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Impact of rhinovirus on hospitalization during the COVID-19 pandemic: A prospective cohort study.

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\textbf{ABSTRACT}

\textbf{Background:} Although the clinical course of the COVID-19 in adults has been extensively described, the impact of the co-detection of SARS-CoV-2 and rhinovirus on severity outcomes is not understood.

\textbf{Objectives:} This study aimed to compare the risk of hospitalization of outpatients with COVID-19 with and without the co-detection of rhinovirus in southern Brazil. Secondly, such risk was also compared between all individuals with COVID-19 and those with single rhinovirus infection.

\textbf{Study design:} Outpatients (>18 years) with acute signs of cough, fever, or sore throat were prospectively enrolled at two emergency departments from May to September 2020. Sample collection was performed to detect SARS-CoV-2 and other 20 respiratory pathogens. Participants were followed for 28 days through telephone interviews.

\textbf{Results:} 1,047 participants were screened and 1,044 were included. Of these, 4.9% were lost during follow-up, and 993/1,044 (95.1%) were included in severity-related analysis. Rhinovirus was the most prevalent pathogen (25.0%, 248/993), followed by SARS-CoV-2 (22.6%, 224/993), with coinfection of these two viruses occurring in 91/993 (9.2%) participants. The risk of COVID-19-related hospitalizations were not different between individuals with and without co-detection of rhinovirus (9.9% vs. 7.6%, respectively, \(P = 0.655\)). Conversely, subjects with COVID-19 had a higher hospitalization risk than single rhinovirus infection (8.3 vs 0.4%, respectively, \(P < 0.001\)).

\textbf{Conclusions:} The co-detection of SARS-CoV-2 and rhinovirus did not change the risk of hospitalizations in adults. Furthermore, COVID-19 was more severe than single rhinovirus infection.

1. \textbf{Introduction}

The COVID-19 pandemic has been the most significant health crisis of the 21st century. Despite all global efforts made to contain the spread of the Severe Acute Respiratory Syndrome Coronavirus type 2 (SARS-CoV-2) since 2020, more than 6 million people have died up to date [1]. Older age and obesity are the most important risk factors for respiratory failure and death [2–7].

The preventive measures to contain the pandemic, which included the widespread use of face masks, hand hygiene, travel restrictions, school closures, and physical distancing, had an important impact on the epidemiology of many infectious diseases [8]. A substantial global decline in the detection of common respiratory viruses, such as influenza and respiratory syncytial virus, was reported, especially during the most rigid restrictions [9–12]. However, the rhinovirus detection was shortly reduced or even not reduced in some settings, making this virus the main
co-circulating with SARS-CoV-2 during the COVID-19 pandemic [13–16]. Despite the accumulating epidemiological evidence mentioned above, the impact of respiratory viral co-detections, including rhinovirus, in the severity of COVID-19 is not completely understood.

The rapid development of highly effective vaccines against SARS-CoV-2 through many different platforms had an important impact in the course of the COVID-19 pandemic, reducing the risks of both severity-related outcomes and transmission [17–20]. However, many factors such as the inequality regarding vaccine distribution, vaccine hesitancy and the emergence of variants of concern may perpetuate SARS-CoV-2 as a global threat [21–23].

All factors mentioned above reinforce the need to evaluate the interactions of SARS-CoV-2 and other respiratory viral infections. The main aim of this study is to evaluate the impact of rhinovirus co-detection on the risk of hospitalization in adults enrolled as outpatients with COVID-19. As a secondary aim, the risk of hospitalization in individuals with single rhinovirus detection and all those with COVID-19 was also compared.

