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TIME-COURSE OF UPPER RESPIRATORY TRACT VIRAL INFECTION AND COPD EXACERBATION

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TAKE HOME MESSAGE: The presence of viruses in patients with stable COPD is rare. Viruses detected at URTI, were not *per-se* associated with an increased risk for exacerbation. URTI is associated with worsening of quality of life and lung function independently of exacerbation.

ABSTRACT

Viral respiratory tract infections have been implicated as the predominant risk factor for acute exacerbations of COPD (AECOPD). We aimed to evaluate, longitudinally, the association between upper respiratory tract infections (URTI) caused by viruses and AECOPD.

Detection of 18 viruses was performed in naso- and oropharyngeal swabs in 450 COPD patients (GOLD 2-4), followed for a mean of 27 months, at stable periods (n=1909), at URTI onset (n=391), 10 days after the URTI (n=356) and at AECOPD (n=177) using a multiplex nucleic acid amplification testing.

Evidence of at least one respiratory virus was significantly higher at URTI onset (52.7%), at 10 days following a URTI (15.2%) and at exacerbation (38.4%), compared with the stable period (5.3%, $p<0.001$). At stable visits rhinovirus accounted for 54.2% of all viral infections, followed by coronavirus (20.5%). None of the viruses could be identified in two consecutive stable visits. Patients with viral infection at URTI onset did not have a higher incidence of exacerbation, compared with patients without viral infection ($p=0.993$). The incidence of any viral infection at AECOPD was similar between URTI-related AECOPD and non-URTI-related AECOPD ($p=0.359$). Only 24% of the patients that had a URTI-related AECOPD had the same virus at URTI and AECOPD. Detection of parainfluenza 3 at URTI onset was associated with higher risk of AECOPD ($p=0.003$). Rhinovirus and coronavirus were the most frequently detected viruses at AECOPD visits accounting for 35.7% and 25.9% of all viral infections, respectively.

The prevalence of viral infection at the stable period of COPD is low. The risk of exacerbation following the onset of URTI symptoms depends on the particular virus associated with the event and was significant only for parainfluenza 3.

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Introduction

Chronic obstructive pulmonary disease (COPD) is the third leading cause of death worldwide with an expectancy of a further increase in its prevalence and mortality in the next decades [1]. Exacerbations are significant events in COPD that affect the progression of the disease and contribute to morbidity, mortality, hospital admission and healthcare costs [2]. It is widely recognized that bacterial and/or viral infections may trigger acute exacerbations of COPD (AECOPD) since 40%-80% of AECOPD that frequently require hospitalization are attributed to viral respiratory tract infections [3]. AECOPD associated with symptoms of a common cold have been shown to have more sudden onset and longer recovery times than AECOPD without cold symptoms [4]. COPD patients having more frequent exacerbations experience nearly double the number of colds compared to patients experiencing fewer exacerbations [5]. It has also been shown that the presence of cold symptoms is associated with an increased risk of AECOPD [6].

Despite the recent development of more specific and sensitive diagnostic methods for the detection of respiratory viruses utilizing PCR, the presence of a virus is usually identified in less than half of upper respiratory tract infections (URTI), raising the question about the incidence of viral infections in URTIs and to what extent they contribute to AECOPD. There are only scarce longitudinal data evaluating COPD both at stable and exacerbation periods [7, 8], but these reports appoint human rhinoviruses as the most common aetiology for viral exacerbations [9]. Several other viruses may also cause disease in patients with COPD, including coronavirus, influenza, parainfluenza 1-3, RSV, adenovirus, and human metapneumovirus [10-12]. However, since URTI symptoms often precede COPD exacerbations it is difficult to define precisely the proportion of exacerbations caused by viruses that were originally associated with URTI. Therefore, clinical studies restricted to sampling for viruses during COPD exacerbations may fail to detect viruses despite highly sensitive PCR technology [10].

In the present study, a well-characterized cohort of 450 patients was evaluated longitudinally, for the presence of viral infections by multiplex PCR, at stable state, at the onset of URTI, 10 days after the URTI and at exacerbations. The aim of the study was to evaluate, longitudinally, the association between URTI caused by viruses and AECOPD. We hypothesise that patients with URTI have a higher incidence of AECOPD.

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METHODS

STUDY DESIGN

In the present study we analysed the co-primary aim of the PREVENT study, an investigator-initiated and driven, multicentre, controlled trial (ISRCTN45572998) [13] that is to evaluate, longitudinally, the association between URTI caused by viruses and AECOPD. The primary co-aim of the PREVENT study was to evaluate whether intensified combination therapy with inhaled corticosteroids (ICS) and long acting beta2 agonists (LABA) at the onset of URTI symptoms as compared to placebo decrease the incidence of AECOPD in patients receiving low maintenance dose of ICS/LABA [13]. The study was approved by each of the Institutional Review Boards (EKBB 306/10) and was conducted in accordance with the ethical principles stated in the Declaration of Helsinki and the guidelines on good clinical practice. All patients provided written informed consent for their participation in the study.

PATIENTS

450 COPD patients were enrolled, who were above 40 years of age and with a smoking history of at least 10 pack-years. Post-bronchodilator forced expiratory volume in 1 second (FEVi) of the patients was less than 80% of the predicted value and post-bronchodilator ratio of FEVi to forced vital capacity (FVC) was less than 0.7, (GOLD 2-4) and a clinically relevant disease, as defined by a history of at least one exacerbation in the previous 12 months. Exclusion criteria were: the presence of a pulmonary condition other than COPD as the main respiratory disease, e.g. bronchiectasis or asthma; a rapid lethal disease, e.g. bronchial carcinoma, advance heart failure, end-stage renal failure; severe immunosuppression including manifested AIDS, organ transplantation or neutropenia ($< 500 \times 10^9$/L); pregnancy or breast feeding; Known allergy or intolerance to the study medication.

PROCEDURES

At inclusion, all patients were assigned to low maintenance dose of ICS/LABA (budesonide 200 g/formoterol 6 g, twice-daily). This treatment was continued for the whole duration of the study, while other concomitant medication, including LAMA, was left unchanged. In addition, each patient was block-randomised 1:1 to receive either intensified dosage of the combination ICS/LABA (budesonide 400 g/formoterol 12 g) or placebo. Patients were instructed to start using the intensified dosage of ICS/LABA at the onset of URTI, twice daily, for 10 days.

At baseline, clinical history, vital signs and oxygen saturation were evaluated. Patients were asked to answer to the following symptoms and quality of life questionnaires: COPD assessment test (CAT); Modified Medical Research Council Dyspnoea scale (MMRC); Wisconsin Upper Respiratory Symptom survey -21 (WURSS-21); St. George's Respiratory Questionnaire (SGRQ). Serum and EDTA-plasma samples, spontaneous or induced sputum and nasopharyngeal swabs were collected. Bodyplethysmography, 6MWT and FeNO measurements were administered by trained technicians according to American Thoracic Society guidelines. Nasopharyngeal and oral swabs were also collected at the study centre at stable visits.

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When patients had a URTI that was defined as new or increased coryzal symptoms (one or more of runny or blocked nose, post-nasal drip and sneezing) for at least 12h, they were instructed to collect nasopharyngeal and oral swabs using a technique that was demonstrated to the patients. Swabs were sent to the study centre by mail. Patients were also asked to contact the study centre within 10 days of URTI onset or in case of exacerbation.

A clinical visit was foreseen 10 days following the onset of URTI. At this URTI-follow-up visit, vital signs, oxygen saturation, symptoms scores, medication and health care resources used were assessed. Spontaneous or induced sputum, nasopharyngeal swabs and serum/EDTA-plasma samples were collected. Lung function and FeNO were performed by body plethysmography according to ERS/ATS guidelines using properly calibrated instruments and well-trained specialized personnel.

In case of AECOPD, the patient was free to seek medical care at the GP office or at the study centre. Symptoms recorded were termed major (dyspnoea, sputum volume or sputum purulence) and minor (cough, wheeze, sore throat or coryza). Exacerbations were defined as mild (requiring medical care and increase dose of short-acting β2-agonist); moderate (requiring either antibiotics and/or parenteral corticosteroids) and severe (requiring hospitalization). In any case, patients were asked to contact immediately the study centre and attend a clinical visit. At this visit, vital signs, oxygen saturation, symptoms scores, medication and health care resources used were assessed. Spontaneous or induced sputum, nasopharyngeal swabs and serum/EDTA-plasma samples were collected. Lung function tests and FeNO measurements were performed. In all but mild exacerbations, obtaining a chest-x-ray for pneumonia exclusion was strongly suggested. Data of the patients seeking care outside the study centre were obtained by revision of medical records of the GP and/or other health care institutions. Management of exacerbations of COPD, e.g. antibiotic and steroid prescriptions, was left up to the discretion of the treating physician and was not influenced by study personnel. All patients who had an AECOPD had a follow up visit 21 days after the AECOPD. At this follow-up visit all patients were asked to fill in the CAT, the MMRC and the WURSS-21 questionnaires.

Every 6 weeks the health status of patients was verified by consulting the health-care records of all involved providers and the patient's diaries (recording any URTI, exacerbation, and use of steroids and antibiotics). Regular, scheduled interviews were performed every 6 weeks over the phone and every 6 months on-site throughout the study period.

