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# Brain injury pathophysiology study by a multimodal approach in children with sickle cell anemia with no intra or extra cranial arteriopathy

Valentine Brousse,<sup>1,2,3,4</sup> Corinne Pondarre,<sup>5,6</sup> Manuelle Kossorotoff,<sup>7</sup> Cecile Arnaud,<sup>5</sup> Annie Kamdem,<sup>5</sup> Mariane de Montalembert,<sup>1,2</sup> Benedicte Boutonnat-Faucher,<sup>1</sup> Slimane Allali,<sup>1,2,8</sup> H el ene Bourdeau,<sup>9</sup> Keyne Charlot,<sup>10</sup> Sebastien Bertil,<sup>11</sup> Lydie da Costa,<sup>2,9,12,13</sup> Philippe Connes,<sup>2,14,15#</sup> David Gr event<sup>16#</sup> and Suzanne Verlhac<sup>17,18#</sup>

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<sup>1</sup>AP-HP, H pital Necker Enfants Malades, Service de P diatrie G n rale et Maladies infectieuses, Paris; <sup>2</sup>LABEX GR-Ex, Paris; <sup>3</sup>Institut National de la Transfusion Sanguine, UMR\_S1134, INSERM, Paris; <sup>4</sup>AP-HP, H pital Universitaire Robert Debr , Immuno-H matologie, Paris; <sup>5</sup>Centre Intercommunal de Cr teil, Service de P diatrie, Cr teil; <sup>6</sup>Paris XII University, INSERM U955, Cr teil; <sup>7</sup>AP-HP, H pital Necker Enfants Malades, Service de Neurologie P diatrique, Paris; <sup>8</sup>Laboratory of Cellular and Molecular Mechanisms of Hematological Disorders and Therapeutical Implications, Paris Descartes – Sorbonne Paris Cit  University, Imagine Institute, INSERM U1163, Paris; <sup>9</sup>AP-HP, H pital Robert Debr , Service d’H matologie Biologique, Paris; <sup>10</sup>Unit  de Physiologie des Exercices et Activit s en Conditions Extr mes, D partement Environnements Op rationnels Institut de Recherche Biom dicale des Arm es, Br tigny-sur-Orge; <sup>11</sup>AP-HP, Service d’H matologie Biologique, H pital Europ en Georges Pompidou, Paris; <sup>12</sup>Service d’H matologie Biologique, Universit  de Paris, Paris; <sup>13</sup>Hematim UR 4666, Amiens; <sup>14</sup>Laboratoire LIBM EA7424, Equipe « Biologie Vasculaire et du Globule Rouge », Universit  Claude Bernard Lyon 1, Villeurbanne; <sup>15</sup>Institut Universitaire de France, Paris; <sup>16</sup>AP-HP, H pital Necker Enfants Malades, Service d’Imagerie P diatrique, Paris; <sup>17</sup>Centre Hospitalier Intercommunal de Cr teil, Service d’Imagerie M dicale, Cr teil and <sup>18</sup>AP-HP, H pital Universitaire Robert Debr , Service d’Imagerie M dicale, Paris, France

#PH, DG and SV contributed equally as co-senior authors

## ABSTRACT

Despite its high prevalence in children with sickle cell anemia (SCA), the pathophysiology of silent cerebral infarcts (SCI) remains elusive. The main objective of this study was to explore the respective roles of major determinants of brain perfusion in SCA children with no past or current history of intracranial or extracranial vasculopathy. We used a multimodal approach based notably on perfusion imaging arterial spin labeling (ASL) magnetic resonance imaging (MRI) and near infra-red spectroscopy (NIRS), as well as biomarkers reflecting blood rheology and endothelial activation. Out of 59 SCA patients (mean age 11.4±3.9 yrs), eight (13%) had a total of 12 SCI. Children with SCI had a distinctive profile characterized by decreased blood pressure, impaired blood rheology, increased P-selectin levels, and marked anemia. Although ASL perfusion and oximetry values did not differ between groups, comparison of biological and clinical parameters according to the level of perfusion categorized in terciles showed an independent association between high perfusion and increased sP-selectin, decreased red blood cell deformability, low hemoglobin F level, increased blood viscosity and no  $\alpha$ -thalassemia deletion. NIRS measurements did not yield additional novel results. Altogether, these findings argue for early MRI detection of SCI in children with no identified vasculopathy and suggest a potential role for ASL as an additional screening tool. Early treatment targeting hemolysis, anemia and endothelial dysfunction should reduce the risk of this under diagnosed and serious complication.