2. Materials and methods

2.1. Participants’ selection

A prospective cohort study was done at emergency rooms (ERs) of Hospital Moinhos de Vento and Hospital Restinga e Extremo Sul in Porto Alegre, Southern Brazil. Hospital Moinhos de Vento, which is a tertiary and a private hospital, has 372 general wards and 113 ICU beds. Hospital da Restinga Extremo Sul is a tertiary hospital with 485 beds and is a reference for Restinga district, providing care exclusively through the Brazilian public health system. From May to September 2020, adults (≥18 years old) presenting at least one sign or symptom suggestive of COVID-19 (cough, fever, or sore throat) within 14 days were screened. The failure in sample collection was the exclusion criteria. Clinical and demographic data, comorbidities, and signs or symptoms suggestive of COVID-19 infection were collected at baseline. After the inclusion at ER, the participants were followed for 28 days through phone calls (on the 7th, 14th, and 28th day) and hospitalization outcomes (hospital admission, use of supplemental oxygen, admission at intensive care unit (ICU), use of invasive mechanical ventilation, and death) were assessed, as described elsewhere [4]. The review of medical records was performed at these hospitals when no information was obtained through the phone calls. The losses were defined if no contact was possible within the 28-day follow-up or no admission at both institutions.

2.2. Pathogen detection

At enrollment, oropharyngeal and bilateral nasopharyngeal swab collection for SARS-CoV-2 detection was performed for all participants and analyzed through qualitative reverse transcription polymerase chain reaction (RT-PCR) assay, as described elsewhere [24]. Participants with detection of SARS-CoV-2, regardless of the occurrence of other respiratory pathogens co-detections, were classified as having COVID-19.

A real-time PCR respiratory panel was done from a second bilateral nasopharyngeal swab, collected at enrollment, and stored at -80 °C. It was assessed the presence of Bordetella pertussis; Chlamydia pneumoniae; Mycoplasma pneumoniae; adenovirus; bocavirus; coronavirus types HKU1, 229E, NL63, and OC43; influenza A virus types H1 and H3; influenza B virus; human enterovirus; human metapneumovirus; parainfluenza virus types 1, 2, and 3; RSV types A and B; and rhinovirus. Acid nucleic extraction was done using the MagMax Viral/Pathogenic Nucleic Acid Isolation (Applied Biosystems) in the KingFisher Duo Prime System platform (ThermoFisher, USA). NanoDrop™ Lite Spectrophotometer (ThermoFisher, Wilmington, Delaware, USA) was used to sample quantification, and then, the samples were diluted between 0.5 and 2.0 ng/µL. RT-PCR assay was done using the Path™ 1-Step RT-qPCR Master Mix CG (A15299, Applied Biosystems) and TaqMan® Microbial Assays-single tube assay (Applied Biosystems, Pleasanton, California, USA). The probe access codes for the target pathogens are listed in Supplementary Table 1. As the reaction control, we used the TaqMan® Respiratory Tract Microbiota Amplification Control (A39178, ThermoFisher).

All the samples were analyzed in the Molecular Biology Laboratory at Hospital Moinhos de Vento.

2.3. Statistical analysis

Data normality assumptions were verified for continuous variables, and values were presented as median and interquartile ranges (IQR). Categorical variables were described as percentages, and Pearson’s Chi-square test or Fisher’s Exact test were used to evaluate the association of clinical and demographic data, as well as severity-related outcomes, between individuals with SARS-CoV-2 single detection and those with both SARS-CoV-2 and rhinovirus co-detection. The same comparisons were also performed between those with single rhinovirus detection and all individuals with COVID-19, regardless of respiratory pathogens co-detection. The cycle threshold (ct) values of ORF1ab, S and N SARS-CoV-2-specific targets were analyzed by the two-tailed Mann–Whitney–Wilcoxon test between the groups (SARS-CoV-2 single detection and the co-detection with rhinovirus). The power estimation was performed using the observed values of the hospitalization outcome according to the groups cited above. The “ES.w2” function was used to calculate the effect size, and then “pwr.chisq.test” function with α = 0.05 (both from the “pwr” package [25]) was applied. Multivariable logistic regression analyses were performed to evaluate the risk of hospitalization, using the backward stepwise method with P value cutoff < 0.20, considering the results from univariate analyses presented in Table 2, in addition to other relevant predictors, as obesity [4]. All data preprocessing and analyses were performed in R 4.1.1 statistical software.

