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# Etiology, clinical characteristics, and risk factors associated with severe influenza-like illnesses in Mexican adults

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ABSTRACT

*Objective:* The aim of this study was to determine the risk factors associated with severe influenza-like illness (ILI) in Mexican adults that could be useful to clinicians when assessing patients with ILI.
*Methods:* Data from adult patients enrolled from 2010 through 2014 in ILI002 – a prospective hospital-based observational cohort study – were analyzed. Etiology and clinical characteristics were compared between cases of severe ILI (defined as hospitalization and/or death) and cases of non-severe ILI.
*Results:* Overall, 1428 (39.0%) out of a total 3664 cases of ILI were classified as severe. Adjusted analyses showed a higher risk of severe ILI associated with signs and symptoms related to lower tract infection, i.e. cough with sputum (odds ratio (OR) 2.037, 95% confidence interval (CI) 1.206–3.477; $P = 0.008$), dyspnea (OR 5.044, 95% CI 2.99–8.631; and shortness of breath (OR 5.24, 95% CI 3.0839.124; $P < 0.001$), and with increases in lactate dehydrogenase (OR 4.426, 95% CI 2.321–8.881; $P < 0.001$) and C-reactive protein (OR 3.618, 95% CI 2.5955.196; $P < 0.001$). Further, there was an increased risk of severe ILI with a longer time between symptom onset and inclusion (OR 1.108, 95% CI 1.049–1.172; $P < 0.001$) and with chronic steroid use (OR 14.324, 95% CI 8.059-26.216; $P < 0.001$).
*Conclusions:* Respiratory viruses can cause severe ILI. The results of this study highlight the importance of evaluating data compatible with lower tract involvement and previous use of immunosuppressants at baseline, because patients meeting these conditions may develop severe illness.

## 1. Introduction

Influenza-like illness (ILI) is a clinical case definition that has been used to standardize the definition of the event in order to establish effective worldwide surveillance programs, understand the burden of acute respiratory infections, and monitor annual changes in disease severity [1]. Acute respiratory infections remain one of the leading causes of mortality worldwide and may evolve into epidemics or pandemics [2].

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The use of novel molecular diagnostic techniques has revealed that a large proportion of severe respiratory infections are due to viruses or subsequent bacterial infections [3]. More than 20 viruses have been identified as the cause of ILI among adults and children, including respiratory syncytial virus (RSV), influenza A and B viruses, coronaviruses (CoV), human rhinoviruses (HRV), adenoviruses (HAdV), metapneumovirus (HMPV), and bocavirus (HBoV) [4].

Most individuals with an ILI experience a mild and self-limiting illness. However, some develop severe disease leading to hospitalization or death. The severity of these infections varies according to the age group, comorbidities, and causal agents, with children being the most studied population. In adults, influenza A H1N1pdm2009 has been associated with a more severe disease, particularly affecting obese patients, pregnant women, and patients with chronic pulmonary disease [5]. In addition, the impact of previous steroid use on the severity of influenza has been controversial [6,7].

It is known that other non-influenza respiratory viruses also affect young children, the elderly, and the immunocompromised [8]. Some individuals can develop pneumonia and severe complications without having these or any identified risks. The substantial contribution of respiratory viruses as a cause of hospitalization is being recognized increasingly [9]. It has been estimated that viruses cause 27.5–39.2% of community-acquired pneumonia in immunocompetent individuals [10]. Recent evidence suggests a high incidence of complications in non-influenza-associated viral pneumonia [11]. However, data on the risk factors for severe disease (hospitalization or death) in regard to other non-influenza respiratory viruses are still poorly understood in adults.

The Mexican Emerging Infectious Diseases Clinical Research Network (LaRed) was created in 2009 at the time of the H1N1 influenza pandemic to evaluate the transmission, clinical course, and disease severity in individuals seeking medical attention for an ILI. The network received funding from the Mexican Ministry of Health, and the US National Institutes of Health (NIH). The first study performed by this network was ILI002, which aimed to evaluate the etiology of ILI in children and adults. The aim of this study was to evaluate the risk factors for severe ILI in Mexican adults.

