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MAJOR ARTICLE

Comparison of Rates of Hospitalization Between Single and Dual Virus Detection in a Mexican Cohort of Children and Adults With Influenza-Like Illness

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Background. Molecular detection methods allow for the simultaneous detection of several infectious agents. This study assesses whether co-infection with 2 viruses as compared with 1 is associated with increased hospitalization in those with acute respiratory infections.

Methods. We prospectively enrolled a cohort of pediatric and adult participants with influenza-like illness during 2010–2014 in Mexico. Clinical information and respiratory samples were collected at enrollment. Respiratory viruses were detected with multiplex polymerase chain reaction (PCR) and influenza-specific reverse transcription PCR assays. Participants were followed for 14 and 28 days after inclusion. Severity of disease, as measured by hospitalization with acute respiratory infections, was compared between single and dual viral infections.

Results. Among 5662 participants in the study, either 1 (n = 3285) or 2 (n = 641) viruses were detected in 3926 participants. Rhinovirus (n = 1433), influenza (n = 888), and coronaviruses (n = 703) were the most frequently detected viruses (either alone or in co-infection). Bocavirus, respiratory syncytial virus (RSV), metapneumovirus, and rhinovirus cases were hospitalized more often than other viruses. Bocavirus+rhinovirus cases were hospitalized more often than those with rhinovirus alone (but not bocavirus alone). RSV cases were more likely to be hospitalized than cases with co-infections of RSV and parainfluenza virus or coronavirus. Metapneumovirus cases were hospitalized more often than those co-infected with metapneumovirus+coronavirus.

Conclusions. In this study, detection of 2 viruses did not significantly increase hospitalizations compared with single virus infections. Larger studies will allow for distinguishing between sequential and simultaneous infection and for a better understanding of the role of each virus during the evolution of acute respiratory episodes.

Keywords. acute respiratory infections; coinfection; hospitalization; influenza; severity.

Current estimates indicate that lower respiratory tract infections (LRTIs) are the fifth leading cause of death in the world, accounting for 2.74 million deaths in 2015 [1]. The etiology of acute respiratory infections is diverse, and respiratory viruses are increasingly recognized as important causes of severe respiratory infections. Before the introduction of molecular detection methods, the etiology of a large proportion of acute respiratory infections could not be ascertained. The increasing use of reverse transcription polymerase chain reaction (RT-PCR) and other molecular methods has allowed for detection of respiratory viruses in a large proportion of cases. In addition, during the last 2 decades, previously unrecognized agents, such as human metapneumovirus (HMPV), human bocavirus (HBoV), rhinovirus C, and several coronaviruses, have been identified as new causes of respiratory infection [2–5]. As such, the use of currently available diagnostic techniques allows detection of at least 1 pathogen in the majority of patients [6]. Additionally, the rates of hospitalization are significantly different based on the virus isolated. Mexican children 5 years of age and younger presenting with influenza-like illness (ILI) caused by human respiratory syncytial virus (RSV) and HMPV have been shown to be at greater risk of hospitalization compared with other viruses [7].

As a result of the increasing use of molecular detection of respiratory viruses and the frequent detection of some of these

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viruses in asymptomatic individuals, there is a need to clarify their role in the etiology of LRTI [8]. In addition, the availability of diagnostic platforms that allow for the simultaneous detection of many pathogens has resulted in the identification of ≥2 agents in a large number of patients with respiratory infections [9–11]. Before the use of molecular methods, detection of viral co-infections was relatively rare [12]. In contrast, most recent studies report detection of >1 virus in approximately one-fourth of patients (22.1%–22.7%) [9–11]. This has created new opportunities for studying the contribution of each virus in the development, intensity, and duration of symptoms, as well as complications (eg, pneumonia) and death. To address this, many studies have sought to determine whether co-infection with ≥2 viruses contributes to the severity of an infection. Some of these studies have reported that the presence of >1 virus is associated with more severe infections, whereas others have not [9–11, 13–15].

In a systematic review and meta-analysis of studies carried out in children <5 years of age, no association between co-infection and increase in disease severity was found, but the need for further studies on this matter was identified [16]. Variability in the results of these studies might be a reflection of the populations included in each study, the definition of severity used, or the viruses that were compared. Of particular relevance is the definition of co-infection, as many studies compare those infected with a specific virus to infection with >1 virus [9–11, 15].

When assessing the effect of the presence of 2 viruses, various combinations may have differential effects on severity. In the present study, we investigated whether severity, defined as hospitalization with acute respiratory infections, increases with 2 viruses over that of each single virus. We analyzed data from a large prospective ILI cohort during 4 consecutive years in Mexico (ILI-002 study).

METHODS

Study Population

This analysis is based on data from ILI-002, a hospital-based prospective observational cohort study of ILI [17-19]. The present analysis includes all participants enrolled in the ILI-002 study in whom 1 or 2 viruses were detected.

