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LETTER TO THE EDITOR

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Coinfection with COVID-19 and coronavirus HKU1—The critical need for repeat testing if clinically indicated

To the Editor,

COVID-19 is the latest global pandemic caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). There have been seven pathogenic human coronaviruses (HCoVs). The four endemic HCoVs (HCoV-229E, HCoV-OC43, HCoV-NL63, HCoV-HKU1) can cause respiratory infections ranging from common cold and croup to lower respiratory tract infections.[1] SARS-CoV-1, MERS-CoV, and SARS-CoV-2 are zoonotic emerging epidemic pathogens with significant morbidity, mortality and economic impact.

The endemic HCoVs have been known to cause coinfections, sequential infections or can be codetected with each other or with other respiratory viruses.[2-5] In general, respiratory viral coinfections are recognized more commonly today with the use of respiratory multiplex molecular diagnostic panels.

We describe a case of endemic HCoV coinfection with SARS-CoV-2 in a patient with COVID-19.

1 | CASE DETAILS

A 34-year-old Filipino domestic worker presented with dry cough and sore throat of 4 days duration on 28th February 2020. She also had a low grade fever on the previous day, with maximal temperature recorded at 38°C. She had travelled to Batam, Indonesia, 3 days before the admission with seven other people from two separate households. Three of them subsequently were found to be confirmed cases of COVID-19 disease.

At presentation, she was afebrile, and her throat was mildly injected. Otherwise, the physical examination, including respiratory exam was unremarkable. Full blood counts, renal and liver function tests and lactate dehydrogenase were within normal limits. Of note, no leukopenia, lymphopenia, or thrombocytopenia was observed. Nasopharyngeal swabs were sent on day 1 of admission, one was positive for HCoV-HKU1 on the FilmArray Respiratory Panel (RP) (BioFire Diagnostics, bioMerieux) but another was negative for SARS-CoV-2 by reverse-transcription polymerase chain reaction (in-house-laboratory-developed test detecting the N and ORF1ab genes by primers, with LightCycler 2.0 instrument from Roche, RotKreuz, Switzerland[a]).[6] Chest radiography revealed ill-defined air space opacities bilaterally in the lower zones. As her travel companions had been confirmed to have COVID-19, and she remained symptomatic, we persisted in testing her for SARS-CoV-2 despite having an "alternative" diagnosis for her initial symptoms. SARS-CoV-2 was eventually detected on day 3 of admission. She made an uneventful recovery without any desaturation and had improvements in her chest radiography. She was discharged on day 20 of her admission; 24 days after onset of symptoms. (Table 1)

2 | DISCUSSION

Endemic HCoVs cause mild to severe respiratory infections or severe pneumonia with acute respiratory distress syndromes primarily in immunocompromised patients.[5] During 2002-2003, the first epidemic HCoV (SARS-CoV-1) emerged in Guangdong province, China. In January 2004, HCoV-HKU1 was discovered in a patient with community acquired pneumonia in Hong Kong.[5] Later HCoV-NL63 was reported in a 7-month-old baby with bronchiolitis in 2004 in Netherlands.[5] Then in 2012, the second epidemic HCoV (MERS-CoV) was reported in the middle east.[5] In December 2019, the third epidemic HCoV (SARS-CoV-2) was found in Hubei province, China and is now a global pandemic.

HCoVs are known to coinfect human hosts with other respiratory viruses, including influenza A/B, RSV, metapneumovirus, enterovirus, adenovirus.[2-5] Even among the four endemic HCoVs, coinfections may sometimes occur.[2-5] In this case, our immediate concern was whether there was cross reactivity of SARS-CoV-2 virus with the CoV-HKU1 target on the FilmArray RP. This was definitively ruled out when a repeat nasopharyngeal swab positive for SARS-CoV-2 on day 18 of admission was subjected to testing again on the FilmArray RP and found to be negative for all pathogens on

[a]For the detection of SARS-CoV-2, real-time reverse-transcription polymerase chain reaction targeting the N and ORF1ab genes were used. The sequence for the N gene forward primer was 5′-CTCAGTCCAAGATGGTATTTCT and reverse primer was 5′-AGCACCATAGGGAAGTCC). The probe sequence was 5′ FAM-ACCTAGGAACTGGCCCAGAAGCT-BHQ1. Thermal cycling was performed at 50°C for 20 minutes for reverse transcription, 95°C for 15 minutes for denaturation and then 50 cycles of 94°C for 5 seconds and 55°C for 1 minute.The sequence for the ORF1ab gene forward primer was 5′-TCATTGTTAATGCC TATATTAACC and reverse primer was 5′-CACTTAATGTAAGGCTTTGTTAAG). The probe sequence was 5′ FAM-AACTGCAGAGTCACATGTTGACA-BHQ1. Thermal cycling was performed at 50°C for 20 minutes for reverse transcription, 95°C for 15 minutes for denaturation followed by 50 cycles of 94°C for 5 seconds, 50°C for 20 seconds, and 72°C for 20 seconfs.For both assays, each 20 μL reaction contained 5 μL RNA template, 1 μL each of forward and reverse primer, 0.5 μL probe and 0.2 μL QuantiTect RT mix (QuantiTect Probe RT-PCR kit, Qiagen). Detection of endogenous RNAse P gene was used as an internal control. Both reactions were performed on LightCycler 2.0 instrument (Roche, RotKreuz, Switzerland).

TABLE 1 Results of SARS-CoV-2 and FilmArray RP testing by the day of admission

Day of admission	SARS-CoV-2	FilmArray RP
1	Not detected	HKU1 CoV detected
2	Not detected	Not done
3-17	Detected	Not done
18	Detected	No respiratory viruses detected
19-20	Not detected	Not done

Abbreviations: RP, respiratory panel; SARS-CoV, severe acute respiratory syndrome coronavirus-2.

the panel. Hence, we believe that our patient experienced coinfection with both endemic HCoV-HKU1 and pandemic SARS-CoV-2.

It is also possible that she had sequential infections. The patient was infected first with HCoV-HKU1 then later with SARS-CoV-2 with the FilmArray RP picking up the remnant or colonizing HCoV-HKU1 RNA that subsequently disappeared.

Our case illustrates the importance of maintaining a high degree of suspicion and to note that positivity for a known virus on any respiratory multiplex assay does not exclude the possibility of coinfection with SARS-CoV-2 in a patient with a compatible clinical presentation and epidemiological history.

3 | CONCLUSIONS

Clinicians need to be aware of coinfections among HCoVs. Testing protocols need to allow for repeat testing (our patient only turned positive on her third test) and testing in individuals with a possible alternative diagnosis. A high degree of suspicion in this rapidly evolving outbreak is required to make the diagnosis. This is vital if we are to try and contain and control the spread of the COVID-19.

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