## Correspondence:

VALENTINE BROUSSE  
valentine.brousse@gmail.com

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## Introduction

Brain injury is a major complication in sickle cell anemia (SCA) patients because of its high prevalence and devastating consequences. Historically, overt stroke used to be the most distressing clinical complication in childhood but its prevalence has dropped from 10% to 1-3% in high-income countries since the implementation of a preventive screening strategy by transcranial Doppler (TCD) coupled with chronic transfusion therapy in case of abnormal results.<sup>1,2,3,4</sup> In sharp contrast, the contribution of silent cerebral infarcts (SCI) to global neurovascular burden has emerged, following the improved ability to detect subclinical lesions by magnetic resonance imaging (MRI). SCI are found in up to 37% of SCA children before the age of 14 years, can be detected as early as 1 year old and their prevalence increases with age.<sup>3,5,6</sup> In addition, SCI are frequently associated with neurocognitive impairment.<sup>7,8</sup> While overt ischemic stroke mainly occurs in the arterial territory of internal carotid or middle cerebral artery (usually with an associated stenosis or occlusion), silent infarcts tend to occur in the border zone areas of the brain, mainly in the deep white matter, including in patients without large vessel arteriopathy. This observation highly suggests that SCI may be related to alterations of perfusion and oxygenation.<sup>9</sup> However, no clear pathophysiological process in SCI genesis has been demonstrated thus far, although anemia, both chronic and acute, seems to be a significant contributor.<sup>6</sup> Conventional MRI shows brain injury in a pattern suggestive of hemodynamic compromise but it does not directly assess cerebral hemodynamics. By contrast, perfusion analysis by arterial spin labeling (ASL) allows to better evaluate the cerebral hemodynamic risk at a regional level, in a non-invasive manner, and may serve as a screening tool to identify children at risk of SCI.

Blood rheology is a key determinant of blood flow, vascular resistance and tissue perfusion.<sup>10</sup> Increased blood viscosity in both adults and children with SCA has been associated with a risk of frequent vaso-occlusive crises. Moreover, SCA individuals with the greatest reduction in red blood cell (RBC) deformability would be at higher risk for developing leg ulcers, glomerulopathy and priapism.<sup>11,12,13</sup> These studies also reported increased RBC aggregates strength in SCA patients with glomerulopathy or frequent priapism. Although a contribution of blood rheology has been speculated in overt stroke in SCA,<sup>14</sup> there is no study focusing on SCI and blood rheology in this disease in the literature. Surprisingly, a study by Brown *et al.*<sup>15</sup> performed in patients with paraproteinemia or leukemia observed no association between cerebral blood flow and blood viscosity. Instead, the authors reported an inverse relationship between cerebral blood flow and arterial oxygen content, showing that regulatory mechanisms can maintain cerebral oxygen transport despite increased blood viscosity. The same negative association has been reported in children with SCA.<sup>16</sup> However, the associations between brain oxygenation, cerebral blood flow in the different brain areas and blood rheology have never been investigated, and more particularly in the context of SCI. In order to better decipher the mechanisms involved in subclinical cerebral injury in children with SCA, we sought to explore the respective roles of hemolysis, abnormal blood rheology, cerebral oxygen supply and brain perfusion characteristics in children with no past or current history of intracranial or extracranial vasculopathy. We used a multimodal approach in a multi-centric pilot study where we i)

compared several biomarkers (markers of endothelial injury and hemorheology), level of brain oxygenation (near infra-red spectroscopy [NIRS]) and perfusion imaging (TCD and arterial spin labeling [ASL] MRI) between children with newly discovered and without SCI and ii) tested the association between these parameters.

## Methods

Consecutive patients from two French referral centers for sickle cell disease (University Hospital Necker-Enfants Malades and Centre Hospitalier Intercommunal de Creteil) were screened during routine visits according to the following inclusion criteria: i) SS or S- $\beta^0$ thalassemia genotype; ii) no past history of abnormal or conditional transcranial and extracranial TCD; iii) at steady state (> 3 months from any vaso-occlusive event, transfusion or infection); iv) age between 5 and 17 years. The study was offered to all children meeting the inclusion criteria and regularly followed up since neonatal screening in the participating centers, between March 2015 and July 2016, with a target of 60 children (based on the expected number of SCA patients meeting the inclusion criteria in these centers and the pilot design of the study). Treatment by hydroxyurea was not an exclusion criterion, but chronic transfusion was. After written informed consent was obtained, a visit during which all investigations were performed was scheduled. The protocol was approved by the ethics committee "Comité pour la Protection des Personnes Ile de France III" (014-A01575-42) and registered in clinicaltrials.gov. Identifier: NCT 02909283.

Clinical parameters as well as relevant past medical events were collected and are available in the *Online Supplementary Appendix*. Biological parameters were measured for each patient: routine hematological and blood rheological parameters, and markers of endothelial activation (see the *Online Supplementary Appendix*).

TCD imaging (TCDI) was performed using a LOGIQ E9 XDclear 2.0 ultrasound system (GE Healthcare, Milwaukee, WI, USA) at Necker – Enfants Malades and an Acuson S 2000 ultrasound system (Siemens Healthineers, Erlangen, Germany) at Centre Hospitalier Intercommunal de Creteil.

MR imaging was performed with a GE Signa HDxt 1.5-T system (GE Medical Systems, Milwaukee, WI, USA) and a 12-channel head-neck-spine coil in non-sedated children.

SCI were defined on MRI as hypersignals of cerebral parenchyma of at least 3 mm on T2 FLAIR sequence. Details on MRI sequences and image analysis including calculation of hypersignal volume are available in the *Online Supplement Appendix*.

Cerebral blood flow was measured using a three-dimensional pseudo-continuous arterial spin labeling (pCASL) sequence (repetition time msec/echo time msec, 4,428/10.5; postlabeling delay =1,025 msec; 80 axial partitions; field of view, 240x240x4 mm; acquisition matrix, eight spiral arms in each three-dimensional partition with 512 points per arm; flip angle, 155°; acquisition time, 4 minutes 17 seconds).

