

The AMAZES Study: Asthma and Macrolides: The Azithromycin Efficacy and Safety study

RESEARCH PROTOCOL

Version 15 – 25 February 2014

CONFIDENTIAL

A large-scale, multicentre, double-blind, placebo controlled randomised trial to compare the efficacy (and safety) of the addition of oral azithromycin for 48 weeks with fixed dose maintenance therapy on the incidence of asthma exacerbations and clinical asthma status in people with persistent asthma

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Sponsor: National Health and Medical Research Council, ID 569246

SYNOPSIS

Title: A large-scale, multicentre, double-blind, placebo controlled randomised trial to compare the efficacy (and safety) of the addition of oral azithromycin for 48 weeks with fixed dose maintenance therapy on the incidence of asthma exacerbations and clinical asthma status in people with persistent asthma

Sponsor: National Health and Medical Research Council, ID 569246

Primary Outcomes:

Asthma exacerbations, Asthma-related Quality of Life

Secondary Outcomes:

Asthma symptoms, lung function, lung function decline, safety outcomes

Number of participants / centres:

420 / 8

Study Duration:

March 2009 – 16 December 2016

Study Population:

Aged ≥ 18 years, male and female participants with symptomatic asthma.

Never or ex-smokers

FEV₁ > 40% predicted

Confirmed variable airflow obstruction

No exacerbation for 4 weeks prior to study entry

Prescribed ICS/LABA maintenance asthma therapy

Study Drug:

Azithromycin 2 x 250mg oral tablets three times weekly or matching placebo

Study Design:

A blinded (randomisation, treatment allocation, outcome assessment), multi-centre randomised placebo controlled trial

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LIST OF ABBREVIATIONS AND DEFINITIONS

| | |
|------------------|---|
| AE | Adverse Event |
| ACQ | Asthma Control Questionnaire |
| ADR | Adverse Drug Reaction |
| AHR | Airway hyperresponsiveness |
| AQLQ | Asthma Quality of Life Questionnaire |
| CCQ | Common Cold Questionnaire |
| CI | Chief Investigator |
| CONC | Concord Repatriation General Hospital |
| COPD | Chronic obstructive pulmonary disease |
| EA | Eosinophilic asthma |
| ECG | Electrocardiogram |
| eNO | Exhaled nitric oxide |
| FEV ₁ | Forced expiratory volume in 1 second (in litres) |
| FVC | Forced vital capacity (in litres) |
| HAPS | Hunter Area Pathology Service |
| GINA | Global Initiative for Asthma |
| ICH | International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use |
| ICS | Inhaled corticosteroids |
| IL | Interleukin |
| INR | International Normalized Ratio |
| JHH | John Hunter Hospital |
| LABA | Long-acting beta agonist |
| MDI | Metered dose inhaler |
| MMP | Matrix metalloproteinase |
| NEA | Non-eosinophilic asthma |
| NHMRC | National Health and Medical Research Council |
| NHPA | National Health Priority Area |
| PAH | Princess Alexandra Hospital |
| TPCH | The Prince Charles Hospital |
| PR | Phone Review |
| RAH | Royal Adelaide Hospital |
| SAE | Serious Adverse Event |
| SCGH | Sir Charles Gairdner Hospital |
| SOP | Standardised Operating Procedure |
| TGA | Therapeutic Goods Association |
| VC | Vital Capacity |
| WAP | Written Asthma Action Plan |
| WIMR | Woolcock Institute of Medical Research |

1 PURPOSE OF STUDY

Asthma is a National Health Priority Area (NHPA) for Australia. The NHPA listing recognises the high burden of illness associated with asthma as well as the potential to achieve significant health gains for the Australian population if asthma can be effectively controlled.

Recent asthma management approaches have targeted airway inflammation, which is a key pathophysiological process resulting in airway hyperresponsiveness (AHR) and episodic respiratory symptoms. The allergen induced Th2-lymphocyte, interleukin (IL)-5 mediated, eosinophilic response in asthma is now well characterised and both effective and specific treatment approaches (including inhaled corticosteroids (ICS)) have been developed to suppress this inflammatory process.¹ However, due to heterogeneity of the inflammatory response, symptomatic asthma may persist despite these therapies. We, and others, have identified that asthma is composed of several subtypes with up to 50% of all asthma cases showing no evidence of eosinophilic inflammation.^{2, 3} The presence of non-eosinophilic exacerbations has been well documented in studies of acute asthma where viral infection induces airway neutrophilia.⁴ Importantly, patients with persistent asthma experience more non-eosinophilic exacerbations than eosinophilic exacerbations and non-eosinophilic exacerbations are not prevented by corticosteroid treatment.^{4, 5} New approaches to treatment are required.

Macrolide antibiotics, including azithromycin, have been extensively investigated in recent years for their therapeutic anti-inflammatory role in respiratory disease, as distinct from their anti-microbial actions. These agents have become first line therapy in panbronchiolitis and many reports are now emerging which indicate benefits in a range of conditions including cystic fibrosis, bronchiolitis obliterans and chronic obstructive pulmonary disease. These agents reduce the expression of pro-inflammatory cytokines and improve lung macrophage function. We recently completed a small placebo-controlled clinical trial in asthma that showed beneficial effects from a macrolide antibiotic taken for 8 weeks with reduced non-eosinophilic airway inflammation: sputum IL-8, neutrophils and neutrophil elastase. Significant improvements in some quality of life parameters were seen. In a separate study in chronic obstructive pulmonary disease (COPD) (where neutrophils also predominate), we found again that azithromycin reduced inflammatory mediators and improved macrophage function.

These findings strongly suggest that macrolides may complement existing therapies, especially in that large group of patients with non-eosinophilic asthma and for those suffering non-eosinophilic exacerbations.

2 AIMS

2.1 Study objectives

To conduct a large-scale, multicentre, double-blind, placebo controlled randomised trial comparing the efficacy (and safety) of the addition of oral azithromycin for 48 weeks to fixed dose maintenance therapy on the incidence of asthma exacerbations and clinical asthma status in people with persistent asthma.

The specific hypotheses that will be addressed are:

1. Addition of azithromycin will result in a 30% reduction in asthma exacerbations; and
2. Azithromycin treatment will significantly improve health status and reduce asthma symptom scores, and this will be most evident in patients with non-eosinophilic asthma.

In addition, a number of biological parameters will be assessed, and hypothesis-driven sub-studies conducted capitalising upon the expertise of the individual Chief Investigators (CI) and adding value to the data-set.

This study will be conducted by leading Australian researchers who have extensive experience in asthma biology and clinical trials, including recent specific experience with macrolides in this context. The study results have a high likelihood of providing data that will lead to changes in clinical practice and directly address a major Australian healthcare priority.

3 BACKGROUND AND PRELIMINARY STUDIES

Stable persistent asthma is characterised by eosinophilic and non-eosinophilic forms of the disease. Symptomatic non-eosinophilic asthma is poorly responsive to corticosteroid^{2, 3} and requires a different treatment approach. In addition, exacerbations of asthma can be accompanied by a transient eosinophil or neutrophil response, depending on the trigger (allergen or virus, respectively). A recent randomised trial showed that adjusting treatment to suppress sputum eosinophils reduced the number of eosinophilic exacerbations, but importantly, did not influence non-eosinophilic exacerbations or day-to-day asthma symptoms.⁵ Non-eosinophilic exacerbations are the dominant type in asthma and are characterised by increased symptoms and sputum neutrophilia.⁵

There is a need firstly to identify effective therapy for stable non-eosinophilic asthma that may improve health status, and secondly to identify effective therapy to reduce asthma exacerbations, especially non-eosinophilic exacerbations. Macrolide antibiotics achieve both of these effects in neutrophilic airway diseases such as cystic fibrosis and bronchiectasis, and hold promise for therapy in asthma.

3.1 Non-eosinophilic Asthma (NEA)

NEA was initially described in uncontrolled asthmatics with normal sputum eosinophil counts,⁶ and since this time, it has been identified in both stable⁷ and acute asthma,^{8, 9} severe corticosteroid dependent asthma¹⁰ and persistent asthma.¹¹

NEA is associated with a poor response to inhaled corticosteroid.^{3, 12} NEA also occurs in steroid-free individuals,¹³ and the absence of eosinophils in NEA has also been confirmed in bronchial tissue by both endobronchial biopsy^{3, 10} and post-mortem examination.¹⁴

We have recently established that neutrophilic asthma has a different mechanistic pathway to eosinophilic asthma (EA). There is dysfunction of the innate immune response with increased gene expression for toll-like receptors 2 and 4, increased IL-8 and IL-1 β ,¹⁵ and increases in proteolytic enzymes neutrophil elastase and total matrix metalloproteinase (MMP)-9.¹⁶ Studies are proceeding to determine whether this represents the effects of an increased inflammatory cell burden in the airway,¹⁷ or the effect of increased innate activation of individual inflammatory cells. While it is often assumed that NEA can be equated to non-atopic asthma, our own data (Prof Gibson; Dr Simpson) indicates that the presence or absence of atopy does not distinguish patients with NEA from those with EA.

3.2 A potential role for macrolide antibiotics in asthma treatment

The optimal treatment of NEA is not known, however corticosteroids have little efficacy in this subtype of asthma.^{3, 12} This is consistent with the dominant action of corticosteroids to reduce eosinophilic inflammation, and their ability to potentiate neutrophilia by inhibition of neutrophil apoptosis. Macrolide antibiotics represent an exciting treatment option for the non-eosinophilic pathway in asthma. As antibacterial agents, macrolides treat intracellular pathogens such as *Chlamydia pneumoniae* and *Mycoplasma pneumoniae*. Macrolides have anti-inflammatory effects that include suppression of IL-8 release from human epithelial cells and neutrophils,¹⁸ promotion of neutrophil apoptosis¹⁹ and suppression of MMP-9 activity.²⁰ Macrolides have clinical efficacy in several neutrophil mediated airway diseases. In diffuse panbronchiolitis,²¹ sinusitis, and nasal polyposis²² macrolide treatment results in a significant reduction in neutrophils and IL-8. In cystic fibrosis and bronchiectasis, macrolides improve quality of life and reduce the rate of exacerbations.²³⁻²⁶ Macrolides also improve quality of life in patients with acute exacerbations of chronic bronchitis²³ and in stable COPD.²⁴ Recent data also show that a ketolide (structurally related to macrolides) can be effective in treating acute exacerbations of asthma, significantly reducing symptom scores by 40%.²⁵ Since NEA is also a neutrophil mediated airway disease, these data suggest macrolides may have clinical efficacy in NEA.

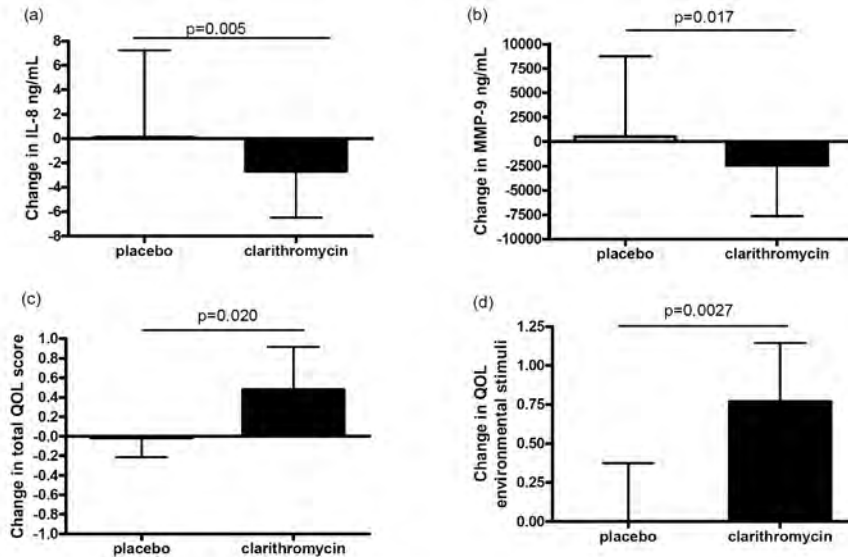
3.3 Pilot data

We have generated significant pilot data to show that macrolides effectively target inflammatory mechanisms in NEA and COPD. A/Prof Reynolds and A/Prof Hodge have identified an accumulation of apoptotic material and impaired clearance of this material by airway macrophages in COPD.²⁷⁻³⁰ The resultant net increase in apoptotic material may exacerbate airways inflammation via secondary necrosis.³¹ Furthermore, they have also established that low-dose azithromycin significantly improved these defects in COPD patients³⁰ suggesting that part of the anti-inflammatory action of macrolides may be through restoration of phagocytosis and removal of apoptotic cells prior to secondary necrosis. As part of the AMAZES collaboration, we have extended these investigations to sputum from NEA. A/Prof Reynolds and A/Prof Hodge have optimised the phagocytosis assay for sputum and shown **decreased phagocytic ability in sputum-derived macrophages in NEA** (10% of macrophages from NEA patients ingested apoptotic cells compared to 22% of macrophages from controls, the same differential we have found in COPD; n=5).

3.4 Pilot randomised control trial of macrolides in asthma

Prof Gibson and Dr Simpson have recently conducted a randomised, double-blind, placebo-controlled trial to investigate the anti-inflammatory effects of macrolides (clarithromycin 500mg twice daily for 8 weeks) in severe refractory asthma. Macrolide therapy significantly reduced airway concentrations of IL-8 and total MMP-9, particularly in NEA (Figure 1a and b) and improved quality of life scores (Figure 1c and d).³² Clinical outcomes were secondary variables in this study, and while the study was not powered to detect changes in clinical outcomes, they observed improvements in quality of life (p=0.020), and non-significant effects on lung function and airway hyperresponsiveness.

Pilot Data Figure 1: Beneficial Effects of macrolides on (a) IL-8; (b) MMP-9; and (c, d) Quality of Life in Asthma³²



4 POPULATION AND STUDY DESIGN

A blinded (randomisation, treatment allocation, outcome assessment), multi-centre randomised placebo-controlled trial will be conducted in participants with symptomatic asthma, adherent to current fixed dose maintenance therapy with ICS in combination with long-acting beta agonists (LABA). Participants will be recruited over 2 years and undertake a 2-week run-in before being randomised to azithromycin 2 x 250mg three times weekly or matching placebo tablets for 48 weeks. At the end of the 48 weeks treatment period, participants will attend a final visit 4 weeks after ceasing treatment. Participants will provide written informed consent. Approvals will be obtained from Human Research Ethics committees, the Therapeutic Goods Administration (CTN) and the trial registered (www.anzctr.org.au).

The study will recruit a national total of 420. See discussion of sample size calculations below.

5 ASSESSMENTS

5.1 Study Schedule

| | | | | | | | | | | | | | | | | | | | | | |
|-------------------------|--------|-----------|-----------|---|-------|----|-------|-------------|-------|----|-------|-------------|-------|----|-------|-------------|-------|----|----------------|--|----|
| Visit # or Phone | 1 | 2 | Phone | 3 | Phone | 4 | Phone | Phone (PR5) | Phone | 6 | Phone | Phone (PR7) | Phone | 8 | Phone | Phone (PR9) | Phone | 10 | 11 | 12 | 13 |
| Weeks | -2 | 0 | 3 | 6 | 9 | 12 | 15 | 18 | 21 | 24 | 27 | 30 | 33 | 36 | 39 | 42 | 45 | 48 | 52 | 72 | 96 |
| Description | Screen | Randomise | Treatment | | | | | | | | | | | | | | | | Post Treatment | JHH and WIMR sites only. Post Treatment. | |

5.2 Schedule of visits and study procedures

| Design | Visit Number (PR = Phone review, visit only if required) | 1 | 2 | 3 | 4 | PR5 | 6 | PR7 | 8 | PR9 | 10 | 11 ^f |
|------------------------|---|-----------------|----------------------|------------------|----------------|-----|----------------|-----|----------------|-----|----------------|-----------------|
| | Weeks | -2 ^d | 0 ^d | 6 | 12 | 18 | 24 | 30 | 36 | 42 | 48 | 52 ^d |
| | (±days) ^a | n/a | n/a | ±7 | ±7 | ±7 | ±7 | ±7 | ±7 | ±7 | ±7 | +7 |
| | Visit Type | Screen | Random ⁿ | Treatment Period | | | | | | | | Post treatment |
| Visit duration (hr) | 2.5 | 2.5 | 1 | 1 | 0.25 | 1 | 0.25 | 1 | 0.25 | 2.5 | 2 | |
| Questions | Informed consent ^b | ✓ | | | | | | | | | | |
| | Medical assessment (new pts only) | ✓ | | | | | | | | | | |
| | Smoking History | ✓ | | | | | | | | | | |
| | Asthma treatment optimisation | ✓ | | | | | | | | | | |
| | Allergy history | ✓ | | | | | | | | | | |
| | Asthma med. use | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| | Concomitant med. use | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| | Hearing status (°Audiology JHH) | ✓ ^e | ✓ ^c | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ ^c | |
| | AQLQ + ACQ6 | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| | Common Cold Questionnaire | ✓ ^e | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| | Epworth Sleepiness Scale | | ✓ | | | | | | | | ✓ | |
| | Symptoms Scale | ✓ | ✓ | ✓ | ✓ | | ✓ | | ✓ | | ✓ | ✓ |
| | Infection and GI questions | | ✓ | | | | ✓ | | | | ✓ | ✓ |
| | Asthma medication adherence | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| | Study medication adherence | | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | |
| | Exacerbations | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| AE assessment | | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | |
| Procedures | Skin prick allergy test | ✓ | | | | | | | | | | |
| | Height and Weight (height v2 + 10 only) | ✓ | ✓ | ✓ | ✓ | | ✓ | | ✓ | | ✓ | ✓ |
| | DLCO (if ex-smoker >10pk yrs) | ✓ | | | | | | | | | | |
| | Exhaled CO | ✓ | | | | | | | | | | |
| | ECG (visit 6 PCH/RAH only) | ✓ ^e | (✓) if not at v1 | ✓ | | | (✓) | | | | ✓ | |
| | eNO (JHH, SCGH, PAH only) | | ✓ | | | | | | | | ✓ | ✓ |
| | Spirometry (pre/post) | ✓ | ✓ | ✓ | ✓ | | ✓ | | ✓ | | ✓ | ✓ |
| | Saline Challenge (if no VAO evidence) | ✓ | | | | | | | | | | |
| | Sputum Induction | ✓ | ✓ | | | | | | | | ✓ | ✓ |
| | Throat & Nose swab (JHH+SCGH only) | | ✓ | | | | | | | | ✓ | ✓ |
| Pregnancy test (urine) | | ✓ | ✓ | ✓ | | ✓ | | ✓ | | ✓ | ✓ | |
| Bloods / Lab tests | EUC/LFT (Safety blood) | ✓ ^e | | | ✓ | | ✓ | | | | ✓ | |
| | FBE/FBC (Safety + inflamm SS) | ✓ | | | | | | | | | ✓ | |
| | Serum/Clot 6mL (inflamm. SS + micro (v2)) | ✓ | ✓ ^(micro) | | | | | | | | ✓ | ✓ |
| | EDTA 3 x 4mL (genetics SS) | ✓ | | | | | | | | | | |
| | Lith.Hep 2x10mL 1x4mL (TPCH/JHH/PAH only) (SS) | | ✓ | | | | | | | | ✓ | ✓ |
| | Sputum Microbiology (all sites) | | ✓ | | | | | | | | ✓ | ✓ |
| Other | Issue WAP (review) | | ✓ | | | | (✓) | | | | (✓) | |
| | Issue diary (+review) | ✓ ^e | (✓) | (✓) | (✓) | | (✓) | | (✓) | | (✓) | |
| | Issue study drug (^g Dispense 2 bottles) | | ✓ | ✓ | ✓ ^g | | ✓ ^g | | ✓ ^g | | | |

Footnotes:

^a Visit timing may be altered ± 7 days of the scheduled visit to accommodate unexpected events, with one exception, Visit 11 must be at least (≥) 4 weeks post treatment +7 days (ie. Timing of visit 11 cannot be shorted to less than 4 weeks).

^b Consent must be obtained prior to any protocol procedures. The study information sheet must inform participant of requirements for withholding or stopping medications in preparation for entry into the study.

- ^c Audiometry at JHH, Visits 2 and 10, ONLY if participant advises of significant hearing loss, in 1st 10 participants randomised.
- ^d The run-in period between the screening (Visit 1) and randomisation (Visit 2) may be extended to a maximum of 8 weeks in the event of unstable asthma, poor medication adherence, respiratory tract infection. If the run-in period extends to greater than 8 weeks, the screening visit must be repeated (Visit 1 Rpt).
- ^e Procedures which are NOT required in participants who, at screening, are deemed ineligible for randomisation due to prohibited concomitant medications or long QTc. All other assessments and procedures are to be completed on all participants screened, this allows collection of complete screening population data.
- ^f In addition to visit 11 (4 weeks after treatment end), some participants at the JHH/WIMR sites may be followed up at 6 months (visit 12) and 12 months (visit 13) after finishing treatment (spirometry and symptoms). (See section 5.1.8).
- ^g Two bottles of study drug are to be dispensed at visits 4, 6 and 8, to allow 12 week interval until the next visit.

5.2.1 Screening Visit (Visit 1)

Participants will be asked to withhold their asthma medications according to Table 2 and antihistamines according to Table 3.