## 2. Methods

ILI002 was a hospital-based prospective observational cohort study conducted at six hospitals in Mexico City and San Luis Potosí, Mexico from April 11, 2010 to April 10, 2014. ILI002 included children and adults with ILI. Inclusion criteria and laboratory methods have been described previously [12,13]. Briefly, patients with ILI seeking medical attention at one of the centers participating in the study, who had at least one respiratory symptom (e.g., shortness of breath, cough) and either fever (≥38°C or subjective feverishness) or one or more non-respiratory symptoms (e.g., malaise, headache), were included. A follow-up interview was performed at 14 ± 3 days and a visit at 28 ± 5 days after inclusion, in order to verify vital status and clinical evolution. Clinical characteristics and general laboratory values were recorded. The results of blood tests on samples obtained as part of clinical care were collected from the medical records, and a nasopharyngeal swab (Copan, Brescia, Italy) for multiple PCR pathogen detection was taken for all participants.

Nasopharyngeal swabs and nasal aspirates were stored at 4°C at each site and sent to a central facility, the Molecular Biology Laboratory of the Infectious Diseases Department at the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán (INCMNSZ) in Mexico City, for testing. The remaining respiratory specimens were stored at −70°C in the central specimen repository. All samples were tested by real-time reverse transcriptase PCR for influenza A following the US Centers for Disease Control and Prevention (CDC) protocol [14].

For multi-pathogen detection, the samples were tested with either the RespiFinder19 or RespiFinder22 kit (PathoFinder BV, Maastricht, The Netherlands). The RespiFinder19 PCR test can detect and differentiate 15 viruses (coronavirus NL63, OC43, and 229E, human metapneumovirus, influenza A, influenza A H5N1, influenza B, parainfluenza virus types 1–4, respiratory syncytial virus types A and B, rhinovirus/enterovirus, and adenovirus), as well as four bacteria (*Bordetella pertussis, Chlamydophila pneumoniae, Legionella pneumophila*, and *Mycoplasma pneumoniae*). The RespiFinder22 kit covers the same pathogens as the RespiFinder19 kit, with the addition of coronavirus HKU1, bocavirus, and influenza A H1N1pdm2009, and removal of influenza A H5N1 .

The study was conducted following the principles of the Declaration of Helsinki, ICH Good Clinical Practice, and Mexican General Health Law, and approval was obtained from the ethics committee at each institution. All data were analyzed anonymously, and all participants gave informed consent. The project has been registered at ClinicalTrials.gov (NCT01418287).

Eligible participants were adults over 18 years of age, who were recruited from four of the LaRed centers specializing in adult populations: Instituto Nacional de Enfermedades Respiratorias Ismael Cosío Villegas (INER), a respiratory diseases oriented tertiary care hospital (240 beds); INCMNSZ, a medical/surgical services tertiary care hospital (180 beds); Hospital General Manuel Gea González (HGMGG), a general hospital (210 beds); and Hospital Central Dr. Ignacio Morones Prieto (HRIMP), a general hospital in the north of Mexico (250 beds). Severe disease was defined as hospitalization (i.e., >24 hours of in-hospital care decided by the attending physician, within 14 days of enrollment). Age, comorbidities, the viral etiology, and clinical characteristics of participants at entry were described and compared to define risk factors for severity.

The median and interquartile range (IQR) were used to summarize quantitative variables, and the frequency and percentage were used to summarize qualitative variables. Univariate logistic regression analyses were performed to evaluate the effect of each baseline covariate on the risk of severe disease. The odds ratios (OR) and their corresponding 95% confidence intervals (CI) were reported. Multivariate logistic regression analyses were performed to explore adjusted significant univariate associations; model selection was performed using bi-directional stepwise selection minimizing the Akaike information criterion (AIC) on a dataset restricted to complete cases of candidate predictors. One model was selected that was restricted to baseline signs, symptoms, and laboratory values, and a second model that was restricted to pathogen frequencies. All laboratory measures were standardized. All *P*-values were two-sided, with no correction for multiple comparisons; a two tailed *P*-value <0.05 was considered statistically significant. All analyses were performed using R version 4.0.4 (The R Foundation, Vienna, Austria).

## 3. Results

Of the 3664 adult patients enrolled in the study, 1428 (39.0%) were classified as having a severe case of ILI. The baseline status of these 1428 patients was as follows: 290 (20.0%) were in the emergency room, 1088 (76.2%) were hospitalized, 29 (2.0%) were in the intensive care unit, and 21 (1.5%) were outpatients (Figure 1). Table 1 reports the clinical characteristics of the study participants and the results of the unadjusted comparisons between the severe ILI and non-severe ILI groups.

