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Cross-reaction and co-infection with DENV and SARS-CoV-2 in Brazil: a case series

Reação cruzada e coinfecção por DENV e SARS-CoV-2 no Brasil: uma série de casos

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ABSTRACT
Co-circulation of Dengue and COVID-19 represents a serious medical concern: although both diseases have similar clinical and laboratory findings, they require different clinical management. The objective of this study was to report three cases of suspected SARS-CoV-2 and dengue virus (DENV) coinfection based on clinical and laboratory evidence. However, only one patient was laboratory confirmed by RT-qPCR positive for SARS-CoV-2 and repeated rapid assay and Enzyme-Linked Immunosorbent Assay (ELISA) for DENV. The other two cases were suggestive of cross-reaction with negative ELISA. All three patients recovered favorably. This highlights the importance of accurate and timely diagnosis, particularly in dengue-endemic countries where the prevalence of cross-reactivity may be high.

Keywords: COVID-19. Cross-reaction. Dengue Virus. SARS-CoV-2.

RESUMO
A Co circulação de Dengue e COVID-19 representa uma séria preocupação médica: embora ambas as doenças tenham achados clínicos e laboratoriais semelhantes, elas exigem um manejo clínico diferente. O objetivo deste estudo foi relatar três casos de suspeita de coinfecção entre o SARS-CoV-2 e o vírus da dengue (DENV) com base em evidências clínicas e laboratoriais. No entanto, apenas um paciente foi confirmado laboratorialmente por RT-qPCR positivo para SARS-CoV-2 e ensaio rápido repetido e ensaio de imunoabsorção enzimática (ELISA) para DENV. Os outros dois casos foram resultados sugestivos de reação cruzada com ELISA negativo. Todos os três pacientes se recuperaram favoravelmente. Isso destaca a importância do diagnóstico preciso e oportuno, principalmente em países endêmicos de dengue, onde a prevalência de reatividade cruzada pode ser elevada.

Palavras-chave: COVID-19. Reação cruzada. SARS-CoV-2. Vírus da Dengue.

INTRODUCTION

On May 5, 2023, the World Health Organization (WHO) declared the end of COVID-19 a Public Health Emergency of International Concern[1]. However, the causative agent, SARS-CoV-2, continues to evolve with new variants jeopardizing the effectiveness of vaccines and available antibody-based therapies[2].

In Brazil, with the introduction of two doses of the COVID-19 vaccine, there was a decrease in the number of cases, hospitalizations, and deaths from COVID-19; nevertheless, other diseases returned to the scenario such as dengue. Currently, the country has returned to the dengue epidemic status with 1,530,940 reported cases (incidence rate of 753,9 cases per 100 inhabitants) and 946 confirmed deaths from dengue[3].

Although we know the periodic circulation of dengue, this upward trend in the number of cases is believed to be related to the COVID-19 pandemic, which culminated in the mobilization of health and epidemiological surveillance teams, as well as the allocation of most health resources to fight the coronavirus, resulting in the delay or underreporting of dengue cases[4, 5].

According to the Pan American Health Organization (PAHO), 2023 had the highest historical record of dengue cases, with more than 4 million new cases, and Brazil was the country with the highest number of cases and the highest number of severe cases. Given this, in 2023, PAHO issued an epidemiological alert on the sustained circulation of dengue in the Americas and its impact on endemic areas, reinforcing the need for preventive measures and the intensification of actions to prepare health services. It also advised against the use of rapid tests (NS1 and/or antibodies) due to their low sensitivity, which can interfere with the timely diagnosis of infection and proper patient management[6].

Dengue and COVID-19 are difficult to distinguish because they have shared clinical and laboratory features; moreover, both can present themselves as a coinfection, which can lead to the underdiagnosis of dengue[7]. In Singapore, Yan *et al*. (2020) observed that patients infected with SARS-CoV-2 were misdiagnosed with dengue by means of a rapid test, evolving into more severe clinical conditions[8].

Studies are still needed to elucidate the clinical consequences of this co-infection, and raise awareness among health professionals about this possibility. Morais, Neto and da Silva (2022)[9] emphasized that arboviruses remain a major challenge for health managers and professionals, especially in epidemic years, so epidemiological studies are essential to help understand the problems faced, as well as possible interferences, remembering that timely diagnosis is essential for proper patient management.

Here, we outlined three cases of patients infected with SARS-CoV-2 who had a reactive dengue rapid test. This was a random survey by convenience sampling, for which we considered the time of symptoms of COVID-19 longer than 10 days since we wanted to evaluate the cross-reaction of antibodies. This study was approved by the Permanent Research Ethics Committee of the State University of Maringá (Protocol 4.696.822).