The following assessments and procedures will be performed:

- Written informed consent will be obtained.
- Medical assessment
- Height and weight without shoes
- Skin prick allergy test with exposure to a range of common aeroallergens, including grasses, house dust mite, cat, dog and moulds (see Table 3 for antihistamine withholding times)
- Medications: asthma and concomitant (Following screening, participants will be advised to consult study staff prior to taking ANY new medications – including over the counter medications).
- Asthma optimisation plan: eligible participants will be considered to be on optimal treatment in the run-in period if their current treatment matches treatment recommended by GINA guidelines (at least GINA Step 3 low dose ICS plus LABA). Acceptable combination therapy formulations are outlined in Table 1.
- Smoking history and exhaled carbon monoxide: if smoking history is >10 pack years, carbon monoxide diffusing capacity (DLCO) will be measured. DLCO/VA (KCO) will be reported.
- Allergy history
- Information about hearing status. Audiometry will be performed on the first 10 randomised participants at the JHH site (at randomisation and post treatment) if, at screening, they indicate that they have previously sustained significant hearing loss
- Juniper Asthma Quality of Life Questionnaire (AQLQ) (included in APPENDIX P)
- Juniper Asthma Control Questionnaire – 6 item (ACQ6) (included in APPENDIX P)
- Common Cold Questionnaire (CCQ) (included in APPENDIX P)
- Symptoms Scale (included in APPENDIX P)
- History of variable airflow obstruction (within 10 years prior)
- Spirometry (Pre bronchodilator FEV₁ and FVC or slow VC). Participants will be advised to withhold their asthma medication for the times outlined in Table 2.
- For participants who do not have a documented history of variable airflow obstruction within the preceding 10 years (according to the criteria set in Section 11.1 Inclusion Criteria), bronchial responsiveness to 4.5% hypertonic saline will be performed in conjunction with sputum induction (medication withholding times as outlined in tables 2 and 3). In the absence of bronchial responsiveness to 4.5% saline, 400µg Salbutamol will be administered and post bronchodilator spirometry performed after 10 minutes to assess reversibility (FEV₁ change >12% or 200mL). If reversibility is still not observed and the participant would otherwise be eligible for inclusion, a peak flow monitor may be provided and peak flow monitored over a 2 week period to assess variability.
- For participants who have documented history of variable airflow obstruction (according to the criteria set in Section 11.1 Inclusion Criteria), post bronchodilator spirometry (FEV₁ and FVC or slow VC) will be performed 10 minutes after administration of 400µg Salbutamol.
- Sputum induction using 4.5% hypertonic saline, to determine airway inflammatory cell type. If participant is able, a spontaneous sputum sample will be collected to determine airway inflammatory cell type in absence of an induced sputum sample.
- Serum for inflammatory markers substudy
- Whole blood test to assess baseline full blood count (safety + inflammation substudy)
- Peripheral venous blood for genetics substudy
- ** Safety blood tests to assess baseline liver and renal function (EUC/LFT)
- ** ECG (visit 1 OR 2 – must be prior to supply of study medication at randomisation). A recent ECG (within previous 6 months) may be cited.
- ** Issue diary card. Participants will record medication use and asthma symptoms during the 2 week run-in phase

*** Procedures that are not required for participants deemed ineligible during screening, on account of prohibited concomitant medications, long QTc or inability to attend study visits. All other assessments and procedures must be performed on all participants who attend a screening visit.*

Table 1: Acceptable combination therapy formulations*

| Combination Medication | Inhaler & Dose Options | Ingredients |
|-------------------------------|-----------------------------------|---|
| Seretide | Seretide MDI 125/25 | 125 mcg fluticasone propionate 25mcg salmeterol xinafoate |
| | Seretide MDI 250/25 | 250 mcg fluticasone propionate 25 mcg salmeterol xinafoate |
| | Seretide Accuhaler 100/50 | 100 mcg fluticasone propionate 50mcg salmeterol xinafoate |
| | Seretide Accuhaler 250/50 | 250 mcg fluticasone propionate 50 mcg salmeterol xinafoate |
| | Seretide Accuhaler 500/50 | 500 mcg fluticasone propionate 50 mcg salmeterol xinafoate |
| Symbicort** | Symbicort Turbuhaler 100/6 | 100 mcg budesonide 6 mcg eformoterol |
| | Symbicort Turbuhaler 200/6 | 200 mcg budesonide 6 mcg eformoterol |
| | Symbicort Turbuhaler 400/12 | 400 mcg budesonide 12 mcg eformoterol |

* Pulmicort (budesonide) in conjunction with Spiriva (Tiotropium Bromide) may be acceptable in relevant participants in consultation with investigator.

** Participants taking Symbicort using the 'SMART' regime (ie. As both reliever and preventer) are not eligible for inclusion. However, if appropriate, such participants may have their Symbicort therapy optimised by the investigator at the screening visit and could then be eligible for inclusion.

Table 2: Withholding times for asthma medications prior to bronchial hyper-responsiveness assessment and spirometry (ALL VISITS)

| 6 hours | 12 hours | 24 hours |
|----------------|-----------------|-----------------|
| Airomir | Austyn | Neulin SR |
| Asmol | Foradile | Slo-bid |
| Atrovent | Neulin | Theo-Dur |
| Atrovent Forte | Oxis | |
| Bricanyl | Seretide | |
| Combivent | Serevent | |
| Epaq | Singulair | |
| Intal | Spiriva | |
| Intal Forte | Symbicort | |
| Respolin | | |
| Tilade | | |
| Ventolin | | |

Table 3: Withholding times for antihistamines prior to skin prick test and bronchial hyper-responsiveness assessment (VISIT 1)

| 5 Days | 5 Day (continued) | 6 weeks |
|---------------|--------------------------|----------------|
| Aller G | Panquil | Hismanal |
| Andrumin | Periactin | |
| Avil | Phenergan | |
| Avil Retard | Polaramine | |
| Benadryl | Sinutab | |
| Claratyne | Sudagesic | |
| Demazin | Teldane | |
| Disolyn | Telfast | |
| Dramamin | Vallergan | |
| Panadol Sinus | Zadine | |
| | Zyrtec | |

5.2.2 Randomisation (Visit 2)

Participants will be advised to withhold their asthma medication for the times outlined in Table 2.

The following assessments and procedures will be performed:

- Medications: asthma and concomitant
- Pre- and Post-bronchodilator spirometry [FEV₁ and FVC or slow vital capacity (VC)].
- Questionnaires: AQLQ, ACQ6, Symptoms Scale, Epworth Sleepiness Questionnaire, CCQ, Exacerbations, Infection and gastrointestinal symptoms questions
- Height and weight without shoes
- eNO (JHH, PAH, SCGH)
- ECG (if not completed at visit 1 or in the previous 6 months)
- Throat and nose swab (subset of sites)
- Urine pregnancy test to be performed on all female participants of child bearing potential, regardless of contraceptive methods. Not required on participants who are surgically sterile.
- Audiometry (first 10 randomised participants at JHH, if indicated at screening that they have previously sustained significant hearing loss)
- Asthma medication adherence. Assessment will be made from dose counters in maintenance medication where available and from diary entries. Participants may repeat the 2-week run-in period where adherence is <80%.
- Issue individualised written asthma action plan (WAP) (asthma management plan) (Appendix A)
- Sputum induction using 4.5% hypertonic saline to determine airway inflammatory cell type. Participants will be pre-treated with 400µg salbutamol 10 mins prior to sputum induction. If participant is able, a spontaneous sputum sample will also be collected to determine airway inflammatory cell.
- Serum blood test to detect *Chlamydia pneumonia* and *mycoplasma pneumoniae*
- Lithium heparin blood for NF-κB substudy
- Stool sample (JHH only) for Inflammation and Microbiome substudy
- Review diary and issue new diary cards for 6-8 weeks of sheets.
- Issue study drug. Participants who are clinically stable (no increase in symptom frequency or asthma medication use) and who meet the inclusion and exclusion criteria will be randomised to receive either azithromycin 250mg or placebo, 2 tablets three times weekly, for 48 weeks

5.2.3 Telephone Follow up

As set out in section 5.1 Study Schedule, participants will be contacted by telephone, at regular intervals between visits and asked about their asthma symptoms, exacerbations, medication use, and side effects. ACQ6 and CCQ will be administered during the telephone follow up. If required, at the discretion of the investigator, participants may be asked to attend an additional face to face review to assess worsening asthma symptoms or adverse events.

5.2.4 Treatment visits (Visit 3 to 9)

- Participants will attend 4 visits during treatment, at intervals of either 6 or 12 weeks (±7 days) (see Section 5.1).
- Visit 3 and 4 will occur at 6 week intervals, visit 6 and 8 will occur at 12 week intervals.
- During the 12 week intervals participants will be reviewed over the phone every 6 weeks (PR5, PR7, PR9, previously named visits 5, 7 and 9) and questioned about their asthma symptoms, medication use and side effects. If required, participants may be asked to attend a face to face review to assess worsening asthma symptoms or adverse events. These additional face to face reviews will then be named 'Visit 5', 'Visit 7', or 'Visit 9' respectively. Further details of the additional face to face reviews are specified in Section 5.2.5.

The following assessments and procedures will be performed:

Participants will be advised to withhold their asthma medication according to Table 2 for all visits, unless worsening asthma symptoms make withholding too difficult.

- Medication use: asthma and concomitant
- Information about hearing status
- Pre- and Post-bronchodilator spirometry (FEV₁ and FVC or slow VC).
- Questionnaires: AQLQ, ACQ6, CCQ, Symptoms Scale, Exacerbations
- Infection and Gastrointestinal symptom questions (Visit 6)
- ECG (all sites Visit 3, PCH and RAH Visit 6)
- Safety blood test (Liver and renal function tests) (Visits 4 and 6)
- Asthma medication adherence
- Study drug adherence
- Adverse event assessment
- Review WAP (visit 6)
- Issue study drug (one bottle (44 tablets) at visit 3, two bottles (88 tablets) at visits 4, 6 and 8).
- Review/collect diary and issue sufficient new cards for 6 or 12 week intervals

5.2.5 Additional Face to Face Review

If required or deemed necessary at a telephone follow up, participants may attend a face to face review to assess worsening asthma symptoms or adverse events during the treatment phase. The assessments and procedures that are required will be determined by the investigator on a case by case basis, these may include any of the regular assessments detailed in Section 5.2.5 Treatment Visits, or additional follow-up where clinically appropriate.

5.2.6 End of treatment visit (Visit 10)

Participants will attend this visit 48 weeks (± 7 days) after commencing treatment (Visit 2).

The following assessments and procedures will be performed:

Participants will be asked to withhold their asthma medications according to Table 2.

- Medication use: asthma and concomitant
- Information about hearing status
- Pre- and Post-bronchodilator spirometry (FEV₁ and FVC or slow VC). Participants will be advised to withhold their asthma medication for the times outlined in Table 2
- Questionnaires: AQLQ, ACQ6, CCQ, Symptoms Scale, Epworth Sleepiness Scale, Infection and Gastrointestinal symptom questions
- Height and weight without shoes
- eNO (JHH, PAH, SCGH)
- ECG
- Throat and nose swab (subset of sites)
- Exacerbations
- Safety blood test (Liver and renal function tests)
- Whole blood test to assess full blood count (inflammation substudy)
- Serum for inflammatory markers substudy
- Lithium heparin blood for NF- κ B substudy
- Stool sample (JHH only) for Inflammation and Microbiome substudy
- Asthma medication adherence
- Study drug adherence
- Review WAP
- Review/collect diary and issue new cards
- Sputum induction using 4.5% hypertonic saline to determine airway inflammatory cell type. Participants will be pre-treated with 400 μ g salbutamol 10 min prior to sputum induction. If participant is able, a spontaneous sputum sample will also be collected to determine airway inflammatory cell type.
- Adverse event assessment
- Schedule Post Treatment visit for at least 4 weeks from visit 10 date (+ 7days).

5.2.7 Post treatment (Visit 11)

Participants will attend this visit at least 4 weeks (+7 days) after stopping treatment (Visit 10 date).

The following assessments and procedures will be performed:

Participants will be asked to withhold their asthma medications according to Table 2.

- Asthma medication use
- Pre- and Post-bronchodilator spirometry (FEV₁ and FVC or slow VC). Participants will be advised to withhold their asthma medication for the times outlined in Table 2.
- Questionnaires: AQLQ, ACQ6, CCQ, Symptoms Scale
- Height and weight without shoes
- eNO (JHH, PAH, SCGH)
- Throat and nose swab (subset of sites)
- Serum for inflammatory markers substudy
- Lithium heparin blood for NF- κ B substudy
- Stool sample (JHH) for Inflammation and Microbiome substudy
- Exacerbations
- Adverse event assessment
- Review/collect diary
- Sputum induction using 4.5% hypertonic saline to determine airway inflammatory cell type. Participants will be pretreated with 400 μ g salbutamol 10 min prior to sputum induction. If participant is able, a spontaneous sputum sample will also be collected to determine airway inflammatory cell type.

5.2.8 Study Withdrawal Visit

To be undertaken if participant withdraws from study during treatment or follow-up phase. The following assessments and procedures will be performed:

- Study medication
- Medications: Asthma and concomitant
- Questionnaires: ACQ6, AQLQ, CCQ, Symptoms scale
- Exacerbations
- Adverse event assessment
- Review/collect diary card
- Pre- and Post-bronchodilator spirometry (FEV₁ and FVC or slow VC). Participants will be advised to withhold their asthma medication for the times outlined in Table 2.
- (*JHH site, OPTIONAL for other sites*). Sputum induction using 4.5% hypertonic saline to determine airway inflammatory cell type (Visit 14). Participants will be pretreated with 400 µg salbutamol 10 min prior to sputum induction. If participant is able, a spontaneous sputum sample will be collected to determine airway inflammatory cell type in absence of an induced sputum sample.
- *In consultation with investigator, safety bloods, ECG etc. may be performed depending on the circumstance of withdrawal and at the time point during which the study the participant has withdrawn.*

5.2.9 Additional post-treatment follow up (visits 12 and 13) (JHH and WIMR sites)

Some participants may attend visit 12 (6 months after finishing treatment) and visit 13 (12 months after finishing treatment) for further follow up.

- Asthma medication use
- Height and weight (without shoes)
- Pre- and Post-bronchodilator spirometry (FEV₁ and FVC or slow VC). Participants will be advised to withhold their asthma medication for the times outlined in Table 2.
- Questionnaires: Symptoms Scale, ACQ6, AQLQ.

6 STANDARD OPERATING PROCEDURES

6.1 Skin Prick Test SOP (see Appendix B)

6.2 Hypertonic Saline Challenge SOP (see Appendix C)

6.3 Sputum Induction SOP (see Appendix D)

6.4 eNO SOP (see Appendix E)

6.5 Bacterial Nose and Throat Swab SOP (see Appendix F)

7 SELECTION AND WITHDRAWAL OF PARTICIPANTS

7.1 Inclusion criteria

1. Able to provide informed written consent
2. Adults (≥ 18 years of age) with symptomatic asthma: ACQ6 ≥ 0.75 at screening
3. Confirmed variable airflow obstruction at screening visit or documented within the past 10 years:
 - Bronchodilator response (BDR) $>200\text{ml}$ OR $> 12\%$ (post-bronchodilator FEV₁ following administration of 400 μg salbutamol, pMDI with spacer; after 10 minutes, or following administration of nebulised salbutamol). BDR ($>200\text{mL}$ or 12%) observed following recovery bronchodilator administration during a saline challenge or induction can also be used to qualify for inclusion.
$$\% \text{ improvement} = \frac{\text{FEV}_1 \text{ post-bronchodilator} - \text{FEV}_1 \text{ baseline}}{\text{FEV}_1 \text{ baseline}} \times 100$$
 - Airway hyper-responsiveness (AHR) in response to any standard challenge agent
 - Peak flow variability $>12\%$ when monitored over at least 1 week.
$$\% \text{ variability} = \frac{(\text{MaxPEF} - \text{min PEF})}{\text{Min PEF}} \times 100$$
 - FEV₁ variability $> 12\%$ (between two FEV₁ values measured within 2 months of each other)
 - Hypertonic saline challenge pD₁₅ $<15\text{mL}$ at visit 1. (If no documented evidence is available prior to screening visit, then hypertonic saline challenge is to be performed).
 - If pD₁₅ $< 15\text{mL}$ saline not observed;
 - Check for BDR at end of saline challenge (400 μg salbutamol, 10 mins).
 - If no BDR observed, provide peak flow meter and chart for 2 weeks monitoring- 4. Stable disease with no respiratory infection, asthma exacerbation, or change in maintenance therapy (respiratory medications) in the 4 weeks preceding screening
- 5. Maintenance combination therapy (at least a minimum low dose ICS and LABA, equivalent to GINA step 3). Asthma subtype will be determined using induced sputum and analysis stratified by sputum eosinophils (\geq or $< 3\%$).

7.1.1 Randomisation Inclusion

1. Asthma treatment optimised according to GINA guidelines (Table 1), (must be $\geq 80\%$ adherence during run-in, repeat 2 week run in if adherence is $<80\%$)
2. Stable asthma (ie. no increase in ACQ score >0.5 from visit 1, no exacerbation or respiratory infection during 2 week run in. Run in can be extended to up to 8 weeks from visit 1 to accommodate unstable asthma during run-in).
3. Confirmed variable airflow obstruction

7.2 Exclusion criteria

1. Investigators or site personnel directly affiliated with the study and their immediate families
2. FEV₁ $< 40\%$ predicted post-bronchodilator (excludes sputum induction based on safety grounds)
3. Current smoker
4. Ex-smokers who have quit within the last year
5. Significant smoking related airspace disease (ex-smokers with more than 10 pack year history AND DLCO/VA (KCO) $<70\%$ predicted OR smoking history >10 pack years and exhaled CO >10 ppm)
6. Treatment with any macrolide or tetracycline 4 weeks prior to screening.
7. Treatment with oral corticosteroids 4 weeks prior to screening (unless a low dose is being taken on a long-term basis: $\leq 10\text{mg}$ for >3 months)
8. Hypersensitivity to macrolides
9. Prolonged QTc $> 0.48\text{s}$ at screening or during treatment
10. Taking medication that will interact with azithromycin in regard to QTc prolongation (Appendix G)
11. Taking medication that will interact with azithromycin in regard to rhabdomyolysis (Appendix G)
** Fluvastatin (Lescol) or Rosuvastatin calcium (Crestor) may be prescribed instead.
12. Existing ECG abnormalities that may lead to arrhythmias
13. Pregnancy/breast feeding, likely to become pregnant or unwilling to use an additional form of contraception for the first 2 months of treatment if taking the oral contraceptive pill
14. Respiratory disease other than asthma (e.g. active tuberculosis, bronchiectasis, emphysema, pulmonary fibrosis) that, at investigator's discretion, would adversely impact on study conduct.

15. Current lung cancer or other blood, lymphatic or solid organ malignancy
16. Inability to attend study visits
17. Impaired liver function at screening as shown by AST, ALT, alkaline phosphatase or total bilirubin > 1.5 times the upper limit of normal (During treatment if > 2 times the upper limit of normal)
18. Impaired renal function at screening as shown by Creatinine Clearance < 30mL/min
19. ACQ6 < 0.75 at screening
20. Unstable asthma: asthma exacerbation within 4 weeks of study entry
21. Cold or respiratory tract infection within 4 weeks of study entry
22. Ocular surgery within 3 months of study entry
23. Abdominal, chest or brain surgery within 3 months
24. Known cerebral, aortic, or abdominal aneurysm
25. Females of child-bearing potential, not using reliable contraception or unwilling to use a second method of contraception during the first 2 months of treatment if taking the oral contraceptive pill.
26. Participants who have participated in another investigative drug study parallel to, or within 4 weeks of study entry

7.3 Smoking History

Tobacco smoking is common in asthma (up to 30%) and induces an additional neutrophilic inflammatory response that is associated with non-responsiveness to corticosteroids. Smoking may also induce incomplete reversibility of airflow obstruction (COPD) that is associated with an intense inflammatory response in the small bronchioles that involve neutrophils and lymphocytes.³³ It is unclear whether macrolides may or may not be effective in these processes. However, we and others are currently evaluating the efficacy of macrolides in COPD.³⁰ In this study it will be important to establish whether macrolides are equally effective in asthmatics with a prior smoking history, where macrolides may also target the airway inflammatory response induced by previous smoking. Since the presence of emphysema may confound the efficacy assessment, we will exclude participants with significant airspace disease (emphysema) diagnosed by a gas transfer test. Ex-smokers will be included and randomisation stratified.

7.4 Withdrawal Criteria

In accordance with the Declaration of Helsinki, each participant is free to withdraw from the study at any time. A CI also has the right to withdraw a participant from the study in the event of intercurrent illness, adverse event or other reasons concerning the health or well-being of the participant, or in the case of lack of cooperation.

Participants will be withdrawn if they are unable to tolerate the study medication, develop a severe adverse event that is potentially drug related, become pregnant during the study, develop abnormal liver function tests (test results > 2 times the upper limit of normal), or withdraw consent. Study medication will be immediately withdrawn and where necessary medical assessment will be provided. Participants will attend a study withdrawal visit after cessation of the study medication and clinical care will be provided by study physicians or the participant's usual physician.

7.5 Adverse Events

Any serious adverse event will be reported in accordance with each study centre's institutional ethics committee guidelines.

7.6 Recruitment

Participants will be recruited from respiratory clinics, clinical research databases and by advertisement. Each centre has a large respiratory caseload to draw from for study participants, and each centre has a track record of successful recruitment for asthma trials. We plan that each site will identify and screen 5-6 patients per week and randomise 1-2 participant per week, a similar recruitment rate to that achieved in previous studies.³² A full-time clinical research assistant is required at each site to achieve this recruitment rate.

8 STUDY MEDICATION

8.1 Drug handling

Azithromycin (azithromycin dihydrate 250mg) and matching placebo (lactose, cellulose, magnesium dihydrate) tablets will be prepared and packaged in opaque plastic bottles by Stenlake Compounding Chemist (Sydney NSW). Drugs will be stored at room temperature (15 – 25 °C; recommended storage temperature), and protected from light, heat and humidity.

Each dose will consist of two tablets (azithromycin dihydrate, 250mg or placebo) taken orally, three times a week. If possible, study medication should be taken at the same time each day. Study medication can be taken with or without food.

Subjects who miss a dose of study medication will be instructed to take the dose as soon as they remember (even if this means that a double dose will be taken on a single day).

Subjects will be instructed to bring any unused medication with them to the clinic at Visits 3 - 10. At all clinic visits, subjects will be reminded of the importance of taking study medication on a regular basis.

8.2 Labelling and dispensing

The study medication will be labelled with:

- participant's randomisation number
- study name
- instructions for use
- medication expiry date
- batch number (date of manufacture)
- bottle number
- prescriber name (clinical investigator at each site)
- bottle contents ("azithromycin or placebo")
- emergency contact number (in the event that un-blinding is required).

The emergency contact number (Prof Peter Gibson) will be for use in case of inadvertent overdose. This person will have emergency access to the medication randomisation code. We have chosen azithromycin in preference to clarithromycin due to superior safety profile (fewer gastrointestinal side effects, less QTc prolongation), proven efficacy in reducing exacerbations in other airway diseases, and our successful use of AZM in COPD.³⁰

Random allocation to treatment will be stratified by centre and past smoking using permuted blocks of 4 or 6. The data manager from the lead site will generate the randomisation allocations using the Randomisation Generator Software.³⁴ The randomisation tables for each stratification list will be provided to Jennifer Hunt at Stenlake who will supervise manufacture, packaging and labelling of active or placebo medication. Medication will be prepared in batches as required by centres. Stenlake will require 2 to 3 months notification for a new batch preparation. New batches will be planned and ordered from Stenlake by the central study coordinator. Stenlake will deliver the study medication to the designated point/person at each site (e.g. hospital research pharmacist/study co-ordinator) as advised by the site-specific co-ordinators.

