Protocol Title

Respiratory virus infections in acutely hospitalized adult patients with pulmonary and extrapulmonary complications

Principal Investigator
Dr. Kelvin To
MBBS, FRCPath, FHKCPath, FRCP (Edin), MD

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Table of content

1. PROTOCOL SYNOPSIS:

Protocol title	Respiratory virus infections in acutely hospitalized adult patients with pulmonary and extrapulmonary complications
Hypothesis	The incidence of respiratory virus infection were underestimated
Primary objective	To determine the incidence and adjusted population attributable fraction of respiratory virus infection among patients with pulmonary and extrapulmonary complications
Study participants	1. Adult hospitalized patients admitted to the acute medical ward of Queen Mary Hospital 2. Adult out-patients of Queen Mary Hospital
Study design	This is a prospective cohort study involving hospitalized adult patients admitted to the acute medical ward of Queen Mary Hospital, an acute care university-affiliated hospital with 1,700 beds in Hong Kong. Consecutive patients will be screened for eligibility based on inclusion and exclusion criteria. Written informed consent will be obtained from all study participants. Saliva specimens will be collected from study participants, and will be tested for respiratory viruses by a multiplex PCR panel. Patients' demographics, underlying diseases, and outcome measures will be collected. The crude incidence will be assessed. Adjusted population attributable fraction will be calculated by adjusting the crude incidence of acutely hospitalized adult patients with the background rate among adult out-patients.
Primary outcome	Incidence of respiratory viruses
Secondary outcome	1. Length of hospital stay 2. Length of stay in general medical ward 3. Length of stay in high dependency unit 4. Length of stay in intensive care unit 5. Proportion of patients requiring oxygen supplementation 6. Proportion of patients requiring positive pressure ventilation 7. Proportion of patients requiring intubation 8. Proportion of patients admitted to intensive care unit 9. Proportion of patients admitted to coronary care unit 10. Proportion of patients admitted to high dependency unit 11. Proportion of patients who die during hospitalization 12. Initial blood test results on admission 13. Radiological investigation results 14. Microbiological investigations results
Data analysis	The fraction of hospitalization that can be attributed by a particular respiratory pathogen will be calculated using adjusted population attributable fraction.

	Continuous variables and categorical variables will be compared using Mann Whitney U test and Fisher's exact test, respectively. A *P* value of <0.05 with two sided testing will be considered statistically significant. Statistical test will be performed using SPSS 23.0.0.

2. BACKGROUND AND RATIONALE

2.1. Background and study rationale

Respiratory viruses cause severe infections, and contribute to a substantial number of hospitalizations, admission to intensive care units and deaths (1). Many hospitalizations due to respiratory virus infection are related to pneumonia or exacerbation of chronic lung disease. In addition, many hospitalizations are related to extrapulmonary complications, such as acute coronary syndrome or stroke (2).

Previous studies have reported the incidence of respiratory viruses among patients with pulmonary complications (3), or the association of respiratory viruses with acute coronary syndrome or stroke (4). However, there are several problems associated with these studies. First, many of these studies are retrospective in nature, and therefore testing was only performed in selected patients with respiratory symptoms. Hence, many patients without respiratory symptoms were not recruited. Second, respiratory virus can be detected in some asymptomatic individuals. Therefore, the presence of respiratory virus may be an incidental finding rather than the cause of the complication. Third, many studies only focus on a few respiratory viruses, especially on influenza virus.

This study aims to address these issues. We propose to conduct a prospective cohort study. We will recruit hospitalized adult patients with exacerbation of underlying lung disease, acute coronary syndrome or stroke. As controls, we will recruit outpatients follow-up for chronic heart disease, chronic lung disease or neurological conditions. We will collect saliva from study participants and perform respiratory virus testing using a multiplex PCR panel. Our previous studies have shown that there is a high concordance between results from respiratory virus testing on saliva and nasopharyngeal specimens (5, 6).
Our study will provide accurate data on the epidemiology of respiratory viruses in pulmonary and extrapulmonary complications. These data are important for clinicians, public health practitioners and scientists.

