Supplemental Online Content

Juhn YJ, WI CI, Takahasi PY, et al. Incidence of respiratory syncytial virus infection in older adults before and during the COVID-19 pandemic. *JAMA Netw Open*. 2023. 6(1):e2250634. doi:10.1001/jamanetworkopen.2022.50634

This supplemental material has been provided by the authors to give readers additional information about their work.

eMethods.

1. Study setting and population: The community-based prospective cohort study was designed to follow adults aged ≥50 years residing in southeast Minnesota (SEMN) during two RSV seasons (October 1, 2019, to April 30, 2020, and October 1, 2020, to April 30, 2021). The study was extended to a non-RSV season (May 1, 2021- September 30, 2021) given the COVID-19 pandemic impact on RSV epidemiology and indeed, the first COVID-19 case in Olmsted County was reported on March 11, 2020. We enrolled patients paneled in the Mayo Clinic primary care practice, Rochester, Minnesota. For this study, we identified all individuals who resided in southeast Minnesota (SEMN) (largely from 4 counties including Olmsted, Dodge, Wabasha, and Goodhue counties) on April 1, 2019, using electronic health records (EHRs). Characteristics of study participants for the pandemic RSV season were similar to those for the pre-pandemic RSV season (data not shown). Our study showed a reasonable retention rate of the enrolled subjects (attrition rate was only 5.7% by the end of the pandemic RSV season), and about 72% of the original study cohort re-participated in the study assessing the incidence of RSV-positive ARI during pandemic non-RSV season. The study was approved by the Mayo Clinic Institutional Review Board (IRB# 19-004142), and we obtained the consent from participants. The study was approved by the Mayo Clinic Institutional Review Board, and we obtained the consent from participants.

2. Instructions for self-collecting swabs of anterior nares and oropharynx: We developed instructions and workflow for self-collecting swabs of anterior nares and oropharynx (see eTable 5). Detailed written and accompanying pictorial and video instructions for self-collection of swab specimens were provided to participants. Participants were instructed to place swab specimens in viral transport media (MicroTest™ M4RT® Multi-Microbe Media; Remel, Inc.,

Lenexa, KS) after self-collection prior to pick-up. Helpful reminders to subjects included refrigeration of samples once collected and until courier pick-up, as well as review of logistics and confirmation of courier pick-up time. At-home self-collection of both anterior nasal and oropharyngeal swabs from each participant to test for RSV was implemented for this study as reported by others.(1-4) Concordance rates for positive PCR test results for both influenza and RSV between swabs performed by research staff and self-swabs was 99% (95% CI: 94-100) in pre-pandemic RSV Season (n=98). In addition, at Mayo Clinic, two independent studies were performed to determine the concordance between self-collected and health care professional-collected pharyngeal and nasal swabs. The results showed high concordance rates in streptococcal [95% (95% CI: 86-98)] and influenza [96% (95% CI: 90-98)] testing between patients and clinic staff.(5, 6) During the pandemic RSV season and the pandemic non-RSV season, the institutional process for handling patients with potential COVID-19 symptoms was activated for positive screening results and possible subsequent COVID-19 testing (see eFigure 1 below for swab workflow). Patients were referred to the COVID-19 nurse line for further screening and evaluation. During this time, if participants did not meet the screening criteria for COVID-19 testing, a home-pick up of self-collected swabs by a courier service was scheduled to adhere to social distancing, and study participants were instructed to self-collect swabs at home of both the anterior nares and oropharynx (throat) using packaged testing kits distributed to consented participants. Additionally, if the COVID-19 test results were negative, then study participants were instructed to self-collect swabs at home using packaged testing kits as described above.

3. Study cohort: Our study cohort was composed of 2,325 subjects followed by primary care providers at Mayo Clinic, recruited as a community-based prospective cohort study of eligible

adults ≥ 50 years old residing in southeast Minnesota. This cohort represents a sample by age, sex, race/ethnicity, and SES of populations residing in SEMN counties.

4. RSV test: Testing for RSV was performed using the Simplexa Flu A/B and RSV Direct assay (DiaSorin Molecular, Cypress, CA), which is FDA-approved for detection of these viruses from nasopharyngeal (NP) swab samples in viral transport media. In this study, both nasal and throat swabs were performed to increase detection rate. The following studies were completed to verify the performance characteristics of the Simplexa Flu A/B and RSV Direct assay:

I. Accuracy

Clinical (n=13) or spiked (n=57) nasopharyngeal swab samples were tested, and the results compared to another commercial, FDA-approved method (Hologic ProFlu+ assay). Samples showing discordant results between these methods were tested by a third method (a validated lab developed real-time PCR) for influenza A/B and RSV. Accuracy studies showed the following adjusted sensitivity and specificity data:

A. Influenza A: 100% sensitivity (30/30); 97.5% specificity (37/38)

B. Influenza B: 100% sensitivity (30/30); 100% specificity (40/40)

C. RSV: 100% sensitivity (30/30); 100% specificity (40/40)

II. Precision

Both inter- (between run) and intra- (within run) precision was verified for the Simplexa Flu A/B and RSV assay using spiked nasopharyngeal swab specimens. All expected positive results were achieved, with 100% agreement in qualitative results both within and between runs. Crossing point values showed a percent coefficient of variation (C.V.) of <5%.

