UNIL | Université de Lausanne
Faculty of Biology and Medicine

CHUV | CENTRE HOSPITALIER UNIVERSITAIRE VAUDOIS

Mémoire de Maîtrise en médecine N° 4533

Neuropsychological impact of sickle-cell disease in children and adolescents

Etudiant
Elisa Santos

Tuteur
Maja-Beck Popovic
Unité d'Hémato-Oncologie pédiatrique

Co-tuteurs
Claire Mayor
Unité de Neurologie et Neuroréhabilitation pédiatrique
Raffaele Renella
Unité d'Hémato-Oncologie pédiatrique

Expert
Eliane Roulet Perez
Unité de Neurologie et Neuroréhabilitation pédiatrique

Lausanne, 01.03.2017

INTRODUCTION

Generalities

Sickle-cell disease (SCD) is a chronic systemic condition that gathers a cluster of erythrocyte disorders, affecting specifically the hemoglobin gene[1-2]. This disease is characterized by acute pain events and gradual organic dysfunction and it is the most common hereditary monogenic condition in the world, mostly prevalent in Sub-Saharan Africa[1-4].

SCD physiopathology

This autosomal recessive condition is due to a mutation in the β-globin gene, located in chromosome 11, in which the 17th nucleotide is mutated from thymine to adenine converting the sixth amino acid, glutamic acid, into valine[1, 3, 5] and producing an abnormal hemoglobin form called Hemoglobin S (HbS). As a consequence of this alteration, a hydrophobic site in the deoxygenated HbS isoform is generated, creating a link between two β-globin chains, and thus causing polymerization[1], which leads to erythrocyte rigidity and fragility[2-4, 6-7].
Three main genotypes cause the SCD: the most common is homozygosity for the β^s allele (HbSS) also called sickle-cell anemia (SCA), followed by heterozygous states HbSC and HbS/β-thalassemia[1, 8]. There are other genotypes combining the β^s allele with other β-globin point mutations leading to sickling of erythrocytes and to SCD, but these forms are less prevalent. All of the above mentioned types are grouped into SCD. The protective advantage of the β^s carrier state over malaria explains the endemic nature of SCD in Sub-Saharan Africa[4]. There are five main haplotypes of the β^s-globin gene according to the geographical origin. They are named Benin, Cameroun, Senegal, Central African Republic and Arab-Indian, which are related with different levels of fetal hemoglobin (HbF)[9]. A higher HbF level is a protective factor of SCD complications[4].

In vivo, the SCD erythrocyte deformation causes vaso-occlusion and hemolysis, both main actors of SCD pathophysiology[1, 2, 7]. Abnormal erythrocytes and leukocytes get trapped into the microcirculation causing vaso-occlusive crises (VOCs) and consequently tissue ischemia[2]. This process, often initiated by inflammation[1, 2], results in acute vaso-occlusive pain, the most common symptom of SCD[1, 7]. Furthermore, following these episodes of vascular occlusion and ischemia, blood flow restoration occurs causing tissue injury[2]. These recurrent successions of ischemia and reperfusion lead to damage to the vessel walls and to a continuous inflammatory state and vasculopathy in children with SCD[2, 7].

As they circulate through the vascular system, sickling erythrocytes are damaged resulting in hemolytic anemia, the second pathophysiological process in SCD[1, 7]. This leads not only to anemia and hypoxia[1, 7], but also to the release of erythrocyte content and membranes into the circulation. The discharge of free hemoglobin and its derivatives into the blood flow contributes to endothelial cell dysfunction, activation of hemostatic cascades and systemic inflammation[1].

The SCD physiopathology, which affects the entire organism, drives to a wide spectrum of clinical complications[10], including the abovementioned VOCs, susceptibility to infection,

acute chest syndrome, chronic lung disease, renal failure, splenic dysfunction, growth impairments, osteomyelitis, bone necrosis and neurological disorders (including stroke) [1, 4, 6-8, 10-11]. Infections and stroke are the two most common causes of mortality[6].

Neurological complications of SCD

Regarding the nervous system, ischemic cerebrovascular accidents (CVA) are the most common neurological complications in children suffering from SCD[5, 12]. Indeed, according to the Cooperative Study of SCD, approximately 10% of the patients will suffer an overt infarct before the age of 20 years[1, 6, 11, 13-14], after which they usually experience significant neurological and cognitive deficits[15].
There are two types of ischemic CVA, overt stroke and silent infarct.
Overt strokes are generally caused by an occlusion of the main supplying arteries of the brain, particularly the internal carotid arteries[11-12, 15], resulting in extensive lesions of white and grey matter of the corresponding territories on magnetic resonance imaging (MRI)[16, 17].
In silent infarcts, vessel occlusion occurs in distal smaller arteries located in border zones, which are more vulnerable to hypoperfusion[14]. Sixteen to 22% of patients suffering from SCD may experience silent infarcts, revealed by brain MRI. These lesions are frequently located in the deep white matter of the frontal lobes[17-21], and do not lead to observable neurological deficit[14], but subtle cognitive impairment[18, 22], especially in attention and executive functions[15, 18, 20].
Overt and silent CVA can be detected by MRI or by transcranial Doppler ultrasonography (TCD) that measures blood flow velocity through the cerebral blood vessels. TCD is thus used to identify children at risk for stroke, with a 90% of sensitivity, lower than MRI, and a 100% of specificity [23-24].
Hemorrhagic CVA, which results from vessel wall weakness, can also occur in SCD but are less frequent in children than in adults[22].

Besides stroke, other pathological processes, which cannot be detected by neurological examination or cerebral imaging, are thought to contribute to cognitive deficits in this population. [12, 25-26]

Cognitive impairment in children with SCD in literature

1) Generalities

Numerous studies have highlighted cognitive deficits in children with SCD with overt stroke or silent cerebral infarcts[4, 12-13, 15, 18, 20, 23, 27-28]. It is well known that cerebrovascular injuries have a significant impact on cognitive and academic performances[29]. Indeed, compared to controls or a standard baseline average, children with SCD and visible brain injuries usually demonstrate lower general intelligence, as measured with IQ-scales[30-32], and exhibit deficits in specific domains such as executive functioning, attention, visuo-motor integration and working memory[6, 20, 28-29], all of which affect academic achievement. Executive functions include cognitive inhibition, planning, working memory and cognitive flexibility. Furthermore, the rise of neurocognitive difficulties seems to increase with age[13, 21, 28-29].

Older children tend to have greater neuropsychological impairment in specific functions[30], which might be the consequence of cumulative aggressions to the brain over time[8].
However, little is known regarding the neuropsychological impairment of children with SCD without any visible brain injury.

2) Neurocognitive difficulties in children free of infarction

Studies about neurocognitive impairment in children without signs of vascular damage compared children suffering from SCD with healthy controls with similar socio-economic status or with standard baselines for age[14, 16-17, 19, 25-26, 30-34]. Most studies evaluated the general intelligence, others also assessed specific cognitive functions such as language, executive functioning, attention, memory or visuo-motor functioning, correlated with MRI and TCD and also laboratory biological markers. All studies, except one, were prospective studies. All, except two on infants, report that, in this population, the IQ values are generally lower than in the healthy control groups or to standard baselines. These children also tend to have difficulties in executive functions, memory, attention or verbal skills function[16, 25-26, 30, 32], although not all studies agree regarding memory and attention[16-17]. Furthermore, this population tends to have a greater variability in cognitive functioning and perform worse in general[17, 30].
In summary, the existing studies focusing on this specific population assess only a small portion of neuropsychological functions, mainly IQ[35]: discordant results may be explained by the small sample sizes, different age ranges and different neurocognitive testing. Moreover, only few studies have correlated cognitive results with SCD-related markers of disease severity. The different studies are summarized in annex 1.

3) Possible causes of cognitive impairment in children free from infarction

The causes of neurocognitive impairment in children with SCD showing no visible brain injury are not well understood. It may include direct effects of SCD on brain function or indirect effects of chronic illness[29].

Regarding direct effects of the SCD on the brain, several causes are possible: accumulation of micro-insults undetectable with the MRI[8, 13, 29] or chronic hypoxia due to chronic anemia[8, 10, 12, 26-27, 29, 32, 35]. Indeed, animal models show that low hemoglobin is correlated with reduced brain metabolism[7]. These models show that hypoxia cause changes in the hippocampus and thus to cognitive difficulties, particularly learning and memory processes[7]. Another study[35] reports that a reduction of 1% of hemoglobin oxygen saturation correlates with a decrease of 0.75 IQ point. Moreover, as a response to chronic hypoxia, increased cerebral blood flow velocity, and subsequently a disturbed cerebral circulation, could cause significant cognitive dysfunction[35].
Finally, children with SCD may suffer from chronic nutritional deficiencies associated with increased metabolism demands, secondary to malnutrition or anemia, that could compromise brain development[8, 13, 26, 29].

