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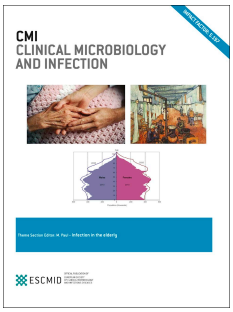

Lower respiratory tract infection in the community: associations between viral aetiology and illness course

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Lower respiratory tract infection in the community: associations between viral aetiology and illness course

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ABSTRACT

OBJECTIVES. This study determined associations between respiratory viruses and subsequent illness course in primary care adult patients presenting with acute cough and/or suspected lower respiratory tract infection (LRTI).

METHODS. A prospective European primary care study recruited adults with symptoms of lower respiratory tract infection between Nov-Apr 2007-2010. Real-time in-house polymerase chain reaction (PCR) was performed to test for six common respiratory viruses. In this secondary analysis, symptom severity (scored 1=no problem, 2=mild, 3=moderate, 4=severe) and symptom duration were compared between groups with different viral aetiologies using regression and Cox proportional hazard models, respectively. Additionally, associations between baseline viral load (cycle threshold (Ct) value) and illness course were assessed.

RESULTS. The PCR tested positive for a common respiratory virus in 1,354 of the 2,957 (45.8%) included patients. The overall mean symptom score at presentation was 2.09 (95%CI 2.07-2.11) and the median duration until resolution of moderately bad or severe symptoms was 8.70 days (interquartile range 4.50-11.00). Patients with influenza virus, human metapneumovirus (hMPV), respiratory syncytial virus (RSV), coronavirus (CoV) or rhinovirus had a significantly higher symptom score than patients with no virus isolated (0.07-0.25 points or 2.3-8.3% higher symptom score). Time to symptom resolution was longer in RSV infections (adjusted hazard ratio (AHR) 0.80, 95%CI 0.65-0.96) and hMPV infections (AHR 0.77, 95%CI 0.62-0.94) than in infections with no virus isolated. Overall, baseline viral load was associated with symptom severity (difference 0.11, 95%CI 0.06-0.16 per 10 cycles decrease in Ct value), but not with symptom duration.

CONCLUSIONS. In healthy, working adults from the general community presenting at the general practitioner with acute cough and/or suspected LRTI respiratory viruses other than influenza impose an illness burden comparable to influenza. Hence, the public health focus for viral respiratory tract infections should be broadened.

INTRODUCTION

From the few studies describing the aetiology of acute lower respiratory tract infections (LRTIs) in primary care patients, we know that most LRTIs in the general community are caused by viral pathogens, in particular rhinovirus, influenza virus, coronavirus (CoV), respiratory syncytial virus (RSV), human metapneumovirus (hMPV), and parainfluenza virus (PiV)(1,2). The illness course of LRTIs in adults presenting in this setting - a relatively healthy, working population - is mostly self-limiting and complications are rare(3). However, with an average of 3.5 days sick leave per year, LRTIs cause a substantial socio-economic burden(3,4). In adults, influenza virus, bacteria, and viral-bacterial coinfections are assumed to cause the most severe illnesses, with most systemic symptoms, longest illness durations, and most complications(5–7). However, evidence on associations between aetiology and severity are mainly derived from hospital care settings with vulnerable patient populations(8–10). In this setting, a focus on pathogens with the highest complication rates is obvious. Quite often, however, this focus is also applied in the general community, with public health interventions as the annual influenza vaccinations targeted at the most vulnerable people with the aim of reducing the risk of complications and death(11). Although data on the impact of respiratory viruses in the primary care setting are limited due to restricted microbial testing and absence of a standardized, validated outcome measure to evaluate illness severity(12), there are studies suggesting that the burden of disease from infections due to respiratory viruses other than influenza – i.p. rhinovirus, coronavirus and RSV - may be greater overall(13). In this study, we aimed to explore the associations between respiratory viral pathogens, including viral load, and illness course in the adult primary care community, thereby opening up possibilities to base the public health focus on the impact of respiratory viruses in primary care, rather than on extrapolated data from hospital settings. This study was conducted in a large European cohort consisting of prospectively enrolled adult patients with acute cough and/or a clinical suspicion for LRTI.

METHODS

Design and study population

This prospective study in primary care is part of the GRACE study (Genomics to combat Resistance against Antibiotics in Community-acquired LRTI in Europe). Participants were recruited between November 2007 and April 2010 by general practitioners (GPs) from 16 primary care networks in 11 European countries (*Supplementary Figure 1*). Patients aged ≥18 years presenting with acute cough (duration of ≤28 days) and/or suspected LRTI, were asked to participate in this study, i.e. to fill out study materials and provide written informed consent(14). Exclusion criteria were pregnancy, breast-feeding, any serious immunocompromised condition and antibiotic use in the previous month(14). About one third of these patients agreed to being randomised to either the intervention (amoxicillin) or placebo arm of the original randomized controlled trial(14). Remaining patients were not randomly assigned, but were included in the observational part of the study(1). In the current study, both trial and observational patients were analysed together, but patients without PCR and/or serology results on viral aetiology (all due to practical reasons) were excluded. Ethical approval was obtained for all participating networks.

