POS-ARI-PC CORE – STUDY SPECIFIC APPENDIX 1 (SSA1):

POS-ARI-PC-001

Study on the incidence of medically-attended respiratory syncytial virus, human metapneumovirus, human parainfluenza virus and rhinovirus amongst older adults in Europe

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1. LIST OF ABBREVIATIONS

ARI	Acute Respiratory Infections
CI	Confidence interval
ECDC	European Centre for Disease Prevention and Control
Ecraid	European Clinical Research Alliance on Infectious Diseases
F2F	Face-to-face
GP	General Practitioner
HMPV	Human metapneumovirus
HPIV	Human parainfluenza virus
NCT	National coordinating team
PC	Primary Care
PCR	Polymerase Chain Reaction
POS	Perpetual Observational Study
PPAS	Point Prevalence Audit Survey
RSV	Respiratory syncytial virus
RTI	Respiratory Tract Infection
RV	Rhinovirus
SAP	Statistical Analysis Plan
SSA1	Study-specific Appendix 1
VALUE-Dx	The value of diagnostics to combat antimicrobial resistance by optimising antibiotic use
VAS	Visual analogue scale

2. INTRODUCTION, RATIONALE AND PROTOCOL STRUCTURE

Respiratory syncytial virus (RSV), human metapneumovirus (HMPV), human parainfluenza virus (HPIV) and rhinovirus (RV) are viral pathogens associated with respiratory tract infections. They are a common cause of worldwide respiratory illness burden and morbidity and with mortality particularly in older adults and immuno-compromised individuals. Despite their public health impact, no effective licensed vaccines or antiviral therapies for the prevention or treatment of these viral infections are currently available[1]. There is an increasing amount of published and ongoing research into the incidence and clinical burden of RSV in older adults[2–6]. However, there is a paucity of data on the incidences of HMPV, HPIV and RV in elderly population -particularly in those attending their general practitioner with respiratory complaints-, and of health impact data related to infection with these viruses. Such information has great relevance to decisions about vaccine development, evaluation, and procurement.
This POS-ARI-PC-001 study will be conducted as a prospective observational study to estimate the incidence of medically-attended RSV, HMPV, HPIV and RV infections in an elderly population in up to five European countries.

The POS-ARI-PC Core protocol describes the overall structure and key study procedures, while this Study Specific Appendix (SSA) outlines relevant changes and details of additional data collection for POS-ARI-PC-001.

3. OBJECTIVES

POS-ARI-PC-001 has the following specific objectives:

Primary objective:

To estimate overall incidences of medically-attended RSV, HMPV, HPIV and RV in adults 60 years and over, and compare between sites and countries, by:

a. Determining incidence rates of medically-attended RSV, HMPV, HPIV and RV infection from sampling included patients;
b. Working with pre-known/determined numbers of adults aged 60 years and over in the total practice population, and the overall age distribution in the country, or, where needed, per region/district or province;
c. Determining attendance for acute RTI in the registered practice patients of 60 years and over per month, over 1 year.

Secondary objectives:

1. To describe clinical and demographic characteristics of included patients, overall, by country and by aetiology.
2. To describe the current management, and clinical outcomes, overall, by country and by aetiology.

3. To describe course of illness (including day feeling recovered, course of individual symptoms, and participant's self-rated health (VAS-score)) related to medically-attended RSV, HMPV, HPIV and RV infection over 14 days (and potentially for other pathogens of interest).
4. To describe healthcare resource utilization (GP visits, hospital visits, hospitalisation and antibiotic prescribing for the RTI) related to medically-attended RSV, HMPV, HPIV and RV infection over 28 days (and potentially for other pathogens of interest).

4. STUDY DESIGN

POS-ARI-PC-001 is a prospective, multi-centre, multi-country observational study in up to 5 European countries. Priority in country selection will be: UK, France and Germany. Other countries of interest are Spain, Netherlands, or a Scandinavian country. The study will start in the UK initially, with the aim to confirm and optimise the study's operational feasibility before expanding to other countries.

National coordinating teams (NCT) will be requested to select participating sites:

- Serving a fixed and known number of registered patients;
- Able to quantify the number of patients aged 60 or older in their registered patients;
- Able to capture, during the course of the study, data on first attendance of the target population for RTI. For countries where first consultations for RTI complaints are F2F, this refers to numbers of practice and home visits. For countries where virtual consultations for first RTI complaints are in place, this refers to numbers of practice, home and virtual consultations. There are two options to this aim: 1) from electronic medical files, or 2) by monthly counting this number.