2.4. Ethical approval

The study was performed following the Decree 466/12 of the National Health Council [26] and Good Clinical Practice Guidelines, after approval by the Hospital Moinhos de Vento Institutional Review Board (IRB n° 4.637.933). All participants included in this study provided written informed consent.

3. Results

In this study, 1047 outpatient participants were screened, and three were excluded (for failure in sample collection). As shown in Fig. 1, 1044 adults were included. Of these, 34 (3.3%) subjects required hospitalization. The 28-days follow-up was successfully obtained for 993 (95.1%) participants.

Among the 993 participants that completed the follow-up, 25.0% (248) were diagnosed positive for rhinovirus, followed by SARS-CoV-2 (22.6%, 224) and Mycoplasma pneumoniae (0.4%, 4) as single detection, as shown in Table 1. The coinfection of rhinovirus and SARS-CoV-2 occurred in 91 (9.2%) participants, and one had also Mycoplasma pneumoniae co-detection associated. Other multiple detections, such as SARS-CoV-2 and other pathogens (coronavirus types HKU1 or NL63; enterovirus; adenovirus; metapneumovirus; or Mycoplasma pneumoniae), and rhinovirus and Mycoplasma pneumoniae, were observed in 16 (1.6%) and 10 (1.0%) participants, respectively. No pathogen detection occurred in 400 (40.3%) individuals.

No detection of Bordetella pertussis; Chlamydia pneumoniae; bocavirus; coronavirus types 229E, and OC43; influenza A virus types H1 and H3; influenza B virus; parainfluenza virus types 1, 2, and 3; RSV types A and B was found.

The median age of the included participants was 36.4 years (IQR,
28.8–44.9, range 18.0–89.1), 62.7% (655/1044) were female, and the median days of symptoms onset to inclusion was 3.0 (IQR, 2.0–5.0, range 0.0–14.0), as shown in Supplementary Table 2. The co-detection of rhinovirus did not influence the risk of hospital admission in individuals with COVID-19 (9.9% vs. 7.6%, P = 0.655), as shown in Table 2. However, there was a higher association with hospitalization, use of supplemental oxygen and admission at intensive care unit (ICU) in participants with COVID-19, regardless of co-detection status, than in those with rhinovirus single detection, as depicted in Supplementary Table 3. Adults with rhinovirus co-detection were younger than those with COVID-19 only (P = 0.030), and the prevalence of hypertension was positively associated with subjects with COVID-19 single detection (16.5% vs. 6.6%, P = 0.032). In multivariable modeling, participants who required hospitalization were significantly older and obese (OR = 1.00, 95%CI 1.00–1.01, P = 0.001; OR = 1.13, 95%CI 1.07–1.20, P < 0.001, respectively). Whereas the presence of hypertension and virus co-detections had no relevance for hospitalization in our cohort (OR = 0.98, 95%CI 0.89–1.08, P = 0.694; OR = 1.02, 95%CI 0.96–1.09, P = 0.567, respectively).

The median, IQR and the comparison between ct values of the probes considering the SARS-CoV-2 single detection and the co-detection with rhinovirus are, respectively: ORF1ab (20.7, IQR 17.7–24.9 and 20.7, IQR 17.2–26.2; P = 0.886), N (20.4, IQR 16.2–24.5 and 19.9, IQR 16.4–24.8;

| Table 1 Pathogen detection of included participants. |
|-----------------------------------------------|
| Pathogen                          | Total(n – 1044) | Completed follow-up(n – 993) |
|-----------------------------------------------|
| Single detection                       |
| Rhinovirus                           | 262 (25.1)     | 248 (25.0)                  |
| SARS-CoV-2                            | 225 (22.5)     | 224 (22.6)                  |
| Mycoplasma pneumoniae                | 4 (0.4)        | 4 (0.4)                     |
| Multiple detections                   |
| Rhinovirus and SARS-CoV-2             | 93 (8.9)       | 91 (9.2)                    |
| SARS-CoV-2 and others                 | 18 (1.7)       | 16 (1.6)                    |
| Rhinovirus and Mycoplasma pneumoniae  | 10 (1.0)       | 10 (1.0)                    |
| No pathogen detection                | 422 (40.4)     | 400 (40.3)                  |

Fig. 1. Study flowchart.
4. Discussion

This is the first prospective study assessing the impact of rhinovirus co-detection on hospitalizations of COVID-19 in adults. Our results strongly suggest that rhinovirus detection does not affect the risk of hospitalization in individuals with COVID-19. Moreover, the risk of hospitalization in adults with COVID-19 was much higher than in those with single rhinovirus detection.

