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# Relationship between hemoglobin, hemolysis, and transcranial Doppler velocities in children with sickle cell disease: Results from a long-term natural history study in Italy in the era of multimodal therapy

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## Abstract

**Background:** Stroke and cerebral vasculopathy are leading causes of morbidity and mortality in patients with sickle cell disease (SCD). Transcranial Doppler (TCD) is a reliable and validated predictor of stroke risk. Children with conditional or abnormal TCD are at an increased risk for stroke, which can be mitigated by red blood cell transfusion or hydroxyurea. Elucidating the relationship between cerebral hemodynamics and hemolytic anemia can help identify novel therapeutic approaches to reduce stroke risk and transfusion dependence.

**Methods:** This long-term, real-world study was designed to evaluate the prevalence of TCD imaging (TCDi)-assessed flow velocities in children and to interrogate their relationship with markers of anemia and hemolysis.

**Results:** In total, 155 children (median follow-up 79.8 months, 1358.44 patient-years) had 583 evaluable TCDi results. Only patients with HbSS or HbS$\beta^0$ had abnormal (1.6%) or conditional (10.9%) TCDi. Children with abnormal or conditional TCDi had lower hemoglobin (Hb) and higher hemolysis markers. A linear correlation was detected between TCD velocity and Hb: an Hb increase of 1 g/dL corresponded to decreases in velocity in the internal carotid and middle cerebral arteries (6.137 cm/s and 7.243 cm/s). Moreover, patients with Hb >9 g/dL presented a lower risk of TCDi-associated events.

**Conclusion:** These results support the need to optimize disease-modifying treatments that increase Hb and reduce hemolysis for stroke prevention in young children with SCD.

KEYWORDS
anemia, hemolysis, sickle cell disease, transcranial Doppler

**Novelty statement**

**What is the new aspect of your work?**

We evaluated the distribution of transcranial Doppler (TCD) velocities in a long-term natural history cohort of children with sickle cell disease (SCD).

**What is the central finding of your work?**

A 1 g/dL increase in hemoglobin (Hb) corresponded to a decrease in velocities in the internal carotid and middle cerebral arteries, and patients with Hb >9 g/dL presented with a lower risk for TCD imaging (TCDi)-associated events.

**What is the specific clinical relevance of your work?**

The clinical relevance pertains to maintaining Hb >9 g/dL in children with SCD, which reduces stroke risk as assessed using TCDi.

## 1 | INTRODUCTION

It is well known that children with sickle cell disease (SCD) are at high risk for ischemic stroke in the first decade of life.[1,2] Of the many acute and chronic complications of SCD, stroke can be particularly devastating for pediatric patients, given its potential to cause physical and neurocognitive deficits.[3,4] Over the past decade, the optimization of comprehensive care and treatment has led to some advances in the management of pediatric SCD.[5,6] Of note, the effectiveness of transcranial Doppler (TCD) ultrasonography in stratifying stroke risk in patients with SCD has been confirmed by randomized controlled trials, which has established the use of TCD screening and monitoring for stroke prevention in children with SCD.[2,7,8] Generally, abnormal (≥200 cm/s) or persistently high conditional (≥170 to <200 cm/s) cerebral artery blood flow velocities, as assessed by TCD, are an indication to start a chronic transfusion regimen and to consider disease-curative options.[2,4,8,9] In case of stable normalization of TCD velocities after 1 year of transfusions, switching to hydroxyurea (HU) treatment can be considered in the absence of stenosis with magnetic resonance angiography (MRA).[10] In addition to the introduction of these clinical practice guidelines, there is also an improved understanding of the complex pathophysiological mechanisms of large-vessel cerebral vasculopathy and stroke in SCD, such as how anemia, hemolysis, and vaso-occlusive events activate inflammatory and coagulation cascades and lead to cellular adhesion, hypercoagulation, endothelial cell activation, hypoxemia, nitric oxide depletion, and impaired blood rheology, thereby increasing stroke risk.[3] Despite this progress, stroke and abnormal or conditional TCD remain a burden on children with SCD in many national cohorts[11–13] and continue to contribute to overall chronic organ damage and early mortality.