III. Limit of Detection

The limit of detection was established by the manufacturer as the following:

A. Influenza A (H1N1): 1 x 10-2 TCID50/mL

B. Influenza A (H3N2): 1 x 101 TCID50/mL

C. Influenza B (Great Lakes): 1 x 100 TCID50/mL

D. RSV A2: 1 x 100 TCID50/mL

E. RSV B: 1 x 100 TCID50/mL

IV. Analytical Specificity

A comprehensive cross-reactivity panel (bacteria [n=30], viruses [n=13] and human DNA) was tested by the Simplexa Flu A/B and RSV assay and all results were negative, indicating 100% specificity.

V. Specimen Stability

Nasopharyngeal swabs in viral transport media (VTM) were demonstrated to be stable for 7 days when stored at either 4°C or -20°C. These samples are likely stable for longer periods of time, but extended stability was not verified by Mayo Clinic.

5. RSV-A and -B subtyping: RSV positive swabs were further tested to determine the RSV subtype. Briefly, an in-house–developed kit was used. RSV-A and -B were detected and quantified by duplex RT-PCR using specific amplification primers and fluorescent probes designed to detect the RSV N gene. The process involves extraction of nucleic acids, conversion of RNA to complementary DNA by reverse transcription, and detection by real-time PCR reaction using a calibration curve (absolute quantitation). Two hundred microliters of M4RT from nasal swab samples was used for the nucleic acid extraction (KingFisher, MagMax Core kit). Nucleic acids were eluted in a volume of 80 µL, and 2.5 µL of the elution was used per RT-PCR amplification. Limits of detection (LODs) were determined via probit approach, as

recommended in the Clinical and Laboratory Standards Institute EP17-A2 guidance. Several dilutions of surrogate samples (M4RT transport medium spiked with different concentrations of RSV-A and RSV-B strains) were used for their determinations. The RSV-A RT-PCR has an LOD of 304 copies/mL, whereas the LOD for the RSV-B RT-PCR is 475 copies/mL. Clinical samples were considered positive when the load was higher than the respective LODs

6. Vital signs and frailty measures: All vital signs and physical frailty measures were completed in-person. Briefly, hand grip strength was done 3 times and an average was calculated via an equation. The dominant hand was used and documented. For walking test, first the pace was measured in seconds by walking 5 meters, which was done twice, and the fastest pace was used for the analysis. Next the 6-minute walk was measured in a hallway in the research office building. Measurements were marked on the wall for consistency, and the total segments walked were counted and entered in the data. Height/weight were also measured in person along with other vital signs (tympanic temperature, blood pressure, pulse, and respiratory rate at the time of the visit).

7. Power calculation and sample size: RSV incidence: Based on the two prospective cohort studies, the annul incidence rate of RSV infection has been reported to be 36-55/1000PY among older adults (36/1000PY for 60+ and 55/1000PY for 65+ years old).(7, 8) Given that the current study includes subjects who age between 50 and 60 (25%), we expect the overall annual incidence rate may be lower that the two studies. Assuming similar incidence rates in this study (36-55/1000 PY) over 7 months of follow-up, our planned sample size (1750=3000x7/12) will provide the margin of error of no greater than 0.009 and 0.011 for incidence rate of 36/1000 PY and 55/1000PY, respectively. The margin of errors (i.e., half the width of the 95% CI) were calculated using the normal approximation to the Poisson distribution with each specified

incidence rate. The 95%CI of the two reported incidence rates for different sample sizes are summarized in the table below. In addition, 95% CIs of 0.01/PY were also presented in the table below which is close to the incidence rate reported by McClure et al (15/1,000PY).(9) Based on our sample size calculation, each of three specified incidence rates will be estimated with an acceptable precision (≤ 0.01) using our target sample size (n=3000) and even the sample size below this target sample size (n=2000) to address the study aim.

The 95% CIs of three reported incidence rates with of detectable incidence rates for different sample sizes over 7 months of follow-up			
	Rate: 0.036/PY	Rate: 0.055/PY	Rate: 0.01/PY
N=1000	(0.021, 0.051)	(0.036, 0.074)	(0.002, 0.018)
N=2000	(0.025, 0.047)	(0.042, 0.069)	(0.004, 0.016)
N=3000	(0.027, 0.045)	(0.044, 0.066)	(0.005, 0.015)

Outcomes of RSV: For outcomes with repeated measures, a previous cross-sectional study reported that the presence of respiratory syncytial virus antibodies was a significant predictor for reduced FEV1 (standardized FEV1 mean±SE) for subjects with non-RSV infection and those with RSV infection were -0.28±0.05 (n=560) vs. -0.61±0.1 (n=163), respectively).(10) In the current study, baseline data will be collected after the first RSV season, which will be used for an intermediate-term outcome data for those who develop RSV infection during the first season. Therefore, we expect to have intermediate-term (6-7 months post-RSV infection) outcome data for all anticipated subjects (108-165 RSV cases with 216-330 controls). In addition, we will collect long-term outcome data (i.e., 12-13 months post-RSV infection). Assuming the anticipated RSV cases and the doubled number of comparison group with Type I error of 0.05, we will have at least 80% power to detect the minimum difference of 0.41 in FEV1 (an intermediate-term outcome) using 108 cases and 216 controls (based on 36/1000PY), and 0.33 using 165 cases and 330 controls (based on 55/1000PY), respectively. The power calculation

was based on comparing two changes in mean FEV1 scores between two time points under normal distributions with 2-sided t-test (estimated standard deviation for RSV infection vs non-RSV infection: 1.28 vs 1.18, respectively) (Russell Lenth's power calculation tool).(11) The observed difference in the literature is 0.33. Therefore, we may lack statistical power to detect the reported difference with 106 cases and doubled controls. However, we expect higher sample size given that our study will follow up for 2 RSV seasons. If we assume that intermediate-term and long-term outcome data will be collected for at least 80% of the subjects, we can expect to have 172 cases and 345 controls based on 36/1000PY. This sample size will provide at least 80% power to detect the minimum difference of 0.32, which is similar to the observed difference in the literature. Therefore, we will have adequate statistical power to address the study aim.