VIRAL AND BACTERIAL DETECTION

Detection of 18 viruses (Adenovirus, Influenza A-B, H1-H3, Parainfluenza 1-4, Respiratory Syncytial virus (RSV) A-B, Rhinovirus/enterovirus, Coronavirus NL63, -OC43, -229E, -HKUl, Bocavirus and Metapneumovirus) was performed in oral and naso-pharyngeal swabs that were collected at stable periods (n=1909), at URTI onset (n=391), at URTI follow-up (10 days after the onset of URTI) (n=356) and at exacerbations (n=177) using a commercial multiplex nucleic acid amplification testing (respiratory pathogen panel NxTAG-RPP, Luminex, MV's-Hertogenbosch, The Netherlands) (14), adding up to over than 50,000 individual viral PCRs. The analysis was performed at the Department of Virology, University Hospital Basel by well-trained and certified personnel. A microorganism was defined as pathogenic, if detected by the commercial multiplex PCR or by the culture-based method at the minimum concentration of

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10^3CFU/ml or above 10^4CFU/ml (excluding mouth flora) depending on the bacterial species. Bacterial growth in sputum was performed semi-quantitatively, as described by Podbielsky et al. (15).

STATISTICAL ANALYSIS

The study data bank contained information on patient clinical characterization, clinical tests and on the instruments used to determine their health status. All involved study centers entered the respective information of their patients in electronic case report forms (eCRF) and transferred them by password-secured website to the coordinating centre. The following software was used for data analyses: Statistical Analysis System 9-4 (SAS® Institute, Cary, NC, USA), R for Windows (R Foundation for Statistical Computing, www.r-project.org) and STATA 14 (Stata Corp, College Station, Texas).

For the descriptive statistics categorical variables were summarized as counts and proportions (percentages) and continuous variables as means, standard deviations, medians and ranges (min to max and interquartile range). The characteristics for different visits (stable visits, URTI, URTI-follow-up and AECOPD) with and without viral infections were compared using a Chi-square test of a Fisher exact test as applicable if the characteristic was of categorical nature. For continuous factors, the Mann-Whitney U-test was used for the comparison between viral infection and no infection.

The association of a viral infection (yes or no) or the presence of a specific virus and respiratory symptom at different visit types tests was determined using mixed logistic regressions (because a patient has several visits of the same type). The outcome for the models used in these regressions was a viral infection or the presence of a specific virus and the predictor was the respiratory symptom test score. The model was adjusted by age and sex as fixed effects and the effect of the patient was added as a random effect.

RESULTS

PATIENTS

From a total of 589 patients with COPD who were screened, 450 patients were eligible to be included in the study and 445 patients were sampled for PCR and included in the analysis (Figure 1). Throughout the study, 49 patients died, 8 patients were relocated, 52 patients withdrawn consent and 12 patients lost to follow-up. From the remaining patients, 198 patients reported a total of 403 URTIs. A total of 187 exacerbations were observed in 118 patients. From these exacerbations, 42 were severe and required hospitalization and 4 of them required stay in the intensive care unit. Patients' mean follow-up was 714 $\pm$ 339 days, totaling 870 person-years.

Characteristics of the 445 patients that were included in the analysis are shown in Table 1. Patients were mostly male, above 40 years of age, with a smoking history of at least 10 pack-years, GOLD 2-4 and a clinically relevant disease, as defined by a history of at least one exacerbation in the previous 12 months [13].

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VIRAL INFECTIONS AT DIFFERENT VISITS

The prevalence of viral infections at different visits is depicted in Figure 2. The incidence of individual viruses at each visit is shown in Figure 3 and Supplementary Table 1. **Baseline visit:** From the 445 patients included in the analysis, 441 had a PCR at baseline visit, revealing an incidence of 5.0% (n=22) of at least one respiratory virus at the time of inclusion. The viruses detected at baseline were: rhinovirus (50.0%), coronavirus (18.2%), influenza (9.1%), parainfluenza (4.5%), metapneumovirus (4.5%), RSV (4.5%), adenovirus (4.5%) and 1 patient (4.5%) had both coronavirus and influenza.

STABLE VISITS: From all stable visits (n=2043), PCR was performed at 1909 visits and revealed that the incidence of at least one respiratory virus was 5.3% (n=102). Rhinovirus accounted for 54.2% of all viral infections, coronavirus for 20.5%, followed by adenovirus (8.4%), parainfluenza (6.5%), influenza (5.5%), RSV (2.8%), bocavirus (0.9%) and metapneumovirus (0.9%) (Supplementary Table 2). None of the above viruses could be identified in two consecutive stable visits (Supplementary Figure 1A), indicating an infection rather than colonization. However, in one case infection with rhinovirus was followed by corona virus in 2 consecutive stable visits.

URTI VISITS: From 403 URTI visits, PCR was performed in 391 visits and revealed that in 52.4% of the visits there was evidence of at least one respiratory virus. The incidence of viral infections at URTI visits was significantly higher than at stable visits ($p<0.001$). Rhinovirus and coronavirus were the most frequently detected viruses at URTI visits accounting for 47.7% and 24.3% of all viral infections, respectively. Influenza accounted for 9.3%, parainfluenza for 8.7% and RSV for 7.8% of all viral infections (Supplementary Table 2). In one case, parainfluenza virus and in 2 cases rhinovirus were identified both at stable state visits and at consecutive URTI visits (Supplementary Figure IB). In 5 cases, patients with a viral infection at a stable visit also had a viral infection at a consecutive URTI visit, however, not with the same virus. In one case, RSV was detected at URTI visit and influenza in a subsequent stable visit (Supplementary Figure 1C).

URTI-FOLLOW-UP VISITS: From all URTI-follow-up visits (n=400), that took place 10 days after the onset of URTI symptoms, PCR was performed in 356 visits and revealed that in 15.2% of the visits, there was evidence of at least one respiratory virus. The incidence of viral infections at URTI-follow-up visits was higher than stable visits but lower than URTI visits

($p<0.001$ for all). Rhinovirus accounted for 67.9% of all viruses detected at URTI-follow-up, followed by coronavirus (14.3%), influenza (7.2%), RSV (5.4%), parainfluenza (3.6%) and metapneumovirus (1.8%) (Supplementary Table 2).

In 6 cases, patients with a viral infection at URTI visit had the same viral infection at a consecutive URTI-follow up visit (two cases with corona virus and rhinovirus, one case with influenza virus, one case with parainfluenza virus, one case with RSV and one case with rhinovirus). In one case, the patient was infected with metapneumovirus at URTI visit and with rhinovirus at a consecutive URTI-follow up visit (Supplementary Figure ID).

AECOPD VISITS: From 187 AECOPD visits, PCR was performed in 177 visits and revealed that in 38.4% of the visits there was evidence of at least one respiratory virus. The prevalence of viral infections at AECOPD visits was higher than stable visits ($p<0.001$) and lower than URTI visits

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(p=0.002). Rhinovirus and coronavirus were the most frequently detected viruses at AECOPD visits accounting for 35.7% and 25.9% of all viral infections, respectively. Influenza accounted for 21.4%, parainfluenza for 8.6%, RSV for 8.6% and metapneumovirus for 8.6% of all viral infections (Supplementary Table 2). Even though rhinovirus was the most common virus detected at all study visits, only 11.1% of all rhinoviruses' detections were associated with exacerbation.

In 6 cases, the same virus was detected in URTI and in a subsequent AECOPD visit; 2 cases with rhinovirus, one case with influenza virus, one case with parainfluenza virus, one case with RSV and one case with corona virus (Supplementary Figure IE).

60 AECOPD occurred within 21 days following a URTI (Figure 4A). PCR was performed in 56 of these events and revealed that in 32 of AECOPD there was no virus detected, in 6 there was a different virus compared with the preceding URTI and in 18 AECOPD the same virus was detected as in the preceding URTI. Figure 4B depicts the sequence of viral infections within 21 days following a URTI. Most of URTI-related AECOPD occurred within the first 10 days following a URTI.

Patients presenting a positive viral PCR at URTI onset did not have a higher incidence of exacerbation as compared to those showing negative viral PCR results (p=0.993). Likewise, the incidence of any viral infection at AECOPD was similar between AECOPD followed URTI and AECOPD independent of URTI (p=0.206) (Table 2). However, detection of parainfluenza 3 at URTI onset was associated with higher risk of AECOPD (p=0.003) (Table 3). There were 9 positive tests for parainfluenza 3 at URTI visits, accounting for an incidence of 2.53% of parainfluenza 3 in URTI visits and for 4.13 % of all detected viruses in URTI visits. From these 9 URTI visits that were positive for parainfluenza 3, 4 (44.4%) turned into AECOPD events within 21 days from the URTI.

Out of 187 AECOPD, in 124 cases patients provided sputum and 108 sputum samples were of good quality and assayed for bacteria growth. Out of 108 good quality sputum samples, 61 cases (56.5%) were positive for bacteria growth (few, moderate or lots of growth) and 47 cases (43.5%) considered negative. The bacteria that were identified were: Streptococcus pneumonia in 2 cases (1.85%), Streptococcus pneumonia + Acinetobacter baumanii in 1 case (0.92%), Streptococcus pneumonia + Enterobacteriacea spp. in 1 case (0.92%), Haemophilus influenzae in 3 cases (2.77%), Haemophilus influenza + Moxarella catarrhalis in 2 cases (1.85%), Moraxella catarrhalis in 8 cases (7.41%), Moraxella catarrhalis + other in 3 cases (2.77%), Moraxella catarrhalis + Enterobacteriace + other in 1 case (0.92%), Staphylococcus aureus + E. coli in 1 case (0.92%), Staphylococcus aureus in 1 case (0.92%), Klebsiella pneumoniae in 2 cases (1.85%), E. coli in 1 case (0.92%), Enterobacteriacea spp. in 15 cases (13.89%), P. aeruginosa in 4 cases (3.7%), Moraxella catarrhalis + Enterobacteriacea spp. in 1 case (0.92%), Enterobacteriacea spp. + other in 3 cases (2.77%), Pseudomonas aeruginosa + other in 1 case (0.92%), Pseudomonas aeruginosa + Achromobacter xylosoxidans in 1 case (0.92%), Pseudomonas aeruginosa + Acinetobacter junii + Enterobacteriacea spp. in 1 case (0.92%), Acinetobacter baumannii in 3 cases (2.77%), Stenotrophomonas maltophilia in 1 case (0.92%) and other in 5 cases (4.63).

