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TITLE:
Respiratory Syncytial Virus and Other Respiratory Viral Infections in Older Adults With Moderate to Severe Influenza-like Illness

ABSTRACT:
Background. Few studies have prospectively assessed viral etiologies of acute respiratory infections in community-based elderly individuals. We assessed viral respiratory pathogens in individuals ≥65 years with influenza-like illness (ILI). Methods. Multiplex reverse-transcriptase polymerase chain reaction identified viral pathogens in nasal/throat swabs from 556 episodes of moderate-to-severe ILI, defined as ILI with pneumonia, hospitalization, or maximum daily influenza symptom severity score (ISS) >2. Cases were selected from a randomized trial of an adjuvanted vs nonadjuvanted influenza vaccine conducted in elderly adults from 15 countries. Results. Respiratory syncytial virus (RSV) was detected in 7.4% (41/556) moderate-to-severe ILI episodes in elderly adults. Most (39/41) were single infections. There was a significant association between country and RSV detection (P = .004). RSV prevalence was 7.1% (2/28) in ILI with pneumonia, 12.5% (8/64) in ILI with hospitalization, and 6.7% (32/480) in ILI with maximum ISS > 2. Any virus was detected in 320/556 (57.6%) ILI episodes: influenza A (104/556, 18.7%), rhinovirus/enterovirus (82/556, 14.7%), coronavirus and human metapneumovirus (each 32/556, 5.6%). Conclusions. This first global study providing data on RSV disease in ≥65 year-olds confirms that RSV is an important respiratory pathogen in the elderly. Preventative measures such as vaccination could decrease severe respiratory illnesses and complications in the elderly.

Study Design and Participants ::: METHODS:
This laboratory-based, epidemiological study assessed the prevalence of RSV and other respiratory viruses in samples collected during the first surveillance year of the INFLUENCE65 clinical trial [11]. INFLUENCE65 (www.clinicaltrials.gov NCT00753272) was a Phase III randomized, controlled study evaluating the relative efficacy of the AS03-adjuvanted trivalent split-virion influenza vaccine compared to unadjuvanted vaccine during the 2008–2009 and 2009–2010 influenza seasons, in 43 802 adults aged 65 years and older. The study was conducted in 3 continents in the northern hemisphere: America (Canada, Mexico, and the US), Europe (Belgium, Czech Republic, Estonia, France, Germany, Norway, Poland, Romania (see Table 2 footnote), Russia, the Netherlands, UK), and East Asia (Taiwan). Study participants were community-based or living in retirement homes, which allowed mixing in the community. Bedridden elderly individuals were not eligible.
Informed consent was given to participate in INFLUENCE65. Samples from US subjects were not included in the present study as ethics approval for additional virologic studies would have been required, leaving a study population of 36 132 subjects.

Surveillance and Case Definition ::: METHODS:
Active surveillance undertaken between the 15th of November and 30th of April comprised weekly or biweekly telephone contacts or home visits. Participants were instructed to report any ILI. Nose and throat swabs were collected from subjects who developed an ILI during the surveillance period.
An ILI was defined as the simultaneous occurrence of at least 1 respiratory symptom (nasal congestion, sore throat, new/worsening cough, dyspnea, sputum production, or wheezing) and one systemic symptom (headache, fatigue, myalgia, feverishness [feeling hot or cold, having chills or rigors], fever [oral temperature of ≥37.5°C]). Because moderate-to-severe ILIs have the most clinically significant implications in terms of complications and associated healthcare costs, we evaluated the etiology of ILI episodes meeting predefined severity criteria. Thus, for the present study, only samples from episodes of moderate-to-severe ILI were selected: defined as associated with pneumonia (based on signs and symptoms with a chest radiograph demonstrating a new or progressive infiltrate), or with hospitalization, or with a maximum (ie, the highest total score achieved on days 0–14) daily Influenza Symptom Severity (ISS) score >2.

Influenza Symptom Severity Questionnaire ::: METHODS:
The ISS questionnaire assesses the severity of 10 symptoms including 3 respiratory (cough, sore throat, and nasal congestion) and seven systemic (headache, feeling feverish, body aches, fatigue, neck pain, interrupted sleep, and loss of appetite) symptoms using a 3-point grading

system (0 = none, 1 = mild, 2 = moderate, 3 = severe) and has been validated for influenza infection [12]. The 10 individual scores (systemic and respiratory) were averaged daily with a final score ranging from 0 to 3. The questionnaire was completed for 15 days.