Indirect causes include genetic factors and environmental causes, such as low socio-economic status, frequent hospitalizations and reduced school attendance. These may also be critical for developmental outcomes and result in decreased performance in cognitive

testing[13, 19, 27, 29, 33, 35]. Additionally, sleep disorders and sleep apneas may also play a role, as shown by a study in which oxygen desaturation is associated with executive difficulties and reduced general intelligence[32].

4) The role of correlating biomarkers with cognitive impairment

Biomarkers are measurable biological characteristics that can be useful tools for detecting early manifestations of a chronic disease. They can be helpful for screening, diagnosis, prognosis or treatment choice.

As mentioned previously, anemia is a main actor of SCD pathophysiology and could be a source of cognitive deficits. Previous studies have associated neurocognitive testing with markers of anemia, such as hemoglobin or hematocrit[14, 19, 25-26, 31, 33]. For example, Steen et al. (2003)[25] demonstrated that a low hematocrit was related to low IQ in children with SCD without a history of CVA. Another study also correlated white blood cell (WBC) and platelet count, as markers of inflammation, a process that is part of the disease, with neurocognitive measures: high WBC count was also associated with low IQ[15]. Except for those aforesaid, no other hematological or chemical markers have been studied.

Investigating new biological markers related to the severity of SCD and reflecting its main pathophysiological processes, such as hemolysis and inflammation, could possibly help to detect children without overt or silent infarct at risk for cognitive difficulties.

Aims of this study

Compared to studies on patients with SCD and reported cognitive impairments in presence of visible cerebral vascular injury, children and teenagers who never had a stroke or silent infarcts have been less explored until now. Thus, the primary aim of this retrospective study was to establish a descriptive neuropsychological profile of this specific population, hypothesizing that it will allow to highlight deficits not only in general intelligence but also in specific cognitive domains, such as executive functioning, attention, memory or fine motor skills functioning.

The second aim was to correlate neuropsychological data with a wide range of medical parameters, such as clinical, hematological and biochemical indices of disease activity, in order to identify biological parameters associated with neurocognitive disorders and to try to understand pathophysiological processes causing cognitive deficits in children with SCD in the absence of neurovascular events. We assumed this would reveal new biomarkers, for example HbF, as possible indicators of the neuropsychological impact of the disease, in order to detect patients at risk of cognitive disorders.

METHODOLOGY

Participants

Twenty-nine children with SCD aged from 5 to 17 years at the time of the neuropsychological evaluation were included into the study. These children are regularly followed at the Pediatric Hematology-Oncology Unit of the Centre Hospitalier Universitaire Vaudois (CHUV). None of these patients had a prior overt stroke or underwent hematopoietic stem cell transplantation.

Procedure

This is a retrospective study. The local Institute of Research in Biomedicine (Commission Cantonale d'éthique de la recherche sur l'être humain CER-VD) approved the protocol (n° 2016-02088, on the 07.02.2016). Written informed consent was obtained from the participants and their parents/legal representative.
Data derived from clinical follow-up in paper or electronic charts were collected. Neuropsychological tests were performed, from 2011 until 2017, by neuropsychologists from the same team. For most participants, these assessments were made after the onset of academic difficulties, and for others, as part of a disease monitoring protocol at 6 and 10 years old. Medical information was collected from the patients' files at the time of their neuropsychological assessment.
Of the 29 children, 12 underwent a second cognitive evaluation. In this study, however, only the first assessment was taken into account, as not all the participants had a longitudinal evaluation. Moreover, in this cross-sectional setting, no group control was used.

Measures

1) Cognitive measures

Standardized tests were used to assess general intelligence, fine motor functioning, memory, executive functions and attention in the participating children with SCD without overt stroke or silent infarct, as documented by MRI or DTC. The Wechsler Preschool and Primary Scale of Intelligence (WPPSI III) designed for children aged from 2 years and 6 months to 7 years and 7 months old, and the Wechsler Intelligence Scale for Children (WISC IV) for children between 6 and 16 years and 11 months old were used to evaluate general intelligence. All the other specific tests are standardized from 6 years old, except for the Stroop, Color trail and Card sorting tests, which are standardized from 8 years old. Table 1 provides the detailed neuropsychological testing.

The average intelligence quotient (IQ) is 100 with a standard deviation (SD) of 15. Intellectual disability is defined as an IQ below 70. For other individual tests, such as visuo-motor precision (NEPSY II), working memory and verbal long-term memory, the standard scores that were used have a global average of 10 and a SD of 3. A value under 6 is defined as pathological. *Z scores,* which assess a performance compared to the average, were also used: a negative *z score* is below average, and a pathological threshold was set at -1.65. Visuo-motor functioning (Purdue Pegboard test), mental flexibility, inhibition, planning

(mazes test) and visual long-term memory (encoding) were expressed in *Z scores*. Attention measures were assessed with the Conners' Continuous Performance Test (CPT). In the CPT, the higher the percentile, the larger the deficit. Vigilance and inattention, given in percentile, are considered pathological when above the 90th percentile. The final "clinical significance" score relates to the probability, expressed in percent, that the patient suffers from a significant attention deficit.

Table 1. **Neuropsychological testing used in children with SCD.**

Wechsler Intelligence Scales: WPPSI III and WISC IV • Total Intellectual Quotient (TIQ) • Verbal Comprehension (VCI) • Perceptual Reasoning (PRI) • General Ability (GAI, compound of VCI and PRI) • Working Memory (WMI) • Processing Speed (PSI)
Motor functioning • Fine motor and manual dexterity: Purdue Pegboard • Visuo-motor precision: NEPSY II (VMP)
Memory • Verbal long-term memory: CMS (word list) • Visual long-term memory: Rey Visual Learning test (15 signs) • Visuo-spatial short-term memory: Corsi test • Visuo-spatial long-term memory: Rey complex figure test
Executive functions and attention • Verbal initiation: Verbal fluency • Nonverbal initiation: Figurative fluency • Verbal working memory (WMI: digit span, reverse order) • Inhibition: Stroop test and CPT motor inhibition • Mental flexibility: Color trail and Card sorting Test • Planning: mazes test WISC III • Sustained attention: CPT II

TIQ: total intellectual quotient, VCI: verbal comprehension index, PRI: perceptual reasoning index, GAI: general ability index (=VCI+PRI), WMI: working memory index, PSI: processing speed index. VMP: visuo-motor precision. CMS: Children's memory scale. CPT: continuous performance test.

Besides isolated scores, composite measures were also employed to correlate with the biological parameters. These composite measures are a mean of different tests assessing closely related functions and they are used to group processes common to complex functions, such as attention, mental flexibility or inhibition. For example, the CPT that includes measures of attention, vigilance, focal attention and impulsivity is summarized in the above described "clinical significance" score. The composite score of mental flexibility represents the mean of the results obtained in three tests: Color Trail, Card Sorting Test and Verbal Fluency Alternation. Finally, the composite score of inhibition processes includes the Stroop Tests and the component of the CPT measuring motor impulsivity.

2) Medical characteristics

We considered patient-specific characteristics such as age, gender, and ethnicity. We also accounted for disease-related factors, for example genotype, number of VOCs or whether the patients had been treated with hydroxyurea or not (Table 2a). Regarding imaging, of the 29 children of the study, 15 underwent MRI and 28 DTC. The one who had no DTC had an MRI.

3) Biomarkers

We collected hematological values such as hematocrit, hemoglobin, HbF, platelets, leukocytes, reticulocytes, and chemical values, such as total bilirubin. All the data collected were part of the standard medical care provided in the hospital within the preceding 12 months of the neuropsychological testing. All data are listed in Table 2b.

To further correlate the results observed in neuropsychological investigations with pathophysiological states rather than isolated biological parameters, we tested novel clinical indices to reflect two main SCD conditions: inflammation and hemolysis. A neutral index was also generated to represent a balanced model, where inflammation and hemolysis are equally contributive to the observed changes. Indexes were the composites of total leukocyte and platelet counts, being indicators of inflammation, and the reticulocyte count relating to hemolysis. To normalize raw total leukocyte, platelet and reticulocyte counts, individual values were expressed in quotients of normal values for age. Equations to calculate the indexes are shown below:

Inflammatory index: (((VarP+VarL)/2)*2+VarR)/3

Hemolytic index: (((VarP+VarL)/2)+VarR*2)/3

Neutral index: (((VarP+VarL)/2)+VarR)/2

VarP: platelet variance. VarL: leukocytes variance. VarR: reticulocytes variance.
A variance is obtained by dividing the score of platelets, leucocytes or reticulocytes by the baseline average for age. For example, a child with 9.7 g/L leukocytes, 213 g/L platelets and 80 G/l reticulocytes, has an *index I* of 1.94, an *index H* of 2.57 and an *index N* of 2.26. In this case, it can be observed that hemolysis plays a bigger role than inflammation; indeed, the larger the index, the more important is the condition (inflammation or hemolysis).

These are new, not validated and not yet published indices that we created aiming to combine biological variables reflecting the same pathophysiological mechanism, in order to express disease-specific processes, which are here inflammation and hemolysis.