Transcranial NIRS was performed using a dual-channel absolute cerebral oximeter FORE-SIGHT® (Branford, CT, USA).

## Statistics

We first compared the different measured parameters in patients with and without SCI using an unpaired student *t* test.

The cohort was divided into terciles of ASL values: hypo-perfusion (Low), normal perfusion (Middle) and hyper-perfusion (High) groups. The three groups were then compared using a one-way ANOVA with Tukey *post hoc* test. A  $\chi^2$  test was used for the qualitative analyses. The significance level was defined as  $P < 0.05$  (SPSS, v. 20, IBM SPSS Statistics, Chicago, IL).

## Results

### General characteristics of the population

Fifty-nine SCA patients (mean age  $11.4 \pm 3.9$  yrs) at steady state were enrolled, of which 34 (57.6%) were treated with hydroxyurea (HU). Patients main clinical and biological characteristics are summarized in Table 1.

### Transcranial Doppler and magnetic resonance imaging analysis

At the time of assessment, all TCD velocities were within normal ranges ( $<170$  cm/s) and there were no arte-

rial abnormalities on MRA analysis. Twelve SCI (3-5 mm:  $n=8$ ; 5-15 mm:  $n=4$ ,  $>15$  mm:  $n=0$ ) were observed in eight of 59 (13%) children. The median volume of SCI was 41,57 mm (range, 15,8-362 mm). SCI were located in the right anterior border zone in six patients. Five patients had a single lesion, while three patients had  $>1$  lesion, including two with bilateral lesions.

### Analysis of patients with silent cerebral infarcts

Children with SCI had a lower past vaso-occlusive crisis (VOC) rate compared to children with no SCI (Table 1). Despite a comparable frequency of HU treatment in both

Table 1. General characteristics of the population.

	All (n=59)	no-SCI (n = 51)	SCI (n = 8)	P
Sex (M/F)	20/39	16/35	4/4	0.301
Age (yrs)	$11.4 \pm 3.9$	$11.4 \pm 3.7$	$11.6 \pm 4.9$	0.865
Ongoing HU ttt at inclusion (N; %)	33; 56	30; 59	3; 38	0.259
Time since HU initiation (yrs)	$3.1 \pm 3.1$	$3.2 \pm 3.2$	$1.6 \pm 1.3$	0.170
Daily dose (mg/kg/day) mean (SD)	$23 \pm 4.6$	$24.1 \pm 3.3$	$12.8 \pm 0.5$	0.001
Age at beginning of HU initiation (yrs)	$8.3 \pm 4.3$	$8.2 \pm 3.9$	$9.0 \pm 5.4$	0.170
Alpha thalassemia (N; %)	24; 41	23; 46	1; 13	0.074
G6PD deficiency (N; %)	4; 7	4; 8	0; 0	0.412
VOC rate (events/yr)	$0.58 \pm 0.58$	$0.65 \pm 0.60$	$0.17 \pm 0.20$	0.001
ACS rate (events/yr)	$0.08 \pm 0.10$	$0.09 \pm 0.10$	$0.03 \pm 0.05$	0.157
Transfusion history (%)	23.7 %	23%	25%	0.96
Mean number of transfusions (n, SD)	8 (11)	8 (10)	12 (17)	0.16
SpO <sub>2</sub> (%)	$99.0 \pm 1.1$	$99.1 \pm 1.0$	$98.6 \pm 2.0$	0.599
DBP (mmHg)	$68 \pm 11$	$69 \pm 11$	$61 \pm 7$	0.047
SBP (mmHg)	$110 \pm 11$	$111 \pm 11$	$106 \pm 10$	0.283
MAP (mmHg)	$82 \pm 9$	$83 \pm 9$	$76 \pm 8$	0.047
Pulse Pressure (mmHg)	$42 \pm 12$	$42 \pm 12$	$45 \pm 6$	0.437
Hb (g/dL)	$8.8 \pm 1.2$	$8.9 \pm 1.1$	$8.2 \pm 1.1$	0.047
Hct (%)	$25.0 \pm 3.3$	$25.3 \pm 3.2$	$23.0 \pm 3.0$	0.025
MCV (fl)	$82 \pm 12$	$83 \pm 12$	$77 \pm 10$	0.186
MCHC (g/dL)	$35.0 \pm 1.5$	$34.9 \pm 1.5$	$35.8 \pm 0.9$	0.037
Reticulocyte count ( $10^9/L$ )	$225 \pm 102$	$224 \pm 105$	$229 \pm 88$	0.91
Platelets ( $10^9/L$ )	$343 \pm 154$	$331 \pm 141$	$418 \pm 220$	0.141
WBC count ( $10^9/L$ )	$8.98 \pm 3.61$	$8.78 \pm 3.64$	$10.29 \pm 3.36$	0.277
HbF (%)	$16.0 \pm 10.1$	$16.0 \pm 10.4$	$16.1 \pm 8.2$	0.986
ASAT UI/L	$49 \pm 16$	$48 \pm 16$	$52 \pm 13$	0.548
LDH UI/L	$484 \pm 164$	$485 \pm 173$	$479 \pm 108$	0.938
Soluble E-selectin (ng/mL)	$73.4 \pm 29.6$	$73.2 \pm 21.7$	$74.5 \pm 39.7$	0.911
Soluble P-selectin (ng/mL)	$59.3 \pm 21.3$	$57.1 \pm 20.7$	$73.5 \pm 21.7$	0.047
CD34* cell (/mL)	$9185 \pm 6738$	$9803 \pm 6773$	$5788 \pm 6229$	0.301
RBC aggregation (%)	$52 \pm 6$	$52 \pm 7$	$51 \pm 4$	0.811
RBC disaggregation threshold ( $s^{-1}$ )	$378 \pm 201$	$359 \pm 191$	$491 \pm 236$	0.037
RBC deformability (a.u.)	$0.49 \pm 0.09$	$0.50 \pm 0.09$	$0.44 \pm 0.07$	0.025
Blood viscosity (cP)	$5.35 \pm 0.93$	$5.40 \pm 0.94$	$5.04 \pm 0.83$	0.312
Hematocrit/blood viscosity (a.u.)	$4.72 \pm 0.59$	$4.73 \pm 0.58$	$4.62 \pm 0.69$	0.627
Right ctSO <sub>2</sub> (%)	$63 \pm 10$	$63 \pm 10$	$61 \pm 9$	0.662
Left ctSO <sub>2</sub> (%)	$63 \pm 10$	$63 \pm 10$	$64 \pm 9$	0.828

