

Official title: Exacerbation in severe asthma patients: mechanisms and biomarkers

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Background

Exacerbations are important events in asthma and represent an important burden for the patient. The risk of experiencing an exacerbation is greatest in the patient with severe asthma, who already experiences daily symptoms, impaired lung function and adverse effects from corticosteroid therapy despite using maximal amounts of anti-asthma therapies particularly corticosteroids(1;2). In the SARP severe asthma cohort, 40% of patients required one or more emergency department visits, compared to 20% and 15% in moderate and mild asthma groups respectively(3); for those using >3 oral corticosteroid bursts in the past year, this was 54% in severe, 13% in moderate and 5% in mild. In the UK BTS cohort, up to 89% of severe asthma patients required one or more courses of high dose prednisolone in the previous year(4). Exacerbations are occurring even while on maximal doses of therapy.

According to the ERS/ATS Task Force on Asthma Control and Exacerbations(5), the definition of an asthma exacerbation is based on deterioration of symptoms that needs urgent action with the need to use of systemic corticosteroids defining severe exacerbations, and those without the needing for corticosteroids or increasing corticosteroids defining moderate exacerbations. However, these definitions while useful in clinical trials are not adequate for health care professionals and for patients, where a prospective definition is needed. There has been relatively few studies examining the circumstances of an exacerbation. In patients with mild-to-moderate asthma, exacerbations were characterized by a gradual fall in peak expiratory flows (PEF) over several days, followed by more rapid changes over 2 to 3 days with a parallel increase in symptoms and rescue beta-agonist use(6), which was unaffected by the use of inhaled corticosteroids or of a long-acting beta-agonist. Very little is known about the course and severity of these exacerbations in severe asthma. We hypothesise that the causes of an exacerbation in severe asthma may be different than those in non-severe asthma.

Causes of exacerbations

Viral respiratory tract infections have been recognised as triggers for exacerbations of asthma with rhinoviruses being the most common virus(7). Bacteria have also been associated with exacerbations including atypical *Mycoplasma pneumonia* and *Chlamydiae pneumonia*, in 27% of

exacerbations(8). In stable patients with severe asthma from RBH, ~50% of sputum cultures were positive for bacteria, and this was more likely in those with an exacerbation in the previous year (9). Microbiome studies of the lower airways of asthma have indicated a more diverse microbial population compared to non-asthma subjects(10;11). In collaboration with Professors Cookson and Moffatt (NHLI), we performed microbiome studies on sputum samples and bronchial brushings in patients with severe asthma and found that *Streptococcus* operational taxonomic units were more abundant in severe asthma sputum samples than in non-severe asthmatics and healthy subjects(12). The potential role of bacterial infections during exacerbations is supported by the observation that a macrolide antibiotic provided a small but significant benefit in accelerating the recovery period (13). It is possible that bacterial infections may play a greater part in the exacerbation observed in patients with severe asthma. However viral infections may also be important. We have recently shown that blood CD8⁺ T cells from severe asthma were markedly activated in terms of expression of T cell activation markers and of MAP kinases, JAK/STAT and the transcription factor, NF-κB (14), with one potential explanation being the activation from viruses. Viral infections may also increase the chance of a bacterial reactivation, as has been shown in virus-induced COPD exacerbations (15).

Exacerbations in severe asthma may also be related to the deranged innate immune responses to infective agents. A reduced interferon response to rhinovirus infections of the asthmatic airways has been described (16), allowing for increased viral replication leading to an increased inflammatory response. In severe asthmatic, we have found a reduction in the ability of the alveolar macrophage and of the monocyte-derived macrophage (MDM) in phagocytosing beads coated with *Staph aureus* and *Haem influenzae* (unpublished; Fig 1). Rhinoviruses could also impair phagocytosis of bacteria by macrophages(17). A defective response in the phagocytosis of apoptotic cells ('efferocytosis') of macrophages from severe asthma and from neutrophilic asthma has also been described(18;19). The impaired phagocytosis has been associated with an intracellular imbalance of glutathione sulphide homeostasis, together with a reduction in histone deacetylase activity (20). In adult asthmatics, an exacerbation is characterised by a neutrophilic inflammation in sputum (21), and rhinovirus-induced asthma exacerbations is also accompanied by a neutrophilic response (22), although rhinovirus has also been associated with an activation of Th-2 mechanisms. The high Th2 phenotype characterised by eosinophilic asthma is highly prone to exacerbations (23). The potential for an interaction between viral infections, allergen exposure and atopic sensitisation as conferring the greatest risk has been proposed(24).