One limitation of the research landscape is that most recent studies evaluating cerebral vasculopathy in children with SCD are either cross-sectional studies or clinical trials with limited years of follow-up.[8,14] To develop more-effective strategies for preventing or reducing large-vessel vasculopathy and permanent stroke-induced brain damage in children with SCD, it is important to collect data from longitudinal studies. Long-term longitudinal data collection from patients with rare diseases, particularly those being treated at specialized centers that offer advanced, high-level care and continuous, standardized data collection, can yield fundamental insights into the natural history of a disease and help identify specific factors that influence clinical manifestations.[15] Here, we leverage data gathered prospectively as part of a single-center SCD natural history study that has been active since 2007, to address some of the existing knowledge gaps related to stroke incidence in pediatric SCD. The main objective of this study was to determine the incidences of abnormal and conditional TCD flow velocities in pediatric patients with SCD and to investigate their correlation with hematologic variables. Using real-world data to study the relationship between anemia and hemolysis and large-vessel cerebral vasculopathy, as assessed via TCD, could help devise strategies to manage risk and optimize treatment for stroke prevention in young children with SCD.

## 2 | METHODS

### 2.1 | Study design

A retrospective study was performed using data from children with SCD who, at age of diagnosis, were prospectively enrolled in the SCD Natural History Study at the Veneto Region Pediatric Sickle Cell Disease Reference Center of Padua University Hospital since October 2007. Patients with TCD-based assessments of cerebral vasculopathy from January 1, 2009, to December 31, 2020, were included in the analysis. The censoring date was December 31, 2020, or the date of death, enrollment in a randomized clinical trial assessing new therapeutic drugs, transfer to a new center, or bone marrow transplantation. The study was approved by the Research Ethic Committee of Padua University Hospital, and written informed consent was obtained by each caregiver.

## 2.2 | Study population

The study population comprised patients with SCD (HbSS, HbSC, HbSβ$^+$, or HbSβ$^0$) aged 0–18 years at study enrollment. HU treatment, chronic red blood cell transfusion, and bone marrow transplantation were prescribed according to the Italian Association of Pediatric Hematology Oncology national guidelines and clinical practice.[16,17] The follow-up standard of care included clinical and hematological evaluation either every 3 months, or more frequently if the patient was on a disease-modifying treatment. Routine monitoring for central nervous system diseases included annual TCDi (included evaluation of right and left terminal internal carotid artery [TICA], anterior cerebral artery, middle cerebral artery [MCA], posterior cerebral artery, top basilar artery, and basilar artery starting at 2 years of age). TCD both with and without imaging were performed on every patient until 2016; thereafter, patients were only tested using TCDi because no substantial differences were detected between results obtained using the two techniques.[18]

## 2.3 | Study outcomes

Patient demographic and disease characteristics were assessed at baseline. Hematological and TCDi data from each patient visit were also collected from the study database. To evaluate the distribution of TCDi velocities in the patient cohort, patients were categorized according to the Stroke Prevention Trial in Sickle Cell Anemia (STOP) criteria of TICA and MCA time-averaged maximum mean velocities (TAMMVs): abnormal for TAMMV ≥200 cm/s, conditional for TAMMV 170–199 cm/s, normal for TAMMV 70–169 cm/s, and low for TAMMV <70 cm/s. Only exams with evaluable right and left measures available for both vessels were included in this analysis. First and successive TCDi assessments for each patient were included. Patients with HbSS and HbSβ$^0$ genotypes were analyzed separately from those with HbSC and HbSβ$^+$ genotypes. Additionally, levels of hemoglobin (Hb) and hemolysis markers, including reticulocyte count, lactate dehydrogenase (LDH), indirect bilirubin, and aspartate aminotransferase, were compared for patients with abnormal and conditional TCDi velocity.