The ILI-002 study was carried out at 6 public hospitals, 5 of them located in Mexico City and 1 in San Luis Potosí. Participating hospitals included 2 general hospitals (1 located in Mexico City and 1 in San Luis Potosí), 2 tertiary care pediatric hospitals, and 2 tertiary care hospitals (1 of them dedicated to the treatment of respiratory disorders, whereas the other provides medical care in a wide range of medical specialties). Adults and children seeking medical attention with ILI, defined as a respiratory symptom (eg, cough, dyspnea) plus a systemic symptom (eg, fever, malaise), were invited to participate in the ILI-002 protocol (ClinicalTrials.gov identifier: NCT01418287). For those enrolled, a follow-up telephone or face-to-face interview was performed at 14+/- 3 days, and a visit happened 28+/-5 days after inclusion. The study protocol was approved by the ethics committee at all participating institutions, and all participants or guardians signed an informed consent or an assent form when pertinent.

Pathogen Detection

A nasopharyngeal swab for multiple PCR pathogen detection was obtained at enrollment. Samples were stored in transport media at 4°C at each site (for sites located in Mexico City) and sent daily to a central facility (Molecular Biology Laboratory, Infectious Diseases Department, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City) for testing. Samples obtained in San Luis Potosí (a site outside of Mexico City) were stored at −70°C and sent weekly to the central facility for pathogen detection testing.

Samples were tested with either the RespiFinder19 kit or the RespiFinder22 kit (PathoFinder BV, Maastricht, the Netherlands). The 19-pathogen PCR test can detect and differentiate 15 viruses (coronavirus [CoV] NL63, OC43, and 229E; HMPV, influenza A, influenza A H5N1, influenza B, parainfluenza virus [HPIV] types 1–4; RSV types A and B; rhinovirus [RV]/enterovirus; and adenovirus [HAdV]) and 4 bacteria (*Bordetella pertussis, Chlamydophila pneumoniae, Legionella pneumophila*, and *Mycoplasma pneumoniae*). The 22-pathogen assay added CoV HKU1, HBoV, and influenza A (H1N1) pdm09 while removing influenza A H5N1. As reported by the manufacturer, the analytical limit of detection of the assay varies between 5 and 50 copies per reaction for most targets. Samples that were tested originally with the RespiFinder19 kit were subsequently tested for HBoV detection with the use of virus-specific primers. In addition, all samples were tested by real-time RT-PCR for influenza A following the Centers for Disease Control and Prevention (CDC) protocol [20].

Study Variables

Participants hospitalized during the 28 days of the study follow-up were considered to have severe disease. Hospitalization was defined as participants who were admitted to the hospital or remained in the emergency departments for at least 24 hours.

Participants with a detected bacterial pathogen (*Bordetella pertussis, Chlamydophila pneumoniae, Legionella pneumophila*, and *Mycoplasma pneumoniae*), no virus, or >2 viruses were excluded. Comorbidities were defined as 1 of the following: chronic obstructive pulmonary disease, cardiovascular disease, diabetes mellitus, previous use of systemic steroids, obesity, overweight, and underweight.

Statistical Analysis

For this analysis, we grouped similar genera of viruses and examined the 8 most frequent groups of viruses isolated:

influenza (A, A (H1N1)pdm09, and B grouped as influenza), HMPV, HPIV, RSV, RV, HAdV, CoV, and HBoV. Comparisons of baseline factors were made between hospitalization and nonhospitalization groups. All potential risk factors that were not categorical were grouped into categories. Chi-square statistics were used to make the univariate comparisons of the risk factors.

Logistic regression models were used to compare hospitalization between all pairs of viruses and the combinations of the 2 viruses. Combinations with <10 participants were not analyzed. Each logistic regression model included sex, age (grouped into 3 categories), days since symptom onset (grouped into 3 categories), and comorbidity (yes/no). Odds ratios and 95% confidence intervals were calculated.

RESULTS

From 2010 to 2014, 5662 participants were included in the ILI-002 study. From these, 96.89% had a 28-day interview, 1619 had no virus isolated, and 32 had no sample; additionally, 85 were excluded for other reasons such as bacterial infections (18 subjects), missing covariate information (11 subjects), and >2 viruses (56 subjects). The final data set had 3926 participants.

Of the 3926 participants, 1856 (47.3%) were hospitalized, 1411 (35.9%) were <11 years old, and 308 (7.8%) were >60 years old; 1673 (42.6%) were males (Table 1). Of the 1856 hospitalized cases, 65 died with 1 virus and 12 died with 2 viruses detected. Table 2 shows the distribution of participants with a single virus diagnosis across the covariates used in the logistic regression models. Influenza, HPIV, CoV, and RV were detected more frequently in participants 11–60 years old, whereas RSV, HMPV, HAdV, and HBoV were more common in children <11 years old. One virus was detected in 3285 and 2 viruses were detected in 641 participants (Table 3). RV (n = 1433), influenza (n = 888), and CoV (n = 703) were the most frequently detected viruses (either alone or in co-infection). The most frequent combination was influenza+CoV (116 of 641 dual infections; 18%). Influenza was found in combination with other agents in 237 participants (37% of 641 dual infections).