1. Prevention TCfDCa. Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons for Coronavirus Disease 2019 (COVID-19). In: Services DoHaH, editor. 2020.

2. McCulloch DJ, Kim AE, Wilcox NC, Logue JK, Greninger AL, Englund JA, et al. Comparison of unsupervised home self-collected midnasal swabs with clinician-collected nasopharyngeal swabs for detection of SARS-CoV-2 infection. JAMA Network Open. 2020;3(7):e2016382-e. doi:10.1001/jamanetworkopen.2020.16382

3. Altamirano J, Govindarajan P, Blomkalns AL, Kushner LE, Stevens BA, Pinsky BA, et al. Assessment of sensitivity and specificity of patient-collected lower nasal specimens for sudden acute respiratory syndrome coronavirus 2 testing. *JAMA Network Open*. 2020;3(6):e2012005-e. doi:10.1001/jamanetworkopen.2020.12005

4. Tu Y-P, Jennings R, Hart B, et al. Swabs collected by patients or health care workers for SARS-CoV-2 testing. *N Engl J Med*. 2020;383(5):494-496. Medline:32492294 doi:10.1056/NEJMc2016321

5. Murray MA, Schulz LA, Furst JW, et al. Equal performance of self-collected and health care worker-collected pharyngeal swabs for group a streptococcus testing by PCR. *J Clin Microbiol*. 2015;53(2):573-578. Medline:25502528 doi:10.1128/JCM.02500-14

6. Dhiman N, Miller RM, Finley JL, et al. Effectiveness of patient-collected swabs for influenza testing. *Mayo Clin Proc*. 2012;87(6):548-554. Medline:22551906 doi:10.1016/j.mayocp.2012.02.011

7. Nicholson KG, Kent J, Hammersley V, Cancio E. Acute viral infections of upper respiratory tract in elderly people living in the community: comparative, prospective, population-based study of disease burden. *BMJ*. 1997;315(7115):1060-1064. Medline:9366736 doi:10.1136/bmj.315.7115.1060

8. Falsey AR. Respiratory syncytial virus infection in elderly and high-risk adults. Exp Lung Res. 2005;31(suppl 1):77. Medline:16395866

9. McClure DL, Kieke BA, Sundaram ME, et al. Seasonal incidence of medically attended respiratory syncytial virus infection in a community cohort of adults ≥50 years old. PLoS One. 2014;9(7):e102586. Medline:25025344 doi:10.1371/journal.pone.0102586

10. How COVID-19 is changing the cold and flu season. Nature. Accessed December 6, 2022. https://www.nature.com/articles/d41586-020-03519-3?WT.ec_id=NATURE-20201217&utm_source=nature_etoc&utm_medium=email&utm_campaign=20201217&sap-outbound-id=EBFB0C4879098937E8DAC2752A238F8530277058#ref-CR3

11. Mahoney FI, Barthel DW. Functional evaluation: the Barthel index. Md State Med J. 1965;14:61-65. Medline:14258950

eTable 1. Case definitions of ARI, LRTD and severity definitions	
Endpoint	**Case definition**
ARI	• At least 2 upper respiratory symptoms OR • At least 2 signs/symptoms from different locations (upper respiratory/lower respiratory/systemic)
RT-PCR-confirmed RSV-ARI	An event meeting the case definition of ARI with at least one RSV-positive swab detected by RT-PCR.
LRTD	An event meeting the case definition of ARI with presence of: • oxygen saturation < 92% or decrease by 5% or more if pre-illness baseline is ≥95%, if available, OR • at least 2 lower respiratory signs/symptoms for at least 24 hours
RT-PCR-confirmed RSV-LRTD	An event meeting the case definition of LRTD with at least one RSV-positive swab detected by RT-PCR
Clinical symptom-based severity assessment of RSV infection	**Mild:** No fever or other systemic symptoms or signs of infection but only upper respiratory symptoms. **Moderate:** Systemic manifestations of infection with upper respiratory symptoms, or symptoms requiring a medical appointment (outpatient) for evaluation, **Severe:** Systemic manifestations of infection with lower respiratory symptoms, or symptoms requiring emergency department visit or hospitalization.
ARI: Acute respiratory infection; LRTD: Lower respiratory tract disease; RSV: Respiratory syncytial virus RT-PCR: Reverse transcription polymerase chain reaction	