Viral and bacterial infections can both contribute to oxidative stress and have also been associated with the generation of reactive oxygen species through the induction of inflammation, with down-regulation of antioxidant defences. Elevated levels of 8-isoprostanes have been measured in sputum infected with bacteria(25). Oxidative stress occurs after allergen challenge, with a rapid loss of superoxide dismutase activity(26). Lower airway levels of GSH with increased GSSG in bronchoalveolar lavage fluid has been reported in asthmatic children with elevated levels of malondialdehyde, 8-isoprostanes and H₂O₂(20). Increased oxidant stress has been seen as a constant feature of severe asthma(27). During an exacerbation, there may be a further augmentation of oxidative stress that may in turn contribute to airway smooth muscle contraction, inflammation and corticosteroid insensitivity(28). We have demonstrated the presence of CS insensitivity in macrophages and airway smooth muscle cells from patients with severe asthma(29;30). This may contribute to the lack of effect of corticosteroids in controlling the deterioration in asthma. Finally, activation of MAP kinases, JAK/STAT and the transcription factor, NF-κB, in blood CD8⁺ T-cells of patients with severe asthma may contribute to greater risk of exacerbations(14).

Heterogeneity and Biomarkers of exacerbations

Because of the different potential causes associated with exacerbations in patients with severe asthma, it is likely that there are different phenotypes. This is important to determine because more specifically-targeted treatments for exacerbations may lead to better resolution without the need to use or increase corticosteroid use. There are also no biomarkers identified for the detection or for quantifying the severity of an exacerbation, and that would also be valuable in patients with severe asthma who are already taking medication that could influence the levels of biomarkers. One potential improvement may result from an earlier detection of the exacerbation and therefore a biomarker for an earlier detection of exacerbation in severe asthma would be useful, particularly for initiation of treatments.

Hypothesis and objectives

We *hypothesise* that (i) exacerbations in severe asthma are causally heterogeneous and (ii) represent a corticosteroid-insensitive event caused by an excess of oxidants and inflammation resulting from respiratory viral and bacterial agents.

The *objectives* of this project are to:

- (i) determine the symptoms and lung function characteristics and course of asthma exacerbations in patients with severe asthma
- (ii) evaluate the role of infective agents (respiratory viruses and bacteria)

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- (iii) define the biomarkers in exhaled breath, sputum and in blood
- (iv) study the presence of oxidative stress, phagocytic capacity of macrophages and corticosteroid insensitivity.

Major *outcomes* of this study will be (i) to assess the feasibility of diagnosing exacerbations earlier (ii) to determine the heterogeneity of the presentation and course of exacerbations (iii) to identify exacerbation phenotypes in terms of causes and (iv) to look for potential targets for treatment.

Study design

We have a database of 300 current patients with severe asthma defined according to ATS criteria(1) with exacerbation rate of 1.5 ± 2.8 (SD)/year. To achieve 1.7 exacerbations after 16 weeks will require 36 patients at 90% power and 5% significance. Adjusting for a possible dropout of 30% would require 56 patients. Therefore, 60 patients will be recruited. To be on the safe side, we will enrol patients with a history of ≥ 3 exacerbations/year for a prospective observational study.

At entry Visit 1, we will enrol and characterise these patients. They will be observed over 12 months during which the number of exacerbations will be recorded on the basis of objective measures with evaluation of ACQ and daily morning and evening PEF. At the earliest onset of each exacerbation, the patient will be requested to contact by phone the Asthma BRU Unit. For one of these events, patients will be asked to attend the laboratory for a series of tests at Visit 2 similar to Visit 1. Visit 3 will be planned within 14 days after Visit 2, when the patient has recovered.

