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Assessment of threat of concurrent SARS-CoV-2 and DENV infection in the COVID-19 pandemic in Brazil in 2020: diagnostic and immunological findings

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Introduction: The first peak of COVID-19 in Brazil was between April and May 2020, at a time of the year when outbreaks of other tropical diseases, such as dengue, would be expected. COVID-19 and dengue have similar pathogenesis. In general, both may lead to mild symptoms but may also cause severe and even fatal symptoms, especially in patients with comorbidities and probably in cases of overlapping infections. The general objective of this study was to assess whether, during the 2020 pandemic, there were cases of concomitant infection between SARS-CoV-2 and DENV.

Methods: For this, we evaluated the specificity and sensitivity of commercial serological anti-SARS-CoV-2 kits using plasma samples from patients with dengue and healthy donors recruited before COVID-19. In the case of confirmed cases of COVID-19/dengue, we evaluated the clinical evolution of these coinfected patients, compared with mono-infected patients; and quantified chemokines CCL2 and CXCL8 by ELISA in COVID-19 patients in order to correlate them with COVID-19/dengue severity and cases.

Results and Discussion: Our results showed that commercial IgA and IgG anti-SARS-CoV-2 kits presented high sensitivity and specificity. This allowed us to see a low rate of co-detection or coinfection between SARS-CoV-2 and DENV in Rio de Janeiro. Among the 57 COVID-19 patients, anti-DENV IgM was detected in

five (8.8%). COVID-19/dengue coinfected patients showed no clinical worsening of COVID-19 and cases in which COVID-19 patients had previous exposure to DENV did not influence the clinical severity of COVID-19. Lastly, CCL2 and CXCL8 appeared to be good markers of COVID-19 severity and did not show increased levels in COVID-19/dengue cases.

KEYWORDS

COVID-19, dengue, coinfection, diagnosis, human

1 Introduction

Dengue is endemic in more than 100 countries in tropical and subtropical areas, and is prevalent in Southeast Asia, the Americas, and the Western Pacific region (1). Brazil has the highest dengue incidence rates in South America, and three severe countrywide epidemics in 2002, 2008 and 2010 can be highlighted (2, 3). Brazilian authors conducted a retrospective study between 2014 and 2019 based on secondary data collected from the National Resources Notification System (SINAN) and the Department of Informatics of the Unified Health System (DATASUS). In this analysis, 5,868,413 suspected cases of dengue were identified. Most women were between 20 and 39 years old. Most patients recovered, but 3,444 died (4). Furthermore, Brazil was also brutally affected by the COVID-19 syndemic, in which the initial cases were described in February 2020 (5, 6). It was only on April 22, 2022, that the Brazilian Ministry of Health was able to declare the end of the Public Health Emergency of National Importance caused by the COVID-19 pandemic in Brazil, with 27,425,743 cases and 638,048 deaths registered (7). The spread of COVID-19 occurred just before dengue reached its seasonal peak, which led to a simultaneous outbreak of both in the first weeks of 2020 (5). From December 30, 2019, to March 12, 2020, cases of dengue increased by 70% year-over-year in Brazil (8). During this period, the infection curve of COVID-19 grew, new strains emerged, and the risk of overlapping with the burden of dengue in the healthcare system increased. Nicolelis et al. (2020) demonstrated that the incidence of COVID-19 and the rate of infection and mortality among people with IgM antibodies for dengue were inversely correlated in different states of the country. Thus, the Brazilian states where a large part of the population contracted dengue in 2019-2020 recorded fewer cases and deaths from COVID-19, and these states took a long time to reach exponential community transmission because the growth in SARS-CoV-2 infection rates was slower. This profile was also observed in other Latin American countries, Asian countries, and islands in the Pacific and Indian Oceans. These findings raised the possibility of cross-reactivity between DENV and SARS-CoV-2 (9).

Great effort was directed toward diagnosing COVID-19, which may have impacted the diagnosing dengue. On the other hand, some authors described cases misdiagnosed as dengue but later confirmed as COVID-19 (10, 11). Nonetheless, there is a risk of coinfection, which would make differential diagnosis difficult (8), with clinical consequences that remain unknown (12).

Although COVID-19 and dengue mostly cause mild symptoms, they also can lead to severe and fatal illnesses, especially in patients with comorbidities. It is already known that dengue and COVID-19 have common clinical and laboratory characteristics. Furthermore, an inefficient antiviral immune response and overproduction of inflammatory mediators are common mechanisms of the immunopathogenesis of the two viruses (13), which makes it possible for the clinical condition of coinfected patients to worsen.