There were 52 subjects with a combination of >3 viruses. The numbers were too small to perform any statistical analyses but were analyzed descriptively. In the subjects with 3 viruses detected, 56% had RV, 54% had influenza, and 48% had CoV. The combination of 3 viruses that occurred the most often (in 6 subjects) was influenza, HMPV, and HBoV.

Hospitalization of Single Viral Diagnosis

The adjusted odds ratios (ORs) for hospitalization rates between each of the viruses included in the study are shown in Figure 1. Participants infected with HBoV were more likely to be hospitalized than those infected with influenza, CoV, HPIV, and RV (Figure 1A). Those with HAdV, HMPV, and RSV had similar but not statistically significant results. Participants infected with RSV were more likely to be hospitalized compared with cases of influenza, CoV, RV, HPIV, HAdV, and, to a lesser extent (not statistically significant), HMPV (Figure 1B). Participants with HMPV were more likely to be hospitalized compared with cases of influenza, CoV, and RV, but were not statistically significantly different compared with HPIV and HAdV (Figure 1C). HPIV cases were more likely to be hospitalized compared with cases of influenza, CoV, and, to lesser extent, HAdV (as assessed by point estimates) (Figure 1D), although none of these comparisons were statistically significant. Participants with HAdV were more likely to be hospitalized compared with cases of influenza and CoV (as assessed by point estimates), although neither of these comparisons reached statistical significance (Figure 1E). Participants with RV were more likely to be hospitalized as compared with CoV cases (Figure 1F). Although not statistically significant, CoV cases were less likely to be hospitalized as compared with those with influenza (Figure 1G). Figure 1H shows comparisons with influenza cases (already described).

Hospitalization of Dual Viral Diagnosis

When 2 viruses were isolated, those with combinations of RSV+HPIV, CoV+HMPV, and CoV+RSV were less likely

Table 1. Comparison of Demographic Characteristics of the Study Population

	Variable	All (n = 3926), No. (%)	Outpatient (n = 2070; 53%), No. (%)	Hospitalized (n = 1856; 47%), No. (%)	*P**
Sex	Female	2253 (57.4)	1315 (63.5)	938 (50.5)	<.001
	Male	1673 (42.6)	755 (36.5)	918 (49.5)	
Comorbidity	No	2412 (61.4)	1617 (78.1)	795 (42.8)	<.001
	Yes	1514 (38.6)	453 (21.9)	1061 (57.2)	
Age	<11 y	1411 (35.9)	386 (18.6)	1025 (55.2)	<.001
	11–59 y	2207 (56.2)	1577 (76.2)	630 (33.9)	
	>60 y	308 (7.8)	107 (5.2)	201 (10.8)	
Days from symptom onset	0–1	2238 (57)	1623 (78.4)	615 (33.1)	<.001
	2–3	751 (19.1)	232 (11.2)	519 (28)	
	>3	937 (23.9)	215 (10.4)	722 (38.9)	

* *P* values are from the chi-square test, simultaneously testing all categories of a demographic variable.

Table 2. Distribution of Covariates Within Each Single Virus Group

Variable		HAdV	HMPV	RV	RSV	CoV	HPIV	Influenza	HBoV
	No.	112	200	1143	422	435	250	651	72
Age	<11 y	0.52[a]	0.57	0.27	0.73	0.18	0.44	0.21	0.49
	11–60 y	0.43	0.35	0.65	0.21	0.74	0.45	0.71	0.43
	>60 y	0.05	0.08	0.09	0.06	0.09	0.1	0.08	0.08
Days from symptom onset	0–1	0.4	0.43	0.65	0.35	0.73	0.61	0.55	0.57
	2–3	0.24	0.2	0.17	0.31	0.11	0.19	0.18	0.14
	>3	0.36	0.38	0.18	0.34	0.16	0.2	0.27	0.29
Comorbidity	Yes	0.41	0.4	0.4	0.39	0.33	0.48	0.35	0.49

Abbreviations: CoV, coronavirus; HAdV, adenovirus; HBoV, human bocavirus; HMPV, human metapneumovirus; HPIV, parainfluenza virus; RSV, respiratory syncytial virus; RV, rhinovirus.
[a]Proportion for each virus.

to be hospitalized than participants infected with individual viruses (Figure 2A–C). The point estimates for individual viruses in HMPV and RV demonstrated a higher likelihood of these patients being hospitalized than those with combinations, but all confidence intervals included 1 (Figure 2D). Participants with HBoV+RV were more likely to be hospitalized than those with RV, but were hospitalized as frequently as those with HBoV alone (Figure 2J). The point estimate for severity in CoV+RV was greater than that for individuals for CoV or RV alone (Figure 2K), but the confidence interval included 1. The confidence intervals for all other combinations included 1, but the point estimates indicated that some of the combinations could be more severe than 1 of the single agents (Figure 2L–R).