In 30 of the 60 patients, we will request for an additional attendance of a subsequent exacerbation (Visit 4) with follow-up (Visit 5). For other exacerbations not studied, the patient will be asked to keep a detailed diary record of symptoms with severity scoring and spirometric and PEF measurements (Exacerbation Diary) over a period of 2 weeks after the onset of exacerbation.

Patients will have their exacerbations treated in the usual way. The only exception will be the use of antibiotics which will be withheld and initiated (if indicated) after the patients has been through Visit 2. Those who have to be hospitalised will not be studied, and only those who can attend the Clinical Research Unit will be studied. The more severe exacerbations will therefore be missed but will be recorded in the patient's diary.

Tests to be performed Visits 1, 2 and 3:

1. Diary of symptom scores, use of beta-agonist reliever and other treatments for 2 weeks

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2. PEFr and spirometry twice daily for 2 weeks
3. Markers of systemic inflammation in blood: CRP, IL-8, IL-6, together with Meso-Scale-Discovery (MSD) platform for other cytokines.
4. Markers of oxidative stress in blood, exhaled breath condensate (EBC) and urine: malondialdehyde (MDA) and 8-isoprostanes (31).
5. Nitric oxide (NO) levels in exhaled breath measured twice daily for 2 weeks using a portable hand-held NO meter (NO_{breath}). EBC for pH & free iron (32)
6. Induced or spontaneous sputum for inflammatory cells and assays of cytokines in supernatants using Meso-Scale-Discovery (MSD) platform, bacteriological culture and for microbiome analysis. H₂S which is elevated in asthma in relation to sputum neutrophil counts (33) will also be measured. If spontaneous sputum is not available, sputum will be induced using ultrasonic nebulization of isotonic saline (34), which is safe in patients during an exacerbation (35).
7. Sputum for bacteriological culture and colony forming units.
8. PCR for respiratory viruses from nasopharyngeal swabs and sputum RNA and reverse transcription performed for respiratory viruses including rhinovirus, influenza virus types A and B, parainfluenza viruses 1 to 3, coronaviruses and respiratory syncytial virus types A and B, and Chlamydia pneumonia, and Mycoplasma pneumonia using real-time 'TaqMan' assays in collaboration with Professor Sebastian Johnston, NHLI.
9. Sputum microbiome: 16S rRNA PCR and pyrosequencing will be performed on sputum with amplification of the V3-V5 regions (36) in collaboration with Professors Cookson and Moffatt, NHLI.
10. Studies on blood cells:
 - (i) MDM's phagocytosis of fluorescently-labelled *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Professor Louise Donnelly, NHLI) on macrophage-derived monocytes as described previously (37)
 - (ii) Transcriptomic analysis of blood CD4⁺ and CD8⁺ T-cells and monocytes, as previously described (14)
 - (iii) Studies of corticosteroid insensitivity in peripheral blood mononuclear cells and in whole blood (Dr Pank Bhavsar, NHLI) (38)

Definition of exacerbation and monitoring

Because these patients with severe asthma are usually well-experienced 'exacerbators', they will be asked to 'diagnose' their own exacerbations. Each has an individualised way of recognising an

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exacerbation. We will discuss with each patient their way of recognising a deterioration and try and work out whether earlier signal warning is possible. One of our aims is to find out what the objective measures of deterioration are. We will also ask patients to record their symptoms and lung function as soon as they feel the onset of an exacerbation, since exacerbations are recognised by the patient as events that are 'clinically identified by being outside the patient's usual range of day-to-day asthma variation'(5). They will also contact the Study Coordinator (Nurse or Clinical Research Fellow) to determine if they can attend for Visit 2. The patient will receive or administer treatments for the exacerbation as usual without interference from the Research team. Except for the exacerbation that will be studied (Visit 2 or Visit 4), the patient will be requested to refrain from starting any antibiotic therapies, which will be started (if this has been prescribed) as soon as Visit 2 studies have been completed.

