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Spotlight

Reducing misclassification bias in severe malaria research

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By incorporating platelets and *Pf*HRP2 into standard case definitions of severe falciparum malaria, Watson et al.[1] increased diagnostic specificity to 93%. The approach trades off sensitivity and might introduce a selection bias but offers a helpful way to handle misclassification.

Severe malaria, which continues to claim over a half million lives annually, has diverse clinical presentations that lack any pathognomonic feature, thus defying reliable diagnosis in highly malarious areas of Africa where incidental parasitemia is common. Because it can resemble other severe illnesses—both infectious and non-infectious, involving virtually any organ system—clinicians and researchers have no easy way to determine whether a given patient's parasitemia indicates true malarial pathophysiology or is a bystander to a non-malarial cause of severe disease.

The conventional case definition of severe malaria relies on one or more of several clinical and laboratory findings in the presence of *Plasmodium falciparum* parasitemia and in the absence of an alternative cause. For several reasons, such as the under-resourcing of clinical laboratories in high-burden areas and the imperfect sensitivities of the tools that are available (e.g., bacterial blood culture systems), it is usually not possible to evaluate every criterion or to reliably exclude even the most common "alternative causes."

This poses a major problem in studies of severe falciparum malaria in moderate- and high-transmission areas, where it is estimated that as many as one-third of cases may be misattributed to malaria. Studies of interventions, disease mechanisms, and epidemiology, or those that measure severe malaria as an outcome (such as vaccine or other preventive studies), are therefore vulnerable to an information bias that can distort, dilute, or conceal true relationships in the observed data.

Methods to improve the specificity of severe malaria research definitions are often guided by expert opinion and lack consensus. For example, early World Health Organization guidelines set a threshold of parasitemia to diagnose severe malarial anemia for research purposes, which was discarded in later editions.[2] Others since then have incorporated additional laboratory or bedside metrics, imaging, and autopsy validations.

In the latest issue of *Science Translational Medicine*, Watson et al. reported on the diagnostic test characteristics of adding platelet count and *P. falciparum* histidine-rich protein II (*Pf*HRP2) concentration to conventional case definitions of severe malaria.[1] Prior work by some of the same investigators described the diagnostic use of *Pf*HRP2 alone and platelets in conjunction with leukocytes.[3,4] In this new study, they are the first to apply latent class analysis to the problem, a widely accepted statistical approach to estimating diagnostic accuracy in the absence of a gold standard. Using data from four malaria cohorts totaling 2,622 patients, the investigators estimated a diagnostic sensitivity of 74% and specificity of 93% when applying a platelet count threshold of $\leq$150,000/μL and *Pf*HRP2 threshold of $\geq$1,000 ng/mL.

The utility of platelet count and *Pf*HRP2 in boosting diagnostic specificity for severe malaria reflects newly discovered aspects of malaria pathobiology. Platelets are increasingly recognized for their role not only in malaria vascular pathogenesis but in the immune response,[5] and although it has long been known that mature *P. falciparum* forms sequester in the end organ capillaries, hidden from peripheral smear review, more recently it was also shown by Kho et al. that a vast portion of the infecting parasites can be contained, intact, in the extravascular spaces of the spleen.[6] Platelet counts therefore provide a measure of the host response, while *Pf*HRP2 concentrations more accurately assess the parasite biomass than peripheral parasite densities.

With the study's several strengths are important caveats. The authors' approach, which they developed for severe malaria diagnosis in African children, mixed pediatric and adult patients from Kenya, Uganda, and Bangladesh. The rationale for including the Bangladeshi cohort was its low transmission dynamics; however, there are low transmission settings in Africa that could have contributed the data needed to triangulate their modeled estimates. The authors report that a sensitivity analysis excluding the Bangladeshi cohort yielded similar results to the main analysis, so it is not clear what the inclusion of that group added to the analysis and at what potential cost to validity in the target population.

Validity also depends on model design. Latent class models are highly sensitive to their specifications such that small adjustments to model parameters can lead to large differences in estimation.[7] The

authors sourced their parameter specifications for platelet count and *Pf*HRP2 from two separate studies, both single-center, set in Malawi and Tanzania.[4,8] Parameterization of models using literature estimates is a delicate exercise, even more fraught in the malaria field because of the paucity of data.

Screening out non-malarial causes of severe disease achieves the goal of reducing misclassification bias but might come at the expense of introducing a selection bias. Watson et al. estimate that their approach will exclude as many as one in three patients with true severe malaria. How this group might differ from the included group needs follow-up; severe malaria is clinically and pathologically diverse, and different phenotypes may differ mechanistically or respond differently to interventions in meaningful ways.

Malaria is grievously understudied and underfunded for a disease of such historical and lethal proportion. The number of scientific papers on malaria is a fraction of those for the other major infectious diseases, and only half its target research and programming budget is reached each year despite mounting evidence of a backslide in progress and rise in parasite drug resistance.[9] Watson et al. have helped move the dial by delivering a simple, elegant tool for refining the precision and reproducibility of malaria research at a particularly vulnerable time.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

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