This will allow for extrapolation of incidence rates to national level.
Participating sites can be: general practices, primary health care facilities, or primary care homes.

NCTs or sites with previous research experience are strongly preferred, and performance in the Point Prevalence Audit Surveys (PPAS) of acute RTI in primary care will be taken into consideration during site selection. Four yearly PPASs were performed from January 2020 onwards as part of the EU-funded VALUE-Dx and RECOVER projects[7,8]. Consultations of patients with acute RTI were anonymously registered by general practitioners. This was a highly effective approach to capture presentation, illness characteristics and severity, and GPs' management (clinical investigation, diagnostic testing, prescribed medication, and provided advice) of nearly 10,000 patients across 18 European countries.

4.1 Study Period

The study will be conducted for 12 consecutive months starting as soon as possible, with patient inclusion continuing throughout one full year to allow for varying seasonality of the

viruses of interest. To ensure representative sampling throughout the 12-months period from each country and each site, a pre-defined number of patients will be recruited each month.

4.2 Eligibility

Inclusion criteria:

Eligible patients will be 60 years of age and over consulting (telephone, video, or face-to-face in the practice, or home visit) with a participating primary health care facility with:

- Symptoms suggestive of an acute lower RTI with new or increased cough as the predominant symptom, with illness duration <=7 days;
  AND/OR
- Symptoms suggestive of an acute upper RTI with sore throat and/or coryza as the predominant symptom, with illness duration <=7 days;
  AND
- Willing and able to provide informed consent and have a swab taken.

Participants who are willing to have a combined throat/nose swab taken, but not willing to provide follow-up data can still be recruited into the study.

Exclusion criteria:

As per POS-ARI-PC Core protocol, with one exception: patients needing hospitalization can be recruited into the study if timing allows. Patients can provide a swab and decide not to participate in follow-up procedures.

4.3 Sample Size Estimation

POS-ARI-PC-001 will recruit a total of approximately 2,000 patients. Details of the sampling schedule are given in section 5.

5. STUDY PROCEDURES

5.1 Screening and Eligibility Assessment

As per POS-ARI-PC Core protocol.

Additionally, daily registration of numbers of patients fulfilling the case definition, in case this cannot be extracted from electronic medical files (monthly, or at the end of the study period).

5.2 Informed Consent

As per POS-ARI-PC Core protocol.

5.3 Baseline Visit (Day 0)

As detailed in POS-ARI-PC Core protocol.

In addition, the following information will be gathered for POS-ARI-PC 001:

- Participant's self-rated health using the EQ-5D (the 5 descriptive questions and visual analogue scale (VAS));
- Employment status: employed, unemployed, retired/pension;

- Rockwood Clinical Frailty score (9-point scale).

5.4 Follow-up with Participant Diaries and/or Phone Call

As detailed in POS-ARI-PC Core protocol.

In addition, the EQ-5D will be added to the diary: the complete EQ-5D on Day 7 and 14, and the VAS daily.

As outlined in the Core protocol, participants who have not returned their diary will be phoned at Day 14 (+7 days) to capture a minimal outcome set (Day 14 phone questionnaire with complete EQ-5D added). In addition, participants who indicated in their diary not feeling recovered from the RTI will be phoned at Day 28 (+/-7 days) using the Day 28 phone questionnaire, with the same questions as the Day 14 phone questionnaire.

5.5 Follow-up with the Recruiting Site

As detailed in the POS-ARI-PC Core protocol.

6. SAMPLING

Sample collection and handling as per POS-ARI-PC Core protocol.

To account for the variable seasonality of the pathogens of interest, and variation in overall medically-attendance for acute respiratory illness, a pre-defined sampling frame will be implemented at country level. Assuming a sample size of 2,000 and 4 or 5 countries in total, each country is expected to enroll 400-500 patients throughout a 12-month study period. Patients will be enrolled consecutively at each study site from the first and third week of the month until the predefined number of samples have been obtained. The sampling frame for representativeness will be agreed upon with each site individually.

