Potential Antigenic Cross-reactivity Between Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) and Dengue Viruses

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Background. Coronavirus disease 2019 (COVID-19) and dengue fever are difficult to distinguish given shared clinical and laboratory features. Failing to consider COVID-19 due to false-positive dengue serology can have serious implications. We aimed to assess this possible cross-reactivity.

Methods. We analyzed clinical data and serum samples from 55 individuals with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. To assess dengue serology status, we used dengue-specific antibodies by means of lateral-flow rapid test, as well as enzyme-linked immunosorbent assay (ELISA). Additionally, we tested SARS-CoV-2 serology status in patients with dengue and performed in-silico protein structural analysis to identify epitope similarities.

Results. Using the dengue lateral-flow rapid test we detected 12 positive cases out of the 55 (21.8%) COVID-19 patients versus zero positive cases in a control group of 70 healthy individuals ($P = 2.5E{-}5$). This includes 9 cases of positive immunoglobulin M (IgM), 2 cases of positive immunoglobulin G (IgG), and 1 case of positive IgM as well as IgG antibodies. ELISA testing for dengue was positive in 2 additional subjects using envelope protein directed antibodies. Out of 95 samples obtained from patients diagnosed with dengue before September 2019, SARS-CoV-2 serology targeting the S protein was positive/equivocal in 21 (22%) (16 IgA, 5 IgG) versus 4 positives/equivocal in 102 controls (4%) ($P = 1.6E{-}4$). Subsequent in-silico analysis revealed possible similarities between SARS-CoV-2 epitopes in the HR2 domain of the spike protein and the dengue envelope protein.

Conclusions. Our findings support possible cross-reactivity between dengue virus and SARS-CoV-2, which can lead to false-positive dengue serology among COVID-19 patients and vice versa. This can have serious consequences for both patient care and public health.

Keywords. COVID-19; dengue; West Nile.

Coronavirus disease 2019 (COVID-19), the illness caused by the recently identified severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has rapidly spread worldwide. As of 1 August 2020, over 10 million patients have been reported globally (https://covid19.who.int/). The symptoms of SARS-CoV-2 infection vary widely, from asymptomatic disease to multisystem organ failure [1, 2].

Symptoms of COVID-19 can also be nonspecific and be misdiagnosed. For instance, both dengue fever and COVID-19 are difficult to distinguish as they share similar clinical and laboratory features [3–5]. Moreover, in 2 cases from Singapore, it has been reported that rapid serological testing for dengue can show false-positive results among COVID-19 patients [6]. This clinical diagnostic challenge is particularly important in regions around the world with dual outbreak of both COVID-19 and dengue [7, 8].

The global burden of dengue has grown dramatically in recent decades as half of the world's population lives in dengue-endemic areas [9]. Approximately 100–400 million cases occur each year (https://www.who.int/en/news-room/fact-sheets/detail/dengue-and-severe-dengue). Laboratory diagnosis of dengue is established directly by detection of viral components in serum or indirectly by serologic means.

During the febrile phase detection of the virus-expressed soluble nonstructural protein 1 (NS1) or dengue-specific immunoglobulin M (IgM) antibodies by means of enzyme-linked immunosorbent assay (ELISA) or the lateral-flow rapid test is sufficient for a confirmatory diagnosis [9]. Lateral-flow based tests are the most prevalent diagnostic method especially in dengue endemic countries because they do not require special knowledge or equipment and are not affected by high ambient tropical temperatures or humidity [10, 11]. Given that a possible cross-reactivity between dengue virus and SARS-CoV-2 could lead to false-positive results for both diseases, we aimed to assess the magnitude of such possible-cross reactivity in a large cohort of individuals who were known to have either SAR-CoV-2 or dengue virus.

METHODS

Study Participants

We included in the study clinical data and serum samples obtained from individuals with proven SARS-CoV-2 infection who were tested for COVID-19 in the Israeli Ministry of Health Central Virology Laboratory. The assay is based on the World Health Organization standard. It targets the SARS-CoV-2 envelope (E), N and RdRp genes [12]. Diagnosis was based on nasopharyngeal swabs, which were tested for SARS-CoV-2 by reverse transcription polymerase chain reaction. Only laboratory-confirmed cases were included. The institutional review board of Sheba Medical Center approved the study and waived the requirement for informed consent on the basis of preserving participants' anonymity.