## 2.4 | Statistical analysis

Descriptive statistics were reported for patient baseline characteristics and for classification of patients into cerebral hemodynamic profiles. Annual means were calculated for Hb and hemolysis markers. Two-sample and Welch *t*-tests for unequal variances were used to compare annual mean Hb values and annual mean hemolysis markers in patients with and without abnormal or conditional TCDi. Fisher and chi-square tests were used to compare categorical variables. Correlations between TCDi (categories and/or velocities) and hematologic variables were also investigated within the subgroup of HbSS and HbSβ$^0$ patients. Additionally, the relationship between TCDi velocity and the presence or absence of a specific disease-modifying treatment (HU, transfusions) was evaluated, considering a 5% type I error and 80% power; an incidence of 15% of abnormal/conditional TCDs has been taken into account, with an expected decrease of 10% in treated patients. Linear regression models were implemented to assess the effects of MCA and TICA TAMMVs as continuous variables on Hb. In particular, the means of right and left velocities for both arteries were used as dependent variables. For the following analyses, only abnormal and conditional TCDi categories were considered. Odds ratios (ORs) for abnormal and conditional TCDi results at different Hb levels were estimated using generalized estimated equations (GEE) with a binomial distribution, logistic function, and exchangeable correlation structure, allowing for correlation among repeated observations for the same patient. Multivariable GEE including clinical characteristics and treatment variables were used to evaluate the association between abnormal or conditional TCDi and mean Hb as a continuous variable and as a categorical variable (Hb <8 g/dL, Hb 8–9 g/dL, Hb >9 g/dL). The SAS software version 9.4 was used to perform all statistical analyses.

**TABLE 1** Characteristics of patients ($N = 155$) with evaluable TCDi[a].

| | *N* | % |
|---|---|---|
| Sex | | |
| Female | 77 | 49.7 |
| Male | 78 | 50.3 |
| Age at SCD diagnosis, months | | |
| Mean (range) | 39.4 | 0–191.8 |
| Median (IQR) | 24.4 | 8.3–57.3 |
| Age group at SCD diagnosis | | |
| ≤1 year | 46 | 29.7 |
| 1 to <2 years | 28 | 18.1 |
| 2 to <5 years | 47 | 30.3 |
| 5 to <10 years | 25 | 16.1 |
| ≥10 years | 9 | 5.8 |
| Genotype | | |
| HbSS | 121 | 78.1 |
| HbSβ$^0$ | 9 | 5.8 |
| HbSC | 22 | 14.2 |
| HbSβ$^+$ | 3 | 1.9 |
| Geographic origin | | |
| Northern Africa | 6 | 3.9 |
| Sub-Saharan Africa | 126 | 81.3 |
| Europe | 16 | 10.3 |
| Caribbean | 3 | 1.9 |
| Other | 4 | 2.6 |

[a]Data are *N* (%) unless shown otherwise.
Abbreviations: HbSβ$^+$, sickle beta plus thalassemia; HbSβ$^0$, sickle beta zero thalassemia; HbSC, hemoglobin sickle cell disease; HbSS, sickle cell anemia; IQR, interquartile range; SCD, sickle cell disease; TCDi, transcranial Doppler imaging.

## 3 | RESULTS

### 3.1 | Patients

A total of 182 patients with SCD were enrolled in the natural history cohort study. At the censoring date, the median follow-up of the entire cohort was 79.8 months (range: 2.1–298.6 months; interquartile range [IQR]: 36.9–126.3 months), corresponding to 1358.44 patient-years of follow-up. Overall, 60.4% of patients had received HU, 16.5% had received chronic transfusions, 11% underwent a bone marrow transplantation, 33% received no disease-modifying therapy, and 20.3% were treated with more than one type of disease-modifying therapy during follow-up. Of the 182 patients in the cohort, 155 had evaluable TCDi and were included in the analyses. Baseline characteristics and steady-state hematological data of patients with evaluable TCDi are described in Tables 1 and 2, respectively.

### 3.2 | TCDi findings

Complete transcranial color-coded duplex sonography (TCCS) exams with all 4 velocities (MCA right/left and TICA right/left) were 584 out of 777 (75.2%); 193 incomplete TCCS exams with missing velocities were excluded from the analysis. A total of 583 complete TCDi exams (range: 1–11 exams per patient) were performed for 155 patients between January 2009 and December 2020. The mean age at TCDi was 8.8 years (range: 1.5–25.6 years), and the median age was 7.8 years (IQR: 5.0–11.7 years); the mean age at first TCDi was 6.6 years (range: 1.5–18.2 years), and the median age was 5.3 years (IQR: 3.2–9.4 years). Most patients (130 of 155 [83.9%]) had the HbSS or HbS$\beta^0$ genotype.