CASE REPORT

The first case is a 56-year-old woman with no contact history with individuals positive for SARS-CoV-2, who presented herself to a regional hospital on January 25, 2021, with painful muscle spasms. She remained in the general medical clinic for 9 days, when she was transferred to HUM's COVID wing, after complaints of cough, headache, hypoxemia, runny nose, and odynophagia of two days onset. A chest tomography showed the presence of a peripheral ground-glass infiltrate, and the RT-PCR was positive for SARS-CoV-2.

She had non-insulin-dependent diabetes mellitus and reported tiredness with 92-95% O_2

saturation, requiring low-flow oxygen support. She did not have arthritis, skin lesions, oral ulcers, or pharmacological alterations. Other laboratory results showed a drop in hematocrit (29.8%) and hemoglobin (9.6 g/dL), neutrophilia with left shift, and lymphopenia in the first 5 days of hospitalization, with progressive recovery. There were no other signs of plasma leakage, bleeding, shock, or other complications (Table 1). Moreover, other laboratory parameters, like D-dimer (1,844 ng/mL FEU) and CRP (24.9 mg/dL), were abnormal. A repeat dengue rapid test (Dengue Duo ECO teste, ECO Diagnostic LTDA) was positive for dengue IgM and IgG on the 23rd day of hospitalization (17th day of respiratory symptoms onset). The same sample tested positive for IgM anti-Dengue by IgM capture ELISA (Panbio™ Dengue IgM Capture ELISA, Abbott Diagnostics Korea Inc.). The patient survived and was discharged.

Table 1. Laboratory Profile in the first patient

Laboratory	Day(s) of admission									
	1	4	5	8	11	12	13	15	20	25
Hematocrit (%)	39.3	36.1	34.7	34.4	31.7	31.9	30.4	29.8	31.8	34.9
Hemoglobin (g/dL)	12.5	12.0	11.7	11.2	10.5	10.6	9.8	9.6	10.3	11.3
White blood cells (mm^3)	3,800	3,6850	6,187	4,691	5,473	5,949	6,978	4,890	4,971	4,697
Segmented neutrophils (%)	66	65	87	57	52	54	80	44	50	43
Banded neutrophils (%)	0	0	3	0	1	3	10	0	0	0
Lymphocytes (%)	28	26	11	29	27	28	19	40	36	45
Lymphocyte count (mm^3)	1,064	1,001	680,6	1,360.4	1,477.7	1,665.7	1,325.8	1,956	1,789.6	2,113.6
Monocyte (%)	4	7	2	10	14	13	1	10	9	7
Eosinophils (%)	2	1	0	3	5	4	0	4	3	3
Basophils (%)	0	1	0	1	2	1	0	2	2	2
Platelets (mm3)	217,000	183,000	201,200	299,600	415,200	513,700	477,100	454,800	349,800	295,000
Aspartate aminotransferase (U/L)	39	-	-	-	-	-	-	-	-	-
Alanine transaminase (U/L)	28	-	-	-	-	-	-	-	-	-
Glucose (mg/dL)	180	146	139	80	120	140	200	147	210	138
Sodium (mmol/L)	144	143	140	143	138	139	139	140	138	138
Potassium (mmol/L)	4.1	4.1	3.3	4.4	3.5	3.3	3.4	4.5	4.5	4.0
Chloride (mmol/L)	111	109	110	114	111	109	108	109	104	108
D-Dimer (ng/mL FEU)	-	-	-	-	-	-	1,844	-	-	-
C Reactive Protein (PCR) (mg/dL)	2.8	15.6	24.9	8.8	-	1.8	1.1	-	<0.5	<0.5
Sed rate (Erythrocyte Sedimentation Speed) VHS (mm)	21	-	-	-	-	-	-	-	-	-

The second case was a 42-year-old man presenting a 14-day onset of continuous fever, as well as myalgia, arthralgia, and cough. Other vitals signs were normal upon admission, and initial blood work showed lymphopenia (520.4/mm^3) and slightly elevated levels of aspartate

aminotransferase (AST, 69 U/L) and alanine transaminase (ALT, 60 U/L). Other laboratory parameters, like D-dimer (606 ng/mL FEU), CRP (6 mg/dL), and chest X-ray, were abnormal. A duplicate Dengue Duo ECO test performed 19 days after symptoms' onset was positive for anti-dengue IgM and negative for anti-dengue IgG. The Panbio™ Dengue IgM Capture ELISA assay was negative for the same sample. COVID-19 antigen rapid assay and RT-qPCR (GENEXPERT-CEPHEID) of the nasopharyngeal swab were positive. The patient was diagnosed with COVID-19, and he was discharged on the 6th day of hospitalization.