Proposed recruitment schedule with 95% confidence intervals for expected numbers of positive cases

Month	Proposed recruitment schedule	%	Pathogen											
			RSV				HMPV				HPIV			
			Prevalence	Expected cases	Range		Prevalence	Expected cases	Range		Prevalence	Expected cases	Range	
1	230	11.5%	6.30%	14.49	8.11	23.60	1.70%	3.91	1.05	9.98	2.60%	5.98	2.20	12.83
2	210	10.5%	3.10%	6.51	2.53	13.52	3.00%	6.30	2.40	13.24	1.60%	3.36	0.78	9.17
3	180	9.0%	1.20%	2.16	0.30	7.37	5.00%	9.00	4.16	16.70	1.50%	2.70	0.50	8.18
4	175	8.8%	0.66%	1.16	0.04	5.76	4.40%	7.70	3.29	15.03	2.30%	4.03	1.11	10.10
5	130	6.5%	0.37%	0.48	0.00	4.57	2.60%	3.38	0.79	9.12	3.30%	4.29	1.25	10.40
6	110	5.5%	0.17%	0.19	0.00	4.00	1.10%	1.21	0.05	5.81	3.90%	4.29	1.25	10.35
7	110	5.5%	1.60%	1.76	0.17	6.68	8.00%	8.80	4.05	16.21	4.70%	5.17	1.74	11.55
8	110	5.5%	1.70%	1.87	0.20	6.86	4.80%	5.28	1.80	11.69	3.00%	3.30	0.76	8.97
9	130	6.5%	0.79%	1.03	0.03	5.52	0.68%	0.88	0.01	5.28	3.10%	4.03	1.11	10.04
10	180	9.0%	2.30%	4.14	1.17	10.26	0.53%	0.95	0.02	5.42	3.40%	6.12	2.29	12.97
11	210	10.5%	7.00%	14.70	8.28	23.80	0.41%	0.86	0.01	5.27	3.10%	6.51	2.53	13.52
12	225	11.3%	7.60%	17.10	10.12	26.72	0.98%	2.21	0.31	7.46	2.70%	6.08	2.26	12.96
Overall	2000		3.28%[2]	65.58	50.86	83.12	2.52%[2]	50.48	37.64	66.20	2.79%[2]	55.85	42.30	72.24
			All pathogens											
			8.60%[2]	171.914	148.08	198.22								

[1] Clopper-Pearson Exact Method used to determine 95% confidence interval for the prevalence

[2] Overall estimated by taking the total expected number of cases divided by recruitment

The table shows a proposed sampling frame based on patient presentation data provided by the UK Royal College of General Practice on Communicable and Respiratory Disease and the incidences of the different viruses of primary interest. This recruitment frame was determined based on a value in-between the incidence weighted values and equal recruitment across the year. Expected numbers positive for each virus of primary interest are displayed alongside estimated 95% CIs. Overall, we would expect 172 positive cases to be detected across 2,000 samples over 12 months with a range of 148 to 198.

The sampling framework may be adjusted throughout the study depending on final number of sites included and observed epidemiological patterns throughout the study period.

The table below shows the estimated confidence intervals (CI) based on a sample size of 2,000 for different estimates of viral incidences. For example, if the true incidence is 4%, with a CI of 3.2%-5%, between 64 and 100 of the 2,000 recruited can be considered positive.

Estimated confidence intervals based on different incidences

Confidence Level	Sample size	Proportion	Confidence Interval Width[1]	95% confidence interval[1]
0.95	2,000	1%	0.9%	0.6% to 1.5%
0.95	2,000	2%	1.3%	1.4% to 2.7%
0.95	2,000	4%	1.8%	3.2% to 5.0%
0.95	2,000	4.6%	1.9%	3.7% to 5.6%
0.95	2,000	5.7%	2.1%	4.7% to 6.8%
0.95	2,000	10%	2.7%	8.7% to 11.4%

[1] Clopper-Pearson Exact Method

Sample analysis

Samples will be sent from the local laboratory to the central laboratory in Antwerp for analysis monthly.

Upon arrival in the central laboratory in Antwerp, samples will be aliquoted.