Thus, we sought to assess the frequency of SARS-CoV-2 and DENV coinfection during the initial phase of the COVID-19 syndemic in 2020 while considering the risks of cross-reactivity of diagnostic kits. In cases of coinfection, we sought to assess the risks of complications over the clinical course and to assess changes in the plasma levels of CCL2 and CXCL8 since both have already been detected in patients with SARS-CoV-2 (14) and in dengue patients, particularly in severe cases (15).

2 Materials and methods

2.1 Study population

Fifty-seven adult patients were included in this study between March and June 2020. The diagnosis of COVID-19 was confirmed based on clinical and molecular diagnoses, through detecting viral RNA using RT-PCR. The patients recruited were either attending outpatient care or had been hospitalized in the intensive care unit of the Hospital Rede Casa and Hospital Universitário Antônio Pedro, both in Rio de Janeiro (RJ), Brazil. Questionnaires were applied to obtain demographic, clinical, and laboratory data, and the patient's medical records were consulted to obtain historical and current data. Among the vaccines offered by the Brazilian Ministry of Health for adults, we highlight the vaccine against yellow fever since our team has worked with arboviruses for years.

The criteria for classifying COVID-19 were as follows. Cases of mild/moderate COVID-19 were defined as those with clinical manifestations compatible with COVID-19, regardless of the number of days of illness, which were attended in the outpatient clinic without hospitalization. Cases of severe COVID-19 were

defined as those that presented clinical manifestations compatible with COVID-19, regardless of the number of days of illness requiring hospitalization. Cases of death due to COVID-19 were defined as those that had clinical manifestations compatible with COVID-19, regardless of the number of days of illness, which required ICU admission, with subsequent death.

In addition, 12 patients with acute DENV-4 from Hospital Plantadores de Cana, Campos dos Goytacazes, RJ, and 12 healthy blood donors, also from RJ, were included as control groups. Plasma from the healthy blood donors had been obtained some years before the pandemic. None of the healthy blood donors had had any febrile episodes or other illnesses within the last three months before blood collection.

2.2 Ethics Statement

The study followed the procedures of the Declaration of Helsinki. All data were kept confidential and anonymous, following the guidelines of the National Research Ethics Committee of the Plataforma Brasil (number 57221416.0.1001.5248 version 6 and number 13318113.7.3001.0021), the Ethics Committee of the Universidade Federal Fluminense (number 30623520.5.0000.5243) and the Research Ethics Committee of Hospital Casa Rio-Botafogo (number 47885515.8.0000.5279). The study participants signed an informed consent statement before undergoing any procedure.

2.3 Blood Sample Collection

About 10 mL of peripheral blood was collected from each participant through venipuncture, into acid-citrate-dextrose tubes (Cat. 364606, BD Vacutainer™). Plasma was obtained after centrifugation of the blood. Aliquots of plasma were stored at -70°C until analysis.

2.4 DENV RNA extraction followed by quantification for molecular diagnosis

Plasma or blood samples from patients with dengue were evaluated for the four DENV serotypes (DENV 1-4). The PureLink viral RNA/DNA mini kit (Cat. # 12280050, Invitrogen) was used to extract viral nucleic acids, in accordance with the protocol described by the manufacturer. After extraction, viral RNA was kept at -70 °C until use. Viral nucleic acids were quantified in the NanoDrop 2000 equipment (Thermo Fisher) of the Immunopharmacology Laboratory (IOC/Fiocruz). For the molecular diagnosis, real-time RT-PCR was executed on the GoTaq® Probe 1-Step RT-qPCR system (Cat. # A6121, Promega) (16) using the Applied Biosystems 7500 Real-Time PCR System (Flavivirus Laboratory, IOC/Fiocruz). The thermocycling parameters consisted of one cycle of 50°C for 30 minutes, one cycle of 95°C for 2 minutes, 50 cycles of 95°C for 15 seconds, and, lastly, 60°C for 1 minute.

RT-PCR for molecular diagnosis of SARS-CoV-2 was performed at the hospital where the patients were recruited, using swabs of oropharyngeal and nasopharyngeal secretions.

2.5 Serological analysis on anti-SARS-CoV-2 IgA and IgG, NS1 DENV viral antigen and anti-DENV 1-4 IgM and IgG, using enzyme-linked immunosorbent assay

SARS-CoV-2 S1 protein-specific binding IgA and IgG (Cat. EI 2606-9601 A and EI 2606-9601 G, Euroimmun) were analyzed using ELISA. The Platelia™ dengue NS1 Ag kit (Cat. 72830, Bio-Rad Laboratories) was used to detect the NS1 DENV viral antigen, using capture ELISA. In addition, anti-dengue serotype 1-4 IgM and IgG (Cat. EI 266b-9601 and Cat. EI 266b9601 G, Euroimmun) were used. All kits were used following the manufacturer's recommendations. The results were expressed as the ratio between the sample absorbance value (donors and healthy patients) and the absorbance value of the kit calibrator.

