

# Regular Transfusion Lowers Plasma Free Hemoglobin in Children With Sick-Cell Disease at Risk for Stroke

Nelson E. Lezcano, PhD; Nadine Odo, CCRC; Abdullah Kutlar, MD;  
Donald Brambilla, PhD; Robert J. Adams, MS, MD

**Background and Purpose**—Intravascular hemolysis releases large amounts of free hemoglobin (PFH) in plasma of sickle-cell disease (SCD) patients. PFH has been associated with harmful endothelial actions including scavenging nitric oxide (NO). Whether PFH plays a role in stroke in SCD has not been examined.

**Methods**—Serum levels of PFH, lactate dehydrogenase, and total bilirubin were measured in stored sera from children at risk for stroke treated in a randomized controlled trial of regular red cell transfusion (STOP study). Baseline and post-treatment ( $\approx 1$  year of transfusion) were compared to determine whether treatment (which reduces stroke risk by 90%) was associated with reduction in markers of hemolysis.

**Results**—Baseline serum PFH values did not differ between treatment groups. PFH declined with repeated transfusion from  $78.7 \pm 8.2$  mg/dL to  $34.4 \pm 3.4$  mg/dL ( $P < 0.001$ ). With only episodic or no transfusion the drop was smaller:  $80.9 \pm 7.5$  to  $62.8 \pm 5.0$  ( $P = 0.019$ ). The decrease was larger in those with regular transfusion (56% versus 22%;  $P < 0.001$ ). Reduction of lactate dehydrogenase and total bilirubin was observed only in those on regular transfusion.

**Conclusions**—Regular transfusion which lowers stroke risk is associated with a significant reduction in PFH. A role for PFH in promoting stroke in SCD should be investigated. (*Stroke*. 2006;37:1424-1426.)

**Key Words:** children ■ hemolysis ■ stroke

Stroke is a major clinical complication of sickle-cell disease (SCD).<sup>1</sup> Clinically evident infarctions are usually associated with a large vessel intracranial vasculopathy.<sup>2</sup> The Stroke Prevention in Sickle Cell Anemia (STOP) trial demonstrated that Transcranial Doppler (TCD) can detect children at high risk and that regular red cell transfusion (about every 3 to 4 weeks) reduced risk by 90%.<sup>3,4</sup> Serum frozen at baseline and quarterly visits on STOP patients provides an opportunity to explore mechanisms for stroke in SCD by comparison of control (persistent high risk) and on-treatment (low risk) samples.

SCD patients have high plasma free hemoglobin (PFH) resulting from high intravascular hemolysis.<sup>5</sup> PFH has been associated with pulmonary hypertension in SCD.<sup>6</sup> PFH is a scavenger of nitric oxide (NO)<sup>7</sup> and has other deleterious vascular effects.<sup>8</sup> NO is an important regulator of vascular tone in the cerebral circulation,<sup>9</sup> but a role for NO or PFH in the development of stroke in SCD has not been examined. Our objective was to determine whether regular blood transfusion was associated with significant reduction in PFH.

## Methods

The details of the STOP study were previously published.<sup>4</sup> Briefly, children without stroke but at high stroke risk based on TCD, time

average mean of the maximum of 200 cm/s or more, were randomized to receive regular red cell transfusions or standard care which allowed episodic transfusion for pain episodes or other events. Venous blood was drawn and allowed to clot at room temperature, and then shipped to the Medical College of Georgia (MCG)-Hemoglobinopathy laboratory in wet-ice within 48 hours where samples were centrifuged, serum removed and frozen ( $-70^{\circ}\text{C}$ ). Informed consent was obtained in accordance with the STOP study requirements.

All 130 randomized STOP patients were eligible, but subjects with grossly hemolyzed or 1 sample only were excluded. The total number of available cases was 112. Actual treatment rather than treatment assignment at randomization was used. Patients were classified in the Transfusion group if they have at least 9 transfusions/year, and in the Episodic/No Transfusion group (included cases with only episodic transfusion) if they received no more than 4 transfusions/year. Levels of hemoglobin S (HbS) were also used as the criterion for treatment classification as follows: patients with HbS  $< 40\%$  were included in the transfusion group and those with HbS  $> 50\%$  were classified in the episodic or not transfusion group. Both methods resulted in identical classification of cases ( $n = 50$  Transfusion and 62 No Transfusion). Baseline and follow-up samples were  $\approx 1$  year apart ( $> 6$  but  $< 36$  months). Statistical analyses were performed using Microcal Origin 6.0-software; a paired  $t$  test was used between baseline and follow-up samples or an Independent  $t$  test (between treatment groups). A 2-tailed Spearman test was used to correlate time between samples with PFH.