Sample Collection ::: METHODS:
Nasal and throat swabs were collected by a study nurse or physician using standardized methods, within 5 days after the onset of each ILI episode, then stored at −70°C. Nucleic acids were extracted from each sample and stored below −60°C at GlaxoSmithKline Vaccines' laboratory [13].

Sample Selection ::: METHODS:
There were 4582 samples collected from 5389 ILI episodes during the INFLUENCE65 2008–2009 surveillance period. We tested 556 (12%) of these samples from subjects who fulfilled the criteria for a moderate-to-severe ILI. One sample per episode was included, but subjects could be included more than once if they had more than 1 ILI episode meeting severity criteria and if there were at least 7 symptom-free days between episodes.

Respiratory Virus Identification ::: METHODS:
The xTAG® Respiratory Viral Panel (US IVD 96 tests; Luminex, Abbott Molecular, Ottignies-Louvain-la-Neuve, Belgium) is a qualitative multiplex RT-PCR used to simultaneously detect and identify nucleic acids from different pathogens. The following respiratory viruses and virus subtype genomes were amplified: Influenza A and subtypes H1, H3, and H5, Influenza B, RSV subtypes A and B, parainfluenza 1, 2, 3, and 4, human metapneumovirus, rhinovirus/enterovirus (the test does not differentiate between the 2), adenovirus, coronaviruses 229E, OC43, NL63, HKU1, and SARS as described elsewhere [14, 15]. The analytical sensitivity ranged from 0.1 to 1 TCID50, corresponding to approximately 50 to 250 genome equivalents [14].

Statistical Methods ::: METHODS:
The primary objective was to estimate the prevalence of RSV in nasal/throat swabs in community-based elderly individuals with moderate-to-severe ILI. Secondary objectives were to estimate the prevalence of other respiratory viruses in this population, and in subgroups defined by pneumonia occurrence, hospitalization status, and ISS score >2. Statistical analyses used SAS Version 9.1 or later. Prevalence was calculated as a proportion with its 2-sided exact 95% confidence interval (CI) [16]. Possible associations between prevalence and baseline characteristics/clinical symptoms were explored using logistic regression with significance level set at $P < .05$.

Role of the Funding Source ::: METHODS:
GlaxoSmithKline Biologicals SA designed the study, collected and analyzed data, interpreted the results, and wrote the report. All authors had access to the data and had final responsibility for the analysis, interpretation, and decision to submit for publication.

Prevalence of RSV in Nasal and Throat Samples ::: RESULTS:
RSV was detected in 41/556 (7.4%) moderate-to-severe ILI episodes (95% CI, 5.3%–9.9%). Most were single infections (39/41). The prevalence of RSV detection was 7.1% (2/28) in episodes with pneumonia, 12.5% (8/64) in episodes with hospitalization, and 6.7% (32/480) in episodes with a maximum ISS score >2 (Table 2). The median length of hospitalization for RSV was 6 days (range, 3–20 days) vs median 10 days (range 2–46) for hospitalized cases without RSV (ie, 24/64 cases with another virus detected and 32/64 with no virus detected).

RSV was detected in 11/179 (6.1%) episodes in subjects 65–69 years of age (95% CI, 3.1–10.7), 13/182 (7.1%) episodes in 70–74 years olds (95% CI, 3.9–11.9), 11/124 (8.9%) episodes in 75–79 year olds (95% CI, 4.5–15.3), and in 6/71 (8.5%) episodes in ≥80 year olds (95% CI, 3.2–17.5). Subjects with and without RSV were of similar age and gender distribution, had a similar proportion of pneumonia episodes, and mean ISS score (data not shown). Hospitalization occurred in 19.5% (8/41) of RSV positive episodes and in 10.9% (56/515) of RSV negative episodes (56/506 [11.1%] excluding 9 with unknown hospitalization status). There was no significant association between hospitalization status and RSV detection (Fishers exact $P = .12$). No RSV was detected among 98 moderate-to-severe ILI cases in the Russian Federation, or in several of the countries with fewer than 20 moderate-to-severe ILI cases: Estonia (N = 17), Taiwan (N = 4), and the United Kingdom (N = 13; Table 3). RSV prevalence in individual countries ranged

from 2.0% (1/50) in Mexico, to 17.1% (12/70) in Czech Republic. The highest prevalence was observed in the Czech Republic (17.1%, 12/70), Norway (15.4%, 2/15), and Germany (14.9%, 7/47). There was a significant association between country and RSV detection (P = .004). Both RSV subtypes (A and B) were detected, although the numbers of each subtype in each country were too small to identify trends (Table 3).