2.6 Sensitivity and specificity

Sensitivity and specificity describe the proportions of positive or negative results among individuals known to be sick. Sensitivity is the probability of a positive outcome in patients (true positive) and is calculated as a/(a+c). Specificity is the probability of a negative result in non-patients (true negative) and is calculated as d/(b+d) (Supplementary Material, Table 1).

2.7 Quantification of CCL2/MCP-1 and CXCL8/IL-8

The plasma levels of CCL2/MCP-1 and CXCL8/IL-8 were quantified using ELISA kits in accordance with the manufacturer's instructions: C-C motif chemokine ligand 2 (CCL2/MCP-1) (cat. 900-T31, Peprotech) and C-X-C motif chemokine ligand 8 (CXCL8/IL-8) (cat. DY208, R&D Systems). Standard curves were used to convert optical density (OD) into concentration units (pg/mL). OD readings were carried out using the Biochrom EZ Read 400 microplate reader.

2.8 Statistical analysis

Data in error bars or tables were presented as the median and interquartile range (IQR-25%-75%). Comparisons between groups for quantitative variables were performed using nonparametric Mann-Whitney or Kruskal-Wallis tests, followed by Dunn's multiple comparisons. Fisher's exact test was used to evaluate qualitative variables represented in percentages (%). The analysis was performed using GraphPad PRISM (version 9) (GraphPad Software). P values < 0.05 were considered statistically significant.

2.9 Data availability

The data sets used and analyzed during the current study are available.

3 Results

3.1 Evaluation of cross-reactivity of commercial anti-SARS-CoV-2 IgA and IgG detection kits

Many new anti-SARS-CoV-2 specific antibody detection kits have been commercialized. To assess the sensitivity and specificity of one of the several kits, plasma samples from healthy donors from 2016 and 2017 (n = 12), dengue patients from 2013 (n = 12) and COVID-19 patients confirmed through RT-PCR (n = 57) were selected. None of the samples from healthy donors showed anti-SARS-CoV-2 IgA or IgG. Among the acute dengue patients, only one patient (8.3%) had anti-SARS-CoV-2 IgA, and none had anti-SARS-CoV-2 IgG. Among the cases of COVID-19 confirmed through RT-PCR, 54 patients (94.7%) presented anti-SARS-CoV-2 IgA (Figure 1A) and 47 (82.5%) anti-SARS-CoV-2 IgG (Figure 1B).

For the sensitivity and specificity calculations of anti-SARS-CoV-2 IgA and IgG kits, we used 56 COVID-19 patients (one other patient was withdrawn from the test because the OD ratio calculation was inconclusive), 8 to 10 healthy donors (also because of inconclusive results) and 12 acute dengue patients. For the IgA kit, the sensitivity was calculated to be 95% and the specificity 96% (Figure 1C). For the IgG kit, the sensitivity was estimated at 84% and specificity at 100% (Figure 1D).

3.2 Evaluation of COVID-19/dengue coinfection rates

For this purpose, we analyzed markers of acute or recent DENV infection by detecting viral genetic material through RT-qPCR, DENV NS1 antigen and anti-DENV IgM antibodies in COVID-19 patients and control groups (healthy controls and dengue patients).

Concerning healthy controls, we did not detect DENV through RT-qPCR, NS1 DENV or anti-DENV IgM in the samples tested. In 83.3% of them, anti-DENV IgG was detected, thus indicating previous exposure to the virus (Figure 2A). Regarding dengue patients, RT-qPCR for DENV was positive in 91.7% of the cases. In addition, NS1 DENV was detected in 41.7%, and only four patients (33.3%) had anti-DENV IgM. Detection of anti-DENV IgG occurred in 75% (Figure 2B). Lastly, in acute patients with clinically confirmed COVID-19 through RT-PCR, DENV was not detected using qRT-PCR or NS1 DENV. However, five of the 57 COVID-19 patients (8.8%) presented anti-DENV IgM, thus indicating that COVID-19 patients could have become infected with DENV close to or concomitantly with SARS-CoV-2. Among the COVID-19 patients, 89.5% had been exposed to DENV at least once since anti-DENV IgG was detected (Figure 2C).

