THE RSV-SAM STUDY: Validation of Nasal and Bronchial Absorption Sampling Methods for the Assessment of Respiratory Syncytial Virus (RSV) Bronchiolitis in Babies and Children

Imperial College-Pulmocide RSV Collaboration:

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Abbreviations

MLF: mucosal lining fluid
NPA: nasospharyngeal aspirate
NS: nasosorption with a SAM, consisting of soft absorptive material
SAM: synthetic absorptive matrix
RSV: respiratory syncytial virus
PICU: paediatric intensive care unit
qPCR: quantitative polymerase chain reaction
NP: nasopharyngeal
URTI: upper respiratory tract infections
NAC: nasal allergen challenge
RTI: respiratory tract infection
BS: bronchosorption
DNA: deoxyribonucleic acid
mRNA: messenger ribonucleic acid

INTRODUCTION

Epidemiology of RSV

Since the discovery of the respiratory syncytial virus (RSV) in 1955, it has been recognised as the single most important cause of severe lung infections in infants of less than 12 months of age (1). Most children admitted to hospital with RSV are previously healthy although prematurity is the main known risk factor for severe disease (2). Globally RSV is the main cause of acute lower respiratory tract infections and a significant cause of death (3).

RSV at St Mary's Hospital

St. Mary's Hospital is responsible for the clinical care of many babies with RSV infection. Of the babies that attend the Accident and Emergency department with upper respiratory tract infections (URTI) each winter, over 100 are admitted to the paediatric wards with RSV bronchiolitis. Around 40 babies a year require mechanical ventilation on the Paediatric Intensive Care Unit (PICU). Although the clinical burden is large, most babies respond well to treatment and mortality is relatively low.

Current sampling methods to diagnose RSV

Studies of the upper and lower airways of human infants with RSV bronchiolitis are required to definitively understand pathological mechanisms. Studies in infants are difficult to undertake, especially as the child may be clinically unstable and conventional procedures to obtain samples may be too invasive. Infants who require mechanical ventilation for severe RSV bronchiolitis are tracheally intubated, sedated and have indwelling vascular catheters. This allows access to the respiratory tract and enables blood sampling.

In infants naturally infected with RSV, the presence of virus is generally detected in nasopharyngeal aspirate (NPA) samples (5;9). In the UK it is standard clinical practice to perform NPA sampling by direct suction of secretions. However, because this involves the introduction of a suction catheter into the nostril and then to the back of the nose, this procedure may be relatively unpleasant. Sampling by NPA has been found superior to nasal swabs manufactured from natural products (10;11).

Using nasal swabs in young children is rapid and painless and also accurate for RSV diagnosis (12). There is concordance between nasal and nasopharyngeal (NP) swabs for viral testing (13). In a comparison with NPA, nose and throat swabs were more comfortable and have less risk of harm in a clinical setting, but NPA is still recommended in a hospital context (14). Flocked nylon swabs gave higher viral loads than cotton swabs when using qPCRs for detection of human rhinovirus (15).

Viral load may be obtained from these samples by quantitative polymerase chain reaction (qPCR) of infectious samples. This correlates with disease severity in infected infants (4;5). In adults infected experimentally with RSV, the viral load peaks before maximal clinical symptoms (6). It is a major parameter in quantitating the magnitude of infection (7). In assessing the effects of novel therapeutics in experimental infection, the area under the curve (AUC) of viral load is generally the primary end point (8). To determine this, daily samples over the disease course are required. However, there are concerns regarding the accuracy of viral load detection from routine NPA samples as this is dependent of the amount of mucous suctioned at the same time, which varies enormously from patient to patient.

The current methods of sampling described above have a number of limitations:

- Samples are often diluted in saline with can lead to poor sensitivity
- There is a lack of consistency in the location the sample is obtained from which can affect both diagnostic specificity and reproducibility
- Sampling can be uncomfortable and invasive

Proposal for novel absorption sampling methods

In conjunction with a specialist medical device manufacturing company (Hunt Developments (Midhurst, West Sussex) we have produced novel nasosorption and bronchosorption kits that have CE marking. Both nasosorption and bronchosorption methods use synthetic absorptive matrix (SAM) strips: that look and feel like blotting paper, and will be placed onto the mucosal surface. These are comfortable to use and can be used at frequent intervals over extended periods of time. This non-invasive technique is ideal for infants and children, and it is possible to obtain neat mucosal lining fluid (MLF) even from normal healthy noses. The eluates contain cytokines and chemokines at high detectable levels on multiplex immunoassay.