Prevalence of Other Respiratory Viruses in Nasal and Throat Samples ::: RESULTS:
Any virus, including RSV and influenza virus, was detected in 320/556 (57.6%) of moderate-to-severe ILI episodes. The highest prevalence of respiratory viruses was observed in the Czech Republic (53/70 [75.7%]), whereas the lowest was in Taiwan (1/4) (Table 3). Detection rates of individual viruses differed between countries but did not display the same pattern as RSV (Supplementary Table 1). The most frequently reported respiratory viruses were influenza A (104/320 positive samples [32.5%]), rhinovirus/enterovirus (82/320 positive samples [25.6%]), RSV (41/320 positive samples [12.8%]), and coronavirus and human metapneumovirus (each with 32/320 positive samples [10.0%]; Table 4).

Multiple viral infections were detected in 11 ILI episodes. Influenza A and/or B and/or rhinovirus/enterovirus were implicated in all but 1 coinfection (Table 4).
Non-RSV viruses detected in pneumonia episodes were influenza A (all H3; 3/28 [10.7%]), human metapneumovirus (1/28 [3.6%]), and rhinovirus/enterovirus (3/28 [10.7%]; Table 5).

Non-RSV viruses were detected in 24/64 hospitalized cases (Table 5). Hospitalization among RSV-positive moderate-to-severe ILI episodes (8/41 [19.5%]) was about 2-fold more common than hospitalization among episodes positive for any other virus (24/279 [8.6%]) and 5-fold more common compared to influenza A (4/104 [3.8%]), respectively.
The median duration of ILI episodes was 15 days. No clear patterns were seen in terms of episode duration according to virus type, although the numbers were small (data not shown).

Frequency of Respiratory Symptoms According to Virus ::: RESULTS:
The frequency of individual respiratory symptoms in subjects with a single infection with 1 of the 5 most prevalent virus types showed no striking pattern of symptoms according to virus type (Table 6). Fever was most frequent in influenza A infection (72.4%) and least frequent in infections due to rhinovirus/enterovirus (40%). Dyspnea and wheezing were most frequent in RSV infections (51% and 46%, respectively). Cough was present in >90% for all virus types.

Associations Between Risk Factors and Viral Detection ::: RESULTS:
Exploratory univariate analysis showed no association between RSV detection and age, smoking status, gender, or randomization group (adjuvanted vs nonadjuvanted trivalent influenza vaccine) of the primary INFLUENCE65 study. Factors found to be associated with RSV detection among moderate-to-severe ILI episodes were “high-level care” as defined in Table 1 (odds ratio [OR], 3.5; 95% CI, 1.4–9.2; P = .0103), congestive heart failure (OR, 2.9; 95% CI, 1.1–7.4; P = .0223), other cardiopulmonary diseases (OR, 2.3; 95% CI, 1.2–4.7, P = .0143), and noninflammatory cerebrovascular/neurological disorders (OR, 2.3; 95% CI, 1.2–4.3, P = .0102). These factors, however, were not independently associated with RSV detection. No independent predictor of RSV detection was determined in the multivariate analysis.

DISCUSSION:
We used multiplex RT-PCR to detect viral etiologies of moderate-to-severe ILI in elderly influenza-vaccinated community-dwelling elderly adults during 1 winter season from November 2008 to April 2009 in a large international study. Viruses were detected in 57.6% (320/556) of moderate-to-severe ILI episodes, of which approximately one-third were influenza A. RSV, rhinovirus/enterovirus, coronavirus, and human metapneumovirus were also important pathogens in this age group, with RSV detected in 7.4% of samples. Moderate-to-severe ILI episodes that were RSV-positive were more likely to result in hospitalization than other viral respiratory infections; however, influenza vaccination may have attenuated the severity of influenza A in the study population.
The virus detection rate of 57.6% in this study is higher than what has been reported in a number of previous studies [6] and can be at least partially explained by the use of a sensitive multiplex RT-PCR, which can detect 18 different viruses and subtypes. However, it is important to recognize