The results of the univariate analyses showed a higher frequency of female patients in both groups; however, male patients had a higher risk of severe illness. Current and passive smokers had a significantly higher risk of severe ILI than participants who had never smoked, but there was no significant difference between former smokers and participants who had never smoked. In addition, significantly increased odds of severe ILI were found for higher age (OR 1.05 for each year increase) and a longer length of time between symptom onset and seeking medical care (OR 1.37 for a 1-day increase). Participants with comorbid medical conditions such as chronic obstructive pulmonary disease (COPD) and cardiovascular disease, as well as those with known or unknown previous use (when compared to no use) of systemic steroids for any medical condition (including autoimmune, immunodeficiency, cancer, HIV, and others), had an increased risk of severe ILI. Diabetes mellitus

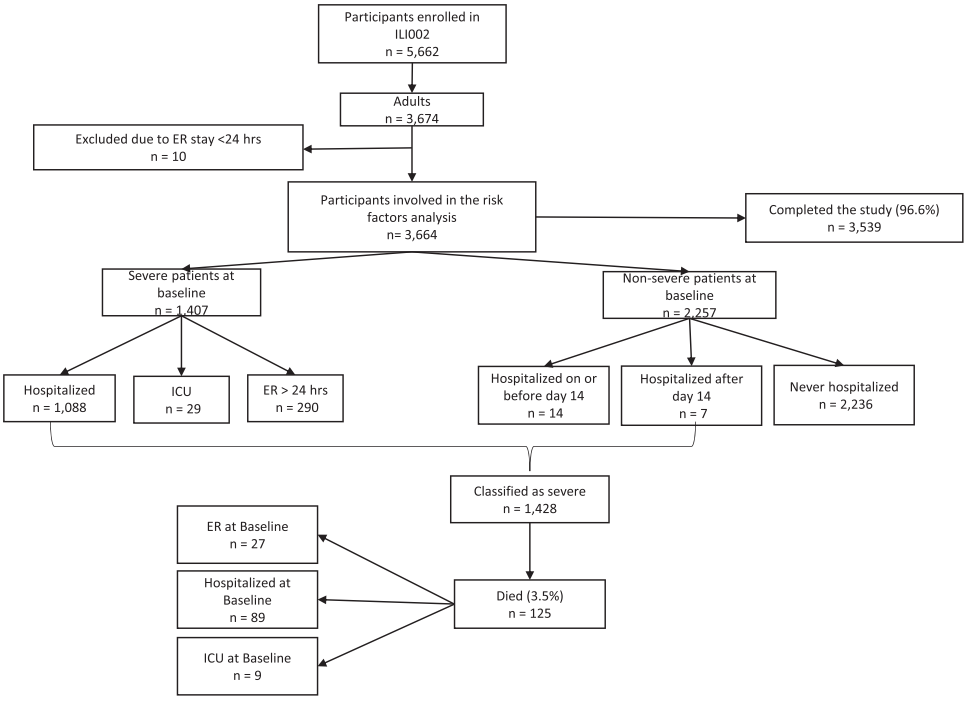


**Figure 1.** Study flow chart.
ER, emergency room; ICU, intensive care unit.

**Table 1**
Demographic characteristics of the enrolled patients

| | Severe ILI | Non-severe ILI | Missing (%) | OR (95% CI) | *P*-value |
|---|---|---|---|---|---|
| Number | 1428 | 2236 | | | |
| Sex (%) | | | 0 (0) | | |
| Female | 815 (57.1) | 1516 (67.8) "*ref*" | | | |
| Male | 613 (42.9) | 720 (32.2) | | 1.58 (1.38–1.82) | <0.001 |
| Smoking history (%) | | | 0 (0) | | |
| Never smoked | 216 (15.1) | 477 (21.3)"*ref*" | | | |
| Passive smoker | 91 (6.4) | 89 (4.0) | | 2.26 (1.62–3.15) | <0.001 |
| Former smoker | 701 (49.1) | 1336 (59.7) | | 1.16 (0.96–1.4) | 0.12 |
| Currently smokes | 420 (29.4) | 334 (14.9) | | 2.78 (2.24–3.45) | <0.001 |
| Age (years), median (IQR) | 47.00 (34.00, 62.00) | 34.00 (25.00, 47.00) | 0 (0) | 1.05 (1.04–1.05) | <0.001 |
| Days from onset of symptoms to inclusion, median (IQR) | 2.00 (1.00, 6.00) | 0.00 (0.00, 0.00) | 7 (0.2) | 1.37 (1.33–1.41) | <0.001 |