Medication will be dispensed by the site pharmacist, Chief Investigator or a qualified delegate at Stenlake, following receipt of a study prescription authorised by the Chief Investigator or medically qualified delegate, according to the participant randomisation code (included on the prescription). Participants will be assigned the next available site-specific randomisation code according to smoking status (Never smoker or Ex-smoker) at the randomisation visit.

Medication accountability: a drug accountability record (Appendix N) will be kept by each dispensing pharmacy or study centre, with a central copy kept by Stenlake. Unused medication will be returned by participants to site research officers who will then return unused medication to the site pharmacy or Stenlake for accountability recording and destruction.

The investigator, research team and participants will be blinded i.e. not have access to the randomisation schedule during the study. At visits and phone review, participants will be asked about all new medications commenced, changes in the use of regular medications, and study medication adherence. To aid adherence, medication will be dispensed at each visit and reminders will be used to facilitate adherence.

8.3 Blinding

Concealed allocation and use of a blinded intervention will maintain blinding of investigators and participants throughout the study and during outcome assessment.

8.4 Concomitant asthma therapy

Participants will continue their usual maintenance and reliever asthma therapy according to their treatment requirements during the baseline observation period. This will be fixed dose maintenance ICS-LABA combination. Rescue medication in the form of a short-acting beta agonist will be used for symptom relief. Each participant will receive an individualised written asthma action plan (Appendix A) at screening or randomisation that will detail maintenance asthma therapy, including the study medication, as well as when and how to increase reliever therapy use and seek medical assistance. IC therapy will remain the same in response to deteriorating asthma. Severe exacerbations will be treated using prednisolone according to current asthma therapy guidelines. Additional use of macrolides and tetracyclines will be prohibited. Participants will be advised to consult study staff before taking ANY new medications (including over the counter medications).

8.5 Side Effects of Azithromycin

Prior clinical trials of azithromycin have found that adverse events are either non-existent or mild to moderate and were reversible on discontinuation of the drug (0.7%). Increases in liver enzymes (ALT, AST) and potassium are reported in some participants. These will be monitored at the initial visit and periodically during treatment. Abnormal tests will be repeated in one week, and if abnormalities worsen, the drug will be ceased.

8.6 Interaction with oral contraceptive

Due to the reported gastrointestinal side-effects of azithromycin and the effect these may have on the absorption of the oral contraceptive pill, we will ask participants who are using the oral contraceptive pill as their primary method of contraception to use an additional method for the first 2 months of treatment. Women of child-bearing potential will be warned about the risk of pregnancy and counselled concerning appropriate measures to prevent this.

8.7 Interaction with Warfarin/Coumarin and derivatives

Macrolide antibiotics are known to interact with warfarin and may result in increased anticoagulant effects of warfarin. The mechanism of interaction of azithromycin with warfarin is unknown as azithromycin is not known to inhibit cytochrome P450 iso-enzyme which metabolises warfarin. Azithromycin may be used with warfarin if used with caution and via close monitoring of the International Normalized Ratio (INR). Participants taking long term warfarin (commenced at least 3 months prior to entering the study and to be taken for at least the duration of the study) may be eligible. Participants taking warfarin will have their INR tested 1 week after commencing the study medication. If the INR is therapeutic, it will be checked again 1 week later. If the INR is still therapeutic, the original testing schedule will be reinstated. If, after starting the study medication, the INR is not therapeutic, the warfarin dose will be adjusted and the INR re-tested weekly until the INR is therapeutic, at which point the original testing schedule will be reinstated. Participants who commence warfarin treatment during the study will have their INR levels monitored according to routine clinical practice. INR should be carefully monitored again upon ceasing the study medication.

8.8 Interaction with Digoxin

Serum levels of digoxin may be *increased* by concomitant administration of erythromycin (and possibly other antibiotics). Participants taking digoxin may be eligible for this study. Participants taking digoxin will have their serum digoxin levels tested 2 weeks post-commencing study medication.

9 OUTCOMES

9.1 Efficacy

9.1.1 Asthma Exacerbations

While current optimal asthma therapy with ICS and LABA reduces exacerbations, non-eosinophilic exacerbations persist.⁵ The dominant pattern of severe asthma exacerbations is non-eosinophilic. We have chosen asthma exacerbations as the primary outcome for this study because non-eosinophilic exacerbations can be frequent, severe,⁹ and potentially macrolide responsive.^{23, 25, 35} It will be important to establish whether long-term macrolide treatment can reduce the frequency of asthma exacerbations.

9.1.2 Health status and symptoms

As a co-primary outcome, we will evaluate health status using the Asthma Quality Of Life Questionnaire (AQLQ). This has been chosen based on the results of our previous study³² where we found a significant improvement in the overall quality of life score in patients with asthma treated with clarithromycin. There was differential responsiveness of the items in the AQLQ to clarithromycin treatment. When the mean change in the score for each item was calculated (end of treatment – baseline) and then the mean difference in score for that item was calculated (clarithromycin – placebo), a clinically important treatment effect (improvement >0.5) was observed in 7 items (Table 4: Pilot Data). The largest treatment effects were seen in items that are not well represented in the asthma control questionnaire (ACQ).²⁴ The one item in the ACQ that relates to these items, daytime symptoms, did show a large treatment effect (change=0.61). These results were also corroborated by a second symptom questionnaire.

Table 4: Pilot Data: Quality of life score comparison

| Quality of life (AQLQ) item description | Mean (SD) change with Clarithromycin | Mean difference Clarithromycin - placebo |
|--|--------------------------------------|--|
| Limited in moderate activities | 0.61 (1.41) | -0.66 |
| Limited in social activities | 0.57 (1.24) | -0.60 |
| Discomfort from chest tightness | 0.65 (1.34) | -0.52 |
| Feeling of chest heaviness | 0.52 (1.41) | -0.94 |
| Feel the need to clear throat | 0.65 (1.9) | -0.74 |
| Asthma symptoms due to dust exposure | 0.74 (2) | -0.53 |
| Asthma symptoms due to weather / air pollution | 0.74 (1.25) | -0.60 |

9.2 Safety

Macrolides are generally well tolerated for the treatment of infections and we and others have shown good tolerance of longer-term use of azithromycin in chronic infections and COPD.³⁰ There are class-specific side-effects that warrant evaluation in this project, and clinicians and patients require reassurance that these are minimal or can be managed before macrolides can be generally recommended for use in asthma. These effects include:

- Induction of antibiotic resistance in oropharyngeal and airway colonising bacteria;
- Prolongation of QTc interval and torsades de pointes (refractory ventricular tachyarrhythmia); and
- Interaction with HMGC_oA reductase inhibitors (statins) causing rhabdomyolysis.

The use of antibiotics can be associated with induction of antimicrobial resistance in colonising bacterial flora. The use of macrolides, including azithromycin, rapidly induces macrolide resistant oral streptococcal flora in healthy volunteers.³⁶ In cystic fibrosis, long term azithromycin therapy increased resistance to both *Staphylococcus aureus* and *Haemophilus influenzae*.³⁷ No adverse clinical effects were observed, and it is unclear if this occurs to a greater (or lesser) degree in patients with chronic airway diseases such as asthma, or whether there are any effects on lower airway colonisation.

Macrolide antibiotics delay myocardial cell repolarisation through potassium channel inhibition, resulting in a prolongation of the QTc interval.³⁵ Azithromycin is the least toxic in this regard, and this is one of the reasons we have chosen azithromycin rather other macrolides. The clinical adverse effect is recurrent torsades de pointes, and this occurs through a combination of patient related factors, drug effects and drug interactions. This problem can be minimised by screening for risk factors and drug interactions (see www.torsades.org).

Rhabdomyolysis is a rare but potentially serious effect of the use of HMGC_oA reductase inhibitors (statins) that results through their inhibition of hepatic cytochrome P450 clearance enzymes, in particular CYP3A4. Macrolides that also inhibit CYP3A4 can lead to a 10-fold increase in the incidence of statin-induced rhabdomyolysis. This effect will be managed by exclusion of patients using statins, or changing to a statin that does not inhibit this liver enzyme (fluvastatin).

Reversible sensorineural deafness due to damage of the outer canal hair cells has been rarely reported with higher doses of some macrolides, such as erythromycin or clarithromycin.³⁸ In a multi-centre study of azithromycin in cystic fibrosis there was no increase in the presence of hearing loss following 6 months of azithromycin therapy.³⁹ This potential adverse effect, while not observed with azithromycin, will be monitored in this study by questioning participant about hearing loss at every visit. A sub-group of participants at the JHH site will have audiometry performed before and after treatment if, at screening, they indicate that they have previously sustained significant hearing loss.

9.3 Reporting procedures for adverse events and intercurrent illness

9.3.1 Screening visit (Visit 1)

Current medical illnesses and medications used to treat such conditions will be recorded at visit 1. Recurring symptoms associated with these pre-existing conditions will not be considered adverse events during the study unless they have a clinically significant increase in severity and/or frequency.

9.3.2 Treatment period (Visits 2-10)

Participants will be questioned at each visit and telephone call about the occurrence of new or worsening symptoms. In addition, participants will be provided with a diary to record respiratory symptoms and asthma medication usage.

9.4 Adverse Event (AE)

An adverse event (AE) is “any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment” (ICH Good Clinical Practice).

Therefore, an AE can be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of azithromycin product, whether or not related to the Zithromax (azithromycin).

9.5 Adverse Drug Reaction (ADR)

A response to azithromycin which is noxious and unintended, and which occurs at doses normally used in man for prophylaxis, diagnosis, or therapy of diseases, or for modification of physiological function.

An Unexpected Adverse Drug Reaction is an adverse reaction, the nature or severity of which is not consistent with the applicable Product Information for azithromycin.

Medical emergencies that involve study participants will be handled by the appropriate clinical staff at each study centre.

The Investigator will evaluate all adverse events as to:

- Maximum intensity
 - Mild (awareness of sign or symptoms, but easily tolerated)

- Moderate (discomfort causes interference with usual activity)
- Severe (incapacitating, or unable to do usual activities)
- Seriousness (see Serious Adverse Events)
- Duration: onset and stop dates
- Action taken
- Relationship to study drug

9.5.1 Recognition of adverse events

- Adverse events will be reported in the case report form in screened subjects during the run-in, where there is a protocol-specified intervention, including washout or discontinuation of usual therapy, or procedure.
- Adverse drug reactions will be defined at each review. These may include, but are not limited to, gastrointestinal side-effects including: nausea, diarrhoea, abdominal discomfort; new or changed cardiac or ECG abnormalities; and hearing impairment.
- Exacerbations of asthma. An exacerbation of asthma will be defined as new onset or worsening of a complex of respiratory symptoms (refer to section (9.7.1 below). Details on severity and characterisation of each exacerbation will be recorded in the participant's case report form.
- Illness during the study unrelated to asthma, if appropriate, will be managed by the referring doctor.
- Unexplained worsening in spirometry that requires intervention or further evaluation.

9.6 Serious Adverse Event (SAE)

9.6.1 Definition of SAE

An SAE is any medical occurrence that, at any dose:

- results in death;
- is immediately life-threatening (Note: the term 'life-threatening' in the definition of 'serious' refers to an event/reaction in which the patient was at risk of death at the time of the event/reaction; it does not refer to an event/reaction which hypothetically might have caused death if it were more severe);
- results in inpatient hospitalisation or prolongation of existing hospitalisation;
- results in a persistent or significant disability/incapacity;
- is a congenital anomaly / birth defect; or
- is an important medical event or reaction.

Medical and scientific judgement should be exercised in deciding whether other situations should be considered serious, such as important medical events that might not be immediately life-threatening or result in death or hospitalisation but might jeopardise the patient or might require intervention to prevent one of the other outcomes listed in the definition above. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalisations, or development of drug dependency or drug abuse.

9.6.2 Follow-up and characterisation of SAE

- Any serious or significant AE, whether or not considered related to the study drug, and whether or not the study drug has been administered, must be reported immediately to: 1) Prof Peter Gibson by telephone / fax on fax number (02) 4985 5850; and 2) each study centre's Human Research Ethics Committee in accordance with their reporting guidelines.
- Any serious and Unexpected Adverse Drug Reactions must be reported immediately to) Prof Peter Gibson by telephone / fax on fax number (02) 4985 5850; and 2) each study centre's Human Research Ethics Committee in accordance with their reporting guidelines. In addition, ADRs are to be reported directly to the Therapeutic Goods Association (TGA) Adverse Drug Reaction Unit (ADRU).
- An SAE, which persists or occurs within 30 days after cessation of the study treatment, will be followed up until the event or its sequelae resolve or stabilise at a level acceptable to the investigator.
- For all SAEs, the investigator is obliged to pursue and provide further information as requested by the Hunter Medical Research Institute. In general, this will include a description of the SAE in sufficient detail to allow for a complete medical assessment of the case and independent determination of possible causality. Information on other possible causes of the event, including other medications and illnesses will be provided. The Chief Investigator's assessment of causality will also be provided. In the case of a subject's death, a summary of available autopsy findings must be submitted as soon as possible to the manufacturer or its designated representative.
- With respect to the reporting of AEs, it is important to distinguish between listed (expected) and unlisted (unexpected) AEs that relate to the study medication. A listed of AEs that relate to azithromycin can be found in the Product Information for azithromycin.

9.7 Outcome assessment

9.7.1 Asthma exacerbations

The primary outcome for hypothesis 1 will be asthma exacerbations.

Asthma exacerbations will be defined as 'severe' or 'moderate' according to the ATS/ERS Asthma Outcomes Taskforce guidelines.⁴⁰

A 'severe' exacerbation will be defined as a participant who requires:

- Use of systemic corticosteroids, or an increase from a stable systemic corticosteroid maintenance dose, for at least 3 days. (Courses of corticosteroids separated by 1 week or more will be treated as separate severe exacerbations);
- Hospitalisation or an emergency department visit requiring systemic corticosteroids.

A 'moderate' exacerbation will be defined as a participant who has/requires:

- Emergency department visit for asthma, not requiring systemic corticosteroids OR;
- A temporary change in preventer treatment (ICS, OR ICS/LABA) or commencement of antibiotics
AND at least one of the following:
 - Deterioration in asthma symptoms for at least 2 days;
 - Deterioration in lung function for at least 2 days;
 - Increased rescue bronchodilator use for at least 2 days

Total number of exacerbations will constitute 'severe' plus 'moderate' exacerbations. Asthma exacerbation data will be collected at 6-weekly clinical assessments, follow-up phone calls, patient diary and by review of medical records.

9.7.2 Health status

Health status will be assessed as a co-primary outcome (hypothesis 2) using the AQLQ.⁴¹ Asthma symptoms will also be assessed using the ACQ6.

9.7.3 Symptom Diary (Appendix Q)

Participants will record daytime and night-time symptoms, night-time waking, and occasions of reliever use in a symptom diary according to the ATS/ERS Asthma Outcomes Taskforce guidelines.⁴² We will report the number of symptom and reliever free days as well as the group mean difference in symptom score. (APPENDIX Q).

9.7.4 Spirometry and sputum induction

Sputum induction will be performed as previously described.⁴³ Following baseline spirometry (CPFS/D™ USB Spirometer + BREEZESUITE software, Medgraphics), hypertonic (4.5%) saline will be nebulised using a De-Vilbiss ultrasonic nebuliser (Somerset, PA USA) and aerosol delivered via a one-way non-rebreathing Hans Rudolph valve box. (Site specific equipment details are included in Appendix C). Each participant will undergo a maximum of 16 minutes nebulisation time and nebulisation time within participants will be kept constant. Sputum will be analysed as described⁴³ with selection and dispersion of muco-cellular clumps and performance of total and differential cell counts.

10 TIMELINE

- Recruitment: March 2009-September 2014
- Treatment: March 2009 – September 2015
- Analysis & Publication: January 2015 – December 2015

The recruitment schedule requires a minimum of 420 participants be randomised over the study period. The planned recruitment period of 2 years has been extended, with ethics approval for the study until December 2016. Since only a proportion of screened patients will be eligible, screening may require 5-6 potential participants to be screened per month.

11 SUB STUDIES

11.1 Use of exhaled nitric oxide to detect NEA

Exhaled nitric oxide will be measured at 3 centres (JHH, PAH, SCGH) to identify the value of this measure to predict the response to azithromycin and to identify NEA. This will extend the generalisability of the results to centres where sputum induction is not available.

In pilot data of 134 adults with asthma, we have established that 75% of patients with NEA have an exhaled nitric oxide of <30 ppb. The median (interquartile range) exhaled nitric oxide was 19.6 (13.2-29.0) in NEA compared to 38.5 (20.6-76.2) in eosinophilic asthma, $p < 0.0001$. Exhaled nitric oxide will be measured using the on-line NIOX chemiluminescent analyser (Aerocrine AB; Solna, Sweden) according to ATS guidelines⁴⁴ (Appendix H).

11.2 Markers of inflammation and Airway and Gut Microbiome

Inflammation will be assessed by the measurement of IL-8 and neutrophil elastase levels (commercial ELISA) in sputum.³² Total blood leukocyte count and C-reactive protein will be measured in peripheral blood, based on our findings of improvements in these parameters following administration of azithromycin therapy in COPD.³⁰ Peripheral blood will also be screened for the presence of novel biomarkers (ELISA and/or western blot) to validate the correlation of specific biomarkers with asthma subtype and potentially correlate these biomarkers with response to azithromycin treatment. Airway sputum gene expression analysis will be used to investigate differential gene expression in asthma subtypes and responses to azithromycin treatment. (Appendix I).

The Microbiome study will assess airway and gut bacteria, and investigate the relationship between microbiome and inflammation.

11.3 Macrophage phenotype and function in NEA; modulation by azithromycin

The effects of azithromycin on the phagocytic ability of sputum-derived macrophages will be assessed in 80 participants (recruited from two centres (JHH, PAH)). Adequate power (88-98%) has been determined using variance estimates from our previous studies of 30-50 never smoker controls (mean phagocytosis 22.6%, SD4.4, %mean tested 27.12%). Briefly, our *in vitro* flow-cytometric assay²⁷⁻³⁰ quantifies the ability of macrophages (resuspended at 4×10^5 macrophages/ml and purified by adhesion to plastic) to phagocytose target cells (16HBE epithelial cell line). Apoptosis is induced in the target cell by exposure to UV and ingested cells identified using flow cytometry and co-staining with macrophage and epithelial markers. Macrophage phenotype (M1, M2), which may be modified by AZM⁴⁵ will also be assessed by flow cytometric markers (Appendix J).

11.4 Genetic studies

During one visit, peripheral venous blood will be collected in EDTA tubes for DNA extraction and storage. These DNA samples will be used to address the pharmacogenomic predictors of the clinical response to azithromycin in asthma, via genetic association or genome-wide association studies (Appendix K).

11.5 NF κ B function and dendritic cell subsets

NF κ B plays a crucial role in the regulation of inflammatory pathways, especially those triggered by innate stimuli associated with microbial infections and products of cell injury. There is some evidence that NF κ B function is abnormal in asthma, though whether the NF κ B pathway is altered by macrolides is not known. Blood will be collected in lithium heparin tubes to test the hypothesis that NF κ B function in blood leukocytes is altered in patients with asthma, and may be especially important in non-eosinophilic forms of asthma. It will also be analysed to explore whether the defect in NF κ B function is partly corrected by treatment with azithromycin and whether changes in the relative numbers of dendritic cell subsets are associated with asthma, and the pattern varies in different forms of asthma (Appendix L).

12 STATISTICAL CONSIDERATIONS

12.1 Sample size

12.1.1 Exacerbations

The sample size calculation is based on an analysis of the primary endpoint using a negative binomial model as specified in the analysis section. Based on Jayaram et al. patients with asthma experience an average of 0.58 exacerbations per year.⁵ Using the formula given by Keene et al. and a dispersion parameter of 0.1, we estimate 194 patients in each treatment arm will be needed to have 80% power to detect a 35% reduction in the rate of exacerbations among participants treated with azithromycin compared to those treated with placebo at the 5% level. Allowing for a 20% drop-out rate, we will randomise 420 participants. Since both EA and NEA participants are at risk of exacerbations, the whole study sample will be used to investigate the effect of azithromycin on asthma exacerbations.

12.1.2 Health Status (Quality of Life, AQLQ)

The effect of azithromycin on health status will be examined in the 2 strata of EA and NEA. Based on (32), 20% of the control group have a clinically significant improvement in QOL (i.e. a > 0.5 unit improvement in their AQLQ score) the study will have 80% power to detect a clinically significant improvement in 33% of patients in the active treatment group (i.e. a 13% difference).³² In order to detect the same effect on health status as,³² 80 subjects per group are required. The AMAZES study will therefore have sufficient power to detect quality of life changes in our planned subgroups of EA and NEA.

12.2 Analysis (supervised by Associate Investigator Dr. Patrick McElduff)

Hypothesis 1 will be examined using a negative binomial model.⁴⁶ This is a flexible extension of the Poisson regression model with allowance for extra Poisson variation (over dispersion) that is usually observed in count data, including over dispersion of counts of exacerbations among asthma patients. The outcome of interest in the model will be the number of exacerbations experienced by each patient. The predictor of interest in the model will be treatment group with length of follow-up included as an offset. The model assumes that the event rates do not vary with time and therefore patients can be included in the analysis even if they only contribute data for part of the follow-up period. The offset variable adjusts for the difference in follow-up times.

Additional analysis will explore differences in the time to first exacerbation between patients treated with Azithromycin and those treated with placebo. The data will be presented graphically using Kaplan-Meier plots and differences in survival curves tested using log-rank test. The hazard ratio between treatment groups will be estimated using a Cox proportional hazards model.

Hypothesis 2 will be tested using analysis of covariance. Separate models will be fitted with health status (AQLQ) and asthma symptom score at 48 weeks as the outcome of interest. The predictor variable of interest in the analysis of variance models will be treatment with baseline level of health status or asthma symptom score included as a covariate. To examine if there is a greater benefit of treatment among patients with NEA, NEA status and an interaction term between treatment and NEA status will be included in the model. The p-value of the interaction term will indicate if there is a statistically significant difference in the treatment effect between eosinophilic and non-eosinophilic patients.

Other outcomes of interest such as ICS dosage, β_2 -agonist use, and questionnaire results will be also be analysed using analysis of covariance. Data will be analysed on an intention-to-treat basis using 2-sided tests with p values <0.05 considered statistically significant. The analysis will be repeated on the per protocol population and all analyses will be repeated by NEA status.

