THE ROLE OF HEMOGLOBIN AND HEMOLYSIS ON TRANSCRANIAL DOPPLER VELOCITIES IN CHILDREN WITH SICKLE CELL DISEASE: DATA FROM A NATURAL HISTORY COHORT

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BACKGROUND

Children with sickle cell disease (SCD) are at increased risk of cerebrovascular events that can impact neurocognitive development and quality of life (Colombatti 2016). Transcranial Doppler ultrasound (TCD) is a validated screening tool to identify pediatric SCD patients with the highest risk of stroke to start on a preventive chronic blood transfusion regimen (Estcourt 2020; Inusa 2019). Conditional and abnormal TCD velocities are an indication to start disease-modifying treatments or consider disease-curative options in children with SCD (Khemani 2019). However, real-world pediatric data on the correlation between hematological variables and TCD results are scarce (Salama 2020). **We aimed to evaluate the distribution of TCD velocities in a pediatric natural history cohort and investigate their correlation with hematological variables and treatments.**

METHODS

We performed a **retrospective analysis** on data from a **prospective pediatric cohort** followed from January 1, 2009, to December 31, 2020 (censoring date). Standard care includes annual TCD from 2 years of age.

We used **transcranial Doppler imaging** (TCDi) and classified results according to STOP criteria, considering terminal internal carotid artery (TICA) and middle cerebral artery (MCA) time-averaged maximum mean velocities (TAMMVs). Only complete exams with right and left measures available for both vessels were included. **Hematological, clinical, and treatment variables** were available from the natural history cohort database. Patients were **divided according to genotype**: HbSS/HbSβ^0 or HbSC/HbSβ$^+$.

Two-sample and Welch t-tests for unequal variances were used to compare mean hemoglobin (Hb) values and hemolysis markers in patients with and without abnormal/conditional TCDi results. **Fisher and chi-square** tests were used to compare categorical variables.

Linear regression models were used to assess the effects of MCA and TICA TAMMVs as continuous variables on Hb. Odds ratios (ORs) for neurological events 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 characteristics and treatment variables were used to evaluate the association between neurological events and Hb.

RESULTS

Of the 182 SCD patients in the cohort, 169 had assessments of cerebral vasculopathy, and 155 had evaluable TCDi (583 exams). 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. The median age at the censoring date was 13.4 years (IQR: 9.1-17.5 years); 130 were HbSS/HbSβ^0, and 25 were HbSC/HbSβ$^+$. Basic demographic characteristics of the cohort are in **Table 1**.

	N	%
Sex		
Female	86	50.9
Male	83	49.1
Age at diagnosis of SCD		
Median: 24.4 months (IQR 8.4-56.5 months) Mean: 41.0 months (range 0-228.4 months)		
≤1 year	49	29.0
1-2 years	31	18.3
2-5 years	52	30.8
5-10 years	26	15.4
≥10 years	11	6.5
Genotype		
HbSC	2	14.8
HbSS	132	78.1
HbSβ$^+$	3	1.8
HbSβ^0	9	5.3
Geographical area of origin		
Northern Africa	6	3.6
Sub-Saharan Africa	139	82.3
Europe	17	9.9
Caribbean	3	1.8
Other	4	2.4

Table 1. Characteristics of 169 Patients with ≥1 Neurological Instrumental Examination during Follow-up.
HbSC, hemoglobin SC disease; HbSS, sickle cell anemia; HbSβ$^+$, sickle beta plus thalassemia; HbSβ^0, sickle beta zero thalassemia; IQR, interquartile range; SCD, sickle cell disease.

The distribution of TCDi results was significantly different between genotypes (P<0.0001) (**Table 2**). Only 37/138 (26.8%) low TCDi results were confirmed as stenosis at the nearest magnetic resonance angiography.

Patients with abnormal/conditional TCDi results had lower Hb (8.4 vs 8.9 g/dL, P≤0.0001) and **higher reticulocyte counts** (317,766 vs 262,750/mm^3, P≤0.0001), **lactate dehydrogenase** (843 vs 690 U/L, P=0.0012), **and aspartate aminotransferase** (61 vs 54 U/L, P=0.0007) compared with patients with normal/low TCDi results (**Figure 1**).

TCDi result	Genotype				N exams	N patients
	HbSC/HbSβ$^+$		HbSS/HbSβ^0			
	N exams	N patients	N exams	N 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**	

Table 2. TCDi Results According to Genotype.
TCDi, transcranial Doppler imaging; HbS, sickle hemoglobin; HbSC, hemoglobin SC disease; HbSS, sickle cell anemia; HbSβ$^+$, sickle beta plus thalassemia; HbSβ^0, sickle beta zero thalassemia.