Serological Assays

Serology Testing to Detect Dengue, West Nile Virus, and SARS-CoV-2 Antibodies

Antibodies against dengue virus were detected by the following commercially available immunoassays: lateral-flow rapid diagnostic test (RDT), which detects antibodies against the envelope protein (RDT; SD BIOLINE Dengue IgG/IgM WB (Abbott, Chicago, IL, USA)) and shown to be highly specific [13, 14] and by ELISA against the envelope ((Panbio® Dengue IgG and IgM Indirect ELISA (Abbott, Chicago, IL, USA)) and nonstructural (NS)-1 (anti dengue NS1 type 1–4 IgG, Euroimmun, Germany) proteins. Detection of IgM or immunoglobulin G (IgG) antibodies against West Nile virus (WNV) in serum samples was performed using ELISA (WNV IgM capture DxSelect ELISA and IgG DxSelect ELISA kits by Focus Diagnostics Inc., Cypress, CA, USA). Samples were tested in all the immunoassays according to the manufacturer's instructions. WNV neutralization was performed as described [15] using a WNV strain (S42H) isolated in Israel in 2015. A dilution equal to 1:10 or above was considered neutralizing. SARS-CoV-2 immunglobulin A (IgA) and IgG antibodies were detected by 2 assays: (1) A semi-quantitative

ELISA (anti-SARS-CoV-2 ELISA IgG, Euroimmun, Germany) performed according to the manufacturer's recommendation [16, 17]. (2) Mount Sinai Hospital Clinical Laboratory COVID-19 ELISA Antibody Test [18], which was modified accordingly: a 96 well microtiter Polysorb plate (Nunc, Thermo, Denmark) coated overnight with 1 µg/mL of Receptor Binding Domain (RBD) antigen for detection of IgG and 2 µg/mL for detection of IgA antibodies was blocked with 5% skimmed milk at 25º C for 60 minutes, and human serum samples (diluted 1:100 with 3% skimmed milk) were added to antigen coated wells. The plate was incubated at 25º C for 120 minutes, washed, and goat anti-human IgG horseradish peroxidase (HRP) conjugate (Jackson ImmunoResearch, Philadelphia, PA, USA) (diluted 1:15000) or anti human IgA HRP conjugate (Abcam) was added to each well for 60 minutes after addition of tetramethylbenzidine (TMB) substrate and stop solution (1M HCl) the OD of each well was measured at 450 nm. ELISA index value above or equal to 1.1 was considered positive. SARS-CoV-2 RBD IgM was detected by a electrochemiluminescence serological test as described (medRxiv preprint doi: https://doi.org/10.1101/2020.06.28.201 41838).

Bioinformatics

Protein Structure Analysis

We hypothesized that cross-reactivity between SARS-CoV-2 and dengue virus could be the result of similarities in the structures of outer proteins of these 2 viruses. Such similarities could potentially cause cross-reactivity if the chains that appear in the outer surface of the virus are exposed to the immune system and trigger the expansion of similar antibodies. Accordingly, we identified the outer chains of selected flaviviruses and of SARS-CoV-2 and compared their structures (Supplementary Table 1S). Cross-reactivity between the different flaviviruses is well established [19]; accordingly we used this information as a positive control to test whether such cross-reactivity could be observed also at the epitope similarity level. We used the 0–1 template modeling (TM)-score scale to measure similarity: TM-score < 0.30 is random structural similarity, whereas 0.5 < TM-score implies the structures have the same fold (ie, are similar in shape) [20]; here, we use the threshold of 0.45 to identify similar structures. TM-score reports were normalized by both aligned chains; we use the maximum among these scores. To study all similar protein chains in these viruses, we organize the data as a network and use Cytoscape [21] to visualize it.

Statistical Analysis

Descriptive statistics were used to summarize the data; results are reported as means and standard deviations (SD), as appropriate. Categorical variables were summarized as counts and percentages. P values were calculated using the 2-tailed Fisher exact test.

RESULTS

Clinical Findings

During the period from 17 March through 13 April, we identified 55 patients with confirmed COVID-19 infection. The demographic and clinical characteristics of the patients are shown in Supplementary Table 2S. The mean (±SD) age of the patients was 45 ± 20 years (range, 14–84); 53% were men. The mean duration of symptoms before COVID-19 diagnosis was 7 ± 8 days. Thirty-one patients were hospitalized (56%), and 24 were managed as outpatients (44%). Full clinical and laboratory details of the former group are shown in Supplementary Table 2S. None of the patients had recently traveled to a dengue virus endemic region.