Clinical measurements

For the collection of clinical data on the day of presentation (baseline), standardized case report forms (CRFs) were used. GPs completed the CRF on the following 12 symptoms rated by the patients using a 4-point Likert-scale (1=no problem, 2=mild, 3=moderate, 4=severe): cough, sputum production, shortness of breath, wheeze, blocked or runny nose, fever, chest pain, muscle aching, headache, disturbed sleep, feeling generally unwell, and interference with normal daily activities. Additionally, the symptoms confusion/disorientation and diarrhoea were rated. Following initial presentation, patients were asked to fill out a symptom diary at home on a daily basis until they had no more symptoms or until the end of follow-up (day 28). Patients were asked to rate the same 12 symptoms by using a 7-point Likert-scale (0=normal, 1=very little problem, 2=slight problem, 3=moderately bad, 4=bad, 5=very bad, 6=as bad as it could be). This diary was internally reliable, valid, and sensitive to change for acute LRTI(15).

Microbiological measurements

At baseline, two nasopharyngeal flocked swabs were taken by trained staff within 24 hours after recruitment and before any antimicrobial treatment had started. Swabs were placed in universal transport medium immediately, frozen locally, and transported on dry ice to the central laboratory (University of Antwerp). Real-time in-house polymerase chain reaction (RT-PCR) testing was performed either as four multiplex RT-PCRs (combining INF-A, INF-B, and RSV; PIV1-4; HRV, hMPV, and the EAV internal control; and finally the human CoV: 229E, OC43, NL63, and HKU1), or as monoplex (all other viruses) (16). RNA/DNA extractions and amplification methods were described previously(1,16). Based on the results from our study comparing the prevalence of viral pathogens between symptomatic and asymptomatic matched controls(1), we evaluated rhinoviruses, influenza viruses, coronaviruses, RSV, hMPV and PiV. Since (pan-)adenovirus (1.3% vs. 1.1%, p=0.33), bocavirus (0.6% vs. 0.8%, p=0.43) and WU/KI polyomaviruses (2.2% vs. 2.5%, p=0.02) were not detected more frequently in symptomatic patients than in controls, they were not considered pathogenic respiratory viruses and therefore excluded from our analyses(1). A cycle threshold (Ct) value - an inverse, logarithmic, quantitative measurement of viral load – below 45 was chosen as cut-off for a positive result. We adjusted our analyses for bacterial infections, which were defined as having at least one of the following pathogens detected in a sputum or nasopharyngeal sample: *Streptococcus* species*,* Gram-negative species, or *Aspergillus* (fungus). Commensals and *Candida* species were considered contaminants for which analyses were not adjusted. Microbiologists who determined the results were blinded to clinical information.

Outcome parameters

We focused on two main outcome parameters: symptom severity at presentation and illness duration. Symptom severity was measured as the mean CRF score for all 12 symptoms (scored 1-4) at baseline(14,17–19). Illness duration was defined as the duration until absence of any symptoms rated moderately bad or severe (score 3 or above) in the symptom diary following initial presentation(14,17–19). Additionally, the severity of all individual symptoms was analysed, dichotomizing symptom severity at no/mild/moderate versus severe.

Statistical analysis

Baseline characteristics were reported as N (%), means (SD) or medians (IQR) as appropriate. Symptom severity at baseline was analysed with linear regression models and expressed as differences in mean symptom severity with a 95% confidence interval (CI). In an additional step, we analysed the presence of individual symptoms with logistic regression, expressed as odds ratios (OR). Duration until absence of symptoms rated moderately bad or severe were analysed with cox proportional hazard models. For the latter analysis, patients were censored at the end of follow-up or if less than ten symptoms were filled out in the symptom diary. If patients already met the event criteria at baseline (n=104), we defined their time to event as one day. Results were expressed as hazard ratios (HR).

For all analyses, we adjusted for the potential confounders defined beforehand (*Supplementary Text 1*). Statistical analyses were performed using SPSS v.25.0 for Windows and the “survival” and “survminer” packages in R v.4. Details of the statistical analysis are described in *Supplementary Text 1*.