Out of 187 AECOPD, 102 cases (54.4%) were tested for both viruses and bacteria. Viral and bacterial infections were identified in 14 cases (13.7%), only viral infections in 33 cases

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(32.3%), only bacterial infections in 22 cases (21.6%) and no infection in 33 cases (32.3%). Out of 14 cases that were identified positive for both viruses and bacteria, coronavirus infections were associated with Moraxella catarrhalis in 3 cases (21.6%); Influenza infections were associated with Haemophilus influenza (1 case, 7.1%) and with Moraxella catarrhalis (1 case, 7.1%); Rhino/-Enterovirus infections were associated with Haemophilus influenza + Moraxella catarrhalis (1 case, 7.1%), Klebsiella pmeumoniae (1 case, 7.1%), Streptococcus pmeumoniae (1 case, 7.1%), and P. aeruginosa (1 case, 7.1%); parainfluenza infection was associated with P. aeruginosa infection (1 case, 7.1%).

FACTORS ASSOCIATED WITH VIRAL INFECTIONS AT DIFFERENT VISITS

We further investigated how patients' characteristics as well as lung function parameters assessed by lung function tests, symptoms and QoL questionnaires (CAT, WURSS, SGRQ) were associated with viral infections at different visits.

STABLE VISITS: At baseline, patients with viral infections were younger and had a higher BMI than patients without viral infections (mean age: 63 vs 67 years, p=0.048; mean BMI: 30.2 vs 27.0, p=0.044) (Table 4, Figure 5). Insulin therapy was associated with a significantly higher incidence of viral infections. Pre- and post-bronchodilator body-plethysmography data showed that patients with viral infections had significantly higher values of FEV1, FEV1% pred, FEV1% pred/VC max%, DLCO SB %, and lower RV%/TLC % and BODE (Table 4, Figure 5). Interestingly, patients with viral infections exhibited a higher post bronchodilator reversibility. Analyzing data from all stable visits with PCR analysis (n=1909), we could demonstrate that cough was more commonly reported in patients with viral infections (p=0.018) and this was also confirmed from the specific question in the CAT score (p=0.002) (Supplementary Table 3, Figure 5). Patients with viral infections produced more sputum volume (p=0.041). In addition, the evidence of potentially pathogenic bacteria in sputum, was higher in patients with viral infections (p=0.026), particularly for P. Aeruginosa (p=0.013).

In a mixed linear logistic regression model, adjusted for age and sex, an increase of 10 units in SGRQ impact score at stable state was associated with an increased chance of 11.6% of having any viral infection (OR: 1.12, 95% CI 1.01-1.23, p=0.032).

URTI VISITS: During the period of the study, there was a direct temporal association between the incidence of URTIs and AECOPD (both following a URTI and independent of the URTI) in winter months (Figure 6). The median annual rate (min to max) per number of patients was for URTIs: 0.29 (0.13 to 0.43) and for AECOPD: 0.12 (0.09 to 0.18).

In a mixed linear logistic regression model, adjusted for age and sex, increase of 10 units in WURSS-21 score at URTI was associated with an increased chance of 15.0% of having any viral infection (OR: 1.15, 95% CI: 1.04-1.27, p=0.003), of 24.6% of having infection with influenza (OR: 1.246, 95% CI: 1.004-1.546, p=0.041) and of 35.0% of having infection with parainfluenza (OR: 1.35, 95% CI: 1.07-1.71, p=0.012) (Supplementary Table 4, results shown are for 1 unit increase).

At URTI-follow-up, viral infections were significantly associated with the age of patients after adjustment for sex and SGRQ symptoms score, SGRQ activity score, SGRQ Impacts score, SGRQ total score, CAT, MMRC Dyspnea scale, WURSS-21 score, FEV1% pred and NO (p<0.005 for all).

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After adjustment for age and sex, an increase of 10 units in WURSS-21 score at URTI-follow-up, was associated with an increased chance of 23.4% of having any viral infection (OR: 1.23, 95% CI: 1.10-1.39, ρ=0.001) and of 22.1% of having rhino/enterovirus (OR: 1.22, 95% CI: 1.06-1.40, p=0.004) (Supplementary Table 5, results shown are for 1 unit increase).

We further analyzed changes in symptoms between the last stable visit before URTI and URTI follow-up, stratified by virus infection at URTI follow-up. Patients with positive viral PCR at URTI follow-up have worse symptoms than patients with negative viral PCR, as assessed by higher values in MMRC (p=0.022), SGRQ activity score (p=0.028) and SGRQ total score (p=0.044) (Supplementary Table 6).

AECOPD VISITS: Cough and sputum production were more common symptoms in patients having a viral infection at AECOPD (p<0.001 and p=0.002, respectively) (Supplementary Table 7, Figure 5). Edema of the extremities was more frequently present in patients without viral infections (p=0.044). Furthermore, patients with viral infection at AECOPD had a higher CAT score (p=0.007) and lower BODE score (p=0.008), as compared with patients without viral infection (Supplementary Table 7, Figure 5).

In a mixed logistic regression model, adjusted for age and sex, an increase of 1 unit in CAT score was associated with an increased chance of 8.4% of having any viral infection at AECOPD visit (OR: 1.084, 95% CI: 1.03-1.14, p=0.005). In an explorative analysis using mixed logistic regression for various associations of viral infections adjusted for the study medication, the risk of having any viral infection at AECOPD was similar between patients that received ICS/LABA and patients that did not receive the study medication (Supplementary Table 8).

Evolution of AECOPD was evaluated by CAT, MMRC and WURSS-21 questionnaires at AECOPD and at follow-up, 21 days after AECOPD. In a linear mixed regression model, adjusted for age and gender (fix effects) and for patient (random effect), there was a decrease in CAT, MMRC and WURSS-21 scores between AECOPD and AECOPD follow up. The decrease in CAT score between AECOPD and AECOPD follow-up was significantly higher in cases of AECOPD with viral infection compared with AECOPD cases without viral infection (-7.12 vs -4.28, p=0.013).

We further explored clinically relevant changes in patients' symptoms, as assessed by changes in total SGRQ score, between the last stable visit and the next follow-up visit that took place 10 days after URTI (Table 5). Change in SGRQ score was significantly higher when the URTI was followed by AECOPD (p<0.001). When the URTI was not followed by AECOPD within 21 days, 46.4% of the patients reported worsening of their symptoms, 14.6% of the patients reported improvement of their symptoms and 29.2% of the patients reported no relevant changes in their symptoms 10 days after the onset of URTI symptoms. When the URTI was followed by AECOPD within 21 days, 60.0% of the patients reported worsening of their symptoms, 5.0% of the patients reported improvement of their symptoms and 18.3% of the patients reported no relevant changes in their symptoms.

All patients who suffered a URTI, showed a significant deterioration of lung function parameters irrespectively if URTI was followed by AECOPD or not (Figure 7).

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DISCUSSION

The present study analyzed the pre-defined co-primary aim of the PREVENT study, ~~that was published in AJRCCM~~ [13], regarding a longitudinal profiling of viral infections in COPD patients and their implication to URTI, COPD exacerbations, symptoms and physiological changes. We report that there is no viral colonization in COPD patients at stable state and that none of the viruses detected at URTI, except parainfluenza 3, was *per-se* associated with an increased risk for exacerbation. Patients presenting a positive viral PCR at URTI onset did not have a higher incidence of exacerbation, compared with patients showing a negative viral PCR.

Invasion of respiratory viruses into the peripheral airways of COPD patients has been well documented during exacerbations as well as in stable conditions [16, 17]. It has been suggested that virus colonization may play a role in maintaining airway inflammation associated with stable COPD [17, 18]. In our study, the incidence of at least one respiratory virus at stable state was low (5.3%), with rhinovirus and corona virus being the most common viruses accounting for 54.2% and 20.5% of all viruses, respectively. The same viruses were also found in a previous study including 68 COPD patients with stable disease, however with a higher prevalence (16.2%) [10]. Interestingly, none of the viruses that we identified in stable state could be detected in two consecutive visits, indicating that there was no viral colonization in COPD patients at stable state.

Recent data indicate that respiratory viral infections influence the bacterial microbiome in COPD patients and vice versa. Infection of COPD subjects and healthy controls with rhinovirus resulted in rise in the bacterial load in sputum and a significant prevalence of H. influenzae [19]. The authors suggested that infection with rhinovirus alters the respiratory microbiome and may induce secondary bacterial infections. Similar findings were also reported by Mallia et al, who observed that 60% of COPD patients experienced a secondary bacterial infection after being infected with rhinovirus [18]. In line with this evidence, in the present study, we have shown that patients with a positive viral PCR at stable state visits, had a higher incidence of potential pathogenic bacteria, particularly for P. Aeruginosa. Adenovirus is known to cause acute airway infections as well as latent infections in the airway epithelium. It has been shown that the expression of adenovirus proteins was increased in airway epithelial cells obtained from subjects with irreversible airflow limitation, compared to epithelial cells from subjects without airflow limitations [20]. Furthermore, studies in guinea pigs have shown that the emphysematous changes induced by cigarette smoke was exaggerated with latent adenoviral infection [21]. These studies imply that latent adenoviral infection is a possible causal factor for COPD. In our study, however, adenovirus was identified only in 0.47% of all stable visits and was never detected in two subsequent visits, indicating that there was no colonization. Similarly, from 102 cases with a viral infection at stable state, only 2 cases with rhinovirus and 1 case with parainfluenza developed a consequent URTI with the same virus.

