SUPPLEMENTAL FILE

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Recruitment

Eligible adults were initially contacted by their general practitioner to inform them about the study by means of an invitation letter. In Belgium and the Netherlands an opt-out procedure was used in which patients were subsequently contacted by the study team unless they indicated they preferred not to be contacted. In the United Kingdom, an opt-in strategy was used in which participants were contacted upon active consent for contact following the initial GP invitation letter. Upon contact, patients were informed about the exact study procedures and in- and exclusion criteria were verified by telephone. Verbal consent was obtained to plan a baseline visit. Written informed consent was signed during the baseline visit in August-September each year. To ensure that all age groups were represented, it was indicated in the study protocol that half of the study population should be aged >75 years. This allowed the sites to deliberately recruit more older participants in the second season to ensure a good representation of all age groups as intended per protocol.

Sensitivity analyses

Sensitivity analyses included imputation of test results of six participants with a missed visit and 13 participants in whom viral testing was delayed. This showed an incidence of 4.4% (+0.2%) in the first, and 7.4% (+0.2%) in the second season. Subsequent addition of participants with probable seroconversion (≥2-fold increase in serum antibodies, n=83) as RSV cases resulted in a total incidence of 8.0% (5.8–10.6%) in the first, and 9.9% (7.5-12.8%) in the second RSV-season.

Lab analyses

Sample collection

During the home visit two nasopharyngeal swabs were collected for viral diagnostics (FLOQSwab™, 3ml UTM Xpert viral transport medium, Copan diagnostics and MicroTest M4RT, Remel). RSV and Influenza were tested within 24 hours after the home visit from the nasopharyngeal sample using the Xpert® Xpress Flu/RSV assay (Cepheid, Sunnyvale, CA, USA)[9], a point of care qualitative real-time PCR. The second nasopharyngeal swab was stored at -80 degrees. After the second RSV-season these second nasopharyngeal samples were tested for RSV (including subtyping) using an in-house quantitative PCR (qPCR). RSV specific pre- and post-fusion as well as neutralizing antibodies in serum were measured at baseline and after the RSV-season. Serology analysis was not performed for influenza. Specification of the specific lab analyses is shown hereafter.

Polymerase Chain Reaction (PCR)

Quantitative Reverse Transcription-Polymerase Chain Reaction (RT-PCR) was used to discriminate RSV A and RSV B subtypes. RSV A and RSV B RNAs extracted from the nasal-swabs are detected and quantified in a duplex RT-PCR format using specific amplification primers and fluorescent probes designed in the RSV N gene, encoding the RSV nucleocapsid protein. The process involves nucleic acids extraction, conversion of RNA to complementary deoxyribonucleic acid (DNA) by reverse transcription and detection by real-time PCR reaction using a calibration curve (absolute quantitation). The limit of detection (LOD) for RSV-A is 304 copies/ml of swab while for RSV-B the LOD is 475 copies/ml of swab. The RSV viral load is reported as copies of RSV RNA per mL of sample.

Serology

Pre-F ELISA:

Streptavidin ELISA plates are coated with biotinylated Pre-F protein [1]. Plates that contain a reference standard and those with the clinical samples are incubated for two hours. Subsequently, an HRP-conjugated mouse anti-human IgG(Fc) detection antibody is added and the antigen specific IgG binding is measured by a luminescent readout. From the mean of the duplicate measurement, Gen5

software is used to calculate the anti-RSV-Pre-F antibody concentration, by referring the samples' luminescence to the 4-PL fit of the standard curve. The antibody concentration is reported in arbitrary EU/L. Lower limit of quantification (LLOQ) of the assay is 14.1 EU/L and upper limit of quantification (ULOQ) is 56224 EU/L.

Post-F ELISA:

Post-F protein coated ELISA plates containing a reference standard and clinical samples are incubated for two hours [1]. Subsequently, an HRP-conjugated mouse anti-human IgG(Fc) detection antibody is added and the antigen specific IgG binding is measured by a luminescence readout. From the mean of the duplicate measurement, Gen5 software is used to calculate the anti-RSV-Post-F antibody concentration, by referring the samples' luminescence to the 4-PL fit of the standard curve. The antibody concentration is reported in arbitrary EU/L. LLOQ of the assay is 6.6 EU/L and ULOQ is 44280 EU/L.