3. STUDY DESIGN

3.1. Study objectives

1. To determine the crude incidence of respiratory virus infection among patients with exacerbation of chronic lung disease, acute coronary syndrome or stroke
2. To determine the adjusted population attributable fraction of respiratory virus infection among patients with exacerbation of chronic lung disease, acute coronary syndrome or stroke

3.2 Study design and organization

3.2.1 Overall study design

This is a prospective cohort study involving hospitalized adult patients admitted to the acute medical ward of Queen Mary Hospital, an acute care university-affiliated hospital with 1,700 beds in Hong Kong. Patients will be screened for eligibility based on inclusion and exclusion criteria. Written informed consent will be obtained from all study participants. Saliva specimens will be collected from study participants, and will be tested for respiratory viruses using a multiplex PCR panel. Patients' demographics, underlying diseases, and outcome measures will be collected.

Since respiratory viruses can be detected in asymptomatic adults, we will also recruit outpatients followed up for a chronic condition to determine the frequency of respiratory viruses among these patients without any acute symptoms requiring hospitalization. We will adjust the crude incidence from this background rate.

3.3 Outcome measurements

3.3.1 Primary outcome measurement

1. Incidence of respiratory viruses

3.3.2 Secondary outcome measurements

1. Length of hospital stay
2. Length of stay in general medical ward
3. Length of stay in high dependency unit
4. Length of stay in intensive care unit
5. Proportion of patients requiring oxygen supplementation
6. Proportion of patients requiring positive pressure ventilation
7. Proportion of patients requiring intubation
8. Proportion of patients admitted to intensive care unit
9. Proportion of patients admitted to coronary care unit
10. Proportion of patients admitted to high dependency unit
11. Proportion of patients who die during hospitalization
12. Initial blood test results on admission
13. Radiological investigation results
14. Microbiological investigations results

3.4. Informed consent

The investigator or his/her representative will explain the nature of the study to the subjects, and answer all questions by the subjects regarding this study. Prior to any study-related screening procedures being performed on the subject, the informed consent statement will be reviewed and signed and dated by the subjects and the person who administered the informed consent.

3.5 Confidentiality of data

Permission for direct access to a subject's data will be sought in writing by the investigator and from the subject as part of the informed consent procedure. This gives permission to examine, analyse, verify and reproduce any records and reports that are important to the evaluation of the study. Any party (e.g., domestic and foreign regulatory authorities, monitors and auditors) with direct access must take all reasonable precautions within the constraints of the applicable regulatory requirements to maintain the confidentiality of the subjects' identities and the Hospital Authority's proprietary

information. It is the monitor's responsibility to verify that each subject has consented, in writing, to direct access.

3.6 Archive of data

The investigator must retain all study documentation pertaining to the conduct of the study at the study site for a period of at least 5 years.

3.7 Ethical and administrative endorsement

The study was approved by the Institutional Review Board (IRB) of the University of Hong Kong and Hospital Authority (IRB reference number: to be added when approved)

4. SELECTION AND ENROLLMENT OF PARTICIPANTS

4.1 Selection of study population at in-patient

4.1.1. Inclusion criteria

1. Admitted to the acute medical ward of Queen Mary Hospital via the accident and emergency department
2. Aged 18 years or above
3. Hospitalized for less than 24 hours at the time of recruitment
4. Presented with exacerbation of underlying lung disease, acute coronary syndrome or stroke
5. Competent and agree to provide written informed consent

4.1.2 Exclusion criteria

1. Admitted to any hospitals in the past 14 days
2. Respiratory virus testing performed in the past 14 days
3. Antiviral against respiratory virus given within the past 14 days
4. Not sufficient saliva

4.2 Selection of study population at out-patient

4.2.1 Inclusion criteria

1. Aged 18 years or above
2. Follow-up at out-patient clinic or at the physiotherapy department of Queen Mary Hospital
3. Competent and agree to provide written informed consent

4.2.2 Exclusion criteria

1. Admitted to any hospitals in the past 14 days
2. Respiratory virus testing performed in the past 14 days
3. Antiviral against respiratory virus given within the past 14 days
4. Onset of new respiratory or non-respiratory symptoms within the past 14 days
5. Not sufficient saliva

4.3 Study enrollment procedures

Patients will be screened for eligibility by a research nurse. Reasons for ineligibility or non-participation of eligible candidates will be documented. A written informed consent will be signed by the patient.