PFH was measured with PFH-kit from Catatchem. The reaction is based on the peroxidase activity of hemoglobin. Lactate dehydroge-

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From the Department of Neurology, Stroke Research Program, Department of Neurology School of Medicine, Medical College of Georgia, Augusta, GA (N.E.L., N.O., R.J.A.); the Department of Medicine and Sickle Cell Center (A.K.), Medical College of Georgia, Augusta, GA; and the New England Research Institutes (D.B.), Watertown, MA.

Correspondence to Robert Adams, MD, Department of Neurology, Stroke Research Program, Department of Neurology, School of Medicine, Medical College of Georgia, 1429 Harper St, Rm HF 1154, Augusta, GA 30912. E-mail rjadams@mail.mcg.edu

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### Comparison Between Baseline and Follow-Up Intravascular Hemolysis Markers in Samples From Children With SCD Receiving Regular Blood Transfusion or Episodic/No Transfusion Treatments

	Transfusion					Episodic/No Transfusion				
	Baseline		Follow-Up			Baseline		Follow-Up		
	Mean	SD	Mean	SD	n	Mean	SD	Mean	SD	n
PFH, mg/dl	78.7	58.0	34.4	24.4**	50	80.9	59.3	62.8	39.5*	62
% HbS	88.1	5.9	20.8	7.1**	50	88.0	5.0	87.1	7.4	62
T-Bili, mg/dl	4.0	2.1	2.6	1.5**	44	3.9	1.6	3.9	1.9	44
LDH, U/L	640.8	144.4	519.6	205.2	44**	714.7	175.9	708.0	206.6	44

Data represents average values, and n=sample size.

T-Bili indicates Total Bilirubin. \* $P<0.05$  and \*\* $P<0.001$  ( $t$  test) in comparison with corresponding baseline value.

nase (LDH) levels, which have been correlated with intravascular hemolysis,<sup>10</sup> were measured using an enzymatic rate method (oxidation of L-lactate to pyruvate, reduction of NAD-NADH and color substrate formation). Total bilirubin was measured using the Diazo method (Synchro LX automatic system; Beckman Coulter, Inc). High pressure liquid chromatography was used for the detection and quantitation of HbS in blood samples; area under the curve method was used for computing HbS percentage.

## Results

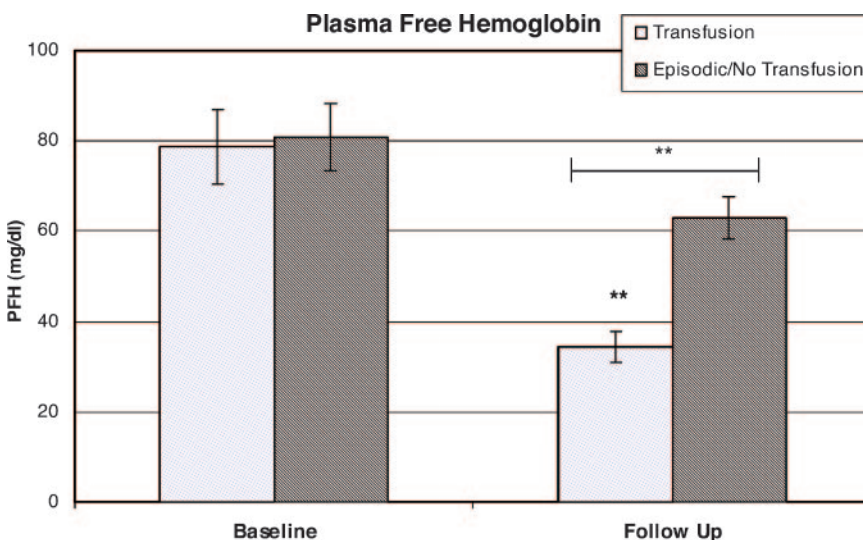
Children in the Transfusion group were  $8.0\pm3.6$  years old (mean $\pm$ SD) at entry and not different from those in the Episodic/No Transfusion group ( $8.1\pm3.0$  years;  $P=0.71$ ). Time between baseline and follow-up samples was also similar (Transfusion:  $13.3\pm4.8$  months, range, 8.8 to 33.3 months,  $n=50$ ; Episodic/No Transfusion:  $13.4\pm4.4$  months, range, 5.1 to 30.3 months,  $n=62$ ,  $P=0.61$ ).