Data analysis

1. The daily variation of symptoms and lung function during the exacerbation visits (2 and 4) will be analysed, and related to the changes observed in the various parameters collected at exacerbation. Comparison of these biomarkers to those at Visit 1 and at the recovery visit will allow for ascertainment of biomarkers that may be associated with exacerbations. Bacterial- or virus-associated, or eosinophil- or neutrophil-associated exacerbation will be defined.
2. Unbiased mathematical methods such as factor and cluster analyses will be used to identify factors at exacerbations and classify groups of similar characteristics looking at all the data collected including the transcriptomic and cytokine profile data. The analysis will be performed by the Bioinformatics group led by Prof Yike Guo of Discovery Science at Imperial College.
3. The stability of the exacerbation profile will be examined within the 30 patients who have had a repeat exacerbation visit. We could also look at this small group as a validation data set.

Timelines and milestones

Oct 2013-Dec 2013: Setting up of study, identification of patients, setting up methods.

Jan 2014-Dec 2014: Recruitment of 30 patients, and follow up to Dec 2014

Jan 2015-Dec 2015: Recruitment of 30 patients, and follow up to Dec 2015

Jan 2016-Sept 2016: Completion of assays, analysis of data, biomarker validation

Future plans of research

1. Information obtained from this study will allow us to plan better any future therapeutic studies on asthma exacerbations and will also provide us with an idea of targets for treatments for

particular types of exacerbations. Therefore, we plan to use the new information gathered to plan for future studies (eg the number of subjects needed for a therapeutic study).

One target of therapy that we are seeking confirmation for is that of oxidant stress. Currently there are no good antioxidants, but N-acetylcysteine or other similar medications is available and could be tested as treatment for exacerbations. We will also test drugs not only for treating exacerbations, but also for reducing exacerbation rate. This may include approaches to reduce corticosteroid insensitivity since the recurrence of exacerbations in patients taking high dose corticosteroids is a reflection of corticosteroid insensitivity. p38 MAPK inhibitors may be such a drug (39).

2. There will be a need for validation of some of the identified biomarkers for each exacerbation phenotype, and this will be done in multicentre studies in collaboration with other Centres within the BTS Severe Asthma Group.
3. This research will also provide information about the best tools to monitor exacerbations in severe asthma patients. This will be examined in newly-recruited patients to our cohort.

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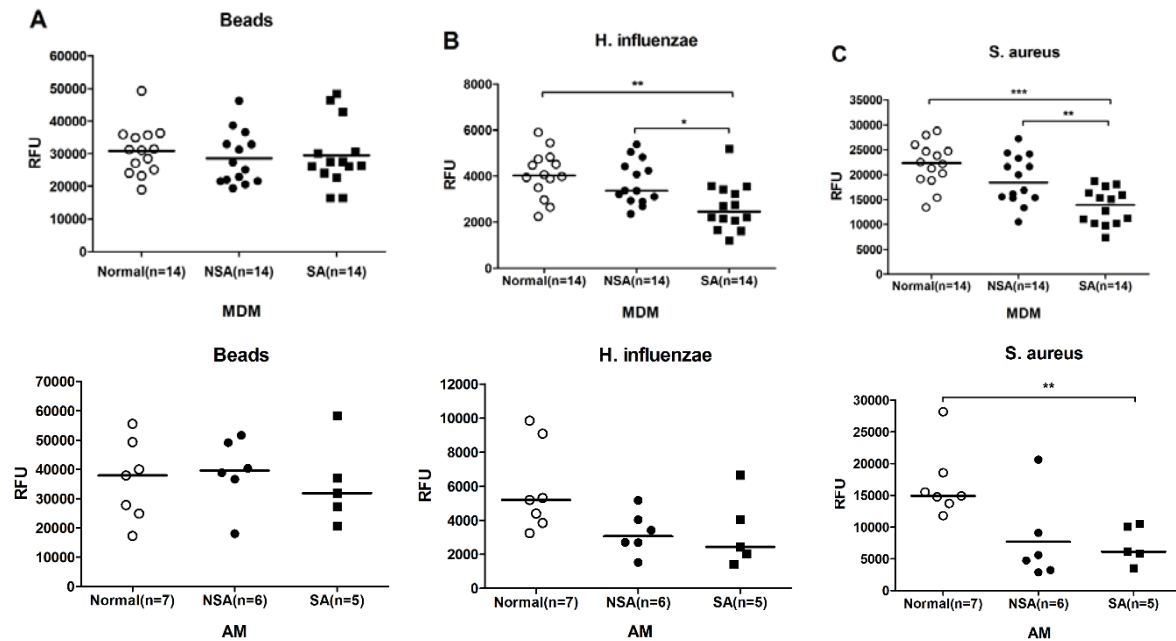