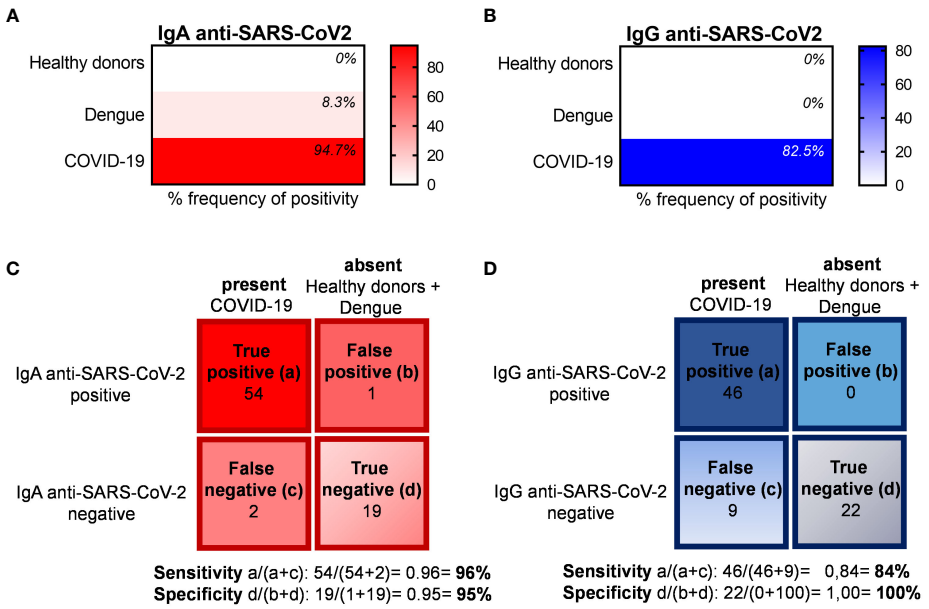

FIGURE 1
Detection of anti-SARS-CoV-2 IgA and IgG antibodies and validity of the commercial kit for serological diagnosis of COVID-19. Detection of IgA **(A)** and IgG **(B)** antibodies was performed in samples from healthy controls (n = 12), dengue patients (n = 12) and COVID-19 patients (n = 57). Statistical significance for frequency of positivity was evaluated using Fisher's exact test. In **(A, B)**, $p < 0.001$. Sensitivity and specificity calculations are shown for the IgA **(C)** and IgG **(D)** detection kits.

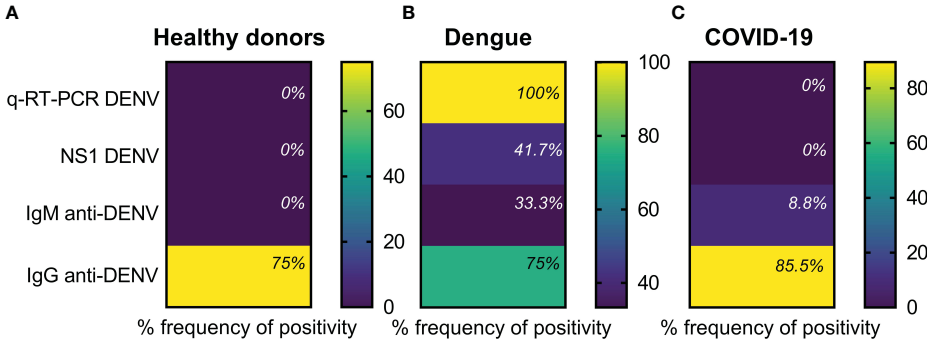

FIGURE 2
Molecular and serological diagnosis of dengue in all study groups. Detection of DENV through RT-qPCR, viral glycoprotein NS1, anti-DENV IgM and IgG antibodies was investigated in plasma from **(A)** healthy donors (n = 12), **(B)** dengue patients (n = 12) and **(C)** COVID-19 patients (n = 57). The frequency of positivity for each test within the group was demonstrated.

3.3 Differential detection pattern of anti-SARS-CoV-2 IgA and IgG antibodies based on the severity of COVID-19 and COVID-19/dengue

The results from detecting anti-SARS-CoV-2 IgA and IgG antibodies were expressed by calculating the ratio of the samples' absorbance values divided by the calibrator's absorbance value, as recommended by the kit manufacturer. All the samples from healthy donors presented mean anti-SARS-CoV-2 IgA and IgG OD ratios in the undetectable ranges of 0.440 (IQR, 0.209–0.630) and 0.265 (IQR, 0.162–0.376), respectively. Regarding the dengue group, the same was observed for IgA (0.359; IQR, 0.243–0.521) and IgG (0.206; IQR, 0.127–0.336). All patients in the COVID-19 and COVID-19/dengue groups presented higher anti-SARS-CoV-2 IgA and IgG averages than those of the healthy controls and dengue group. In this cohort of patients, we did not observe any difference among the COVID-19 group, including the five COVID-19/dengue patients, regarding specific IgA or IgG (Figures 3A, B).

3.4 Comparison of clinical severity between patients with COVID, dengue and COVID-19/dengue

Patients with COVID-19 were classified according to clinical status, and patients with dengue and healthy donors were separated from the others for comparative analyses, as detailed in Table 1.