Patients classified in the Transfusion group had an average of 16 transfusions (range of 9 to 26) and an average HbS of 88.1% HbS at baseline and 20.8% at follow-up (range 7.4 to 39.0% HbS at follow-up). Of 62 classified in the Episodic/No Transfusion group, 39 had no transfusions, 9 received 1 transfusion, 6 received 2 transfusions, 5 received 3 and 3 children received 4 transfusions. Baseline % HbS was 88.0% in this group and 87.1% HbS at follow-up (range 51.5 to 97.8%; Table).

The Table shows baseline and follow-up PFH, % HbS, total bilirubin and LDH for both groups. PFH was lower at follow-up in those in the Transfusion group dropping from  $78.7\pm8.2$  mg/dL at baseline to  $34.4\pm3.4$  mg/dL ( $P<0.001$ ). A smaller decrease from  $80.9\pm7.5$  to  $62.8\pm5.0$  ( $P=0.019$ ) was observed in Episodic/No Transfusion group (Figure). LDH dropped from  $640.8\pm21.7$  U/L to  $519.6\pm30.9$  U/L ( $P<0.01$ ) in the Transfusion group, but no change was seen in the Episodic/No Transfusion group ( $714.7\pm26.5$  U/L at baseline and  $708.1\pm31.1$  U/L at follow-up;  $P=0.76$ ). Similar results were seen for total bilirubin ( $4.0\pm0.3$  mg/dL at baseline and  $2.6\pm0.2$  mg/dL,  $P<0.001$  after regular transfusion and  $3.9\pm0.2$  mg/dL at baseline versus  $4.0\pm0.3$  mg/dL,  $P=0.64$  after episodic or no transfusion; Table). Time between baseline and follow-up samples was not significantly correlated with PFH in the Transfusion group ( $r=0.24$ ,  $P=0.08$ , 2-tailed Spearman test).

## Discussion

The STOP study showed a 90% reduction in first stroke with regular transfusion<sup>4</sup> but how it works is not clear. Being on regular transfusion resulted in about a 10% increase in total hemoglobin, a marked reduction in HbS as a fraction of the total hemoglobin, a reduction in reticulocytes but no significant differences in white blood cells or platelet counts.<sup>11</sup> We



Effect of regular transfusion on PFH levels. Transfused children had lower PFH levels after the treatment period. Values are expressed as mean $\pm$ SEM ( $n=50$  transfusion,  $n=62$  no transfusion). \*\* $P<0.001$  ( $t$  test). Statistic significance between baseline and follow-up samples in transfusion treatment group, and between treatments at follow-up.

now report that regular transfusion is also associated with a significant reduction in PFH which may be in part responsible for its vascular protective effects. Plasma free hemoglobin may exert a number of deleterious effects,<sup>8</sup> and elevated PFH has been correlated to pulmonary hypertension.<sup>6</sup> Lowering PFH may reduce the toxic effects of free heme and heme-derived iron which increases cellular susceptibility to oxidants<sup>12</sup> and may cause endothelial dysfunction from depletion of NO.<sup>7</sup> PFH is elevated in SCD from <2 mg/dL (upper range of normal) to between 17 and 41 (steady state versus "crisis" values respectively).<sup>5</sup> Baseline levels of PFH in this study are clearly elevated by these standards, but this finding should be interpreted with caution because of how the samples were collected and shipped to the central laboratory. Because red cells were not immediately separated from serum, some of the measured PFH may be attributable to postcollection hemolysis. However, the change with treatment, which is the major new finding we report, is not an artifact of collection/shipping as patients were randomized in permuted blocks among the sites to insure that each site had both transfusion and no transfusion patients. Therefore, collection and shipping of samples was not systematically related to treatment assignment. Transfusion also reduces amount of circulating HbS containing cells which are known to bind to endothelium and may promote vasculopathy.<sup>13</sup> The emerging appreciation of the deleterious effects of PFH in SCD and other disorders,<sup>8</sup> and the marked reduction shown in this randomized trial, support further research into PFH as a possibly important factor driving stroke risk in children with SCD and perhaps explaining one of the ways regular transfusion is so effective in preventing stroke in SCD.

### Summary

Regular red cell transfusion is associated with a marked reduction in PFH in children with SCD at high risk for stroke.

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