As expected, the prevalence (52.4%) of viral infections at URTI visits was significantly higher compared with stable state visits. Rhinovirus, the major cause of the common cold in the community, was the predominant virus detected at URTI, however only in one case rhinovirus could be detected in a follow-up visit, 10 days after the URTI, confirming the absence of colonization with rhinovirus in COPD patients. Similar detection rates have also been reported in previous epidemiological studies showing that 20-40% of patients with classic URTI symptoms

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fail to yield an etiological agent even if they were subjected to the most up-to-date viral culture and PCR [22]. Therefore, we may postulate that URTI symptoms may be triggered by other factors, such as allergic rhinitis. It has been suggested in the literature that allergens stimulate Th2 pathways resulting in eosinophilia and that such an allergic inflammation influences, in a synergistic or additive manner, the infection with respiratory viruses [23]. Thus, it is tempting to hypothesize that COPD patients with eosinophilia are more susceptible to viral infections and this may explain why these patients experience more exacerbations.

The importance of colds in COPD exacerbation risk has been previously emphasized [6], however a longitudinal approach was lacking. In the present study, we provide evidence that only 14% of URTIs were followed by AECOPD within 21 days. A viral infection was detected in 43% of URTI-related AECOPD and only in 32.1% of the cases the same virus was detected in AECOPD and URTI. Even if the incidence of viral infections at AECOPD was 38.4%, in agreement with previous studies [6, 24], there was no difference in the incidence of any viral infection at AECOPD that followed a URTI and AECOPD independent of URTI. It is also striking that none of the viruses detected at URTI, except parainfluenza 3, was *per-se* associated with an increased risk for exacerbation. Therefore, the notion that viral AECOPD are usually linked to colds could not be proven in our study. However, viral infections at URTI may cause acute cell damage, release of ROS and activation of NF-kB which may lead to non-viral AECOPD [25, 26].

Rhinovirus was the most prevalent virus at AECOPD accounting for 35.7% of all viral infections and this agrees with a systematic review of 19 original articles reporting on 1728 patients having AECOPD [27]. Even though rhinovirus was the most common virus detected at all study visits, only 11.1% of all rhinoviruses' detections were associated with exacerbation. However, rhinovirus was the most frequently detected agent and is typically circulating during the summer season, when exacerbations were less frequent. Conversely, viruses like influenza, metapneumovirus, para-influenza- and RSV circulate more frequently during the winter season and have a propensity for infecting the lower respiratory tract [22]. Considering that in 34.9% of the events no virus was identified either at URTI or at AECOPD, we could postulate that another viral agent, which is not among the 18 routinely tested in our PCR panel, could be responsible for the infection both at URTI and exacerbation. Of note, patients with stable COPD rarely had a positive virus PCR and that the incidence of virus detection declined abruptly already within 10 days of URTI onset, supporting the notion that any results provided from samples collected with a delay of even a few days may not be representative.

Viral infections have a great impact on the quality of life of COPD patients and therefore it has been suggested that quality of life questionnaires may be used to predict viral infections in COPD patients [28, 29]. To our knowledge, the present study is the first study to demonstrate that an increase of 10 units in SGRQ impact score in COPD patients at stable state and of WURSS-21 score at URTI is significantly associated with an increased chance of having a viral infection. Furthermore, 1 unit increase in the CAT score was significantly associated with an increased chance of having a viral infection at AECOPD. CAT score has been previously shown to correlate with the length of hospitalization in COPD patients at exacerbation, especially for patients with respiratory coinfection [24].

Clinically relevant changes in patients' symptoms between the last stable visit and the next URTI follow-up visit, were evaluated by SGRQ change by 4 points, as previously suggested [30].

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Following a URTI, about half of the patients reported a clinically relevant worsening of their symptoms, even if they did not have a subsequent AECOPD according to the current guideline definition (Table 5). These findings raise the consideration that change in medication in COPD patients with URTI may improve outcomes.

Potential limitations of our study include the low number of exacerbations most likely due to the fact that the diagnosis of an AECOPD was restricted to the events fulfilling the criteria suggested by the official European Respiratory Society/American Thoracic Society recommendations [31]. Another limitation is that we did not collect data on the vaccination status of our patients, however, it has been shown that there is no relationship between the percentage of vaccinated patients and the prevalence of influenza infections [2]. An additional limitation is that events of vasomotor rhinitis or allergic rhinitis, that are also presented with acute symptoms similar to URTI events, may have also been included as URTI events. This may also explain the low prevalence of viral infections at URTI reported in our study.

Strengths of our study include the longitudinal design of the study including patients from URTI to AECOPD and the evaluation of viral detection at stable state, at URTI, 10 days after the URTI and at exacerbations by multiplex PCR that detects 18 viruses, adding up to over than 50,000 individual viral PCRs. Another advantage of our study was the fact that patients were instructed and equipped to collect nasal and oral-pharyngeal swabs at the onset of URTI symptoms allowing for immediate sampling and thus potentially increasing PCR sensitivity. As self-collected swabs are as sensitive as clinician-collected nasal washes for the detection of respiratory viruses by PCR [32] and since the detection of viral pathogens in oropharyngeal swabs is equally or more sensitive than sputum samples for most viruses [32] the results presented in this study are representative for viral infections in COPD patients.

Furthermore, the interventional part of the PREVENT study is not expected to influence the results and conclusions of the present study as an explorative analysis using mixed logistic regression for various associations of viral infections adjusted for the study medication showed that the risk of having any viral infection at AECOPD was similar between patients that received ICS/LABA and patients that did not receive the study medication. In conclusion, our study provides solid evidence that there is a higher incidence of viral infections at URTI and at AECOPD compared with the stable period of COPD. Rhinovirus was the most frequently detected virus; however, we could not confirm viral colonization in any of the visits. The risk of exacerbation following the onset of URTI symptoms depends on the particular virus associated with the URTI since detection of parainfluenza 3 at URTI onset was associated with higher risk of AECOPD. URTI is associated with clinically relevant worsening of quality of life and lung function independently of guideline-defined exacerbation. Only in one-third of the cases, the same virus was detected in URTI and AECOPD and therefore, in the occasion of a viral detection at URTI, physicians should not assume that the same virus is likely to appear in a subsequent AECOPD. This should be considered for the effective treatment of patients as well as for the design of future studies that aim to investigate the effectiveness of anti-viral treatment in COPD patients with URTIs and AECOPD. These findings have potential implications and applicability for general practitioners, internists and respiratory physicians when treating patients with COPD.

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The corresponding author, had full access to all the data in the study and had final responsibility for the decision to submit for publication.

CONTRIBUTORS' STATEMENT

DS, HH, MT conceived and designed the study. DS, DS, RL, JR, LB, EP, MT, WS collected patient's data. HH organized and supervised the multiplex molecular diagnostic testing of respiratory viral pathogens. CS, LG conducted the statistical analyses. All authors contributed to and approved the final manuscript taking complete responsibility for the integrity of the work from initiation until the publication.

DECLARATION OF INTEREST

The authors have no conflict of interest to declare.

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Figure 1. Consolidated Standards of Reporting Trials flow diagram. AECOPD: Acute exacerbation of chronic obstructive pulmonary disease; URTI: upper respiratory tract infection; PCR: polymerase chain reaction; URTI-related AECOPD: AECOPD that occurred within 21 days after a URTI.