We highlight some interesting data. Regarding age, we noticed that patients with COVID-19, regardless of the clinical presentation, were older than the healthy donors and dengue patients. Among the clinical symptoms, headache, myalgia/

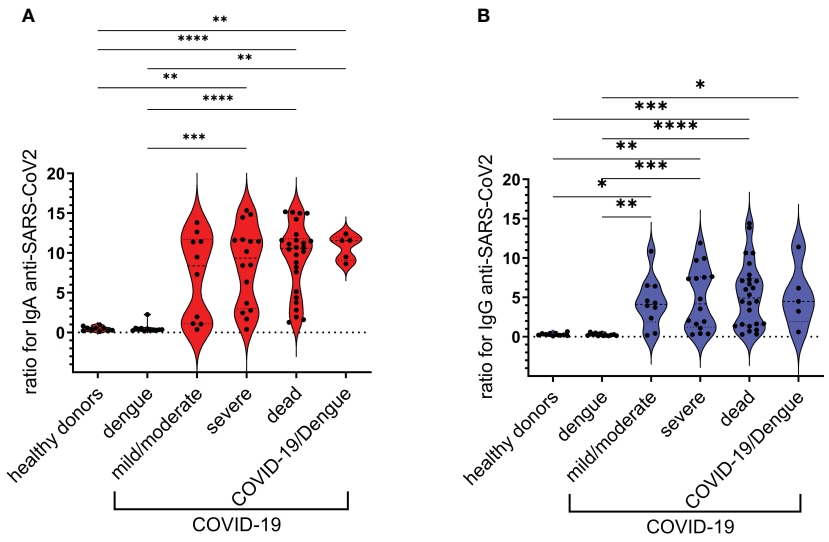

FIGURE 3
IgA and IgG seropositivity for SARS-CoV-2. Assessment of specific **(A)** IgA ratio and **(B)** IgG ratio in healthy donors (n = 12), acute dengue (n = 12), mild/moderate acute symptomatic COVID patients (n = 10), severe acute symptomatic COVID patients (n = 16), deceased COVID-19 patients (n = 26), and COVID-19/dengue patients (n = 5). The violin plots express the median (middle line) and numerical data distributions using density curves. The Kruskal-Wallis test and Dunn's multiple comparison test were used. *p < 0.05, **p < 0.01, ***p < 0.001 and ****p < 0.0001 indicate significant differences.

TABLE 1 Demographic and clinical characteristics of patients with dengue and COVID-19.

	Healthy donors	Dengue	COVID-19 mild/moderate	COVID-19 severe	COVID-19 deceased	COVID-19/dengue	*P**
Total	12	12	10	16	26	5	
Female (%)	42	67	20	38	31	60	**ns**
Age (years) [a]	33 (30-43)	43 (27-51)	66 (47-70)	51 (46-64)	67 (60-74)	66 (50-69)	**< 0.0001**
Days after the onset of symptoms [b]	*NA*	3 (3-3)	7 (4-8)	9 (6-12)	10 (6-16)	10 (7-11)	**0.0009**
Signs/symptoms (%)							
Fever	0	100	80	69	65	75	**ns**
Cough	0	22	90	90	73	50	**0.0063**
Coryza	0	0	10	23	4	25	**ns**
Headache	0	100	0	15	12	25	**< 0.0001**
Myalgia/arthralgia	0	70	10	30	12	25	**0.0055**
Fatigue	0	50	10	30	31	25	**ns**
Vomiting/nausea/diarrhea	0	70	20	30	12	25	**0.0116**
Bleeding [c]	0	70	0	0	0	0	**< 0.0001**
Vascular alteration [d]	0	30	100	85	65	100	**0.0124**
Hospitalization (%)	0	17	0	100	100	80	**< 0.0001**

[a]Median (interquartile range, IQR, 25%-75%); Participants reported acute symptoms.
[b]Number of days after onset of symptoms at the time of hospital admission.
[c]Bleeding included rash, petechiae, and gingival bleeding.
[d]Vascular changes included ascites, pleural effusion, pericardial effusion, and dyspnea.
Values in bold denote statistical significance ($p < 0.05$); the nonparametric Kruskal-Wallis test, followed by Dunn's multiple comparison tests, was used for quantitative variables and Fisher's exact test for qualitative variables to assess the frequency of positivity.
NA, not applicable; ns, not significant.

arthralgia, vomiting/nausea, and hemorrhagic manifestations were more frequent in dengue patients. At the same time, coughing, vascular changes and hospitalizations were more frequently described among COVID-19 patients, including COVID-19/dengue patients.