RESULTS

Study population

We included 2,957 adult patients (*Figure 1*). Demographics and clinical symptoms at presentation are presented in *Table 1*. Patients had a median age of 50 years (IQR 36-63), 1,195 (40.4%) were male and 1,603 (54.2%) were a former or current smoker. The overall mean symptom score at presentation was 2.09 (95%CI 2.07-2.11). Respiratory viruses (1,411) were detected in 1,354 patient samples (*Figure 2*). The proportion of influenza virus positive patients was lower among patients who received the annual influenza vaccination during the preceding fall/winter (38/707, 5.4%) than among non-vaccinated patients (259/2250, 11.5%) ($p<0.001$). Follow-up data were available for 2,393 patients (80.9%). Baseline disease characteristics did not differ between patients who did (n=2,393), or did not (n=564) fill out a symptom diary. Of all 2,393 patients included in the symptom duration analysis, 2,186 patients (91.3%) documented resolution of symptoms rated moderately bad or severe before the end of follow-up, with a median duration of 6.00 days (IQR 4.00-11.00 days). At presentation, only two patients were prescribed antiviral medication (oseltamivir).

Association between respiratory viruses and symptom severity

We evaluated the severity of symptoms at presentation for patients with CoV, hMPV, influenza virus, PiV, rhinovirus and RSV, as compared to patients without these viruses, with adjustment for confounders, bacteria and co-viruses. Influenza virus, hMPV, RSV, CoV and rhinovirus were significantly associated with, respectively, 0.25 (95%CI 0.19-0.31), 0.16 (95%CI 0.07-0.26), 0.12 (95%CI 0.04-0.21), 0.09 (95%CI 0.02-0.16) and 0.07 (95%CI 0.02-0.12) points higher symptom scores at presentation as compared to patients without detected virus (*Table 2*). Among patients in whom a virus was detected, a ten cycles lower Ct value – i.e. a higher viral load – measured at presentation, was associated with a 0.11 (95%CI 0.06-0.16) point higher mean symptom severity as compared to patients without detected virus. After stratification for viral aetiology, we only observed an association between viral load and symptom severity for rhinovirus (increase of 0.12 per 10 cycles reduction in Ct value, 95%CI 0.04-0.20) and for RSV (increase of 0.16 per 10 cycles reduction in Ct value, 95%CI 0.01-0.30). When looking at differences in the severity of individual

symptoms of these viruses (*Figure 3*), influenza virus was independently associated with severe fever (OR 6.3, 95%CI 4.0-9.8), headache (OR 3.1, 95%CI 2.2-4.5), chest pain (OR 2.0, 95%CI 1.3-3.2), muscle pain (OR 2.5, 95%CI 1.6-3.9), disturbed sleep (OR 1.4, 95%CI 1.1-1.9), being generally unwell (OR 2.5, 95%CI 1.8-3.5), and interference with daily activities (OR 2.5, 95%CI 1.8-3.5). RSV was associated with severe headache (OR 2.0, 95%CI 1.2-3.5), disturbed sleep (OR 1.7, 95%CI 1.1-2.5) and a runny nose (OR 2.9, 95%CI 1.9-4.4). hMPV was associated with severe dyspnoea (OR 2.0, 95%CI 1.0-3.7) and headache (OR 2.0, 95%CI 1.1-3.7). Rhinovirus was associated with severe wheeze (OR 1.6, 95%CI 1.0-2.6), a runny nose (OR 1.6, 95%CI 1.2-2.1) and negatively associated with severe cough (OR 0.8, 95%CI 0.6-0.9). CoV was associated with a severe runny nose (OR 2.0, 95%CI 1.4-3.0) and negatively associated with severe chest pain (OR 0.3, 95%CI 0.1-0.9).

Association between respiratory viruses and illness duration

After adjustment for bacterial coinfections, baseline symptom severity and other potential confounders, patients with detected viral pathogen(s) had no significantly different HR (0.93, 95%CI 0.86-1.02) for resolution of moderately bad or severe symptoms compared to patients in which no virus was detected (*Table 3*). We also assessed the duration until resolution of moderately bad or severe symptoms for the six individual viruses as compared to patients without a detected virus (*Figure 4*). Patients with RSV had an adjusted hazard ratio (AHR) of 0.80 (95%CI 0.65-0.96) and patients with hMPV an AHR of 0.77 (95%CI 0.62-0.94) for symptom resolution, indicating a significant longer symptom duration as compared to patients without RSV and hMPV, respectively. All other viral pathogens showed no significant differences in AHRs. Among patients in whom a virus was detected, there was no association between baseline viral load and duration of moderately bad or severe symptoms (AHR per unit lower Ct value 1.01, 95%CI 0.99-1.02). After stratification for viral aetiology, no significant associations were found between viral load and symptom duration.