Neutralizing antibodies RSV-A2 µPRNT50:

Test samples were heat inactivated, initially diluted 1:50, and 2-fold serially diluted in Virus Growth Medium (DMEM-Glutamax, 2% of FBS and 1% penicillin streptomycin). Virus was diluted to obtain 150 PFUs/wells in VGM, and added in a 1:1 ratio to diluted serum. After incubation at 37°C for one hour, plates containing Vero cells were inoculated with this mixture, centrifuged for 10 minutes at 700 x g and incubated at 37°C for one hour. Inoculum was removed and the methylcellulose overlay (0.75% in 1X MEM-2% FBS-2% PS, 8 mM glutamine, 0.2% $NaHCO_3$) is added on wells and plates were incubated at 37°C, 5% CO2 for 40 hours (±2h). After fixation (cold acetone 85%, 4°C for 1h), plates were immunostained with anti-RSV mouse antibody (Abcam 24011) 1h30 at 37°C then goat anti-mouse IgG PE (Invitrogen P852) for 1h30 at 37°C.The plates were enumerated with an Ensight reader (Perkin Elmer) and titers were quantified as the reciprocal serum dilution to obtain 50% virus inhibition.

References

1. Krarup, A., et al., *A highly stable prefusion RSV F vaccine derived from structural analysis of the fusion mechanism.* Nat Commun, 2015. **6**: p. 8143.

Supplemental tables

Table S1: Respiratory tract infections and diagnostics									
	2017-2018				**2018-2019**				**Total**
	NL	**BEL**	**UK**	**Total**	**NL**	**BEL**	**UK**	**Total**	
Participants	148	204	175	527	208	131	174	513	**1040**
ARTI Episodes	136	154	125	415	177	122	130	429	**844**
POCT (Cepheid Xpert) performed	133	133	124	390	177	108	130	415	**805**
Validation qPCR (in-house) performed	126	127	106	359	173	107	120	400	**759**
Serology assays (seroconversion) performed	124	185	142	451	163	116	140	419	**870**
RSV infection									
Molecular tests									
POCT (Cepheid Xpert)	5	3	2	10	8	10	6	24	**34**
PCR (qPCR in-house)	6	2	2	10	9	9	4	22	**32**
RSV A (based on qPCR)	2	2	1	5	0	0	1	1	**6**
RSV B (based on qPCR)	4	0	1	5	9	9	3	21	**26**
Any PCR (POCT or qPCR)	6	3	2	11	9	10	6	25	**36**
Serology									
Neutralizing antibodies (≥4 fold)	3	2	1	6	6	3	4	13	**19**
Pre-Fusion antibodies (≥4 fold)	3	3	3	9	11	4	5	20	**29**
Post-Fusion antibodies (≥4 fold)	3	3	5	11	8	3	5	16	**27**
Seroconversion (≥4 fold)	5	4	6	15	11	6	7	24	**39**
Probable seroconversion (≥2 <4 fold)	8	9	6	23	11	4	6	21	**44**
RSV outcomes									
RSV-illness*	8	6	8	22	15	12	10	37	**59**
RSV (sensitivity)**	13	14	14	41	23	14	13	50	**91**
Influenza infection									
Influenza A (POCT, Cepheid)	3	3	8	14	3	10	4	17	**31**
Influenza B (POCT, Cepheid)	10	12	7	29	0	0	0	0	**29**
Any influenza (POCT, Cepheid)	13	15	15	43	3	10	4	17	**60**
Other infection***	113	115	107	335	163	88	120	371	**706**

NL = Netherlands, BEL = Belgium, UK = United Kingdom Numbers represent either number of cases or positive tests. *Primary outcome of either PCR positive RSV infection or ≥4-fold increase (seroconversion) in any RSV antibody. ** RSV based on positive PCR or seroconversion of ≥2-fold increase in any RSV antibody *** RSV and influenza negative by POCT and PCR.