We would like to use these SAMs to take MLF samples from the nasal and bronchial mucosal surfaces to see if these novel techniques can overcome the problems with current sampling methods. We plan to use these absorption techniques to measure RSV viral load. We also aim to look at the immune response in terms of the anti-viral interferon response (IFN-γ, IFN-λ, IFN-α2a, IP10, ITAC). In therapeutic studies in the future, it may be possible to document levels of drug (pharmacokinetics) in nasal MLF.

Nasosorption (NS) and bronchosorption (BS)

NS involves using a synthetic absorptive matrix (SAM) to absorb mucosal lining fluid (MLF) in a precise manner from the inferior turbinate. At Imperial College we have used NS in children with allergic rhinitis (16), infants with a family history of atopy (17), and in atopic adults after nasal allergen challenge (NAC) (18)**.** SAM has also been used after experimental human rhinovirus challenge in asthma when both NS and BS were performed (19;20). The Stephen Durham group at Imperial have recently compared and validated a variety of SAMs (21), and performed a nasosorption study after cat NAC (22)

The swabs are medical devices, have small handles and are enclosed in a sampling tube. These devices are CE-marked, clean and sterile, and produced in a specialist medical device factory.

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Bronchosorption (BS) involves a similar method to NS with a similar SAM material. The SAM is attached to a plastic reinforced catheter that can be introduced through an endotracheal tube to access the bronchial mucosa. Professor Onn Min Kon, respiratory consultant at St Mary's Hospital, has successfully carried out over 700 BS sampling procedures in adults with a range of respiratory diseases. The technique has been very well tolerated, and has permitted cytokines and chemokines to be measured in the bronchial mucosal lining fluid (MLF).

Using a similar but smaller device we hope to take samples from infants ventilated due to severe RSV bronchiolitis. Babies ventilated with severe RSV infection, have an endotracheal tube (ETT) in position that requires suction clearage at regular intervals. Hunt Developments have developed a specialised device for introduction down the ETT, that can be used for BS in babies ventilated for severe RSV bronchiolitis.

As an initial step we will perform a pilot study on tolerability in ventilated infants, and have strict inclusion criteria which will allow inclusion of infants who are stable on the ventilator with low-moderate ventilation and oxygen requirements. We intend to compare inflammatory mediator levels in mucus that has been directly suctioned from the ETT, with levels of mediators in samples obtained using SAM by nasosorption and bronchosorption.

Genetics

Mendelian single gene deficiencies in the form of primary immunodeficiencies have demonstrated the important role that host genetics play in infectious disease outcome. More recently, single gene disorders predisposing to a narrow spectrum of infections have been described. Examples include defects in the IL12-IFNγ pathway predisposing to atypical mycobacterial infections, IRAK4-MYD88 defects to invasive pyogenic infections, and TLR3-IFN defects to herpes simplex encephalitis. Sequencing of the patient samples by whole exome/genome sequencing or linkage analysis will be carried out. One part of this study aims to identify genetic aetiologies of severe RSV infections which will provide tools for molecular diagnosis and genetic counselling.

Summary

In summary this study will compare the novel methods of NS and BS with the standard technique of nasophayngeal aspiration (NPA) and routine ETT suction. We shall assess the samples for diagnosis of RSV, viral load and immune responses in the airways of babies with RSV infection. We shall also assess the genetics of babies included in this study, to see if they may be vulnerable to RSV infection.

AIMS

The study involves obtaining nasal samples from babies with suspected RSV infection in the emergency department (ED), as well as those admitted to the paediatric wards and PICU at St. Mary's Hospital, London. This will help us to understand the epidemiology of and the host response to RSV infection in infants both hospitalised and in the community. In addition, we shall take nasal samples from infants attending hospital for outpatient appointments, routine elective surgery and admitted to PICU for non-respiratory diagnoses as controls.

The specific aims are:

1. To study different sampling methods; comparing the established method of NPA and tracheal suction to nasosorption and bronchosorption. We will assess sample volumes, clinical tolerability and virology results: especially viral load.

2. To assess the safety and validity of using bronchosorption to take bronchial MLF samples.

3. To study severity of disease and correlate this with quantitative RSV PCR.

4. To study the incidence of RSV A and B infection, different subtypes of RSV infection.

5. To measure the nasal and bronchial mucosal immune response: in terms of cytokines and chemokines, especially those relating to interferons.

6. To identify if there is a genetic predisposition to severe RSV infection

7. To understand feasibility of these sampling processes in preparation for an interventional study potentially in winter 2017-8.