Figure:

Phagocytosis of fluorescently-labelled *Haemophilus influenzae* and *Staphylococcus aureus* by monocyte-derived macrophages (MDM; top panels) and by alveolar macrophages (AM; lower panels) from normal subjects (Normal) and from non-severe asthmatics (NSA) and severe asthmatics (SA). There was no difference in phagocytosis of fluorescently-labelled beads not coated with bacteria (Panel A). However, there was significantly lower phagocytosis of beads coated with *Haemophilus influenzae* and *Staphylococcus aureus* by MDMs from severe asthma patients (Panel B). For macrophages, there was a reduction of phagocytosis of *Haemophilus influenzae* and *Staphylococcus aureus* by AMs from both asthma groups, although this was only significant for *Staphylococcus aureus* phagocytosis by AMs from severe asthma (Panel C). * p < 0.05; ** p < 0.01.

Patient Information and Informed Consent Form

Exacerbations in severe asthma patients: mechanisms and biomarkers

Asthma: Version 1.1 21 June 2013

INTRODUCTION

We would like to invite you to take part in a research study examining exacerbations in severe asthma. Before you decide whether to take part, it's important to understand why the research is being done and what it involves. Please take time to read this information sheet. Talk to your family, friends, doctor or nurse if you wish. If anything is not clear and you require more information before you decide whether or not to take part in the study, please telephone the study team on 0207 351 8051. Additionally, you may find it useful to visit the INVOLVE website (<http://www.invo.org.uk/>) which has information for patients about research in the NHS.

1. WHAT IS THE PURPOSE OF THE STUDY?

Asthma and other wheezing illnesses affect one in ten adults in the UK. Asthma is a condition that affects the airways – the small tubes that carry air in and out of the lungs. The main aim of this research is to better understand the exacerbations (attacks) that occur in severe asthma. Severe asthmatics have difficulty in controlling their disease, despite good medical care and taking all the medicines that usually work well in asthma. Having an exacerbation is quite common in patients with severe asthma, and it is one of the events that patients with severe asthma are usually very concerned about. We need to understand better what happens during an exacerbation of asthma. We would first like to find out whether there are certain viruses and bacteria that are associated with the exacerbation, and secondly we would like to find out what sort of inflammation is occurring during the exacerbation by measuring various proteins in the blood and in the phlegm from the lungs. We also wish to find out whether the type of exacerbation is different from one person to another and we will also find out whether there something that can give an early warning that an exacerbation will be happening in the near future. This research will give us some insight into how to obtain better treatments for the exacerbation and also to prevent them from occurring.

2. WHY HAVE I BEEN INVITED TO PARTICIPATE?

You have been invited to participate because you have severe asthma and you are a patient attending our clinic at the Royal Brompton Hospital.

3. DO I HAVE TO TAKE PART?

Participation in this study is entirely voluntary. It is up to you to decide whether or not to take part. If you do take part, you will be given this information sheet to keep and be asked to sign a consent form. You are free to withdraw from the study at any time and without giving a reason. This will not in any way affect the standard of care you receive. The information sheet and consent form for the study have been approved by an Ethics Committee (EC).

4. WHAT WILL HAPPEN TO ME IF I TAKE PART?

If you decide that you want to take part in this research study, we will ask you to participate in a variety of tests, which will look at different aspects of your asthma. The entire study takes approximately 12 months and includes between 3 to 5 visits to the Asthma lab at the Royal Brompton Hospital. As soon as you experience an asthma attack (exacerbation), you will be asked you to contact us, the study team. We will then arrange whether you will be able and if so, when you can come to the study centre to be assessed.