M: male; F: female; SD: standard deviation; SCI: silent cerebral infarct; HU: hydroxyurea; VOC: vaso-occlusive crisis; ACS: acute chest syndrome; DBP: diastolic blood pressure; SBP: systolic blood pressure; MAP: mean arterial pressure; MCHC: mean corpuscular hemoglobin concentration; MCV: mean corpuscular volume; Hct: hemtocr; HbF: hemoglobin F; ASAT: aspartate aminotransferase; LDH: lactate dehydrogenase; RBC: red blood cell; a.u.: arbitrary units; CP: centipoise; ctSO<sub>2</sub>: cerebral tissue hemoglobin oxygen saturation; P-values are given for the comparison between the non-SCI and SCI groups.

Table 2. Cerebral blood flow values in patients with or without silent cerebral infarct.

	no-SCI (n = 51)	SCI (n = 8)	P
<b>Right hemisphere perfusion (mL/100 g/min)</b>			
ACA	116.5 ± 17.8 (112.0-121.0)	126.1 ± 17.4 (114.0-138.0)	0.162
Anterior Junctional	122.9 ± 21.1 (117.0-129.0)	123.8 ± 16.2 (113.0-135.0)	0.909
Superficial MCA	111.9 ± 17.9 (107.0-117.0)	116.5 ± 13.8 (107.0-126.0)	0.489
Caudate nucleus	93.3 ± 16.2 (88.8-97.8)	98.5 ± 16.4 (87.1-110.0)	0.406
Putamen	82.5 ± 14.2 (78.6-86.4)	90.0 ± 13.5 (80.7-99.3)	0.168
posterior Junctional	120.6 ± 23.3 (114.0-127.0)	120.5 ± 16.9 (109.0-132.0)	0.988
<b>Left hemisphere perfusion (mL/100 g/min)</b>			
ACA	116.5 ± 19.4 (111.0-122.0)	132.0 ± 23.9 (115.0-149.0)	0.117
Anterior Junctional	121.6 ± 22.1 (116.0-128.0)	122.8 ± 15.5 (112.0-134.0)	0.887
Superficial MCA	111.3 ± 18.9 (106.0-116.0)	121.0 ± 16.1 (110.0-132.0)	0.174
Caudate nucleus	94.3 ± 16.0 (89.9-98.7)	102.8 ± 14.2 (93.0-113.0)	0.165
Putamen	83.0 ± 14.4 (79.0-87.0)	93.4 ± 11.0 (85.8-101.0)	0.058
Posterior Junctional	119.9 ± 26.4 (113.0-127.0)	122.5 ± 24.3 (106.0-139.0)	0.794
<b>Posterior fossa perfusion</b>			
Right Cerebellar hemisphere	88.1 ± 8.2 (85.8-90.3)	84.1 ± 8.2 (78.4-89.8)	0.295
Left Cerebellar hemisphere	89.6 ± 16.9 (85.0-94.2)	85.4 ± 8.1 (79.8-91.0)	0.277

No significant difference in cerebral blood flow values was evidenced between the two groups. Values are given as means ± standard deviation (95% Confidence Interval); SCI: silent cerebral infarcts; MCA: middle cerebral artery; ACA: anterior cerebral artery.

Table 3. Characteristics of the population according to the level of perfusion assessed by arterial spin labeling in the right anterior cerebral artery and categorized in terciles.