eTable 2: Characteristics at enrollment of participants who reconsented and those who were not reconsented		
	Withdrawn prior to May 1, 2021, or declined reconsent (n=643)	Reconsented (n=1682)
Age in years at enrollment		
Mean (SD)	70.0 (10.7)	66.9 (9.7)
Median (Range)	69 (50, 98)	66 (50, 96)
Age category at enrollment, n (%)		
50-59 years	123 (19.1%)	439 (26.1%)
60-69 years	204 (31.7%)	595 (35.4%)
70-79 years	169 (26.3%)	465 (27.6%)
80 or above	147 (22.9%)	183 (10.9%)
Gender, n (%)		
Female	369 (57.4%)	1011 (60.1%)
Race/Ethnicity, n (%)		
American Indian/Alaskan Native	0 (0.0%)	4 (0.2%)
Asian	3 (0.5%)	18 (1.1%)
African American	2 (0.3%)	10 (0.6%)
Hispanic or Latino	6 (0.9%)	8 (0.5%)
Unknown	16 (2.5%)	18 (1.1%)
Non-Hispanic White	616 (95.8%)	1624 (96.6%)
Geographic location, n (%)		
Dodge County	38 (5.9%)	110 (6.5%)
Goodhue County	31 (4.8%)	54 (3.2%)
Olmsted County	569 (88.5%)	1510 (89.8%)
Wabasha County	2 (0.3%)	3 (0.2%)
Other counties, MN	3 (0.5%)	5 (0.3%)
Rurality, n (%)		
Living in rural area	145 (22.6%)	361 (21.5%)
Socioeconomic status (HOUSES in quartile), n (%)		
1 (Lowest)	103 (17.2%)	220 (13.8%)
2	145 (24.2%)	404 (25.3%)
3	172 (28.7%)	448 (28.1%)
4 (Highest)	180 (30.0%)	525 (32.9%)
Missing	43	85
Chronic illness (within 3-year of enrollment), n (%)		
Congestive heart failure	52 (8.1%)	66 (3.9%)
Asthma	64 (10.0%)	187 (11.1%)
Chronic Obstructive Pulmonary Disease	65 (10.1%)	114 (6.8%)
Any other heart or lung disease	417 (64.9%)	977 (58.1%)
Diabetes mellitus	98 (15.2%)	204 (12.1%)
Immunosuppressed conditions	65 (10.1%)	157 (9.3%)
Previous vaccination before enrolment, n (%)		

eTable 2: Characteristics at enrollment of participants who reconsented and those who were not reconsented

	Withdrawn prior to May 1, 2021, or declined reconsent (n=643)	Reconsented (n=1682)
Influenza vaccination (within 1 year)	203 (31.6%)	480 (28.5%)
Pneumococcal vaccination (ever)	367 (57.1%)	858 (51.0%)
History of RSV infection before enrollment*, n (%)	4 (0.6%)	2 (0.1%)

*History of RSV was based on ICD10 codes from medical record which most likely under-represents the true number

eTable 3: Characteristics of participants with RSV-positive ARI and matched participants with RSV-negative ARI during pre-pandemic RSV Season for measurement of outcomes at the time of enrollment					
	Subjects with RSV-positive ARI (n=58)	**Subjects with RSV-negative ARI (n=116)**	**Univariate analysis**		
			Odds ratio	**95% Confidence Interval**	**P-value**
Age at enrollment (years)					
Mean (SD)	65.6 (10.3)	65.6 (9.8)	1.00	0.97 – 1.03	0.98
Median (Range)	65 (50, 87)	65 (51, 87)			
Age category at enrollment, n (%)					
50-59 years	18 (31.0%)	38 (32.8%)	1	ref	
60-69 years	19 (32.8%)	40 (34.5%)	1.00	0.45 – 2.21	0.99
70-79 years	14 (24.1%)	24 (20.7%)	1.23	0.51 – 2.95	0.63
80 or above	7 (12.1%)	14 (12.1%)	1.06	0.36 – 3.10	0.92
Gender, n (%)					
Female	35 (60.3%)	70 (60.3%)	1	ref	
Male	23 (39.7%)	46 (39.7%)	1.00	0.52 - 1.92	0.99
Race/Ethnicity, n (%)					
Non-Hispanic White	57 (98.3%)	109 (94.0%)	1	ref	
Other	1 (1.7%)	7 (6.0%)	0.27	0.03 - 2.33	0.23
Marital Status, n (%)					
Married/Living with someone in a marriage-like relationship	46 (90.2%)	75 (74.3%)	1	ref	
Single	5 (9.8%)	26 (25.7%)	0.31	0.11 - 0.89	0.03
Missing	7	15			
Socioeconomic status (HOUSES in quartile), n (%)					
Q1 (lowest SES)	9 (16.4%)	12 (11.0%)	1	ref	
Q2	9 (16.4%)	19 (17.4%)	0.63	0.19 - 2.07	0.44
Q3	23 (41.8%)	35 (32.1%)	0.88	0.31 - 2.44	0.79
Q4 (highest SES)	14 (25.5%)	43 (39.4%)	0.43	0.15 - 1.26	0.12
Missing	3	7			