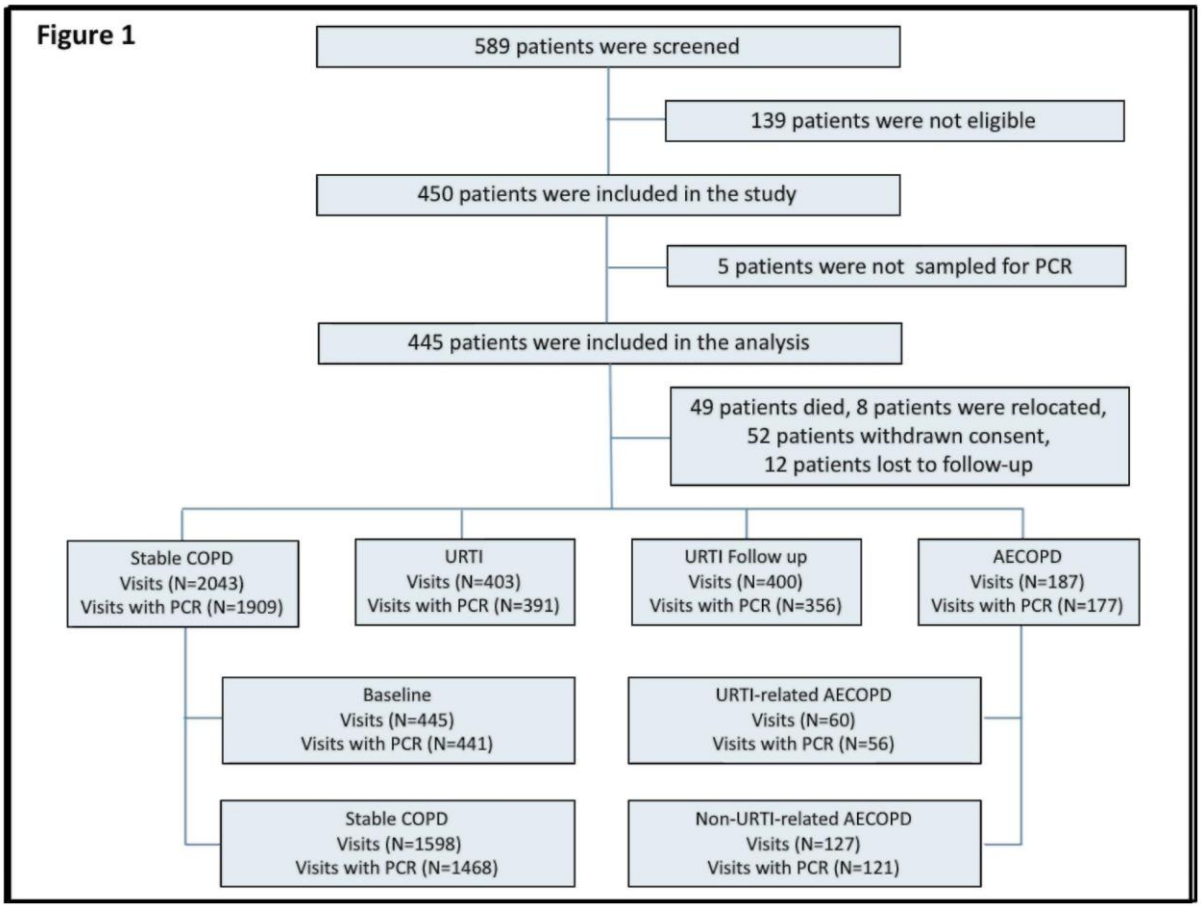

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Figure 2. Percentages of patients with and without viral infections as assessed by PCR at different visits. URTI: upper respiratory tract infections; URTI follow-up: 10 days after URTI; AECOPD: exacerbations of COPD.

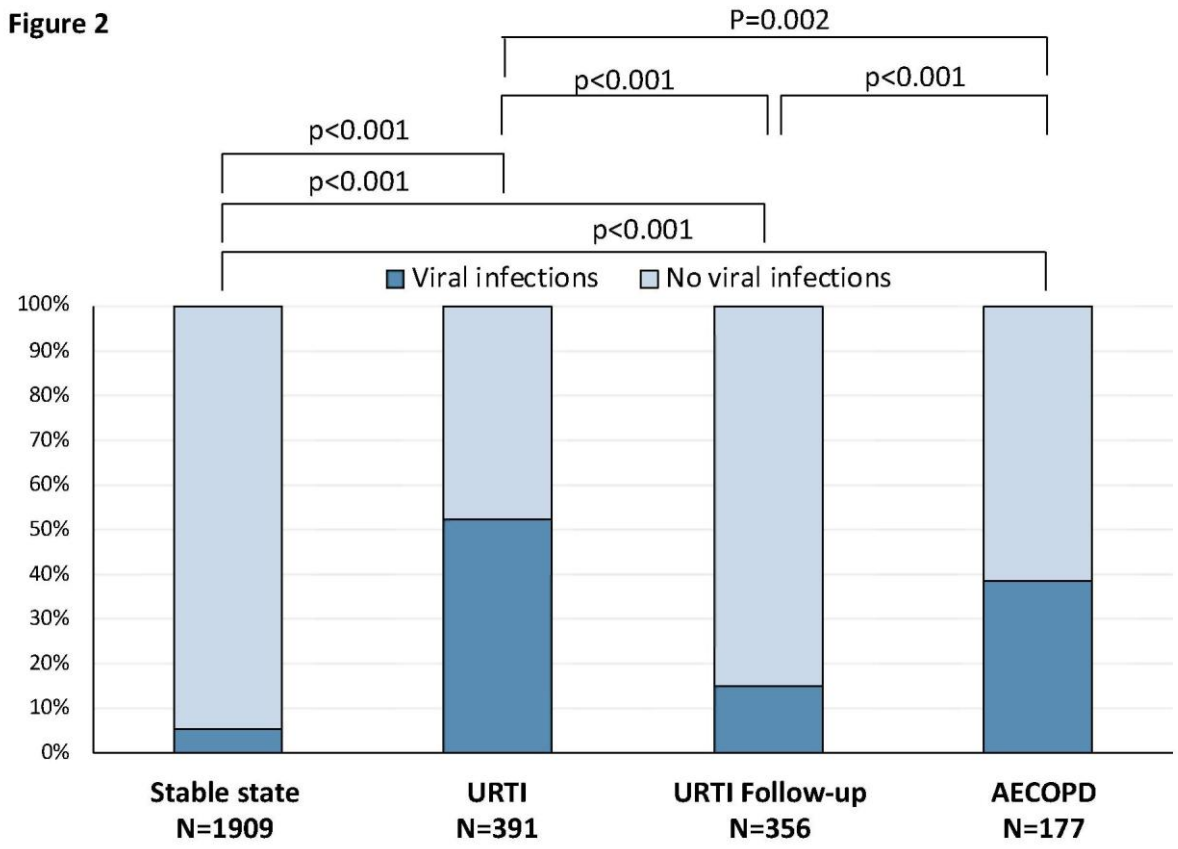

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Figure 3. Numbers and percentages of the individual respiratory viral infections at each visit throughout the study duration. The bar charts depict the number of virus aggregated as follows: influenza (influenza A H1N1, influenza B, influenza HI and influenza H3); parainfluenza (para-influenza 1, para-influenza 2, para-influenza 3, para-influenza 4); coronavirus (coronavirus 229E, coronavirus HKUl, coronavirus NL63, coronavirus OC43); and RSV (RSV A and RSV B). URTI: upper respiratory tract infections.

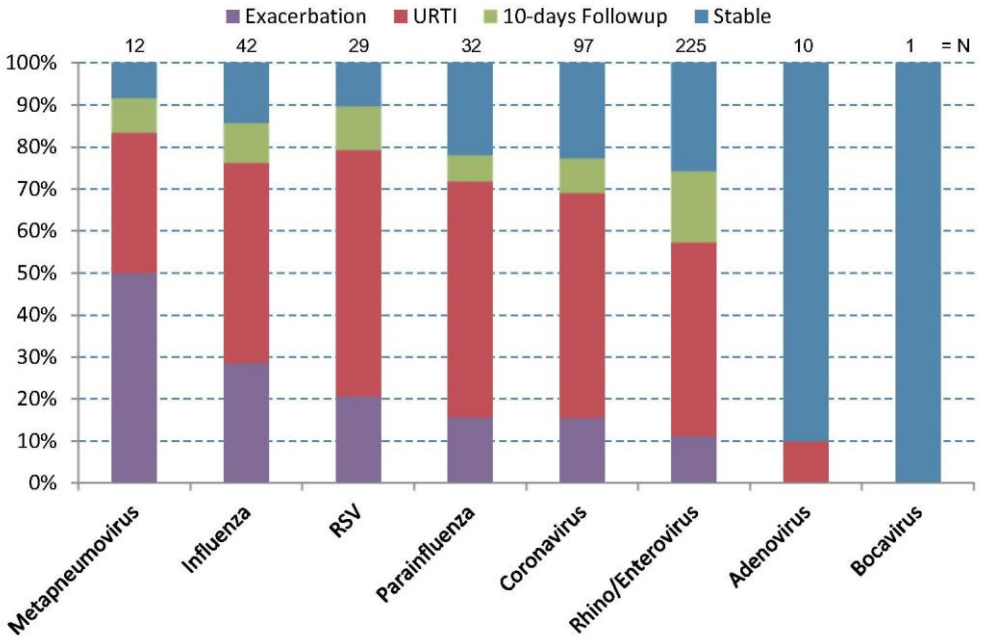

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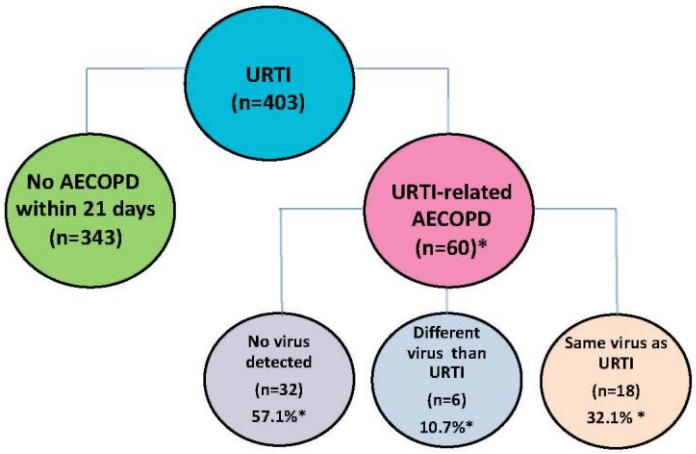
Figure 4. (A) Scheme depicting the number of URTI-related AECOPD (AECOPD occurring within 21 days following a URTI). (B) Viral infections in URTI-related AECOPD. RhV: Rhinovirus; CoV: corona virus; PIV: parainfluenza virus; RSV: respiratory syncytial virus; MPV: metapneumovirus; IV: influenza virus; URTI: upper respiratory tract infection; FU: follow-up 10 days after a URTI.