The distribution of TCDi flow velocities was significantly different between the genotype subgroups ($p < .0001$; Table 3). None of the patients in the HbSC and HbS$\beta^+$ group had abnormal or conditional results, whereas 20 of them had at least one low flow result. In the HbSS and HbS$\beta^0$ group, 5 patients had at least one abnormal result, 22 patients had at least one conditional result, and 74 patients had at least one low flow result. Most abnormal and conditional velocities were detected in the MCA (87.5% and 88.7%, respectively), whereas most low velocities were detected in TICAs (88.2%). Only 37 of 138 (26.8%) low TCDi results were confirmed as stenosis at the nearest MRA.

### 3.3 | Correlation between TCDi flow velocities and hematologic variables

Because only patients with severe SCD genotypes presented abnormal or conditional TCDi results, this analysis was performed for the HbSS and HbS$\beta^0$ subgroup only. Compared with normal and low TCDi results, abnormal and conditional TCDi results were associated with lower mean Hb levels (8.9 vs 8.4 g/dL, respectively, $p < .0001$) and higher reticulocyte counts (262 750 vs. 317 766/mm$^3$, respectively, $p < .0001$), LDH values (690 vs. 843 U/L respectively, $p = .0012$), and aspartate aminotransferase values (54 vs. 61 U/L, respectively, $p = .0007$) (Figures 1A–D).

A linear correlation was detected between the TICA and MCA TAMMVs and Hb: an increase of 1 g/dL in Hb corresponded to a decrease in velocity of 6.137 cm/s in the TICA TAMMV (Figure 2A)

**TABLE 2** Hemoglobin and hemolysis markers for patients with evaluable TCDi ($N = 155$).

| | N | Mean | Median | Range | IQR | SD |
|---|---|---|---|---|---|---|
| Hb, g/dL | 152[a] | 8.9 | 8.8 | (5.2–13.8) | (8.0–9.8) | 1.3 |
| Fractionated Hb, % | | | | | | |
| HbS | 152 | 64.3 | 68.7 | (2.9–97.1) | (49.3–79.2) | 18.6 |
| HbF | 152 | 12.7 | 9.9 | (0.2–90.5) | (3.8–19.9) | 10.5 |
| HbC | 144 | 18.2 | 0 | (0–50.0) | (0–43.2) | 21.4 |
| HbA1 | 151 | 16.5 | 0 | (0–93.9) | (0–34.3) | 23.5 |
| HbA2 | 152 | 3.4 | 3.2 | (0–36.0) | (2.8–3.9) | 1.0 |
| Reticulocyte counts, n/mm$^3$ | 149 | 260 175 | 245 500 | (2300–963 600) | (168100–341 150) | 123 590 |
| Bilirubin, µmol/L | 148 | 43.0 | 32.9 | (0.7–312.0) | (22.1–51.3) | 34.1 |
| Direct | 148 | 8.0 | 7.7 | (0.1–218.3) | (5.8–9.7) | 6.0 |
| Indirect | 146 | 35.3 | 24.0 | (1.3–258.7) | (15.5–43.0) | 32.2 |
| ALT, U/L | 150 (96.8%) | 26.1 | 21.0 | (3.0–981.0) | (16.0–28.0) | 32.4 |
| AST, U/L | 149 (96.1%) | 54.3 | 50.0 | (11.0–1302.0) | (40.0–63.0) | 36.8 |
| LDH, U/L | 145 (93.5%) | 709.4 | 598.0 | (113.0–2590.0) | (431.0–899.0) | 386.1 |

[a]No Hb values were available for three patients who were either referred to the study center from peripheral hospitals only to undergo transcranial color-coded sonography or arrived at the study center a few days before the study censoring date.

Abbreviations: ALT, alanine transaminase; AST, aspartate aminotransferase; Hb, hemoglobin; HbA, hemoglobin subunit alpha; HbC, hemoglobin C; HbF, fetal hemoglobin; HbS, sickle hemoglobin; LDH, lactate dehydrogenase; TCDi, transcranial Doppler imaging.