Correlations analysis

Statistical analyses were first realized between isolated clinical parameters and neuropsychological measures and then biological composite measures and neuropsychological measures. The Statistical Package for the Social Sciences (SPSS statistics, version 23.0) was used to analyze the data. A descriptive analysis of the participants' clinical parameters and performance on neuropsychological testing was first realized. Spearman

correlations were then computed aiming to identify associations between clinical parameters and neuropsychological results. A significant correlation is defined with $p < 0.05$.

RESULTS

Demographic and medical characteristics

Tables 2a and 2b describe the detailed demographic and clinical characteristics of the participating children with SCD. The study included 17 boys and 12 girls, and the majority of them (25 children or 86,2%) had the HbSS genotype. They were all from African origin, patients with the HbSC genotype were African and Metis, and finally the only patient with the HbS- genotype was Afro-American. Countries of origin are listed in table 2a, allowing to see the different haplotypes represented. Eighteen out of 29 patients (62%) suffered at least one VOC during the year preceding the neuropsychological testing.

Of the 29 children of the cohort, 15 had an MRI performed, showing no signs of overt or silent CVA; 28 had a DTC, classified as ordinary (i.e. normal cerebral blood flow velocity (CBFV) <170cm/s, showing no risk of infarct,) in 25 of the patients and conditional in 3 (i.e. CBFV = 170-199cm/s, suggesting intracranial stenosis and intermediate risk for infarct,). All 3 patients with conditional risk at DTC had a normal MRI and out of the 15 patients who had an MRI, 3 had a conditional DTC and 1 had no DTC.
All patients tended to have higher inflammatory scores compared to age-related normal values, especially with regard to leukocytes and platelets. Their values were generally superior to the average baseline in healthy controls (leukocytes 4-13 g/L, platelets 150-400 g/l). Similarly, they had higher reticulocyte values than the average baseline (25-75 G/L). As expected, they were anemic and tended to have an elevated percentage of HbF (see Figure 1).

Table 2a. **Demographic characteristics of the participating children with SCD.**

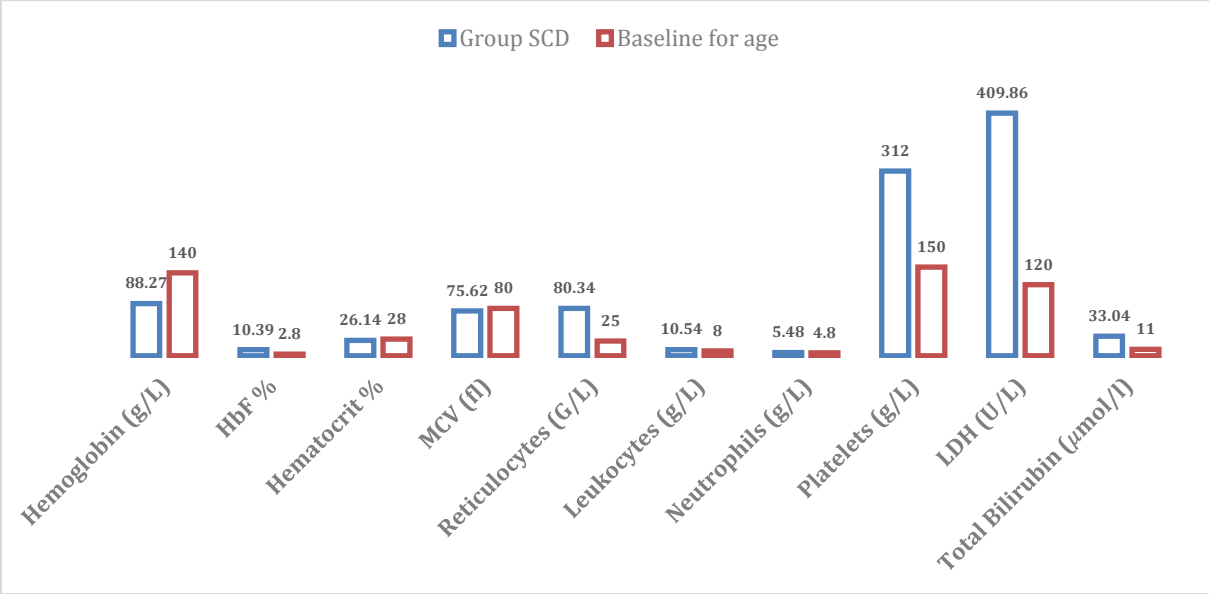
	Children with SCD (N=29)
Age (average, range)	9.83 (4-17)
Sex: Female / Male	12 / 17
Genotype: HbSS / HbSC / HbS-	25 (86,2%) / 3 (10,3%) / 1 (3,4%)
Country of origin	
• **Dominican Republic**	13
• **Angola**	5
• **Cameroun**	5
• **Congo**	2
• **Brazil**	1
• **Ivory Coast**	1
• **Tchad**	1
• **Togo**	1
• **USA**	1

Table 2b. **Clinical characteristics of the participating children with SCD.**

	Children with SCD (N=29)	Baseline average (interval)
Number of VOC episodes	1.62 (0-10)	
Number of ACS episodes	0.24 (0-2)	
Number of inpatient admissions (during the preceding 12 months)	1.89 (0-9)	
Patients on hydroxyurea treatment	11	
Patients on chronic transfusions	6	
Hemoglobin F (HbF)	10.39 (0.6-63.9) %	<2%
Hemoglobin	88.27 (72-114) g/L	120-160 g/L
Hematocrit	26.14 (20-35) %	36-40%
Total leukocytes	10.54 (3.7-18.1) g/L	4-13 g/L
Mean corpuscular volume (MCV)	75.62 (30-98) fl	74-91 fl
Absolute neutrophils	5.48 (1.8-11.5) g/L	1.5-6.6 g/L
Platelets	312 (147-573) g/L	150-400 g/L
Reticulocytes	80.34 (31-162) G/L	25-75 G/L
Lactate dehydrogenase (LDH)	409.86 (165-862) U/L	120-300 U/L
Total bilirubin	33.04 (9-82) µmol/l	5-17 µmol/l
Indices:		
• ***Index H***	2.71 (1.13-5.05)	
• ***Index I***	2.20 (1.01-3.74)	
• ***Index N***	2.45 (1.07-4.34)	

VOC : vaso-occlusive crisis. ACS : acute chest syndrome.
Regarding the column of the group with SCD children, it represents the average and, in the brackets, the range, except for the patients on hydroxyurea treatment and on chronic transfusions, where only the number of patients is given.

Figure 1. **Biological means of hematological variables in children with SCD and baselines average**



The three indices are not represented in this figure, as they are new and there is no existing baseline for age.

Neuropsychological results

All the children in the study underwent all the neuropsychological tests. Table 3 describes the neuropsychological results.

Intelligence

As a group, our patients showed a global intellectual efficiency within the normal range, although, in the lower average. Indeed, almost all the intelligence indexes were at one SD below average. Four of the 29 patients, i.e. 13,8% of our collective, displayed mild intellectual deficiency (GAI<70). As GAI is a mean between VCI and PRI, it indicates these children had difficulties in verbal conceptualization (VCI) and in visual perceptual and reasoning abilities (PRI). Furthermore, working memory (WMI) was weak with scores within the lower limits of the standard (-1,6 SD).
In our descriptive analysis, we observed lower IQ scores in older patients.

Visuo-motor functioning

Fine motor skills were within average, in both the unimanual and bimanual coordination tasks. There were no significant discrepancies in dexterity of the dominant and non-dominant hand. Eight out of the 29 patients, i.e. 27,5%, had a severe visuo-motor precision deficit.

Attention

Despite heterogeneous results, clinical scores showed that our patients had, on average, a profile indicating a 64.5% probability of having attention deficit disorder (CPT clinical significance score). Moreover, vigilance and focal attention abilities were in the lower average. A detailed analysis showed that 6 out of 29 patients, i.e. 20,7%, had a percentile higher than 94 in vigilance and inattention, corresponding with a severe attention deficit.

Executive functioning

Our patients presented disturbed inhibition skills and mental flexibility and working memory were in the low average. Seven of the 29 children, i.e. 24,2%, performed below -2.40 in inhibition tests (-2.44, -2.82, -2.9, -3.86, -3.3), including two who scored -4 and -4.67, which represents a particularly important deficit. Four of the 29 patients, i.e. 13,8%, had scores below -1.95 (-1.95, -2.11, -2.43, -2.87) in mental flexibility showing a severe deficit. Planning abilities (mazes) were within average, except for two children, i.e. 6,9%, who scored -1.96.

Memory

Processes of encoding, recall and recognition were generally preserved in the verbal and visual modality.