DISCUSSION

Reports of the impact of multiple viral infections have been more frequent with the availability of PCR assays that detect multiple pathogens [9–15]. Most reports analyze data by grouping all combinations and compare this group with different single viruses. This has resulted in variable interpretations. ILI-002 is a large study of those with ILI in which a multipathogen PCR assay was performed on samples from all participants. The size of this cohort allowed there to be a sufficient number of participants with various virus combinations to perform separate analyses for some virus combinations and examine whether virus combinations increase severity over individual viruses. Our results highlight the importance of carrying out these separate analyses.

Our data demonstrate that the severity of diseases was higher with specific viruses (eg, HBoV, RSV, and HMPV). However, in no combination was the dual infection significantly worse than in both of the individual viruses. Furthermore, as a class effect, it does not appear that infection with >1 virus increases severity of disease. Many of the confidence intervals for the combinations include no difference for each of the comparisons with the individual viruses. Based solely on point estimates, which may change with increasing numbers of participants, there appear to be some patterns that indicate a leading or governing effect of 1 virus in the combination. An example of this is the combination of CoV+RSV (Figure 2C, panel C); CoV cases and CoV+RSV cases were less severe than cases of only RSV. Moreover, the severity of CoV+RSV cases was no different than that of CoV cases. This could indicate that CoV is the leading/governing agent in the combination. Similarly, HBoV may be governing RV in the combination of these 2 viruses (panel J), RV governing CoV (panel K), influenza governing HBoV (panel N), and RV governing RSV (panel P). The confidence intervals around the combinations are often large and include 1, so these interpretations are not conclusive and could differ if larger numbers of participants were studied. However, the

Table 3. Number of Single and Dual Infections

	HAdV	HMPV	RV	RSV	CoV	HPIV	Influenza	HBoV
HAdV	112[a]	13	37	**8**	13	**6**	15	**7**
HMPV		200	21	**7**	21	**7**	21	**5**
RV			1143	46	73	40	45	28
RSV				422	22	14	22	**7**
CoV					435	21	116	**2**
HPIV						250	**5**	**6**
Influenza							651	13
HBoV								72

Abbreviations: CoV, coronavirus; HAdV, adenovirus; HBoV, human bocavirus; HMPV, human metapneumovirus; HPIV, parainfluenza virus; RSV, respiratory syncytial virus; RV, rhinovirus.
[a]Values in the diagonal correspond to single infections. The bolded categories show combinations that were not analyzed due to small numbers.