DISCUSSION

Adult patients visiting the GP with acute cough or suspected LRTI due to influenza virus, hMPV, RSV, CoV or rhinovirus had a 0.07-0.25 points (or 2-8%) higher mean symptom severity score (range 1-4) at presentation as compared to patients presenting with acute cough or suspected LRTI without detection of one of these respiratory viruses. In translation, patients with RSV - who have a 0.12 point (4%) higher symptom score at presentation than patients in whom no virus is detected - rate one or two symptoms severe instead of moderate, moderate instead of mild, or mild instead of absent. Additionally, RSV and hMPV were associated with a longer duration of moderately bad or severe symptoms, which might be linked to the pattern of immune response to these viruses(21). For all respiratory virus together, a higher viral load measured at presentation, was significantly associated with a higher symptom severity. This was caused by significant associations between viral load and symptom severity for rhinovirus and RSV. There was no association between viral load and the duration of moderately bad or severe symptoms.

Clinical implications

This study does not provide direct clinically actionable insight. However, although we do not provide recommendations on clinical management or treatment, we do think that the large number of patients included in this study provides important information which can be used to prioritize different respiratory viruses in the primary care setting. Currently, public health resources in the general community are guided by the aim to prevent complications in the most vulnerable people, and are focused almost exclusively on influenza(11,22,23). From a socio-economic perspective, however, targeting public health resources only at influenza virus neglects the substantial illness course in the community caused by other respiratory viruses. From our results we conclude that RSV and hMPV impose a disease burden that compares well to that of influenza virus and should therefore receive more attention in the primary care setting, e.g. by supporting the development and implementation of prevention approaches like vaccines(24-26).

Strengths and limitations

Despite the fact that we had a large cohort in which data were collected in a standardized manner, and outcome measures were in line with previous studies(14,17–19), there are several potential sources of bias that might limit the validity of our results. Firstly, it is possible that non-agreement of patients to participate in this observational study was not random. The extent to which this selection might be present is uncertain since we have no information on the number and characteristics of patients who declined participation. Secondly, the use of medication, such as antibiotic treatment, antiviral treatment, (over-the-counter) symptomatic treatment, and prophylactic antibiotics with antiviral effects (as azithromycin) might have influenced outcomes . We consider it unlikely that receiving antibiotics caused biased results, since the in-study amoxicillin trial showed no differences in outcomes between the intervention and placebo group(14). Since only two patients in our cohort were prescribed antivirals (oseltamivir), we consider the effect of antiviral treatment also negligible. Unavailability of data on the use of prophylactic antibiotics and symptomatic medication made adjustment for these factors impossible. Thirdly, there might be bias in the self-report of symptoms by patients. However, previous studies showed a high internal reliability, validity and sensitivity of the symptom diary we used(15). Also, since the 95/207 (46%) patients who did not meet the event criteria and who did not fill out their symptom diary completely were censored for the analysis, we do not expect selection bias due to loss of follow-up. Fourthly, the required sample size for the prospective observational cohort was not determined on the specific requirements of the current study. Hence, inconclusive or non-significant results can therefore not be considered definite to prove the absence of associations. We specifically choose not to correct for multiple testing, as this correction may further hamper statistical power, especially for viruses only detected in a limited number of patients. Fifthly, the relatively low overall percentage of detected viruses might have been caused by the inclusion of patients with quite long duration of symptoms. Since respiratory fluids are renewed quickly in the patient, viral pathogens in patients with longer duration of symptoms might therefore not have been detectable anymore. Finally, a higher viral load was associated with a higher symptom severity at presentation. Looking at specific viruses we only found this association for RSV and rhinovirus, which confirms previous studies(27–29). However, the interpretation of single viral load measurements is difficult. Not only are viral loads of respiratory viruses highly dependent on variation in sampling location and

technique, they also rise and drop rapidly and it is known that symptoms mostly follow the viral load(30,31).

In conclusion, in this study among relatively healthy adult patients presenting in a primary care setting with acute cough and/or a suspected LRTI, influenza virus, hMPV, RSV, CoV and rhinovirus were associated with an increased symptom severity at presentation as compared to patients without a detected virus. In this general community population, RSV and hMPV were associated with a longer duration of moderately bad or severe symptoms. This study emphasizes that public health policies as vaccinations and awareness among GPs should not remain focused on influenza virus exclusively, but should also include other common respiratory viruses like RSV and hMPV that pose a high socio-economic burden to the general adult community.

TRANSPARANCY DECLARATION

Conflict of interest: the authors declare that they have no conflict of interest. Funding: the study was part of the European Union FP6 funded Network of Excellence GRACE. Orion Diagnostics provided the QuikRead instruments and kits for this study. The study sponsors played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the paper for publication. Ethical approval: all procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent: informed consent was obtained from all individual participants included in the study.