Table 3. **Descriptive results.**

Neuropsychological testing	**N**	**Mean of the cohort**	**Minimal value of the cohort**	**Maximal value of the cohort**
General intelligence				
• TIQ	12	89.03	62	116
• VCI	28	85.68	53	120
• PRI	29	94.03	58	124
• GAI	22	86.91	60	118
• WMI	22	75.28	54	100
• PSI	29	86.48	60	109
Visuo-motor functioning				
• Purdue dh (z score)	28	-0.37	-0.37	1.50
• Purdue ndh (z score)	28	-0.30	-1.4	1.95
• Purdue assembly (z score)	27	-0.60	-1.86	1.52
• VMP: NEPSY II (standard score)	26	6.19	2	13
Attention				
• CPT: vigilance (percentile)	25	73.50	25.60	99
• CPT: inattention (percentile)	25	68.12	14	99
• CPT: clinical significance (CM, %)	26	64.54	28	99
Executive functioning				
• Flexibility (CM, z score)	18	-1.25	-2.87	0.65
• Inhibition (CM, z score)	18	-1,56	-6	2.36
• Working memory (RDS, standard score)	22	7.77	3	15
• Planning : mazes test (z score)	27	-0.56	-1.96	1
Memory				
• *Verbal long-term memory: CMS (word list)*				
○ Encoding words (standard score)	25	8.52	5	12
○ Recall words (standard score)	25	8.12	4	13
○ Recognition words (standard score)	25	8	3	13
• *Visual long-term memory: Rey (sign list)*				
○ Encoding RVLT (z score)	14	0.47	-0.85	2.72
○ Recall RVLT (nbr of correct answers/15)	15	8.80	4	15
○ Recognition RVLT (nbr of correct answers/15)	14	14.93	7	15

TIQ: total intellectual quotient, VCI: verbal comprehension index, PRI: perceptual reasoning index, GAI: general ability index (=VCI+PRI), WMI: working memory index, PSI: processing speed index. Dh: dominant hand. Ndh: non-dominant hand. VMP: visuo-motor precision. CPT: continuous performance test. RDS: reverse digit span. CM: composite measure. RVLT: Rey visual learning test. In the second column, are represented the number of participants who received the test.

Correlation between neuropsychological and biological measures

Spearman correlations (Spearman's rho) were computed between clinical data and neuropsychological results. Among biological and clinical data previously quoted, it has been found that platelets, leucocytes, reticulocytes and hydroxyurea therapy significantly correlate with a number of executive measures and/or visual memory. Moreover, it has been found that *index I* significantly correlates with measures of executive functioning, and that all three Indices significantly correlate with visual long-term memory, especially *index H.* As an important amount of correlations was realized, only the significant relevant ones are reported thereafter and are shown in annex 2. No other parameters showed significant association with cognitive measures.

1) Biological data only

Several significant negative correlations were found. Two IQ measures significantly correlated with age (GAI: $r=-0.447$; $p<0.01$; N=22, VCI: $r=-0.392$; $p<0.01$, $N=28$), suggesting older children have a reduced general intelligence.

There was a significant negative association between platelets count and executive functions. Indeed, the higher the platelets count in a child with SCD, the lower the executive performance scores, mainly in inhibition ($r=-0.525$; $p<0,05$; $N=18$) and mental flexibility ($r=-0.761$; $p<0.01$; $N=18$) (Figure 2a and 2b).

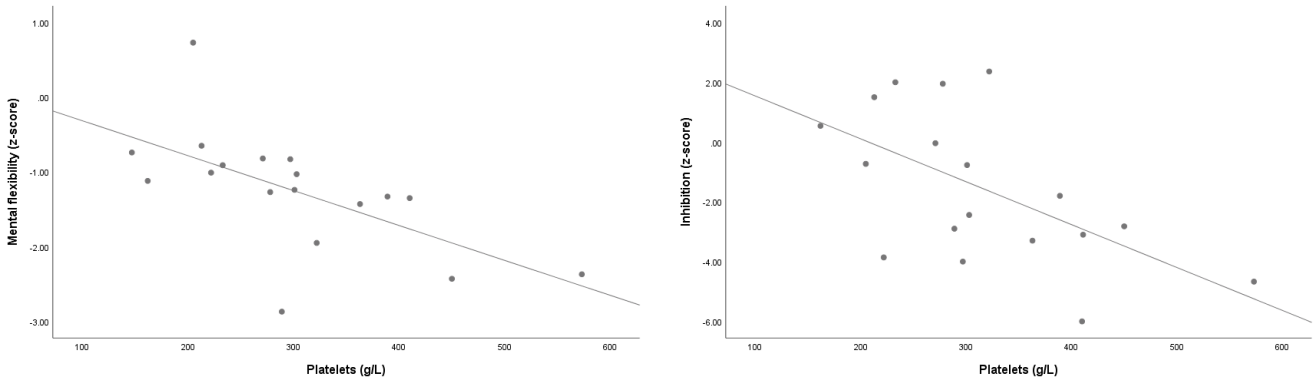


Figure 2a and 2b. **Correlations between platelets and mental flexibility and between platelets and inhibition.**

Interestingly, inhibition skills were also negatively associated with hematocrit count ($r=-0.475$; $p<0.05$; $N=18$), suggesting inhibition is weaker in children without anemia.

Working memory significantly negatively correlated with leukocytes (RDS, $r=-0.561$; $p<0.01$; $N=22$) (Figure 3), implying that children with high leukocyte levels have difficulties to maintain information in mind that they could use for learning or reasoning.

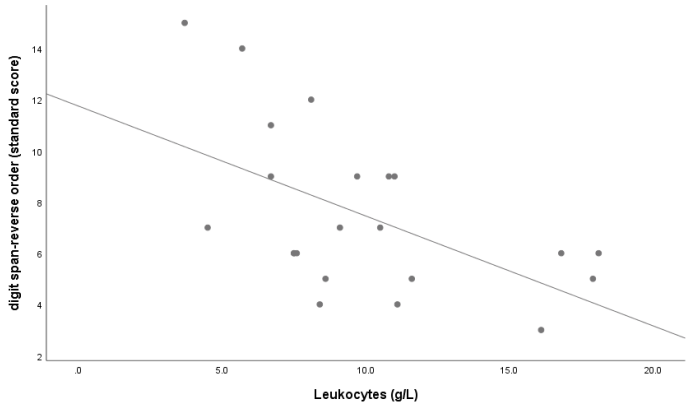


Figure 3. **Correlation between leukocytes and working memory.**

There was a significant negative association between reticulocyte count and memory performances, essentially in encoding (RVLT encoding, *r=-0.862; p<0.01, N=14)* and recall (RVLT retrieval, *r=-0.656; p<0.01; N=15)* (Figure 4a and 4b). There was no memory deficit strictly speaking, but there was a reduced recall measure (less than 9/15 signs recovered in memory) in patients with reticulocyte levels above 75 G/l, which corresponds to the limit of the pathological threshold.

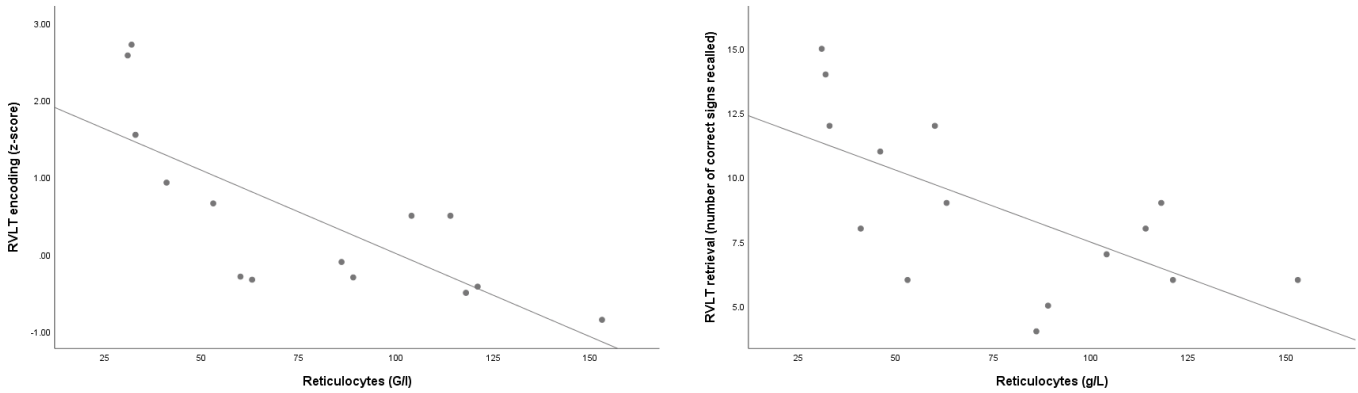


Figure 4a and 4b. **Correlations between reticulocytes and encoding and reticulocytes and recall.**

Regarding disease-related factors, children who were on hydroxyurea therapy showed significant negative correlations with general intelligence measures (GAI (r=-0.518; p<0.05; N=22), VCI (r=-0.540; p<0.01; N=28), PRI (r=-0.418; p<0.05; N=29)).

No significant correlations were observed between measures of attention and visuo-motor functioning and clinical measures.

Figure 5 summarizes the different correlations between biological data and corresponding neuropsychological measures.