| COPD (%) | 66 (4.6) | 7 (0.3) | 0 (0) | 15.43 (7.57–37.07) | <0.001 |
| Cardiovascular disease (%) | 325 (22.8) | 224 (10.0) | 0 (0) | 2.65 (2.2–3.19) | <0.001 |
| Diabetes mellitus (%) | 65 (4.6) | 73 (3.3) | 0 (0) | 1.41 (1–1.99) | 0.05 |
| Use of systemic steroids (%) | | | 0 (0) | | |
| No | 650 (45.5) | 2146 (96.0) "*ref*" | | | |
| Unknown | 7 (0.5) | 2 (0.1) | | 11.56 (2.78–77.68) | <0.001 |
| Yes | 771 (54.0) | 88 (3.9) | | 28.93 (22.94–36.89) | <0.001 |
| BMI classification (%) | | | 37 (1.0) | | |
| Normal | 406 (29.0) | 875 (39.3) "*ref*" | | | |
| Underweight | 75 (5.4) | 46 (2.1) | | 3.51 (2.4–5.2) | <0.001 |
| Overweight | 495 (35.4) | 863 (38.7) | | 1.24 (1.05–1.45) | 0.01 |
| Obese | 423 (30.2) | 444 (19.9) | | 2.05 (1.72–2.45) | <0.001 |
| CRP, median (IQR)[a] | 0.05 (−0.43, 1.42) | −0.49 (−0.56, −0.31) | 473 (12.9) | 4.28 (3.74–4.95) | <0.001 |
| CPK, median (IQR)[a] | −0.19 (−0.29, 0.02) | −0.18 (−0.25, −0.08) | 370 (10.1) | 1.37 (1.23–1.56) | <0.001 |
| Creatinine, median (IQR)[a] | −0.12 (−0.28, 0.08) | −0.17 (−0.26, −0.04) | 198 (5.4) | 2.01 (1.69–2.44) | <0.001 |
| Hemoglobin, median (IQR)[a] | −0.05 (−0.98, 0.53) | 0.13 (−0.27, 0.62) | 180 (4.9) | 0.62 (0.57–0.67) | <0.001 |
| LDH, median (IQR)[a] | −0.07 (−0.31, 0.51) | −0.23 (−0.34, −0.10) | 341 (9.3) | 6.31 (5.12–7.85) | <0.001 |
| Lymphocytes, median (IQR)[a] | −0.86 (−1.21, −0.15) | 0.38 (−0.24, 0.99) | 509 (13.9) | 0.24 (0.22–0.27) | <0.001 |
| Neutrophils, mean ± SD[a] | 0.54 ± 1.03 | −0.38 ± 0.78 | 528 (14.4) | 3.48 (3.14–3.86) | <0.001 |
| Platelets, median (IQR)[a] | −0.19 (−0.87, 0.57) | −0.02 (−0.46, 0.49) | 183 (5.0) | 0.89 (0.83–0.95) | <0.001 |
| Died (%) | 125 (8.8) | 0 (0) | 0 (0) | | |

BMI, body mass index; CI, confidence interval; COPD, chronic obstructive pulmonary disease; CPK, creatine phosphokinase; CRP, C-reactive protein; ILI, influenza-like illness; IQR, interquartile range; LDH, lactate dehydrogenase; OR, odds ratio; SD, standard deviation.
[a] Standardized values. OR corresponds to an increase of one standard deviation.

