CASE DEFINITIONS AND STATISTICAL ANALYSIS PLAN RESCEU OA MANUSCRIPT BURDEN OF DISEASE.

Date: 9th September 2019

General

"RESCEU OA" is a multicenter prospective cohort study aiming to determine the incidence of RSV in older adults aged 60 years and older in the general population. In two consecutive winter seasons (2017-2018, 2018-2019), each year a cohort of older adults was followed to identify respiratory infections during one season. RSV and influenza was tested at the moment of acute respiratory tract infection. This document contains the outcome definitions and statistical analysis plan for the description of the incidence and burden of RSV in older adults. Outcomes and definitions related to risk factor analysis and biomarker analysis from this study will not be covered in this document but discussed in separate analyses plans.

Abbreviations

ARTI = Acute respiratory tract infection
MA = Medically-attended
LRTI = Lower respiratory tract infection
PCR = Polymerase Chain Reaction
POCT = Point of Care Testing
RSV = Respiratory syncytial virus

Summary of study objectives and endpoints

	Objectives	Endpoints
Primary	1. To estimate the incidence of RSV-associated infection and RSV-associated MA-ARTI in both inpatients and outpatients.	Incidence rate of confirmed (PCR or serologic) RSV infections and incidence rate of PCR confirmed RSV infection-associated MA-ARTI in inpatients and outpatients.
Secondary	1. To estimate the incidence of influenza: a) Any influenza-ARTI b) MA-influenza-ARTI	Incidence rate of any PCR confirmed influenza and MA-influenza-ARTI in inpatients and outpatients.
	2. To estimate RSV-associated, influenza associated and all-cause mortality.	Mortality through the winter season associated with RSV, influenza and all-cause mortality.
	3. To determine change in frailty over the course of the study	Change in frailty score between start and end-of-season comparing those with RSV, influenza, other-ARTI and those without infection.
	4. To determine long-term cardiac and respiratory outcomes	Incidence of new cardiopulmonary disease diagnoses between start and end-of-season comparing those with RSV, influenza, other-ARTI and those without infection.
Post-Hoc	1. To compare the burden based on measures of disease severity.	Comparing those with RSV, influenza, other-ARTI – Duration of symptoms – Proportion of patients receiving medication (respiratory, antibiotics, antivirals, corticosteroids) – Proportion of LRTI – Proportion of medical visits – Proportion of hospital admissions – Classification of disease severity as severe, moderate or mild.

Table 1. Objectives and associated endpoints.

OUTCOME DEFINITIONS:

Primary outcome:

Incidence rate of confirmed RSV-infection (RSV-ARTI)

- Combined endpoint of confirmed RSV-infection: if either one of the following tests is positive for RSV, this confirms the RSV case-definition:
    - RSV positive PCR on nasal swab.
    - Seroconversion assay (≥4-fold increase in any RSV antibody titer). Serologic evidence for RSV infection marked by a ≥4 fold increase in **any** RSV antibody by end of season compared to baseline sample. Antibodies tested for are pre-fusion, post-fusion and neutralizing antibodies.

Incidence rate of medically-attended-RSV infection (MA-RSV-ARTI)

- Case-definition based on sample collection and report:
    - RSV-infection based on molecular test result, as cases with only serologic evidence of RSV-infection cannot be linked directly to medical attendance and will therefore not fulfil the definition of MA-RSV infection.
      AND
    - Report of medical visit for respiratory symptoms to a GP, specialist, emergency department or hospital (within 28 days of RSV confirmed infection). Medical attendance will be described for:
        - Inpatients (hospitalization)
        - Outpatients (GP, ED, other doctor visits)

Sensitivity analysis of primary outcome**:**

The sensitivity analysis is performed to assess the uncertainty in the RSV incidence based on the limitations of the diagnostic tests performed. This includes cases in which ARTI episodes did not have molecular testing by means of nasal swab collection. Additionally, some swabs may be false-negative when the time interval between onset of symptoms and sampling was long. When this time interval is more than 7 days, the chance of RSV detection decreases substantially (Walsh et al JID 2013). Second, serum antibodies could be increased over the season but still be below the cut-off of positive infection (≥2 to <4 fold increase). When RSV infection would occur early in the season while convalescence sampling would only occur several months after the infection, antibodies could have decreased again below the 4-fold threshold within this timeframe (Habibi et al Am J Crit Care Med 2015). To correct for the uncertainty of the diagnostic test procedures, the following sensitivity analyses are performed:

1. Multiple imputation will be used to impute the viral test result (RSV positive or RSV negative) in participants with a reported ARTI but without a viral test result, and in those in whom the nasal swab was collected >7 days after onset of symptoms when no data from the seroconversion assays are available. Imputation will be performed per season based using the following characteristics: age, site and month when the infection occurred and exposure to a partner with RSV.
2. Cases in which serum antibodies were increased by ≥2 fold are included as RSV-positive cases.

