Co-infection of SARS-CoV-2 with other respiratory pathogens in patients with liver disease

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Abstract
Respiratory illness caused by SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) was first documented in Wuhan, China, in December 2019, followed by its rapid spread across the globe. Accumulating evidence has demonstrated viral/bacterial co-infection in the respiratory tract could modulate disease severity and its outcome in COVID-19 infection. In this retrospective study, 300 chronic liver disease patients with radiologically confirmed lower respiratory tract infection were enrolled from September 2020 to December 2021. In all of them, along with SARS-CoV-2, other respiratory viral/bacterial pathogens were studied. In total, 23.7% (n=71) patients were positive for SARS-CoV-2. Among the positive patients, 23.9% (n=17) had co-infection with other respiratory pathogens, bacterial co-infections being dominant. The SARS-CoV-2 negative cohort had 39.7% positivity (n=91) for other respiratory pathogens, the most common being those of the rhinovirus/enterovirus family. Ground glass opacity (GGO) with consolidation was found to be the most common radiological finding among SARS-CoV-2 co-infected patients, as compared to only GGO among SARS-CoV-2 mono-infected patients. Accurate diagnosis of co-infections, especially during pandemics including COVID-19, can ameliorate the treatment and management of suspected cases.

INTRODUCTION
In December 2019, an increasing number of pneumonia cases with unknown aetiology emerged in Wuhan, Hubei, China. High-throughput sequencing from lower respiratory tract samples revealed a novel coronavirus that was initially named 2019 novel coronavirus (2019-nCoV) and is now referred to as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1]. The highly transmissible SARS-CoV-2 led to a devastating pandemic and, as of March 17 2022, a total of 4 64 621 732 confirmed cases of coronavirus disease (COVID-19), including 6 082 616 deaths, have been reported globally [2]. Before the emergence of the SARS-CoV-2 pandemic, in 2003, an outbreak of SARS-CoV occurred, followed by the emergence of another outbreak by Middle East respiratory syndrome coronavirus (MERS-CoV) in 2012, both viruses belonging to the family betacoronavirus [3]. The transmissibility of SARS-CoV-2 is higher than that of SARS-CoV and MERS-CoV as is apparent from the number of persons being infected and the extent of the spread of the virus across the globe [4, 5]. Co-infection with bacteria and other respiratory viruses is a common complication of many viral respiratory tract infections, associated with significant morbidity and mortality [6]. Though the COVID-19 disease has spread to almost 222 countries and territories (https://www.worldometers.info/coronavirus/) across the globe, the evidence of co-infection of SARS-CoV-2 with other respiratory pathogens, especially viruses, and their clinical implications in the Indian population is becoming increasingly important [7]. Infection with two or multiple pathogens can influence the overall disease severity and clinical outcome as compared to that of a single infection. In addition to direct interactions between the pathogens (co-infecting a single host), host factors serve as important determinants in modulating overall disease outcomes [8–11]. Co-infections can be favourable, detrimental or have no-effect on the disease outcome, depending on the different levels of host–microbe interactions. The most common observed outcome for viral co-infections is termed viral interference, a phenomenon where one virus inhibits the survival and replication of another co-infecting virus [12]. Viral co-infections are increasingly being reported nowadays. Co-occurrence of respiratory viruses including influenza virus, human metapneumovirus, rhinovirus and respiratory syncytial virus (RSV) in COVID-19 patients affects the length of hospital stay, and
overall mortality and morbidity [13–15]. The prevalence of co-infection with viral pathogens was variable among COVID-19 patients in different studies; however, it could be up to 50% among non-survivors [16]. Moreover, radiological findings among SARS-CoV-2 mono-infected and co-infected cases are limited. Therefore, the present study aimed to determine the rates of co-infection of SARS-CoV-2 with other respiratory viruses/bacteria among patients with radiological evidence of pulmonary involvement on high-resolution computed tomography (HRCT) scan suggestive of lower respiratory tract infection (LRTI) and compare their radiological presentations. Understanding the consequences of co-infections could help in better interpretation and correlation between pathogens and their clinical outcomes.