Figure 4A



*PCR was performed in 56 visits

Figure 4B

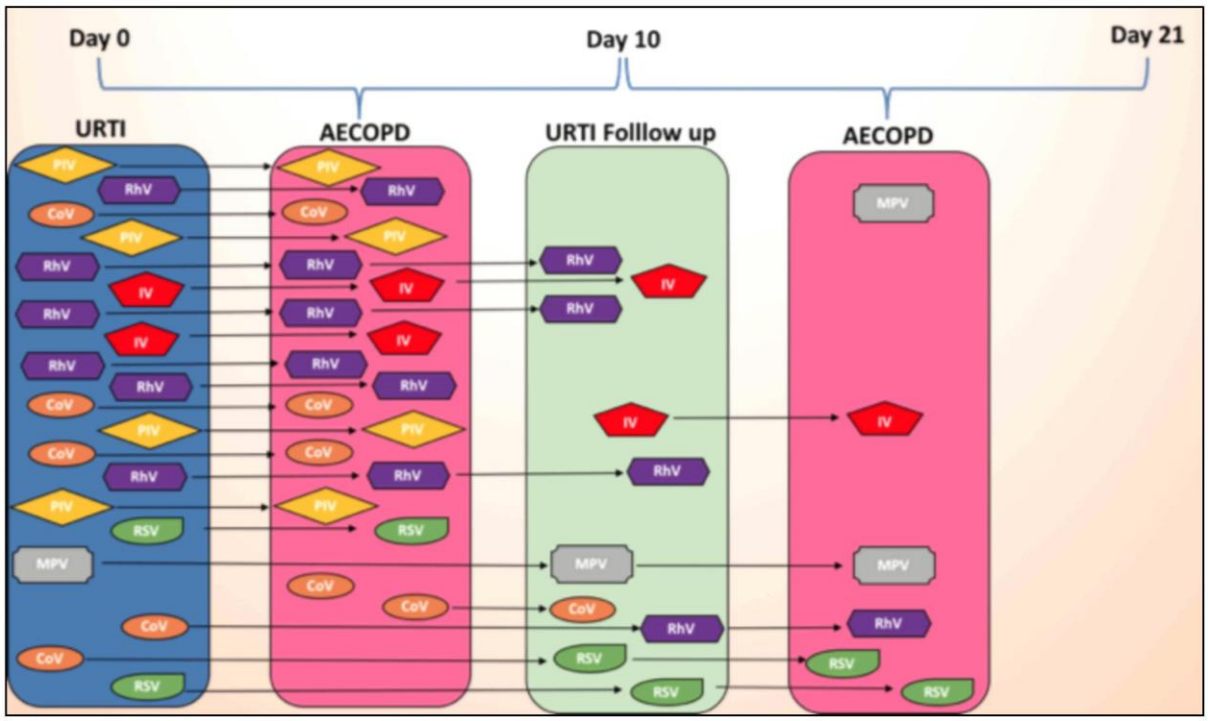


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Figure 5. Factors significantly associated with viral infections at different visits. FEVi: forced expiratory volume in 1 second; FVC: forced vital capacity; TLC: total lung capacity; RV: residual volume. CAT: COPD Assessment Test; BODE: Body mass index-airflow Obstruction-Dyspnoea, and Exercise.

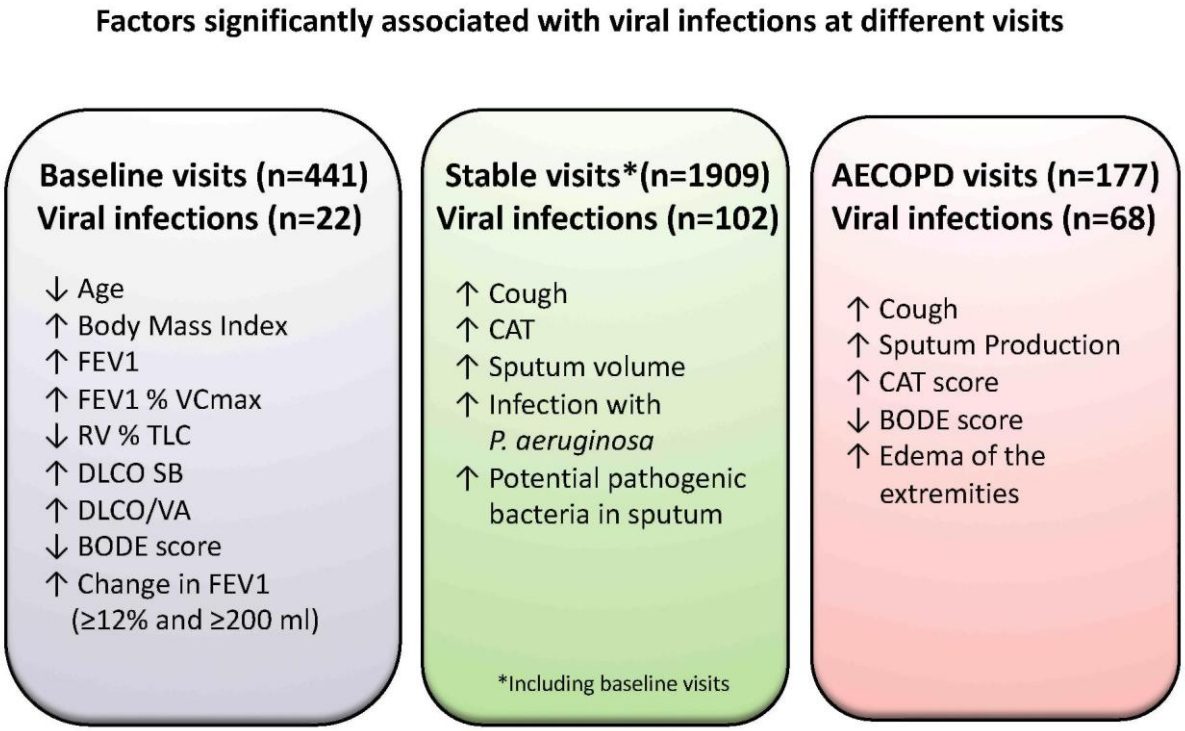

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Figure 6. URTIs and exacerbations over the time of the study. Incidence of URTI and COPD exacerbations (following an URTI or independent of an URTI) in the overall population throughout the study time according to the yearly season (summer and winter).

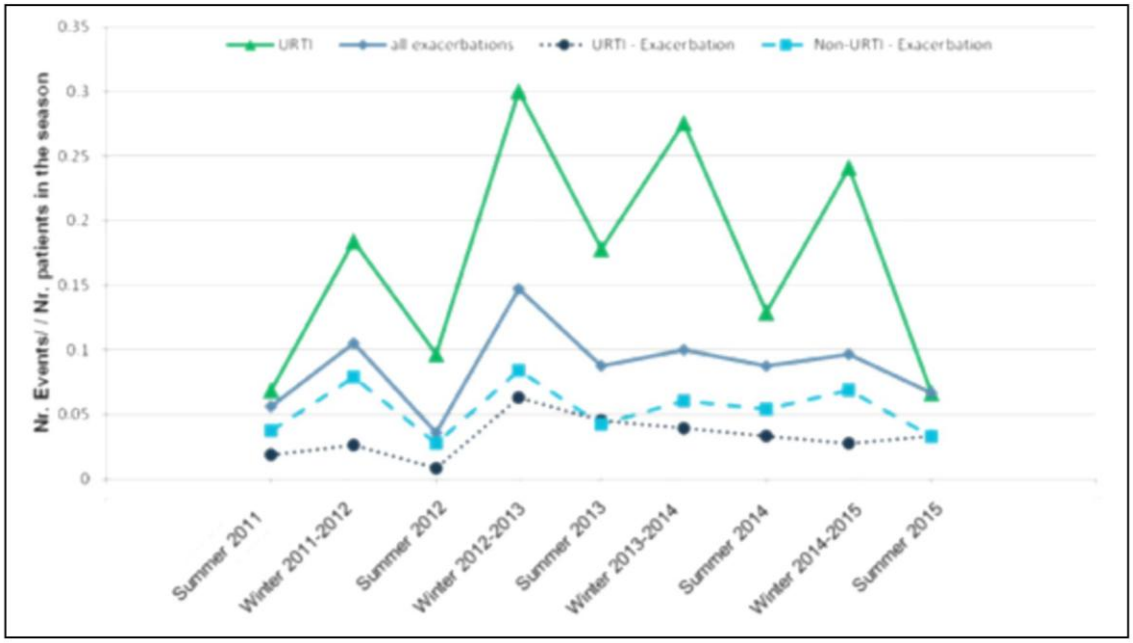

Figure 7. Scheme depicting the impact of URTI on quality of life and lung function parameters. FEVi: forced expiratory volume in 1 second; FVC: forced vital capacity; URTI-related AECOPD: AECOPD occurring within 21 days after a URTI. Lung function measurements at URTI-follow-up were performed in 323 cases (80.1%) with a median elapsed time from URTI to URTI-follow-up of 11 days (inter quartile range: 10-12).

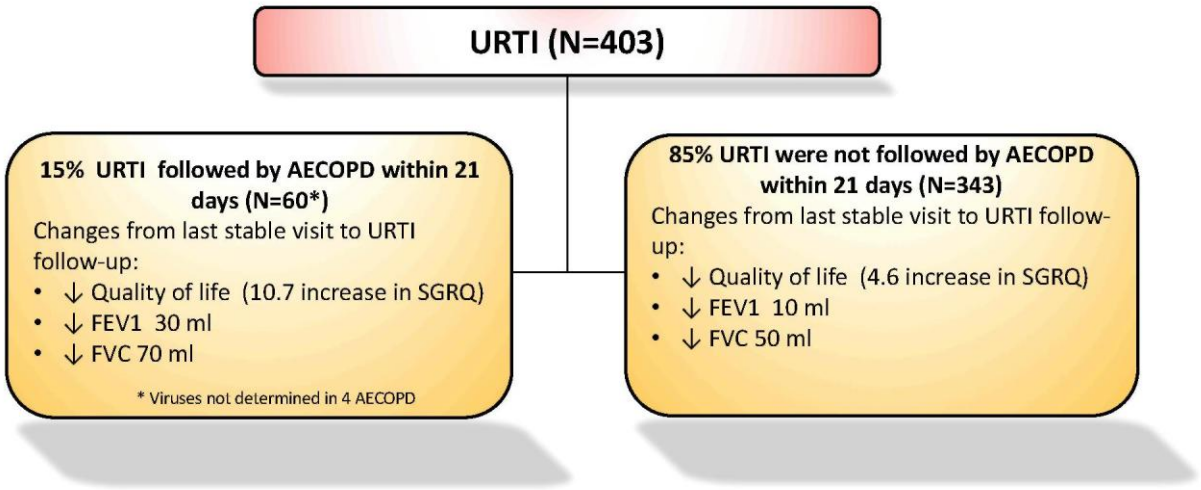

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Table 1. Baseline Characteristics of the Patients Included in the Study*