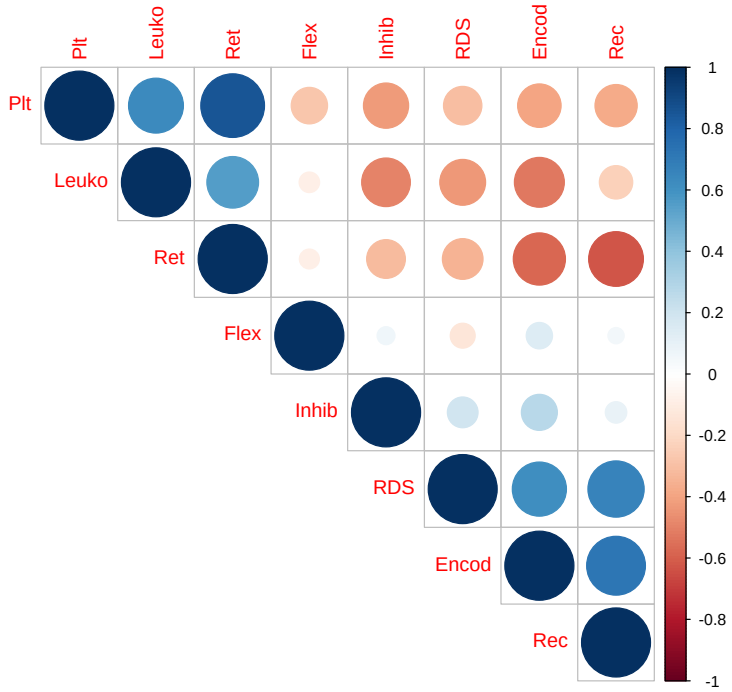


Figure 5. **Correlation matrix.**
Leuko: leukocytes. Plt: platelets. Ret: reticulocytes. Flex: mental flexibility. Inhib: inhibition. RDS: working memory. Encod: encoding. Rec: recall.

The figure is a graphical display of the correlation matrix. The positive correlations are visualized in blue and negative correlations in red color. The color intensity and the size of the circles are proportional to the correlation coefficients. Moreover, the correlation matrix was reordered for hierarchical clustering order. On the right of the graphic, the color legend shows the correlation coefficients and the corresponding colors.
As an example, it has been shown that reticulocyte count correlates with visual long-term memory, in particular with recall. Indeed, in the last column crossing with the third line, we can see a large red circle, representing an important negative correlation between these two variables.
Regarding the large dark blue circles observed diagonally, they represent two same variables correlating with each other, which explains this result of strong positive correlation of 1.

2) Biological composite measures

Index I significantly negatively correlated with mental flexibility *(r=-0.513; p>0.05; N=18)* and working memory *(r=-0.516; p>0.05; N=22)* (Figure 6a and 6b). Interestingly, there was no other significant association with inhibition measures. Moreover, all three indexes correlated negatively with visual long-term memory, especially in the processes of encoding and recalling. Among the indices, the one that correlated the best was the hemolytic index (*Index H*) (encoding: *r=-0.854; p<0.01; N=14* and recall: *r=-0.620; p<0.05; N=15*) (Figure 7a and 7b). The higher the index, the lower are the outcomes of visual learning.

There was no significant correlation between IQ measures, hydroxyurea treatment or any other clinical biological measure.

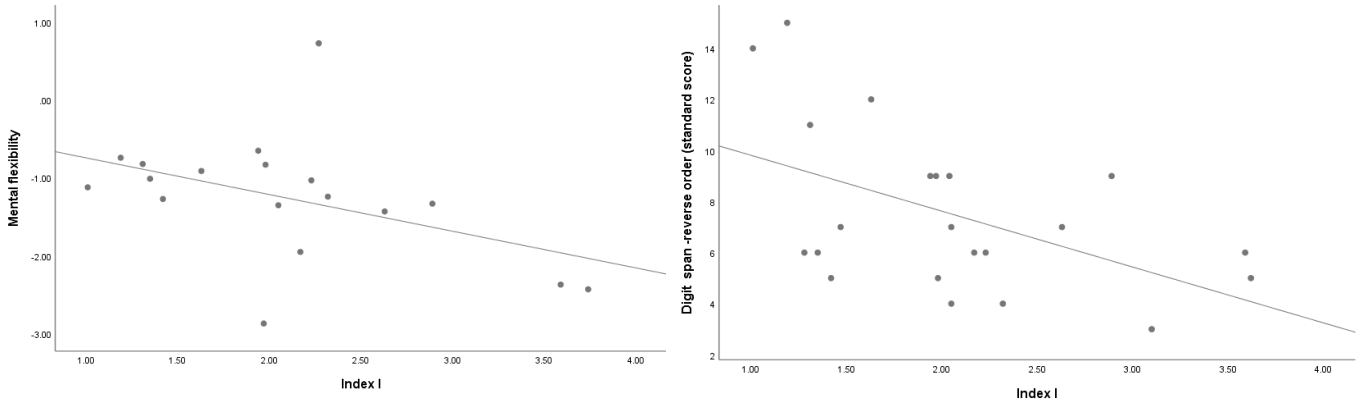


Figure 6a and 6b. **Correlations between *index I* and mental flexibility and *index I* and working memory**

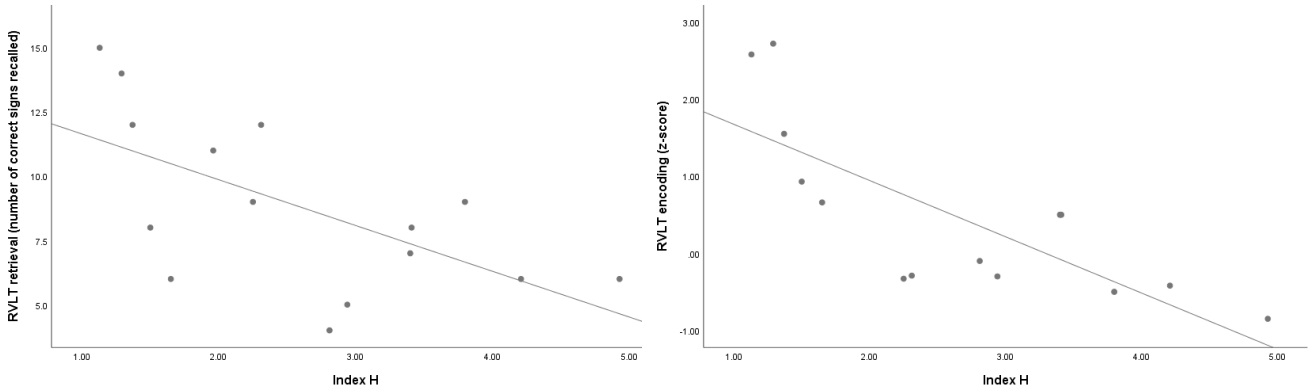


Figure 7a and 7b. **Correlations between *index H* and recall and *index H* and encoding**

DISCUSSION

In agreement with previous literature, this study demonstrates that children with SCD and without evidence of overt stroke or silent infarct suffer from mild cognitive impairments, with a broad individual variability. Indeed, in our sample, the mean IQ scales were in the low average range, mainly between 1 and 1.5 SD below the average population. There is also evidence of executive dysfunction, particularly in inhibition, mental flexibility and working memory. Furthermore, these children perform poorly in visuo-motor precision tasks. Finally, they obtain scores within the average in fine motor skills, and within the low average in

terms of attention and visual and verbal long-term memory. These specific cognitive functions were not associated with IQ measures.

Regarding correlation between neuropsychological results and biomarkers reflecting disease activity, the first three main findings of the present study are that (1) children with SCD with high platelet counts perform poorly in executive functioning, especially in mental flexibility and inhibition tasks; (2) those with high leukocyte counts show clear deficits in verbal working memory; (3) those with elevated reticulocyte counts perform worse in visual long-term memory. These results suggest that platelets and leukocytes, both mediators of inflammation, and reticulocytes, reflecting hemolysis, could be predictors of disturbances in executive functions and visual long-term memory in children and teenagers suffering from SCD. Moreover, despite our small sample size, the association between two IQ measures with age in our cohort suggests that general intelligence declines with increasing age, as showed in previous studies[13, 21, 29]. Accumulations of MRI-undetectable brain injuries over time could explain gradual cognitive impairment.

In order to increase the probability to find an association between a defined biological state and specific cognitive impairments, we developed three composite hematological indices reflecting either a hemolytic (H), or inflammatory (I) or neutral (N) state. We thus found two further significant associations, i.e. between *Index I* and executive functioning (4), particularly flexibility and verbal working memory, mirroring the first (1) and second (2) results. These outcomes suggest that inflammation may play a role in difficulties in executive functioning. Moreover, *index H* noticeably correlates with visual and visuo-spatial memory (5), suggesting that children with SCD with severe hemolysis encounter difficulties in visual and visuo-spatial learning. Hemolysis may hence play a role on cognitive functions independently from inflammation.