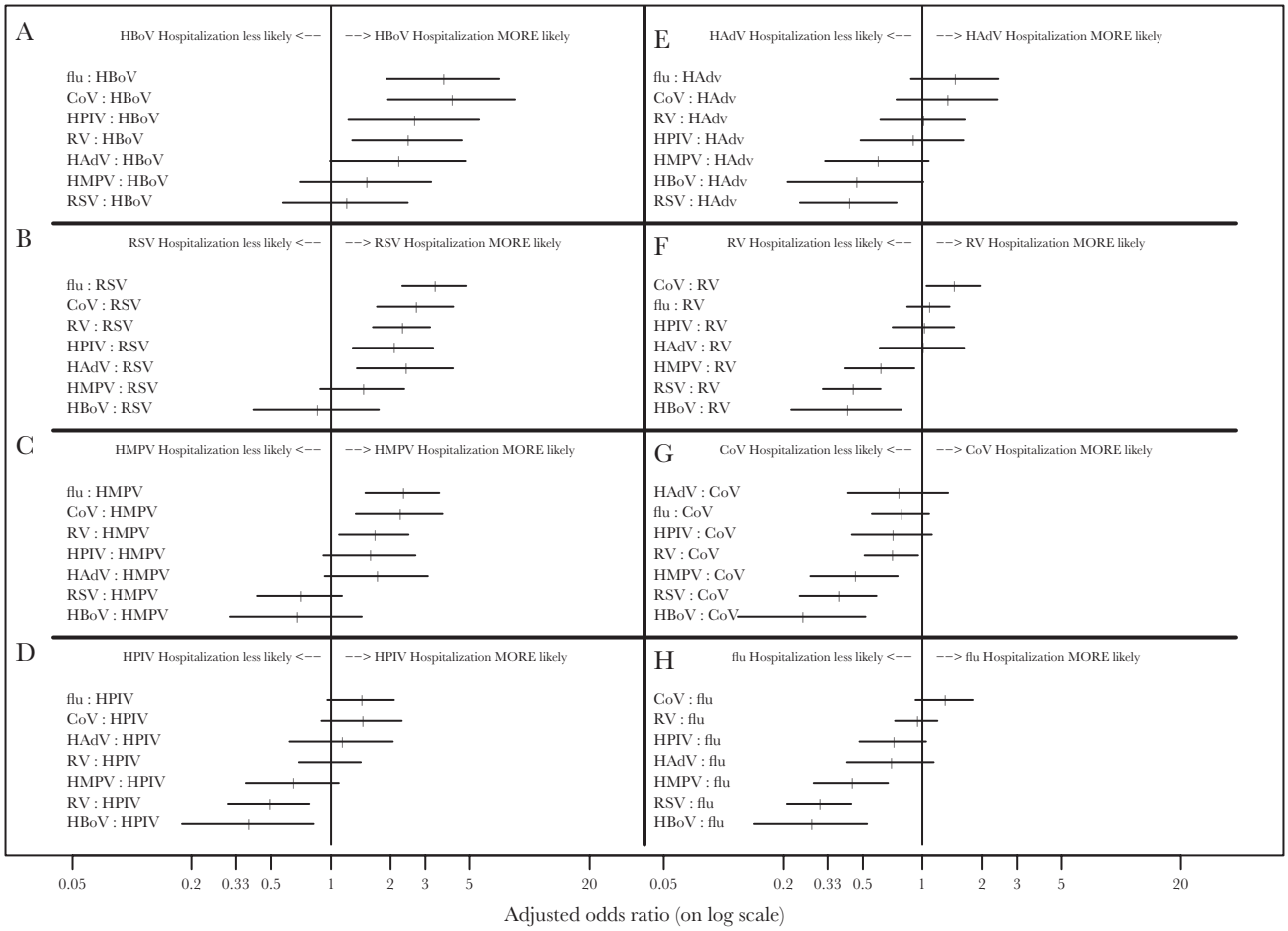

Figure 1. Pairwise comparisons for the likelihood of having a severe case of influenza-like illness. Each panel shows the adjusted odds ratio between a specific virus and each of the other viruses included in the study. The adjusted odds ratios (taking into account age, sex, days from onset of symptoms, and comorbidity) are shown for comparisons between each pair of viruses. Ninety-five percent confidence intervals are included for each estimated odds ratio.

pattern observed in the point estimates is suggestive of the governing effect described above and warrants further study.

One explanation for the governing results is that virological testing was done only once in each participant, which does not distinguish sequential infections with 2 viruses from simultaneous infection with both viruses. It is possible that these results could reflect sequential infections rather than simultaneous infection and that symptoms (and hospitalization) could be the result of only 1 of the 2 viruses. The sample collection time with respect to the course of illness would then be an important factor. In a study carried out in participants with ILI, viral co-infections were detected more frequently in samples obtained during the first 2 days from symptom onset compared with those obtained after 3–7 days [21]. Thus, it is plausible that detection of co-infections might be the result of prolonged shedding of 1 virus with a subsequent infection with the second virus. Also, it could reflect a reduced ability of a second virus to replicate due to an already initiated host response and the production of interferon as a result of an initial viral infection. Interference of 1 virus with another virus has been shown to occur in vitro [22], and epidemiological studies suggest that circulation of 1 virus might affect circulation of another virus in communities [23].

Overall, most previous studies have shown similar severity of single infections when compared with mixed infections [16]. However, some studies have shown significant differences between specific combinations of viruses and single viruses. For example, in cases of co-infection with RSV+RV and RSV+HBoV, the illness appeared to be more severe than in cases of RSV or HBoV infection alone [24]. Our results for RSV+RV follow the same pattern, but we did not have sufficient data to study the combination RSV+HBoV.

Our analysis showed that participants with detection of only influenza virus were less likely to require hospitalization than participants in whom other viruses were detected. This was observed despite the fact that the study included the 2013–2014 winter season, when a severe wave of influenza A(H1N1) pdm09 was registered in Mexico [25]. This result could be derived from inclusion of all influenza subtypes in the analysis. The lower hospitalization rate in participants with influenza

