Accurate Diagnosis of Cerebral Malaria: A Role for Parasite Histidine-Rich Protein 2?

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(See the article by Seydel et al, on pages 309–18.)

The World Health Organization (WHO) definition of cerebral malaria requires *Plasmodium falciparum* parasitemia and coma not attributable to convulsions, sedatives, hypoglycemia, or another detectable non-malarial cause [1]. In a series of elegant autopsy-based studies, Taylor and colleagues demonstrated that as many as 23% of children who meet this WHO definition of cerebral malaria actually die from other causes and that the presence of malaria retinopathy has a >90% sensitivity and specificity for predicting “true” cerebral malaria, in which parasite sequestration is documented in cerebral capillaries [2]. Assessment of malaria retinopathy in cerebral malaria has led to significant research advances, but the assessment requires highly specialized training and equipment, so it has not been practical in clinical settings in most low-income countries.

Parasite histidine-rich protein 2 (pHRP2) is produced by *P. falciparum* throughout its life cycle [3]. It is released from infected erythrocytes as a water-soluble protein [4]. In areas of low to moderate transmission, plasma concentrations of pHRP2 appear to correspond well to body parasite biomass [5]. Elevated pHRP2 concentrations may, therefore, reflect a high level of sequestered parasites. In the current issue of the journal, Seydel and colleagues provide evidence that high pHRP2 concentrations are an excellent marker for biopsy- or retinopathy-confirmed cerebral malaria [6]. The study design has several strengths that boost confidence in the accuracy of its findings, including the use of 3 study groups, which allowed for initial testing, establishment of an optimized pHRP2 cutoff level, and prospective assessment of the sensitivity and specificity of that cutoff level. The first study group consisted of children with WHO-defined cerebral malaria who died. The pHRP2 concentrations in children with autopsy-confirmed parasite sequestration were compared with the pHRP2 concentrations in those without sequestration. The pHRP2 concentrations distinguished almost perfectly between the 2 groups (area under receiver operating characteristic curve, 0.98). The second study group consisted of children from an earlier study who had WHO-defined cerebral malaria and who did (cases) or did not (controls) have malaria retinopathy. The pHRP2 concentrations in this group were used to identify a cutoff pHRP2 concentration that yielded optimal sensitivity and specificity for malaria retinopathy. The third study group was a cohort of children with WHO-defined cerebral malaria who were examined prospectively for malaria retinopathy. The pHRP2 cutoff concentration established in the second study group was assessed in this third study group. The established pHRP2 cutoff concentration (1700 ng/mL) had a positive predictive value of 94% and a negative predictive value of 79% for malaria retinopathy. With these positive and negative predictive values, a pHRP2 value above the cutoff in a child with a diagnosis of cerebral malaria would greatly strengthen confidence in the diagnosis, whereas a value below the cutoff might prompt more vigorous investigation of other potential causes of coma. Because almost a quarter of children classified as having cerebral malaria actually have other reasons for coma, a pHRP2 cutoff test may prompt the clinician to seek and address other diagnoses early in the disease process. This application is one for which a simple pHRP2 concentration-based test holds real promise as a tool that could improve disease outcome.

So, are we ready to move to field use of a pHRP2 concentration-based test to identify true cerebral malaria in children and adults with *P. falciparum* parasitemia and coma? Not quite yet. First, other studies are needed to replicate the findings reported here by Seydel et al. In particular, it will be important to assess whether findings are similar in older children and adults in Southeast Asia who develop cerebral malaria. Second, uniform protocols for measurement of
pHRP2 will need to be agreed upon, as multiple testing methods and protocols currently exist. The cutoff of 1700 ng/mL proposed might not be appropriate for a test using different pHRP2 standards or antibodies. Ideally, identical or highly similar testing kits and protocols should be used in the comparator studies. Third, the presence of pHRP2 deletions must be sought in areas where testing is conducted or proposed. Parasite populations that lack pHRP2 have been documented in the Amazon [7], and populations with deletions in the histidine-rich repeat region of the hrp2 gene have been reported in Mali [8]. In these areas, pHRP2 concentrations may be low or unmeasurable, and a test of pHRP2 concentration would be useless. This potential obstacle underscores the importance of confirmatory studies in other malaria-endemic areas. Furthermore, in areas of high malaria transmission, pHRP2 concentration may reflect recent as well as current infections and may not predict disease severity [9]. Thus, in areas of high transmission, assessment of pHRP2 concentration may have little utility in the diagnosis of cerebral malaria. However, because most cerebral malaria occurs in areas of low to moderate and unstable transmission, this possibility is not a major concern. Finally, the study by Seydel et al was designed specifically to differentiate in children with *P. falciparum* parasitemia and coma those who were retinopathy positive (presumed true cerebral malaria) from those who were retinopathy negative (presumed to have another cause of coma). Children with other forms of severe malaria may have significant parasite sequestration and high pHRP2 concentrations. The findings of the study by Seydel et al do not support the use of a high pHRP2 concentration to differentiate cerebral malaria from other forms of severe malaria—that is a clinical decision—but rather support its use to improve accuracy of the diagnosis of cerebral malaria in the child with *P. falciparum* parasitemia and coma. With all of these caveats, high-concentration pHRP2 is a great candidate for a rapid diagnostic test (RDT), because pHRP2 is already the basis of RDTs widely used in low-income countries for diagnosis of clinical *P. falciparum* malaria [10]. If current pHRP2 RDTs can be modified to detect pHRP2 above a specific concentration, a high-concentration pHRP2 RDT could be available for practical use in hospitals in low-income country in a relatively short period of time.

It will take a few years and several more studies to know whether a point-of-care test based on a cutoff concentration of pHRP2 can improve the diagnosis of cerebral malaria in the field. It is likely that this test will not be useful in all areas where *P. falciparum* malaria is endemic. But to date no other biomarker for cerebral malaria has demonstrated the high positive and negative predictive values seen for pHRP2 in the study by Seydel et al. A test of pHRP2 concentration could be the first field-based test since microscopy to improve the diagnosis of cerebral malaria. That potential makes this study an exciting new contribution to research on severe malaria.

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