Previous literature suggests a strong association between reduced hemoglobin[4, 12, 36] or reduced hematocrit[15, 19, 25] and cognitive performance in individuals with SCD as well as in the general population. Reduced hemoglobin is a marker of reduced brain oxygenation, which could explain suboptimal cognitive function. For example, Hijmans and colleagues showed that lower hemoglobin levels correlated with weaker verbal short-term memory[12]. Since children with SCD are more likely to suffer from anemia, they are more vulnerable regarding neuropsychological disorders than general population. Although the present study revealed a negative correlation between elevated hematocrit and decreased inhibition skills, an artifact due very likely to a bias through a small sample size, it appears important to maintain a satisfactory hemoglobin level to avoid cognitive impairments. Blood transfusions, which improve cerebral blood flow and brain oxygenation, have actually been reported to be beneficial in preventing SCD complications[1-2, 4, 27], especially stroke in those who have had a pathological TCD[2, 5, 17]. However, blood transfusions are also fraught with several complications, such as increased viscosity, communicable infections, alloimmunization or iron overload[1-2, 4, 17, 27].
Cognitive improvement is also expected with hydroxyurea therapy, indicated when recurrent VOCs or STAs, in order to improve clinical symptomology. Surprisingly, our study

showed a negative correlation between test results and hydroxyurea administration, suggesting that children under treatment perform worse than the others. This could be explained by the fact that children receiving this therapy are more likely affected biologically and hence neuropsychologically. One could also suspect a deleterious effect of hydroxyurea itself on cognition. A recent study evaluated the influence of blood transfusion and hydroxyurea treatment versus no treatment on cognitive function[37] showing no significant differences. Future studies should evaluate the benefits of blood transfusion and hydroxyurea therapy on cognitive functions in children with SCD without brain lesions with a prospective and longitudinal design.

Although we hypothesized to find an association between HbF or hemoglobin and specific neuropsychological testing results, the present study failed to reveal any significant correlations. A sufficiently high (mean= 88.27g/L) hemoglobin level in our cohort, could explain the absence of anemia related deficits. Regarding HbF, probably no association emerged because of the diversity of haplotypes represented in our sample and its small size.

Children with SCD who present silent infarcts on MRI are more likely to suffer impairment in executive functions and attention due to the frequent frontal lobe location of silent infarcts[4, 18, 20, 27]. Most studies have focused on sustained attention, mental flexibility and working memory leaving aside other specific measures of attention and executive functioning such as planning or inhibition. Regarding correlation with biomarkers, one study demonstrated anemia and low HbF levels were related to deficits in executive functioning[4]. Similarly to another study of children without overt stroke or silent CVAs[32], we found impairment in executive functioning in our cohort. A broader range of executive functions was evaluated, allowing to highlight deficits in mental flexibility, working memory and particularly in inhibition skills. Our finding of a correlation between higher platelet and leukocyte counts (1 and 2), as well as a more elevated *index I* (4), and weaker executive skills is interesting but not easy to explain. It will have to be confirmed by further studies.
Attention deficits were also found in our patients. However, the present study failed to show any significant correlation between attention measures and clinical or biological parameters. This may be explained by the small sample size and age heterogeneity of our cohort.

Regarding the third (3) and fifth (5) results, high level of reticulocytes reflects hemolysis and anemia. The correlation of higher levels of reticulocyte count and *Index H* with weaker long-term visual memory may suggest that hemolysis and anemia may alter memory performances. However, no significant deficits in terms of memory were found. This could again be an artifact. Nonetheless, several studies reported that hemolysis is a critical element of SCD severity[3, 38-40] and plays a role in the development of SCD complications, including pulmonary hypertension, priapism, leg ulcers and cerebrovascular disease[38-40]. These complications are partly the result of nitric oxide deficiency, mediated by increased hemolysis, which contributes to vasculopathy[3, 38-39]. Memory deficits are present in children after overt and silent strokes[41], but less is known about children with SCD who do not have any detectable brain damage. Iampietro and colleagues[7] gathered several studies focusing on episodic memory. They reported that in animal and human studies, the hippocampus,

center of learning and memory, is particularly vulnerable to hypoxia and systemic inflammation[7] and that this vulnerability has also been illustrated in studies of human adults without SCD, in the context of cardiac arrest, sleep apnea or high altitude exposure[7]. Animal models of anemic hypoxia have shown an association between low hemoglobin levels and reduced hippocampal metabolism[7], suggesting that learning and memory may indeed be negatively influenced by hypoxia and anemia, which could also explain the results of the present study. Here too, research is needed in order to verify and clarify our hypothesis.

There are several important limitations to this study. First, the small sample size, which was not representative of the entire SCD population, limited the power of the analyses. Second, since it was a retrospective study, we encountered missing biological and neuropsychological data. For example, only 15 of the 29 children (51.72%) had a brain MRI, preventing from being sure that none had a silent infarct. Moreover, the clinical data collected did not always correspond to the time of the neuropsychological examination. Third, we cannot be sure the studied patients had only SCD related cognitive impairment because factors such as chronic disease, genetic background other than SCD or socio-economic status may also contribute to mild cognitive impairments. Fourth, there no matched control group. Fifth, the newly created composite biological indices are not validated yet, limiting the interpretation of our analyses.

A way to strengthen future research would be to conduct prospective studies on a larger patient cohort, taking into account the clinical parameters used here, as well as psychosocial data. This cohort should be followed longitudinally, each patient being its own control, or be compared to healthy matched controls for age, sex, ethnicity and socio-economic status. This could allow early screening of at-risk patients and their specific problems, who may benefit from early neurocognitive remediation programs and/or medical treatment. Additionally, in a longitudinal setting, developmental-related aspects of cognitive functioning and disease progression in children suffering from SCD could be investigated. It would be important to longitudinally follow IQ, executive functioning, attention, memory and visuo-motor skills. As for the biological parameters, it would be interesting to survey hemoglobin, hematocrit, leukocytes, platelets, reticulocytes, LDH and bilirubin, which are parameters reflecting anemia, hemolysis and inflammation, three main pathophysiological processes in SCD.

To conclude, our findings confirm that, children and adolescents suffering from SCD evidence of overt or silent stroke are at risk to present mild cognitive deficits in specific domains. Preliminary results show that hematological markers, principally platelets and leukocytes, reflecting inflammation, and reticulocytes, reflecting hemolysis, correlate respectively with executive functioning and visual long-term memory. This study emphasizes the importance of performing early neurocognitive evaluations in the management of these patients, in order to detect those at risk for cognitive difficulties and to provide them with the best care.

Our results also suggest that pathophysiological processes, which cannot be detected by current imaging techniques, contribute to cognitive impairment in individuals suffering from SCD. In order to better understand these processes and modify the course of the disease in time, further research is needed reliably identify sets of biological markers that best correlate with cognitive functions.

REFERENCES

1. David C. Rees, Thomas N. Williams and Mark T. Gladwin. Sickle cell disease. *Lancet*, 2010, 376: 2018-2031.

2. Kenneth I. Ataga and Jonathan Stocker. The trials and hopes for drug development in sickle cell disease. *British Journal of Haematology*, 2015, 170: 768-780.

3. Sanjay Tewari et al. Environmental determinants of severity in sickle cell anemia. *Haematologica*, 2015, 100(9): 1108-1116.

4. N. Ruffieux et al. Association between biological markers of sickle cell disease and cognitive functioning amongst Cameroonian children. *Child Neuropsychology: a journal on normal and abnormal development in childhood and adolescence*, 2013, 19(2): 143-160.

5. Barbara P. Yawn et al. Management of sickle cell disease, Summary if the 2014 Evidence-Based Report by Expert Panel Members. *JAMA*, 2014, 312(10): 1033-1048.

6. Mary C. Kral, Ronald T. Brown and George W. Hynd. Neuropsychological aspects of pediatric sickle cell disease. *Neuropsychology Review*, 2001, 11(4): 179-96.

7. Mary Iampietro, Tania Giovannetti and Reem Tarazi. Hypoxia and inflammation in children with sickle cell disease: implications for hippocampal functioning and episodic memory. *Neuropsychological Review*, 2014, 24: 252-265.

8. Ronald T. Brown, F. Daniel Armstrong and James R. Eckman. Neurocognitive aspects of pediatric sickle cell disease. *Journal of Learning Disabilities*, 1993, 26(1): 33-45.

9. Milena M. Magalhães et al. Comparative study of sickle cell anemia and hemoglobin SC disease: clinical characterization, laborytory biomarkers and genetic profiles. *BMC Hematology*, 2017, 17: 15.

10. Vivien A. Sheehan et al. Genetic modifiers of sickle cell anemia in the BABY HUG cohort : influence on laboratory and clinical phenotypes. *American Journal of Hematology*, 2013, 88: 571-576.

11. Sarah Helps et al. Psychosocial and neurocognitive aspects of Sickle Cell Disease. *Child and Adolescent Mental Health*, 2003, 8(1): 11–17.

12. Channa T. Hijmans et al. Neurocognitive deficits in children with sickle cell disease are associated with the severity of anemia. *Pediatric Blood & Cancer*, 2011, 57(2): 297–302.

