Assessing and Minimizing the Effect of Malaria on SARS-CoV-2 Serodiagnostics

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Malaria may affect the reliability of SARS-CoV-2 seroassay performance and limit understanding of SARS-CoV-2 epidemiology in malaria-endemic regions. We present our experience conducting SARS-CoV-2 serosurveillance in seasonal malaria-affected communities in Mali and discuss relevant literature regarding the effect of malaria on the performance of SARS-CoV-2 serodiagnostics, including approaches to minimize the effect of malaria-associated assay interference.

Keywords: malaria, COVID-19, SARS-CoV-2, serology, antibody, cross-reactivity

INTRODUCTION

There are many possible interfaces for COVID-19 and malaria in malaria-endemic regions, including pandemic-related program disruptions, shifts in clinical disease burden, and the effect of malaria on SARS-CoV-2 test performance (1, 2). In partnership with the Malian Ministry of Health, the Malaria Research and Training Center (MRTC) and the National Institutes of Health sought to assist in the public health response to COVID-19 in Mali. MRTC maintains clinical and research laboratory infrastructure in many regions of Mali to develop tools and strategies for malaria control and elimination. This infrastructure was leveraged to develop COVID-19 serosurveillance testing capacity, and forms part of a large in-country response to swiftly redirect existing research resources to address the pandemic (3). It is critical to ensure that serosurveillance tools provide reliable data for use in the local public health response. We discuss relevant literature regarding the effect of malaria on the performance of SARS-CoV-2 serodiagnostics, present our experience qualifying assay performance and conducting SARS-CoV-2 serosurveillance in seasonal malaria-affected communities in Mali, West Africa (4, 5), present new data regarding the effect of recent malaria infection on SARS-CoV-2 serostatus, and outline approaches to optimize assay performance in malaria-endemic regions.

MALARIA AND SARS-COV-2 SERODIAGNOSTICS

For serological surveillance to be useful in the public health response to the COVID-19 pandemic, test selection and validation in the target population must be addressed. High rates of false-positivity have been described for multiple commercial SARS-CoV-2 serological assays in sub-Saharan Africa

(6–11), and in other regions (12, 13), conceivably due to cross-reactivity with other coronaviruses or other endemic infections, including malaria. This is not a new phenomenon, for example high rates of false-positivity in HIV antibody tests have been previously described in Africa (14–16). Poor SARS-CoV-2 test specificity, particularly in the low or unknown prevalence setting, risks overestimation of community burden and may cause unnecessary harm, including diversion of limited public health resources and inaccurate estimations of population-level exposure or immunity. To ensure reliable serosurveillance data, the primary aim of assay qualification must be understanding the pattern and degree of background reactivity to SARS-CoV-2 antigens in order to optimize assay performance, while understanding the nature and causes of this reactivity an important secondary aim. As a result, we conducted extensive assay qualification to optimize test performance in Mali prior to conducting SARS-CoV-2 community serosurveillance (5). This included evaluating negative control samples collected prior to 2020 for background IgG reactivity to commonly tested SARS-CoV-2 antigens, other betacoronaviruses, and a panel of *P. falciparum* antigens, assessing *in vitro* SARS-CoV-2 pseudovirus neutralizing activity in negative controls, and evaluating the performance of several test configurations using local positive and negative controls.

While there has been minimal correlation between exposure to other human coronaviruses and SARS-CoV-2 seropositivity in pre-pandemic samples (5, 6, 11, 12, 17), malaria has been variably associated with poor assay performance (5, 7, 9, 17). In malaria-endemic regions including Mali, background antibody reactivity to SARS-CoV-2 antigens is common, increases with age group, and varies regionally (5, 17). Increased reactivity with age may suggest cumulative exposures may be responsible for background signal, and that assay performance may vary in different age groups. Background SARS-CoV-2 antigen reactivity may also vary seasonally, although year-round samples were not available for assessment in a Malian community with highly seasonal malaria transmission (**Supplementary Figure 1**). These observations implicate malaria as a potential confounder when considering SARS-CoV-2 seroassay performance.