	Lower CBF (N=20)	Middle CBF (N = 19)	Higher CBF (N = 20)
Age (yrs)	12.4 ± 4.2	10.5 ± 4.2	11.2 ± 3.2
HU (%)	71	56	43
α thalassemia (%)	46	29	25*
G6PD deficiency (%)	31	35	35
VOC rate (events/yr)	0.68 ± 0.53	0.47 ± 0.43	0.63 ± 0.73
ACS rate (events/yr)	0.09 ± 0.11	0.06 ± 0.08	0.09 ± 0.11
SpO <sub>2</sub> (%)	99.3 ± 1.0	99.1 ± 1.2	98.8 ± 1.1
DBP (mmHg)	70 ± 12	67 ± 11	66 ± 10
SBP (mmHg)	114 ± 12	108 ± 12	108 ± 10
MAP (mmHg)	85 ± 10	81 ± 9	80 ± 9
Hb (g/dL)	9.2 ± 1.2	8.9 ± 1.1	8.3 ± 1.0*
Hct (%)	26.3 ± 3.4	25.2 ± 3.3	23.6 ± 2.6*
MCV (fL)	82 ± 13	81 ± 11	84 ± 11
MCHC (g/dL)	35.0 ± 1.3	35.2 ± 1.0	34.9 ± 2.0
Reticulocyte count (10 <sup>9</sup> /L)	193 ± 84	229 ± 112	255 ± 105*
Platelets (10 <sup>9</sup> /L)	315 ± 123	314 ± 141	388 ± 159
White blood cells (10 <sup>9</sup> /L)	7.99 ± 3.37	9.23 ± 3.89	9.91 ± 3.51
HbF (%)	19.4 ± 11.3	18.4 ± 10.2	11.9 ± 6.5*#
ASAT UI/L	42 ± 8	50 ± 15	55 ± 18*
LDH UI/L	407 ± 126	515 ± 154	545 ± 186
CRP mg/L	5.8 ± 8.4	6.0 ± 5.0	5.4 ± 9.2
E-selectin	67.0 ± 22.3	68.5 ± 35.0	85.0 ± 28.4
P-selectin	49.3 ± 14.7	58.2 ± 21.3	68.9 ± 23.1**
RBC aggregation (%)	52 ± 6	53 ± 7	50 ± 5
RBC disaggregation threshold (s <sup>-1</sup> )	390 ± 222	399 ± 225	356 ± 174
RBC deformability (a.u.)	0.53 ± 0.06	0.51 ± 0.09	0.46 ± 0.08*#
Blood viscosity (cP)	5.75 ± 1.03	5.23 ± 0.80	5.18 ± 0.93*
Hematocrit/blood viscosity (a.u.)	4.66 ± 0.70	4.80 ± 0.40	4.67 ± 0.67
Right ctSO <sub>2</sub> (%)	64 ± 10	62 ± 9	63 ± 10
Left ctSO <sub>2</sub> (%)	64 ± 13	64 ± 8	64 ± 10

a.u.: arbitrary unit; G6PD: glucose-6-phosphate dehydrogenase; HU: hydrurea; VOC: vaso-occlusive crisis; ACS: acute chest syndrome; DBP: diastolic blood pressure; SBP: systolic blood pressure; MAP: mean arterial pressure; Hb: hemoglobin; Hct: hematocrit; MCV: mean corpuscular volume; MCHC: mean corpuscular hemoglobin concentration; ASAT: aspartate aminotransferase; LDH: lactate dehydrogenase; RBC: red blood cell; ctSO<sub>2</sub>: cerebral tissue hemoglobin oxygen saturation. Significantly different from the lower cerebral blood flow (CBF) group: \*P<0.05; \*\*P<0.01. Significantly different from the Middle CBF group: #P<0.05.

groups, significant differences in biological profiles were evidenced: children with SCI had increased plasma P-selectin level and RBC disaggregation threshold, and lower hemoglobin (Hb) level and RBC deformability. Of note, daily dose of HU was significantly lower in patients with SCI compared to those with no SCI at the time of data collection but the sample size was too small ( $n=3$  of 8) to further interpret this finding.

In addition, diastolic blood pressure (DBP) and mean arterial pressure (MAP) were lower in patients with SCI. Oximetry data (right and left cerebral oxygenation levels) showed no significant difference between patients with

and without SCI. Of note, there was no association between cerebral blood flow (CBF) values and the presence of SCI (Table 2).

#### Arterial spin labeling perfusion imaging

In the whole group of patients, CBF values showed no major asymmetry and correlated across all territories ( $r$  ranging from 0.46 to 0.91;  $P$  ranging from  $<0.01$  to  $<0.001$ ). Interestingly, comparison of patient subgroups according to CBF terciles in the arterial territories showed marked differences (see Table 3 for the right anterior cerebral artery territory, as an illustration). The group with