13. Winfred Wang et al. Neuropsychologic performance in school-aged children with sickle cell disease: A report from the Cooperative Study of Sickle Cell Disease. *The Journal of Pediatrics*, 2001, 139: 391–397.

14. John J. Strouse et al. Inverse correlation between cerebral blood flow measured by continuous arterial spin-labeling (CASL) MRI and neurocognitive function in children with sickle cell anemia (SCA). *Blood*, 2006, 108(1): 379-381.

15. Françoise Bernaudin et al. Multicenter prospective study of children with sickle cell disease: radiographic and psychometric correlation. *Journal of Child Neurology*, 2000, 15(5): 333-343.

16. Nadia Scantlebury et al. White matter integrity and core cognitive function in children diagnosed with sickle cell disease. *Journal of Pediatric Hematology/Oncology*, 2011, 33(3): 163-71.

17. Reem A. Tarazi et al. Neuropsychological functioning in preschool-age children with Sickle Cell Disease: the role of illness-related and psychosocial factors. *Child Neuropsychology: a journal on normal and abnormal development in childhood and adolescence*, 2007, 13(2): 155-172.

18. Jeffrey Schatz et al. Poor school and cognitive functioning with silent cerebral infarcts and sickle cell disease. *Neurology*, 2001, 56(8): 1109-1111.

19. Jeffrey Schatz et al. Language processing deficits in Sickle Cell Disease in young school-age children. *Developmental Neuropsychology*, 2009, 34(1): 122-136.

20. Ronald T. Brown et al. Neurocognitive functioning and Magnetic Resonance Imaging in children with sickle cell disease. *Journal of Pediatric Psychology*, 2000, 25(7): 503-513.

21. Elliott P. Vichinsky et al. Neuropsychological dysfunction and neuroimaging abnormalities in neurologically intact adults with sickle cell anemia. *JAMA*, 2010, 303(18): 1823-1831.

22. Christopher L. Edwards et al. Neuropsychological assessment, neuroimaging, and neuropsychiatric evaluation in pediatric and adult patients with sickle cell disease (SCD). *Neuropsychiatric Disease and Treatment*, 2007, 3(6): 705–709.

23. Jeffrey Schatz et al. EXAMINER Executive function battery and neurologic morbidity in pediatric sickle cell disease. *Journal of the International Neuropsychological Society,* 2014, 20: 29-40.

24. Mireille J Bakker et al. Cerebrovascular function and cognition in childhood: a systematic review of transcranial Doppler studies. *BMC Neurology,* 2014, 14: 43.

25. R. Grant Steen et al. Cognitive impairment in children with hemoglobin SS Sickle Cell Disease: relationship to MR Imaging findings and hematocrit. *AJNR Am J Neuroradiol,* 2003, 24: 382–389.

26. Andrea V. Swift et al. Neuropsychologic impairment in children with Sickle Cell Anemia. *American Academy of Pediatrics,* 1989, 84(6): 1077-1085.

27. Eve Puffer, Jeffrey Schatz and Carla W. Roberts. The association of oral hydroxyurea therapy with improved cognitive functioning in sickle cell disease. *Child Neuropsychology,* 2007, 13: 142-154.

28. Channa T. Hijmans et al. Neurocognitive deficits in children with Sickle Cell Disease: a comprehensive profile. *Pediatr Blood Cancer,* 2011, 56: 783–788.

29. Jeffrey Schatz et al. Cognitive functioning in children with Sickle Cell Disease: a meta-analysis. *Journal of Pediatric Psychology,* 2002, 27(8): 739–748.

30. Robert B. Noll et al. Neuropsychological functioning of youths with Sickle Cell Disease: comparison with non-chronically ill peers. *Journal of Pediatric Psychology,* 2001, 26(2): 69–78.

31. Alexandra M. Hogan et al. An exploratory study of physiological correlates of neurodevelopmental delay in infants with sickle cell anemia. *British Journal of Haematology,* 2006, 132: 99-107.

32. Matthew J. Hollocks et al. Nocturnal oxygen desaturation and disordered sleep as a potential factor in executive dysfunction in sickle cell anemia. *Journal of the International Neuropsychological Society*, 2012, 18: 168-173.

33. Robert J. Thompson et al. Neurocognitive development of young children with Sickle Cell Disease through three years of age. *Journal of Pediatric Psychology*, 2002, 27(3): 235–244.

34. R. Grant Steen et al. Cognitive deficits in children with Sickle Cell Disease. *Journal of Child Neurology,* 2005, 20(2): 102—107.

35. Jamie M. Kawadler and al. Intelligence quotient in paediatric sickle cell disease: a systematic review and meta-analysis. *Developmental Medecine & Child Neurology*, 2016, 58: 672-679.

36. Brian Daly, Mary C. Kral and Reem A. Tarazi. The role of neuropsychological evaluation in pediatric sickle cell disease. *The Clinique Neuropsychologist, 2011, 25(6): 903-925.*

37. Luise Burkhardt et al. Cognitive and fine motor deficits in a pediatric sickle cell disease cohort of mixed ethnic origin. *Ann Hematol*, 2017, 96: 199-213.

38. Kato GJ, McGowan VR, Machado RF, Little JA, Taylor VJ, Morris CR, and al. Lactate dehydrogenase as a biomarker of hemolysis-associated nitric oxide resistance, priapism, leg ulceration, pulmonary hypertension and death in patients with sickle cell disease. *Blood Reviews,* 2006, 107: 2279–2285.

39. Kato GJ, Gladwin MT and Steinberg MH. Deconstructing sickle cell disease: Reappraisal of the role of hemolysis in the development of clinical subphenotypes. *Blood Reviews,* 2007, 21: 37–47.

40. Kato GJ, Hebbel RP, Steinberg MH, and Gladwin MT. Vasculopathy in Sickle Cell Disease: Biology, Pathophysiology, Genetics, Translational Medicine and New Research Directions. *Am J Hematol,* 2009, 84(9): 618–625.

41. Leslie D. Berkelhammer et al. Neurocognitive sequelae of pediatric Sickle Cell Disease: A review of the literature. *Child Neuropsychology: a journal on normal and abnormal development in childhood and adolescence,* 2007, 13(2): 120-131.

42. William J. Savage et al. Evidence gaps in the management of sickle cell disease : A summary of needed research. *American Journal of Hematology,* 2015, 90(4): 273-275.

43. Kofi A. Anie. Psychological complications in sickle cell disease. *British Journal of Haematology,* 2005, 129: 723–729.

ANNEXES

Annex 1. **Studies including neuropsychological testing in children with SCD free of infarction**

AUTORS	METHODOLOGY	NEUROPSYCHOLOGICAL TESTING	CLINICAL DATA	RESULTS
Swift and al. (1989) [18]	Prospective study N= 42 (SCD=21, controls=21) Age: 7-16 years old Control group (healthy sibling pairs) No MRI/TCD was performed *May contain silent infarcts* Hydroxyurea/transfusions: unspecified	General intelligence: WISC-R: Full-scale IQ, Verbal IQ, Performance IQ Kaufman factor scores (verbal comprehension, perceptual organization, freedom from distraction) Verbal subtest scores (information, similarities, arithmetic, vocabulary, comprehension, digit span) Performance subtest scores (picture completion, picture arrangement, block design, object assembly, coding)	Educational history/socio-economic information/family information Physical and medical information (height, weight, Hb concentration, HbS %, HbF %, number of VOCs, number of hospitalizations) Social education/school progress/attendance	SCD group performed worse than control group in IQ measures (-1 SD). SCD showed cognitive deficits in most cognitive functions (-1,5 SD), except for one measure of verbal memory. No association was found between IQ and hemoglobin, % of HbS or % of HbF.
Robert B. Noll et al. (2001) [24]	Prospective study N= 62 (SCD=31, controls=31) Age: 9-16 years old Control group (matched	General intelligence: WISC-R Academic achievement (reading, spelling, arithmetic): WRAT-R Spatial/constructional		Lower total and verbal IQ scores, and attention/memory deficits in SCD group. Mean scores of cognitive functions were generally lower in the SCD group than

	control by age, sex, race) No MRI/TCD was performed *May contain silent infarcts* Hydroxyurea/transfusions: unspecified	abilities: VMI Sustained attention + impulse control: MFFT Ability to learn and memorize information: WRAML Fine motor speed & manual dexterity: Purdue Pegboard Test		controls.
Robert J. Thompson et al. (2002) [27]	Prospective study N= 89 Age: 6-36 months old No control group No MRI/TCD was performed No hydroxyurea, no blood transfusions	Bayley scales of Infant Development II: Mental Developmental Index, Psychomotor Developmental Index	Illness severity factors: SCD phenotype, hematocrit % Parenting risks: cognitive, knowledge of child development, psychological adjustment Family functioning (FES)	Decrease in cognitive functioning between 12 and 24 months old. At 24 months, deficits in cognitive functions were related to parenting risk and HbSS phenotype.
R. Grant Steen et al. (2003) [17]	Prospective study N= 49 Age: 4-19 years old No control group MRI was performed (28 normal, 21 abnormal)	General intelligence: WISC-R + WISC-III: Full-scale IQ, Verbal IQ, Performance IQ. Verbal comprehension/ Perceptual organization/ Freedom from distraction/Processing	Imaging technique: MRI Laboratory markers: Hematocrit	Children with imaging abnormalities had greater deficits in verbal IQ and verbal comprehension than children with normal MRI. Low hematocrit was associated with lower Full-Scale IQ, verbal comprehension and