13 STUDY GROUP AND GOVERNANCE

The study group comprises established clinical investigators who have successfully conducted and completed asthma efficacy and asthma mechanisms studies over many years (AMAZES Organisational Chart Appendix M). Each centre is experienced in the recruitment and conduct of a randomised trial. Prof Gibson and Dr Simpson have experience in the design and management of randomised controlled trials, coordinating sample collection and assessment of inflammatory mediators.³² Prof Gibson and Dr Simpson co-designed and supervised the first RCT of macrolide antibiotics in severe refractory asthma.³² Prof Upham has 16 years of experience in human clinical research, pre-clinical studies of novel therapeutics, together with pharmaceutical sponsored studies of inhaled steroids, LABA and roflumilast in asthma and COPD. A/Prof Reynolds with A/Prof Hodge has recently designed, implemented and completed an investigator-initiated phase-2 study of azithromycin in COPD.³⁰ Prof Reynolds has contributed to several industry-sponsored clinical trials in asthma and COPD. He has had extensive experience in participant recruitment for investigator-initiated trials that looked at airway cell biology. A/Prof Hodge is experienced in the recruitment of participants for investigator-initiated studies in adults and in study design and interpretation.

Study techniques will be standardised between centres, and quality control monitored regularly throughout the study. In 2007, Prof Gibson and Dr Simpson provided training in sputum induction to two sites; the remaining two sites have well established induction methods.

14 MONITORING AND DATA ACCESS

All study sites will be monitored on-site on a regular basis and will be notified in writing of planned monitoring visits. Monitoring will be completed by the central coordinator at each site. During monitoring, any relevant source data and all study data collected will be made available. Any corrections or clarifications arising from the monitoring visit will be clarified/corrected by site staff prior to the next scheduled monitoring visit. Site coordinators will report study progress to the central coordinator on a monthly basis.

De-identified study data will be copied/scanned/uploaded and forwarded to the central coordinator on a regular basis or when requested by the central coordinator or coordinating investigator. Upon site close-down, all study data collected will be provided to the central site for long-term storage and archiving. If required, additional copies or original site-specific study data can also be archived at each study site in accordance with each site's local protocols.

15 INNOVATION AND SIGNIFICANCE

- **The AMAZES trial will establish whether azithromycin has a role in the treatment of persistent asthma in adults.**

This represents a new approach to asthma therapy that uses an approved, off-patent product that is accessible and can be implemented rapidly based on the results of the AMAZES study. The study will therefore have an important and immediate impact on asthma management in Australia and internationally. The safety and long-term efficacy issues that

we will address in the AMAZES study mandate that the treatment is not implemented until the results of this study are known. The high chances of success require the urgent conduct of the study.

- **Preliminary data indicate there is a strong likelihood of a successful efficacy outcome.**

From prior NHMRC funded project grants, the AMAZES investigators have established a strong case for biological plausibility of this new approach to asthma treatment. We have demonstrated innate immune defects in patients with NEA and COPD, and shown that these are improved by macrolide therapy. The AMAZES trial will establish the clinical consequences of this intervention.

The AMAZES trial represents Australian innovation in clinical medicine that offers the possibility of a globally significant outcome leading to better asthma care.

16 ETHICAL CONSIDERATIONS

Written informed consent will be obtained from each participant prior to the collection of any data or samples.

16.1 Study Drug

Azithromycin is registered and approved for long-term use in Australia. It has been used widely in patients with bacterial infections and in the long-term treatment of cystic fibrosis. Overall, at the dose we propose to use, azithromycin is usually free of significant side effects. Potential side effects and toxicity concerns are described in detail above.

16.2 Sputum Induction

Sputum induction will be undertaken using 4.5% hypertonic saline. Lung function will be monitored using a spirometer before and after the administration of each saline dose to maximise safety. The test will be stopped at the participant's request or if lung function falls below a safe level. Sputum induction can cause cough and minor chest discomfort, and wheeze. This is brief and promptly responds to salbutamol, which will be provided for participants' comfort.

16.3 Allergy tests

Prick skin tests to a range of common allergens such as house dust mite, grass, animal hair and mould will be undertaken. This involves placing some liquid containing these substances on the forearm and lightly pricking the skin. The skin test may cause transient (20-30 min) itch if the participant is allergic to these substances. Participants will be offered the use of a cream to relieve the itch.

16.4 Blood tests (safety and sub-studies)

A small amount of blood (25 – 40 mL or approximately 1-2 tablespoons) will be collected. The collection of blood may cause slight localised bruising.

16.5 General considerations

All information obtained in this study will be available to the participant's general practitioner or specialist at their request. All participation is voluntary and participation will not affect the participant's current or future management. All information collected will be confidential and only accessible to the research team. Results of the study will be collated and communicated to the scientific community in a de-identified manner in which the identification of individual participants is not possible.

16.6 Serious Adverse Events

The respective Human Research Ethics Committees will be notified of any serious adverse event in accordance with local reporting guidelines.

17 ANALYSIS AND REPORTING OF RESULTS

17.1 Data Management

(see also Section 16: 'Statistical considerations')

Al Dr Patrick McElduff (B Math, Grad Dip Med Stats, PhD; 48 pubs since 2003) and the HMRI/SMPH statistical and clinical trials support unit have the expertise and track record to provide the technical leadership to supervise the data management and analysis. Al McElduff is senior statistician with this group that includes 2 professors (D'Este, Attia), statistical assistants and data programmers. Al McElduff has 14 years experience as a statistician. His first statistical job was as the data manager/statistician for the Newcastle MONICA project, which collected detailed clinical data on 14,000 people who had a heart attack in the Lower Hunter Region from 1983 to 1994 as well as data from 3 risk factor surveys over the same period. Al McElduff has also worked with other large datasets including constructing the Salford Diabetes register in the UK, a register of all hospital, pathology, GP and death data for over 10,000 patients over a 10-year period. Since returning to Australia, Al McElduff has worked as the Senior Statistician for Datapharm, Australia, a contract

research organisation and in that position was involved in the trial design, data management and analysis of many clinical trials in Australia and overseas. He continues to work as a senior statistical consultant for Datapharm, Australia. Al McElduff will supervise the full-time data manager employed on this grant and the PSP3 (in year 5) to perform the statistical analysis.

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19 APPENDICES

APPENDIX A - INDIVIDUALISED WRITTEN ASTHMA ACTION PLAN (WAP)

The AMAZES Study: Asthma and Macrolides: the AZithromycin Efficacy and Safety study

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| |
|----------------|
| MRN: _____ |
| Name: _____ |
| Address: _____ |
| DOB: _____ |

ASTHMA ACTION PLAN

| | |
|---|---|
| WHEN WELL | |
| Ventolin(Asmol) _____ | Dose: 2 puffs when needed for asthma symptoms |
| Preventer _____ | Dose: _____ |
| Dose: _____ | |
| Take Ventolin 2 puffs 10 minutes before exercise | |
| WHEN NOT WELL | |
| <ul style="list-style-type: none"> • If your peak flow reading does not reach 60 % of your best value, which is _____ following your medication for a 24 hr period. OR • If you are waking at night due to your asthma. OR • If you require your Ventolin more frequently than usual and are not getting the same effect. | |
| <i>Then</i> | |
| <ul style="list-style-type: none"> • Increase your Ventolin: Take 2 extra puffs as needed up to 6 times per day • Contact the AMAZES study team to arrange a review (ph: _____) • See your doctor if your symptoms are severe or not responding to Ventolin | |
| FOR A SEVERE ATTACK | |
| <ul style="list-style-type: none"> • If your peak flow does not reach 40 % of best value, which is _____ OR • If you have a severe shortness of breath and can only speak in short sentences. OR • If you are having a severe attack of asthma and are frightened. OR • If you need to take your Ventolin more than 4 hourly and do not gain an effect. | |
| <i>Then</i> | |
| <ul style="list-style-type: none"> • Take Ventolin 4 puffs : _____ repeat if you do not improve • Take _____ mg of prednisone • Seek medical attention immediately by calling an ambulance on 000 • Continue to use your Ventolin until help arrives | |

Signature _____

Date _____

APPENDIX B - PRICK SKIN TEST SOP

The allergy skin prick test is used to determine whether a patient/participant is atopic or not. A positive reaction to one or more allergens indicates atopy while no positive reaction to allergens implies the patient is not atopic. Mechanisms of action: the immediate reaction is mediated by degranulation of mast cells. Histamine is the main mediator responsible for the wheal and flare response.

The purpose of this method is to describe the method of carrying out a skin allergy test safely and ensuring that a reliable result is obtained.

1. Equipment:

1.1. Reagents (allergens stored in fridge)

- 1.1.1. Histamine: positive control 10mg/ml histamine hydrochloride
- 1.1.2. Negative control (Glycerin)
- 1.1.3. Allergens for testing *Important: Allergens should be at room temperature prior to testing. Remove from refrigerator 30 minutes prior to testing.*

1.2. Materials

- 1.2.1. Biohazard bin for lancet disposal
- 1.2.2. Holder for allergens
- 1.2.3. Sigmacort Corticosteroid Cream
- 1.2.4. Alcohol wipes
- 1.2.5. Gloves
- 1.2.6. Flat surface such as a table
- 1.2.7. Skin Prick Test measuring slide, or ruler
- 1.2.8. Pillow armrest with 'Bluey' underpad placed under the arm
- 1.2.9. Skin Prick Lancets, Order from Bayer Diagnostics ph 1800 039 076, Code 2054254 box of 200)
- 1.2.10. Biro for marking subject's arm, marker pen for the skin
- 1.2.11. Tissues for removing allergen drops
- 1.2.12. Soap and water
- 1.2.13. Paper towel
- 1.2.14. Skin Prick Test Worksheet
- 1.2.15. Stopwatch/timer
- 1.2.16. Razor

2. Method

2.1. Pre Procedure

Request that the participant withholds antihistamine medication for **5** days (or 6 weeks for Hismanal) prior to the test. Supply them with the "Withholding Medication" list for Skin prick testing (Table 3).

2.2. Procedure

The antigen is placed on the skin and introduced in the epidermis (prick test)

- 2.2.1. Allergens should be at room temperature prior to testing. Remove from refrigerator 30 minutes prior to testing.
- 2.2.2. Explain the test to the participant ie, place drops of solutions onto their forearm, lightly scratch them and see if they come up in a lump. Explain side effects that may be experienced: itchiness, redness and a lump like a mosquito bite and that any responses usually subside in about 20 minutes.
- 2.2.3. Consider contraindications to the test, question whether the participant has a known anaphylactic reaction to any allergen
- 2.2.4. As per worksheet question the participant as to whether they have had any antihistamines in the past **5** days. If yes, then reschedule skin prick test instructing the participant to withhold antihistamine use for at least **5** days before their visit.
- 2.2.5. Instruct the participant to lay their exposed arm (inner arm up) on top of a bluey underpad which has been positioned on a pillow on the table.
- 2.2.6. Gently wipe the arm with an alcohol swab to clean after putting on your gloves.
- 2.2.7. Using a biro, draw a grid (2 squares width by 6 squares length in the centre of the right forearm at least 5 cm above the wrist and 3 cm below the cubital fossa. The squares should be at least 2 cm apart to avoid false-positive reactions. Ensure there is minimal hair; shave if necessary.
- 2.2.8. Areas of thick hair, wounds, abrasions, scar tissue and veins should be avoided if possible.
- 2.2.9. Place a small drop of each allergen into a square. Allow a small amount of allergen to form at end of dropper. Touch liquid lightly to forearm. Surface tension will pull the allergen to the skin. Follow the order of the allergens on the results sheet. Do not change the order of allergens once determined.
Important: Do not allow dropper to touch skin as this will result in contamination of the allergen when the dropper is returned to the bottle.

19.1.1.1 Right forearm

| | | Antecubital fossa |
|---------------|-----------------------|-------------------|
| Histamine (+) | Glycerin (-) | |
| Grass Mix #7 | D. Pter. | |
| Cat Hair | Dog Hair | |
| Mould Mix #10 | Aspergillus fumigatus | |
| | | Wrist |

- 2.2.10. Apply one drop of each allergen next to the appropriate mark on the arm taking care to have the participant's arm flat to prevent allergen drop from running. Also take care not to touch the skin with the dropper to prevent potential contamination of the allergen bottle.
- 2.2.11. Release the lancet by twisting the plastic joint and pulling at the same time.
- 2.2.12. With the lancet exposed, prick the skin (using either the upward pressure or down pressure method) in the middle of the allergen drop and release skin gently. **DO NOT** flick the lancet across the skin as the skin opening should not be visible or draw blood.
- 2.2.13. Use a separate lancet for each allergen. Dispose of lancet in 'sharps' container.
- 2.2.14. Allow 1 minute and then blot excess allergen using tissues. Blot gently and ensure that allergens are not transferred to sites other than the position on the grid.
- 2.2.15. Ask the subject not to scratch the area and remind if necessary.
- 2.2.16. The histamine result (positive control) is measured at **10 minutes** (9 minutes after blotting) while the allergen results and negative control are read at **15 minutes** (14 minutes after blotting).
- 2.2.17. Record diameter of wheal (figure 2). For a wheal that is irregular in shape take the mean of the length of the longest diameter and the perpendicular length of the shortest diameter through the point of abrasion (figure 3). Add the two measurements together and divide by 2 to determine the mean. If the result of the wheal is circular, one measurement of the diameter (in mm) is sufficient. If you have difficulty determining the margin of the wheal, run a finger lightly over the area and observe the point at which the skin blanches. Where there is no response to a particular allergen a **zero** must be recorded on the Data Collection Sheet. Blank spaces and dashes may lead to confusion during data analysis. *Any weal that has a "tail" called a pseudopod can be regarded as a strong reaction. The length of the pseudopod is included in the measurement recorded.* **IMPORTANT:** Measure the WHEAL (lump) **NOT** area of erythema (redness).

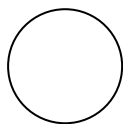


Figure 2

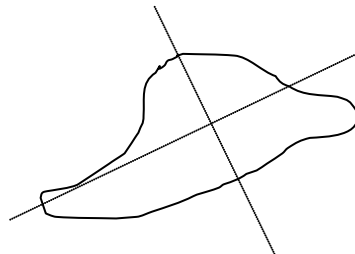


Figure 3

- 2.2.18. Confidence in the test result is determined by a positive reaction to the positive control (ie, histamine of at least 3mm x 3mm) and negative reaction to the negative control (ie, saline). If a negative reaction to positive control or a positive reaction to negative control is recorded the test must be repeated on the opposing arm, or at the next study visit.
- 2.2.19. When all measurements have been taken and on completion of recording results, remove biro marks with alcohol wipe.
- 2.2.20. Have the participant move over to the sink and wash their arm with the water and soap. Dry with paper toweling and apply corticosteroid cream to the area of itching and discomfort.
- 2.2.21. Explain to the participant that the reaction may last up to 24 hours after the test.
Important: Return allergens to refrigerator (4°C) at completion of testing session.

3. Maintenance:

- 3.1. Ensure that the allergens are in date (this is recorded on the allergen bottle)
- 3.2. Ensure the exposed arm is clean and free of wounds, etc (as detailed above)
- 3.3. The allergens are located in a particular order in the tray for the Pulmonary Function (PFT) Lab work, therefore ensure allergens are returned to tray in the order removed

4. Safety Precautions:

- 4.1. Return the allergens to the refrigerator.
- 4.2. Return the pillow, measuring slide and stopwatch to the correct locations
- 4.3. Document skin prick test worksheet into participant's file
- 4.4. Clean the work area with alcohol and paper toweling (alcohol is in spray bottle located on the sink bench)

- 4.5. As described above lancets are to be disposed of into a sharps container
- 4.6. Bluey and cup are to be placed into the contaminated bin.
- 4.7. Avoid any allergens where the patient knows they have a reaction to.
- 4.8. Explain to the participant that if they still have a reaction to any of the allergens after 24 hours to notify staff or seek medical attention

5. Troubleshooting:

- 5.1. Suspect or incorrect results require the test to be repeated.
- 5.2. Positive responses are known to decline after 50 years of age
- 5.3. Responses may be less with immunotherapy (record if the patient has received this treatment for any allergens to be tested).
- 5.4. Sensitive skin may induce false positives but should be screened by negative control

6. Allergen supply:

Link Pharmaceuticals: T: 1800 824 166 F: 1800 824 199

| Allergen | REF # |
|-------------------------|------------|
| Grass Mix #7 | HS 0850 TR |
| D. Pteronyssinus | HS 6692 UP |
| Cat Hair | HS 4815 TR |
| Dog Hair | HS 4084 ED |
| Mould Mix #10 | HS 5137 ED |
| Aspergillus fumigatus | HS 5021 ED |
| Glycerin: '-ve control' | HS 6806 ED |

AUSPMAN MANUFACTURING FACILITY, PRINCESS MARGARET HOSPITAL, PERTH, WA

Histamine 10mg/ml '+ve control'

ALLERGY SKIN PRICK TEST WORKSHEET

Has the participant taken Antihistamines within the past 5 days?
(Hismanal 6 weeks)

Yes No

*If **yes** provide details do not continue test and reschedule appointment.*
How long since last antihistamine taken?

Start Time: _____

| Test | Wheal Size (mm) x (mm) | Mean wheal size (mm) | Test | Wheal Size (mm) x (mm) | Mean wheal size (mm) |
|--|------------------------|----------------------|---|------------------------|----------------------|
| CONTROLS | | | | | |
| Histamine/ +ve Control (measure at 10 mins) | ___ X ___ | ___ | Glycerin/ -ve Control (measure at 15 mins) | ___ X ___ | ___ |
| ALLERGENS (measure at 15 mins) | | | | | |
| Grass mix #7 | ___ X ___ | ___ | D. Pteronyssinus | ___ X ___ | ___ |
| Cat Hair | ___ X ___ | ___ | Dog Hair | ___ X ___ | ___ |
| Mould Mix #10 | ___ X ___ | ___ | Aspergillus fumigatus | ___ X ___ | ___ |

** If the subject does not experience a reaction to an allergen, 0 should be entered for length and width.*

ATOPY positive if (any allergen wheal \geq 3mm)

Yes No

Performed By: _____

APPENDIX C - HYPERTONIC SALINE CHALLENGE SOP

Note: Sputum Induction can be conducted in combination with hypertonic saline challenge. A subject can be encouraged to provide a sputum sample throughout a challenge and after a β_2 agonist has been given. The challenge ceases once β_2 agonist has been given. Hypertonic saline inhalation after β_2 agonist is an induction (see SOP sputum induction).

1 Equipment:

- Refrigerator
- ULTRA-NEB™ ultrasonic nebuliser, DeVilbiss, Model 2000 (JHH, PCH, RAH)/ NE-U12 Omron Ultrasonic nebuliser (SCGH)
- Hans Rudolph valve box, large 2 way No. 2700 (JHH, PCH, SCGH)
- Saliva trap (green)
- Vacumed 1001 mouthpiece
- Nebuliser cup and lid + tubing
- Spirometer + mouth pieces (CPFS/D™ USB Spirometer + BREEZESUITE software, Medgraphics)
- Volumatic/Spacer
- Stopwatch
- Calculator
- Nose clip
- Bench top Digital scales

2 Materials:

- 4.5% hypertonic saline – 200mL bottle
- Disposable mouthpiece for spirometer
- Salbutamol
- Saline Challenge Worksheet (Appendix C)
- Absorbent sheets (i.e. Blueys or underpads)
- Tissues

3 Set Up:


3.1 Calibrate spirometer

Assemble valve box

With clean hands, open sterile packet containing valve box (4 parts) and valves (2 valves wrapped in blue cloth, do not dispose of blue cloth). See Illustration 1 section 11.

Take the main body of the valve box (No.3 on Illustration 1, largest piece, contains the writing 2700 Series Large 2-Way NRBV and arrows) and 1 valve (No.4 on Illustration 1).


Ensure the valve is sitting firmly on frame (if not pull valve to completely cover frame).

Turn the main body of the valve box upright with the writing up or arrow  and place the valve facing up or out of the main body (Illustration 2). Take the corresponding piece (No. 5 Illustration 1 clear – each piece only fits one spot) and screw (over the valve) to the main body.

Take the remaining valve (No.2 Illustration 1) and place downwards within the opposing end of the main body (follow flow arrows). Attach the corresponding piece (No. 5 Illustration 1, white) to the main body of the valve box. Attach the final piece (No. 6 Illustration 1 for mouthpiece) to the lower portion of the main body of the valve box (Illustration 3).

Take a Vacumed mouthpiece and attach to final piece attached to valve box. This can be hard to attach and may require stretching over connection at the base of valve box. Vacumed mouthpieces can be found on shelves opposite TB office or in the right hand draw of trolley within Pulmonary Function Lab.

Attach saliva trap (green attachment found on sink in drainer in clinical lab) to clear end of valve box or end displaying

 on main body of valve box.

Wrap blue cloth (original sterile wrapping cloth for valves) around Vacumed mouthpiece. Subjects will then recognize someone else has not recently used that mouthpiece. Place assembled valve box on absorbent sheet in front of nebulizer.

3.2 Prepare nebuliser cup

Select a nebuliser cup and lid from drainer on sink in clinical lab.

Remove a 200mL bottle of 4.5% hypertonic saline solution from the refrigerator in the clinical lab. Check the expiry date. The hypertonic saline solution should be at room temperature when used.

Pour the hypertonic saline solution into the nebuliser cup ensuring the minimum level has been reached and the maximum level has not been exceeded. These levels are marked on the side of the nebuliser cup.

Place lid firmly on nebuliser cup, matching arrow on lid to arrow on cup.

Select a length of tubing (grey) hanging from rack above the sink in the clinical lab.

Attach one end of the tubing to the nebuliser cup lid (place grey rubber end of tubing over larger/higher outlet port on nebuliser cup lid).

Take nebuliser cup and attached tubing to digital scales and weigh to 1 decimal point. Record weight and cup selected on the Saline Challenge Worksheet in the 'Nebuliser cup pre-test weight' section.

3.3 Prepare nebuliser

Place previously prepared nebuliser cup and tubing into the nebuliser, ensuring arrows on cup and lid are aligned with that on the nebuliser and elements on the base of the cup are aligned with those of the nebuliser

The nebuliser should be on the maximum output setting (dial on front of nebuliser turned fully to the right).

Attach white tubing on nebuliser to the nebuliser cup lid (smaller inlet port).

Attach the loose end of the tubing (grey) on the nebuliser cup to the free (opposite to saliva trap) end of the valve box. Secure firmly with micropore tape around tubing and valve box or use blue connector piece (in drainage tray).

Check the position of the valves and the tubing is connected correctly by reviewing the flow direction noted by the arrows on the valve box before commencing challenge.