Details of the tests to be performed are attached at the end of this document.

- **Visit 1: Screening visit** – The Nurse or member of the study team will explain the study in detail. If you have any questions the nurse or study staff will be able to answer them. If you are satisfied with the answers and would like to take part in the study you will be asked to sign a consent form.

A number of tests will then be carried out. This visit will last for up to 3 hours. During this visit we would like to do the following:

Medical history, Physical examination, Questionnaires, Blood test, Urine, Throat swab, Nasal wash and nasal brush, Lung function testing, Forced oscillation technique, Collecting breath condensates, Sputum induction, Exhaled Nitric Oxide, Methacholine challenge test, Diary card

- **Visit 2, Exacerbation visit, which will take up to 3 hours**

Exacerbation visit – this should take place within the first 6 months from the time of visit 1. This visit will take place at the Asthma lab at the Royal Brompton Hospital; you will not be required to stay overnight, unless there is any reason for you to need further treatment in hospital. You will receive treatment as usual for your exacerbation. We will arrange transport for you to attend our lab.

At this visit we would like to do the following:

Physical examination, Medication history, Lung function testing, Forced oscillation technique, Blood sample, Urine sample, Nasopharyngeal swab, Sputum induction, Exhaled Nitric Oxide, Exhaled Breath Condensate

- **Visit 3, Exacerbation recovery visit at 14 days after the exacerbation visit, which will take up to 3 hours.**

We will repeat the same tests as at Visit 2.

- **Visit 4, Second Exacerbation visit (Similar to Visit 2) –**

This is optional as we are looking for half of the subjects undergoing this. The same tests will be performed as above. This visit will take place over not more than 2-3 hours). You will not be required to stay in hospital overnight.

- **Visit 5, Second recovery Exacerbation visit (Similar to Visit 3)-** This is optional and will only be done if you have gone through Visit 4. The same tests will be performed again.

5. WILL EXPENSES BE PAID?

Reasonable time and travel expenses you may have incurred as a result of participation in this study will be reimbursed.

6. WHAT ARE THE ALTERNATIVES FOR DIAGNOSIS AND OR TREATMENTS?

Regardless of whether you join this study, you will be treated with the current best treatment. This is particularly with respect to your treatment for exacerbations. This should be treated as usual

7. WHAT ARE THE SIDE EFFECTS OF ANY TREATMENT RECEIVED WHEN TAKING PART?

This study does not include a new or experimental drug. You will continue with your prescribed asthma medication and your usual treatments for your exacerbations.

8. WHAT ARE THE POSSIBLE BENEFITS OF TAKING PART IN THIS STUDY?

We cannot promise the study will help you but the information we get from this study may help improve the treatment and monitoring of people with asthma, and particularly for treating exacerbations of asthma.

9. WHAT IF THERE IS A PROBLEM?

Imperial College London holds insurance policies which apply to this study. If you experience serious and enduring harm or injury as a result of taking part in this study, you may be eligible to claim compensation without having to prove that Imperial College is at fault. This does not affect your legal rights to seek compensation.

If you are harmed due to someone's negligence, then you may have grounds for a legal action. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been treated during the course of this study then you should immediately inform the Investigator [Professor K F Chung at 0207 351 8995 \(Secretary: Nicola Bell\)](#). The normal National Health Service complaint mechanisms are also available to you. If you are still not satisfied with the response, you may contact the Imperial AHSC Joint Research Office.

10. WILL TAKING PART IN THIS STUDY BE KEPT CONFIDENTIAL?

All information which is collected about you during the course of the study will be treated confidentially. All study data related to you will be coded. This means that you will be given a code number that will be used to identify you and information about you without having to use your name, medical record number, or other common identifiers. If you give your consent to participate in this study, you also give your consent for access to your medical file.

We do need to let your GP and hospital consultant know you are taking part in this study. If any clinically relevant information is obtained as a result of your involvement in the study we will discuss this with you. Your permission will be sought to share this information with the relevant doctor involved in your care.