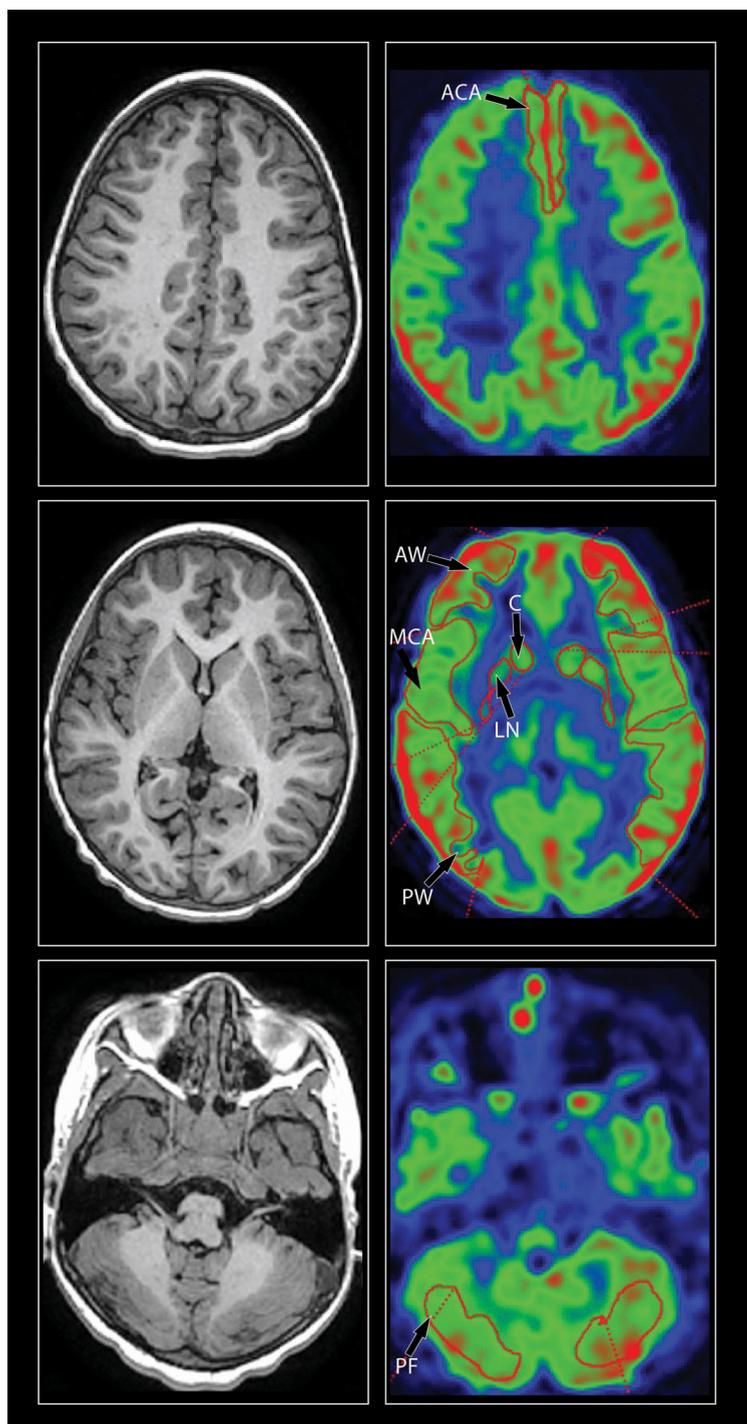


Figure 1. Regions of interest on the arterial spin labeling sequence. Left column: T1 weighted imaging. Right column: arterial spin labeling (ASL) imaging. First row: axial section tangential to the upper wall of the lateral ventricles, second row: axial section crossing the anterior and posterior white commissures (AC-PC), third row: axial section at the level of the internal auditory canals. All axial images are parallel to CA-CP. ACA: anterior cerebral artery area; AW: anterior watershed area; MCA: middle cerebral artery area; PW: posterior watershed area; C: caudate nuclei; LN: lenticulostriate nuclei; PF: posterior fossa.

higher CBF had increased markers of hemolytic anemia (lower Hb, increased aspartate aminotransferase (ASAT), lactate dehydrogenase [LDH] and reticulocyte count), lower HbF level, increased marker of endothelial activation (sP-selectin value), lower RBC deformability and blood viscosity compared to the group with lower CBF. In addition, the frequency of patients with co inherited  $\alpha$ -thalassemia was lower. An ordinal multivariate analyses was performed to test the independent associations between the perfusion level (tercile groups) and the main parameters influencing blood flow. Blood viscosity was retained for the model because it is a key determinant of blood flow.<sup>10</sup> Since blood viscosity is highly dependent on hematocrit/Hb and colinearity was indeed very strong between blood viscosity and hematocrit and Hb (variance inflation factor [VIF] = 36.6 and 37.4, respectively), hematocrit and Hb were not included in the multivariate model. Likewise, ASAT and reticulocyte count were not considered for the model as, like LDH, they reflect hemolysis. In the multivariate model, which was highly significant ( $P < 0.001$ ), all the parameters except LDH ( $P = 0.058$ ), were independently associated with the level of perfusion (sP-selectin:  $P < 0.01$ ; RBC deformability:  $P < 0.01$ ; HbF level:  $P < 0.05$ ; blood viscosity:  $P < 0.05$ ;  $\alpha$ -thalassemia:  $P < 0.05$ ).

### Oximetry measurements

In the whole population, cerebral oximetry results showed a significant positive correlation with Hb, SpO<sub>2</sub>, RBC deformability, blood viscosity and HbF and a negative correlation with markers of hemolysis (total bilirubin and LDH) as well as the RBC disaggregation threshold (Table 4). Of note, blood pressure, age, reticulocyte count, level of selectins and TCD velocities did not correlate with oximetry results. A multivariate analysis was performed for the right and left cerebral tissue hemoglobin oxygen saturation including SpO<sub>2</sub>, Hb, RBC deformability, HbF, LDH and the RBC disaggregation threshold. Hb was preferred to blood viscosity as tissue oxygenation is thought to be highly dependent on the number of RBC and Hb concentration. None of the parameters was significant ( $P = 0.38$  and  $P = 0.35$  for the right and left ctSO<sub>2</sub>, respectively), which may be explained by the fact that all are interrelated to affect brain oxygenation.