that multiplex RT-PCR may have different analytic sensitivities for the different viruses, which can affect the diagnostic yield. In addition, viral load may vary for specific viral infections with RSV viral load typically low in the upper airways of adults [5, 17, 18]. Another factor that may explain the variability of virus detection rates in the literature is the clinical definition used to trigger swab collection and further laboratory testing. There is currently no universally accepted definition of all ILI or moderate-to-severe ILI, in particular in the elderly population where the clinical presentation may be atypical. We attempted to define moderate-to-severe ILI using objective and subjective criteria, which we felt were relevant to respiratory illnesses rather than minor colds. Fever occurs less commonly in the elderly; our definition of ILI, which did not require the presence of fever, is an important difference from other studies using ILI definitions to evaluate noninfluenza viruses. The inclusion of fever as an obligatory part of the ILI case definition would have excluded around 30%–60% of cases, depending on virus type. The majority of subjects with moderate-to-severe ILI were identified by an ISS >2. Although every attempt was made to capture all cases, proportionally more swabs (up to 44%) were missed from episodes with pneumonia and hospitalization. This may mean that subjects presenting with an ILI that resulted in pneumonia deteriorated so rapidly that no swab could be collected in time. Thus, our data may have missed part of the most severe spectrum of disease (no deaths occurred in the test cohort). Of note, as per protocol, swabs were only to be taken if an ILI occurred. A subject presenting with pneumonia without a preceding ILI as per the protocol definition, would have had no swab taken.

We observed an effect of country on RSV prevalence driven mainly by results from 2 countries (Czech Republic and Russia). This effect probably reflected seasonal variability during the study period, although a cultural effect of family/household structure could also have contributed to the regional differences we observed. Studies in individual countries over multiple seasons and considering different disease severities are needed to more fully understand the regional burden of RSV in the elderly.

One of the limitations of our study is that the primary INFLUENCE65 study was designed to evaluate ILI associated with influenza infection, not all causes of respiratory infection. Even though the definition of an ILI was broad, study physicians may have been biased in their clinical assessment and decision whether or not to take a swab depending on whether influenza was active in their community. Other potential limitations of the study include that all subjects received influenza vaccination, which is likely to have reduced influenza infection rates. We were not able to distinguish simple viral infection from combined viral and bacterial infections. Small numbers of RSV positive subjects meant that subanalyses by pneumonia and hospitalization status were unable to identify meaningful associations. Finally, hospitalization status was unknown for 9 (<2%) moderate-to-severe ILI episodes.

We identified no independent predictor of RSV detection. However, another study found that RSV infection was more frequent among adults with congestive heart failure or chronic pulmonary disease [8]. Elderly individuals with chronic cardiac or respiratory disease are therefore likely to benefit most from RSV prevention.

Few prospective studies have evaluated etiologies of acute respiratory infections in community-based elderly individuals. In one prospective US study conducted over 4 winter seasons, RSV was detected (using RT-PCR) annually in up to 3%–10% of elderly individuals living in the community, with the illness preventing normal daily activities in 42% of patients [8]. A prospective study conducted in community-dwelling elderly persons in the United Kingdom detected RSV antibodies (using complement fixation) in 3% of 497 illnesses among the 533 persons followed for 2 winter seasons [10]. A prospective study in Brazil that relied on passive reporting of acute respiratory infections by community-dwelling elderly detected a virus (using RT-PCR) in 15/49 samples (30.6%) [19]. Rhinovirus/enterovirus was detected in all cases but 1 (human metapneumovirus), and no RSV was identified.

In our study, RSV was detected by RT-PCR in 7.4% of severe ILIs in elderly community-dwelling adults and was more prevalent in hospitalized patients than in patients with pneumonia or an ISS score >2. Although our study was not designed to determine incidence rates or evaluate disease burden, the results are consistent with previous reports suggesting that RSV is an important cause of moderate-to-severe ILI and may be associated with hospitalization in the elderly [20–22]. The results of our study cannot be generalized to all ILI in the elderly because moderate-to-severe ILI represented only 10% of all ILI identified in eligible study sites. In addition, our study was limited to 1 year, and evaluations over multiple seasons may more accurately reflect global RSV impact. We observed country-to-country variability in RSV detection, with the highest proportion of RSV-positive episodes occurring in the Czech Republic and Poland. Such variation will require further study over time to understand the implications of these findings. Despite these limitations,

this is the first global study to our knowledge providing data on RSV disease in the elderly. Consistent with reports from North America, our study confirms that RSV is an important respiratory pathogen in older adults.

In conclusion, assessment of RSV prevalence using multiplex RT-PCR in influenza-vaccinated subjects aged ≥65 years from 14 countries with moderate-to-severe ILI showed that RSV was implicated in 7.4% of respiratory illnesses. Our study illustrates the importance of RSV and other respiratory viruses as causes of serious respiratory illness affecting older adults around the globe. Thus, in addition to influenza prevention, prevention of viral infections such as RSV could decrease severe illnesses in the elderly, particularly those at increased risk of the complications of viral respiratory tract infections.