AUTHOR CONTRIBUTIONS

The larger GRACE observational study was designed by CCB, TJMV, PL, DC and HG, and sampling protocols by MI, CL, KL and HG. MI, CL, PL, TV and HG supervised the day-to-day management at study sites. PCR and serological analyses were performed by KL, AMVL, CL, KZ, ECJC, MV and FC. Data were analysed by LMV, RB, NPAZ, BDLB, JJO and FC. The manuscript was designed and drafted by LMV, NPAZ and FC, and was reviewed by all authors.

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Table 1. *Baseline characteristics included patients (n=2957).*

Demographics	**Patients (n=2957)[a]**
Age (years)	50 (36-63)
Gender (male)	1195 (40.4%)
Caucasian ethnicity	2862 (96.8%)
Comorbidities[b]	
COPD	176 (6.0%)
Asthma	307 (10.4%)
Other lung disease	62 (2.1%)
Heart failure	57 (1.9%)
Ischemic heart disease	159 (5.4%)
Other hearth disease	111 (3.8%)
Diabetes	190 (6.4%)
Smoking past or current	1603 (54.2%)
Disease related characteristics at presentation	
Severe cough	983 (33.2%)
Sputum production	309 (10.4%)
Shortness of breath	215 (7.3%)
Wheeze	115 (3.9%)
Blocked or runny nose	355 (12.0%)
Fever	122 (4.1%)
Chest pain	155 (5.2%)
Muscle aching	163 (5.5%)
Headache	226 (7.6%)
Disturbed sleep	542 (18.3%)
Feeling generally unwell	349 (11.8%)
Interference with normal daily activities	344 (11.6%)
Confusion/disorientation	6 (0.2%)
Diarrhoea	16 (0.5%)
One or more abnormalities at lung auscultation	1165 (39.4%)
Breaths (per minute)	16 (15-18)
Heart rate (beats per minute)	76 (70-83)
Systolic blood pressure (mmHg)	127 (117-140)
Diastolic blood pressure (mmHg)	80 (70-85)
Oral temperature (degrees Celsius)	36.7 (36.4-37)
Medication prescribed for illness[c]	2086 (70.5%)

[a] Demographics are given as absolute numbers with % for categorical variables or as median with interquartile range (IQR) for continuous variables. [b] Some patients had multiple comorbidities. [c] Prescribed medication included antibiotics, antitussives, mucolytic drugs, antihistamines, bronchodilators and anti-inflammatory drugs.

Table 2. *Symptom severity[a] at presentation in patients consulting in primary care with a detected virus or no detected virus (n=2957).*

	Mean (SD) symptom score at presentation	**Unadjusted difference between groups (95% CI)**	**Adjusted difference between groups (95% CI)[b]**
No virus(es) (n=1603)	2.02 (0.49)	(ref)	(ref)
≥1 virus(es) (n=1354)	2.18 (0.52)	0.17 (0.13-0.20)	0.13 (0.10-0.17)
No virus(es) (n=1603)	2.02 (0.49)	(ref)	(ref)
1 virus (n=1297)	2.18 (0.51)	0.16 (0.13-0.20)	0.13 (0.09-0.16)
2 viruses (n=57)	2.27 (0.54)	0.13 (0.06-0.19)	0.22 (0.09-0.35)
CoV (n=205)[c]	2.15 (0.48)	0.10 (0.03-0.18)	0.09 (0.02-0.16)[d]
hMPV (n=121)[c]	2.18 (0.52)	0.16 (0.06-0.25)	0.16 (0.07-0.26)[d]
Influenza virus (n=297)[c]	2.32 (0.55)	0.30 (0.23-0.36)	0.25 (0.19-0.31)[d]
PiV (n=73)[c]	2.13 (0.51)	0.10 (-0.01-0.22)	0.07 (-0.04-0.19)[d]
Rhinovirus (n=572)[c]	2.15 (0.50)	0.12 (0.07-0.16)	0.07 (0.02-0.12)[d]
RSV (n=143)[c]	2.17 (0.53)	0.14 (0.05-0.22)	0.12 (0.04-0.21)[d]

[a] Calculated as the mean (standard deviation) symptom severity score for all 12 symptoms at presentation.

[b] Estimates controlled for age, gender, pulmonary comorbidities (asthma, COPD and other lung diseases), hearth failure, current smoking, influenza vaccination during the preceding fall or winter, coinfection with at least one respiratory bacterium or with Aspergillus and duration of symptoms before presentation.

[c] Reference group is no CoV, hMPV, influenza virus, PiV, rhinovirus or RSV respectively.

[d] By including all six viruses in the model, estimates were additionally controlled for coinfection with another respiratory virus.

