaar met andere recent gepubliceerde neonatale screeningscohorten zoals het Dallas newborn cohort en cohorten in Frankrijk en het Verenigd Koninkrijk.[2,44,45] De morbiditeit in ons cohort was aanzienlijk lager in vergelijking met deze cohorten, met een laag aantal opnames voor dactylitis, pijnlijke crisen en acuut chest syndroom.
Dit kan waarschijnlijk voor een deel worden verklaard door verschillen in opname criteria aangezien in Nederland de meeste complicaties (zoals dactylitis en pijnlijke crisen) thuis worden behandeld. De mortaliteit en morbiditeit van ons cohort is daarnaast aanzienlijk lager in vergelijking met de grootste longitudinale cohort studie van de Verenigde Staten, de CSSCD die patiënten tussen 1978 en 1988 heeft geïncludeerd. [46] Dit is met name een uiting van de verbetering in de zorg van de afgelopen jaren. Dit benadrukt tevens de noodzaak om ook recente follow-up studies te verrichten, zodat de huidige problemen in diagnose, prognose en behandeling kunnen worden opgespoord. Hiervoor is nationale en internationale samenwerking erg belangrijk.

Tot nu toe hebben wij nog niet de cerebrale complicaties in deze patiëntengroep kunnen onderzoeken vanwege de jonge leeftijd van de patiënten (0-7 jaar), met uitzondering van screening voor intracraniële stenose met transcraniële Doppler. Screening op wittestofafwijkingen zal vanaf de leeftijd van 8 jaar plaatsvinden, evenals neurocognitief onderzoek. Het is daarom nog niet bekend of de lage morbiditeit van onze cohort zal ook leiden tot een lage incidentie van wittestofafwijkingen en een goede neurocognitieve uitkomst. Acute anemische episodes zijn een risicofactor voor SCIs, en een lage incidentie van deze complicatie kan mogelijk leiden tot minder SCIs.[31,32]

Naast het evalueren van mortaliteit en morbiditeit, hebben wij specifiek het gewichtsverloop in dit cohort geëvalueerd. Eerdere studies hebben een ernstige groeivertraging aangetoond bij kinderen met sikkelcelziekte, variërend tussen 0.5 tot 2 standaarddeviaties onder het gemiddelde van de bevolking.[47,48] Deze studies zijn echter enkele decennia geleden uitgevoerd toen de zorg voor kinderen met sikkelcelziekte van een ander niveau was en vóór de invoering van neonatale screening. In ons neonatale screeningscohort was er geen sprake van ondergewicht. Echter, bij een aanzienlijk deel van de jonge kinderen was er sprake van obesitas: variërend van 12 tot 23% bij de leeftijdsgroepen 1 tot 5 jaar. Obesitas kwam meer voor bij patiënten met een mild genotype, maar zelfs bij patiënten met een ernstige genotype zat het gemiddelde BMI (body mass index) boven het bevolkingsgemiddelde. Dat er bij zoveel jonge patiënten met sikkelcelziekte sprake was van obesitas is zeer opmerkelijk maar sluit wel aan bij de trend naar toegenomen gewicht in de algemene populatie. Een recent gepubliceerde crosssectionele studie in kinderen met sikkelcelziekte heeft aangetoond dat er bij ruim 22% van de 665 kinderen sprake was van overgewicht of obesitas.[49] Onze resultaten

dienen nog bevestigd te worden in een grotere patiëntengroep en met een langere follow-up duur.

De trend naar overgewicht en obesitas zal van groot belang zijn voor de lange termijn complicaties bij patiënten met sikkelcelziekte. Obesitas en de daaruit voortvloeiende complicaties zoals hypertensie en diabetes zijn extra risicofactoren voor neurologische, cardiovasculaire en renale ziekte. Patiënten met sikkelcelziekte hebben reeds een verhoogd risico op deze complicaties, die verantwoordelijk zijn voor aanzienlijke morbiditeit en mortaliteit later in het leven. Verder onderzoek zal zich moet zich richten op de determinanten op overgewicht en obesitas bij kinderen met sikkelcelziekte, en daarnaast moeten de behandeling opties nog worden onderzocht. Om te beginnen moeten artsen, ouders en patiënten zich bewustzijn van het risico op obesitas.