Several mechanisms for malaria-associated SARS-CoV-2 antigen cross-reactivity have been proposed. SARS-CoV-2 nucleocapsid antibody reactivity by commercial assay has been associated with the presence of IgG antibodies to some *Plasmodium* sp. antigens, and several other neglected tropical diseases (9), although this is not consistent between SARS-CoV-2 assays. We did not demonstrate any substantial correlation between SARS-CoV-2 antigen reactivity using our reference ELISA and a panel of 11 *P. falciparum* antigens responsible for both short-lived and long-lived serological response following malaria infection (5, 18). A weak positive correlation has been reported between the same SARS-Cov-2 antigen constructs and AMA-1 ELISA reactivity in malaria-experienced Cambodian samples (12). While it remains possible that specific antibodies to *Plasmodium* sp. antigens could cross-react with SARS-CoV-2 antigens, the effect may not be substantial or predictable across

assays. Non-specific polyclonal antibodies arising from acute malaria may also contribute to poor assay performance. Symptomatic and asymptomatic malaria have been associated with transient SARS-CoV-2 spike protein antibody reactivity, attributed to cross-reactive antibodies targeting N-linked glycans (17). Additionally, commercial spike protein and nucleocapsid based SARS-CoV-2 assay false-positivity has been associated with higher parasitemia malaria infections, although the presence of malaria alone versus no malaria did not reach statistical significance in this study (7). While acute malaria may affect SARS-CoV-2 assay performance, similar to cross-reactive *Plasmodium* sp. antibodies, this phenomenon may not be consistent across all groups and assays. As a result, there may not be a single distinct mechanism through which malaria induces SARS-CoV-2 assay reactivity. Irrespective of the stimulus, antibodies responsible for SARS-CoV-2 antigen background reactivity in malaria-endemic areas do not demonstrate functional activity *in vitro* (5, 7, 12, 17).

OPTIMIZING TEST PERFORMANCE IN MALARIA-ENDEMIC AREAS

The concept of malaria-associated cross-reactivity presents several challenges to SARS-CoV-2 assay implementation, where performance must be balanced with cost and the timeliness of results. Specific collaboration with groups conducting intensive malaria studies may be warranted to obtain control samples and address the issue of malaria-associated assay interference. Ultimately any testing approach must be pragmatic and suited to local conditions and available resources. Approaches that may improve test performance include: comparison of different antigens to select constructs with lower background reactivity (5, 11, 13, 17), cutoff adjustment to set population-specific thresholds or relative to local background reactivity (5, 19), dual antigen testing (5, 19, 20), and avidity testing to eliminate low-affinity cross-reactive antibodies (9) (**Table 1**). Groups responsible for testing in malaria-endemic regions should consider determining background reactivity/assay false positivity rates in pre-pandemic samples and make compensatory adjustments if required before implementing high volume testing. This approach is outlined in **Figure 1** and may include selection of one or several methods to optimize test specificity.

In our study, a two-antigen ELISA assessing mammalian cell-expressed SARS-COV-2 spike protein and RBD antigens with population-specific cutoffs was selected based on the performance of this configuration in a Malian control population and the availability of in-country laboratory facilities and expertise (4, 5). Background ELISA reactivity in Malian pre-pandemic samples was most pronounced to SARS-CoV-2 nucleocapsid protein. While spike protein and RBD signals were comparatively lower, neither was sufficiently specific for a single-antigen approach without using prohibitively high assay cutoffs (5). Interestingly, there was

TABLE 1 | Effect of several approaches to improve SARS-CoV-2 serology test performance in malaria-endemic regions.

Reference	Population	Assay(s)	Approach	Sensitivity range (%)	Specificity range (%)	Maximum relative improvement in false positive rate
Emmerich et al. (11)	Ghana (NC n=283) Nigeria (NC n=150)	Various commercial				Ghana: 3.9 Nigeria: 6.5
			EuroImmun IgG (NCP) ELISA	N/A	Ghana: 83.0 Nigeria: 72.0	
			EuroImmun IgG (spike) ELISA	N/A	Ghana: 94.3 Nigeria: 90.7	
			EDI IgG (NCP) ELISA	N/A	Ghana: 77.7 Nigeria: 39.3	
			Mikrogen IgG (NCP) ELISA	N/A	Ghana: 89.7 Nigeria: 82.7	
Cota et al. (13)	Brazil	Various commercial				24.5
	(NC n=116, PC n=173)		Vircell IgG (spike and NCP) ELISA	76.8	53.4	
	(NC n=116, PC n=109)		EuroImmun IgG (NCP) ELISA	58.7	95.8	
	(NC n=116, PC n=166)		Mbiolog Allserum IgG ELISA	60.2	98.1	
Woodford et al. (5)	Mali (NC n=312, PC n=23)	In-house IgG (RBD) ELISA	Cutoff adjustment	78.3-82.6	76.5-94.5	4.3
		In-house IgG (spike) ELISA	Cutoff adjustment	82.6-82.6	92.9-95.5	1.6
		In-house IgG ELISA	Two-antigen*	73.9-82.6	95.5-99.4	7.5
Uyoga et al. (20)	Kenya (NC n=900, PC n=147)	In-house IgG (RBD) ELISA	Cutoff adjustment	80.5-91.3	88.3-99.0	11.7
		In-house IgG (spike) ELISA	Cutoff adjustment	92.5-94.3	90.0-99.0	10.0
		In-house IgG ELISA	Two-antigen*	91.4-94.3	99.0-99.7	3.3
Steinhardt et al. (9)	Nigeria (NC n=213, PC n=32)	EuroImmun IgG (NCP) ELISA	Urea wash (0M to 4M)	72.7-95.5	84.1-98.5	10.6