**Table 2**
Signs and symptoms reported at enrollment by severity status

| | Severe ILI | Non-severe ILI | Missing | OR (95% CI) | *P*-value |
|---|---|---|---|---|---|
| Number | 1428 | 2236 | | | |
| Upper respiratory | | | | | |
| Sneezing (%) | 341 (23.9) | 1459 (65.3) | 0 (0.0%) | 0.17 (0.14–0.19) | <0.001 |
| Runny nose (%) | 637 (44.6) | 1833 (82.0) | 0 (0.0%) | 0.18 (0.15–0.21) | <0.001 |
| Nasal congestion (%) | 391 (27.4) | 1610 (72.0) | 0 (0.0%) | 0.15 (0.13–0.17) | <0.001 |
| Sore throat (%) | 591 (41.4) | 1816 (81.2) | 0 (0.0%) | 0.16 (0.14–0.19) | <0.001 |
| Dry cough (%) | 431 (30.2) | 1169 (52.3) | 0 (0.0%) | 0.39 (0.34–0.45) | <0.001 |
| Lower respiratory | | | | | |
| Dyspnea (%) | 968 (67.8) | 338 (15.1) | 0 (0.0%) | 11.82 (10.08–13.89) | <0.001 |
| Shortness of breath (%) | 736 (51.5) | 471 (21.1) | 0 (0.0%) | 3.99 (3.45–4.61) | <0.001 |
| Cough with sputum (%) | 997 (69.8) | 1065 (47.6) | 0 (0.0%) | 2.54 (2.21–2.93) | <0.001 |
| Chest congestion (%) | 279 (19.5) | 360 (16.1) | 0 (0.0%) | 1.27 (1.06–1.5) | 0.01 |
| Non-respiratory | | | | | |
| Fever (%) | 959 (67.2) | 1119 (50.0) | 0 (0.0%) | 2.04 (1.78–2.34) | <0.001 |
| Headache (%) | 777 (54.4) | 1751 (78.3) | 0 (0.0%) | 0.33 (0.29–0.38) | <0.001 |
| Confusion/difficulty thinking (%) | 46 (3.2) | 323 (14.4) | 0 (0.0%) | 0.2 (0.14–0.27) | <0.001 |
| Malaise (%) | 739 (51.8) | 1715 (76.7) | 0 (0.0%) | 0.33 (0.28–0.38) | <0.001 |
| Fatigue (%) | 846 (59.2) | 1711 (76.5) | 0 (0.0%) | 0.45 (0.39–0.51) | <0.001 |
| Muscle aches (%) | 516 (36.1) | 1383 (61.9) | 0 (0.0%) | 0.35 (0.3–0.4) | <0.001 |
| Red eyes (%) | 177 (12.4) | 961 (43.0) | 0 (0.0%) | 0.19 (0.16–0.22) | <0.001 |
| Watery eyes (%) | 291 (20.4) | 1351 (60.4) | 0 (0.0%) | 0.17 (0.14–0.2) | <0.001 |
| Diarrhea (%) | 85 (6.0) | 226 (10.1) | 0 (0.0%) | 0.56 (0.43–0.73) | <0.001 |
| Nausea (%) | 206 (14.4) | 636 (28.4) | 0 (0.0%) | 0.42 (0.36–0.5) | <0.001 |

CI, confidence interval; ILI, influenza-like illness; OR, odds ratio.

was present in a similar proportion in both groups and showed no significant relationship with the risk of severe ILI. There was a higher risk of severe ILI for participants who were underweight (OR 3.51, 95% CI 2.4–5.2), overweight (OR 1.24, 95% CI 1.05–1.45), or obese (OR 2.05, 95% CI 1.72–2.45), when compared to participants with a normal body mass index (BMI). Increases in C-reactive protein (CRP), creatine phosphokinase (CPK), creatinine, lactate dehydrogenase (LDH), percent neutrophils, and platelets were associated with an increase in the risk of severe ILI, whereas increases in hemoglobin and the percent lymphocytes were associated with a decrease in the risk of severe ILI (Table 1).

In severe cases, the median length of hospital stay from study enrollment was 9.00 days (IQR 5.00–17.00 days). A small proportion of participants from this group were in the intensive care unit ($n$ = 29, 0.79%), and at total of 125 participants died (3.41% of the total included population, 8.8% of the severe cases, all of whom were in the severe patient category at baseline).

On univariate analysis, upper respiratory symptoms (sore throat, nasal congestion, sneezing, etc.) were associated with a lower risk of severe ILI, whereas lower respiratory symptoms were associated with a higher risk of severe ILI. Further, non-respiratory symptoms (headache, malaise, etc.) were associated with a lower risk of ILI, with the exception of fever, which was associated with a higher risk of severe ILI (Table 2). Upper respiratory tract symptoms were found to be more frequent in female patients ($P < 0.05$) ( **Supplementary Material** Table S1).

The most frequently reported virus was rhinovirus/enterovirus (252 cases in the severe group and 556 cases in the non-severe group). The proportion of rhinovirus/enterovirus was higher in non-severe cases (24.9% vs 17.6% in severe cases) and this pathogen was associated with a lower risk of severe ILI ($P < 0.001$). Infection by seasonal coronavirus was higher in participants with non-severe disease as well (10.2% vs 4.6% in severe cases) and this pathogen was associated with a lower risk of severe ILI ($P < 0.001$). The proportion of influenza viruses was similar in the two groups (12.5% in the severe ILI group vs 12.6% in the non-severe ILI group). The frequencies of the other isolated viruses are reported in **Supplementary Material** Table S2.