	Hydroxyurea: Unspecified Possible blood transfusions.	speed: WISC-III		freedom from distractibility.
R. Grant Steen et al. (2005) [29]	Prospective study N= 108 (SCD=54, controls=54) Age: 6 months-18 years old Control group (healthy matched controls by age, sex, race) MRI was performed (30 normal, 24 abnormal) Hydroxyurea/transfusions: Unspecified	General intelligence: WISC-III: • Verbal IQ, Full-scale IQ, Performance IQ • Verbal comprehension • Perceptual organization • Freedom from distraction • Processing speed		Deficits in Full-Scale IQ, Verbal IQ, and Performance IQ in SCD group in comparison to controls. Full-Scale IQ was 12.9 points lower than controls and declined with age.
Hogan et al. (2005) [21]	Prospective study N= 28 (SCD=14, controls=14) Age: 3-12 months old Control group (healthy matched controls by age, race) No MRI was performed, TCD was performed	The Bayleys Infant Neurodevelopmental Screener (BINS)	Imaging technique: TCD Disease factors: clinical event, SCA complications Laboratory markers: Hb Oxymetria Birth weight	SCD group obtained BINS scores suggestive of higher risk of neurodevelopmental delay in comparison with controls. Between 3 and 9 months, the number of moderate–high BINS risk scores significantly augmented. At 9 months, BINS scores were positively associated with hemoglobin and negatively associated with TCD velocity.

	No hydroxyurea, no blood transfusions.			
John J. Strouse et al. (2006) [13]	Prospective study N= 24 Mean age: 8.5 +/_ 2 years old No control group MRI was performed (22 normal, 2 abnormal), TCD was performed 2 received hydroxyurea, no blood transfusions	General intelligence: Full-scale IQ, Verbal IQ, Performance IQ	Imaging technique: TCD, MRI Laboratory markers: PCV, WBC count, platelet count	Increase in CBF was correlated with IQ measures. High leukocyte count was correlated with lower Full-Scale IQ.
Tarazi et al. (2007) [39]	Prospective study N= 26 Age: 3-5 years old No control group 7 had DTC or MRI (normal) Hydroxyurea/transfusions: unspecified	General intelligence: WPPSI-III Language: WPPSI-III, DAS Immediate memory/brief attention: NEPSY, DAS Visual-spatial/visual-construction: WPPSI-III Motor/visual-motor: NEPSY, Purdue Pegboard Test	Disease factors: Hb, number of hospitalizations, number of documented pain episodes Psychosocial risk factors: mother's education, family income, FES, PIP (measure of parenting stress: communication, emotional functioning, medical care, role function)	Mean Full-Scale IQ for SCD group was 89. Performances on memory and brief attention were greater than other cognitive functions. Socioeconomic status was correlated with deficits in most cognitive tests. Number of children living at home and parental stress levels were negatively correlated with motor/visuo-motor. Good school attendance was positively correlated with language.
Schatz et al.	Prospective study	Language: TOLD-P:3	Laboratory markers:	High-risk SCD presented deficits in the 3

(2009) [20]	N= 108 (high-risk SCD=33, low-risk SCD=21, controls=54) Age: 5-7 years old Control group (healthy matched control by age, sex, ethnicity and family income) No MRI/DTC was performed *May contain silent infarcts* Hydroxyurea/transfusions: unspecified	Short-term word span/Processing speed/ Academic and pre-academic skills: WJ Visual-motor skills: WMI + KABC (Hand Movements subtest)	hematocrit	language domains.
Scantlebury et al. (2011) [31]	Retrospective study N= 25 (SCD=15, controls=10) Age: 6-17 years old Control group (healthy matched controls by age) MRI was performed (normal appearing) Hydroxyurea/transfusions: unspecified	General intelligence: WISC-III/IV + WAIS-III Working memory: WISC/WAIS (WMI) Processing speed: WISC/WAIS (PSI) Sustained visual attention: CPT-II	Imaging technique: MRI (axial FLAIR and DWI)	Increases in apparent diffusion coefficient and deficits in processing speed and verbal working memory in SCD group without visible lesion Deficits in working memory and processing speed. Microstructure of the right frontal lobe and cerebellum correlated with processing speed.
Hollocks et al.	Prospective study	General intelligence: WASI: Full-scale IQ	Polysomnography	Mean IQ of SCD sample was 1 SD below

(2012) [22] Prospective	N= 10 Age: 8-16 years old No control group MRI was performed (9 normal, 1 abnormal), TCD was performed (normal) Hydroxyurea/transfusions: unspecified	Executive functioning: D-KEFS, BRIEF		population average. Falls of hemoglobin oxygen saturation was correlated with cognitive deficits. Deficits in executive functions.

WISC (-R): Wechsler Intelligence Scale for Children (-Revised). Hb: Hemoglobin. HbF: fetal hemoglobin. WRAT-R: Wide Range Achievement Test-Revised. VMI: The Beery Developmental Test of Visual-Motor Integration-3rd revision. MFFT: Matching Familiar Figures Test. WRAML: Wide Range Assessment of Memory and Learning. FES: Family Environment Scale. TCD: Transcranial Doppler. MRI: magnetic resonance imaging. SCA: sickle-cell anemia. PCV: packed-cell volume. WBC: white blood cell. WPPSI: Wechsler Preschool and Primary Scale Intelligence. DAS: Differential Abilities Scales. TOLD-P-3: Test of Language Development-Primary, 3rd edition. WJ(-R): Woodcock-Johnson (Revised) Tests. WMI: Working memory index. KABC: Kaufman Assessment Battery for Children. WAIS: Wechsler Adult Intelligence Scale. CPT: Conners Continuous Performance Test. DWI: Diffusion-weighted imaging. WASI: Wechsler Abbreviated Scale of Intelligence. D-KEFS: Delis-Kaplan Function System. BRIEF: Behavior Rating Inventory of Executive Functions.

This table shows the neuropsychological tests used in children suffering from SCD to assess several cognitive functions. In the fourth column are listed different clinical data used to correlate the neurocognitive functions.

Annex 2. **Spearman's rho correlations between neuropsychological measures and clinical data**

			IAG	ICV	IRP	Flex	Inhib	RDS	RVLT encoding	RVLT recalling
Spearman's rho	**Hydroxyurea**	Correlation Coefficient	-.518*	-.540**	-.418*	.187	-.033	.147	.151	.176
		Sig. (2-tailed)	.013	.003	.024	.458	.897	.513	.606	.531
		N	22	28	29	18	18	22	14	15
	Age	Correlation Coefficient	-.447*	-.392*	-.298	.148	-.094	.237	.345	.675**
		Sig. (2-tailed)	.037	.039	.117	.558	.711	.288	.227	.006
		N	22	28	29	18	18	22	14	15
	Platelets (g/L)	Correlation Coefficient	-.247	-.041	-.066	-.761**	-.525*	-.515	-.772**	-.634*
		Sig. (2-tailed)	.267	.836	.733	.000	.025	0.14	.001	0.15
		N	22	28	29	18	18	22	14	15
	Leukocytes (g/L)	Correlation Coefficient	.007	.174	.051	-.303	-.394	-.561**	-.791**	-.390
		Sig. (2-tailed)	.975	.376	.791	.222	.106	.007	.001	.168
		N	22	28	29	18	18	22	14	15
	Reticulocytes (%)	Correlation Coefficient	-.021	.166	.065	-.408	-.207	-.359	-.862**	-.656**
		Sig. (2-tailed)	.925	.397	.737	.093	.409	.101	.000	.008
		N	22	28	29	18	18	22	14	15
	Index N	Correlation Coefficient	-.011	.158	0.29	-.497*	-.263	-.372	-.852**	-.620*
		Sig. (2-tailed)	.960	.421	.909	.036	.291	.089	.000	.014
		N	22	28	29	18	18	22	14	15
	Index I	Correlation Coefficient	-.063	.145	.015	-.513*	-.335	-.516*	-.838*	-.501
		Sig. (2-tailed)	.781	.462	.938	.030	.174	.014	.000	.057
		N	22	28	29	18	18	22	14	15

Index H	Correlation Coefficient	.001	.166	.063	-.431	-.203	-.374	-.854**	-.620*
	Sig. (2-tailed)	.997	.744	.662	.074	.418	.113	.000	.014
	N	22	28	29	18	18	22	14	15

**. Correlation is significant at the 0.01 level (2-tailed). *. Correlation is significant at the 0.05 level (2-tailed).