Dengue Virus Rapid Test Serology Was Positive in 12 out of 55 Patients (22%) With SARS-CoV-2 Infection

We first tested the study cohort for IgG and IgM antibodies using the rapid dengue virus diagnostic test (SD BIOLINE Dengue). We found 9 patients positive for IgM, 3 positive for IgG, and 1 positive for both IgM and IgG out of the 55 individuals with confirmed COVID-19 (Table 1). In addition, we tested serum samples from a control group of 70 healthy individuals, which we obtained prior to September 2019. In this cohort the dengue rapid test was negative in all samples ($P = 2.5E-5$). Subsequently, we assessed dengue virus serostatus using ELISA, which detects antibodies directed against dengue E protein. This analysis demonstrated that out of the 12 COVID-19 patients who were positive using the dengue rapid test, only 1 had a weak positive IgG result and none were positive for ELISA IgM (Table 1). In addition, dengue IgM and IgG E based ELISA were positive in 1 and 5 patients, respectively, out of the 43 COVID-19 patients who were negative using the initial dengue rapid test. Dengue has known cross-reactivity with the E protein of WNV. Additionally, WNV seropositivity in the Israeli population is estimated at approximately 11% [22]. Therefore, we tested these IgG dengue E positive samples for WNV E based IgG and IgM ELISA and subsequently performed WNV neutralization. Out of the 5 dengue positive samples, 3 were positive for WNV E IgG. These samples were also positive in WNV-neutralization, further providing evidence that these dengue fever positive ELISA samples represented antigenic cross-reactivity with past infection with WNV. Because dengue is not endemic in Israel,

Table 1. Serology Characteristics of 14 Patients With Coronavirus Disease 2019 (COVID-19) and False-Positive Dengue Serology

Index Case No.	Positive COVID-19 PCR to Sample Collection (days)	Dengue Rapid IgM	Dengue Rapid IgG	Dengue E-ELISA IgM	Dengue E-ELISA IgG	Dengue NS1-ELISA IgG	WNV E-ELISA IgM	WNV E-ELISA IgG	West-Nile Neutralization Assay [d]	Euroimmun SARS-CoV-2 ELISA IgA	Euroimmun SARS-CoV-2 ELISA IgG	Electro-chemiluminescence SARS-CoV-2 ELISA IgM	Mount-Sinai ELISA IgA	Mount-Sinai ELISA IgG
3 [a]	4	Positive	Neg	Neg	Neg	Neg	Neg	Positive	<1:10	Neg	Neg	Neg	Neg	Neg
515	24	Positive	Neg	Neg	Neg	Neg	Neg	Neg	-	Positive	Positive	N/A	Positive	Positive
35 [a]	4	Positive	Neg	Neg	Neg	Positive	Neg	Positive	1:320	Neg	Neg	Neg	Equ	Neg
67 [a,c]	6	Positive	Neg	Neg	Neg	Neg	Neg	Neg	-	Positive	Neg	N/A	Positive	Positive
523	34	Positive	Neg	Neg	Neg	Equ	Neg	Neg	-	Neg	Neg	Positive	Neg	Neg
41 [a,b]	0	Positive	Neg	Neg	Neg	Neg	Neg	Neg	-	Positive	Neg	Positive	Positive	Positive
516	20	Positive	Neg	Neg	Neg	Neg	Neg	Neg	-	Neg	Neg	Equ	Neg	Neg
518	20	Positive	Neg	Neg	Positive	Neg	Neg	Positive	1:320	Neg	Neg	Equ	Neg	Neg
514	22	Positive	Neg	Neg	Neg	Neg	Neg	Neg	-	Equ	Positive	Positive	Positive	Positive
519	19	Positive	Positive	Neg	Neg	Neg	Neg	Neg	-	Positive	Positive	Positive	Positive	Positive
62 [a,b]	3	Neg	Positive	Neg	Neg	Neg	Neg	Neg	-	Positive	Neg	Equ	Positive	Neg
2 [a]	17	Neg	Positive	Neg	Neg	Neg	Neg	Neg	-	Neg	Neg	Positive	Neg	Neg
527	19	Neg	Neg	Neg	Positive	Neg	Neg	Neg	-	Positive	Positive	Positive	Positive	Positive
517	6	Neg	Neg	Equ	Positive	Neg	Neg	Neg	-	Neg	Neg	Equ	Neg	Neg

Abbreviations: COVID-19, coronavirus disease 2019; ELISA, enzyme-linked immunosorbent assay; IgG, immunoglobulin G; IgM, immunoglobulin M; Neg, negative; Equ, equivocal; N/A, not available; PCR, polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

[a] These patients were hospitalized.