The epidemiology of many communicable diseases was markedly changed during the COVID-19 pandemic. Detections of highly prevalent agents of respiratory infections such as influenza and respiratory syncytial viruses decreased worldwide during 2020 [9,10]. In the same way, other viruses such as adenovirus, para-influenza, and metapneumovirus also were less detected compared to previous seasons [27, 28]. Conversely, rhinovirus was reported as the main co-circulating virus during the pandemic. A decrease of its detection during the most restrictive periods of social isolation but an earlier relapse as soon as restrictions were relaxed were reported in many countries, mainly through surveillance studies [15,16,29]. Our findings reinforce with prospectively collected data all these epidemiological changes during the first year of pandemic. In addition, age and obesity were the most critical risk factors associated with hospital admission [4].

Throughout the COVID-19 pandemic, much was discussed about the comparison regarding the severity of the infection due to SARS-CoV-2 and other respiratory viruses, as well as the role of respiratory coinfections. However, as the spread of most prevalent respiratory viruses was reduced, as mentioned above, many of these comparisons were mostly based on historical or retrospective data [30,31]. Our findings provide a direct comparison between the single infections and the co-detections of the two main co-circulating viruses in 2020. Furthermore, respiratory symptoms were more associated with rhinovirus single detection, whereas general symptoms were observed in individuals with SARS-CoV-2 infection. Although rhinovirus might be only a bystander in some situations, it has been consistently related to lower respiratory tract disease in older adults and asthma exacerbation [32–34]. However, the risk of hospitalization due to rhinovirus was much lower than those secondary to COVID-19 in our study.

The role of coinfection of SARS-CoV-2 with other respiratory viruses is not fully understood. Some experimental data suggested that the simultaneous infection by rhinovirus might protect individuals from severe COVID-19 due to Interferon stimulation [35]. Nonetheless, our results did not support any changes in the severity of COVID-19 when rhinovirus was co-detected. Before the COVID-19 pandemic, most of the available evidence suggested that detection of multiple respiratory viruses was not associated with changes in severity and our findings are in line with previous evidence [36,37]. However, the severity of multiple infections, including SARS-CoV-2, might be different, although our findings do not suggest this association in SARS-CoV-2 and rhinovirus co-detection.

Regarding the SARS-CoV-2 variants, the B.1.1.28, B.1.1.33, B.1.91, B.1.1 and B.1.1.143 lineages were reported to predominante at the time of the study [38,39], with special attention to the emergence of the B.1.1.28 lineage (which originated the Gamma lineage – P.1) that was described in November 2020. Although our enrollment was completed in September, it is unlikely that the Gamma lineage was represented in our samples.

This study has some limitations. First, as the incidence of most respiratory viruses sharply decreased during the pandemic, only the impact of SARS-CoV-2 and rhinovirus co-detection was possible to assess. Second, as participants were mostly middle-aged outpatients enrolled at the beginning of the clinical course with mild disease, evaluation of severity-related outcomes other than hospitalization was not possible. Third, technically it is not possible to differentiate between actual rhinovirus infection and its detection as a bystander. Fourth, the use of antihistamines at inclusion might mask the prevalence of atypical bacteria. Fifth, this study was done before the emergence of variants of concern. On the other hand, no previous study directly compared prospectively the impact of rhinovirus co-detection on the risk of hospitalization due to COVID-19 in adults, evaluating co-detections using a broad viral/bacterial panel, a large sample size, and 28 days of follow-up.

In summary, our results strongly suggest that rhinovirus co-detection has no prognostic role in COVID-19. Moreover, rhinovirus single
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