METHODS

Study design and enrolled participants

This was a retrospective study in which chronic liver disease (CLD) admitted patients with available HRCT findings suggestive of LRTI at our tertiary care hospital between September 2020 to December 2021 were enrolled. Archived once thawed specimen (nasopharyngeal and oropharyngeal swabs in viral transport medium) collected from these enrolled patients was retrieved from −80 °C storage. All the archived specimens were tested, in a single freeze–thaw cycle, in parallel by reverse transcriptase real-time PCR (RT-PCR) for SARS-CoV-2 and multiplex real-time PCR for other respiratory viral pathogens. Complete demographic and clinical details of all enrolled patients were collected from the Hospital Information System. CLD is defined as a progressive deterioration of liver functions for more than 6 months. The Covid-19 disease severity was classified based on recommended guidelines: mild, symptomatic (fever, cough, fatigue, headache, nausea, loss of taste and smell) without evidence of viral pneumonia or hypoxia; moderate, clinical signs of pneumonia and SpO2 ≥90% on room air at sea level; severe, clinical signs of pneumonia (fever, cough, dyspnoea) plus one of the following – respiratory rate >30 breaths min⁻¹, severe respiratory distress, or SpO2 <90% on room air (https://www.icmr.gov.in/).

Real-time PCR for detection of SARS-CoV-2

Nucleic acid extraction was performed with 300 µl specimen using a Chemagic viral DNA/RNA kit (PerkinElmer) on a Chemagic 360 instrument (PerkinElmer), following the manufacturer's instructions. An aliquot of 10 µl extracted RNA elute was further subjected to RT-PCR for the qualitative detection of SARS-CoV-2 using a RealStar SARS-CoV-2 RT-PCR kit, targeting the E gene and S gene.

Multiplex PCR for the detection of other viral respiratory pathogens

Detection of varied viral respiratory pathogens was performed using an automated multiplex BioFire FilmArray Respiratory panel assay (bioMérieux), targeting 18 viral pathogens with a turnaround time of about 45 min, including sample preparation and DNA/RNA extraction. The panel was run on the FilmArrayVR Torch system, according to the manufacturer's instructions. The panel included adenovirus, coronavirus 229E, coronavirus HKU1, coronavirus OC43, coronavirus NL63, human metapneumovirus, human rhinovirus/enterovirus, influenza A, influenza A/H1, influenza A/H1-2009, influenza A/H3 (Flu A/H3), influenza B, MERS-CoV, para-influenza 1, para-influenza 2, para-influenza 3, para-influenza 4 and RSV.

Detection of bacterial respiratory pathogens

The data of various bacterial respiratory pathogens detected among enrolled patients were recorded from the Hospital Information System.

Statistical analysis

We performed descriptive statistics and calculated median with interquartile range (IQR) and/or number (n) and percentage (%) of the variables, wherever it was appropriate, using SPSS.

RESULTS

Baseline characteristics of patients

A total of 300 admitted CLD patients with HRCT findings suggestive of LRTI were enrolled during the study period (Fig. 1). The mean age of the studied population was 47.5 years, with a male predominance of 82% (n=246). Among these 300, 71 (23.7%) patients were found to be positive for SARS-CoV-2 and the remaining 229 (76.3%) were negative, as depicted in our schematic workflow (Fig. 1). The detailed baseline characteristics of enrolled patients are described in Table 1.

Respiratory pathogens in SARS-CoV-2 positive patients

Among the total of 71 SARS-CoV-2 infected patients, 17 (23.9%) of them had co-infection with other respiratory pathogens (bacteria or virus) (Table 2). Among these 17 patients, co-infection with a viral pathogen was recorded in 4 (23.5%). Co-infection
of SARS-CoV-2 with rhinovirus/enterovirus was seen in two patients (11.8%), RSV in one (5.9%) and influenza virus in one (5.9%). Bacterial co-infection was recorded in the remaining 13 (76.5%) patients. *Klebsiella pneumoniae* (*n*=4, 23.5%) was observed to be the most common co-infecting bacteria, followed by *Staphylococcus aureus* (*n*=3, 17.6%). No viral–bacterial co-infection was found in this cohort.

