Adding sputum and saliva to nasopharyngeal swab samples for PCR detection of Respiratory Syncytial Virus in adults hospitalized with acute respiratory illness may double case detection

Julio Ramirez[1], Ruth Carrico[1], Ashley Wilde[1], Alan Junkins[1], Stephen Furmanek[1], Thomas Chandler[1], Paul Schulz[1], Robin Hubler[2], Paula Peyrani[2], Sonali Trivedi[2], Qing Liu[2], Sonal Uppal[2], Bradford Gessner[2], Elizabeth Begier[2] and the NIDI RSV Study Group[1]

[1]Norton Infectious Diseases Institute, [2]Pfizer, Inc.

INTRODUCTION

Respiratory Syncytial Virus (RSV) is a leading cause of respiratory illness in adults, with older adults and those with compromised cardiac, pulmonary, or immune systems most at risk of severe disease. RSV is primarily diagnosed by RT-PCR testing of nasopharyngeal (NP) swabs. The collection and testing of additional specimen types to NP swab RT-PCR has been documented to increase RSV detection in pairwise comparisons, however no study has looked at the cumulative effect of adding multiple specimen types tested for detection of RSV.

The objective of this study was to compare RSV detection rates between NP swab RT-PCR alone versus NP swab RT-PCR with addition of saliva, sputum, and serology testing.

METHODS

This was a prospective cohort study of patients hospitalized with acute respiratory illness (ARI) in four adult acute care-hospitals in Louisville, KY from December 27, 2021 to April 1, 2022. Patients were eligible for inclusion if they were:

1) aged 40 years or older
2) hospitalized with ARI, defined as at least one of the following:
   a) new onset or increase from baseline in any of following signs/symptoms: nasal congestion, rhinorrhea, sore throat, hoarseness, cough, sputum production, dyspnea, wheezing, hypoxemia
   b) admitting diagnosis suggestive of ARI
   c) exacerbation of underlying cardiopulmonary disease involving acute respiratory symptoms

After informed consent was obtained, we sought to collect NP swab, saliva, sputum, and an acute blood specimen on all subjects. Respiratory samples on any given subject were collected on the same day (day of enrollment). Patients were scheduled for a follow up between 30 and 60 days to collect convalescent blood specimens.

RSV detection from NP swab, saliva and sputum specimens was defined as a positive result on RT-PCR test. RSV detection from serology was defined as a four-fold rise between acute and convalescent paired blood specimens in antibody to any RSV antigen. RSV detection rate by NP swab alone was calculated as the number of patients RSV detected from NP swab specimens divided by the number of patients in the study. RSV detection rate by NP swab plus other specimens was calculated as the number of patients with RSV detected from any specimen divided by the number of patients in the study.

RSV detection rates by NP swab alone vs NP swab plus other specimen were compared.

RESULTS

A total of 639 patients were enrolled into the study. RSV was detected in 35 (5.5%) patients. Patient characteristics for RSV patients are depicted in **Table 1**. **Figure 1** depicts the sample collection rates for respiratory and serology specimen. The final clinical diagnosis for RSV detected patients is shown in **Figure 2**. **Figure 3** depicts RSV detection by RT-PCR of respiratory specimen, and **Figure 4** depicts RSV detection by all sample types. The increase in RSV detection, using NP swab RT-PCR as the baseline, are depicted in **Figure 5**.

Table 1. Patient Characteristics for RSV Patients	N (%)
Overall	35 (100%)
Demographics	
Age (years), Median [Q1 - Q3]	65 [57 - 74]
Gender, Male	19 (54.3%)
Race, White or Caucasian	25 (71.4%)
Race, Black or African American	9 (25.7%)
Current Tobacco use	8 (22.9%)
Comorbidities*	
Chronic heart disease, other	22 (62.9%)
Obesity (BMI ≥ 30)	20 (57.1%)
Diabetes mellitus (DM)	17 (48.6%)
Coronary artery disease (CAD)	14 (40.0%)
Chronic heart failure (CHF)	12 (34.3%)
Chronic obstructive pulmonary disease (COPD)	11 (31.4%)
Immunocompromised**	10 (28.6%)
Chronic kidney disease (CKD)	9 (25.7%)
Obesity (BMI > 40)	9 (25.7%)
Asthma	5 (14.3%)
Stroke	5 (14.3%)
Neurologic and neurodevelopment conditions	5 (14.3%)

*Comorbidities are not mutually exclusive, and patients may have more than one comorbidity. **Immunocompromised includes persons with autoimmune disorders, active immunosuppressant drug therapy, immunodeficiency, HIV, AIDS, Cancer, solid tumor or hematologic malignancy, or organ transplant