4 Method:

Ensure that the subject has withheld their asthma and allergy medication for the required times (Tables 2 and 3).

If a subject has not withheld their medications for the required length of time, the subject where possible should be rescheduled.

Document the type, amount and time of last asthma medication on the Saline Challenge Worksheet

Explain the purpose of the test, how it is to be conducted and possible side effects e.g. coughing, dry mouth, gagging, chest tightness, wheezing, dyspnoea, nausea and excess salivation

Measure the baseline spirometry FEV₁ and FVC as per ATS guidelines. Record highest FEV₁ and FVC on Saline Challenge Worksheet.

Calculate a 15% fall of the highest FEV₁ and record on Saline Challenge worksheet (FEV₁ x 0.85 = 15% fall).

**Ask the subject to apply the nose clip and insert the valve box mouthpiece into their mouth, in a similar manner to a snorkel.

Advise the subject to breathe normally (tidal breathing) and if they need to cough, to cough into the mouthpiece rather than removing the mouthpiece.

Also advise the subject, if they are uncomfortable at any time whilst on the nebuliser, they should raise their hand and the nebuliser will be stopped.

When the subject is comfortable, turn on the nebuliser (green switch on nebuliser press to left) and start the stopwatch when the first breath appears from the saliva trap end of the valve box.

Turn the nebuliser off (green switch press to right) after 30 seconds have elapsed.

Remove the mouthpiece from the subject's mouth and place the mouthpiece, opening down, on the absorbent sheet so any excess residue can be drained. This prevents a potential backwash of pooled secretions through the one-way valve.

Wait 1 minute after each nebulisation episode in order to record maximal bronchoconstriction.

Measure FEV₁ at the end of 1 minute and record on Saline Challenge Worksheet. Calculate the percentage of the FEV₁ fall $(\text{Baseline FEV}_1 - \text{FEV}_1) \times 100 / \text{Baseline FEV}_1$ and record on worksheet.

If the FEV₁ percentage fall is less than 15%, nebulisation should resume immediately the percentage of the FEV₁ has been calculated. If the FEV₁ percentage fall is greater than 15% the challenge is complete and nebulisation ceased.

If the FEV₁ percentage fall is less than 15% repeat steps**, increasing the nebulisation time by doubling the time period: 1 minute, 2 minutes and 4 minutes. A 4-minute period is the maximum time for continuous nebulisation. Continue up to a cumulative time of 15.5 minutes.

Once a FEV₁ percentage fall of greater than 15% has been achieved, the nebulisation ceased, the subject should be given 400µg of salbutamol via a spacer. Wait 10 minutes and then measure FEV₁. Ensure the subject's FEV₁ has returned within 10% of the baseline measurement and that they are experiencing minimal discomfort.

If combining the saline challenge with a sputum induction, nebulization should resume immediately, until 15.5mins cumulative nebulisation time is reached, or the patient has another fall in FEV₁ of great that 15% of baseline. If this second fall in FEV₁ occurs, the subject should be given 200µg of salbutamol via a spacer. Wait 10 minutes and then measure FEV₁. Ensure the subject's FEV₁ has returned within 10% of the baseline measurement and that they are

experiencing minimal discomfort, the saline challenge + sputum induction test is now completed. Refer to the flow chart in section 10 Illustrations for further information.

At the end of the procedure, weigh the nebuliser cup, attached tubing (grey) and remaining saline using digital scales to 1 decimal point. Record in 'Nebuliser cup post test weight' section on Saline Challenge Worksheet. The nebuliser output can be calculated by: (nebuliser cup pre test weight – nebuliser cup post test weight) / cumulative time.

5 Maintenance:

Nebuliser output quality assurance should be completed monthly
Nebulisers and cups to be serviced by Engineering yearly or as needed

6 Shutdown:

Following post-test weighing, the nebuliser cup, lid and tubing can be cleaned using chlorohexidine surgical handwash or similar, rinsed thoroughly and allowed to air dry.

Dispose of used spirometer mouthpiece in contaminated waste bin.

Valve box and saliva to be washed and sterilized according to site-specific methods. Nose clips and spacers to be sterilized according to site specific methods where applicable, or disposed of appropriately.

The laptop/desktop computer should be shutdown as appropriate.

Bench tops and chairs should be wiped with 70% ethanol (surface disinfectant) solution after each procedure

7 Safety Precautions:

All saline challenge procedures should be conducted in areas where in case of emergency resuscitation equipment is available. A doctor should also be available for assistance and to answer any queries during the procedure.

Saline challenge should not be performed on subjects with a FEV₁% predicted of < 40%

If the subject is clinically unstable or becomes symptomatic during the procedure, caution should be exercised when determining the length of each nebulisation. Monitor the FEV₁ at 1 to 2 minute intervals during each nebulisation if there is reason for concern.

8 Troubleshooting:

If the subject wishes to cough during the procedure, they should be encouraged to cough into the nebuliser and not remove the mouthpiece

Remember the saline effect is cumulative, timing between nebulisations should be kept to 1 minute intervals according to subject comfort

Reassurance and encouragement is essential throughout the challenge

An absorbent sheet can be placed on the subject's lab to protect clothing (drip tray)

9 Spare Parts:

DeVilbus nebulisers can be purchased from Sunrise Medical Pty Limited, Castle Hill, Ph: 02 – 98993144.

Nebuliser cups can be purchased from Sunrise Medical Pty Limited, Castle Hill, Ph: 02 – 98993144.

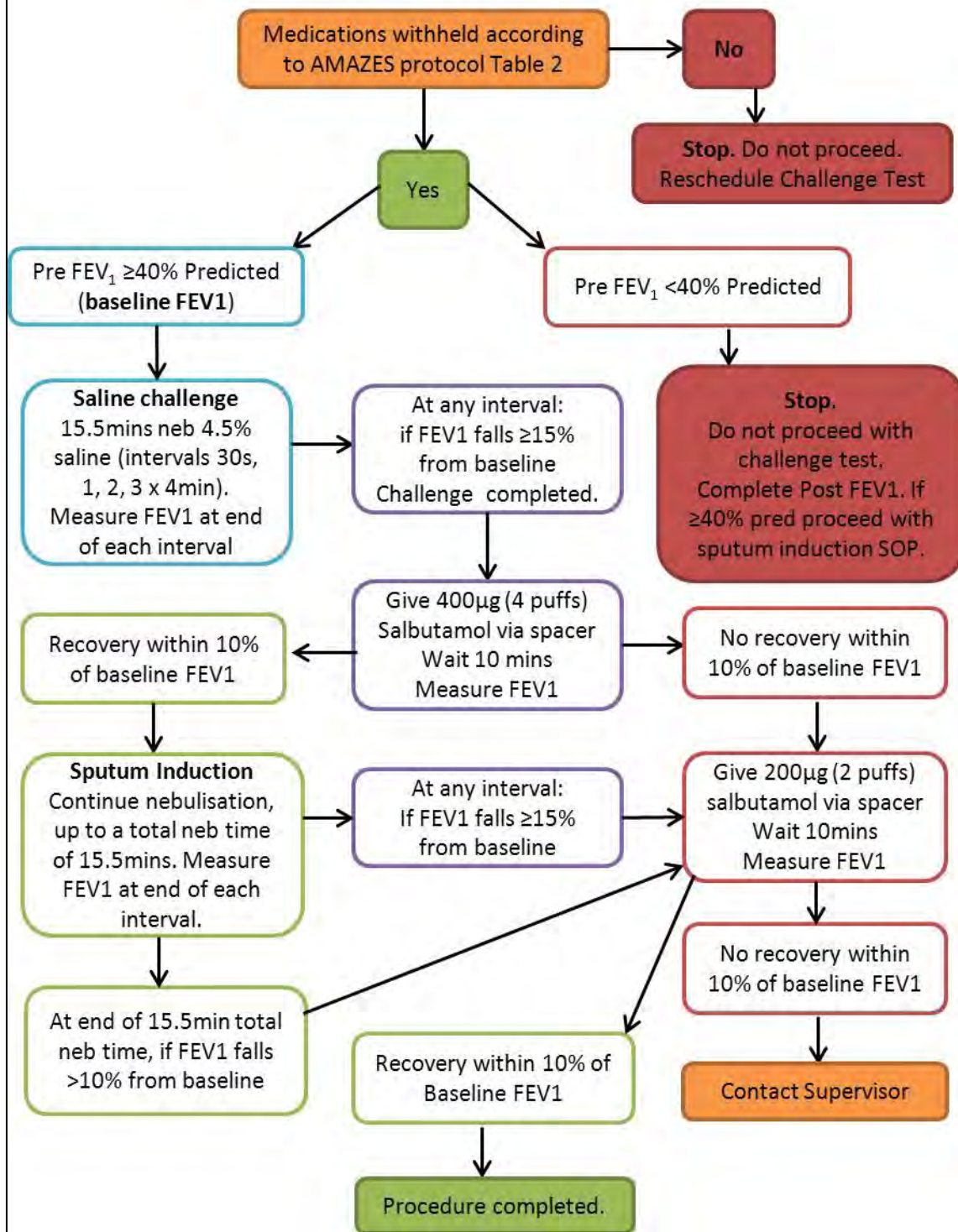
Hans Rudolph valve boxes can be purchased from RJ & VK Bird, 54 Canterbury Road Middle Park VIC 3206, Ph: 03 – 96909898.

Vacumed mouthpieces can be purchased from RJ & VK Bird, 54 Canterbury Road Middle Park VIC 3206, Ph: 03 – 96909898.

Quotes should be acquired before ordering.

10 Illustrations: continued over page;

**AMAZES Saline Challenge + Induction Flowchart
(Visit 1 - Screening)**



SPUTUM INDUCTION (& HYPERTONIC SALINE CHALLENGE - IF APPLICABLE)

Induction Only **Saline Challenge + Induction**
(if no documented variable airflow obstruction within last 10 years)
 NB. **DO NOT** give bronchodilator (B₂) prior to saline challenge.

☞ Please refer to sputum induction flowchart for safety guidelines.

Mouth rinsed (x3)? Yes Nebuliser Make: _____
 Asthma medications withheld? Yes 4.5% saline 0.9% saline

| | |
|---|--|
| IS THE BEST FEV ₁ , L (POST B ₂ IF INDUCTION ONLY) ≥ 40% PREDICTED? <u>YES</u> <input type="checkbox"/> NO <input type="checkbox"/> | 15% FALL FROM BASELINE FEV ₁ , L = 85% X BASELINE FEV ₁ , L _____ |
|---|--|

Nebuliser Cup
 Pre-weight (g): _____ Post-weight (g): _____ Pre-weight – Post-weight = Delivered dose (g): _____(mL)

| Saline nebulised time <i>(adjust times if required)</i> | FEV ₁ , L effort | | | % fall from baseline FEV ₁ | Saline Induced Sputum (SIS) produced (✓) | B ₂ required? Time Dose paused | | Recovery FEV ₁ , L (post B ₂) |
|---|-----------------------------|---|---|---|---|--|--|--|
| | 1 | 2 | 3 | | | | | |
| Baseline Best FEV ₁ , L (post B ₂ – induction only) | | | | | Spontaneous Sputum (SS)?* | | | |
| 30 sec | | | | | | | | |
| 1 min | | | | | | | | |
| 2 min | | | | | | | | |
| 4 min | | | | | | | | |
| 4 min | | | | | | | | |
| 4 min | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |

* NB. If spontaneous sputum (SS) is collected prior to induction, use new specimen jar for saline induced sputum (SIS).

Total cumulative induction time (mins) : _____ **Time sputum sample collection completed :** _____

HYPERTONIC 4.5% SALINE CHALLENGE ONLY:

Was a drop in FEV₁ of 15% observed? YES NO If NO → salbutamol + post-B₂ spirometry

If YES, was this within 15mL saline? YES NO If YES → participant ELIGIBLE for study.

📄 Complete and attach a PD₁₅ sheet to show volume of saline required for 15% drop in FEV₁.

If NO to above Qs & FEV₁ reversibility <12%, issue peak flow meter for participant monitoring over 2 weeks

APPENDIX D - SPUTUM INDUCTION SOP

Note: Sputum Induction can be conducted in combination with hypertonic saline challenge.

1 Equipment:

- Refrigerator
- ULTRA-NEB™ ultrasonic nebuliser, DeVilbiss, Model 2000 (JHH, PCH, RAH)/ NE-U12 Omron Ultrasonic nebuliser (SCGH)
- Hans Rudolph valve box, large 2 way No. 2700 (JHH, PCH, SCGH)
- Saliva trap (green)
- Vacumed 1001 mouthpiece
- Nebuliser cup and lid + tubing
- Spirometer + mouth pieces (CPFS/D™ USB Spirometer + BREEZESUITE software, Medgraphics)
- Volumatic/Spacer
- Stopwatch
- Calculator
- Nose clip
- Bench top Digital scales

2 Materials:

- 4.5% hypertonic saline – 200mL bottle
- Disposable mouthpiece for spirometer
- Salbutamol
- Sputum Induction/Saline Challenge Worksheet
- Disposable cups x2, 1 with water
- Absorbent sheets (i.e. Blueys or underpads)
- Tissues
- Sputum collection jar and specimen bag

3 Set Up:

Calibrate spirometer.

Assemble valve box (Appendix C).

Prepare nebuliser and cup (Appendix C).

Check the position of the valves and the tubing is connected correctly by reviewing the flow direction noted by the arrows on the valve box before commencing induction.

4 Method:

Explain the purpose of the test, how it is to be conducted and possible side effects e.g. coughing, dry mouth, gagging, chest tightness, wheezing, dyspnoea, nausea and excess salivation.

Measure the baseline spirometry FEV₁ and FVC as per ATS guidelines.

Give the subject 4 puffs of salbutamol via a spacer. Wait 10 minutes and repeat spirometry.

Calculate a 15% fall of the highest FEV₁ post salbutamol and record on worksheet (FEV₁ post salbutamol x 0.85 = 15% fall).

Provide the subject with 1 cup of water and 1 empty cup. Ask the subject to rinse and gargle and spit in empty cup (x3). This will minimise squamous cell contamination of the specimen.

Dispose of cup, subject may retain a cup of water to drink during the procedure.

Instruct and demonstrate how to obtain sputum from the lungs by coughing and clearing the throat (deep cough and hack!).

**Ask the subject to apply the nose clip and insert the valve box mouthpiece into their mouth, in a similar manner to a snorkel.

Advise the subject to breathe normally (tidal breathing) and if they need to cough, to cough into the mouthpiece rather than removing the mouthpiece.

Also advise the subject, if they are uncomfortable at any time whilst on the nebuliser, they should raise their hand and the nebuliser will be stopped.

When the subject is comfortable, turn on the nebuliser (green switch on nebuliser press to left) and start the stopwatch when the first breath appears from the saliva trap end of the valve box.

Turn the nebuliser off (green switch press to right) after the required time as per protocol. This maybe 30 seconds if combined Saline Challenge/Sputum induction or up to 5 minutes if sputum induction alone.

Remove the mouthpiece from the subject's mouth and place the mouthpiece, opening down, on the absorbent sheet so any excess residue can be drained. This prevents a potential backwash of pooled secretions through the one-way valve.

Wait 1 minute after each nebulisation period.

Record FEV₁ at the end of 1 minute and record on worksheet.

During the 1-minute after nebulisation or after recording the FEV₁ encourage the subject to cough in order to obtain a sputum sample. Ask them to cough, clear their throat and deposit all or any oral contents into the specimen container. Listen to the subject to ensure the sample is from the lungs and not post nasal secretions.

Calculate the percentage of the FEV₁ fall (Post B2 FEV₁ - FEV₁) x 100 / Post B2 FEV₁ and record on worksheet.

Refer to section 10 Illustrations for flow chart of procedure.

If the FEV₁ percentage fall is less than 15%, nebulisation should resume immediately the percentage of the FEV₁ has been calculated. Repeat steps ** either increasing or repeating nebuliser time according to the protocol. Continue up to a cumulative time of 15.5 minutes.

If the FEV₁ percentage fall is greater than 15% and there is not enough sputum produced, give the subject 4 puffs of salbutamol via a spacer. Wait 10 minutes and then measure FEV₁. Ensure the subject's FEV₁ has returned within 10% of the baseline measurement before recommencing nebulisation (maximum cumulative nebuliser time = 15.5 minutes).

At the end of the procedure, label the specimen jar as per protocol (including subject initials, subject number, SIS for saline induced sputum or SS for spontaneous sputum, date and time), place in specimen bag and refrigerate.

Contact lab staff for immediate pick up of specimen.

At the end of the procedure, remove the nebuliser cup and tubing (grey) without the valve box from the nebuliser and take to digital scales and again weigh to 1 decimal point. Record in "Nebuliser cup post test weight" section on worksheet. The nebuliser output can be calculated by: (nebuliser cup pre test weight – nebuliser cup post test weight) / cumulative time.

5 Maintenance:

Nebuliser output quality assurance should be completed monthly.

Nebulisers to be serviced by Biomedical Engineering yearly or as needed.

Nebuliser cups may be taken to Biomedical Engineering for repair.

6 Shutdown:

See Appendix C

Bench tops and chairs should be wiped with 70% ethanol (surface disinfectant) solution after each procedure.

7 Safety Precautions:

Stop the nebulisation if:

- FEV₁ has dropped 15% on two occasions
- An adequate specimen has been produced and the cumulative nebulisation time is 15.5 minutes
- The subject requests the nebulisation to stop

Ensure the subject's FEV₁ has returned to within 10% of the baseline measurement and that they are experiencing minimal discomfort before allowing them to leave a supervised area.

All sputum induction procedures should be conducted in areas where in case of emergency resuscitation equipment is available. A doctor should also be available for assistance and to answer any queries during the procedure.

If the subject is clinically unstable or becomes symptomatic during the procedure, caution should be exercised when determining the length of each nebulisation. Monitor the FEV₁ at 1 to 2 minute intervals during each nebulisation, if there is reason for concern.

Sputum induction procedures should be conducted in negatively ventilated rooms

8 Troubleshooting:

Many subjects will cough immediately the nebulisation finishes. This opportunity can be used to obtain sputum but exercise moderation so as not to tire the subject or cause a sore throat.

It usually takes a cumulative time of about 11 minutes to produce an adequate specimen. Listen for a moist cough as a sign the subject is ready to produce sputum.

Remember the saline effect is cumulative, timing between nebulisations should be kept to 1 minute intervals according to subject comfort.

Reassurance and encouragement is essential throughout the induction.

Communication with lab staff is essential to ensure consistent and adequate samples are being provided

Never dispose of a specimen (even if you think it is no good), this judgment should be made by the lab staff only.

If a subject provides a spontaneous sputum specimen (i.e. prior to induction), the specimen should be labeled as such and a new specimen jar used for the induced sputum specimen.

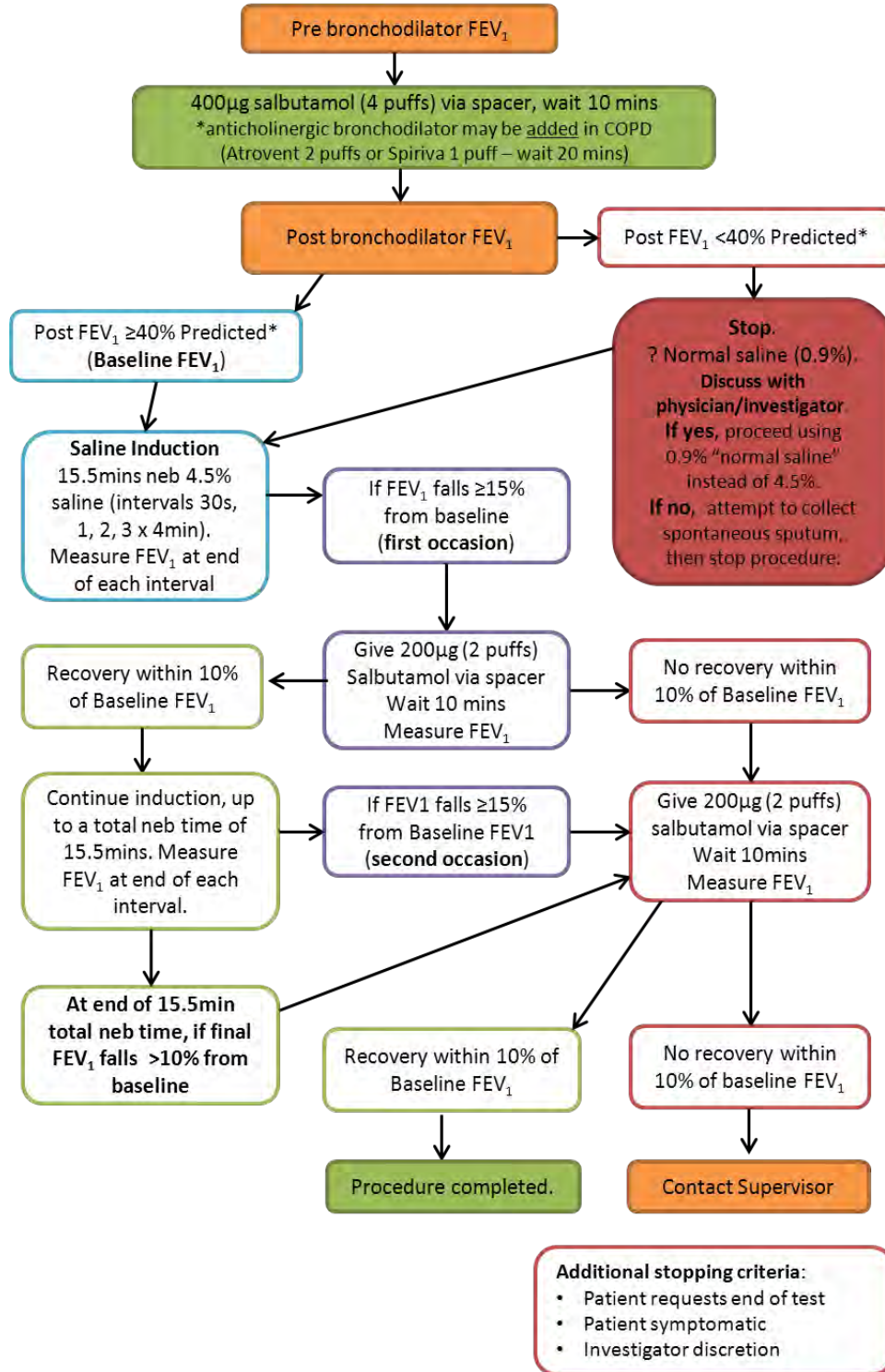
Subjects may prefer to provide a sputum specimen alone or standing.