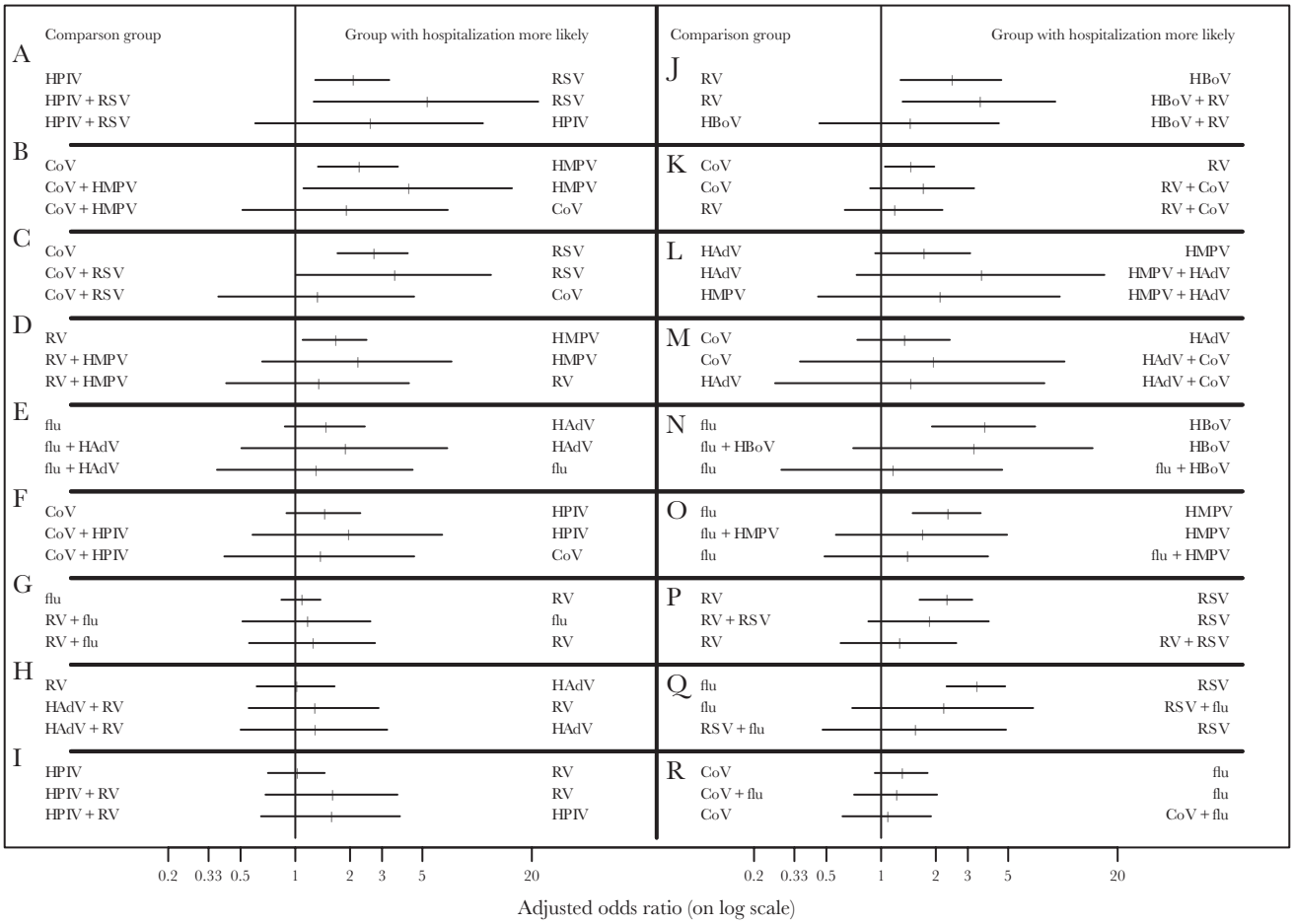

Figure 2. Adjusted odds ratios and 95% confidence intervals for the comparisons of severity between patients with co-infection and patients with each of the viruses as single agents. Each panel includes a comparison of each of 2 viruses with the co-infection with both viruses. Within each comparison, the more severe group is on the right side. All odds ratios are adjusted for age, sex, days from onset of symptoms, and comorbidity.

virus infection compared with those with other viruses may also be explained by influenza vaccination. Since 2009, influenza vaccination coverage in Mexico has been high [26], and influenza vaccination has been reported to reduce influenza hospitalizations [27]. Unfortunately, it was not possible to obtain detailed data regarding influenza vaccination status for study participants to assess this.

We also found that for single virus comparisons, HBoV, RSV, and HMPV were associated with severe infections. The RSV and HMPV finding is consistent with other reports that show these viruses to be leading causes of LRTI in children and adults [28–30]. In contrast, the role of HBoV as a cause of severe infections is less well established. Because HBoV infection is very common and frequently found in asymptomatic participants, the pathogenic role of this virus has been questioned [31, 32]. Children with higher viral loads tend to have more severe infections [33], longer hospitalization duration [34, 35], and are found to have co-infection by other viruses [36, 37] less frequently than those with lower viral loads. However, in children in daycare, viral load had no apparent association with severity of illness [31], which also might reflect challenges in reproducibly measuring viral load in secretions. In all, these studies suggest that HBoV is frequently present in children as an asymptomatic or chronic infection with low viral loads, whereas some infections in which a high viral load is present may be associated with severe infections requiring hospitalization. Our study did not measure viral load, so it is unclear if our HBoV participants represented a sample of high–viral load participants, as we did not include asymptomatic participants with HBoV.

The strengths of this study include the large numbers of participants and the consistent baseline testing and follow-up. Limitations include the definition of severity, as hospitalization was a surrogate for actual patient health status. Patients may be hospitalized for causes other than their respiratory illness, such as worsening of comorbidities, observation in high-risk participants, or social reasons. Further studies should be done using direct measures of patient health status such as intensity and duration of participants' symptoms or complications (eg, pneumonia). We have developed a symptom scale for influenza (FLU-PRO) as part of the study ILI-002 that could be used in future studies [38].

The presence of chronic underlying conditions is an important factor that is associated with risk of developing severe respiratory infections. In addition, it is possible that chronic conditions may increase the risk of acquiring infections by multiple pathogens. A recent study reported that patients with coinfection caused by 2 or 3 different influenza virus strains were more likely to have underlying cardiovascular disorders than those with single influenza infections [39]; however, no differences were observed in the prevalence of other underlying conditions. Although some other studies have found a higher prevalence of chronic disorders in patients with multiple viral pathogens, these appear to be limited to specific conditions, and no differences have been observed for other disorders [15, 40]. In addition, many studies have not found an association between the presence of chronic disorders and detection of multiple viruses [9, 14, 21, 24]. The main objective of our study was to determine if codetection of 2 viruses was associated with worse outcome, and we did not analyze which factors may have led to acquisition of ≥2 viruses; nevertheless, our analysis included the presence of chronic conditions as a covariate, in order to account for potential confounding.