3.5 Comparison between COVID-19 patients with and without previous exposure to DENV.

To observe the possible impact of prior immunity to DENV in COVID-19 patients, we made a comparative investigation taking into consideration the detection of anti-DENV IgG (Table 2). Among the parameters analyzed, we observed that COVID-19 patients exposed to DENV had longer duration of COVID-19 infection and the incidence of coryza was significantly higher in the same group.

3.6 Measurement of inflammatory mediators CCL2 and CXCL8 in COVID-19 and dengue patients.

As shown in Figure 4, CCL2/MCP-1 levels in patients who died from COVID-19 were very high, but this was only statistically significant compared with the dengue group (Figure 4A). CXCL8/IL-8 levels were higher in the severe COVID-19 and death groups than in the healthy controls and dengue patients. One interesting finding was that CXCL8/IL-8 distinguished mild COVID-19 from fatalities (Figure 4B).

4 Discussion

A recent study found that about 45% of Brazilian COVID-19 patients had already been infected with DENV, and 38.4% also had active DENV infection based on anti-DENV IgM detection. Thus,

TABLE 2 Demographic and clinical characteristics of dengue-immune COVID-19 patients.

	COVID-19		*P value*
	DENV anti-IgG-positive	DENV anti-IgG-negative	
Total	51	6	
Female (%)	35	17	**ns**
Age (years) [a]	65 (49-70)	63 (48-68)	**ns**
Days after onset of symptoms [b]	10 (6-12)	5 (3-9)	**0.0541**
Signs/symptoms (%) [b]			
fever	71	50	**ns**
cough	78	25	**0.0219**
coryza	12	0	**ns**
headache	10	0	**ns**
myalgia/arthralgia	14	25	**ns**
fatigue	33	25	**ns**
vomiting/nausea/ diarrhea	18	0	**ns**
bleedings	0	0	**ns**
vascular alteration	67	100	**ns**
hospitalization (%)	80	83	**ns**
deaths (%)	51	33	**ns**
anti-SARS-CoV-2 IgA ratio	10.70 [5.14-11.60]	5.64 [1.96-10.72]	**ns**
anti-SARS-CoV-2 IgG ratio	4.50 [1.61-7.36]	3.31 [1.09-9,02]	**ns**

[a]Median (interquartile range, IQR, 25%-75%); Participants reported acute symptoms.
[b]Number of days after onset of symptoms at the time of hospital admission.
[c]Bleeding included rash, petechiae and gingival bleeding.
[d]Vascular changes included ascites, pleural effusion, pericardial effusion and dyspnea.
Values in bold denote statistical significance ($p < 0.05$); the nonparametric Mann-Whitney test was used for quantitative variables and Fisher's exact test for qualitative variables.
ns, not significant.

according to those authors, a significant proportion of COVID-19 patients in the central-western region of Brazil had SARS-CoV-2/DENV coinfection (11). In the present study, among COVID-19 patients with detectable RT-PCR in Rio de Janeiro, southeastern Brazil, DENV coinfection was not confirmed by RT-qPCR or NS1 DENV detection. However, 8.8% of the COVID-19 patients presented anti-DENV IgM, thus indicating that these patients could have been infected with DENV at a time very close to having COVID-19 or concurrently. Thus, the frequency of COVID-19/DENV coinfection may be quite different between the different regions of Brazil.

On the other hand, studies support the hypothesis of cross-reactivity between anti-DENV antibodies and SARS-CoV-2 antigens. These data could explain the false positive results observed in rapid serological tests for dengue (17). Along these lines, Yan et al. reported on the cases of two patients in Singapore who had COVID-19 but were diagnosed with dengue after a positive rapid test result for NS1, IgM, and IgG anti-DENV. Both patients returned to the hospital some days later with persistent fever and worsening clinical conditions. When an RT-PCR nasopharyngeal swab was performed, the result was positive for SARS-CoV-2 (18).

No study has described any relationship between DENV serotypes and COVID-19 severity. To the best of our knowledge, only two studies have reported a close relationship between DENV and SARS-CoV-2, one with the DENV-1 serotype and the other with DENV-2, and neither of them investigated any direct relationship with the severity of either serotype.

The first study, by Nath et al., predicted that human antibodies against the DENV serotype 2 envelope can bind to the SARS-CoV-2 Spike RBD protein. Those authors made some speculations based on these data. Among these speculations was the possibility that immunological memory triggered by previous dengue infections in endemic countries could reduce the severity and spread of COVID-19. Moreover, anti-SARS-CoV-2 antibodies could minimize future dengue incidence. The worst possibility would be that anti-SARS-CoV-2 antibodies could increase the incidence and severity of DENV infection by inducing antibody-dependent enhancement of infection (19).