9 Spare Parts:

See SOP Saline Challenge
Specimen jars and bags

10 Illustrations:

AMAZES Saline Induction ONLY Flowchart
(Visit 2, 10, 11 + optional at withdrawal visit)



11 References:

ATS spirometry guidelines
Sputum Sample Collection video - Aventis
ERS Sputum Induction Guidelines

SPUTUM INDUCTION

☞ Please refer to sputum induction flowchart for safety guidelines.

Mouth rinsed (x3)? Yes Nebuliser Make: _____
 Asthma medications withheld? Yes 4.5% saline 0.9% saline

| | |
|---|--|
| IS THE BEST FEV ₁ (L) (POST B ₂) ≥ 40% PREDICTED? <u>YES</u> <input type="checkbox"/> NO <input type="checkbox"/> | 15% FALL FROM BEST BASELINE FEV ₁ , L = 85% X BEST BASELINE FEV ₁ , L _____ |
| Nebuliser Cup Pre-weight (g): _____ Post-weight (g): _____ Pre-weight – Post-weight = Delivered dose (g): _____ | |

| Saline nebulised time <i>(adjust times if required)</i> | FEV ₁ , L effort | | | % fall from baseline FEV ₁ | Saline Induced Sputum (SIS) produced (✓) | B ₂ required? Dose Time paused | | Recovery FEV ₁ , L (post B ₂) |
|--|-----------------------------|---|---|---|---|--|--|--|
| | 1 | 2 | 3 | | | | | |
| Baseline FEV ₁ , L (post B ₂ administration) | | | | | Spontaneous Sputum (SS)?* | | | |
| 30 sec | | | | | | | | |
| 1 min | | | | | | | | |
| 2 min | | | | | | | | |
| 4 min | | | | | | | | |
| 4 min | | | | | | | | |
| 4 min | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |

Cumulative induction time (mins) : _____ **Time sputum sample collection completed :** _____

* NB. If spontaneous sputum (SS) is collected prior to induction, use new specimen jar for saline induced sputum (SIS).

APPENDIX E - EXHALED NITRIC OXIDE SOP (Aerocrine Niox® eNO)

1 Purpose of the test

Aerocrine Niox® eNO is to be used at JHH and SCGH for measurement of eNO.

The Sievers Nitric Oxide Analyzer (NOA) 280i will be used at PAH for measurement of eNO.

Nitric oxide (NO) is a small, highly reactive molecule, which can be produced by airway endothelial, epithelial and inflammatory cells.

NO triggers airway smooth muscle relaxation, helps to kill tumour cells, inhibit viral replication and other pathogens (bacteria, fungi). It is a marker of airway inflammation.

The Aerocrine Niox® eNO (exhaled nitric oxide) device uses chemiluminescence to detect, in the parts per billion (ppb) range, the amount of NO in exhaled air. Elevated eNO values indicate airway inflammation or other pathological conditions.

NO levels are elevated, compared with normal subjects in the following respiratory conditions: asthma; eosinophilic bronchitis; rhinitis; bronchiectasis; active pulmonary sarcoidosis; active fibrosing alveolitis; upper and lower respiratory infections.

Elevated eNO levels are correlated with airway hyperresponsiveness to methacholine

eNO levels are reduced, compared with normal subjects in the following conditions: current smoking; severe COPD; cystic fibrosis; conditions where the motility of cilia is defective; pulmonary hypertension; alpha-1 antitrypsin deficiency.

The following additional factors may affect eNO levels:

- circadian rhythm. Measurements should be taken at a standardised time of day
- food and beverages. Ingestion of foods that contain nitrogen increase eNO levels, whilst alcohol has been shown to reduce eNO levels. Therefore, subjects should refrain from eating and drinking for at least one hour prior to the test
- Spirometry may transiently reduce eNO levels. Therefore spirometry should be performed after eNO measurement
- Strenuous exercise should be avoided one hour before the measurement
- Some medications affect eNO levels, therefore all medications administered, dose and time of last dose should be recorded.

Important: Read the Aerocrine Niox® manuals prior to operation of the device. Mobile telephones must be switched off in the testing area

2 Equipment

Refer to Section 4 Technical Manual, p23-32.

Equipment List:

- eNO packaging. RETAIN for future transportation
- eNO instruction manuals (Technical and Daily Use manual)
- eNO device (45 kg) (sampling tube)
- Power lead (eNO device to mains)
- Keyboard and mouse, LCD Monitor and leads (to mains and to computer)
- N2 cylinder and trolley
- Nose pegs and Mouthpieces
- Tissues
- Chair
- Floppy disks or network cables and router for back up of data
- Biohazard bags and bin to dispose of mouthpieces

Connect and set up the equipment (Niox Technical manual Section 2 p23-32).

Important: Ensure that gas cylinder is properly secured at all times.
Device must be kept as close to upright as possible (< 10° deviation from vertical) during transport.
Device must be operated in a stable (air conditioned), temperature-controlled environment (20 + 0.5 °C).
Do not place eNO device in direct sunlight.
Leave device on between testing sessions. Shut down system only when the device needs to be transported to another centre.
A filter must be positioned at the end of the sampling tube at all times.
Replace room air filter every 30 days (write date for replacement on filter).

3 Daily start up

Important: Device must be turned on for a minimum of 24 hours prior to calibration and data acquisition. System requires 30 minutes to “warm up” prior to each testing session. Move mouse to initiate warm up. Click **OK** to continue once warm up process completed. A self-test will be performed. Click **Calibrate**, if there is a request for calibration

4 Calibration

Refer to Niox Daily Use Manual, Chapter 5; p35-51. Once system is set up and calibrated, leave in clinical mode (from drop down menu bar select **File, Clinical mode**).

4.1 Gas cylinder connection

Refer to Niox Daily Use Manual, Chapter 5; p35-43.

4.2 Calibration procedure

Important: A minimum pressure of 10 bar in cylinder is required prior to calibration.

NB: Gas cylinders must be imported from Sweden. Order replacement 1 month in advance of expected date required.

Calibrations must be carried out upon request by the system. These include:

1. Fortnightly;
2. After the system has been shut down;
3. Drift of FE_{NO} 0-point or FE_{NO} sample flow; and
4. Significant changes of temperature in testing area.

Following transportation refer to Niox Daily Use Manual, Chapter 5, p41-51.

Fortnightly calibrations: refer to Niox Daily Use Manual, Chapter 5, p44-51.

NB: Follow screen prompts to calibrate.

To initiate calibration manually, select **Options, Calibrate** from menu bar at top of screen.

Flush regulator and gas tubes prior to calibration (10-20 times if device has been transported - Niox Daily Use Manual, Chapter 5, p40-42).

Enter calibration gas concentration given in the **analysis** column (refer to “Certificate of Analysis” attached to cylinder), click **Continue**.

System will flush.

Follow screen prompt and mount breathing handle (+filter) in calibration port (behind service port) p47. Click **continue**. Ensure valves 2 and 3 are closed. Open valve **1**. Open valve **3**. Open valve **2** and adjust gas pressure so that the marker in the **Calibration Gas Adjustment** dialogue fluctuates in green field and the **OK** indicator turns green.

Follow screen prompts. Click **Continue**. Click **OK** when calibration is finished and successful.

Important: Close valves in the following order: 1, 2, 3. Click OK.

Remove breathing handle from calibration port. Close service port.

Leave device into **Standby Mode** until ready to collect patient data.

5 Laboratory Procedure

Refer to Niox Daily Use Manual, Chapter 8, p69-88.

Important: Operate in clinical mode as settings adhere to ATS guidelines. Do not use in “standby mode”. Perform after FOT test and prior to spirometry. Ensure subject has switched off their mobile telephone. Subject must refrain from eating for 1 hr (ATS recommendations) prior to test.

NB: Smoking, respiratory tract infections, nitrate-rich food & beverages and strenuous exercise affect eNO levels.

5.1 New Subject File

In **Enter Patient Information** window:

1. Enter **ID number** in **Patient ID** field;
2. Enter **subject's initials** in **Patient Name** field; and
3. Select name of examiner from the drop down list in **Examiner** field.

Click **OK**.

NB: All three fields must be complete to progress.

Click **No** to use default **Niox standard settings**.

Click **OK** in **Patient Data** dialogue box (i.e. no additional information to fields).

Self Test dialog box will be displayed.

Click **OK**. The clinical mode window will be displayed.

In **Clinical Mode** window select **balloon** (children) or **meter** (adults) icon. The **sound** indicator may be used for visually impaired subjects.

Remove room air filter and place new filter on to end of breathing handle. Do not place hands over the end of the filter. Pass subject the breathing handle.

5.2 Instructions for Subject (data collection)

Background: The test measures nitric oxide in exhaled air to provide information about the airways in the lungs.

Important: Subjects must not wear nose pegs during the manoeuvre.

Seat subject comfortably on a chair (back erect, shoulders relaxed). Explain test to subject.

Ask subject to:

1. breathe out normally (to FRC);
2. place the mouthpiece in their mouth seal their lips around it
3. breathe in as deeply as possible (to TLC); and
4. breathe out with slight force and at a constant rate - 50 ml/s (green area for meter display; between 2 horizontal lines for balloon display) until asked to stop.

As subject breathes out, the meter/balloon display window is triggered.

NB: If subject has difficulty triggering the display window an alternative procedure can be used. Ask subject to take a big breath in (to TLC), place the mouthpiece in their mouth and blow out with slight force and at a constant rate - 50 ml/s. Select **Options, Start measurement** from the menu bar as subject breathes out. This manoeuvre is exhalation only (annotate on data collection sheet). Once the screen has switched over to the meter/balloon and **prior** to exhalation ask them to release their nose/remove nose peg.

Subject has up to 6 attempts to produce 3 valid measurements.

Repeat test.

When 3 valid measurements have been obtained **Save** dialogue box will be displayed. Click **OK** to save data.

At end of testing session leave device in **Standby** mode.

Important: Wipe down breathing handle with between subjects.
Discard subject's filter after measurements and replace with room air filter.

To start a new measurement, select **New Patient** from the **Patient** menu or click icon.

The following dialogue box will be displayed *"Do you want to close the current patient and open/create a new patient?"*

Click **Yes**.

NB: Exhalation time should be reduced for children. Change settings under **Options** menu. Return to default setting when testing adults.

6 Cleaning and Maintenance

A new "single use" mouthpiece, with filter, must be used for each subject. Care must be taken not to touch the mouth part of the mouthpiece. Mouthpieces should be disposed off as biological waste.

Wipe down areas that come into contact with subjects, and thoroughly (breathing handle, benchtop and chair) between testing sessions using a cloth and Viraclean (or similar product).

Inspect hoses, connectors and cables routinely for kinks, poor connections etc.

Replace the room air filter on the breathing handle **every 30** days (write the date for replacement on filter).

Replace NO scrubber every 6 months (Sept 2005; Mar, Sept 2006; Mar, Sept 2007).

Service must be performed every 18 months (Sept 2006; Mar 2008). Device must be repacked and transported to Germany.

7 Infection Control

The Niox[®] breathing handle may be cleaned up to 10 times in the event of contamination. Refer to Chapter 3 Daily Use manual, p 26.

8 Data Backup

Refer to Chapter 6 Technical manual, p 64-66 or Chapter 11 Daily Use Manual, p113-119.

Important: Back up data daily, at the end of each testing session.

Insert floppy disk into port at front of Niox[®] device. Designate 1 floppy disk per day for 2 weeks, label with name of day. Rotate through floppy disks on a 2-week cycle.

Alternatively, the device may be networked to the primary laptop using cables and wireless router.

From **Options** menu select **Backup, Backup data**.

Select a location in **Backup Patient Database** dialogue box.

Click **Backup**. Back up data files to another **PC** (not laptop); Burn a CD **weekly** – label, include date.

9 Shut down and transportation

Refer to Niox Technical Manual, Chapter 11, p 83-85.

Retain and store all packaging as it will be required for future transportation.

Ensure all data is backed up.

Select **File, shut down**. Click **OK**.

Wait for flushing of system to complete. Turn off switch to mains at rear of device.

Leave device for 30 minutes prior to disassembly.

Remove in-let hose for transportation. The spring-loaded collar requires a screwdriver to remove. Plug hole with a cork.

Place hoses in plastic bag (to preVENT dust/dirt entering).

Disconnect cylinder and remove regulator.

10 Trouble-shooting

| Problem | Solution |
|--|--|
| RC pressure warning | Contact Ray Jarvis |
| Non-critical cabinet temperature warning | Check room temperature. Ensure airconditioning is operational and maintained. If critical, software will prompt for calibration. |
| Power interruption | Niox software will restart and prompt for recalibration. Connection to uninterruptible power supply (UPS) unit will avoid power disruption unless >12 hours. |
| Back-up Unable to establish connection with G:\Woolcock\FOT | Occurs when Niox unit is turned off. Ensure FOT computer is turned on and network cable is connected. Prompt saying unable to restore connection to G:\ will be displayed. Click continue. Niox software will start by default. Allow system to warm up (~ 30 minutes). Click cancel when prompted for calibration. Open any test subject. On completion of self-test choose exit to "NT settings" from the "Options" tab. Niox software will close Double click on "My computer" on desktop to list all drives connected to the Niox device. Select G:\Woolcock drive (red cross through icon) to restore network connection. Start Niox software and perform calibration procedure when prompted. |

Niox contact details:

Ray Jarvis

rjarvis@zynergymedical.com.au

T: 02 9344 8311

F: 02 9344 8411

M: 0416 025 868

11 References

American Thoracic Society/European Respiratory Society. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. Am J Respir Crit Care Med 2005; 171: 912-930.

APPENDIX F - BACTERIAL NOSE AND THROAT SWAB SOP

Important Note 1:

This is a clinical research-only protocol for Respiratory Medicine, HMRI studies.

This is NOT a protocol used by Hunter New England Area Health for bacterial detection.

Important Note 2:

Swabs should be transported to pathology for processing as soon as practical. Swabs can be stored and transported at room temperature.

1. Purpose of this SOP:

To describe the procedure for collecting nasal and throat swabs for bacterial detection.

2. Equipment: PPE: personal protective equipment (safety glasses, gloves)

3. Materials:

- 3.1. Swabs (plastic shaft in blue-lid container in AMIES medium) Interpath Pty Ltd ph 1800 626369 Box of 50 Cat #COPAN 108 CIS
- 3.2. Alcohol hand gel

4. Set Up:

- 4.1. Explain to the patient the procedure and the number of samples being taken. Also warn the patient that some minor discomfort may be felt but that no damage will result from the swabs.
- 4.2. Label the swab containers and check the patient identity against the case report form (CRF)
- 4.3. Clean hands with alcohol gel and put on PPE

5. Method:

5.1. Nasal swab

- 5.1.1. Twist the lid off the tube and withdraw the swab from the packaging
- 5.1.2. Sample a single anterior nostril by gently abrading the nasal mucosa
- 5.1.3. Replace the swab firmly in its container
- 5.1.4. If doing 2 swabs, repeat **steps 5.1.1 - 5.1.3** in the other nostril using a different swab
- 5.1.5. Place the completed labelled swab(s) in the specimen bag and store at room temperature

5.2. Throat swab

- 5.2.1. Twist the lid off the tube and withdraw the swab from the packaging
- 5.2.2. With the patient seated, place their head in a neutral position with their mouth open and tongue protruding. If necessary, use a wooden tongue depressor. The chance of gagging is reduced by asking the patient to focus on breathing steadily throughout the procedure.
- 5.2.3. Sample both tonsils and the posterior oropharynx with the swab. Avoid touching the swab on the tongue or other parts of the mouth.
- 5.2.4. Replace the swab firmly in its container
- 5.2.5. If doing 2 swabs, repeat **steps 5.2.1 – 5.2.4** using a different swab
- 5.2.6. Place the completed labelled swab(s) in the specimen bag and store at room temperature

6. Safety Precautions:

- 6.1. **Contact supervisor immediately if any problems occur.**

APPENDIX G - PROHIBITED CONCOMITANT MEDICATIONS LIST

| Generic name | Proprietary Name | Indications |
|--|----------------------------------|-----------------------|
| Azithromycin | Zedd | Macrolide Antibiotic |
| | Zithromax | |
| | Zithromax IV | |
| Clarithromycin | Clarac | |
| | Clarihexas | |
| | Clarithro | |
| | Clarithromycin | |
| | Kalixocin | |
| | Klacid | |
| Erythromycin | Erythromycin | |
| | E-Mycin | |
| | EES | |
| | Eryc | |
| | Erythrocin IV | |
| Roxithromycin | Blaxsig | |
| | Roxar | |
| | Roxide | |
| | Roximycin | |
| | Roxithromycin-RL | |
| | Rulide | |
| | Rulide D | |
| Amiodarone | Cordarone | Anti-arrhythmic |
| | Pacerone | |
| Disopyramide | Norpace | |
| Dofetilide | Tikosyn | |
| Ibutilide | Corvert | |
| Procainamide | Pronestyl | |
| | Procan | |
| Quinidine | Cardioquin | |
| | Quinaglute | |
| Sotalol | Betapace | |
| Amlodipine besylate; Atorvastatin calcium | Caduet | Hypolipidaemic agents |
| Atorvastatin calcium | Lipitor | |
| Pravastatin sodium | APO-Pravastatin | |
| | Chemmart Pravastatin | |
| | Cholstat | |
| | GenRx Pravastatin | |
| | Lipostat | |
| | Liprachol | |
| | Pravachol | |
| | Pravastatin-DP | |
| Pravastatin sodium | Pravastatin-GA | |
| | Pravastatin-RL | |
| | Pravastatin generichealth | |
| | Pravastatin Winthrop | |
| | Terry White Chemists Pravastatin | |
| | Vastoran | |
| Simvastatin | Chemmart Simvastatin | |
| | Genepharm Simvastatin | |
| | GenRx Simvastatin | |
| | Lipex | |

| Generic name | Proprietary Name | Indications |
|---------------------|----------------------------------|-----------------------------|
| | Ransim | Hypolipidaemic agents |
| | Simvabell | |
| | Simvahexal | |
| | Simvar | |
| | Simvastatin-DP | |
| | Simvastatin-RL | |
| | Simvastatin generichealth | |
| | Simvastatin Winthrop | |
| | Simvasyn | |
| | Terry White Chemists Simvastatin | |
| | Vytorin | |
| | Zimstat | |
| | Zocor | |
| Arsenic trioxide | Trisenox | Anti-cancer/Leukaemia |
| Astemizole | Hismanal | Anti-histamine |
| Bepridil | Vascor | Anti-anginal |
| Chloroquine | Aralen | Anti-malarial |
| Halofantrine | Halfan | |
| Chlorpromazine | Thorazine | Anti-psychotic |
| Haloperidol | Haldol | |
| Mesoridazine | Serentil | |
| Pimozide | Orap | |
| Thioridazine | Mellaril | |
| Cisapride | Propulsid | |
| Sparfloxacin | Zagam | Antibiotic |
| Domperidone | Motilium | Anti-nausea |
| Droperidol | Inapsine | Sedative |
| Levomethadyl | Orlaam | Opiate agonist |
| Methadone | Methadose | |
| | Dolophine | |
| Buprenorphine | Norspan Transdermal Patch | Opioid agonist |
| Pentamidine | NebuPent | Anti-infective |
| | Pentam | |
| Cyclosporin | Cicloral | Immunosuppressive |
| | Neoral | |
| | Sandimmun | |
| Zidovudine | Combivir | Anti-viral |
| | Retrovir | |
| | Trizivir | |
| Ergot alkaloid | Cafergot | Migraine |
| | Ergometrine injection | Agents acting on the uterus |
| | Syntometrine | |

APPENDIX H - SUB STUDY: Use of exhaled nitric oxide to detect NEA

Exhaled nitric oxide will be measured at 3 centres (JHH, PAH, SCGH) to identify the value of this measure in predicting the response to azithromycin and identifying NEA. This will extend the generalisability of the results to centres where sputum induction is not available. Exhaled nitric oxide will be measured at visits 2 (randomisation), 10 (end of treatment period) and 11 (4 weeks post-treatment).

In pilot data of 134 adults with asthma, we have established that 75% of patients with NEA have an exhaled nitric oxide of <30 ppb. The median (interquartile range) exhaled nitric oxide was 19.6 (13.2-29.0) in NEA compared to 38.5 (20.6-76.2) in eosinophilic asthma, $p < 0.0001$. Exhaled nitric oxide will be measured using the on-line NIOX chemiluminescent analyser (Aerocrine AB; Solna, Sweden) according to ATS guidelines.⁴⁴

APPENDIX I - SUB STUDY: Markers of inflammation and Airway and Gut Microbiome

To be carried out in participants from each study site as outlined below.

This substudy will evaluate additional mechanistic outcomes testing the hypothesis that AZM is effective at reducing markers of airway inflammation and systemic inflammation in non-eosinophilic asthma. In addition it will collect samples to characterise the airway and gut microbiome in these patients.

Inflammation will be assessed by measuring IL-8 and neutrophil elastase levels (commercial ELISA) in sputum.³² Total blood leukocyte count and C-reactive protein (CRP) will be measured in peripheral blood, based on improvements in these parameters following administration of azithromycin therapy in COPD.³⁰

Peripheral blood will also be screened for the presence of novel biomarkers (ELISA and/or western blot) to validate the correlation of specific biomarkers with asthma subtype and potentially correlate these biomarkers with response to azithromycin treatment.

Total sputum and faecal DNA will be extracted (Qiagen, Hilden, Germany) and the V8 and V9 regions of the 16 S rRNA gene amplified. Amplicons will be sequenced from the reverse primer using the GS-FLX platform at the Australian Centre for Ecogenomics. Identified bacterial species using real time PCR using commercially available kits (Qiagen).

Airway gene expression will be assessed using Illumina Bead Arrays and real-time PCR to investigate differential gene expression in asthma subtypes and responses to azithromycin treatment.

Additional analyses may be performed subject to funding and review by the management committee.

PROTOCOL:

Inflammatory markers in sputum (*all sites*)

1. Sputum supernatant obtained at visits 2, 10 and 11 is to be stored at -80°C.
2. Samples to be grouped and shipped frozen, twice annually to JHH for analysis of IL-8 and neutrophil elastase levels.
3. Additional markers may be assessed if funding is available.

Airway gene expression analysis (*all sites to collect, analysis dependent on budget*)

1. 100µL of sputum obtained at visits 2, 10 and 11 is to be stored in 700µL RLT Buffer (QIAGEN, catalogue #79216) at -80°C.
2. Samples to be grouped and shipped frozen, twice annually to JHH for gene expression analysis.