All samples that are provided by you will be uniquely identified with a sample identifier and your code. If you change your mind about participating in the research, the code and link to your identity will allow us to locate your samples and destroy them so they cannot be used for further research.

11. WHAT WILL MY STUDY DATA ARE USED FOR?

Your study data will be used for research into asthma and may be used in research related to the development of pharmaceutical products, diagnostics or medical aids. The handling of your study data will be in accordance with applicable Data Protection law(s).

12. WHAT WILL HAPPEN TO THE SAMPLES I GIVE?

The samples you provide will be examined within the first 2 years of their collection. Any remaining samples will be stored for up to 20 years, at special facilities that are designed to store samples safely and securely. No information will be stored on the sample labels that may be used to identify you. Any movement and storage of samples will be carried out in accordance with the Human Tissue Act 2004. At the end of the storage time any remaining samples will be destroyed.

13. WHAT WILL HAPPEN IF I DO NOT WANT TO CARRY ON WITH THE STUDY?

You can decide to withdraw from the study at any time. Refusal to take part or if you withdraw after giving your consent will have no consequences for your present or future treatment by your doctor. Information collected may still be used. If you ask, we will destroy any stored samples that can still be identified as yours. This will not impact on the treatment and care that you are or will be receiving in any way.

14. WHAT WILL HAPPEN TO THE RESULTS OF THE RESEARCH STUDY?

The result of the study will be considered for publication or presentation at (scientific) symposia or congresses. You will not be identified in any report/publication or presentations of the study. During the study and at the end of the study all participants will be informed on the general but not individual results.

You have the right to request information about your study data be provided to you with a copy of them. You also have the right to request that any inaccuracies in such data be corrected. If you wish to make a request, then please contact your clinic at Royal Brompton Hospital.

15. WHO IS ORGANISING AND FUNDING THE RESEARCH?

The research is being organised under Imperial College London and under the Biomedical Research Unit of the Royal Brompton Hospital. We have applied to Asthma UK to fund this study.

16. CONTACT DETAILS & SOURCE FOR ADDITIONAL INFORMATION.

For the duration of the study, you will be under the care of Professor Chung. If at any time between your visits you feel that any of your symptoms are causing problems, or you have any questions during the study, please contact your study team. The telephone number to reach the study team is 0207 351 8051 (ask for Mr Joao Rocha, the Study Nurse).

APPENDIX:

Visit details:

Visit 1 – Screening visit – The Nurse or member of the study team will explain the study in detail. If you have any questions the nurse or study staff will be able to answer them. If you are satisfied with the answers and would like to take part in the study you will be asked to sign a consent form.

A number of tests will then be carried out. This visit will last for up to 3 hours. During this visit we would like to do the following:

- **Medical history** - you will be asked about your medical history including respiratory and non respiratory related history, smoking, allergies and what medications you use.
- **Physical examination** – we will perform assessments such as record your height, weight, blood pressure and listen to your chest
- **Questionnaires:**
- **Blood test** – we would like to obtain a sample (10 teaspoons, 50 mls) of your blood to keep for measurement of various markers. Some of the blood will be used to measure how cells in the blood can engulf particles and bacteria. We will separate various cells in the blood to study them in the test-tube. We will extract genes from the blood cells to examine the genes expressed. Obtaining the blood may cause some local discomfort and bruising, rarely there is a risk of irritation of the nerves.
- **Urine** - we would like to collect a sample of your urine. We will use this to check for indicators of asthma and exposure to cigarette smoke.
- **Throat swab:** This will be taken by using swab to scrape the back of the throat. This will be used to detect any bacteria.
- **Nasal wash and nasal brush:** This will be done by push some salt water into one nostril and collecting the return back, Then one side of the nose will be gently scraped with a tiny brush to retrieve some cells from the wall of the nose. These will also be used to isolate any bacteria.
- **Lung function testing** – we will check your breathing with spirometry. It is a test to measure the amount of air you have in your lungs by breathing in and how well you can push the air back out by blowing it hard into a tube. If your asthma diagnosis has not been confirmed within 12 months of this visit we will repeat the spirometry ('reversibility' test) before and after you inhale salbutamol. This is to measure how much your airways respond to salbutamol. Spirometry may cause you to cough or make your chest feel tight. Salbutamol will be available if this occurs. Salbutamol relaxes muscles in the air passages of the lungs. It helps to keep the airways open, making it easier to breathe. Salbutamol may make you feel a little shaky and increase your heart rate.
- **Forced oscillation technique** - this test is used to measure lung movement during normal breathing. You will be asked to breathe normally through a mouthpiece into a tube during which time measurements are made. This will be repeated 3 times.