In the multivariate logistic regression model ($n$ = 2751), a statistically significant higher risk of severe ILI was found to be associated with increases in the time between symptom onset and inclusion (OR 1.108, 95% CI 1.049–1.172; $P < 0.001$), CRP (OR 3.618, 95% CI 2.59553.196; $P < 0.001$), and LDH (OR 4.426, 95% CI 2.321-8.881–10.074; $P < 0.001$), as well as the presence of cough with sputum (OR 2.018, 95% CI 1.195–3.466; $P = 0.009$), dyspnea (OR 5.044, 95% CI 2.99–8.631; $P < 0.001$), shortness of breath (OR 5.24, 95% CI 3.083-9.124; $P < 0.001$), with chronic steroid use (OR 14.324, 95% CI 8.059-26.216; $P < 0.001$ and enrollment at INER when compared to INCMNSZ (OR 21.77, 95% CI 11.388–43.381; $P < 0.001$). Conversely, a decrease in the risk of severe ILI was found to be associated with increases in hemoglobin and platelets, as well as the presence of sore throat, muscle aches, headache, nasal congestion, sneezing, and enrollment at HGMGG when compared with INCMNSZ (Figure 2, **Supplementary Material** Table S3). There was no significant difference between HRIMP and INCMNSZ (Figure 2, **Supplementary Material** Table S3).

**Supplementary Material** Tables S4 and S5 report the demographic characteristics and signs and symptoms of the patients in the two groups, with univariate analysis, restricted to the participants who were included in the multivariate model. **Supplementary Material** Table S6 shows the pathogen frequencies (single pathogen detection) by severity status for the participants who were included in the multivariate model. The most frequently reported virus was rhinovirus/enterovirus (172 cases in the severe group and 429 cases in the non-severe group). The proportion of rhinovirus/enterovirus was higher in non-severe cases (24.4% vs 17.3% in non-severe cases) and this pathogen was associated with a lower risk of severe ILI ($P < 0.001$). Infection by seasonal coronavirus was higher in participants with non-severe cases as well (10.0% vs 4.6% in severe cases) and this pathogen was associated with a lower risk of severe ILI ($P < 0.001$). The proportion of influenza viruses was similar in the two groups (14.2% in the severe ILI group vs 13.2% in the non-severe ILI group).

## 4. Discussion

This study compared severe and non-severe ILI in a large group of adult patients in Mexico City across four post 2009 influenza pandemic seasons.

In this study, 63.6% of the patients seeking care for ILI were female. In the univariate analysis, male sex was associated with severe illness; however this association was not statistically significant in the multivariate analysis. Differences in respiratory tract infection severity by sex have been reported previously. For example, there is evidence that the