5. STUDY PROCEDURES

5.1 Schedule of evaluations

5.1.1 Participants at the acute medical ward (in-patients)

On the day of enrollment:
- Inclusion and exclusion criteria
- Written informed consent
- Demographic information
- Questionnaire on vaccine history and symptoms
- Collection of saliva specimen

Follow-up after patient discharge
- Hospitalization data (length of stay, laboratory and radiological tests, treatment)

5.1.2 Participants at the out-patient clinic

On the day of enrollment:
- Inclusion and exclusion criteria
- Written informed consent
- Demographic information
- Questionnaire on vaccine history and symptoms
- Collection of saliva specimen

5.2 Assessment and clinical data collection

Initial symptom assessment will be performed by trained research nurses at the medical or at outpatient clinics. At the time of enrollment, the following information will be collected from the patient by the research staff using a standard questionnaire:
- Symptoms and date of symptom onset
- Prior use of oseltamivir or zanamivir within 14 days
- Prior use of antibiotics within 14 days
- Attended out-patient clinic for the same illness
- Close contacts with respiratory symptoms in the past 7 days
- Receive influenza vaccine in the past season
- Receive pneumococcal vaccine

The following data will be extracted from the clinical management system:
- Age, sex, comorbidities
- National Early Warning Score (NEWS) at A&E (20) (respiration rate, oxygen saturation, need for supplemental oxygen, temperature, systolic blood pressure, heart rate, level of consciousness)
- Time of hospital admission and discharge
- Clinical findings
    - Underlying disease

- Clinical symptoms and signs during hospitalization
- Hematological, biochemical, microbiological, immunological, and radiological investigation results on admission
- Need for supplementary oxygen, positive pressure ventilation, intubation, admission to intensive care unit, coronary care unit or high dependency unit during the hospital stay
- Final diagnosis

5.3 Detection of respiratory viruses

5.3.1 Specimen collection

Patients will be asked to spit out saliva (~1 ml) into a sterile container and viral transport medium (VTM) will be added as we described previously (5, 6). The specimens will be transported to the laboratory and stored at -80°C until testing.

5.3.2 Molecular testing of respiratory virus

Saliva will be tested for respiratory viruses using a commercially available multiplex PCR panel.

6. STATISTICAL METHODS

6.1. Sample size

The sample size is based on the feasibility of the budget. A total of 100 hospitalized patients and 100 out-patients will be recruited.

6.2 Analysis of the study

Crude incidence of each respiratory virus and overall respiratory virus infection among hospitalized patients and out-patients will be estimated. To adjust for the background rate of respiratory virus detection among out-patients, we will calculate adjusted population attributable fraction (aPAF) as described previously (22). Conceptually, aPAF represents the fraction of hospitalization that can be attributed to a particular pathogen. Death rate of each detected respiratory virus and overall respiratory virus infection among patients in the acute medical wards will be estimated.

For symptomatology of respiratory viruses, frequencies and proportions of respiratory and non-respiratory symptoms for each respiratory virus and overall respiratory virus infection will be calculated.

Statistical test will be performed using SPSS version 23.0. A P value of <0.05 with two-sided testing will be considered statistically significant.

7. STUDY TEAM and STUDY SITE

7.1 Study team

Principal investigator

- Design of study, coordination and assessment of subjects for study enrollment, analysis of data, writing up

Co-investigators
- Coordination and assessment of subjects for study enrollment
Study nurse
- Subject enrollment, collection of clinical specimens, data collection

7.2 Study site

Queen Mary Hospital, Pokfulam Road, Pokfulam, Hong Kong Special Administrative Region, China

8. REFERENCES

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