Table 3. *Symptom duration[a] (days) in patients consulting in primary care with detected virus or no detected virus (n=2393). A hazard ratio <1 indicates a disadvantageous effect on symptom resolution.*

	Median (IQR) time to resolution of symptoms rated moderately bad or worse	**Unadjusted hazard ratio (95% CI)**	**Adjusted hazard ratio (95% CI)[b]**
No virus(es) (n=1288)	6 (4-10)	(ref)	(ref)
≥1 of six viruses (n=1105)	7 (5-11)	0.93 (0.86-1.01)	0.93 (0.86-1.02)
No virus(es) (n=1288)	6 (4-10)	(ref)	(ref)
1 of six viruses (n=1056)	7 (5-11)	0.94 (0.87-1.03)	0.94 (0.86-1.03)
2 of six viruses (n=49)	8 (5-15)	0.74 (0.55-1.00)	0.76 (0.56-1.03)
CoV (n=177)[c]	7 (4-11)	0.92 (0.78-1.09)	0.95 (0.80-1.12)[d]
hMPV (n=108)[c]	8 (6-12)	0.80 (0.65-0.98)	0.77 (0.62-0.94)[d]
Influenza (n=243)[c]	7 (5-10)	1.12 (0.97-1.28)	1.08 (0.93-1.24)[d]
PiV (n=60)[c]	8 (5-11)	0.98 (0.75-1.28)	0.97 (0.74-1.26)[d]
Rhinovirus (n=445)[c]	7 (5-11)	0.90 (0.81-1.01)	0.93 (0.83-1.04)[d]
RSV (n=121)[c]	8 (5-14)	0.79 (0.65-0.96)	0.80 (0.65-0.96)[d]

[a] Calculated as the median (IQR) number of days with symptoms rated moderately bad or worse by the patient following initial presentation.

[b] Estimates controlled for age, gender, pulmonary comorbidities (asthma, COPD and other lung diseases), heart failure, current smoking, influenza vaccination during the preceding fall or winter, coinfection with at least one respiratory bacterium or with Aspergillus and duration of symptoms before presentation.

[c] Reference group is no CoV, hMPV, influenza virus, PiV, rhinovirus or RSV respectively.

[d] By including all six viruses in the model, estimates were additionally controlled for coinfection with another respiratory virus.

Figure 1. *Flow-chart patient exclusion as compared to the total number of patients included in the GRACE cohort*(1).