[b] These patients required invasive ventilation.

[c] This patient passed away.

[d] A dilution equal to 1:10 or above was considered neutralizing.

it is highly unlikely that these COVID-19 patients with positive E IgG dengue and negative E IgG WNV were previously infected with dengue. Nevertheless, these samples were tested negative with a NS1 based ELISA, which detects antibodies against dengue NS1 and therefore efficiently evaluate past exposure to dengue virus [23]. Overall, these results suggest that cross-reactivity between ELISA dengue E IgG and SARS-CoV-2 may also exist (Table 1). Testing the 14 samples from dengue cross-reactive COVID-19 patients with 2 ELISAs and an electrochemiluminescence serological test for IgA, IgM and IgG demonstrated that all but 1 sample had equivocal or positive antibodies against SARS-CoV-2.

SARS-CoV-2 Serology Status Among Patients With Dengue Fever

To test the possibility of false-positive (FP) SARS-CoV-2 serology in patients diagnosed with dengue fever, we assessed SARS-CoV-2 serology status in serum samples obtained prior to September 2019 from patients with clinical and serological diagnosis of acute (IgM) and past (IgG) dengue fever. Using the Euroimmun ELISA, which detects antibodies against the S protein, we found 21 out of 95 samples to be positive or equivocal for SARS-CoV-2 IgG or IgA antibodies. This is significantly higher FP rates as compared with rates detected in 102 healthy controls (4% vs 22%, $P = 1.6E-4$) and in prior studies [16, 17] (Supplementary Table 3S). We next examined if the cross-reactivity observed was a general or more specific phenomenon by testing samples from acute (IgM) and past (IgG) dengue fever patients with an ELISA that detects antibodies against the RBD antigen. Results (Supplementary Table 3S) show higher, although not statistically significant, FP rates for samples obtained from past dengue fever patients as compared with healthy controls. To further explore such cross-reactivity between SARS-CoV-2 and dengue virus, we hypothesized that although they belong to different families of viruses they might share structural similarities.

In-silico Protein Structure Analysis Reveals Possible Similarity Between SARS-CoV-2 Spike Protein and Dengue Envelope Protein

Because sequence similarity could not explain the observed cross-reactivity (Supplementary Figure 1S), we hypothesized that structure similarity might expose regions in the outer protein chains that can explain the immune cross-reactivity. We focused our analysis on the comparison of folds of chains and structures that are known to be in the outer surface of a protein, because it is estimated that antibodies identify epitopes that are in specific folds. Based on these assumptions, we propose that structure similarity might correlate with positivity rate. Indeed, very high similarity (TM score 0.75–1.0) was found between many chains of the different flaviviruses (Supplementary Figure 2S, Figure 1A). The same analysis between flaviviruses and SARS-CoV2 identified high similarity between the SARS-COV-2 6LVN chains, which are part of the HR2 domain of the

spike protein and several chains in the envelope protein of both dengue (4CBF, 4UIF, 5A1Z, etc) and Zika viruses (6JFI, 5YGH, etc). The similarity between SARS-CoV-2 6LVN chains and WNV were not with chains of the envelope protein but rather with chains of the NS1 protein (4O6C) of WNV (Figure 1B). Few other SARS-CoV-2 chains from the spike protein (6LXT, 6VSB, 6VXX etc) demonstrated high similarity with chains from the envelope protein of both Zika and dengue viruses, although such similarity could not be apparent with chains from WNV (Figure 1B). Altogether, we identified structure similarities between chains of the SARS-CoV-2 spike protein and chains of the envelope protein of both Zika and dengue but not with WNV. Such similarities could potentially explain the cross reactivity between SARS-CoV-2 and dengue virus.

DISCUSSION

Here we provide lines of evidence to support cross-reactivity between dengue and SARS-CoV-2, which can lead to false-positive dengue serology among patients with COVID-19. Failing to consider COVID-19 infection because of a positive dengue result can have serious consequences for both patient care as well as for public health policies.