Characteristics	All Patients (N=445)
Age -years	66.94 ± 9.41
Male sex-no. (%)	300 (67.41)
BMI-kg/m2	27.22 ±6.401
Current smoker - no. (%)	156 (35.05)
Pack-years- mean	52.31 ±27.82
Duration of COPD - yearsr	7.77 ±1.55
Mean number of exacerbations in the previous year - mean	1.13 ±0.84
Any LABA - no. (%)	351 (78.87)
Any LAMA - no. (%)	292 (65.61)
Any inhaled glucocorticoids - no. (%)	321 (72.13)
MMRC Dyspnoea scale †	1.50 ±0.99
Total score on the SGRQ‡	37.48 ± 17.89
Score on the CAT§	14.88 ± 6.86
Post-bronchodilator FEV_1 - % of predicted value	54.66 ±16.87
Post-bronchodilator ratio of FEV_1 to FVC	45.47 ± 13.40
Post-bronchodilator TLC - % of predicted value	112.78 ±20.52
Post-bronchodilator RV - % of predicted value	151.37 ±48.94
Reversibility- no. (%)¶	28 (6.70)
Fractional exhaled nitric oxide - ppm	20.56 ±13.40
Atopy-no. (%)	87 (23.45)
Total score on the BODE ||	2.51 ±2.03
Potential pathogenic bacteria in the sputum - no. (%)	75 (16.85)

* Values are presented as means ± SD. COPD denotes chronic obstructive pulmonary disease. LABA: long-acting β_2-agonist; LAMA: long acting muscarinic antagonists; FEV_1: forced expiratory volume in 1 second; FVC: forced vital capacity; TLC: total lung capacity; RV: residual volume.

† Score on the Modified Medical Research Council Dyspnoea scale (MMRC) ranges from 0 to 4, with a score of 4 indicating that the patient is too breathless to leave the house or becomes breathless when dressing or undressing.

‡ Scores on the St. George's Respiratory Questionnaire for COPD (SGRQ-C) range from 0 to 100, with higher scores indicating worse health status; the minimum clinically important difference is 4 points.

§ COPD Assessment Test (CAT) ranges from 0 to 40, with higher scores denoting a more severe impact of COPD on a patient's life. The difference between stable and exacerbation patients was five units. No target score represents the best achievable outcome.

¶ Reversibility was defined as a change in FEV_1 ≥12% and >200 ml when comparing pre and post-bronchodilator values.

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Table 2. Viral infections at AECOPD stratified by the presence of URTI in the previous 21 days before AECOPD

	AECOPD following URTI (n=56) N(%)	**AECOPD independent of URTI (n=121) N**(%)	**p- value**
Any viral infection	24 (44.6)	44 (36.4)	0.206
Adenovirus	0 (0.0)	0(0.0)	-
Bocavirus	0 (0.0)	0(0.0)	-
Coronavirus	5 (8.9)	10(8.3)	0.811
Influenza	3 (5.4)	9 (7.4)	0.631
Metapneumovirus	2(3.6)	4(3.3)	0.928
Parainfluenza	4(7.1)	1(0.8)	0.054
RSV	3 (5.4)	3(2.5)	0.341
Rhino/enterovirus	7(12.5)	18 (14.9)	0.851

*Using mixed logistic regression models to consider the effect of a patient having more than one visit

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Table 3. Risk of an exacerbation within 21 days of an URTI with the presence of a certain detected virus at the time of URTI (total observations: 391)

Risk factor*	Value	Estimate	StdErr	StdErr Ratio	**Hazard ratio**	**95% CI for HR**	**p-value**
Coronavirus 229E	No viral infection	0.135	0.290	1.084	1.145	0.65-2.02	0.641
(Reference is other viruses detected)	Coronavirus 229E	-0.529	1.033	1.015	**0.589**	**0.08 - 4.46**	**0.608**
Coronavirus HKUl	No viral infection	0.169	0.290	1.074	1.184	0.67-2.09	0.561
(Reference is other viruses detected)	Coronavirus HKUl	0.165	0.787	1.068	**1.179**	**0.25-5.51**	**0.834**
Coronavirus NL63	No viral infection	0.217	0.308	1.133	1.243	0.68-2.28	0.481
(Reference is other viruses detected)	Coronavirus_NL63	0.892	0.666	1.063	**2.440**	**0.66** - 9	**0.180**
Coronavirus OC43	No viral infection	0.182	0.296	1.085	1.199	0.67-2.14	0.539
(Reference is other viruses detected)	Coronavirus OC43	0.257	0.640	1.046	**1.293**	**0.37-4.53**	**0.688**
Influenza B	No viral infection	0.202	0.286	1.058	1.224	0.7 -2.14	0.480
(Reference is other viruses detected)	Influenza B	1.006	0.839	1.128	**2.734**	**0.53-14.1**	**0.231**
Metapneumovirus	No viral infection	0.181	0.290	1.083	1.198	0.68-2.12	0.533
(Reference is other viruses detected)	Metapneumovirus	1.039	0.889	0.865	**2.827**	**0.49-16.1**	**0.242**
Parainfluenza 1	No viral infection	0.207	0.291	1.076	1.231	0.70-2.18	0.475
(Reference is other viruses detected)	Parainfluenza 1	1.231	0.867	1.179	**3.426**	**0.63 - 18.7**	**0.155**
Parainfluenza 3	No viral infection	0.278	0.289	1.050	1.320	0.75-2.33	0.336
(Reference is other viruses detected)	Parainfluenza 3	1.831	0.620	1.147	**6.238**	**1.85 - 21.0**	**0.003**
RSV A	No viral infection	0.164	0.289	1.081	1.178	0.67-2.08	0.571
(Reference is other viruses detected)	RSV A	0.172	1.020	0.994	**1.187**	**0.16-8.76**	**0.866**
RSV B	No viral infection	0.190	0.282	1.046	1.210	0.7 **-2.1**	0.500
(Reference is other viruses detected)	RSV B	0.625	0.794	1.081	**1.869**	**0.39 - 8.86**	**0.431**
Rhino Enterovirus	No viral infection	-0.129	0.336	1.122	0.879	0.45 -1.7	0.701
(Reference is other viruses detected)	Rhino/Enterovirus	-0.647	0.437	1.153	**0.524**	**0.22-1.23**	**0.139**

*Regressions for which the risk factor was Adenovirus, Bocavirus, Influenza A H1N1, Influenza H1 subtype, Influenza H3 subtype, Parainfluenza 2 and Parainfluenza 3 did not converge due to few or no detections

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Table 4. Descriptive statistics for characteristics at BASELINE visits*

Characteristic/ Factor		Baseline visits (n=445)§	No Viral Infection (n=419)	Viral Infection (n=22)	p-value
Age (years)	n	445	419	22	0.048
	Mean + SD	66 + 9	67 + 9	63 + 8	
	Median (IQR)	67 (61-74)	67 (61-74)	63 (58-70)	
	Median (Range)	67 (37-89)	67 (37-88)	63 (48-81)	
Body Mass Index	n	445	419	22	0.044
	Mean + SD	27.2 + 6.3	27.0 + 6.3	30.2 + 7.1	
	Median (IQR)	26.1 (22.8-30.6)	25.9 (22.6-30.4)	28.8 (23.5-34.5)	
	Median (Range)	26.1 (14.5-62.2)	25.9 (14.4-62.2)	28.8 (18.9-42.6)	
Malignant lymphoma	no	443 (99.5)	20 (90.9)	419(100)	0.002
	yes	2 (0.4)	2(9.1)	0(0)	
Insulin	no	428 (96.2)	19 (86.36)	405 (96.6)	0.046
	yes	17 (3.8)	3 (13.6)	14(3.3)	
Post-Bronchodilator FEV1 [L]	n	424	399	22	0.002
	Mean ± SD	1.4 ±0.5	1.4 ±0.5	1.8 ±0.5	
	Median (IQR)	1.4(1.0-1.8)	1.4(1.0-1.8)	1.8(1.4-2.3)	
	Median (Range)	1.4(0.3-3.1)	1.4(0.3-3.1)	1.8(0.7-2.7)	
Post-Bronchodilator FEV1 [%]	n	424	399	22	0.002
	Mean ± SD	54.7 ± 16.9	54.0 ± 16.8	65.7 ±15.5	
	Median (IQR)	55.2 (42.6-67.7)	55.0 (42.1-66.8)	70.5 (49.9-75.0)	
	Median (Range)	55.2 (16.0-97.0)	55.0 (16.0-97.0)	70.5 (34.5-97.0)	
Post-Bronchodilator FEV1 % VC MAX [%]	n	424	399	22	0.015
	Mean ± SD	45.5 ± 13.4	45.0 ±13.2	53.2 ±14.6	
	Median (IQR)	44.7 (35.7-55.9)	44.5 (34.9-55.6)	51.7 (40.6-62.5)	
	Median (Range)	44.7 (15.4-84.0)	44.5 (15.4-78.0)	51.7 (27.6-84.0)	
Post-Bronchodilator RV % TLC [%]	n	372	351	19	0.018
	Mean ± SD	51.4 ±9.7	51.7 ±9.5	45.0 ±12.0	
	Median (IQR)	51.2 (44.8-57.3)	51.5 (45.1-58.1)	46.1 (41.3-52.0)	
	Median (Range)	51.2 (2.3-79.9)	51.5 (29.0-79.9)	46.1 (2.3-61.9)	
Post-Bronchodilator DLCO SB [%]	n	357	338	18	0.017
	Mean ± SD	55.9 ±19.4	55.2 ±19.3	66.9 ± 18.7	
	Median (IQR)	55.4 (41.0-70.6)	55.1 (40.0-69.7)	62.9 (54.7-76.4)	
	Median (Range)	55.4(11.8-107.6)	55.1(11.8-107.6)	62.9 (31.4-99.9)	
Post-Bronchodilator DLCO/VA [%]	n	354	335	18	0.043
	Mean ± SD	69.4 ± 24.8	68.7 ± 24.7	80.3 ± 22.5	
	Median (IQR)	66.7 (50.5-88.4)	66.3 (50.1-87.8)	85.9 (63.3-101.0)	
	Median (Range)	66.75 (0.72-140.4)	66.3 (0.72-140.4)	85.9 (41.2-114.1)	
***BODE* score**	n	370	347	21	0.021
	Mean ± SD	2.5 ±2.0	2.6 ±2.0	1.6 ±1.7	
	Median (IQR)	2 (1-4)	2 (1-4)	1 (0-2)	
	Median (Range)	2 (0-9)	2 (0-9)	1 (0-6)	
Reversibility (LF)	no	395 (94.5)	17(77.3)	375 (95.4)	0.005