NC, pre-pandemic negative controls; PC, PCR-confirmed positive controls; RBD, receptor binding domain; NCP, nucleocapsid protein; ELISA, enzyme linked immunoassay.
*Comparison between two-antigen and single-antigen spike approach using same cutoffs. N/A, positive control cohort not assessed.

minimal correlation between SARS-CoV-2 antigen reactivity in pre-pandemic samples (5), consolidating the concept that a two-antigen approach may be useful to minimize background signal.

To understand the practical effect of seasonal malaria on the performance of our assay, we analyzed our Malian serosurveillance data with respect to malaria transmission at a community and individual level. Overall, SARS-CoV-2 community seroprevalence was inversely proportional to seasonal malaria transmission intensity. Both before and after the 2020 malaria season, SARS-CoV-2 seroprevalence was consistently higher urban areas compared to rural regions with higher entomological inoculation rates (4) (**Supplementary Text**). In a population of 1037 individuals of all ages that were SARS-CoV-2 seronegative at the beginning of the 2020 malaria season, subjects underwent comprehensive malaria monitoring as part of an MRTC clinical trial supported by EDCTP (21) at the rural village of Donéguébougou. In this cohort, 33.3% (345/1037) experienced at least one episode of acute malaria between serosurvey visit 1 and visit 2, and 24.3% (252/1037) demonstrated SARS-CoV-2 two-antigen seroconversion during the malaria season. There was no difference in the rate of malaria diagnosis in the SARS-CoV-2 seropositive group compared to the seronegative group (34.9% (88/252) vs 32.7% (257/785), Fisher exact test p=0.54). In a multiple logistic regression model including age, an episode of acute malaria was not

associated with SARS-CoV-2 seropositivity (OR 1.13, 95% CI: 0.83-1.53) (**Figure 2A**). In the subset of 345 individuals experiencing at least one acute malaria episode, both SARS-CoV-2 spike protein and RBD reactivity were not correlated with time since recent malaria diagnosis (Spearman r=-0.04, p=0.46 for spike protein and r=-0.04, p=0.52 for RBD respectively) (**Figure 2B**). Furthermore, in a multivariate analysis of these cases, time since most recent malaria diagnosis (OR 1.00, 95% CI: 0.99-1.01), and number of acute malaria episodes since visit 1 (OR 0.86, 95% CI: 0.51-1.40) were not associated with SARS-CoV-2 seropositivity at visit 2. Paired with the overall observation that SARS-CoV-2 seroprevalence was lowest in communities with the most intense malaria transmission (4) (**Supplementary Text**), the lack of association between recent acute malaria and SARS-CoV-2 serostatus suggests the two-antigen reference ELISA selected for community serosurveillance in Mali minimizes the possible interference of seasonal malaria on SARS-CoV-2 antibody testing.