Table S2. Characteristics of RSV patients								
	PCR positive				**PCR negative***		**No PCR (No ARTI)**	
	Seropositive (≥4-fold) N = 16	Probable seroconversion (≥2 <4-fold) N = 11	No seroconversion N = 4	No serum available N = 5	Seropositive (≥4-fold) N = 16	Probable seroconversion (≥2 <4-fold) N = 20	Seropositive (≥4-fold) N = 7	Probable seroconversion (≥2 <4-fold) N = 12
Age (years)ϕ	76 [67-89]	70 [64-82]	78 [66-82]	74 [63-86]	73 [62-82]	75 [61-88]	78 [73-84]	70 [61-95]
Cardiopulmonary disease	7 (44%)	2 (18%)	1 (25%)	2 (40%)	2 (13%)	8 (40%)	2 (29%)	2 (17%)
Number of infections	16	11	4	5	23	27	0	0
Missed visits (No PCR)	0	0	0	0	3	1	0	0
Medical attendance	5 (31%)	4 (36%)	0 (0%)	2 (40%)	4/23 (17%)	8/27 (30%)	-	-
Time onset disease until PCR sampling (days)ϕ	3 [2-6]	4 [2-7]	3 [2-4]	3 [2-7]	4 [1-10]	4 [1-9]	-	-
Delayed sampling (> 7 days)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	3/20 (15%)	3/26 (12%)	-	-
Time ARTI until convalescence serology (weeks)ϕ	21 [9-29]	24 [6-27]	24 [20-28]	26 [21-29]	23 [3-36]	20 [4-34]	-	-

Values are numbers and percentage of cases with that characteristic unless otherwise indicated by the ϕ indicating median [range]. * Only those with serologic evidence of RSV are included in this table; Patients without a PCR but with a reported (missed) infection are included in these columns.

Table S3. Characteristics patients with RSV-illness compared to the total study population		
	Total study population N = 1040	RSV-illness* N=59
Study site:		
Belgium	335 (32%)	23(38%)
Netherlands	356 (34%)	18 (31%)
United Kingdom	349 (34%)	18 (31%)
Age:		
Years median [range]	75 [60-100]	75 [62-89]
Age above 75	562 (54%)	33 (56%)
Female sex	554 (54%)	36 (61%)
Northwest European†	999 (97%)	56 (95%)
Living situation:		
Alone	341 (33%)	18 (31%)
Only adults in the household	667 (64%)	40 (68%)
Children in the household	32 (3%)	1 (1%)
High educational level‡	394 (38%)	27 (46%)
Comorbidity§		
Cardiovascular disease	212 (21%)	10 (17%)
Lung disease	120 (12%)	6 (10%)
Cardiovascular or lung disease	307 (30%)	16 (27%)
Diabetes	80 (8%)	3 (5%)
Allergies (any) [1]	276 (27%)	15 (26%)
Hay fever	59 (6%)	4 (7%)
House dust mite	32 (3%)	0 (0%)
Respiratory medication	174 (17%)	9 (16%)
Polypharmacy (>4 medicines)	372 (36%)	19 (32%)
Pneumococcal vaccination[2]	118 (13%)	10 (18%)
Influenza vaccination[3]	752 (76%)	47 (81%)
Smoking status		
Current smoker	80 (8%)	7 (12%)
Former smoker	409 (39%)	22 (37%)
Alcohol status		
Current drinker	666 (64%)	30 (53%)
Average amount (mode)	1-7 glasses/week	1-7 glasses/week
Frailty[4]		
GFI score median [range]	2 [0-12]	2 [0-7]
Frail (score > 4 points)	148 (15%)	6 (10%)

Values are numbers and percentage of cases with that characteristic unless otherwise indicated * Based on positive PCR at the moment of acute infection or seroconversion ≥4-fold over baseline. †Defined as university of applied sciences or higher. ‡ Groningen Frailty Indicator (GFI) score of ≥4 points. Missing data <1% is not shown, if more than 1% is missing, the percentages are added as footnote. [1]missing N=52 (5%), [2]missing N=95 (9%), [3]missing baseline N=78 (8%), missing end-of-season N=114 (11%), missing either N=180 (17%) [4]missing N=62.