Inflammatory markers in blood

Full blood count (including total blood leukocyte count) (all sites)

1. Total blood will be collected at visits 1 and 10 for total leukocyte count (4 mL EDTA tube - purple). The total leukocyte count will be a part of the full blood count (FBE) included in the safety bloods. FBE is to be done by site specific pathology services.

Serum CRP test and Biomarker Screening (all sites to collect, CRP test dependent on budget)

1. Serum will be collected at visits 1, 10 and 11 for CRP measurements and biomarker screening (6mL clot activator tube – red).
2. Serum is to be processed at site as follows. After 30 minutes, centrifuge at 3000 rpm for 10 minutes and make 500-1000µL aliquots. Store at -80°C (CRP in serum stable for greater than 1 year when stored as described).
3. Serum samples to be grouped and shipped frozen twice/year to JHH. Hunter Area Pathology Service (HAPS), Newcastle to perform CRP analysis. JHH to perform biomarker screening (500-1000µL serum required).

Airway and Gut microbiome

1. Sputum obtained at visits 2, 10 and 11 is to be stored at -80°C.
2. Sputum samples to be grouped and shipped frozen, twice annually to JHH for analysis
3. Faeces samples will be collected in a sub-group of 20 patients from the Newcastle centre only, at Visits 2, 10 and 11.

APPENDIX J - SUB STUDY: Macrophage phenotype and function in NEA: modulation by azithromycin

This research will address the hypotheses that

1. Analysis of induced sputum may provide a non-invasive technique to characterise macrophage phenotype and function in chronic lung diseases including asthma and COPD
2. Macrophage phenotype and function is impaired in non-eosinophilic asthma. This defect may contribute to the chronic inflammation and persistent airway neutrophilia that characterises this asthma subtype.
3. Azithromycin treatment will significantly improve macrophage phenotype and function and that this will be most evident in patients with non-eosinophilic asthma

Background

Evidence now shows that asthma is composed of several subtypes with up to 50% of all asthma cases showing no evidence of eosinophilic inflammation and a persistence of airway neutrophilia (non-eosinophilic asthma (NEA)). The presence of NEA exacerbations has been well documented in studies of acute asthma where viral infection results in increased influx of cells dominated by neutrophils.

Phagocytosis of accumulated apoptotic cells by alveolar macrophages (a process termed 'efferocytosis') is likely to be important in the airways in patients with NEA because (a) the chronic airway inflammation in asthma has been shown to lead to increased epithelial cell loss; (b) increased neutrophil numbers are present in the airways of these patients; and (c) chronic airway inflammation results in airway hyperresponsiveness and episodic respiratory symptoms. A resultant impairment of clearance of accumulated apoptotic cells has the potential to lead to secondary necrosis of uncleared cells and perpetuation of inflammation, and may be a target for therapeutic intervention.

Most of the studies of phagocytosis in asthma have focused on phagocytosis: the ability of alveolar macrophages to phagocytose bacteria or IgG opsonized yeast in asthma.^{47, 48} Fitzpatrick and colleagues compared normal volunteers to non-asthmatic children with chronic cough and those with moderate and severe asthma. Decreased phagocytic ability of alveolar macrophages to ingest *S. aureus* was noted in the children with poorly controlled asthma. Increased rates of infection by agents that include rhinovirus cause significant exacerbations of asthma. Oliver *et al* recently reported that rhinovirus exposure cause a reduced macrophage phagocytic response to labelled bacterial particles but not to latex beads, suggesting a specific defect in macrophage phagocytic ability in response to rhinovirus infection.⁴⁹ Despite these numerous studies of phagocytosis of bacteria in asthma, there have been few studies of efferocytosis in asthma. Huynh and colleagues compared the ability of alveolar macrophages to phagocytose apoptotic T-cell line Jurkats in normal volunteers, mild to moderate asthmatics, and severe, oral steroid dependant asthmatics.⁵⁰ They initially noted a reduced number of phagocytic bodies in the severe asthmatics but not in the mild-moderate group compared to normals. Further *ex-vivo* studies confirmed that alveolar macrophages from severe asthmatics had reduced ability to phagocytose the apoptotic cells. Interestingly, macrophages from the severe group were resistant to the phagocytosis – stimulating effect of LPS, but were responsive to dexamethasone, whereas macrophages from the mild to moderate asthmatics responded in a similar fashion to the normals.

The potential role of defective phagocytosis of apoptotic cells in the airways in NEA has not been determined.

Our previous studies have focused on the role of apoptosis and macrophage dysfunction in chronic obstructive pulmonary disease (COPD) which is also characterised by defective airway repair, chronic inflammation and an accumulation of neutrophils in the airway. We have shown that alveolar macrophages from subjects with COPD have significantly reduced ability to phagocytose apoptotic bronchial epithelial cells as well as defects in several mediators that are involved in the phagocytic process. We showed reduced expression of lung collectins (surfactant proteins A and D, and mannose binding lectin), and recognition molecules (mannose receptor, LDL receptor related protein [CD91] and PECAM (CD31)). It is likely that the non-eosinophilic form of asthma may follow this pattern as we (in collaboration with J Simpson (Hunter Medical Research Institute, NSW, Australia) have performed preliminary investigations of efferocytosis of apoptotic epithelial cells in sputum from a small number of asthmatics and noted a significant reduction in phagocytic ability of alveolar macrophage from asthmatics with NEA (a similar defect to that found in our COPD subjects) but no changes in the asthmatics with eosinophilic disease.

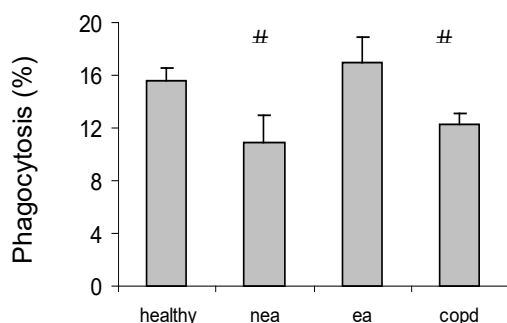


Figure 1. Phagocytosis of apoptotic bronchial epithelial cells by sputum-derived macrophages from healthy never smoker controls, asthmatics with NEA or eosinophilic (EA) disease and patients with COPD. Results are expressed as the percentage of macrophages ingesting apoptotic cells significantly reduced efferocytosis compared to never smoker controls. # significantly reduced efferocytosis compared to never smoker controls

The implications of macrophage phenotype in the airway are also an area of intense research interest at present. 'M1' macrophages are classically activated (by IFN- γ and/or LPS and TNF- α) and produce inducible nitric oxide synthase (iNOS) and promote inflammation and phagocytosis. M2 macrophages in contrast produce arginase and the anti-

inflammatory cytokines IL-10 and TGF- β . Our preliminary studies in COPD have shown a mixed macrophage phenotype with low production of arginase and mannose binding lectin (required for effective efferocytosis), and reduced expression of several molecules involved in macrophage recognition of apoptotic cells [mannose receptor, LDL receptor related protein (CD91), PECAM (CD31), FcR (CD16, CD64)]. Macrophage expression HLA-ABC and HLA-DR [required for effective antigen presentation] was also reduced. Functional capacity was mixed, with reduced phagocytic ability [M2] but capability of producing high levels of pro-inflammatory cytokines in response to stimulus [M1]. Taken together, these data indicate that alveolar macrophages from COPD subjects are functionally impaired, possibly the result of a phenotype switch. The implications of macrophage phenotype in NEA are currently unknown; however, it is possible that dysregulated macrophage phenotype and function (similar the changes noted in COPD) are present in NEA and that these changes may contribute to the neutrophilia and tissue damage that are characteristic of the disease. Already we have noted low expression of mannose receptor in sputum macrophages collected from patients with NEA and COPD compared to those with EA (NEA 37% \pm SEM; EA64% \pm SEM14%; COPD20% \pm SEM).

Treatment of NEA

Recent randomised controlled trials show that adjusting treatment to reduce sputum eosinophils can effectively reduce the number of eosinophilic exacerbations, but more importantly, does not influence asthma control or non-eosinophilic exacerbations, which are the dominant type in asthma. The optimal treatment of NEA is not known, however corticosteroids have little efficacy in this disease. There is therefore a need to characterise more fully the reasons for the chronic inflammation and neutrophilic accumulation and to define effective therapeutic options for NEA.

Macrolide antibiotics represent an exciting treatment option for asthma. In addition to their established anti-bacterial role there is both *in vitro* and *in vivo* evidence for an anti-inflammatory activity of macrolides, and some evidence that they may be efficacious in neutrophil mediated airway diseases. We have shown that in patients with COPD, azithromycin improved the phagocytosis of apoptotic epithelial cell and neutrophils. While the exact anti-inflammatory mechanisms of macrolide antibiotics are unknown our data suggest that part of the inflammatory action of macrolides may be through restoration of phagocytosis and removal of apoptotic cells prior to secondary necrosis.

Research plan:

Population and study design will be essentially as described in NHMRC proposal 569246.

Two centres (Hunter Medical Research Institute - John Hunter Hospital, and Royal Adelaide Hospital) will provide all subjects for this sub-study. Thirty EA and thirty NEA subjects allows adequate power to detect a 30% increase in phagocytic ability following azithromycin therapy (based on previous studies), and to compare macrophage phenotype and function in the two groups. We have already optimized specimen delivery between the centres (on average, a sputum collected and prepared can be delivered within 14h with no adverse effects on macrophage viability or phagocytic function). However we will compare sputum parameters in 3 subjects at 0 and 14h post collection; if differences are found, samples from both sites will be held for 14h before testing.

Cell counts: Only samples with a cell count of greater than 4×10^5 macrophages/mL will be tested for macrophage phagocytic ability and phenotype. Macrophage phenotype only will be assessed in samples with cell counts lower than 4×10^5 macrophages/mL.

Subject groups

1. NEA (PRE AND POST AZITHROMYCIN): n=30
2. EA (PRE AND POST AZITHROMYCIN): n=30
3. Normal controls: non-smokers with no lung disease: n=15

Samples

Induced sputum will be collected in both centres as described in NHMRC proposal 569246. Sputum samples will be collected at visits 1 OR 2 (not required at visit 2 if adequate macrophage numbers are obtained)*, 10 and 11. (An additional sputum sample may be collected approximately 2 weeks following visit 11 if adequate macrophage numbers are not obtained). * In the initial stages, samples from visit 1 and 2 will be used to enable a repeat of assays and assessment of inter-assay variability. Commencement date: June 2009; Expected completion date: June 2011

Methods

Following collection, sputum will be processed, prepared for differential cell counts and cells resuspended in RPMI1640 (1% FCS, 0.5% HEPES, 2% Pen/strep, 1% Amphotericin Fungizone). Samples from JHH will be sent overnight by courier to RAH..

Phagocytic ability of sputum-derived macrophages will be investigated using our established method. Briefly, our *in vitro* flow-cytometric assay quantifies phagocytosis of target cells (apoptotic bronchial epithelial cells) by macrophages (resuspended at a concentration of 4×10^5 macrophages/ml and purified by adhesion to plastic). Apoptosis is induced in the target cell by exposure to UV [these cells are stained with mitotracker red] and ingested cells identified using flow cytometry and co-staining with a macrophage marker and mitotracker red. A comparison between the 'gold standard technique' of measuring phagocytosis in BAL-derived alveolar macrophages and sputum-derived macrophages will be undertaken in 3 healthy individuals from whom both sputum and BAL will be collected within a 24h period.

Phenotypic analyses of macrophages

A limited panel of markers will be applied to investigate M1/M2 polarity phenotype [CD11C, CD64, CD91, mannose receptor]. *Arginase* and *mannose binding lectin* will be measured in sputum supernatant by ELISA.

APPENDIX K - SUB STUDY: Genetic studies - Pharmacogenomics (TPCH)

Ian Yang 12 May 2009

Hypothesis

In patients with asthma, genomic determinants influence response to azithromycin.

Rationale

A number of host factors are likely to influence the clinical and biological response to azithromycin in patients with asthma. Candidates for biological pathways involved include inflammation, neutrophil chemoattractants and chemokines, cell adhesion and innate immunity. Pathways linked to macrolide activity and pharmacokinetics may also be involved. However, there may be many other biological pathways and genes (with known or unknown function) that influence the response to azithromycin – hence, we will use a bias-free pharmacogenomic approach to discover genetic determinants of this clinical response.

Methods

1. Blood collection

Blood for genomic DNA extraction will be taken at Visit 1 during the study. 3 EDTA blood tubes (4 ml each = 12 ml total) will be used to collect blood from a peripheral arm vein. The 3 tubes containing the blood sample will then be frozen and stored in a -20°C freezer. When convenient, a batch of the frozen blood tubes will be couriered frozen (e.g. with dry ice, or with ice packs) to the Thoracic Research Laboratory, The Prince Charles Hospital (TPCH), Brisbane. Additional notes:

- There is no need for any centrifugation of the tubes prior to freezing.
- The tubes should not be stored in a -80°C freezer as the tubes may break.
- If required, the blood sample could be taken at any other visit (instead of Visit 1), if more convenient.
- Only one set of 3 EDTA blood tubes for genomic DNA is required (repeated sampling is not required).
- Ideally, the tubes of blood should be sent to TPCH within 3 months of blood collection, to facilitate processing and avoid excessive storage before processing.

2. DNA extraction and processing

Genomic DNA will be extracted from blood using the salt extraction method. DNA quality and concentration will be measured by optical density spectrometry using a Nanodrop spectrophotometer. DNA in solution will then be aliquoted as a stock solution and working solution, and stored appropriately until use. Initially two of the blood tubes will be extracted for genomic DNA, with the third tube used as a back-up in case re-extraction is required.

3. Genome-wide association study (GWAS)

The GWAS on the samples will be performed using Illumina Infinium Human 610-Quad BeadChip microarrays, containing 620,901 loci (including ~550,000 single nucleotide polymorphisms (SNPs) within genes and ~60,000 copy number variations in known genomic variation regions). We will outsource these microarrays to a commercial facility such as the Australian Genome Research Facility, which has high-throughput capability for such assays. Briefly, the array platform consists of multiplex bead-arrays with fluorescently labelled, SNP-specific primers. 200 ng of DNA template will be amplified overnight at 37°C then fragmented and precipitated with isopropanol. The dried precipitated pellet will be resuspended and hybridised to the BeadChip, to anneal to locus-specific 50-mers on the chip. The arrays will be incubated at 48°C overnight, then enzymatic extension will be performed. The products will be fluorescently stained and the bead fluorescence will be read using an Illumina BeadArray Reader. Quality of spots will be assessed using the Illumina BeadStudio 2.0 software.

Statistical analysis: Association will be tested with SNPs filtered for low call rate (>98%), and we will check assays for problem SNPs. Checks on data quality will be carried out as follows: Hardy Weinberg equilibrium will be tested for in the subjects. Outliers will be identified based on clustering using identity by state (IBS) information, as implemented in the genetics software program, PLINK. *SNP association with azithromycin response:* The program PLINK will be used to perform tests of association for a variety of genetic models (additive, genotypic). Logistic regression-based models in PLINK allow inclusion of clinical covariates. Tests for interaction between loci and for haplotypic models will be tested as required (as implemented in PLINK). *Risk copy number variations:* This platform also allows the discovery of copy number variations (CNVs) in the genome of subjects compared to controls. We will analyse for CNV differences between cases and controls, using recently described methods (implemented in PLINK).

Ethical considerations

The DNA will be stored in secure, locked freezers in the Thoracic Research Laboratory of The Prince Charles Hospital, for at least 7 years after the completion of the trial, in accordance with usual standards for storage of human samples for research. DNA will be stored in tubes that are de-identified but potentially re-identifiable with the use of a coded list; this is to ensure that any additional trial outcomes can be matched with the DNA samples. The DNA collected for this study will only be used for purposes specifically associated with this clinical trial. This genetics research study will examine natural genetic variants (polymorphisms) that are naturally inherited by a proportion of the general population. These polymorphisms, in themselves, do not diagnose an actual disease state. Furthermore, the grouped data will be analysed and applied to estimate the response to azithromycin in groups of patients, rather than using the genetic data in any individual patient. Because the genetic results do not specifically diagnose any disease state, genetic counselling and family screening are not required in this study.

APPENDIX L - SUB STUDY: NF κ B function and dendritic cell subsets (PAH)

Hypotheses

- (a) NF κ B function in blood leukocytes is altered in patients with asthma, and may be especially important in non-eosinophilic forms of asthma.
- (b) The defect in NF κ B function is partly corrected by treatment with azithromycin.
- (c) Changes in the relative numbers of dendritic cell subsets are associated with asthma, and the pattern varies in different forms of asthma.

Rationale

NF κ B plays a crucial role in regulating inflammatory pathways, especially those triggered by innate stimuli associated with microbial infections and products of cell injury. There is some evidence that NF κ B function is abnormal in asthma, though whether the NF κ B pathway is altered by macrolides is not known.

Allergic asthma is associated with changes in numbers of circulating dendritic cell subsets, but it is not known whether similar, or other changes in blood DC are associated with distinct asthma phenotypes as defined by sputum inflammatory cells.

Practical considerations

These studies are best performed on freshly collected blood samples, and would require blood samples to *arrive at PAH lab within 24 hours of collection*. Hence the ideal sites would be Brisbane and Newcastle. It would be difficult for this part of the study to involve Perth and Adelaide.

Blood samples to be collected at the same visits as sputum induction, preferably in the morning.

- At randomisation (visit 2),
- Final week of treatment (visit 10),
- Post treatment (visit 11)

Methods

- Collect 25 mL of blood into lithium heparin Vacutainer tubes (2 x 10 mL + 1 x 4mL green).
- **Process blood in a biohazard hood under sterile conditions.**
- Centrifuge blood tube (2000 rpm, 10 mins) within 2 hours of collection.
- Remove all of the plasma, reserving 1 – 2 mL (see next step). Divide and aliquot remaining plasma into 3 tubes and freeze at -20°C*.
- Transfer reserved 1 – 2 mL of plasma into a sterile 50 mL falcon tube.
- Transfer remaining blood cells to the falcon tube containing the 1 -2 mL of plasma. Fill tube to the top with incomplete RPMI culture medium (additives not required). Replace lid and gently invert to mix cells.
- Ensure tube is labelled with site ID, participant ID, visit number, date AND time of collection. Cover lid securely with parafilm.
- Sample to be sent to PAH via World Courier at room temperature (to arrive within 24 h of collection).

At PAH:

- Mononuclear cells are separated by gradient centrifugation with Lymphoprep.
- Cells to be stimulated *in vitro* with TLR4 and TLR7 ligands.
- Collect supernatant at 6 and 24 hours. Store at -80°C for later measurement of cytokine panel in Adelaide (cytometric bead array).
- Prepare nuclear extracts and measure the five NF κ B subunits by DNA binding ELISA.
- DC subsets stained in whole blood and enumerated by flow cytometry.

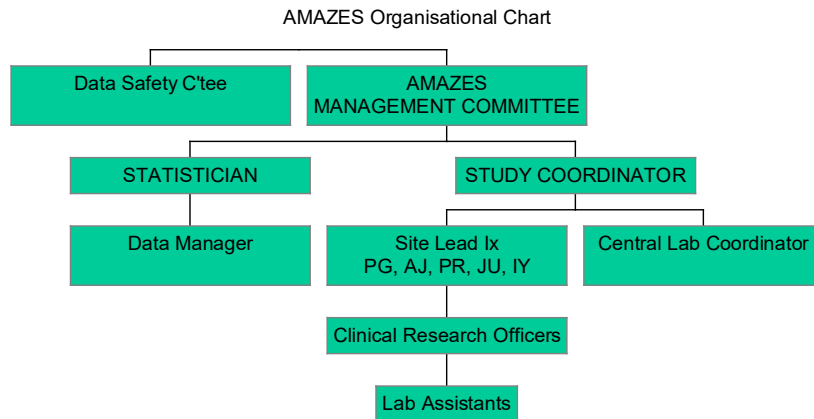
* The frozen plasma from these samples should still be sent to PAH, but can be stored and sent as a batch every 3 months.

An analysis of the initial data would be performed after 12 months. If initial data appears promising, later studies of NF κ B in sputum could be undertaken if management group approves.

John Upham
8 July 2009

APPENDIX M – AMAZES GOVERNANCE

AMAZES Organisational Chart



AMAZES MANAGEMENT COMMITTEE
PG, JS, PR, IY, JU, SH, AJ

DATA SAFETY COMMITTEE
A/Professor Peter Wark
Dr David Arnold
Dr Anne Vertigan

STATISTICIAN: Dr Patrick McElduff

STUDY COORDINATOR: Catherine Delahunty

CLINICAL RESEARCH OFFICERS:
JHH: Gabrielle LeBrocq & Kelly Steel
RAH: Kirsty Herewane
SCGH: Peta Grayson, Robyn Jones
PAH: Michelle Towers & Tina Collins
TPCH: Janet Shaw & Pam Fung
CONC: Tessa Bird
WIMR: Gloria Foxley
LPH: Christina Madzinga

CENTRAL LAB COORDINATOR: Bridgette Ridewood

Laboratory Assistants:

JHH: processing team
PAH: Melanie Carrol, Alice Chen
RAH: Phillip Holt
TPCH: Janet Shaw
SCGH: processing by WestPath
WIMR: Kevin Oreo
CONC: Kevin Oreo
LPH: Melissa McClean, Kevin Oreo

APPENDIX N – DRUG ACCOUNTABILITY RECORD

| | | |
|----------------------------------|-------------------------|----------------------|
| PARTICIPANT RANDOMISATION NUMBER | SUBJECT ID (eg. 01-001) | PARTICIPANT INITIALS |
| | | |

| DISPENSING DETAILS | | | | RETURNED DETAILS | | | |
|--------------------|--------------|---------------|----------|------------------|---------------|-------------------|----------|
| DATE | VISIT NUMBER | BOTTLE NUMBER | INITIALS | DATE | BOTTLE NUMBER | QUANTITY RETURNED | INITIALS |
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APPENDIX O - SAFETY: MICROBIOLOGY SURVEILLANCE

Microbial Resistance as an Adverse Outcome in AMAZES

Possible effects:

Bacterial resistance to macrolides
Transmission of resistant bacteria
Infection with a resistant organism
Antibiotic related adverse event (ARAE): eg colitis, candidiasis
Superinfection with Candida

Event reporting

| Event | Baseline [wk 0, V2] | End of treatment [wk 48, V10] | Post surveillance [wk 96 V14] |
|--------------------------------------|---------------------|-------------------------------|-------------------------------|
| Proportion isolates resistant to AZM | | | |
| Infection related events, n (%) | - | | |
| ARAE, n (%) | | | |

Event definitions

Bacterial resistance to macrolides:

% pathogenic isolates from throat swabs, induced sputum that exhibit macrolide resistance

Infection with a resistant organism

Potential: participant with a significant infectious illness

Confirmed: bacteriology showing isolation of macrolide resistant organism from a participant with a significant infectious illness

Antibiotic related adverse event:

Antibiotic associated diarrhoea

Clostridium difficile colitis: colitis with positive C.difficile toxin test

Candidiasis: oral, vulvovaginal:

Event detection

Bacterial resistance to macrolides

Sample: Throat swab, nose swab induced sputum

Timing: visits 2, 10, 11

Population: sputum all sites [geographic distribution of macrolide resistance], nose/throat swabs at subset of sites.