DISCUSSION

To understand interactions between COVID-19 and malaria it is critical that reliable SARS-CoV-2 surveillance is available in
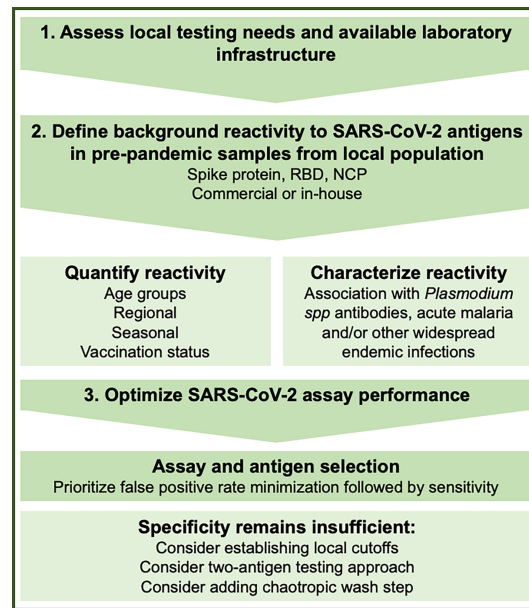
FIGURE 1 | Considerations to understand and minimize the effect of SARS-CoV-2 serology background reactivity in malaria endemic regions.
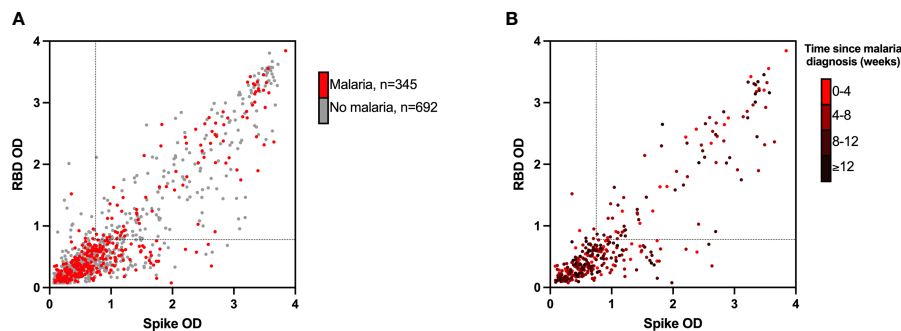

FIGURE 2 | Assay absorbance values (OD) for RBD (y-axis) and spike protein (x-axis) at visit 2 **(A)** in all 1037 participants undergoing intensive malaria surveillance and **(B)** in 345 participants experiencing at least one acute malaria episode, stratified by time since time since malaria diagnosis. RBD, receptor binding domain; OD, optical density. Dotted lines represent assay cutoffs for SARS-CoV-2 seropositivity. In panel **(B)** darker data points represent a longer time since most recent malaria diagnosis (weeks).

malaria-endemic regions. In many cases, these regions may be both disproportionately affected by uncertain assay performance and most in need of reliable serological tools due to limited access to gold-standard molecular diagnostics. Careful assay qualification is required prior to use, particularly where demographics and exposure histories differ significantly from the original assay validation population (5, 22). While it appears that a spike-based assay is likely to have better specificity compared to other antigens, we are unable to recommend a specific SARS-CoV-2 serological assay for use in malaria-endemic regions, as there are limited head-to-head studies, performance varies regionally, and there may be other local confounding factors that need to be addressed. Rather, a tailored

approach is needed based on the target population, where one or several of the approaches presented above are likely to assist in test optimization. By using a two-antigen test, adjusting seropositivity thresholds, and reviewing the effect of intercurrent malaria on serostatus following implementation, we believe that we have minimized the effect of malaria on SARS-CoV-2 seroprevalence interpretation in Mali.

Reliable SARS-CoV-2 serosurveillance in communities with intense seasonal malaria transmission allows for a better understanding of the twin burdens of COVID-19 and malaria in Mali. We have identified high rates of seasonal malaria and COVID-19 infection in Mali, including many individuals with evidence of adjacent or concurrent infections. Despite rapidly

increasing seroprevalence, we did not identify a large burden of COVID-19 attributable clinical disease in community serosurveillance (4). Combined malaria and SARS-CoV-2 surveillance allows for the informed allocation of resources to fight the emergent COVID-19 virus and the long-standing malaria epidemic. Understanding of the COVID-19 pandemic in Mali may also allow for the safe continuation of the malaria elimination agenda, and avoidance of excess malaria mortality due to program disruptions.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Deidentified data collected for this study may be made available to others after approval of and with a signed data access agreement.

ETHICS STATEMENT

The studies contributing to this manuscript were reviewed and approved by the USTTB FMOS/FAPH ethics committee and the NIH IRB. Written informed consent or assent was obtained from all study participants.

AUTHOR CONTRIBUTIONS

JW collated and analyzed the data and drafted the manuscript. All authors contributed to the conception and design of the studies contributing data to the analysis. All authors contributed to manuscript revision, and approved the submitted version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fitd.2021.781586/full#supplementary-material

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