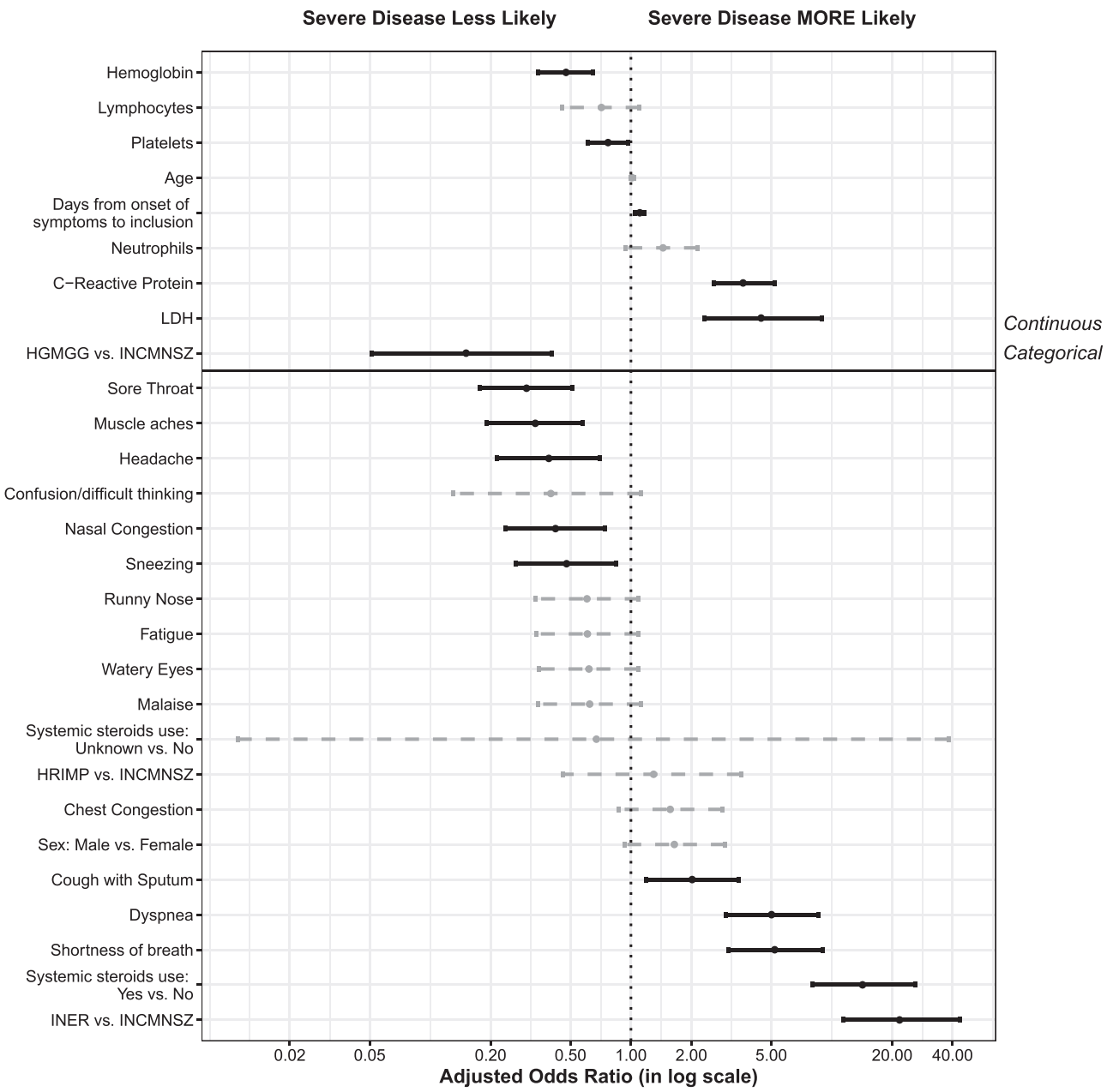


**Figure 2.** Multivariate logistic regression model generated by bi-directional stepwise selection procedure relating the clinical features and severity of influenza-like illness: odds ratios with 95% confidence intervals. The gray dashed lines represent non-significant odds ratios at $\alpha = 0.05$.
LDH, lactate dehydrogenase; HGMGG, Hospital General Manuel Gea Gonzalez; HRIMP, Hospital Regional Dr. Ignacio Morones Prieto,; INCMNSZ, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán; INER, Instituto Nacional de Enfermedades Respiratorias Manuel Cosio Villegas.

severity of influenza infection is worse in females for seasonal, outbreak, and pandemic influenza viruses [15]. However, evidence has been contradictory regarding sex differences in influenza. Many studies have not adjusted for comorbidities such as asthma (which is more prevalent in women) or pregnancy (a known risk factor for severe influenza). The present study showed a higher prevalence of males requiring hospitalization, but male sex was not significant after adjusting for other variables.

Pregnancy has been recognized as a risk factor for severe influenza [16]; however since LaRed sites do not offer specialized obstetric services, it was not possible to explore this condition as a risk factor for severe ILI. In the univariate analysis, cardiovascular disease and COPD were associated with severe ILI, as described by others [17]. In the multivariate analysis, diabetes mellitus, cardiovascular disease, and COPD were not found to be associated with severity. This could be due to the age characteristics of the study cohort, with a younger population on average (median age 34 years for non-severe cases and 47 years for severe cases), in which the prevalence of some chronic conditions is lower [18].

LaRed sites are mostly tertiary care facilities (referral hospitals with highly specialized staff) where there is a higher proportion of patients with chronic diseases requiring steroid therapy than in the general population. This could explain the high proportion of participants exposed to these medications in the study cohort. The evidence on the effect of steroid use on clinical outcomes in patients with influenza remains unclear [19], and the effect of prior exposure to steroids on the severity of illness has not been studied systematically. There is evidence of an association of steroid use with higher long-term rates of infection (ad-

justed hazard ratio 2.10, 95% CI 1.73–2.56) [20]. In the current study, a strong association was found between steroid use and the severity of ILI, suggesting a deleterious effect of this medication in the evolution of ILI. The impact of steroid use on the immune response is well known [21]. This might explain the higher severity in the study participants using steroids. Immunosuppression predisposes the patient to more infections, including viral, bacterial, and fungal infections. Nonetheless, this finding is interesting, because prior evidence has demonstrated that patients on steroids are usually predisposed to opportunistic fungal infections more than viral respiratory infections [22]. Steroids may affect disease outcomes depending on the timing, dosage, and severity of the disease. Further studies on steroid use and its association with severe ILI are needed to determine the dosage and timing that may be associated with more severe ILI and the influence of underlying medical conditions on disease severity.