In another study from the same group published in 2022, 93% of the serum samples from Indian patients with COVID-19 were reported to cross-react with dengue in ELISA assays, although all were negative in RT-PCR and NS1 for DENV. The COVID-19 serum samples neutralized DENV-1, although 57% had no evidence of DENV pre-exposure. The authors concluded that serodiagnosis for dengue would be questionable in endemic areas (20).

Therefore, further studies need to be conducted to understand the consequences of the immunological interaction between dengue and COVID-19, regarding disease severity. To assess the possibility of cross-reactivity between commercial anti-SARS-CoV-2 IgA and IgG ELISA kits, we used plasma from healthy donors collected before the COVID-19 syndemic and dengue cases during the 2013 epidemic. Among all the samples evaluated, only one confirmed case of dengue was positive for anti-SARS-CoV-2 IgA, but no anti-SARS-CoV-2 IgG was detected. Thus, our study indicated that commercial ELISA kits have high specificity and sensitivity for confirming the serological diagnosis of COVID-19. A study by Spinicci et al. in Italy investigated 32 serum samples positive for COVID-19 and found that none detected IgG/IgM for DENV. In contrast, rapid tests for COVID-19 antibodies were falsely positive in the cases of 44 DENV-positive serum samples. Therefore, the accuracy of rapid diagnostic tests is a matter of concern and urgently needs to be ensured (21).

Chen et al. observed that the incidence of dengue decreased by 44.1% in 2020, compared with 2019, in Latin American and Southeast Asian countries. This reduction became even more pronounced from April 2020 onwards, albeit with the exceptions of Singapore, which had an above-average number of cases in 2020, and Ecuador, Brazil and Peru, which had additional seasonal increases towards the end of the year. To assess whether dengue cases were underreported, those authors analyzed the annual dengue mortality rates in each country in the study. Their hypothesis was that dengue underreporting would happen more

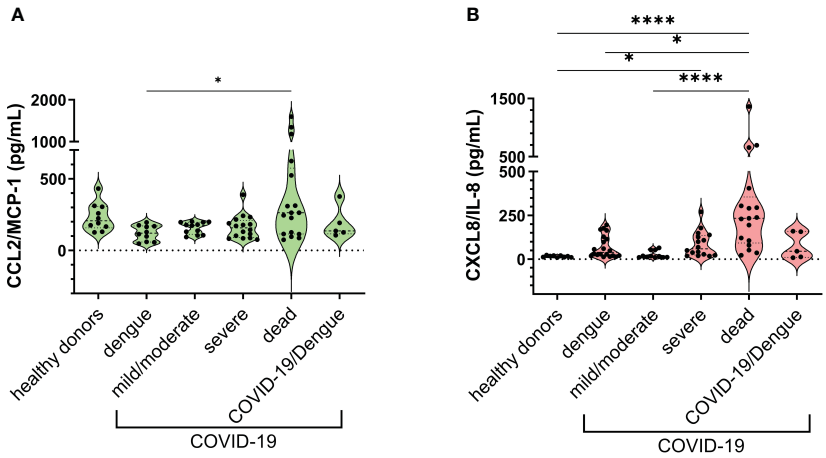

FIGURE 4
Comparison of CCL2/MCP-1 and CXCL8/IL-8 concentrations. **(A)** CCL2/MCP-1 and **(B)** plasma CXCL8/IL-8 were quantified in healthy non-COVID-19 donors (n = 12), acute dengue patients (n = 12), mild/moderate acute symptomatic COVID-19 patients (n = 10), severe acute symptomatic COVID-19 patients (n = 16), COVID-19 patients who died (n = 17) and COVID-19/dengue patients (n = 5). Violin plots show the median (middle line) and numerical data distributions using density curves. The Kruskal-Wallis test and Dunn's multiple comparison test were used. Asterisks indicate significant differences (*$p < 0.05$ and ****$p < 0.0001$).

evidently in mild cases, considering that severe and fatal cases would still seek emergency care, thus resulting in high fatality rates (22). Cardona-Ospina et al. proposed that viral interference occurs between SARS-CoV-2 and DENV, through a process in which viruses would compete for entry and replication. According to these authors, because of the high virulence and pathogenicity of SARS-CoV-2 and its tropism for endothelial cells, like DENV, competitive inhibition may exist between the viruses, which would explain the decrease in the numbers of dengue cases in some countries (23).

The areas with the highest incidence of dengue in Brazil and other countries in Latin America and Asia were less likely to have incidence of and deaths from COVID-19. However, it is unclear whether having previously had dengue might result in some immunity to SARS-CoV-2 (9). On the contrary, in another study in Brazil, it was observed that individuals with preexposure to dengue generally presented additional symptoms, compared with individuals not exposed to dengue. However, a decrease in 60-day mortality among COVID-19 patients with a history of dengue infection was also observed (24).