In a previous analysis of ILI-002 limited to children <5 years of age, the severity of single viruses was compared [7], and the results were similar to our findings for single viruses in the present study. The majority of studies have focused on children, and the effect of mixed viral infections in adults is less clear [40]. One of the strengths of this analysis is that our study population included both pediatric and adult symptomatic participants. Although some comparisons resulted in estimates with wide confidence intervals, our interpretation based on point estimates revealed several patterns and could be hypothesis-generating for future studies.

CONCLUSIONS

Our results suggest that, in general, having >1 virus detected by PCR on a respiratory sample does not increase the severity of disease from ILI. To assess the differences in hospitalization rates of mixed respiratory infections, it is necessary to carry out analyses between specific combinations of viruses. When 2 viruses are detected, it appears that the clinical severity of a respiratory infection, as defined by hospitalization, may be associated with 1 of these agents. Future studies with a larger number of participants designed to distinguish between sequential and simultaneous infection and using direct measures of patient health status should be of help in defining the role of each virus during the evolution of an acute respiratory episode.

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References

1. GBD 2015 LRI Collaborators. Estimates of the global, regional, and national morbidity, mortality, and aetologies of lower respiratory tract infection in 195 countries: a systematic analysis of the Global Burden of Disease Study 2015. Lancet Infect Dis **2017**; 17:1133–61.
2. van den Hoogen BG, de Jong JC, Groen J, et al. A newly discovered human pneumovirus isolated from young children with respiratory tract disease. Nat Med **2001**; 7:719–24.
3. Kahn JS, McIntosh K. History and recent advances in coronavirus discovery. Pediatr Infect Dis J **2005**; 24(11 Suppl):S223–7.
4. Allander T, Tammi MT, Eriksson M, et al. Cloning of a human parvovirus by molecular screening of respiratory tract samples. Proc Natl Acad Sci U S A **2005**; 102:12891–6.
5. Lamson D, Renwick N, Kapoor V, et al. Mass Tag polymerase-chain-reaction detection of respiratory pathogens, including a new rhinovirus genotype, that caused influenza-like illness in New York State during 2004–2005. J Infect Dis **2006**; 194:1398–402.
6. Taboada B, Espinoza MA, Isa P, et al. Is there still room for novel viral pathogens in pediatric respiratory tract infections? PLoS One **2014**; 9:e113570.
7. Ortiz-Hernández AA, Nishimura KK, Noyola DE, et al. Differential risk of hospitalization among single virus infections causing influenza like illnesses. Influenza Other Respir Viruses. **2019**; 13:36–43.
8. Pavia AT. Viral infections of the lower respiratory tract: old viruses, new viruses, and the role of diagnosis. Clin Infect Dis **2011**; 52(Suppl 4):S284–9.
9. García-García ML, Calvo C, Rey C, et al. Human metapnuemovirus infections in hospitalized children and comparison with other respiratory viruses. 2005–2014 prospective study. PLoS One **2017**; 12:e0173504.