**TABLE 3** Distribution of TCDi results according to sickle cell disease genotype.

| | Genotype subgroups | | | | | |
|---|---|---|---|---|---|---|
| | HbSC or HbSβ$^+$ | | HbSS or HbSβ$^0$ | | | |
| TCDi result | No. exams | No. patients | No. exams | No. patients | No. exams | No. patients |
| Abnormal | – | – | 8 (1.6%) | 5 | 8 | 5 |
| Conditional | – | – | 56 (10.9%) | 22 | 56 | 22 |
| Normal | 14 (20.0%) | 9 | 311 (60.6%) | 110 | 325 | 119 |
| Low | 56 (80.0%) | 20 | 138 (26.9%) | 74 | 194 | 94 |
| Total | 70 | | 513 | | 583 | |

Abbreviations: HbSβ$^+$, sickle beta plus thalassemia; HbSβ$^0$, sickle beta zero thalassemia; HbSC, hemoglobin sickle cell disease; HbSS, sickle cell anemia; TCDi, transcranial Doppler imaging.

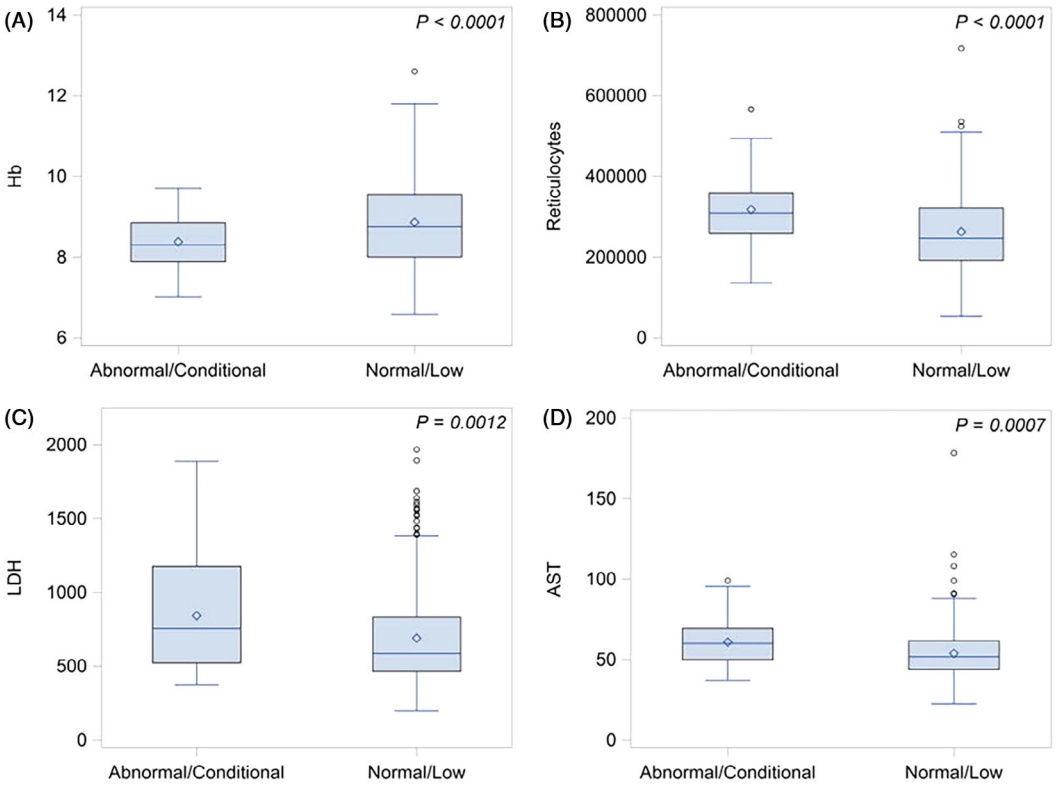


**FIGURE 1** Correlation of Hb and hemolysis markers with TCDi results. Comparisons of (A) Hb (g/dL), (B) reticulocytes (n/mm 3), (C) LDH (U/L) and (D) AST (U/L) in the history of patients with severe SCD genotypes (HbSS or HbSβ0) with and without abnormal or conditional TCDi results are shown. Two-sample and Welch *t*-tests for unequal variances were used for these comparisons. AST, aspartate aminotransferase; Hb, hemoglobin; HbSS, sickle cell anemia; HbSβ 0, sickle beta zero thalassemia; LDH, lactate dehydrogenase; SCD, sickle cell disease; TCDi, transcranial Doppler imaging.

and 7.243 cm/s in the MCA TAMMV (Figure 2B). Linear regression models showing a significant inverse correlation between Hb and TAMMV in the subgroup of patients with HbSC/HbSβ+ genotype and in the whole population (HbSS/HbSβ0 + HbSC/HbSβ+) are reported as supplementary materials (Supplementary Figures S1A,B and S2A,B).

