

Impact of IdeS treatment on HIT antibody-mediated platelet activation. (A) HIT antibodies cause platelet activation by binding to epitopes exposed on PF4 (red tetramers) complexed with heparin (black squiggle) or platelet surface glycosaminoglycans (black, shown branching from surface proteoglycans). Once bound, the constant portion of these antibodies (Fc) binds to and activates the platelet IgG receptor, FcγRIIA (in green), resulting in platelet activation. (B) IdeS-treated antibodies are able to maintain binding to their target via the F(ab')<sub>2</sub> portion, but given the lack of the Fc domain, are unable to bind to FcγRIIA and activate platelets.

patient to be placed on alternative anti-coagulation or IVIg after IdeS therapy (the latter, to help avoid infectious complications resulting from plasma IgG cleavage, and to inhibit platelet activation induced by newly synthesized HIT antibodies). These important issues and questions should be investigated in the context of a prospective clinical trial.

**Conflict-of-interest disclosure:** A.P. has equity ownership in Retham Technologies; serves on the advisory board for Veralox Therapeutics; and receives royalties/patents from Versiti Blood Research Institute. ■

## REFERENCES

1. Kizlik-Masson C, Deveau Q, Zhou Y, et al. Cleavage of anti-PF4/heparin IgG by a bacterial protease and potential benefit in heparin-induced thrombocytopenia. *Blood*. 2019;133(22):2427-2435.
2. Dhakal B, Kreuziger LB, Rein L, et al. Disease burden, complication rates, and health-care costs of heparin-induced thrombocytopenia in the USA: a population-based study. *Lancet Haematol*. 2018;5(5):e220-e231.

3. Kuitunen A, Sinisalo M, Vahtera A, Hiltunen L, Javela K, Laine O. Autoimmune heparin-

induced thrombocytopenia of delayed onset: a clinical challenge. *Transfusion*. 2018;58(12): 2757-2760.

4. Pishko A, Gimotty P, Guevara J, Cuker A. The risk of major bleeding in patients with suspected heparin-induced thrombocytopenia (HIT) [abstract]. *Blood*. 2018;132(suppl 1). Abstract 377.
5. Cuker A, Arepally GM, Chong BH, et al. American Society of Hematology 2018 guidelines for management of venous thromboembolism: heparin-induced thrombocytopenia. *Blood Adv*. 2018;2(22): 3360-3392.
6. Padmanabhan A, Jones CG, Pechauer SM, et al. IVIg for treatment of severe refractory heparin-induced thrombocytopenia. *Chest*. 2017;152(3):478-485.
7. Jordan SC, Lorant T, Choi J, et al. IgG endopeptidase in highly sensitized patients undergoing transplantation. *N Engl J Med*. 2017; 377(5):442-453.
8. Padmanabhan A, Ratner LE, Jhang JS, et al. Comparative outcome analysis of ABO-incompatible and positive crossmatch renal transplantation: a single-center experience. *Transplantation*. 2009;87(12):1889-1896.
9. Winstedt L, Järnum S, Nordahl EA, et al. Complete removal of extracellular IgG antibodies in a randomized dose-escalation phase I study with the bacterial enzyme IdeS—a novel therapeutic opportunity. *PLoS One*. 2015; 10(7):e0132011.
10. Lorant T, Bengtsson M, Eich T, et al. Safety, immunogenicity, pharmacokinetics, and efficacy of degradation of anti-HLA antibodies by IdeS (imlifidase) in chronic kidney disease patients. *Am J Transplant*. 2018;18(11): 2752-2762.