**Respiratory pathogens in SARS-CoV-2 negative patients**

Among 229 SARS-CoV-2 negative patients with radiological evidence of LRTI, viral or bacterial aetiology was reported in 91 (39.7%). Out of these 91 patients, 73 (80.2%) were positive for other viral aetiology, the most common being rhinovirus/enterovirus (*n*=31, 42.5%), followed by RSV (*n*=15, 20.5%). Isolated bacterial infection was seen in 10 (11.0%) patients, *K. pneumoniae* being the most prevalent respiratory bacteria among the SARS-CoV-2 negative group. Viral–bacterial co-infection was seen in eight (8.8%) patients (Fig. 1).

**HRCT analysis**

The findings on the HRCT images were described as the following four patterns: ground glass opacity (GGO), GGO with consolidation, consolidation and others (linear/reticular opacity, pleural effusion and bronchiectasis). In the SARS-CoV-2 positive group,

| Characteristic | SARS-CoV-2 positive (*n*=71) | SARS-CoV-2 negative (*n*=229) |
|---------------|-------------------------------|-------------------------------|
| Number of samples [n (%)] | 17 (23.9) | 54 (76.1) | 91 (39.7) | 138 (60.3) |
| Median age (years) [IQR] | 48 [43–54] | 45 [32–74] | 50 [39–60] | 47 [36–56] |
| Male [n (%)] | 14 (82.4) | 42 (77.8) | 76 (83.5) | 114 (82.6) |
| Female [n (%)] | 3 (17.6) | 12 (22.2) | 15 (16.5) | 24 (17.4) |

### Table 1. Baseline characteristics of the enrolled study population
GGO with consolidation was the most common among the co-infected cohort as compared to GGO only in the SARS-CoV-2 mono-infected group. In contrast, bilateral infiltrates were the most common finding among the SARS-CoV-2 negative group, with no significant difference among viral/bacterial/viral–bacterial patients.

**Clinical outcome among SARS-CoV-2 positive and negative patients**

Among SARS-CoV-2 positive patients, severity in terms of COVID-19 infection was as follows: (a) monoinfected – mild (27%), moderate (37%) and severe (35%); (b) co-infected – mild (35%), moderate (29%) and severe (35%). Overall adverse clinical outcome in terms of mortality was seen in 70 (23.3%). In SARS-CoV-2 positive patients, mortality was seen in six patients (8.45%) each for co-infected and mono-infected groups. Among SARS-CoV-2 negative patients, overall mortality was observed among 58 patients (25.3%). Mortality in patients infected with either respiratory virus or bacteria was seen in 15 (16.5%). Co-infection of bacteria and respiratory virus resulted in three deaths (3.4%). In the remaining 40 (28.1%) cases, no underlying viral or bacterial aetiology could be established. No significant difference in terms of clinical outcomes was observed between SARS-CoV-2 mono-infected and co-infected patients per se.

**DISCUSSION**

This study is, to our knowledge, the first of its kind to determine rates of co-infection with other respiratory pathogens among SARS-CoV-2 infected patients with underlying CLD. The present study showed that 23.9% of SARS-CoV-2 infected patients were co-infected with one or more respiratory pathogens, which is in consonance (0.6–50%) with previous studies [16–18]. A study from India reported a co-infection rate of 46.6% (89/191) among patients infected with SARS-CoV-2 [17]. The lower co-infection rate in the present study might be due to the fact that only adults with underlying liver disease were enrolled.