ENDPOINTS

To compare SAM and nasopharyngeal aspirate (NPA) as sampling methods for measuring RSV viral load.

PRIMARY ENDPOINT: RSV viral load

Virology: How do SAM and NPA compare for RSV viral load determination?

We shall also assess:

- Sensitivity, specificity, quantitation
- Utility for RSV-A vs RSV-B

SECONDARY ENDPOINTS

1. Immune Response: Establishing the use of nasal and bronchial sampling to measure the host immune response to RSV. These techniques could then be applied to the study of drug pharmacokinetics for new therapeutics in future clinical studies.

2. Epidemiology and virology: This study will assess the number of babies with upper respiratory tract infections (URTI) presenting to St Mary's Hospital ED, the number of babies with confirmed RSV hospitalised on the Paediatrics Ward and also the number admitted to the PICU. The study will assess the relative incidence of RSV A and B and allow us to determine whether RSV subtype is associated with disease severity.

3. Genetics: To establish whether there is a genetic predisposition to severe RSV infection. To carry out genetic analysis by targeted gene sequencing, next generation sequencing or whole genome SNP typing in order to evaluate any genetic deficiencies.

4. Correlation of qPCR with clinical severity

Type of Study: Pilot study

Duration: To take place in RSV infection season between October and March, starting in October 2015.

Identification of patients: We shall obtain our study groups from patients attending St. Mary's Hospital, London.

Number and types of Subjects:

A total of 5 participant groups (n = 30 in each group)

Details of each group and respiratory sampling procedures are given below:

- *Group 1:* Babies with suspected respiratory tract infection (RTI) in the ED:

    Nasosorption (NS) in each nostril

    NPA

- *Group 2:* Babies with diagnosed RSV infection admitted to paediatric wards:

    NS (2 to 3 times daily, 4-6 hours apart)

    NPA daily

- *Group 3:* Babies with diagnosed severe RSV infection in PICU requiring mechanical ventilation:

    NS (2 to 3 times daily, 4-6 hours apart)

    NPA daily

    Bronchial aspirate (BA) daily

- *Control Group 1:* Babies without respiratory symptoms, attending routine outpatient appointments or undergoing elective surgical procedures:

    Nasosorption (NS) only once

- *Control Group 2 :* Babies without RSV infection but requiring mechanical ventilation in PICU:

    NS (2 to 3 times daily, 4-6 hours apart)

    NPA daily

Bronchial aspirate (BA) daily

PROCEDURES

Sampling by Absorption:

Nasosorption: SAM strips will be placed inside each of the nostrils for up to 30 seconds. We will place the SAM on the inferior aspect of the inferior turbinate and middle turbinate. There is minimal discomfort in sampling in these areas. This is a well-tolerated, non-invasive procedure without the need for local anaesthetic.

Bronchosorption: Following clearance of excess mucus by suction, we will introduce the SAM device that will be passed down the ETT to collect bronchial MLF. The device will be passed into the ETT in the same way that a routine suction catheter is introduced. The device will be introduced by the consultant paediatric intensivist or lead physiotherapist gently to a maximum distance of 10cm beyond the end of the ETT, or until it impacts in the bronchial tree. It will then be withdrawn by 5cm and the SAM paper will then be extruded. The device will then be kept in this position for 10 seconds to allow for maximal absorption. The SAM will then be retracted into the device and the device will be withdrawn.

NB for the 2016-2017 season we will not be carrying out the bronchosorption procedure as we have concerns regarding the device and this is being revised accordingly.

Bronchial aspirate:

Routine clearance of excess mucus by suction through the endotracheal tube. We will keep this aspirate that is usually discarded.

Nasophargeal aspirate:

NPA will be carried out after the Nasal SAM has been performed. NPAs will be collected using a standardized protocol (23-25). The child is supine and a small suction catheter is used to take fluid from the back of the nose directly with no saline flush is a routine method used to suction babies with a blocked nose in clinical practice.

In babies who are admitted to the ward, sampling will have to consider the impact of nasogastric and other tubes in the airway. If both NS and NPA ~~all~~ samples have to be taken from the same nostril there should be a 10 min interval between each sample

Blood sampling:

If the baby is having a blood test as part of their routine hospital care an additional 1mL will be taken and used to measure markers of infection and inflammation, together with genetic analysis (subject to consent). If the baby requires further blood tests for clinical reasons, we may take further 1ml research samples at up to 2 further time points to see changes in the immune response.