Table S4. Severity of ARTI specified by RSV and influenza subtypes				
	RSV-A N= 6	**RSV-B N= 26**	**Influenza-A N=31**	**Influenza-B N= 29**
Duration of symptoms; median [IQR]	10 [8-18]	19 [13-27]	18 [14-22]	17 [14-25]
Unresolved illness[a]	1 (17%)	5 (19%)	5 (19%)	4 (14%)
Medication[b]	3 (50%)	4 (15%)	13 (43%)	13 (45%)
Respiratory medication	3 (50%)	3 (12%)	7 (23%)	6 (21%)
Antibiotics	0 (0%)	1 (4%)	9 (30%)	9 (31%)
Antivirals	0 (0%)	0 (0%)	1 (3%)	1 (3%)
Corticosteroids	0 (0%)	0 (0%)	1 (3%)	1 (3%)
Medical attendance	1 (17%)	8 (31%)	19 (61%)	17 (59%)
Hospitalization	0 (0%)	0 (0%)	1 (3%)	0 (0%)
Emergency department	0 (0%)	0 (0%)	0 (0%)	0 (0%)
General practitioner visit	1 (17%)	7 (27%)	16 (55%)	16 (55%)
Telephone call to doctor	0 (0%)	1 (4%)	2 (7%)	1 (3%)
LRTI[c]	0 (0%)	0 (0%)	0 (0%)	1 (3%)
Death	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Severity classification				
Mild	3 (50%)	18 (69%)	11 (36%)	9 (31%)
Moderate	3 (50%)	8 (31%)	17 (55%)	19 (66%)
Severe	0 (0%)	0 (0%)	3 (10%)	1 (3%)

No statistical analysis has been done on these subgroups because of low numbers. Abbreviations: IQR=interquartile range; LRTI = Lower respiratory tract infection. 4 RSV cases had unknown subtyping since they were only tested using the POCT and were therefore excluded from this table [a] Illness that persisted beyond the 28 diary days. [b] Enhanced use or newly prescribed inhaled respiratory medication, antibiotics, antivirals or corticosteroids. [c] clinically diagnosed or radiologically confirmed pneumonia.

Table S5. Risk groups and severity of PCR confirmed RSV-ARTI				
		Duration of symptoms median days(range)	Medical attendance	Medication[a]
Cardiopulmonary Comorbidity[b]	Yes (n=12)	19 (6-28)	5 (42%)	4 (33%)
	No (n=24)	20 (4-28)	6 (25%)	6 (25%)
Cardiac comorbity	Yes (n=7)	19 (13-28)	3 (43%)	2 (29%)
	No (n=29)	20 (4-28)	8 (28%)	8 (28%)
Lung comorbidity	Yes (n=5)	20 (6-28)	2 (40%)	2 (40%)
	No (n=31)	19 (4-28)	9 (29%)	8 (26%)
Old age (>75 years)	Yes (n=20)	20 (7-28)	5 (25%)	5 (25%)
	No (n=16)	19 (4-28)	6 (38%)	5 (31%)
Frail (GFI ≥4)	Yes (n=2)	18 (8-28)	0 (0%)	2 (100%)
	No (n=32)	19 (4-28)	10 (31%)	8 (25%)

None of the differences were statistically significant at p-value<0.05. [a] Enhanced use or newly prescribed inhaled respiratory medication, antibiotics, antivirals or corticosteroids. [b] Either cardiac or lung comorbidity. Cardiac comorbidity included all arrhythmias, structural heart diseases, angina and cardiac events such as infarction, percutaneous coronary intervention and bypass surgery. Hypertension was not included in this definition. Lung disease included asthma, COPD, chronic bronchitis and emphysema.

WEEK 1; symptoms each day

For each day, please give every symptom a score from 0 to 6. If you score 0 for all symptoms for two consecutive days, complete the weekly questions for week 1.

Score	Severity of symptom:
0	Normal/not affected
1	Very little problem
2	Slight problem
3	Moderately bad
4	Bad
5	Very bad
6	As bad as it could be

Symptoms	***Day 1***	***Day 2***	***Day 3***	***Day 4***	***Day 5***	***Day 6***	***Day 7***
Cough							
Phlegm (coughing up slime)							
Shortness of breath							
Wheeze (during breathing out)							
Blocked/runny nose							
Muscle Ache							
Headache							
Disturbed sleep							
Feeling generally unwell							
Interference with normal activities/work							
Interference with social activities							

Please score your temperature daily;

Score	Severity of symptom
……°C	If measured, please write down the degrees Celsius
0	Not measured but does not feel warm/feverish
1	Not measured but does feel warm/feverish

	Day 1	***Day 2***	***Day 3***	***Day 4***	***Day 5***	***Day 6***	***Day 7***
Temperature							