In our report, co-infection in SARS-CoV-2 positive patients with other respiratory viruses was found to be 5.6% (n=4), with rhinovirus/enterovirus as the most common. Notably, rhinoviruses are found as commensals residing in the upper respiratory tract, although harmless they could lead to infection in those with compromised immunity [19]. Previous studies by Kim et al. and Ma et al. reported higher rates of co-infection with viruses among COVID-19 patients (24/116, 21%) and (18/250, 7.2%), respectively [13, 18]. In agreement with findings by Marshall et al., we found a low co-infection rate among SARS-CoV-2 positive...
patients [20]. However, a recent study by Contou et al. reported no co-infection related to viruses in SARS-CoV-2 positive patients [21]. Therefore, there are mixed results in terms of co-infection rate among SARS-CoV-2 positive patients. In our study, around 18% (n=13) of SARS-CoV-2 infected patients had bacterial co-infection, the most common bacterial species being *K. pneumoniae*. Previously, a few studies from India have shown bacterial co-infection among SARS-CoV-2 positive patients [22, 23] with implications for disease outcomes. These bacteria may be found as commensals colonizing our respiratory tract without causing any disease. However, under immunocompromised settings, these commensals can turn into pathogens. There is an increasing line of evidence suggesting that respiratory viral infection could predispose patients to bacterial co-infection, resulting in an altered course of clinical outcomes [9]. Viral replication results in damaged lung epithelial cells, recruiting immune cells leading to the release of several cytokines. Usually, our body's immune response clears the infection and patients recover. However, in some cases, a dysfunctional immune response can trigger increased levels of cytokines, resulting in what we term a 'cytokine storm'. This immune state makes the patient highly susceptible to secondary infections [24]. This wide variation in the estimation of rates of co-infection ranging from 0 to 50% might be due to the role of several confounding factors, including geographical and demographic patterns, time period and prevalence of respiratory pathogens in hospital settings [25].

Among SARS-CoV-2 negative patients (n=229), 91 patients showed the presence of respiratory pathogens. Rhinovirus/enterovirus was the most common virus, which is in consonance with other studies [13, 14], and *K. pneumoniae* was the most common bacteria in this cohort. Interestingly, viral–bacterial co-infection was observed in eight patients (8.8%). New studies are imperative to assess the pathogenesis underlying mixed viral–bacterial infections and their implications for host immune responses, especially among co-morbid patients.

GGO with or without consolidation in a peripheral and basilar predominant distribution was found to be the most common finding in our SARS-CoV-2 positive cohort, which is in agreement with previous reports [26, 27]. The differential diagnosis of COVID-19 and other viral/bacterial pneumonia is challenging based on HRCT findings, owing to overlapping computed tomography features, which is in consonance with previous studies [28, 29].

In our study population, the mortality was higher in the SARS-CoV-2 negative population, suggesting that the virus is not associated with mortality per se. Other pre-existing comorbidities may have played a role in the adverse clinical consequences.

Intriguingly, in 60% (n=138) individuals, no respiratory pathogens were detected despite being HRCT-proven LRTI patients. This could be due to the fact that this is a retrospective record-based study and HRCT findings may persist for a longer time while respiratory samples turn negative for PCR or many patients were empirically started on antibiotic therapy leading to negative bacterial culture.

The findings in our study suggest a declining trend of circulation of non-SARS-CoV-2 respiratory pathogens during the COVID era, which could be because of the preventive measures that were taken to avoid COVID-19. Strict lockdowns, healthcare prioritization towards COVID-19 and temporary disruptions in normal routine medical care might have led to a decrease in the expected laboratory reports of other respiratory pathogens. Another possible reason could be viral interference might have played a role in mitigating overall co-infection rate and its associated clinical outcomes in SARS-CoV-2 co-infected patients.

The present study has certain limitations. It is a retrospective study where only adult CLD admitted patients were enrolled. In addition, fungal aetiology associated with varied HRCT findings among patients presenting with LRTI was not evaluated.

**Conclusion**

Viral interference may lead to successful survival of one virus and clearance of others mediated by viral–host immune interactions. This could be one probable reason for reporting lower respiratory viral co-infection in SARS-CoV-2 positive patients. Accurate and timely detection of viral and/or bacterial co-infections in patients with radiological evidence of LRTI would help in efficient targeted treatment strategies and, probably, better clinical outcomes.

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**Author contributions**
E.G.: conceptualization, supervision, methodology and writing – review and editing. J.S.: writing – original draft and project administration. R.A.: methodology and writing – review and editing. A.S.: data curation and formal analysis. A.P.: data curation. S.T.: data analysis and editing manuscript.

**Conflicts of interest**
The authors declare that there are no conflicts of interest

**Ethical statement**
This study was reviewed and approved by our institutional ethical committee. The written consent of patients enrolled was waived (ethical approval number IEC/2021/84/MA10).
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