The last patient was a 57-year-old man who was admitted to the HUM Intensive Care Unit (ICU) on January 25, 2021, after worsening of respiratory condition, with SaO_2 86%, blood pressure of 150x90, heart rate of 110, and afebrile. On the day of admission, laboratory results showed hyperglycemia, elevated lactate, altered gamma-GT, D-dimer (1,038 ng/mL FEU), lymphopenia (5%), neutrophilia (91%) with left shift, drop in hemoglobin (9.1 g/dL) and drop in hematocrit (28.1%). Platelet counts were normal. RT-PCR performed 17 days after symptoms' onset confirmed SARS-CoV-2-positive and chest X-ray revealed an undetermined pattern for pneumonia of viral etiology. He tested positive for IgM/IgG using a Dengue Duo ECO test in duplicate, but the Panbio™ Dengue IgM Capture ELISA came back negative for the same sample. He was discharged on February 11 of the same year.

DISCUSSION

At the beginning of the COVID-19 pandemic, a study conducted in Singapore highlighted the concern in identifying two patients diagnosed with COVID-19 by SARS-CoV-2 RT-PCR with positive serological antibody tests for dengue but negative DENV RT-PCR, suggesting false-positive serological test results for dengue or cross-reactivity[8]. Since then, studies have demonstrated the challenges faced by dengue-endemic countries since both dengue and COVID-19 exhibit non-specific symptoms, including fever, headache, abdominal pain, malaise, and nausea, and share laboratory evidence as well, like leukopenia and thrombocytopenia[10].

Our case report describes two cases of false-positive DENV results, suggestive of cross-reactivity with COVID-19 and a potential misdiagnosis case of human coinfection with SARS-CoV-2 and DENV. In the state of Paraná, Brazil, the diagnosis of DENV is performed by RT-qPCR within the first five days after the symptoms' onset and by IgM MAC-ELISA from the sixth day of symptoms. Moreover, some basic health units use the rapid duo test (NS1, IgM, and IgG) in patient screening[11]. However, although the indication of rapid tests is just for patient screening and cautiously, many clinicians from basic health units in Paraná' municipalities base their therapeutic decisions on these tests.

According to Spinicci *et al.*, (2020), the concern about false-positive dengue serology tests in COVID-19 patients could be downsized, at least when ELISA is performed[12]. So, DENV was detected by repeat dengue rapid and confirmed by IgM capture ELISA. Therefore, our case report could indicate a potential coinfection with dengue fever and COVID-19, following the diagnostic criteria recommended by the Department of Health of the state of Paraná.

We believe that the first patient already had a mild DENV infection when she was hospitalized, suggested by two positive rapid assays for IgG and IgM and confirmed by IgM capture ELISA test. The antigen test was negative, as well as the RT-PCR. IgG and IgM findings do not define the exact moment of infection; the presence of IgM characterizes a recent infection because it is the first immunoglobulin isotype to appear and declining generally to undetectable levels over 2-3 months[13]. Also, nonspecific changes in laboratory parameters were found shortly after admission. Later, around the 13th to the 20th day after admission, we observed a worsening of laboratory parameters and the onset of respiratory symptoms suggestive of a

new viral infection, confirmed by positive RT-qPCR for SARS-CoV-2. Fortunately, the patient did not develop severe respiratory complications resulting from COVID-19, nor did she progress to severe forms of DENV. The diagnosis of dengue was obtained after the patient was discharged.

The other two cases were considered plausible cases of cross-reaction since when repeating the analysis (IgM MAC ELISA for dengue), the results were discordant. The rapid assay used had DENV viral envelope protein as a recombinant antigen. Yaniv Lustig *et al*., (2021), identified structure similarities between chains of SARS-CoV-2 spike protein and chains of envelope protein of both Zika and Dengue virus, which could potentially explain the cross-reactivity between SARS-CoV-2 and DENV[14]. Other studies also reported a false-positive dengue IgM in confirmed COVID-19 patients and raised two hypotheses: antigenic similarity between those viruses triggers the production of anti-DENV antibodies by the immunological memory cells[8], and SARS-CoV-2 antibodies cross-react with DENV antigens used in the dengue serological tests, causing misleading results[15]. To this day, it is not clear how this happens, requiring further studies to clarify these events.

This report points out the complexity of diagnosing viral infections and highlights the importance of accurate and timely diagnosis, with complete laboratory investigation, mainly in dengue-endemic countries. We also emphasize the urgency of government initiatives such as orientation campaigns for the population and health professionals with an emphasis on strengthening surveillance and control systems, always remembering that the diagnosis of one infection does not exclude the possibility of another concomitant infection.

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