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PhD training	Year	ECTS
General courses		
AMC World of Science	2010	0.7
Basic Laboratory Safety	2010	0.4
Introduction Access Database Learning	2010	0.4
Reference Manager, basic and advanced	2010	0.2
Pubmed e-learning	2010	0.1
BROK, including Good Clinical Practise	2011	0.9
Communication and PhD, UvA	2011	0.6
Project Management, UvA	2011	0.6
Clinical Data management	2011	0.2
Oral Presentation in English	2011	0.8
Crash course chemistry	2011	0.4
Systematic Revieuws, Dutch Cochrane Centre	2011	0.3
Clinical Epidemiology	2011	0.4
Practical Biostatistics	2012	1.1
Scientific writing in English	2013	1.5
Specific courses, workshops		
Advanced Annual Sickle Cell and Thalassaemia Course, Guy's Sint Thomas' Hospital, London	2010	1.5
Masterclass, Scientific Symposium, Emma Children's Hospital	2011	0.4
NVK, Dag van de Jonge Onderzoeker	2011	0.4
NVK, Dag van de Jonge Onderzoeker	2012	0.4
Weekly consultation, department of Pediatric Hematology	2010-2014	5.7
Monthly scientific presentations, department of Pediatric Hemathology and Immunology	2012-2014	1.0
International conferences		
Annual congress of Pediatrics, Amsterdam	2011-2015	1.5
Annual congress of the American Society of Hematology	2013	1.5
Annual congress of the American Society of Hematology	2014	1.5

Teaching

Supervising of bachelor students (total of 7)	2012-2014	7.0
Supervising of master student	2012-2013	1.0
Total ECTS points		*30.5*

Clinical work

Resident Pediatric Intensive Care Unit, Emma Children's hospital, 0.25 fte 2012-2014

Committees

Chairman and co-founder of Jonge Onderzoekers Kindergeneeskunde (JOK): a committee of young researchers in pediatrics of the AMC and VUmc hospital, activities include workshops and extra-curricular meetings with invited speakers 2011 – 2013

Member of organizing committee of the 2013 annual scientific symposium in pediatrics in Amsterdam 2012 – 2013

Poster and oral Presentations

Poster presentation, annual scientific symposium Amsterdam, 'Risk factor analysis of cerebral white matter hyperintensities in children with sickle cell disease'. 2015

Poster presentation, American Society of Hematology, San Fransisco, 'Volume of white matter hyperintensities predicts neurocognitive functioning in children with sickle cell disease' 2014

Poster presentation, American Society of Hematology, New Orleans, 'Cerebral Small Vessel Disease in Patients With Sickle Cell Anemia – Initial findings with Ultra-High Field 7T MRI' 2013

Poster presentation, annual scientific symposium Amsterdam, 'White matter lesions at 7T MRI in sickle cell disease: a specific form of small vessel disease?' 2013

Poster presentation, annual scientific symposium Amsterdam, 'Endothelial dysfunction in children with sickle cell disease' 2012

Oral presentation, Annual scientific symposium, Emma Children's Hospital, 'Growth in children with sickle cell disease in the Netherlands.' 2011

Publications

Van der Land V, Zwanenburg JJ, Fijnvandraat K, Biemond BJ, Hendrikse J, Mutsaerts HJMM, Visser F, Wardlaw JM, Nederveen AJ, Majoie CBLM, Luijten PR, Nederkoorn PJ. *Cerebral lesions on 7 Tesla MRI in patients with sickle cell anemia.* Cerebrovascular Diseases **2015**:39:181-189.

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Van der Land V, Peters M, Heijboer H, Van Ommen CH, Cnossen MH, Dors N, Suijker MH, Van der Lee JH, Fijnvandraat K. *First report on sequelae of sickle cell disease in children diagnosed through neonatal screening in the Netherlands.* Manuscript in preparation.

Václavů L, Van der Land V, Heijtel DFR, Majoie CBLM, Fijnvandraat K, Mutsaerts HJJM, Nederveen AJ. *T1 in blood for sickle cell disease for quantification of cerebral blood flow from arterial spin labeling MRI.* Manuscript in preparation.

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CURRICULUM VITAE

Veronica van der Land was born October 12 , 1981 in Wieringerwerf, where she grew up and went to high school. After obtaining her high school diploma she started her study Medicine at VU University in 2001. During this period she participated in an honours programme for students interested in research, which resulted in a first publication. She did an extracurricular internship in Nephrology at St. Elisabeth Hospital Curaçao and a senior internship Pediatrics at VU University Amsterdam.

After obtaining her medical degree in 2008, she worked as a pediatric resident at Zaans Medical Center, followed by the pediatric Intensive Care Unit of Leiden University Medical Center. In 2010 she started her PhD research in the field of sickle cell disease, under the supervision of prof. dr. Taco Kuijpers and dr. Karin Fijnvandraat at the Emma Children's Hospital of the Academic Medical Centre Amsterdam. During her PhD research Veronica also worked as a pediatric resident at the pediatric Intensive Care Unit of the Emma Children's Hospital.

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