eTable 3: Characteristics of participants with RSV-positive ARI and matched participants with RSV-negative ARI during pre-pandemic RSV Season for measurement of outcomes at the time of enrollment					
	Subjects with RSV-positive ARI (n=58)	**Subjects with RSV-negative ARI (n=116)**	**Univariate analysis**		
			Odds ratio	**95% Confidence Interval**	**P-value**
Highest grade or level of school completed, n (%)					
High school graduate or GED	7 (12.1%)	5 (4.3%)	1	ref	
Some college	17 (29.3%)	36 (31.0%)	0.34	0.09 - 1.24	0.10
Four-year college graduate (Bachelor's degree)	19 (32.8%)	40 (34.5%)	0.34	0.09 - 1.23	0.1
Graduate or professional school	7 (12.1%)	20 (17.2%)	0.25	0.06 - 1.07	0.06
Other or missing	8 (13.8%)	15 (12.9%)	0.38	0.09 - 1.62	0.19
Job Status, n (%)					
Working full time for pay (35 or more hours a week)	22 (43.1%)	37 (36.6%)	1	ref	
Working part-time for pay	8 (15.7%)	13 (12.9%)	1.03	0.37 - 2.93	0.94
Not working for pay at present	21 (41.2%)	51 (50.5%)	0.69	0.33 - 1.45	0.32
Missing	7	15			
Chronic illness (within 3-year of enrollment), n (%)					
Congestive heart failure	4 (6.9%)	4 (3.4%)	2.07	0.49 - 8.74	0.31
Asthma	6 (10.3%)	17 (14.7%)	0.67	0.25 - 1.83	0.43
Chronic obstructive pulmonary disease	3 (5.2%)	9 (7.8%)	0.65	0.17 - 2.53	0.53
Any other heart or lung disease	36 (62.1%)	61 (52.6%)	1.48	0.77 - 2.83	0.23
Diabetes mellitus	7 (12.1%)	11 (9.5%)	1.31	0.47 - 3.62	0.59
Immunosuppressed conditions	6 (10.3%)	13 (11.2)	0.91	0.33 - 2.57	0.86
Charlson comorbidity index					
Mean (SD)	1.0 (1.4)	1.0 (1.2)	1.04	0.81 - 1.32	0.77
Median (Range)	0 (0, 6)	1 (0, 4)			
Previous vaccination before enrolment					
Influenza vaccination (within 1 year), n (%)	24 (41.4%)	32 (27.6%)	1.85	0.95 - 3.62	0.07

eTable 3: Characteristics of participants with RSV-positive ARI and matched participants with RSV-negative ARI during pre-pandemic RSV Season for measurement of outcomes at the time of enrollment					
	Subjects with RSV-positive ARI (n=58)	**Subjects with RSV-negative ARI (n=116)**	**Univariate analysis**		
			Odds ratio	**95% Confidence Interval**	**P-value**
Pneumococcal vaccination (ever), n (%)	30 (51.7%)	53 (45.7%)	1.27	0.67 - 2.41	0.45
Time after the latest Pneumococcal vaccination, years					
Mean (SD)	2.8 (3.5)	2.0 (1.9)	1.12	0.92 - 1.36	0.24
Median (Range)	3 (0, 19)	2 (0, 8)			
History of RSV infection before enrollment*, n (%)	0 (0%)	1 (0.9%)	-		
SF-36					
Physical function					
Mean (SD)	85.5 (19.1)	85.6 (16.5)	1.00	0.98 - 1.02	0.97
Median (Range)	90 (10, 100)	90 (25, 100)			
Missing	1	1			
Role limitations due to physical health					
Mean (SD)	89.2 (26.1)	87.3 (28.5)	1.00	0.99 - 1.01	0.66
Median (Range)	100 (0, 100)	100 (0, 100)			
Missing	0	2			
Role limitations due to emotional problems					
Mean (SD)	93.0 (19.7)	90.9 (23.2)	1.00	0.99 - 1.02	0.56
Median (Range)	100 (0, 100)	100 (0, 100)			
Missing	1	2			
Energy/fatigue					
Mean (SD)	68.9 (19.0)	68.4 (17.9)	1.00	0.98 - 1.02	0.84
Median (Range)	75 (20, 100)	70 (10, 100)			
Missing	2	3			
Emotional					
Mean (SD)	84.9 (10.4)	84.5 (13.8)	1.00	0.98 - 1.03	0.84

eTable 3: Characteristics of participants with RSV-positive ARI and matched participants with RSV-negative ARI during pre-pandemic RSV Season for measurement of outcomes at the time of enrollment					
	Subjects with RSV-positive ARI (n=58)	**Subjects with RSV-negative ARI (n=116)**	**Univariate analysis**		
			Odds ratio	**95% Confidence Interval**	**P-value**
Median (Range)	88 (48, 100)	88 (32, 100)			
Missing	1	3			
Social functioning					
Mean (SD)	92.7 (15.2)	93.6 (14.5)	1.00	0.97 - 1.02	0.69
Median (Range)	100 (37.5, 100)	100 (25, 100)			
Missing	0	1			
Pain					
Mean (SD)	81.8 (18.2)	80.4 (18.7)	1.00	0.99 - 1.02	0.62
Median (Range)	90 (22.5, 100)	90 (22.5, 100)			
Missing	0	2			
General health					
Mean (SD)	75.4 (15.2)	74.7 (15.4)	1.00	0.98 - 1.02	0.77
Median (Range)	75 (25, 100)	75 (30, 100)			
Missing	1	2			
Activities of Daily Living (ADL) and frailty at enrollment					
Barthel ADL Index					
Mean (SD)	98.9 (2.9)	99.3 (2.6)	0.95	0.84 - 1.07	0.36
Median (Range)	100 (85, 100)	100 (80, 100)			
Missing	4	2			
Lawton - Brody ADL Instrumental Scale					
Mean (SD)	7.8 (0.9)	7.9 (0.3)	0.63	0.34 - 1.16	0.13
Median (Range)	8 (3, 8)	8 (6, 8)			
Missing	1	2			
FRAIL Scale health status, n (%)					
Robust	47 (81.0%)	84 (73.7%)	1	ref	