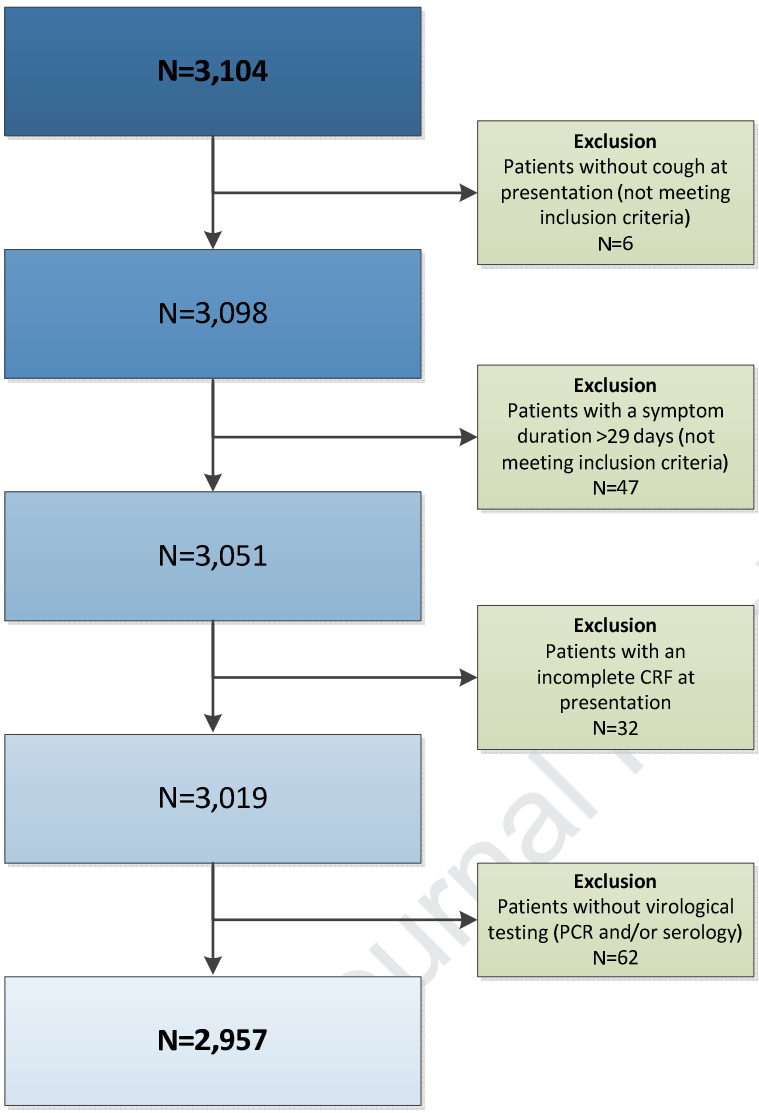
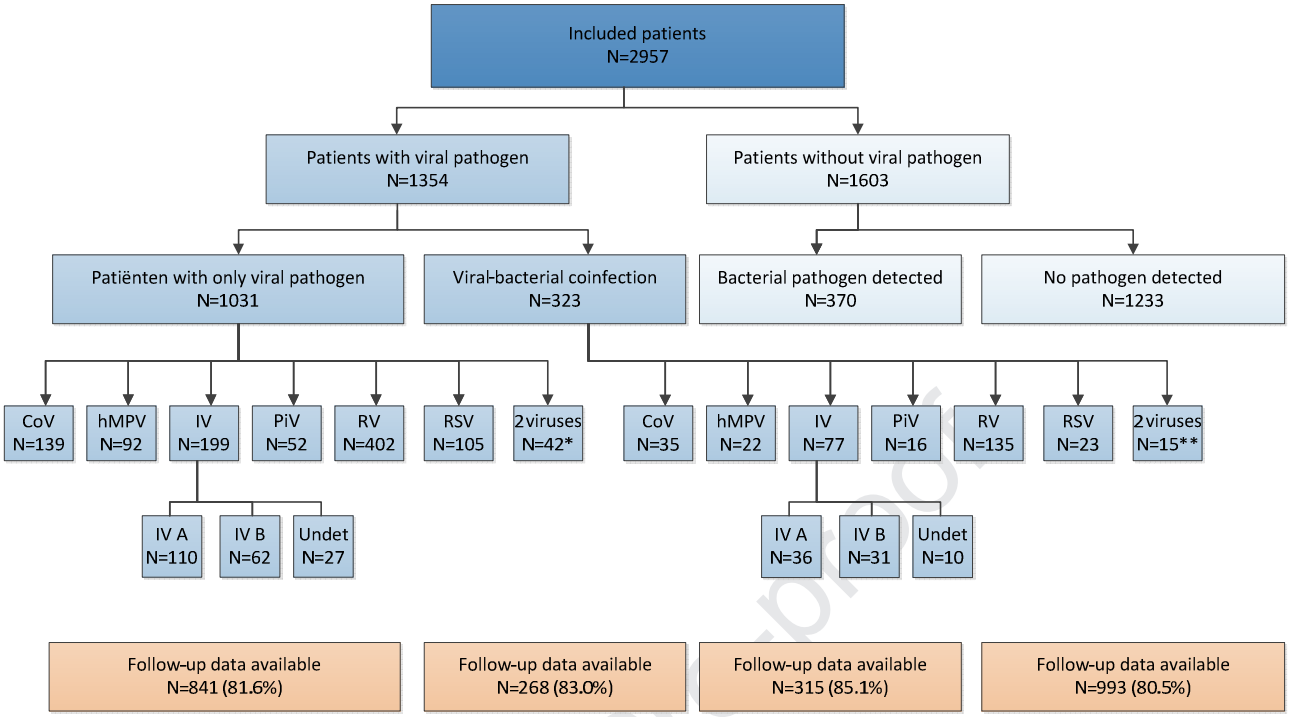


Figure 2. *Detected viral pathogens in included patients (n=2957) and availability of follow-up data.*