Univariate analysis showed a significant inverse correlation between abnormal/conditional TCDi results and Hb as a continuous variable (OR: 0.484, $p < .001$). Notably, when considering Hb as a categorical variable, patients with Hb >9 g/dL presented a significantly lower risk for TCDi events than patients with Hb <8 g/dL (OR: 3.211, 95% confidence interval [CI]: 1.406–7.336, $p = .0016$) and patients with Hb between 8 and 9 g/dL (OR: 4.859, 95% CI: 2.268–10.410, $p = .0008$). In the multivariate analysis, the correlation between TCDi results and Hb, both as continuous and categorical variables, remained significant ($p < .001$ and $p = .0281$, respectively); moreover, the risk of presenting abnormal or conditional TCDi results decreased with age ($p = .0158$). No significant correlation was

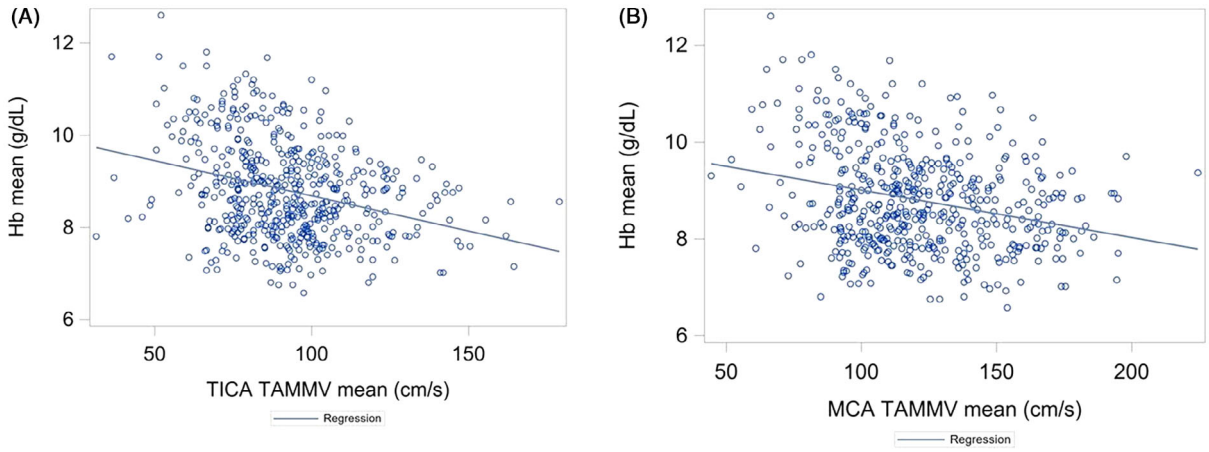


**FIGURE 2** Linear inverse correlation between Hb and TAMMV in the subgroup of patients with an HbSS/HbS β0 genotype. (A) The correlation between mean Hb and TICA TAMMV TCDi values. (B) The correlation between mean Hb and MCA TAMMV TCDi values. Linear regression models were used to assess the effects of MCA and TICA TAMMVs as continuous variables on Hb. Hb, hemoglobin; MCA, middle cerebral artery; TAMMV, time-averaged maximum mean velocity; TCDi, transcranial Doppler imaging; TICA, terminal internal carotid artery.

detected between TCDi results and the presence or absence of a disease-modifying treatment ($p = 0.3973$) in this cohort.