eTable 3: Characteristics of participants with RSV-positive ARI and matched participants with RSV-negative ARI during pre-pandemic RSV Season for measurement of outcomes at the time of enrollment					
	Subjects with RSV-positive ARI (n=58)	**Subjects with RSV-negative ARI (n=116)**	**Univariate analysis**		
			Odds ratio	**95% Confidence Interval**	**P-value**
Pre-Frail	10 (17.2%)	29 (25.4%)	0.62	0.27 - 1.39	0.24
Frail	1 (1.7%)	1 (0.9%)	1.79	0.11 - 30.14	0.68
Missing	0	2			
EQ-5D-3L Single Summary Index (USA)					
Mean (SD)	0.94 (0.09)	0.93 (0.09)	1.12	0.78 - 1.62	0.54
Median (Range)	1.00 (0.63, 1.00)	1.00 (0.52, 1.00)			

*History of RSV was based on ICD10 codes from medical record which most likely under-represents the true number

eTable 4: Assessment of the impact of RSV-positive ARI compared to matched RSV-negative ARI during pre-pandemic RSV Season on short-term frailty outcomes (within 4 weeks after ARI). There was no significant intermediate-term (6-7 months after ARI) and long-term (12-13 months after ARI) impact on frailty at any point in the follow-up duration.

	Subjects with RSV-positive ARI (N=58)	Subjects with RSV-negative ARI (N=116)	Univariate analysis			Adjusted analysis		
			Effect size	95% Confidence Interval	P-Value	Effect size	95% Confidence Interval	P-Value
Activities of Daily Living (ADL) and frailty								
Barthel ADL Index*								
Mean (SD)	98.6 (3.6)	99.0 (2.3)	-0.28	-1.24, 0.69	0.568	-0.34	-1.31, 0.63	0.49
Median (Range)	100 (80, 100)	100 (90, 100)						
Missing	7	14						
Lawton - Brody ADL Instrumental Scale*								
Mean (SD)	7.8 (0.9)	7.9 (0.3)	-0.06	-0.18, 0.07	0.380	-0.03	-0.13, 0.07	0.52
Median (Range)	8 (2, 8)	8 (6, 8)						
Missing	6	10						
FRAIL Scale health status*, n (%)								
Robust	37 (72.5%)	77 (72.6%)	1	ref		1	ref	
Pre-Frail/Frail	14 (27.5%)	29 (27.4%)	1.20[$]	0.51, 2.84	0.673	1.32[$]	0.52, 3.33	0.55
Missing	7	10						
EQ-5D Single Summary Index (USA) *								
Mean (SD)	0.94 (0.08)	0.93 (0.10)	0.09	-0.15, 0.32	0.464	0.10	-0.15, 0.35	0.42
Median (Range)	1.00 (0.83, 1.00)	1.00 (0.46, 1.00)						
Missing	6	10						
Physical frailty results								
Average Grip Strength (kg) *								

Mean (SD)	30.8 (9.8)	32.8 (10.4)	-1.78	-3.79, 0.24	0.083	-1.48	-3.54, 0.58	0.16
Median (Range)	29.9 (11.2, 64.6)	31.5 (14.4, 57.7)						
Missing	7	13						
Gait Velocity (m/sec) *								
Mean (SD)	1.37 (0.32)	1.40 (0.39)	-0.02	-0.13, 0.08	0.681	0.00	-0.12, 0.11	0.95
Median (Range)	1.34 (0.62, 2.50)	1.31 (0.68, 2.50)						
Missing	6	10						
Total Meters Walked (m) *								
Mean (SD)	488.1 (95.3)	505.1 (96.0)	-16.82	-44.47, 10.84	0.231	-10.04	-36.82, 16.73	0.46
Median (Range)	494 (136, 670)	513 (245, 690)						
Missing	6	10						
Spirometry results^								
Predicted FEV1%*								
Mean (SD)	88.2 (17.5)	92.4 (16.2)	-4.27	-9.86, 1.32	0.133	-3.52	-9.49, 2.44	0.24
Median (Range)	87 (48, 127)	95 (44, 122)						
Missing	7	10						
Predicted FVC%*								
Mean (SD)	91.6 (14.7)	95.20 (14.7)	-3.52	-8.31, 1.26	0.147	-3.13	-8.35, 2.08	0.24
Median (Range)	92 (53, 126)	98 (49, 120)						
Missing	7	10						
Predicted FEV1/FVC%*								
Mean (SD)	91.1 (10.6)	91.9 (10.4)	-0.82	-4.08, 2.44	0.619	0.45	-2.99, 3.89	0.80
Median (Range)	91 (74, 109)	95 (64, 108)						
Missing	7	11						
Predicted PEF%*								
Mean (SD)	86.3 (22.3)	93.5 (20.0)	-7.28	-13.75, -0.80	0.028	-7.07	-13.83, -0.30	0.04
Median (Range)	87 (33, 124)	92 (38, 146)						
Missing	8	11						
Predicted FEF 25-75								

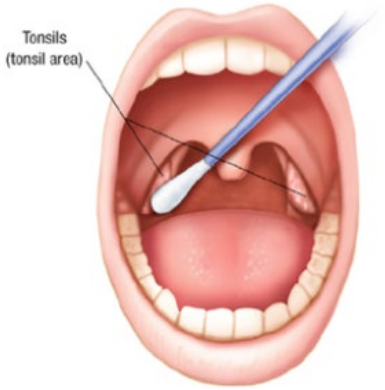
%*								
Mean (SD)	85.2 (30.6)	88.6 (27.6)	-3.48	-13.21, 6.24	0.479	-2.25	-12.38, 7.87	0.66
Median (Range)	86 (31, 150)	90 (22, 157)						
Missing	8	10						