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change in FEV1 of >=12% and >=300ml	yes	23 (5.5)	5 (22.7)	18(4.6)	
Reversibility (LF) change in FEV1 of >=12% and >=200ml	no	390 (93.3)	17(77.3)	370 (94.1)	0.011
	yes	28 (6.7)	5 (22.7)	23 (5.8)	

*Among several parameters that were tested, (sex, smoking status, shortness of breath, cough, wheezing, sputum production, inhaled medication, systemic medication, oxygen therapy, non-invasive ventilation, surgical therapy for COPD, comorbidities, MMRC Dyspnea scale, SGRQ, CAT score, microbiology in sputum, GOLD stage, GOLD group, lung function parameters including DLCO SB, NO in exhaled air, 6 min walking distance) only statistically significant parameters are presented in the Table.

§4 patients had no information on viral infection

Lung function parameters were also assessed pre-bronchodilation and values were similar with the those presented in the Table for post-bronchodilation.

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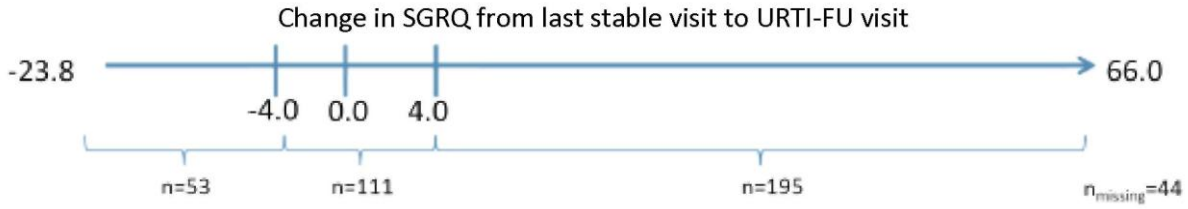
Table 5. Clinically relevant changes in symptoms between last stable visit and URTI follow-up visit, 10 days after a URTI, as assessed by SGRQ change by 4 points

Symptoms	**URTI without subsequent AECOPD within 21 days n=343**	**URTI with subsequent AECOPD within 21 days n=60**	**p-value**
Change in SGRQ, Median (min-max)	4.3 [-23.8-47.1]	11.3 [-14.0 - 66.0]	<0.001[a]
Clinically relevant worsening in symptoms (increase in SGRQ > 4 points) n (%)	159 (46.4)	36(60.0)	0.021[b]
Clinically relevant improvement in symptoms (decrease in SGRQ > 4 points)[d] n (%)	50(14.6)	3(5.0)	
No clinically relevant change in symptoms (change in SGQR between l-3)[e] n (%)	100(29.2)	11(18.3)	
Missing cases (no SGRQ) n (%)	34 (9.9)	10(16.7)	



[a]Mann-Whitney U-test
[b]Chi-square test
[c]Change in SGRQ from 4.0 to 66.0 (n=195)
[d]Change in SGRQ from -23.8 to -4.0 (n=53)
[e]Change in SGRQ from >-4.0 to < 4.0 (n=111)

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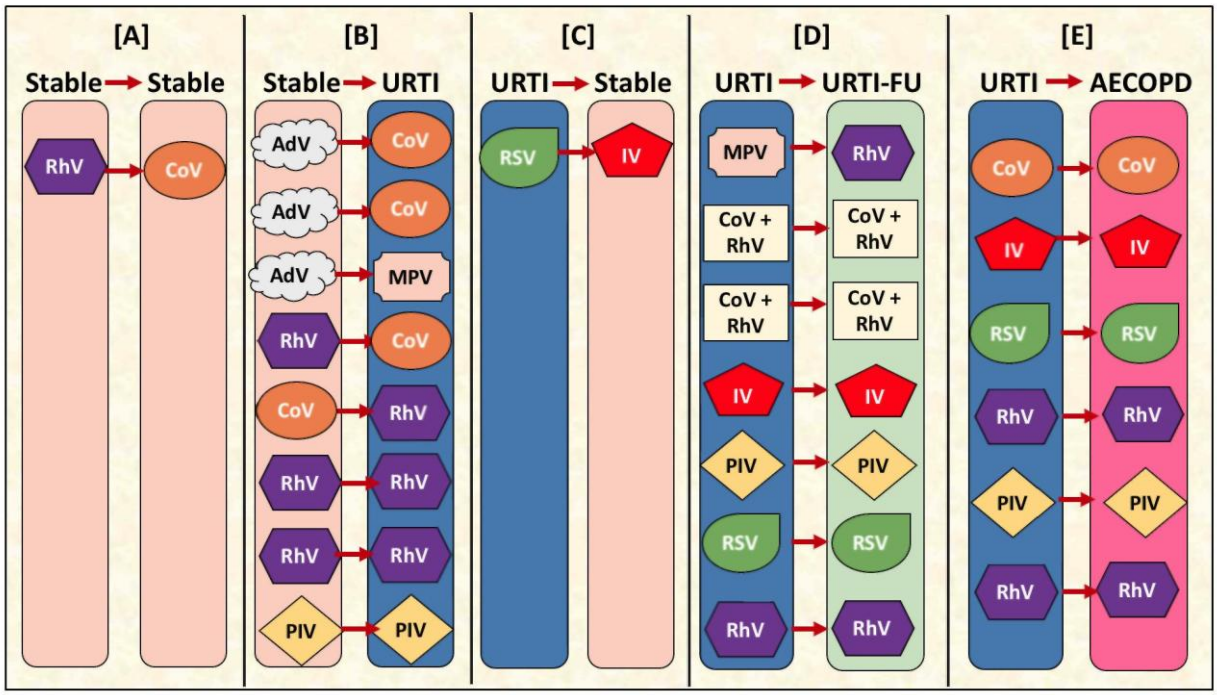
Supplementary Figure 1. Viral infections in consecutive visits.
RhV: Rhinovirus; CoV: corona virus; AdV: adenovirus; PIV: parainfluenza virus; RSV: respiratory syncytial virus; MPV: metapneumovirus; IV: influenza virus; URTI: upper respiratory tract infection; FU: follow-up 10 days after a URTI. Only consecutive visits are shown in the scheme. From the group of patients that had viral infections in different visits (not consecutive): 5 patients had viral infections in two visits but with a different virus; 10 patients had viral infections in two visits with the same virus (4 with CoV, 3 with RhV, 2 with IV and 1 with PIV); 4 patients had diverse viral infections in 3 different visits; 3 patients had the same viral infection in 3 different visits (1 with MPV, and 2 with RhV); 3 patients had the same viral infection in 4 different visits (2 with RhV, and 1 with IV); 1 patient had various viral infections in 5 different visits and 1 patient had viral infection with RV in 5 different visits.



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Supplementary Table 1. Incidence of different viruses at different visit types.

Virus type	All Visits N=2833 n (%)	Stable N=1909 n (%)	URTI N=391 n (%)	URTI FU N=356 n (%)	AECOPD N=177 n (%)
Adenovirus	10(0.35)	9 (0.47)	1(0.28)	0(0.0)	0(0.0)
Bocavirus	1 (0.04)	1(0.05)	0 (0.0)	0(0.0)	0(0.0)
Coronavirus 229E	34(1.20)	15 (0.79)	10 (2.81)	4(1.37)	5(2.82)
Coronavirus HKUl	18 (0.64)	4(0.21)	11 (3.09)	2 (0.69)	1(0.57)
Coronavirus NL63	19 (0.67)	1(0.05)	13 (3.65)	2 (0.69)	3(1.69)
Coronavirus OC43	27(0.95)	2(0.10)	19 (5.34)	0(0.0)	6(3.39)
Influenza A H1N1	8 (0.28)	0 (0.0)	6(1.69)	0(0.0)	2(1.13)
Influenza B	18 (0.64)	4(0.21)	6(1.69)	2 (0.69)	6(3.39)
Influenza H1	2 (0.07)	1(0.05)	1(0.28)	0(0.0)	0(0.00)
Influenza H3	14 (0.49)	1(0.05)	7(1.97)	2 (0.69)	4(2.26)
Metapneumovirus	12(0.42)	1(0.05)	4(1.12)	1 (0.34)	6(3.39)