10. Wong-Chew RM, García-León ML, Noyola DE, et al. Respiratory viruses detected in Mexican children younger than 5 years old with community-acquired pneumonia: a national multicenter study. Int J Infect Dis **2017**; 62:32–8.
11. Wishaupt JO, van der Ploeg T, de Groot R, et al. Single- and multiple viral respiratory infections in children: disease and management cannot be related to a specific pathogen. BMC Infect Dis **2017**; 7:62–73.
12. Drews AL, Atmar RL, Glezen WP, et al. Dual respiratory virus infections. Clin Infect Dis **1997**; 25:1421–9.
13. Chauhan JC, Slamon NB. The impact of multiple viral respiratory infections on outcomes for critically ill children. Pediatr Crit Care Med **2017**; 18:e333–8.
14. Antalis E, Oikonomopoulou Z, Kottaridi C, et al. Mixed viral infections of the respiratory tract; an epidemiological study during consecutive winter seasons. J Med Virol **2018**; 90:663–70.
15. Moe N, Krokstad S, Stenseng IH, et al. Comparing human metapneumovirus and respiratory syncytial virus: viral co-detections, genotypes and risk factors for severe disease. PLoS One **2017**; 12:e0170200.
16. Lim FJ, de Klerk N, Blyth CC, et al. Systematic review and meta-analysis of respiratory viral coinfections in children. Respirology **2016**; 21:648–55.
17. Galindo-Fraga A, Ortiz-Hernández AA, Ramírez-Venegas A, et al; La Red ILI 002 Study Group. Clinical characteristics and outcomes of influenza and other influenza-like illnesses in Mexico City. Int J Infect Dis **2013**; 17:e510–7.
18. Paulin-Prado P, Nishimura K, Freimanis-Hance L, et al. Mexico Emerging Infectious Diseases Clinical Research Network. Clinical characteristics of asthmatic patients with influenza-like illness and risk of severe exacerbations in Mexico. Ann Allergy Asthma Immunol **2016**; 116:402–7.
19. Gamino-Arroyo AE, Moreno-Espinosa S, Llamosas-Gallardo B, et al. Epidemiology and clinical characteristics of respiratory syncytial virus infections among children and adults in Mexico. Influenza Other Respir Viruses **2017**; 11 11:48–56.
20. World Health Organization. CDC protocol of realtime RTPCR for swine influenza A (H1N1). Available at: http://www.who.int/csr/resources/publications/swineflu/CDCrealtimeRTPCRprotocol_20090428.pdf. Accessed 01 March 2019.
21. Peci A, Winter AL, Gubbay JB, et al. Community-acquired respiratory viruses and co-infection among patients of Ontario sentinel practices, April 2009 to February 2010. Influenza Other Respir Viruses **2013**; 7:559–66.
22. Shinjoh M, Omoe K, Saito N, et al. In vitro growth profiles of respiratory syncytial virus in the presence of influenza virus. Acta Virol **2000**; 44: 91–7.
23. Zheng X, Song Z, Li Y, et al. Possible interference between seasonal epidemics of influenza and other respiratory viruses in Hong Kong, 2014–2017. BMC Infect Dis **2017**; 17:772–779.
24. Calvo C, García-García ML, Pozo F, et al. Respiratory syncytial virus coinfections with rhinovirus and human bocavirus in hospitalized children. Medicine (Baltimore) **2015**; 94:1–7.
25. Dávila-Torres J, Chowell G, Borja-Aburto VH, et al. Intense seasonal A/H1N1 influenza in Mexico, winter 2013-2014. Arch Med Res **2015**; 46:63–70.
26. Ropero-Álvarez AM, El Omeiri N, Kurtis HJ, et al. Influenza vaccination in the Americas: progress and challenges after the 2009 A(H1N1) influenza pandemic. Hum Vaccin Immunother **2016**; 12:2206–14.
27. Havers F, Sokolow L, Shay DK, et al. Case-control study of vaccine effectiveness in preventing laboratory-confirmed influenza hospitalizations in older adults, United States, 2010–2011. Clin Infect Dis **2016**; 63:1304–11.
28. Shi T, McAllister DA, O'Brien KL, et al; RSV Global Epidemiology Network. Global, regional, and national disease burden estimates of acute lower respiratory infections due to respiratory syncytial virus in young children in 2015: a systematic review and modelling study. Lancet **2017**; 390:946–58.
29. Falsey AR, McElhaney JE, Beran J, et al. Respiratory syncytial virus and other respiratory viral infections in older adults with moderate to severe influenza-like illness. J Infect Dis **2014**; 209:1873–81.
30. Williams JV, Harris PA, Tollefson SJ, et al. Human metapneumovirus and lower respiratory tract disease in otherwise healthy infants and children. N Engl J Med **2004**; 350:443–50.
31. Martin ET, Fairchok MP, Kuypers J, et al. Frequent and prolonged shedding of bocavirus in young children attending daycare. J Infect Dis **2010**; 201:1625–32.
32. Rhedin S, Lindstrand A, Hjelmgren A, et al. Respiratory viruses associated with community-acquired pneumonia in children: matched case-control study. Thorax **2015**; 70:847–53.
33. Moesker FM, van Kampen JJ, van der Eijk AA, et al. Human bocavirus infection as a cause of severe acute respiratory tract infection in children. Clin Microbiol Infect **2015**; 21:964.e1–8.
34. Zhao B, Yu X, Wang C, et al. High human bocavirus viral load is associated with disease severity in children under five years of age. PLoS One **2013**; 8:1–8.
35. Principi N, Piralla A, Zampiero A, et al. Bocavirus infection in otherwise healthy children with respiratory disease. PLoS One **2015**; 10:1–15.
36. Zhou L, Zheng S, Xiao Q, et al. Single detection of human bocavirus 1 with a high viral load in severe respiratory tract infections in previously healthy children. BMC Infect Dis **2014**; 14:1–8.
37. Jiang W, Yin F, Zhou W, et al. Clinical significance of different virus load of human bocavirus in patients with lower respiratory tract infection. Sci Rep **2016**; 6:1–6.
38. Powers JH 3rd, Bacci ED, Guerrero ML, et al. Reliability, Validity, and Responsiveness of InFLUenza Patient-Reported Outcome (FLU-PRO©) scores in influenza-positive patients. Value Health **2018**; 21:210–8.
39. Gregianini TS, Varella IRS, Fisch P, et al. Dual and triple infections with influenza A and B viruses: a case-control study in Southern Brazil. J Infect Dis **2019**; 220:961–8.
40. Choi SH, Chung JW, Kim HR. Clinical relevance of multiple respiratory virus detection in adult patients with acute respiratory illness. J Clin Microbiol **2015**; 53:1172–7.