Dengue fever and COVID-19 are difficult to distinguish because they share clinical and laboratory features [3–5]. Yan G et al recently described 2 cases that were wrongly diagnosed as dengue but later confirmed to be COVID-19 [5]. In addition, it has been recently reported in Brazil and other regions that both viruses spread simultaneously as copandemics [7, 24]. In these regions, diagnostic misclassification can be highly problematic. Cross-reactivity between SARS-CoV-2 and dengue viruses may interfere with accurate clinical diagnosis and treatment, understanding of undelaying pathomechanisms, and possible development of vaccine.

We cannot exclude the possibility that the phenomenon seen in this study is assay-specific false positive and not true antigenic cross-reactivity. Our results demonstrating cross-reactivity of samples from COVID-19 patients on the dengue RDT as well as samples from dengue patients with the Euroimmun ELISA in addition to our in-silico protein structure analysis suggests that at least some specific antigenic cross-reactivity exists. However, the results of the dengue ELISA and SARS-CoV-2 Mount Sinai RBD-ELISA demonstrating only minimal cross-reactivity suggests that this cross-reactivity is not due to a significant antigenic similarity. It is possible to speculate that the existence of minor structural similarities between dengue and SARS-CoV-2 may result in selected epitopes identity. As a consequence only assays detecting antibodies targeting these epitopes will show differential cross reactivity. Nevertheless, both the RDT and the SARS-CoV-2 ELISA are widely used worldwide, and therefore the issue of cross-reactivity with these assays have huge implications. One interesting example is the RDT dengue by SD bioline
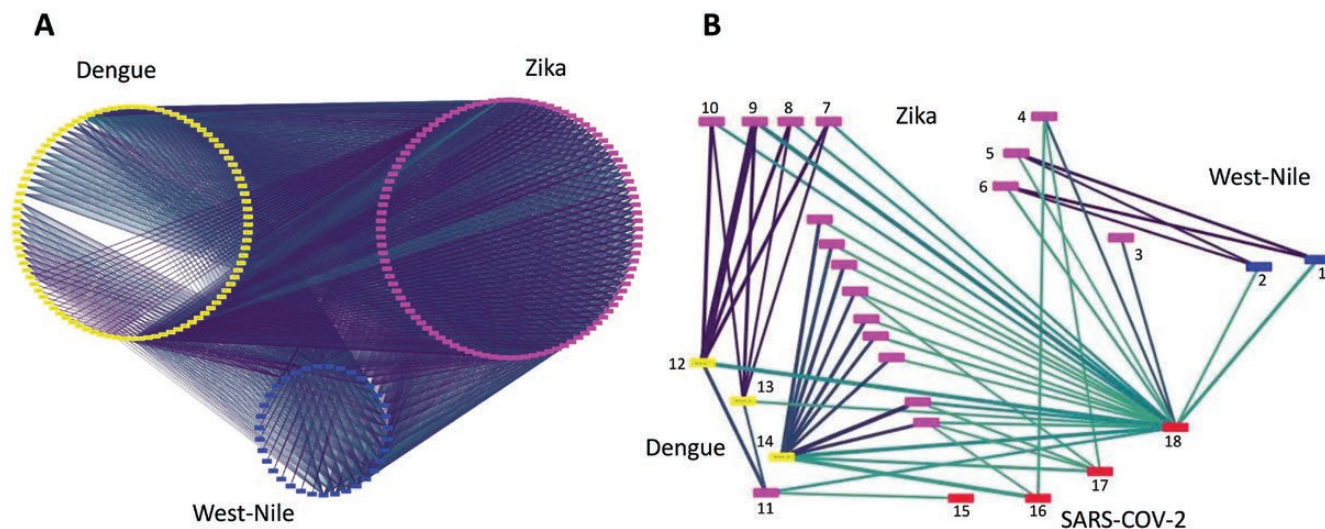
Figure 1. Structural similarities between flaviviruses and SARS-CoV-2. Nodes represent outer protein chains, colored based on their virus. Edges connect chains in two viruses that are structurally similar (max TM-score > 0.45) and colored based on the TM-score, with darker edges representing more similar structures. *A*, Extensive structural similarity among the outer proteins of the 3 flaviviruses. To compare the structural similarities between coronavirus and the 3 flaviviruses, we first cluster the protein chains: 2 chains are grouped into the same cluster if they belong to the same virus, their sequences are >90% identical, and the structures of the aligned residues are essentially the same (<1A RMSD). Supplementary Table 1S lists the number of clusters for each virus. *B*, Network where the nodes are clusters of chains, colored based on their virus, and edges connect 2 clusters of chains, which include structurally similar chains; the width of the edge is proportional to the number of structurally similar chain pairs within the 2 clusters. The chains in each node are described as follows: **nodes for West Nile virus**: (1) 4O6C_A, 4O6C_B, 4O6C_C, 4O6C_D, 4O6C_E, 4O6C_F, 4O6D_B, 4TPL_B; (2) West Nile: 4O6D_A, 4TPL_A; **nodes for Zika virus**: (3) 5YGH_A, 5YGH_B; (4) 6C44_A, 6C44_B; (5) 5GS6_A, 5GS6_B; (6) 5K6K_A, 5K6K_B; (7) 5IRE_E, 6JFI_E,6JFH_E; (8) 5IRE_C, 6JFI_C, 6JFH_C; (9) 5GZR_A, 5GZR_B, 5GZR_C, 5IZ7_A, 5IZ7_B, 5IZ7_C, 5H30_A, 5H30_B, 5H30_C, 5H37_A, 5H37_B, 5H37_C; (10) 5IRE_A, 6JFI_A, 6JFH_A; (11) 5U4W_G, 5U4W_I, 5U4W_K; **nodes for dengue virus**: (12) 3J27_A, 3J27_C, 3J27_E, 3J2P_A, 3J2P_C, 4CBF_A, 4CBF_C, 4CBF_E, 4UIF_C, 4UIF_E, 5A1Z_C, 5A1Z_E; (13) 4UIF_A, 5A1Z_A; (14) 3J27_B, 3J27_D, 3J27_F, 3J2P_B, 3J2P_D, 4CBF_B, 4CBF_D, 4CBF_F, 4UIF_B, 4UIF_D, 4UIF_F, 5A1Z_B, 5A1Z_D, 5A1Z_F; **nodes for SARS-COV-2:** (15) 6LXT_A, 6LXT_B, 6LXT_C, 6LXT_D, 6LXT_E, 6LXT_F; (16) 6VSB_B, 6VSB_C, 6VXX_A,6VXX_B, 6VXX_C, 6VYB_A, 6VYB_C; (17) 6VSB_A, 6VYB_B; (18) 6LVN_A, 6LVN_B, 6LVN_C, 6LVN_D. Abbreviation: SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