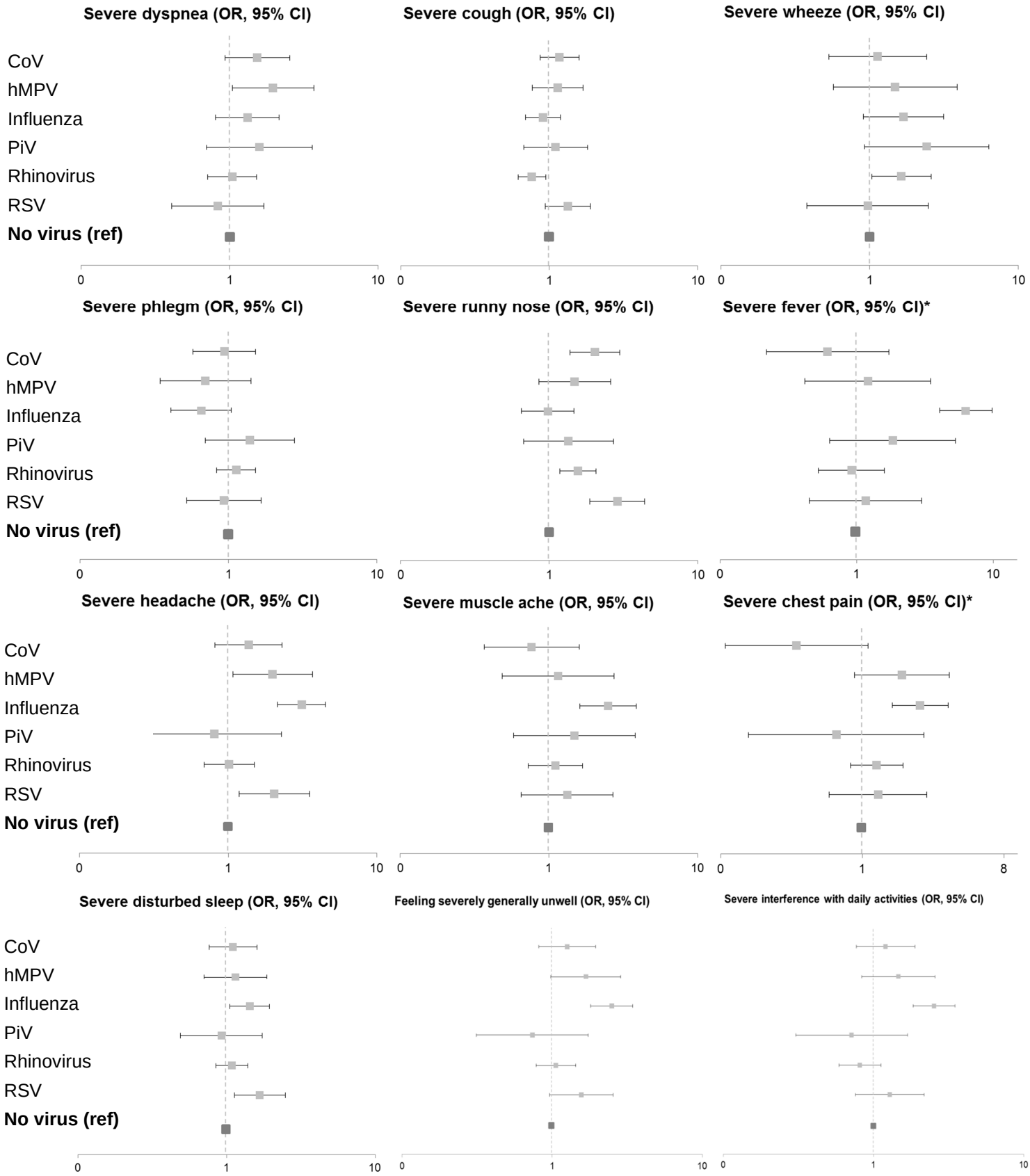


CoV, coronavirus; hMPV, human metapneumovirus; IV, influenza virus; PiV, Parainfluenza virus; RV, rhinovirus; RSV, respiratory syncytial virus; Undet, influenza virus type undetermined.

* The following combinations of viral pathogens were found: CoV + RV (n=10), IV + RV (n=8), CoV + hMPV (n=5), CoV + RSV (n=4), RV + RSV (n=4), IV + RSV (n=3), CoV + IV (n=2), hMPV + RV (n=2), IV + PiV (n=1), CoV + PiV (n=1), RV + PiV (n=1), RSV + PiV (n=1).

** The following combinations of viral pathogens were found: CoV + RV (n=5), IV + RV (n=3), CoV + IV (n=3), CoV + RSV (n=1), RV + RSV (n=1), IV+ RSV (n=1), RV + PiV (n=1).

Figure 3. *Forest plots showing odds ratios (OR) with 95%CI on the log scale for CoV, hMPV, influenza virus, PiV, rhinovirus and RSV for a severe burden of individual symptoms at presentation (highest on 4-point Likert scale). The reference category is no virus isolated. ORs are derived from logistic regression models (one model per symptom) with adjustment for bacterial and viral coinfections, age, gender, pulmonary comorbidities (asthma, COPD and other lung diseases), hearth failure, current smoking, influenza vaccination during the preceding fall or winter and duration of symptoms before presentation.*



** For fever and chest pain the scale on the x-axis was altered for visual purposes.*

Figure 4. *Cox regression survival curves for the duration of symptoms rated moderately bad or worse in patients with LRTI and a viral monoinfection (n=2344), stratified by detected virus. The reference category is no virus detected. Survival curves are derived from multivariate cox regression models with adjustment for bacterial coinfections, age, gender, pulmonary comorbidities (asthma, COPD and other lung diseases), hearth failure, current smoking, influenza vaccination during the preceding fall or winter and duration of symptoms before presentation.*