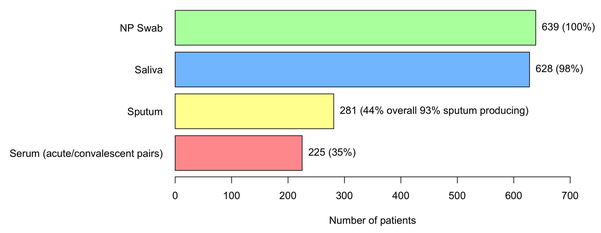

Figure 1. Sample collection by sample type

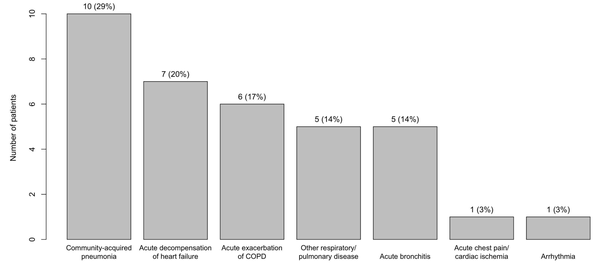

Figure 2. Final Diagnosis of RSV-detected patients

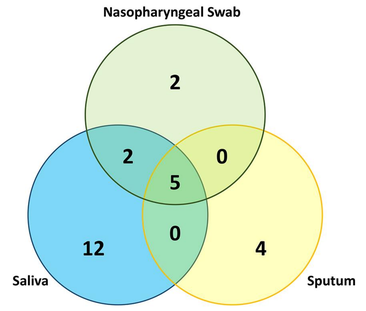

Figure 3. RSV detection by respiratory sample type

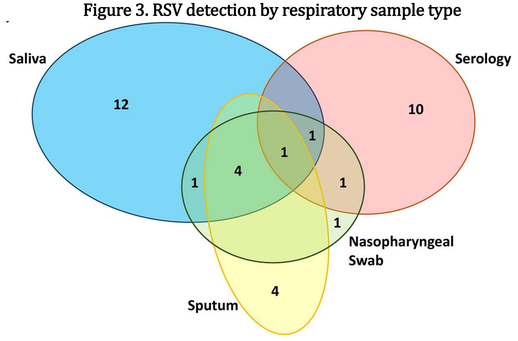

Figure 4. RSV detection by any sample type

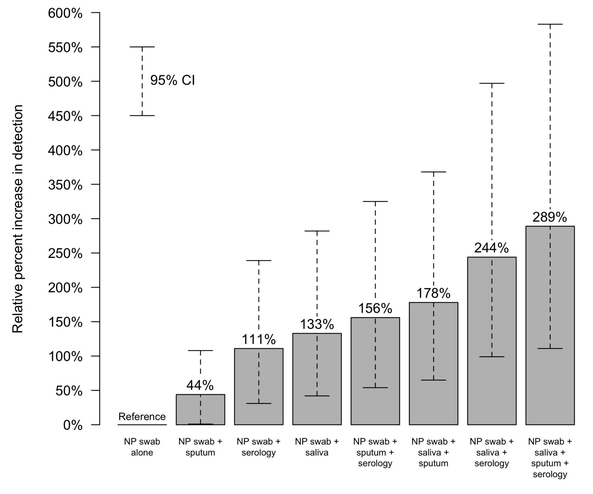

Figure 5. Increase in RSV detection by addition of other samples

DISCUSSION

RSV was detected in 9 (1.4%) patients by NP swab alone, and in 35 (5.5%) patients by NP swab plus additional specimens, corresponding to a 3.9 times higher detection rate (95% CI: 2.2 to 6.8). Even though RT-PCR of NP swab is the most commonly used test to detect RSV in hospitalized patients, our study indicates that a significant number of patients hospitalized with RSV-associated ARI have negative PCR of NP swabs. Prior literature has reported increased detection associated with adding sputum or serology to NP swab, but this is the first study utilizing a wide variety of specimen types, including saliva, and assessing their synergistic effects for RSV detection.

Saliva specimens yielded the highest number of RSV detections among respiratory specimens. There are several potential reasons for this. RSV may replicate in the primary salivary glands such as parotid, submandibular, and sublingual glands, producing a constant flow of the virus or viral genetic material into the saliva. It is possible that saliva may be a more desirable diagnostic sample for the diagnosis of respiratory viruses both for better yield and tolerability to patients.

In patients with a productive cough, sputum was an important specimen for RSV identification. Sputum is known to have higher RSV titers than nasal swabs, allowing for increased detection of RSV when this specimen in available.

Serology is complementary to PCR testing of respiratory samples and its added value depends in part of the length of illness prior to evaluation. Serology increased RSV detection by an additional 40% above that of RT-PCR of respiratory specimens (~100% increase above NP swab alone).

Our data indicate that a more accurate burden of RSV disease in future studies can be achieved by testing multiple specimen types or adjusting for underestimation associated with use of limited specimen types. Furthermore, our study suggests that vaccine studies evaluating efficacy or effectiveness of an RSV vaccine will ideally include multiple specimens for diagnosis of disease.

The primary limitation of our study was the low number of RSV diagnoses, which limited study power and makes definitive conclusions more difficult.

CONCLUSIONS

In our study, RSV detection almost quadrupled with the addition of testing from other specimen types besides NP swab. This study and other literature suggest hospitalized RSV ARI burden estimates in adults based solely on NP swab RT-PCR should be adjusted for underestimation.

FUNDING AND CONFLICTS OF INTEREST

This is an investigator-sponsored study funded by Pfizer, Inc. Those with Pfizer affiliations are employees of Pfizer and may own Pfizer stock.