DOI 10.1182/blood.2019000695

© 2019 by The American Society of Hematology

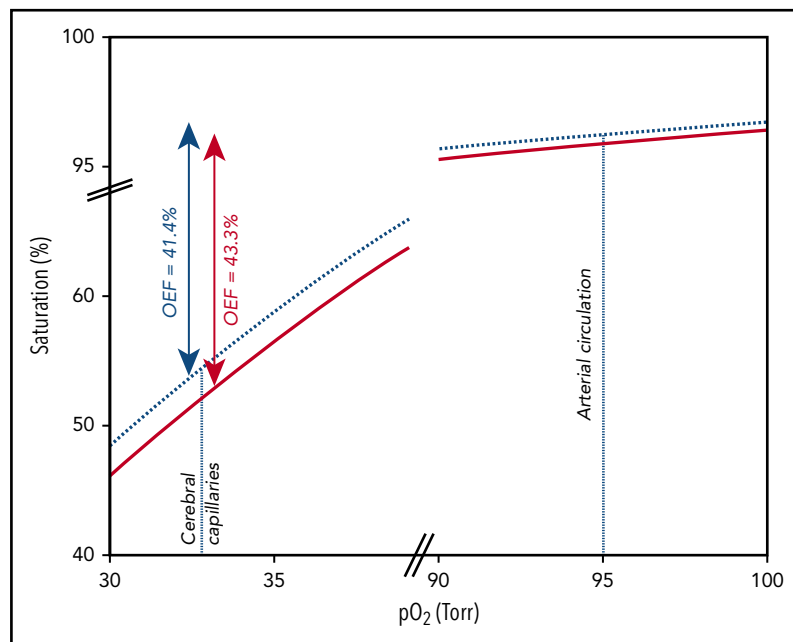
## RED CELLS, IRON, AND ERYTHROPOIESIS

Comment on Fields et al, page 2436

# Brain O<sub>2</sub> reserve in sickle cell disease

John C. Wood | University of Southern California

In this issue of *Blood*, Fields and colleagues demonstrate that patients with sickle cell disease (SCD) on hydroxyurea have lower cerebral oxygen extraction fraction (OEF) than similar patients not receiving disease-modifying therapy.<sup>1</sup> Although hydroxyurea has become the standard of care for young children with SCD, its ability to protect the white matter from silent strokes remains unknown. In the Créteil cohort, SCD patients had access to regular TCD screening since birth, and hydroxyurea was routinely administered to children with frequent vasoocclusive crises. Despite this, the prevalence of silent strokes was 37.4% by the age of 14 in this cohort.<sup>2</sup> With the increasing use of hydroxyurea, in lieu of transfusion therapy, for SCD patients with abnormal transcranial Doppler results,<sup>3</sup> there is appropriate anxiety about hydroxyurea's cerebral protection.



Plot of 2 hypothetical hemoglobin dissociation curves derived from the Hill equation and p50 values of 29.6 (solid line) and 28.6 (dotted line). The horizontal and vertical axes have been interrupted to highlight the small differences in oxygen saturation observed under both arterial and cerebral capillary pO<sub>2</sub> values. OEF can be calculated as the difference in oxygen saturation under arterial oxygen conditions (pO<sub>2</sub> 95 mm Hg) and at the cerebrovascular capillary level (pO<sub>2</sub> 33 mm Hg). The left-shifted hemoglobin dissociation curve (dashed line) yields a lower OEF (41.4%) than the unshifted curve (OEF 43.3%), despite identical tissue pO<sub>2</sub> levels. Thus, OEF, in isolation cannot be considered a marker of cerebrovascular risk.

Cerebral OEF represents the amount of oxygen removed from hemoglobin as it returns from the brain. OEF will increase whenever oxygen delivery is compromised or metabolic activity increases. The difference between resting OEF and maximum OEF is the extraction reserve. Cerebral blood flow will also increase in response to elevated metabolic demand, where the ratio of maximum to resting cerebral blood flow is the cerebrovascular flow reserve. Both of these mechanisms protect the brain from ischemic damage.

The magnetic resonance imaging technique used by the authors, asymmetric spin echo, exploits the magnetic differences between oxygenated and deoxygenated hemoglobin to estimate OEF. It offers similar tissue oxygenation information as provided by cerebral near-infrared spectroscopy, but provides markedly improved spatial localization. Using asymmetric spin echo, the authors have previously documented that silent strokes colocalize with regions of increased tissue OEF<sup>4</sup> in deep white matter structures located in watershed areas. Recent results corroborate these findings: cerebral blood flow and oxygen delivery are lowest in these regions.<sup>5</sup> Tissue OEF is inversely

proportional to hemoglobin level<sup>4,6</sup> and improves following exchange transfusion.<sup>6</sup> The authors conclude that tissue OEF therefore represents a marker of metabolic stress and a direct risk factor for white matter injury.

However, as intuitive and compelling as these arguments are, it is critical to appreciate the challenges of comparing OEF across study populations. The brain regulates its blood flow to maintain adequate oxygen tension (pO<sub>2</sub>), not its blood oxygen saturation.<sup>7</sup> Normal cerebral pO<sub>2</sub> is ~33 mm Hg.<sup>8</sup> The OEF for any given brain pO<sub>2</sub> will be governed by a patient's particular oxygen dissociation curve. For example, consider the 2 hemoglobin dissociation curves (see figure). The dashed curve has a p50 of 28.6 mm Hg, and the solid curve has a p50 of 29.6 mm Hg. At normal arterial oxygen tensions (95 mm Hg), there is <1% saturation difference between the 2 curves. However, the solid curve is significantly more efficient at unloading oxygen to the brain tissue, leading to lower tissue oxygen saturation and higher calculated OEF. Although the extraction reserve is lower for the solid curve (compared with the dashed curve), the flow reserve is higher because

less cerebral blood flow is required to support any given cerebral metabolic demand. Thus, p50 alters the balance between extraction and flow reserves but does not fundamentally change tissue pO<sub>2</sub> (within a homeostatic range).

There are many factors that shift the hemoglobin dissociation curve. Fetal hemoglobin has a p50 ~22.8 mm Hg (left shift), and sickle hemoglobin has a p50 ~30.3 (right shift).<sup>9</sup> Anemia right shifts p50 ~0.3 mm Hg per gram of hemoglobin through upregulation of 2,3-diphosphoglycerate (DPG),<sup>10</sup> whereas transfused blood is left shifted because of 2,3-DPG degradation during storage.<sup>10</sup> In the Fields paper, the hydroxyurea cohort has less anemia, more fetal hemoglobin, and less hemoglobin S than the nonhydroxyurea-treated cohort; thus, a lower OEF is expected based on p50 considerations alone. The curves in were derived using literature p50 values (see figure), and hemoglobin electrophoresis results from the paper, simple mixture assumptions and idealized hemoglobin dissociation parameters for the hydroxyurea treated (dashed curve) and untreated (solid curve) patients. The predicted OEFs of 41.6% and 43.5% are surprisingly close to the observed values of 40.9% and 42.9%, respectively.