A 1ml blood sample will be taken at the first time point for genetic analysis as detailed below.

Blood Genetics Methods

i. **Sample collection** Blood samples from the baby will be collected and genetic material extracted

ii. **Sample processing** To carry out genetic analysis from DNA/RNA isolated from infants by targeted gene sequencing, next generation sequencing or whole genome SNP typing in order to evaluate any genetic deficiencies.

iii. **Data Collection** It will be necessary to collect demographic and clinical data from the families in order to define clearly clinical phenotypes. This will include data on age, sex, ethnic groups and clinical details relating to the phenotype. These clinical data will be provided by collaborating clinicians from participating hospitals.

<h1 style="text-align:center">INCLUSION AND EXCLUSION CRITERIA</h1>

Group 1 and Group 2

Inclusion Criteria

- Infants aged 2 weeks-24 months
- Presentation to the Emergency Department with any upper respiratory tract infection (URTI) in the RSV season (Oct-March).
  OR
- Documented RSV infection, admitted to the paediatric wards at St Mary's Hospital.

Exclusion Criteria

- Any local or systemic factor that would influence the safety of nasal sampling.
- Bilateral indwelling nasal catheters or local nasal pathology preventing access for nasal sampling.
- Bleeding disorders.
- The baby is taking part in another interventional study.
- The parents or guardians not able to sign the informed consent from due to limited English or comprehension despite the use of independent interpreter services.
- Limited life expectancy of the baby,

Group 3

Inclusion criteria

- Hospitalised Infants admitted to the PICU at St. Mary's Hospital, aged 2 weeks-24 months with documented RSV infection (by rapid test and/or PCR).
- Infants of weight >2kg.
- On a conventional ventilator with an Endotracheal Tube (ETT) of >2.0mmm diameter

Exclusion criteria

- Any local or systemic factor that would influence the safety of nasal sampling.

- Bilateral nasal catheters or local nasal pathology preventing access for nasal sampling.

- The baby is taking part in another interventional study.

- Prematurity - corrected gestational age <36 weeks, weight <2kg

- Significant hypoxia or instability precluding ventilator disconnection

- Transcutaneous oxygen saturation of <95% on 60% oxygen

- Risk of bleeding

- Pneumothorax

- Infants receiving oral corticosteroid therapy at any time in past month

- Parents or guardians not able to sign informed consent from due to limited English or understanding despite the use of independent interpreter services.

- Limited life expectancy or a decision to limit management.

- NB – in the event of an exclusion to carrying out bronchial aspirate, (i.e: Significant hypoxia or instability precluding ventilator disconnection

- Transcutaneous oxygen saturation of <95% on 60% oxygen

- Risk of bleeding

- Pneumothorax);

As long as there is no contra-indication to carrying out NPA or nasal SAM sampling, then these children can still be included in the study, but will not require bronchial sampling.

Control Group 1

Inclusion criteria

- Babies, aged 2 weeks-24 months, attending routine outpatient appointments or undergoing elective surgical procedures.

Exclusion criteria

- Any respiratory symptoms
- All other exclusion are the same as Groups 1 and 2

Control Group 2

Inclusion criteria

- Infants aged 2 weeks-24 months.

- Infants ventilated on the PICU for any condition

- Confirmed RSV negative by PCR of respiratory tract samples

Exclusion Criteria

- All exclusions are the same as Group 3
- In addition – any concern about raised intracranial pressure

DATA STORAGE

Anonymised data will be stored in Redcap in the Department of Paediatrics at Imperial College London. The database will be password protected and will be web-based. Only those directly involved in the study will be able to access the data. Individuals will only be identified on the database by their unique alphanumeric identifier; data relating to the subjects' names to this identifier will be stored separately. Any data generated may be stored indefinitely and shared with other researchers outside of the participating sites. This will be done through databases such as ENA and EGA. Any data made available on these databases will be strictly confidential.

DATA ANALYSIS

Levels of inflammatory mediators (e.g. cytokines as pg/ml) across the patient groups will be presented as:

- Raw data: linear and log plots as appropriate
- Box-whisker plots: median/quartile/range
- Heat maps to show correlation between levels of mediators

Statistical analysis will be performed to demonstrate significance of levels of individual biomarkers and molecular signatures.

Data and all appropriate documentation will be stored for a minimum of 10 years after the completion of the study, including the follow-up period.

WITHDRAWAL CRITERIA

Any clinically significant side effects during procedures or withdrawal of consent by the parents or guardians of participants.