## 4 | DISCUSSION

In this long-term, longitudinal, natural history study of pediatric patients with SCD treated in an academic center, the prevalence of abnormal and conditional TCDi results was relatively low (1.6% and 10.9%, respectively) compared with rates reported in other pediatric cohort studies; however, more follow-up is needed to confirm these results.[5,12,19,20] Additionally, the distribution of TCDi results differed based on SCD genotypes: abnormal and conditional velocities were detected only in patients with severe forms of SCD (HbSS and HbSβ$^0$), whereas low TCDi velocity was reported most frequently in patients with HbSC and HbSβ$^+$ genotypes. Although undetected by TCDi, cerebrovascular damage does likely occur in patients with HbSC or HbSβ$^+$ genotypes but is probably reflective of microvascular injury rather than large-vessel vasculopathy.[12,21] Moreover, analyses of the hematological and TCDi data from this study demonstrate the importance of maintaining adequate Hb thresholds to reduce the risk of abnormal and conditional TCDi in the real-world setting.

A positive correlation between anemia and increased cerebral blood flow velocities has previously been shown in some cross-sectional studies and clinical trials with short observation times[20,22] but has not been demonstrated in other studies. A 2011 Brazilian study reported a negative correlation between TAMMV and Hb level in patients with HbSS and HbSβ$^0$ genotypes.[23] Similarly, a 2018 Jamaican pediatric cohort study showed that lower Hb was associated with higher TCD velocities.[20] In a meta-analysis of 41 studies relating Hb concentration to clinical outcomes, Hb concentration was significantly lower in patients with an abnormal TCD velocity (0.6 g/dL [95% CI: 0.3–0.9] lower than that in patients without abnormal TCD velocity).[24] In this longitudinal cohort study, a 1 g/dL increase in Hb corresponded to a decrease of 6.137 cm/s in TICA TAMMV and 7.243 cm/s in MCA TAMMV in patients with HbSS and HbSβ$^0$ genotypes. Moreover, when considering Hb as a categorical variable in both univariate and multivariate regression analyses, an Hb level >9 g/dL was predictive of a lower risk of developing abnormal or conditional velocities than was an Hb level <9 g/dL. Previously, a chart review study of infants aged 2–6 months who underwent TCD screening indicated that patients with Hb <8.5 g/dL had 2.7 times the risk of having an abnormal or conditional TCDi velocity compared with patients with Hb ≥8.5 g/dL.[25] National guidelines and clinical practice generally consider Hb below 8 g/dL as the Hb threshold for prescribing disease-modifying therapies for children with SCD.[16,17] The results reported here suggest that existing Hb thresholds for identifying treatment candidates are probably too low and need to be reconsidered for more effective stroke prevention.

These results also support the use of therapies that increase Hb at a young age to reduce the risk of developing abnormal or conditional TCDi and stroke. HU is the most well-established pharmacologic therapy for increasing Hb in patients with SCD. The beneficial effects of HU-mediated Hb improvements on reducing TCD velocity in children have been demonstrated previously.[22,26] However, studies in both a US longitudinal cohort and an Italian cohort showed that HU treatment is often not optimized, and utilization with the maximum tolerated dose that yields a clinical response is low.[17,27] Optimizing the use of treatments that reduce anemia, either as a monotherapy or in combination, could help reduce the risk of stroke in children with SCD and provide the added benefit of reducing the need for chronic transfusion therapy that is traditionally used for this purpose.

The association between high TCD velocities and elevated hemolysis markers has been previously described in different cohorts.[5,19,25,28,29] A similar trend was observed in the cohort of HbSS and HbSβ$^0$ patients described here; however, the inverse correlation between TCDi results and Hb in the multivariate analysis was stronger. No significant correlation was detected between TCDi results and

the presence or absence of a disease-modifying treatment in this analysis. These findings may suggest that maintaining adequate Hb levels and lowering hemolysis, rather than the type of treatment used to achieve higher Hb and lower hemolysis, could be more important for lowering stroke risk. In this context, the role of chronic transfusion therapy is well known from randomized studies, and the importance of HU compared with transfusion is known from the non-inferiority TwiTch study,[10,30] but further studies with larger populations are needed to investigate the eventual impact of single types of disease-modifying treatments and their magnitude of effect on TCD results. These data generally underscore the potential of multimodal therapy for personalized treatment in SCD, which is important considering the extreme phenotypic variability of the disease. Moreover, as different treatments become available in the real-world setting, larger prospective, comparative studies could be performed.