Signs/symptoms associated with the lower respiratory tract were predominant in participants with severe cases of ILI. Notably, shortness of breath and dyspnea were strongly associated with a higher risk of severe ILI. Viral agents causing severe respiratory disease can propagate not only in the upper respiratory epithelium, but also in the lower airway tissues, as reported in immunocompromised patients [23], and also in adults [24]. Additionally, the presence of lower respiratory tract symptoms may influence the decision of the attending medical staff regarding in-hospital management, which would classify the patient as severe according to the study definition. On the other hand, symptoms related to upper respiratory tract infection (sore throat, muscle aches, nasal congestion, watery eyes, malaise) were found more likely to be associated with mild disease. In the study cohort, nearly 9% of the patients with severe cases of ILI died, as has been described previously for some specific etiological agents (influenza, RSV) [24].

Participants with severe ILI presented with lymphocytopenia and anemia, and with an increased CRP as non-specific markers of systemic disease. This is consistent with disease caused by other severe acute respiratory infections, such as severe acute respiratory syndrome (SARS) [25], and with other viral respiratory infections [26] as recently reported for SARS-CoV-2 [27]. This raises the hypothesis that these tests may be used as tools for the early prediction of severe cases.

As reported elsewhere [24], the proportion of participants with severe cases of ILI who had influenza viruses detected on PCR was approximately 13%, and the proportion was similar in the two severity groups. In recent reports, rhinovirus was recognized as an important agent for severe respiratory disease in adults with comorbidities [28,29]; however in the present study cohort, rhinovirus/enterovirus and seasonal coronavirus infections were more frequent in the non-severe cases. This agrees with the observations of other authors who have conducted studies on respiratory infections and have found non-influenza respiratory viruses to be associated with a lower disease severity [30,31].

The univariate analysis showed risk factors consistent with well-documented risk factors for influenza severity, such as smoking, previous cardiovascular disease, and the time from onset of illness to medical attention [32]. However, in the multivariate analysis, steroid use was also found to be a risk factor strongly associated with severe ILI. Recently, the use of steroids for community-acquired pneumonia was evaluated extensively [33]. A beneficial effect was reported, particularly for some acute parameters (fever, hypotension), but there was no demonstrated impact on mortality [34]. In accordance with the study findings, there are reports of potential deleterious effects of steroid use in viral respiratory infections, such as influenza A H1N1pdm2009 and H5N1 [35] and mild COVID-19 disease [36].

This study has the advantage of comparing a substantial number of patients systematically, prospectively, and simultaneously. One of the limitations of this study is that the PCR multiplex platform used was unable to identify a pathogen in 35% of cases and this was associated with the severity in the multivariate pathogen analysis. It is important to mention that evidence of bacterial infection was not evaluated. However, it is possible that a secondary bacterial infection might have contributed to severe illness, or other viruses not identified by PCR could have been involved.

It was also not possible to differentiate whether any of the factors associated with severity in the analysis influenced the decision of the treating physician to indicate hospitalization, and the risk of potential selection bias is acknowledged. However, the results emphasize the relevance of associations between clinical characteristics, laboratory parameters, and respiratory viruses and severe ILI.

Although the study took place from 2010 to 2014, the topic is still of interest, as studies conducted thus far have yielded partially divergent results. Besides, these results are still currently relevant. In the context of the COVID-19 pandemic, taking into account the overlapping of symptoms between SARS-CoV-2 infection and ILI [37], and the similar seasonal pattern among ILI-causing viral agents [38], it is important to clearly identify risk factors for severe ILI that may apply to the current context.

In conclusion, these results highlight the importance of evaluating data compatible with lower tract involvement and the previous use of corticosteroids in the initial evaluation, since these patients were found to present a higher frequency of severe illness. The role of specific agents and co-infections, as well as other immunosuppressive diseases and therapies, needs further research.

## Declarations

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*Ethical approval:* The study was conducted according to the guidelines of the Declaration of Helsinki and was approved by the institutional review board (or ethics committee) of each participating institution.

*Conflict of interest:* The authors declare that they have no competing interests.

*Patient consent:* Informed consent and assent were obtained from all participants involved in the study.

*Data availability:* The data presented in this study are available on request from the corresponding author. The data are not publicly available because of the requirement for a data-sharing agreement that provides: (1) a commitment to using the data only for research purposes and not to identify any individual participant; (2) a commitment to securing the data using appropriate computer technology; and (3) a commitment to destroying or returning the data after analyses are completed.

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijregi.2023.01.012.

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