Does this mean that the decreased OEF in the Fields study is unimportant? Far from it. Although the hemoglobin left shift with hydroxyurea inhibits oxygen unloading (which could have negative consequences to the brain), it is balanced by the increase in oxygen-carrying capacity due to the elevated hemoglobin levels. Increased hemoglobin in the hydroxyurea-treated patients allows normal brain metabolism despite lower cerebral blood flow and lower OEF, thus preserving both compensatory arms of brain oxygenation.

A key point of this commentary, however, is to emphasize that OEF cannot be considered a marker of brain risk in isolation. If hydroxyurea produced a left shift without its beneficial effects of increased hemoglobin level, it could be deleterious, despite improving OEF values. The brain would be forced to increase its flow to maintain normal pO<sub>2</sub> levels, placing more hemodynamic stress on the vasculature and further exhausting cerebrovascular flow reserve. This is not an idle thought experiment because there

are ongoing clinical trials using agents that increase hemoglobin oxygen affinity. Success of any hemoglobin-dissociation curve modifying agents will depend on their ability to both improve functional oxygen carrying capacity and balance the increased difficulty of oxygen unloading. Changes in OEF can only be interpreted in parallel with changes in brain oxygen delivery,<sup>5</sup> effectively creating maps of tissue oxygen utilization.

A second key point is the emerging theme that anemia, by itself, is fundamentally damaging to the brain. Anemia triggers compensatory cerebral hyperemia, leaving little ability to vasodilate further in response to metabolic stress. Anemia also depletes the oxygen extraction reserve, removing the fastest compensatory response to acute hypoxia. In the present study, and several other works not cited here, hemoglobin level is the key determinant of white matter risk not hemoglobin S percentage. As a result, we may need to reconsider our comfort level with hemoglobin levels previously thought to be safe.

**Conflict-of-interest disclosure:** J.C.W. is a consultant for bluebird bio, WorldCare Clinical, ApoPharma, Celgene, Ionis Pharmaceuticals, Silence Therapeutics, and Biomed Informatics; has received grant funding from the National Institutes of Health, National Heart, Lung, and Blood Institute; and research support in-kind from Philips Healthcare. ■

## REFERENCES

1. Fields ME, Guillems KP, Ragan D, et al. Hydroxyurea reduces cerebral metabolic stress in patients with sickle cell anemia. *Blood*. 2019;133(22):2436-2444.
2. Bernaudin F, Verlhac S, Arnaud C, et al. Impact of early transcranial Doppler screening and intensive therapy on cerebral vasculopathy outcome in a newborn sickle cell anemia cohort. *Blood*. 2011;117(4):1130-1140, quiz 1436.
3. Ware RE, Davis BR, Schultz WH, et al. Hydroxycarbamide versus chronic transfusion for maintenance of transcranial doppler flow velocities in children with sickle cell anaemia-TCD With Transfusions Changing to Hydroxyurea (TWITCH): a multicentre, open-label, phase 3, non-inferiority trial. *Lancet*. 2016;387(10019):661-670.
4. Fields ME, Guillems KP, Ragan DK, et al. Regional oxygen extraction predicts border zone vulnerability to stroke in sickle cell disease. *Neurology*. 2018;90(13):e1134-e1142.
5. Chai Y, Bush AM, Coloigner J, et al. White matter has impaired resting oxygen delivery in sickle cell patients. *Am J Hematol*. 2019;94(4):467-474.
6. Guillems KP, Fields ME, Ragan DK, et al. Red cell exchange transfusions lower cerebral blood flow and oxygen extraction fraction in pediatric sickle cell anemia. *Blood*. 2018;131(9):1012-1021.
7. Morikawa T, Kajimura M, Nakamura T, et al. Hypoxic regulation of the cerebral microcirculation is mediated by a carbon monoxide-sensitive hydrogen sulfide pathway. *Proc Natl Acad Sci USA*. 2012;109(4):1293-1298.
8. Charbel FT, Hoffman WE, Misra M, Ausman JI. Increased brain tissue oxygenation during arteriovenous malformation resection. *Neurol Med Chir (Tokyo)*. 1998;38(Suppl):171-176.
9. Young RC Jr, Rachal RE, Del Pilar Aguinaga M, et al. Automated oxyhemoglobin dissociation curve construction to assess sickle cell anemia therapy. *J Natl Med Assoc*. 2000;92(9):430-435.
10. Sohmer PR, Dawson RB. The significance of 2,3-DPG in red blood cell transfusions. *CRC Crit Rev Clin Lab Sci*. 1979;11(2):107-174.

DOI 10.1182/blood-2019-04-901124

© 2019 by The American Society of Hematology