ADVERSE EVENTS

DEFINITIONS

Adverse Event (AE): any untoward medical occurrence in a patient or clinical study subject.

Serious Adverse Event (SAE): any untoward and unexpected medical occurrence or effect that:

- **Results in death**
- **Is life-threatening** – *refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe*

- **Requires hospitalisation, or prolongation of existing inpatients' hospitalisation**
- **Results in persistent or significant disability or incapacity**
- **Is a congenital anomaly or birth defect**

Medical judgement should be exercised in deciding whether an AE is serious in other situations. Important AEs that are not immediately life-threatening or do not result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above, should also be considered serious.

REPORTING PROCEDURES

All adverse events should be reported. Depending on the nature of the event the reporting procedures below should be followed. Any questions concerning adverse event reporting should be directed to the Chief Investigator in the first instance.

Non serious AEs
All such events, whether expected or not, should be recorded.

Serious AEs
An SAE form should be completed and faxed to the Chief Investigator within 24 hours. However, relapse and death due to hospitalisations for elective treatment of a pre-existing condition do not need reporting as SAEs.

All SAEs should be reported to the REC where in the opinion of the Chief Investigator, the event was:

- 'related', ie resulted from the administration of any of the research procedures; and
- 'unexpected', ie an event that is not listed in the protocol as an expected occurrence

Reports of related and unexpected SAEs should be submitted within 15 days of the Chief Investigator becoming aware of the event, using the NRES SAE form for non-IMP studies. The Chief Investigator must also notify the Sponsor of all SAEs.

Local investigators should report any SAEs as required by their Local Research Ethics Committee, Sponsor and/or Research & Development Office.

Contact details for reporting SAEs

Fax: 020 331 25750, attention Dr Simon Nadel

Please send SAE forms to:

St, Mary's Hospital,

7th Floor, Queen Elizabeth the Queen Mother Building,

Paddington

London

W2 1NY

Tel: 020 78866077 (Mon to Fri 09.00 – 17.00)

ASSESSMENT AND FOLLOW-UP

When sampling is well tolerated there will not be follow up of patients, and the patients will not generally be informed of the result of analyses.

REGULATORY ISSUES

ETHICS APPROVAL

The Chief Investigator has obtained approval from the West Midlands – The Black Country Research Ethics Committee. The study must be submitted for Site Specific Assessment at each participating NHS Trust. The Chief Investigator will require a copy of the Trust R&D approval letter before accepting participants into the study. The study will be conducted in accordance with the recommendations for physicians involved in research on human subjects adopted by the 18th World Medical Assembly, Helsinki 1964 and later revisions.

CONSENT

Consent to enter the study must be sought from each participant's parents or guardians only after a full explanation has been given, an information leaflet offered and time allowed for consideration. Signed consent should be obtained. The right of the parent or guardian to refuse to participate without giving reasons must be respected. After the participant has entered the study the clinician remains free to give alternative treatment to that specified in the protocol at any stage if he/she feels it is in the participant's best interest, but the reasons for doing so should be recorded. In these cases the participants remain within the study for the purposes of follow-up and data analysis. All parents and guardians are free to withdraw at any time from the protocol treatment without giving reasons and without prejudicing further treatment.

CONFIDENTIALITY

The Chief Investigator will preserve the confidentiality of participants taking part in the study and is registered under the Data Protection Act.

INDEMNITY

Imperial College Healthcare NHS Trust London holds negligent harm and non-negligent harm insurance policies which apply to this study. Imperial College Healthcare NHS Trust holds standard NHS Hospital Indemnity and insurance cover with NHS Litigation Authority for NHS Trusts in England, which apply to this study.

SPONSOR

Imperial College Healthcare NHS Trust will act as the main Sponsor for this study. Delegated responsibilities will be assigned to the NHS trusts taking part in this study.

PARTICIPANT REIMBURSMENT

There will be no reimbursement for participating in this study as samples are taken opportunistically.

FUNDING

This study is funded by Pulmocide.

AUDITS

The study may be subject to inspection and audit by Imperial College Healthcare NHS Trust London under their remit as sponsor and other regulatory bodies to ensure

adherence to GCP and the NHS Research Governance Framework for Health and Social Care (2nd edition).

STUDY MANAGEMENT

The day-to-day management of the study will be co-ordinated through Dr. Simon Nadel, consultant paediatrician and intensivist.

PUBLICATION POLICY

Our aim is to ensure the full dissemination of all study results in medical publications of the highest impact and citation factor. Individual subjects will be strictly anonymised.

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