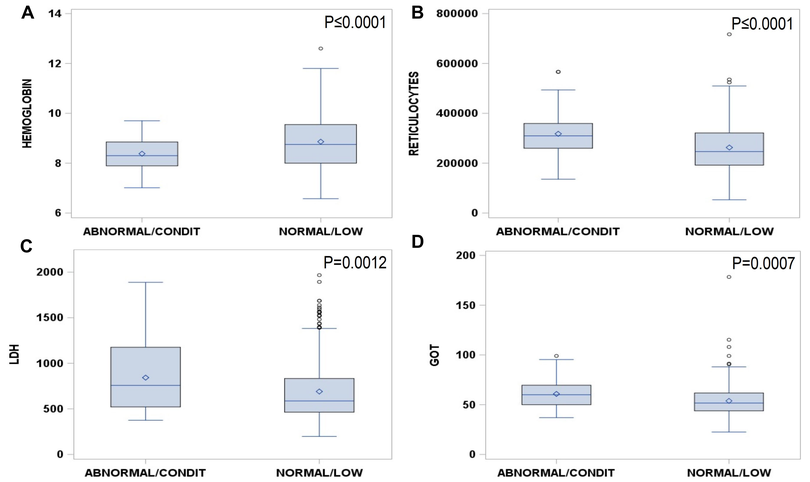
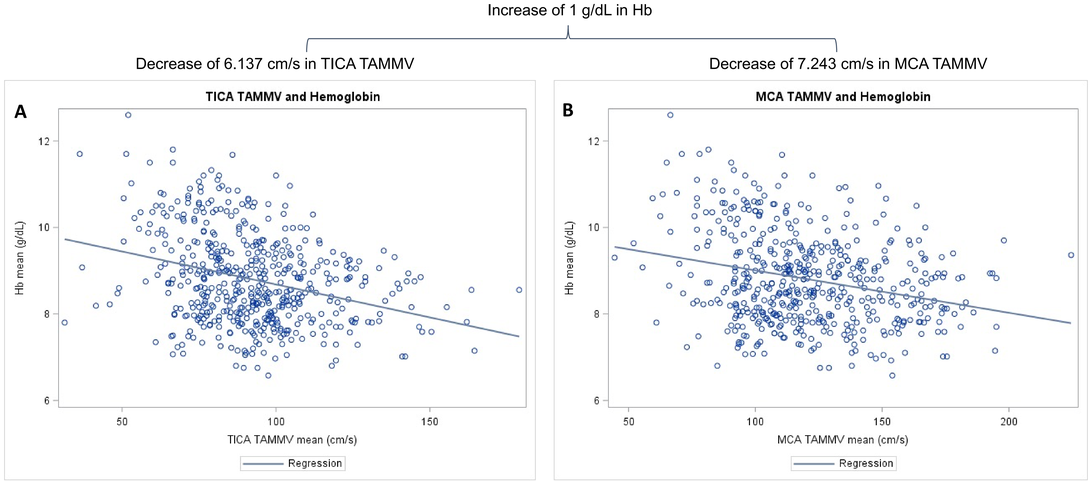


Figure 1. Comparison of (**A**) Hb (g/dL), (**B**) Reticulocytes (n/mm^3), (**C**) LDH (U/L) and (**D**) GOT in the History of Patients with and without Abnormal/Conditional TCDi results.
GOT, aspartate aminotransferase; Hb, hemoglobin; LDH, lactate dehydrogenase; TCDi, transcranial Doppler imaging.



We detected a **linear correlation between TICA/MCA TAMMVs and Hb** (**Figure 2A** and **B**). Univariate analysis showed significant inverse correlation between abnormal/conditional TCDi results and Hb considered as a continuous variable (OR: 0.484, P<0.001).

In the multivariate analysis, the correlation between TCDi results and Hb remained significant; moreover, the risk of presenting abnormal/conditional TCDi results decreased with age (OR: 0.833, P<0.0064).

Figure 2. Inverse Linear Correlation between Hb (Mean of the Year) and TAMMVs Measured in TICA (**A**, P<0.0001) and MCA (**B**, P<0.0001).
Hb, hemoglobin; MCA, middle cerebral artery; TAMMV, time-averaged maximum mean velocity; TICA, terminal internal carotid artery.

CONCLUSIONS

This analysis from our natural history cohort shows a significant inverse correlation between Hb and MCA and TICA/MCA TAMMVs, supporting the **beneficial effect of higher Hb levels in reducing TAMMV**. Disease-modifying therapies increasing Hb and reducing hemolysis could be helpful in reducing TAMMV in children with SCD.

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