### Effect of hydroxyurea treatment

HU treatment did not significantly impact the results of neurovascular explorations in this cohort, except for sE- and sP-selectin levels that were significantly lower in children receiving treatment. Treated children received a daily dose of HU that was not a maximum tolerated dose but was a fairly high dose (23 +/- 4.6 mg/kg/day) and both groups had indeed comparable levels of Hb or HbF. Although compliance was not specifically addressed, the decreased level of selectins in treated children argues for an effect of HU on alleviating endothelial injury, in addition to its known effect on hemolytic anemia. The cross-sectional design of the study, however, does not allow to actually compare groups at baseline, i.e., before treatment initiation and precludes further interpretation. In line, the specific effect of HU in children with SCI (or the lack of, given the significantly low dose) is limited by the small sample size (n=3).

**Table 4. Correlations between cerebral oxygenation (ctSO<sub>2</sub>) and biological parameters**

	Left ctSO <sub>2</sub> (%)	Right ctSO <sub>2</sub> (%)
Age (yrs)	r = -0.12	r = -0.22
SpO <sub>2</sub> (%)	r = 0.35*	r = 0.43**
DBP (mmHg)	r = -0.08	r = 0.14
SBP (mmHg)	r = -0.09	r = -0.08
MAP (mmHg)	r = -0.09	r = 0.08
Hb (g/dL)	r = 0.48***	r = 0.53***
Hct (%)	r = 0.53***	r = 0.56***
MCV (fL)	r = 0.07	r = 0.17
MCHC (g/dL)	r = -0.26	r = -0.30*
Reticulocyte count (10 <sup>9</sup> /L)	r = -0.06	r = -0.12
Platelets (10 <sup>9</sup> /L)	r = -0.17	r = -0.14
White blood cells (10 <sup>9</sup> /L)	r = -0.26	r = -0.24
HbF (%)	r = 0.44**	r = 0.45**
ASAT UI/L	r = -0.37*	r = -0.24
LDH UI/L	r = -0.51***	r = -0.43**
CRP mg/L	r = -0.07	r = -0.24
E-selectin	r = -0.16	r = -0.20
P-selectin	r = 0.12	r = 0.07
RBC aggregation (%)	r = 0.03	r = -0.10
RBC disaggregation threshold (s-1)	r = -0.30*	r = -0.27*
RBC deformability (a.u.)	r = 0.54***	r = 0.51***
Blood viscosity (cP)	r = 0.30*	r = 0.30*
Hematocrit/blood viscosity (a.u.)	R = 0.21	R = 0.17

DBP: diastolic blood pressure; SBP: systolic blood pressure; Hb: hemoglobin; Hct: hematocrit; MCV: mean corpuscular volume; MCHC: mean corpuscular hemoglobin concentration; ASAT: aspartate aminotransferase; LDH: lactate dehydrogenase; CRP: C-reactive protein; a.u.: arbitrary units; RBC: red blood cell; ctSO<sub>2</sub>: cerebral tissue hemoglobin oxygen saturation Significant correlations: \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

### Discussion

In this study of 59 young children highly selected for no present or past history of cerebral and extra cranial vasculopathy, a prevalence of eight of 59 (13%) children with SCI was found, illustrating the residual burden of neurovascular injury in patients having received recommended follow-up. SCI predominated in the fronto-parietal white matter at the junction between the anterior and the middle cerebral artery territories, as previously described.<sup>17</sup> Expectedly, on T2 FLAIR sequence, no SCI >15 mm were found which would have presumably translated in clinically detectable symptoms. Likewise, volumes of SCI were within previously described ranges.<sup>18</sup> Consistent with the inclusion criteria of no past conditional or abnormal TCD or transient vasculopathy, there was no arterial abnormality on MRA analysis. SCI and more specifically their size, volume and localization may impact cognitive function. In this study cognitive testing was not performed and subsequently cognitive consequences related to SCI cannot be inferred. Nevertheless, the frequency of SCI in these highly selected children plead for early identification of silent lesions in order to further explore cognitive functions for early implementation of supportive learning skills. In line with recent recommendations, MRI screening, is therefore highly recommended in all children with SCA, including in those with no identified vasculopathy.<sup>19</sup>

Given the absence of large vessel vasculopathy in these children, SCI are presumably unrelated to ischemia occurring downstream of a large vessel stenosis. SCI may nevertheless share common risk factors with large vessel vasculopathy and result from impaired perfusion. Indeed, we found distinctive clinical and biological features in children with SCI. In line with previous reports, a markedly increased hemolytic and anemic profile favoring SCI was evidenced, consistent with a lower pain rate.<sup>20,21</sup> HU treatment did not significantly impact these data, but interpretation is very limited given the small sample size of treated children with SCI (n=3), and the low daily dose at the time of data collection. Interestingly, despite significantly greater anemia in children with SCI, blood viscosity did not significantly differ across groups because RBC deformability was reduced in the former group, which exerts opposite effects on blood viscosity.<sup>22</sup> Intravascular hemolysis is a major determinant of vascular and endothelial dysfunction.<sup>23</sup> Consistent with this, an increased level of sP-selectin, a marker of endothelial activation, was evidenced and was associated with SCI. sP-selectin could potentially serve as a biomarker of cerebral injury in children.<sup>6</sup> In contrast with previous studies,<sup>24</sup> a lower diastolic and mean arterial pressure was found in children with SCI. Altogether, this data strengthens the hemodynamic pathophysiology of SCI, whereby a further drop in pressure and/or blood flow in children with severe anemia results in ischemia in the border zone region, characterized by terminal arterial supply. Furthermore, hemorheological exploration of these patients suggests that increased RBC aggregate strength and decreased red cell deformability may also influence the risk of SCI. Deformable RBC mostly flow in single file in capillaries and RBC aggregates need to be fully dispersed before entering into the microcirculation.<sup>22,10</sup> Consequently, decreased RBC deformability and increased RBC aggregates strength may both impair blood flow at the entry of small capillaries and affect tissue perfusion.