Testing: bacteriological procedures outlined below.

Infection with a resistant organism:

Direct questioning at visits 6, 10, 11

Investigation and collection of relevant data related to each reported potentially infectious event from hospital records

Antibiotic related adverse event:

Diarrhoea: direct questioning at visits 2,6,10,11

C.difficile colitis: testing if presents with antibiotic associated diarrhoea, investigation and collection of relevant data related to each reported diarrhoea event from hospital records

Oral candidiasis: visual inspection at V2,6,10,11

Vulval candidiasis: direct questioning of female subjects at V2,6,10,11

Cutaneous *Staph. aureus* infection: direct questioning about boils and soft tissue infection events in patients (if positive, extend questions to cover family members)

Infectious adverse event (IAE)

Clinically important infections: if a participant seeks medical attention for an infection or an infection-related adverse event, this will be recorded as an Infectious Adverse Event (IAE). As much information as possible will be gathered about the event including any available microbiological test results. An IAE form will be completed and the investigator will assess any need for additional follow up. The completed IAE form and any de-identified medical information collected will be filed in the participant CRF.

MICROBIOLOGY SUBSTUDY

1. Bacterial colonisation in asthma and its relation to asthma phenotype and pathogenetic features.
Hypothesis: increased bacterial colonisation in neutrophilic asthma
2. Geographical variation of macrolide resistance of respiratory flora in Australian adults with asthma.
Hypothesis: geographical variation in macrolide resistance exists between the 5 centres [? Related to background macrolide use]

3. Induction of microbial resistance by long term AZM therapy in asthma: frequency, predictors, impact, adverse events, molecular mechanisms

Hypothesis: Long term AZM use in asthma induces high prevalence of macrolide resistance that is not associated with adverse infectious outcomes.

Bacteriology procedures:

All sites

- Sputum - identification of pathogens and Azithromycin sensitivities (Visits 2, 10 and 11).

Subset of sites (1 – 2 sites)

- Sputum – identification of pathogens and commensal organisms and Azithromycin sensitivities (Visits 2, 10 and 11).
- Throat swabs – identification of pathogens and commensal organisms and Azithromycin sensitivities (Visits 2, 10 and 11).
- Nose swabs – identification of *Staph aureus* and community acquired Methicillin-resistant *Staph Aureus* (MRSA) (Visits 2, 10 and 11).

Culture

SPUTUM - Selected sputum will be used to produce slides for gram stain. An aliquot of selected dispersed sputum and prepared slides are then to be sent to pathology for qualitative microbiology and Azithromycin sensitivities according to standard procedures of each individual laboratory. Plates will be read at 24 and 48 hours. All pathogens present are to be identified and reported (all organisms present are to be reported for a subset of sites). Biochemical testing for Azithromycin sensitivity will be performed using the Clinical and Laboratory Standards Institute (CLSI) method. Azithromycin discs will be obtained from OXOID. Resistant strains are to be stored in glycerol at -80°C for future characterisation of resistance genes, if required. Stored strains are to be batch shipped to John Hunter site as required (Contact Central Study Coordinator to arrange courier).

THROAT SWABS – Throat swabs are to be sent directly to pathology for qualitative microbiology and Azithromycin sensitivities. Chocolate bacitracin and blood agar plates (with Optochin and Bacitracin discs) will be inoculated with the throat swab. Plates will be read at 24 and 48 hours. All organisms present are to be identified and reported. Biochemical testing for Azithromycin sensitivity will be performed using the Clinical and Laboratory Standards Institute (CLSI) method. Azithromycin discs will be obtained from OXOID. Resistant strains are to be stored in glycerol at -80°C for future characterisation of resistance genes, if required. Stored strains are to be batch shipped to John Hunter site as required (Contact Central Study Coordinator to arrange courier).

NOSE SWABS – Nose swabs are to be sent directly to pathology. Chocolate bacitracin and blood agar plates (with Optochin and Bacitracin discs) will be inoculated with the nose swab. Plates will be read at 24 and 48 hours. *Staph aureus* will be cultured further on chromogenic OXOID Brilliance™ agar for identification of MRSA. *Staph aureus* isolates will be stored in glycerol at -80°C for future characterisation in case further typing/analysis is warranted. Stored strains are to be batch shipped to John Hunter site as required (Contact Central Study Coordinator to arrange courier).

Organisms the pathology service will be asked to identify in sputum and throat swab culture may include (although not be limited to) the following:

- Various alpha-haem Streptococci (Viridans group)
- Haemolytic streptococci (non group A)
- *Strep. Pneumoniae*
- *Staph. Aureus*
- *Moraxella spp.*
- *Moraxella catarhalis*
- *Haemophilus influenzae*
- *Pseudomonas aeruginosa*

APPENDIX P - QUESTIONNAIRES

JUNIPER ASTHMA CONTROL QUESTIONNAIRE © – Used with permission

ASTHMA CONTROL QUESTIONNAIRE

Circle the number of the response that best describes how you have been during the past week.

- 1 On average, during the past week, how often were you woken by your asthma during the night?
- 0 Never
 - 1 Hardly ever
 - 2 A few times
 - 3 Several times
 - 4 Many times
 - 5 A great many times
 - 6 Unable to sleep because of asthma
- 2 On average, during the past week, how bad were your asthma symptoms when you wake up in the morning?
- 0 No symptoms
 - 1 Very mild symptoms
 - 2 Mild symptoms
 - 3 Moderate symptoms
 - 4 Quite severe symptoms
 - 5 Severe symptoms
 - 6 Very severe symptoms
- 3 In general, during the past week, how limited were you in your activities because of your asthma?
- 0 Not limited at all
 - 1 Very slightly limited
 - 2 Slightly limited
 - 3 Moderately limited
 - 4 Very limited
 - 5 Extremely limited
 - 6 Totally limited
- 4 In general, during the past week, how much shortness of breath did you experience because of your asthma?
- 0 None
 - 1 Very little
 - 2 A little
 - 3 A moderate amount
 - 4 Quite a lot
 - 5 A great deal
 - 6 A very great deal
- 5 In general, during the past week, how much of the time did you wheeze?
- 0 Not at all
 - 1 Hardly any of the time
 - 2 A little of the time
 - 3 A moderate amount of the time
 - 4 A lot of the time
 - 5 Most of the time
 - 6 All the time
- 6 On average, during the past week, how many puffs of short-acting bronchodilator (eg Ventolin) Have you used each day?
- 0 None
 - 1 1-2 puffs most days
 - 2 3-4 puffs most days
 - 3 5-8 puffs most days
 - 4 9-12 puffs most days
 - 5 13-16 puffs most days
 - 6 More than 16 puffs most day

TOTAL SCORE (Questions 1-6) = _____

ACQ(6) = Total score/6 = _____

**JUNIPER ASTHMA QUALITY OF LIFE QUESTIONNAIRE (AQLQ)
(STANDARDISED)**

1. Please indicate how much you have been limited by your asthma in strenuous activities (such as hurrying, exercising, running up stairs, sport) during the last 2 weeks. **Green card**
2. Please indicate how much you have been limited by your asthma in moderate activities (such as walking, housework, gardening, shopping, climbing stairs) during the last 2 weeks. **Green card**
3. Please indicate how much you have been limited by your asthma in social activities (such as talking, playing with pets/children, visiting friends/relatives) during the last 2 weeks. **Green card**
4. Please indicate how much you have been limited by your asthma in work related activities (such as tasks that you have to do at work) during the last 2 weeks. **Green card**
5. Please indicate how much you have been limited by your asthma in sleeping during the last 2 weeks. **Green card**
6. How much discomfort or distress have you felt over the last 2 weeks as a result of chest tightness? **Red card**
7. In general how often during the last 2 weeks have you felt concerned about having asthma? **Blue card**
8. How often during the last 2 weeks did you feel short of breath as a result of your asthma? **Blue card**
9. How often during the last 2 weeks did you experience asthma symptoms as a result of being exposed to cigarette smoke? **Blue card**
10. How often during the last 2 weeks did you experience a wheeze in your chest? **Blue card**
11. How often during the past 2 weeks did you feel you had to avoid a situation or an environment because of cigarette smoke? **Blue card**
12. How much discomfort or distress have you felt over these 2 past weeks as a result of coughing? **Red card**
13. How often during the past 2 weeks did you feel frustrated as a result of your asthma? **Blue card**
14. How often during the past 2 weeks did you experience a feeling of chest heaviness? **Blue card**
15. How often during the past 2 weeks did you feel concerned about the need to take medications for your asthma? **Blue card**
16. How often during the past 2 weeks did you feel the need to clear your throat? **Blue card**
17. How often during the past 2 weeks did you experience asthma symptoms as a result of being exposed to dust? **Blue card**

Continued on next page.

18. How often during the past 2 weeks did you experience difficulty breathing out as a result your asthma? **Blue card**
19. How often during the past 2 weeks did you feel you had to avoid a situation or an environment because of dust? **Blue card**
20. How often during the past 2 weeks did you wake up in the morning with asthma symptoms? **Blue card**
21. How often during the past 2 weeks did you feel afraid of not having your asthma medication available? **Blue card**
22. How often during the past 2 weeks were you bothered by heavy breathing? **Blue card**
23. How often during the past 2 weeks did you experience asthma symptoms as a result of the weather or air pollution outside? **Blue card**
24. How often during the past 2 weeks have you been woken at night by your asthma? **Blue card**
25. How often during the past 2 weeks have you had to avoid or limit going outside because of the weather or air pollution ? **Blue card**
26. How often during the past 2 weeks did you experience asthma symptoms as a result of being exposed to strong smells or perfume? **Blue card**
27. How often during the past 2 weeks did you feel afraid of getting out of breath? **Blue card**
28. How often during the past 2 weeks did you feel you had to avoid a situation or environment because of strong smells or perfume? **Blue card**
29. How often during the past 2 weeks has your asthma interfered with getting a good night sleep? **Blue card**
30. How often during the past 2 weeks have you had the feeling of fighting for air? **Blue card**
31. Think of the overall range of activities that you would have liked to have done during the past 2 weeks. How much has your range of activities been limited by your asthma? **Yellow card**
32. Overall, among all the activities that you have done during the past 2 weeks, how limited have you been by your asthma? **Green card**

(Response cards are included on next page)

RED CARD

1. A very great deal of discomfort or distress
2. A great deal of discomfort or distress
3. A good deal of discomfort or distress
4. A moderate amount of discomfort or distress
5. Some discomfort or distress
6. Very little discomfort or distress
7. No discomfort or distress

GREEN CARD

1. Totally limited, couldn't do activity at all
2. Extremely limited
3. Very limited
4. Moderate limitation
5. Some limitation
6. A little limitation
7. Not at all limited

YELLOW CARD

1. Severely limited – most activities not done
2. Very limited
3. Moderately limited – several activities not done
4. Slightly limited
5. Very slightly limited – very few activities not done
6. Hardly limited at all
7. Not limited at all – have done all activities that I wanted to do

BLUE CARD

1. All of the time
2. Most of the time
3. A good bit of the time
4. Some of the time
5. A little of the time
6. Hardly any of the time
7. None of the time

COMMON COLD QUESTIONNAIRE (CCQ)

| COMMON COLD QUESTIONNAIRE | | | |
|--|--|---|----|
| <i>Current cold – complete as requested</i> | | | |
| Do you currently have a cold/flu? | YES | <input style="width: 40px; height: 20px;" type="text"/> | NO |
| If Yes: | | | |
| How many days since your cold worsened? | <input style="width: 100px; height: 25px;" type="text"/> | | |
| If asthma has also been worse: | | | |
| How many days since your asthma worsened? | <input style="width: 100px; height: 25px;" type="text"/> | | |

In the past 2 days, have you experienced any of the following:

| | None | Mild | Moderate | Severe |
|-------------------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| Category 1: General Symptoms | | | | |
| Fever | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Chills | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Muscle Pains | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Category 2: Nasal Symptoms | | | | |
| Watery Eyes | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Runny Nose | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Sneezing | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Category 3: Throat Symptoms | | | | |
| Sore Throat | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Category 4: Chest Symptoms | | | | |
| Cough | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Chest Pain | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |

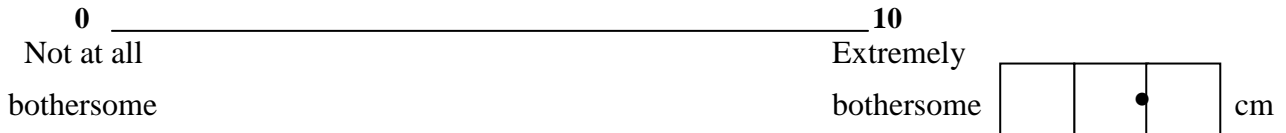
| | |
|---|---|
| <p style="text-align: center;">Probable Virus</p> <p>Moderate (or greater) in at least 2 of the above 4 categories</p> <p>Mild (or greater) in at least 3 of the above 4 categories</p> <p>YES <input style="width: 40px;" type="text"/> NO <input style="width: 40px;" type="text"/></p> <p>Comments</p> | <p style="text-align: center;">Possible Virus</p> <p>Cough + Mild (or greater) in at least 1 of the above 4 categories</p> <p>YES <input style="width: 40px;" type="text"/> NO <input style="width: 40px;" type="text"/></p> |
| FLU VACCINE | |
| <p>Have you had a flu vaccine in the past month/(past year visit 1)? YES <input style="width: 40px;" type="text"/> NO <input style="width: 40px;" type="text"/></p> <p>If yes, date (mm/yy) _____ / 20_____</p> | |

ASTHMA SYMPTOMS SCALE

SYMPTOMS Scale

Please place a cross on the line between 0 and 10 where your symptoms are bothersome for you

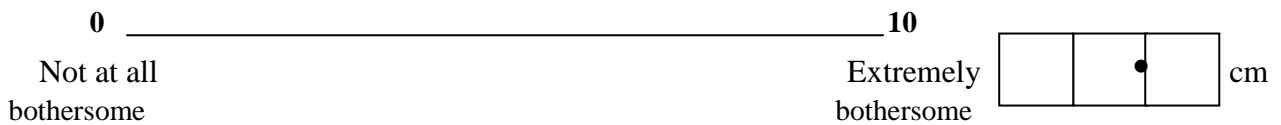
Nasal symptoms (blocked nose, runny nose, itchy nose or sneezing)



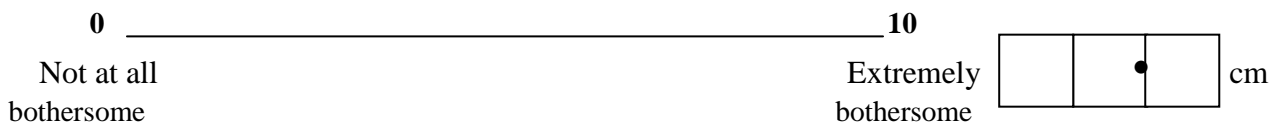
Breathlessness



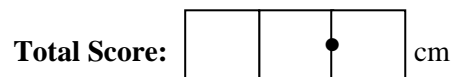
Wheeze



Sputum production



Cough



EPWORTH SLEEPINESS SCALE

Epworth Sleepiness Scale

(*participant to complete*)

How likely are you to doze off or fall asleep in the following situations, in contrast to feeling just tired? This refers to your usual way of life in recent times. Even if you have not done some of these things recently, try to work out how they would have affected you. Use the following scale to choose the most *appropriate number* for each situation:

- 0 = would *never* doze
- 1 = *slight* chance of dozing
- 2 = *moderate* chance of dozing
- 3 = *high* chance of dozing

| Situation | Chance of dozing |
|--|------------------|
| Sitting and reading | |
| Watching television | |
| Sitting, inactive in a public place(e.g. a theatre or a meeting) | |
| As a passenger in a car for an hour without a break | |
| Lying down to rest in the afternoon when circumstances permit | |
| Sitting and talking to someone | |
| Sitting quietly after a lunch without alcohol | |
| In a car, while stopped for a few minutes in traffic | |

APPENDIX Q – PARTICIPANT SYMPTOM DIARY - EXAMPLE ONLY – Please note, formatting has been adjusted for inclusion in protocol document.

| | | | | | | | | | | | | | | | | | | | |
|--|---|-----------------|--------------------------|---|--|----------|--|--|--|--|--|--|--|--|--|--|--|--|--|
| Date Dispensed: ___ / ___ / ___ Visit # ___ | | | | | | | | | | | | | | | | | | | |
| AMAZES ID: ___ - ___ (___) <small>(Site ID) (Subject ID) (Initials)</small> | | Date (dd/mm) | | WEEK ___ | | WEEK ___ | | | | | | | | | | | | | |
| | | Day of the week | | | | | | | | | | | | | | | | | |
| DAYTIME SYMPTOMS - For each question record number as follows: | | | | DAYTIME SYMPTOMS (Record before bed)[#] | | | | | | | | | | | | | | | |
| 1 | How often did you experience asthma symptoms today? <i>None of the time 0 1 2 3 4 5 6 All of the time</i> | | | | | | | | | | | | | | | | | | |
| 2 | How much did your asthma symptoms bother you today? <i>Not bothered at all 0 1 2 3 4 5 6 Severely bothered</i> | | | | | | | | | | | | | | | | | | |
| 3 | How much activity could you do today? ** 3 = normal activity ** <i>More than usual 0 1 2 3 4 5 6 Less than usual</i> | | | | | | | | | | | | | | | | | | |
| 4 | How often did your asthma affect your activities today? <i>None of the time 0 1 2 3 4 5 6 All of the time</i> | | | | | | | | | | | | | | | | | | |
| NIGHT TIME SYMPTOMS - For each question record number as follows: | | | | NIGHT TIME SYMPTOMS (Record when you get up)[#] | | | | | | | | | | | | | | | |
| 5 | Did you wake up with asthma symptoms? <small>(This can be awakening in the middle of the night, or on awakening in the morning).</small> <i>No = 0, Once = 1, More than once = 2, Awake 'all night' = 3</i> | | | | | | | | | | | | | | | | | | |
| Have you had any of the following symptoms at any time (day or night)? <small>For each symptom record a number: None = 0, Mild = 1, Moderate = 2, Severe = 3</small> | | | | SYMPTOM TYPES (Record before bed) <i>None = 0, Mild = 1, Moderate = 2, Severe = 3</i> | | | | | | | | | | | | | | | |
| 6 | Wheeze | | | | | | | | | | | | | | | | | | |
| 7 | Cough | | | | | | | | | | | | | | | | | | |
| 8 | Sputum Production | | | | | | | | | | | | | | | | | | |
| 9 | Shortness of breath or chest tightness | | | | | | | | | | | | | | | | | | |
| 10 | Runny nose or sneezing | | | | | | | | | | | | | | | | | | |
| 11 | Blocked nose | | | | | | | | | | | | | | | | | | |
| 12 | Sore throat or hoarse voice | | | | | | | | | | | | | | | | | | |
| 13 | Head or face ache | | | | | | | | | | | | | | | | | | |
| 14 | Aches, chills or fever | | | | | | | | | | | | | | | | | | |
| MEDICATION USE IN THE LAST 24 HOURS | | | | MEDICATION USE | | | | | | | | | | | | | | | |
| 15 | On how many OCCASIONS did you use a reliever puffer (Ventolin/Asmol/Bricanyl) in last 24hrs: | | (a) TO RELIEVE SYMPTOMS? | | | | | | | | | | | | | | | | |
| | | | (b) PRIOR TO EXERCISE? | | | | | | | | | | | | | | | | |
| 16 | Total NUMBER OF PUFFS of preventer medication used in 24hrs? <small>(Symbicort/Seretide/ _____)</small> | | | | | | | | | | | | | | | | | | |
| 17 | Have you used any other asthma medications? <small>Record name and indicate Yes/No (Y/N) for each day</small> | | | | | | | | | | | | | | | | | | |
| 18 | Study Medication Taken? Tick when taken - 2 tablets, 3 times a week (eg. Mon/Wed/Fri) | | | | | | | | | | | | | | | | | | |

If you develop a cold or your cold worsens, please phone the AMAZES study team on: _____

20 PROTOCOL VERSION CONTROL

| Version | Date | Prepared By | Change Description |
|---------|-------------|---------------------|--|
| 1 | 4 NOV 2008 | JODIE SIMPSON | N/A |
| 2 | 22 DEC 2008 | JODIE SIMPSON | Improvements to protocol under discussion. |
| 3 | 19 JAN 2009 | ERIN HARVEY | Improvements to protocol under discussion. |
| 4 | 24 FEB 2009 | ERIN HARVEY | Improvements to protocol under discussion. |
| 5 | 31 MAR 2009 | ERIN HARVEY | Improvements to protocol under discussion. |
| 6 | 8 APR 2009 | ERIN HARVEY | Improvements to protocol under discussion. |
| 7 | 13 MAY 2009 | ERIN HARVEY | Improvements to protocol under discussion. |
| 8 | 03 JUN 2009 | ERIN HARVEY | Improvements to protocol under discussion. |
| 9 | 17 JUL 2009 | ERIN HARVEY | Improvements to protocol under discussion. |
| 10 | 24 JUL 2009 | ERIN HARVEY | Improvements to protocol under discussion. |
| 11 | 10 SEP 2009 | ERIN HARVEY | Update, inclusion/exclusion criteria clarified, questionnaires included. |
| 12 | 19 APR 2010 | ERIN HARVEY | Update, SOPs clarified, appendices updated. |
| 13 | 28 JUL 2010 | ERIN HARVEY | Follow up period reduced to 4 weeks, staff update, prohibited medications updated. |
| 14 | 03 FEB 2012 | CALIDA GARSIDE | Schedule of assessments updated, prohibited medications update, inclusion/exclusion criteria clarified, documents reformatted. |
| 15 | 25 FEB 2014 | CATHERINE DELAHUNTY | Add airway & gut microbiome to inflammation substudy, addition of faeces sample collection at JHH (V2, V10, V11), update staff contact details |

END OF PROTOCOL