Similar to findings reported in the Créteil newborn cohort that evaluated patients with SCD through childhood up to age 18–20 years,[19] the risk of abnormal and conditional TCDi was higher in young children and reduced with age. Moreover, higher velocities were not always related to stenosis at MRA, confirming that abnormal TCD usually precedes stenosis occurrence and highlighting the need for prevention strategies before the occurrence of stenosis.

In this pediatric natural history cohort, hematological and instrumental longitudinal, systematic data collection was performed as per the national and international guidelines for organ damage monitoring. Additionally, TCDi results were obtained and classified according to the STOP protocol, and therapies were prescribed per standard of care. The median follow-up was therefore relatively longer than what has previously been reported in other cohort studies.[8] As such, this study provides valuable natural history data for a disease for which patient life expectancy is increasing and sets a new paradigm for long-term data collection.[15,31]

This study does have some limitations. TCDi exams were performed by different operators over a long period of time, which could affect the reproducibility of the results of this operator-dependent exam. However, this is a limitation that is likely to be common to most studies conducted in a real-world setting. Secondly, about 25% of the TCDi exams were incomplete and therefore excluded from the analysis; this highlights the need to improve both the operators' technical abilities and patient compliance. The problem of inadequate TCD scans had been reported in Europe and the United States and is indeed a crucial aspect to support in the real-world setting, but an inadequate result per se was not associated with an increased risk of cerebrovascular disease.[32–34] Lastly, the high median age at first TCDi reflects the lack of a national newborn screening program and delays in diagnosis in Italy, which are limitations that demand urgent actions to ensure adequate treatment at a young age for all children with SCD.

In conclusion, analysis of data from this longitudinal natural history cohort demonstrates the importance of maintaining adequate Hb thresholds to reduce the risk of abnormal or conditional TCDi flow velocities in children with SCD, particularly in those with the HbSS or HbS$\beta^0$ genotype (sickle cell anemia [SCA]). More specifically, these findings suggest that a 1 g/dL increase in Hb and maintenance of Hb >9 g/dL have a protective effect for stroke risk. Accordingly, the use of disease-modifying therapies that increase Hb and reduce hemolysis for children with SCA at an early age, aside from the typically recommended prophylactic transfusion, could help reduce stroke risk and prevent permanent cerebrovascular damage. This strategy could reduce the burden and challenges associated with transfusion therapy for young children with SCA. Larger cohorts and longitudinal studies are needed to confirm these results and provide sufficient evidence for the modification of current guidelines.

## AUTHOR CONTRIBUTIONS

**Giulia Reggiani**: Study design, data collection, and writing. **Beatrice Coppadoro**: Statistical analyses. **Vania Munaretto**: Study design, data collection, and manuscript review. **Alessio Pieroni**: Data collection and manuscript review. **Federica Viaro**: Data collection and manuscript review. **Renzo Manara**: Data collection and interpretation, manuscript review. **Anne Beauburn**: Study design, manuscript review. **Claudio Baracchini**: Study design, data interpretation and manuscript review. **Alessandra Biffi**: Manuscript review. **Laura Sainati**: Study design, data interpretation, and manuscript review. **Raffaella Colombatti**: Study design, data interpretation and manuscript review.

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## CONFLICT OF INTEREST STATEMENT

Giulia Reggiani, Beatrice Coppadoro, Vania Munaretto, Alessio Pieroni, Federica Viaro, Renzo Manara, Claudio Baracchini, and Laura Sainati have no conflicts of interest. Anne Beaubrun was an employee of Global Blood Therapeutics, Inc. at the time of the study. Alessandra Biffi is a member of the scientific advisory board and a scientific consultant for Orchard Therapeutics; is founder of and a scientific advisor for Altheia Science; is founder and CEO of Gene Therapy Consulting; has sponsored research agreements with Orchard Therapeutics and Altheia Science; and has received an honorarium for a symposium from Vertex Pharmaceuticals. Raffaella Colombatti is on the advisory boards of BluebirdBio, NovoNordisk, Global Blood Therapeutics, Inc., Novartis, Forma Therapeutics, and Addmedica; and has received research funding from Global Blood Therapeutics, Inc., and BluebirdBio. Global Blood Therapeutics, Inc. is a wholly owned subsidiary of Pfizer Inc.

## DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

## PATIENT CONSENT

Informed consent was obtained from the legal guardians.

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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