In the present study, to investigate the possible impact of prior immunity to DENV in patients with COVID-19, we performed a comparative analysis that took into consideration whether these patients had or had not had prior exposure to dengue. From these two groups, we were unable to differentiate any impact from previous exposure to dengue. Nonetheless, considering that in Brazil and particularly in Rio de Janeiro, a large part of the population has been exposed to dengue, the statistical data of the present study may not have represented the influence of exposure to dengue on COVID-19.

It is known that severe coronavirus patients, including those with SARS-CoV-1, MERS, and SARS-CoV-2, show a significant increase in circulating cytokines that is correlated with greater disease severity and cases of death (25–28).

Elevated plasma levels of various inflammatory mediators, including CCL2 and CXCL8, have already been detected in patients with SARS-CoV-2 infection (14). Our data demonstrated that patients with severe infection or who died from COVID-19 had high levels of CCL2 and CXCL8/IL-8. However, dengue patients had lower levels than cases of death due to COVID-19. One interesting finding was that CXCL8/IL-8 levels distinguished mild COVID-19 from fatalities.

The study by Liu et al. (29) was the main driver for our CCL2 and CXCL8 research. Those authors initially demonstrated in SARS-CoV infected monkey models that the presence of anti-spike IgG antibodies before viral shedding caused fatal acute lung injury because these antibodies distort the healing response, promote overproduction of CCL2/MCP-1 and CXCL8/IL-8 and give rise to recruitment/accumulation of pro-inflammatory monocytes/macrophages in the animals' lungs. In parallel with these findings, the authors observed that patients who eventually died from SARS had a lung pro-inflammatory profile like that of the monkey model, and increased levels of circulating CCL2/MCP-1 and CXCL8/IL-8. Thus, they defined a mechanism in which a virus-specific antibody response can have a pathological consequence, which would provide a potential target for treating SARS-CoV or other virus-mediated lung injuries using CCL2/MCP-1 and CXCL8/IL-8 blockers (29).

In much the same way, other authors have shown that in SARS-CoV-2 infection, the frequency of monocytes initially increases in the bloodstream; and that after the release of CCL2 by memory T cells residing in the tissue of the activated airways, infiltration of these monocytes in the airways occurs (30). Hyperinflammation is associated with greater severity of COVID-19, which is characterized by an unregulated increase in inflammatory cytokines and chemokines, including IL-6, IL-10, TNF-α, CXCL10, CCL2 and CCL3, thereby leading to acute respiratory

distress syndrome (ARDS) (31, 32). Elevated CCL2 has also been observed in dengue patients, particularly in severe cases (15). Another chemokine synthesized during inflammation is CXCL8, which is elevated in COVID-19 and dengue (33). A recent study has suggested that anti-spike IgG likely alters the functional response of macrophages in the lungs during acute infection, thus resulting in increased CXCL8 production and increased infiltration and accumulation of inflammatory monocytes/macrophages and ARDS (34).

There is noteworthy apprehension that the coming of COVID-19 has overlapped with other human viruses, most notably dengue, in several epidemic regions in Brazil. While both diseases may lead only to mild symptoms, they can also cause severe and fatal illnesses, particularly in patients with comorbidities and overlapping infections. Given this possible scenario, more studies should be carried out to address this critical issue promptly.

As conclusions from this study, we emphasize that the commercial kits used for detecting anti-SARS CoV-2 IgA and IgG have high sensitivity and specificity. This allowed us to see a low rate of co-detection or coinfection between SARS-CoV-2 and DENV in Rio de Janeiro. Neither the cases of co-detection of COVID-19/dengue nor the cases in which patients with COVID-19 already had previous exposure to DENV influenced the clinical severity of COVID-19. Lastly, CCL2 and CXCL8 appeared to be good markers of COVID-19 severity and did not show increased levels in COVID-19/dengue cases.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by the National Research Ethics Committee of the Plataforma Brasil (number 57221416.0.1001.5248 version 6 and number 13318113.7.3001.0021), the Ethics Committee of the Universidade Federal Fluminense (number 30623520.5.0000.5243) and the Research Ethics Committee of Hospital Casa Rio-Botafogo (number 47885515.8.0000.5279). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

EA and LD-O-P conceived the study. JR, DF-M, TM, FC, EA, LD-O-P performed laboratory work. JA, AS, LS, PD, LD-O-P carried out the analysis. JR, DF-M, TM, FC, LD-O-P prepared the datasets. JR and LD-O-P wrote the manuscript. EA, FS, LD-O-P contributed to the interpretation of results. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) FS, EA and LD-O-P declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fitd.2023.1249574/full#supplementary-material

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