Samples will be analysed using real-time PCR by using Custom TaqMan® Array Cards (ThermoFisher Scientific) and the Fast advance Master Mix (ThermoFisher Scientific) will be applied according to the instructions of the manufacturer for the qualitative detection of influenza A, influenza B, influenza A-H1, influenza A-H3, human coronaviruses NL63, 229E, OC43 and HKU1, Middle East respiratory syndrome coronavirus, severe acute respiratory syndrome coronavirus 1 and 2, parainfluenza viruses 1, 2, 3 and 4, human metapneumovirus, rhinovirus*, respiratory syncytial virus A and B, adenovirus, enterovirus, enterovirus D68*, parechovirus, bocavirus, *Mycoplasma pneumoniae, Bordetella holmesii, Bordetella pertussis, Chlamydophila pneumoniae, Chlamydia psittaci, Legionella pneumophila, Moraxella catarrhalis, Streptococcus pneumoniae, Haemophilus influenzae* and *Staphylococcus aureus* in a Quantstudio 7 flex instrument (ThermoFisher Scientific).

* The microarray of ThermoFisher can specifically distinguish between rhinoviruses and enteroviruses (e.g. EV-D68) by the specific primers that are used.

7. STATISTICAL ANALYSIS

Descriptive statistics will be used to determine characteristics of those in the study, overall, by country, and by illness aetiology. Characteristics of participants will be presented by means and standard deviations, or median and interquartile range as appropriate for continuous measures, and numbers and percentages for categorical measures. These will be presented both by country and overall. The number of adults 60 years of age and over registered at the practice and numbers of those having attended for acute RTI will be reported per practice, and used for incidence calculations.

Estimated incidence rate of medically-attended RSV, HMPV, HPIV and RV will be calculated, along with 95% CIs using Clopper-Pearson Exact Method, and will be presented by country.

Change in clinical outcomes and quality of life measures, will be presented graphically and analysed using mixed effects models adjusted for any prognostics factors as appropriate. These models will include fixed effects for time in order to estimate change and random effects for participant and practice to allow for clustering of measures. Descriptive statistics will be presented for healthcare resource utilization.

A separate Statistical Analysis Plan (SAP) for POS-ARI-PC-001 will be developed and approved.

8. ETHICAL CONSIDERATIONS

As per POS-ARI-PC Core protocol.

The pseudonymised sample outcomes and participant data that will be collected in this study will be shared within the ECRAID-Base Consortium and with Sanofi. Participants will be informed and asked to give consent for this pseudonymised data sharing.

9. DATA MANAGEMENT

As per POS-ARI-PC Core protocol.

In order to monitor the study and the sampling frame, monthly reports will be generated per country and site including:

- Number of patients 60 years of age and over consulting for acute RTI
- Number of recruited patients
- Microbiology results

Patient population of 60 years of age and over will be the mid-period population, a method also employed by ECDC.

10. LIMITATIONS OF THE STUDY

The aim of the study to make robust incidence estimates of viral aetiologies in patients 60 years and over attending primary care for RTI complaints. The study will only sample of subset of patients (limited sample of the population). Therefore, measures need to be in place to extrapolate the incidence data to the target population, which include:

- The sampling framework to ensure a systematic enrolment and accounting for respiratory virus seasonality;
- Recruiting sites must have a defined catchment area (a fixed number of registered patients and knowing who of those is 60 years or older);
- To quantify per-practice first attendance of patients 60 years or older for RTI complaints.

These data together will enable calculating the burden of RSV, HMPV, PIV and RV in the population. Example of the calculation.

- Practice has 3,000 registered patients, 25% is 60 and over: 750 elderly patients;
- For each month: numbers of presenting patients (for example 20) multiplied by the incidence found in the study (for example 5%) results in 1/750 elderly;
- Sum all months for viral aetiologies apart and together;
- Extrapolate to the population results in health care attended for specific virus aetiology in elderly patients per 100,000 or million elderly inhabitants.

As common in observational studies there is the possibility of inclusion bias, e.g. that more or less severe patients are enrolled based on factors such as ease of sample collection, need for a home visit, or ease of explaining the study and obtain informed consent. This may affect the interpretation of the results and thereby the ability to extrapolate findings to the overall population. To minimize this, sites will report the method of consultation (practice, home visit, virtual) upon inclusion and when capturing first practice attendance of elderly patients with RTI complaints. This can be used for adjustments in the analyses. Sites will undergo training, and re-training if required, on the sampling framework with reinforcement to sequentially enroll patients until the target of the month is reached.

11. MONITORING AND QUALITY ASSURANCE

As per POS-ARI-PC Core protocol.

12. REFERENCES

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