* Frailty and physical frailty outcomes without significant impact on short-term, intermediate-term and long-term frailty measurements and therefore only the short-term outcome is reported; ^Spirometry results were only available at the short term follow up (due to risk of spirometry as an aerosol generating procedure during COVID-19 pandemic) so no further time points were reported even if significant

eTable 5. Instruction for self-swab

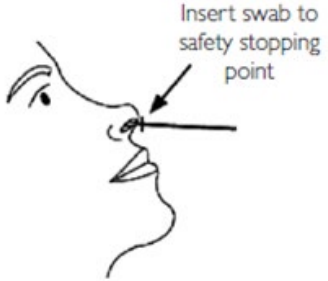
A. Throat swab

1. Wash your hands with soap and water. Dry them with a clean towel.
2. Open the tongue depressor package, if needed.
3. Open the swab package. Keeping the collection container in the package, remove the cap from the collection container.
4. Touching only the plastic stick end, take the swab out of the package.
5. If swabbing yourself, stand or sit in front of a mirror. If swabbing someone else, stand or sit in front of the person.
6. Open mouth wide so you can see the tonsils or the areas where the tonsils used to be.
7. Use the tongue depressor to hold the tongue down, if needed.
8. Carefully guide the swab to the back of the throat. Avoid touching the tongue or roof of the mouth.
9. Rub the swab back and forth at least two times on each tonsil. Or rub the areas where the tonsils used to be.
10. Carefully guide the swab out of the mouth. Do not lay down the swab.
11. Firmly push the swab into the collection container and replace cap.
12. Throw away the tongue depressor, if you used one.
    (Reference: Patient Education MC 1492-07)



B. Nose swab

1. Remove another swab from the plastic sleeve and hold by the handle
2. Position yourself with your head slightly tilted back.
3. Gently insert the swab in either the nostril to about one inch or until you meet resistance.
4. With the swab in place, rotate in a circular motion 2 times and then keep it there for 15 sec.
5. Remove the swab. Do not lay the swab down.
6. Place the swab into the collection container with the other swab (throat swab) and firmly tighten cap
7. Wash your hands with soap and water.
8. Bring the collection container to the location you were told.
    (Reference: Dhiman et al Mayo Clinic Proceeding 2012: modified)



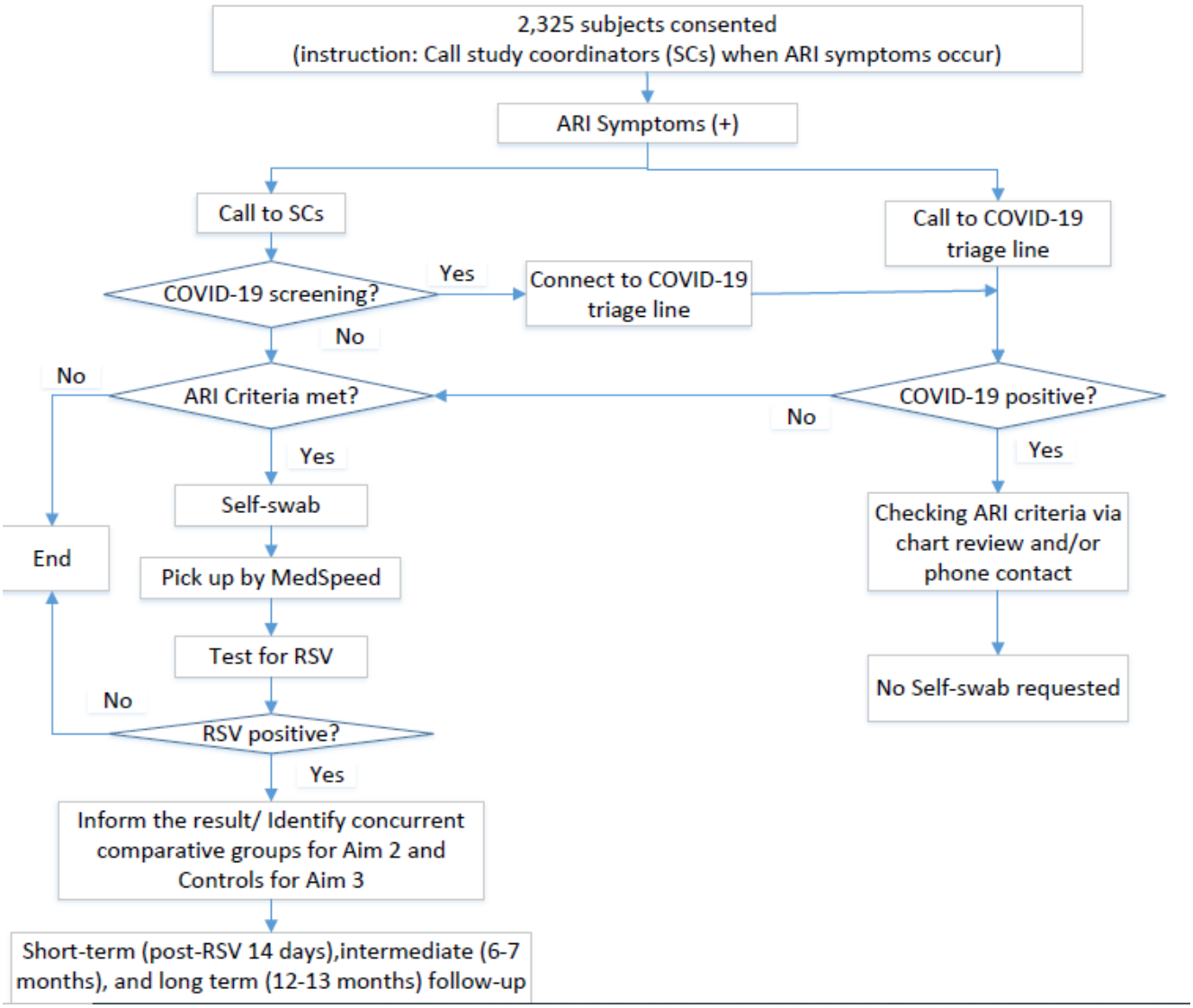
eTable 6. Sources and definitions of variables			
Variable	Source	Determined by:	
RSV and Influenza	Reverse transcription polymerase chain reaction (RT-PCR)	Positive lab test	
Age in years at enrolment, gender, race/ethnicity	Electronic health records (EHRs) and self-report of RSV study	Electronic health records and self-report from questionnaire of RSV study	
Geographic location:	EHRs and self-report of RSV study	Address information at time of enrollment from Electronic Health Records or self-report	
Rurality by living address	Self-reported address information of RSV study	Address information at time of enrollment. 2010 Census bureau based on population size, Land use, etc. (https://www.census.gov/programs-surveys/geography/guidance/geo-areas/urban-rural.html)	
Socioeconomic status (SES, defined by HOUSES index)	Self-reported address information of RSV study and real property data	Address information at time of enrollment. A validated individual socioeconomic status (SES) measure based on housing features (the lower HOUSES, the lower SES). Quartiles were defined by the county population in 2014 using county-specific real property data. HOUSES quartile data will be accompanied with specific counties where HOUSES were calculated.	
History of chronic illness	Any diagnostic codes (ICD10) within 3 years from the enrollment date	**Condition**	**ICD 10 Codes**
		Congestive heart failure	I50.XXX
		Asthma	J45.XXX
		Chronic Obstructive Pulmonary Disease	J40.XXX, J41.XXX, J42.XXX, J43.XXX, J44.XXX, J47.XXX
		Any other heart or lung disease	I05.XXX-I16.XXX, I26.XXX-I28.XXX, I30.XXX-I52.XXX, (except I30.XXX, I33.XXX, I40.XXX), J60.XXX-J92.XXX,