Cerebral blood flow begins at a low level in the perinatal period, increases to a peak value at 3-8 years of age and then gradually decreases to adult levels with a negative correlation with age.<sup>25</sup> Several reports have documented elevated CBF in the grey matter of children with SCA, compared to normal controls,<sup>26-28</sup> a finding attributed to the compensatory increase in blood velocity and flow secondary to baseline anemia. This study allowed further insight by showing that, regardless of concomitant HU treatment, the level of perfusion was independently associated with blood viscosity (which is negatively correlated to anemia), HbF level, endothelial activation,  $\alpha$ -thalassemia status, and marginally associated with the level of hemolysis. ASL perfusion may therefore serve as an additional screening tool that integrates all these parameters to identify children at risk of subclinical injury, beyond the known risk factor, and regardless of cerebral vasculopathy. Thresholds for risk assessment will need to be determined by further prospective studies.

Oximetry analysis yielded expected and coherent results. NIRS is a non-invasive measurement of cerebral oxygenation, that varies with SpO<sub>2</sub> and Hb. In line with previous reports, we show that cerebral oxygenation is low in SCA patients.<sup>29,30,31</sup> Our results also confirmed an association between RBC rheological parameters and cerebral oxygenation, suggesting that patients with the most deformable RBC and less robust RBC aggregates

would have increased cerebral perfusion and oxygenation. However, because NIRS is unable to measure cerebral oxygenation at a depth of interest and is limited to the anterior territories, its additional predictive value remains to be demonstrated, particularly in patients at risk of white matter SCI.

This study has a number of limitations. Its cross-sectional design does not allow associations to be interpreted as causalities and its sample size was small, particularly regarding the number of patients with SCI treated by HU. It is possible that the lack of association between elevated CBF values and the presence of SCI was due to the low power of the study, for instance. Regarding imaging techniques, ASL MRI has also inherent limitations. In particular the transit time is reduced in SCA patients relative to non-anemic children. Thus, the labeling efficiency and postlabeling delay may not be adequate in every patient and may modify the perfusion signal measured by ASL.<sup>28</sup> Another parameter, the fixed value of the T1-longitudinal relaxation time of blood used for CBF quantification may not be adequate in every individual patient, given its dependence on hematocrit and blood composition. Another limitation is that CBF measurements were made in grey matter, while silent infarcts are located preferentially in white matter. At a magnetic field strength of 1.5 Tesla, the signal-to-noise ratio of the resulting perfusion map is too low in the cerebral white matter to allow accurate measurements. Despite these limitations, however, our ASL results adequately reflected the level of perfusion in different territories of the brain. In fact, our results were consistent with previous reports regarding the influence of both anemia and age and further allowed novel coherent results regarding the influence of endothelial activation and blood rheology for instance, and more generally the possible pathophysiology of SCI genesis.

Altogether the findings of this study suggest that SCI may, like overt stroke, preferably occur in otherwise paucisymptomatic children with marked hemolytic anemia and endothelial dysfunction. Cerebral blood flow measurements by ASL MRI may help assess the quality of perfusion at a microvascular level in children with no vasculopathy but nevertheless at risk of subclinical injury. An early disease-modifying treatment like HU, which improves all afore-mentioned factors associated with SCI may therefore decrease SCI risk as well, in addition to its known beneficial effect on TCD velocities and stroke risk.<sup>27,32,33</sup> Decreasing hemolytic anemia, improving RBC rheological characteristics and limiting endothelial injury will help avoid cerebral injury, particularly in case of further aggression such as arterial stenosis, acute hypoxia, drop in Hb or increased metabolic demand. Large prospective trials evaluating the protective effect of HU on SCI are ongoing and will hopefully confirm such beneficial effect. It is possible that new therapeutic approaches such as P-selectin blockade by monoclonal or pan antibodies, may have further protective effects in this particular complication.

#### Disclosures

VB, CP and MdM report honoraria and expert/consultancy testimony for Addmedica.

#### Contributions

VB, MK, DG and SV designed the study; VB, CP, CA, AK, MdM, BBF and SA enrolled patients; HB and LdC were responsible for biological data, except for E- and P-selectins that were measured by SB; SV and DG were responsible for imaging

data; KC and PC were responsible for oximetry and hemorheological data interpretation; VB, PC, CP, MK, DG, and SV analyzed the data; PC was responsible for statistical analysis; VB and PC wrote the manuscript. All authors reviewed, edited and approved the manuscript.

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