(shown here to detect 22% of COVID-19 patients): This assay was shown by us and others to be highly specific with ~95–100% specificity, with sensitivity that depends on the time since symptom onset [13, 14]. The SD bioline test is one of the most used in the world for dengue diagnosis because it is cheap and available to test in resource-poor settings. It is widely used in Southeast Asia and South America—areas endemic to dengue with most dengue infections and much less used in developed countries, which mostly see only returning travelers infected with dengue. Moreover, with the introduction of the Sanofi dengue vaccine and the increased risk for severe disease within dengue naive individuals, it is now mandatory to test for dengue antibodies before immunization [24]. Therefore, in Southeast Asia and South America, which have cocirculation of dengue and COVID-19 [7, 23] 22% of COVID-19 infections may be falsely identified as dengue. This may lead to spread of COVID-19 infections due to lack of isolations, as well as vaccination of dengue naive individuals.

Furthermore, antigenic cross-reactivity between dengue and COVID-19 raises questions regarding the possibility of protective immunity overlap between these 2 diseases, on the one hand, or worsening of the disease through antibody dependent enhancement on the other hand. This question should be addressed in future studies. Finally, our observations of structure similarity of specific SARS-CoV-2 and dengue protein structures could explain the observed cross-reactivity. Interestingly, our bioinformatics structure similarity does not predict cross-reactivity with WNV, and indeed we could not observe such cross-reactivity. Our results also predict potential cross-reactivity with Zika virus, which needs to be further validated. These results suggest that protein structure similarities regardless of genetic distance could predict cross-reactivity between viruses from distant phylogenetic lineages.

Altogether, our results highlight the importance of recognizing false-positive dengue serology results in patients with COVID-19 and vice versa.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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