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A better biomarker for cerebral malaria: In the eye of the beheld?*

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In the developed world, cerebral malaria among travelers or immigrants from malaria-endemic countries is an uncommon cause for intensive care unit admission (1); however, in the developing world, millions of children develop cerebral malaria each year, and the mortality can be 10% to 30% (2, 3). Most children are treated at local health centers, and the health practitioner must diagnose cerebral malaria without the aid of neuroimaging or even basic clinical or microbiological laboratory tests. A key advancement in the diagnosis of cerebral malaria was the Blantyre Coma Score, a six-point summation of a child's vocal and motor responses to a painful stimulus and ability to track visually (4). This score is associated with mortality and is used not only in research studies, but also in clinical practice (5, 6). Cerebral malaria is typically defined as impaired consciousness, not attributable to hypoglycemia or seizure, in a patient with *Plasmodium falciparum* on a peripheral blood smear. Although this definition identifies children at high risk for death, it may also include those who have coma from other causes such as meningitis or intoxication and in whom the parasitemia is incidental.

When the clinical diagnosis of cerebral malaria was compared with postmortem examination of children who died from presumed cerebral malaria, the results revealed that greater than 20% of children thought to have cerebral malaria actually died from nonmalarial causes (7). Although the clinical diagnosis of cerebral malaria may have lacked specificity, bedside retinal examination findings matched almost perfectly with the autopsy findings of parasites sequestered in the brain thought to represent a key step in the pathogenesis of cerebral malaria. Nineteen of 20 patients with autopsy-confirmed cerebral malaria had retinopathic changes, whereas among ten patients lacking parasite sequestration on autopsy (three who had been clinically defined as cerebral malaria and seven with nonmalarial causes of coma), only one had any features of retinopathy. This roughly translates into a sensitivity of 95%

*See also p. 919.

and a specificity of 90% for the presence of retinopathy. In follow-up studies, retinopathy has been prospectively evaluated as a prognostic indicator of fatal outcome (8); however, the use of this important diagnostic examination has not yet been widely adopted in clinical practice.

In the this issue of *Critical Care Medicine*, Conroy et al (9) compared the diagnostic performance of serological markers against the retinal examination (performed by experts) as a gold standard definition of cerebral malaria. They chose to analyze three markers that were previously associated with increased risk of death from cerebral malaria: angiopoietin-1, angiopoietin-2, and sTie-2.

The angiopoietins regulate endothelial barrier integrity in the setting of inflammation, and so may be true biological mediators of cerebral endothelial pathology, not just good markers. Angiopoietin-1 is constitutively expressed by endothelial cells and binds to the receptor Tie-2 where it transduces signals that oppose endothelial cell apoptosis, promote angiogenesis, and maintain endothelial tight junctions (10). Angiopoietin-2, released preformed by exocytosis of Weibel-Palade bodies, binds to endothelial Tie-2 but does not transduce a signal, thereby acting as an antagonist of angiopoietin-1 (11, 12). Angiopoietin-2 permits breakdown of endothelial barrier integrity and expression of endothelial adhesion molecules such as intercellular adhesion molecule-1 in the setting of tumor necrosis factor stimulation (13). These angiopoietin-2-mediated steps are essential for firm adhesion of neutrophils and migration of neutrophils into tissue. The exocytosis of angiopoietin-2 from Weibel-Palade bodies might contribute to the vascular leak, inflammation, and cerebral edema associated with cerebral malaria. Clinical studies of malaria have shown that angiopoietin-2 is associated with decreased nitric oxide bioavailability, severity of malaria infection, retinopathy, and death (14–17).

In this most recent study, Conroy et al calculated the sensitivity and specificity of angiopoietin-2 as a diagnostic marker of malaria retinopathy by analyzing the levels of angiopoietin-2 in children clinically classified as cerebral malaria, comparing those with and without retinopathy. Angiopoietin-2 serum concentrations were significantly elevated in children with retinopathy and correlated with specific retinopathic findings such as the severity of hemorrhages, retinal whitening, and the presence of nonperfused vessels. When analyzed as a dichotomous variable (angiopoietin-2 >3.85 ng/mL), angiopoietin-2 was associated with increased odds of malarial retinopathy and increased odds of death, an association that withstood adjustment for age, respiratory distress, Blantyre Coma Score, and the presence of severe anemia. One of the strengths of this study was the analysis of hard end points such as retinopathy and death; however, this particular study population represented a severe spectrum of cerebral malaria with a mortality of 38% and therefore may not be generalizable to other settings where lower mortality rates are seen.

Identification of a good prognostic marker can require striking a compromise between sensitivity and specificity. The intersection of sensitivity and specificity decision plots is usually a good place to start. In this study, a concentration of angiopoietin-2 of approximately 6 ng/mL seems to perform moderately well (60%) in terms of both sensitivity and specificity. However, the regression tree models used to ascertain the optimal cutoff value for angiopoietin-2 to predict death (3.85 ng/mL) appeared to penalize the false-negative rate, increasing the sensitivity to >90% at the expense of specificity, which fell to <40%. The inclusion of additional clinical variables did not improve this specificity.

How might angiopoietin-2 perform as an end point for interventional studies in cerebral malaria? Angiopoietin-2 has been proposed as an end point for a clinical therapeutic trial of inhaled nitric oxide that aims to restore endothelial cell quiescence during acute cerebral

malaria (18). Exocytosis of Weibel-Palade bodies containing angiopoietin-2 is negatively regulated by nitric oxide, and therefore patients treated with antimalarial drugs plus inhaled nitric oxide are expected to have a more rapid reduction in angiopoietin-2 than patients receiving antimalarial drugs plus placebo. It is a reasonable end point for a pilot study of this promising therapy and should indicate whether inhaled nitric oxide affects microvascular endothelium in patients with malaria. However, given the low specificity of angiopoietin-2 as a predictor of death, it might be hard to predict whether a therapy that lowers angiopoietin-2 will have an effect on outcome until mortality is directly measured in larger studies in the future.

Should angiopoietin-2 be incorporated into the clinical management of cerebral malaria? Point-of-care testing could enable rapid bedside determination of angiopoietin-2 levels, but an elevated level would not be a specific predictor of death. In a resource-limited setting, a test with high specificity is desirable because it allows identification of a smaller number of high-risk individuals for more intensive monitoring or intervention. For both clinical practice and clinical research, the retinal examination will likely remain our clearest window into the soul of cerebral malaria pathology.

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