Secondary outcomes

1. A) Incidence rate of confirmed influenza infection (influenza-ARTI)

    - Positive influenza PCR on nasal swab

    B) Incidence rate of medically-attended-influenza (MA-influenza-ARTI)

    - Case definition based on sample collection and report:
        - Influenza-infection based on PCR on nasal swab
          AND
        - Report of medical visit for respiratory symptoms to a GP, specialist, emergency department or hospital (within 28 days of influenza confirmed infection).
          Medical attendance will be described for:
            i. Inpatients (hospitalization)
            ii. Outpatients (GP, ED, other doctor visits)

2. RSV-associated, influenza-associated and all-cause mortality.

    Mortality during follow-up is calculated for RSV and influenza-associated deaths and all-cause deaths. Death is associated with RSV or influenza if death occurs while the patient still experiences symptoms of that respiratory infection episode. All-cause death will be described for ARTI-related death (occurring while the patient still experiences symptoms of a respiratory infection, stratified per ARTI type), and non-ARTI related death which is defined as all deaths without a respiratory infection component occurring during study follow-up. Mortality rates are compared in those with RSV, influenza, other-ARTI and those without infection

3. Change in frailty over the season

    Change of frailty score (Groningen Frailty Indicator, Peters JAMDA 2012) between start and end-of-season is compared for those with the primary outcome of RSV infection versus those with other types of ARTI and those without an ARTI. Point changes are compared as well as the proportion of patients that are classified as frail indicated by a score of 4 or higher on the Groningen Frailty Indicator questionnaire.

4. To determine long-term cardiac and respiratory outcomes

    New cardiac and pulmonary diagnoses or an increased use of respiratory medication over the season will be compared for those with the primary outcome of RSV infection versus those with other types of ARTI and those without an ARTI.

Post-Hoc analysis

1. Burden of RSV-ARTI, influenza-ARTI and other ARTI

Patients with RSV, influenza and other infection are described regarding severity measures described below. These severity measures have to occur within 28 days after onset of the disease and while disease is still present. RSV patients are compared to those with influenza. No comparison with other (non-RSV, non-influenza) infections is made since this group is likely heterogeneous.

Severity measures:

1. Duration of symptoms defined as the number of days between onset of disease until the symptoms resolve or at 28 days if symptoms still persist at the end of the diary. Median days (interquartile range) are compared, since there is a maximum of 28 consecutive diary days because of the design of the diary.
2. New prescription or increased dosage of medication for respiratory symptoms within 28 days of onset of symptoms. Proportion of patients that got a prescription, or report to have increased their dose/frequency of previously used medication.
    a. Respiratory medicine (inhalants), including short and long acting beta-mimetics and inhaled corticosteroids.
    b. Antibiotics
    c. Antivirals
    d. Corticosteroids (oral).
3. Medical attendance (see page 2 and 3). Proportion of patients with medically-attended infection within 28 days in those with RSV positive or influenza positive infection. This will be stratified for inpatients and outpatients.
4. Lower respiratory tract infection (LRTI), defined as a doctor's diagnosed LRTI (clinical pneumonia) or radiologically confirmed pneumonia. Proportion of LRTI within 28 days in those with RSV positive or influenza positive infection.
5. Hospitalization. Proportion of hospital admissions for respiratory symptoms within 28 days in those with RSV positive or influenza positive infection.

In addition to these measures, we will classify severity of infection as severe, moderate or mild (adapted from Belongia 2018 Open Forum Infect Dis). We defined severe disease as hospitalization with a respiratory component within 28 days after start of the ARTI episode. Moderate disease included all other medically-attended infections, LRTI or new or increased used of inhaled respiratory medication, antibiotics, antivirals or corticosteroids. All other respiratory episodes were classified as mild disease.

GENERAL STATISTICAL ANALYSIS

Handling of missing data

In the sensitivity analysis of the primary outcome, RSV test results are imputed for those with a missing PCR test result and in those with an extended time interval between onset of symptoms and sample collection when no data about seroconversion is available. All other secondary and post-hoc analyses are performed comparing patients with complete data for these specific analyses.

Statistical tests

The incidence rate of (MA-)RSV-ARTI is calculated by dividing the total number of infections by the total study population per study season. The confidence intervals for the (MA-)RSV-incidence estimates are calculated using the Exact Clopper-Pearson method. Person-time is only used to estimate the incidence rate if censoring is above 10% of the study population. Proportions are compared using the Chi-Square test. The Fisher's exact test is used to compare proportions when numbers in any of the cells of the contingency table are below ten. Continuous variables will be checked for normality. If normally distributed, an independent T-test is performed for comparison. If data is not normally distributed a non-parametric Mann-Whitney U test is used. Multiple imputation is performed using 10 iterations in 10 imputed datasets. All analyses are performed in R version 3.5.1 and the mice package is used for multiple imputation.