			J98.XXX (except J98.01)
		Diabetes mellitus	E08.XXX-E13.XXX
		Immunosuppressed Conditions	B20.XXX, D80.XXX-D89.XXX Z94.XXX, T86.XXX C81.XXX-C96.XXX C00.XXX-C80.XXX, C7A.XXX, C7B.XXX (ICD-10) AND 96401-96417, 96420-96425, 96440-96549 (CPT) Immunosuppressive medication: Janus kinase inhibitors: tofacitinib; Calcineurin inhibitors: cyclosporine, tacrolimus Interleukin inhibitors: rilonacept, canakinumab, brodalumab, anakinra, reslizumab, ustekinumab, mepolizumab, tocilizumab, dupilumab, ixekizumab, benralizumab, guselkumab, secukinumab, tildrakizumab, sarilumab, basiliximab,

			risankizumab, siltuximab, daclizumab; Selective Immunosuppressants: alefacept, sirolimus, efalizumab, mycophenolate, belimumab, natalizumab, fingolimod, leflunomide, dimethyl fumarate; TNF alfa inhibitors: etanercept, infliximab, golimumab, certolizumab, rituximab, adalimumab; Other immunosuppressants: pomalidomide, methotrexate, omalizumab, azathioprine, lenalidomide, thalidomide, everolimus, abatacept, vedolizumab, teriflunomide, lymphocyte immune globulin, emapalumab, siponimod, belatacept, baricitinib, muromonab-CD3, eculizumab, anti-thymocyte globulin

			(rabbit), ravulizumab:
History of RSV infection	Any diagnostic code (ICD10) or positive lab test ever (from all medical records)	B97.4, J12.1	
Vaccine history (influenza vaccination, pneumococcal vaccination)	Any Procedural code (CPT) within 1 year from the enrolment date	**Condition**	**CPT Codes**
		Seasonal Influenza	90630 90653 90653 90654 90655 90656 90657 90658 90660 90661 90662 90663 90664 90666 90667 90668 90672 90673 90674 90682 90685 90686 90687 90688 90689 90756 4037F 4274F G0008 G8108 G8109 G8110 G8423 G8424 G8425 G8426 G8482 G8483 G8484 G8636 G8637 G8638 G8639 G8640 G8641 G9141 G9142 Q0034 Q0158 Q2033 Q2034 Q2035 Q2036 Q2037 Q2038 Q2039
		Pneumococcal vaccination (PPSV23 or PCV 13)	90732 90670
All other questionnaire data	Self-reported information collected at the consent visit by study questionnaire		

eFigure 1. Workflow for identifying RSV cases and controls including COVID-19 era



eFigure 2. Flow diagram for subject recruitment and retention