- Collecting breath condensates. We will collect a sample of water condensate from the air you breathe out. We will use this to measure various markers. This is done by breathing normally onto a tube for approx 10 min. There is no discomfort associated with this test.
- Sputum induction - you will be asked to inhale a salty mist of different concentrations through a nebulizer and then asked to cough into a plastic container. This process will be repeated on the second day. Your lung function will be monitored. You may experience an unpleasant salty taste in your mouth, nausea or a sore throat. The salty mist may cause you to wheeze. You will be given salbutamol before the test to prevent the likelihood of wheezing and if necessary further salbutamol can be given if wheezing develops. This will be used to measure the inflammation in your airways. The sputum sample will also be used to culture for bacteria and will also be used to detect bacterial and viral genetic material.
- Exhaled Nitric Oxide -Nitric oxide is a gas present in everyone's breath. You will be asked to exhale into a mouthpiece, breathing out at different speeds. By doing this the nitrogen content of your breath will be measured by a computer. This will measure the inflammation in your airways.

If you are one of those asked to take part because of your asthma, but who has not had your asthma diagnosis confirmed in the last 12 months we will want to carry out another test on a day separate from the previous assessments.

- Methacholine challenge test - before we carry out this test we will ask women of child bearing age to confirm that they are not pregnant. If there is any doubt a pregnancy test will be carried out. You will be asked to inhale a mist that contains different concentrations of methacholine. The mist is produced by a device called a nebulizer and you inhale the mist through a mouthpiece. Before the test begins and after each period of inhalation you will be asked to blow forcefully into a spirometer. This test is used to determine how responsive (or irritable) your airways are and to determine the severity of any asthma. The inhalation of aerosols may be associated with mild shortness of breath, cough, chest tightness, wheezing, chest soreness or headache. Many patients do not have any symptoms at all. Symptoms (if they occur) are mild, last only a few minutes, and disappear following the inhalation of a bronchodilator medication such as salbutamol.
- We will discuss with you your experience of your previous exacerbations and what warning you get before the onset of an exacerbation. We will discuss with you what would be an early warning for you to start recording your symptoms and peak flow measurements at home on a machine that we will provide you. We will ask you to call us as soon as possible when you feel an exacerbation is impending. We will ask you to take or seek treatment for your exacerbation as you would usually do. We do not wish you to withhold treatments for your exacerbation.
- Diary card – we would like you to collect information on your asthma for the next two weeks. We will give you a machine to use twice daily to record your peak flow and spirometry tests. You will be given instructions as to how to use the machine at home.

- If your asthma worsens and you have an 'exacerbation', we will want you to record information as soon as possible for 4 weeks. We will explain to you what you need to record and when to do this. We will then review the information you have recorded with you at your next follow-up visit.

Visit 2 - Exacerbation visit – this should take place within the first 6 months from the time of visit 1. This visit will take place at the Asthma lab at the Royal Brompton Hospital; you will not be required to stay overnight, unless there is any reason for you to need further treatment in hospital. You will receive treatment as usual for your exacerbation. We will arrange transport for you to attend our lab.

At this visit we would like to do the following:

- Physical examination – the same as the Screening Visit.
- Medication history- we will ask you about any other medicines you are taking.
- Lung function testing - We will test your breathing as before, but will not need to confirm your asthma diagnosis.
- Forced oscillation technique - this test is used to measure lung movement during normal breathing. You will be asked to breathe normally through a mouthpiece into a tube during which time measurements are made. This will be repeated 3 times.
- Blood sample – we would like to obtain a sample of up to 50 ml (10 teaspoons) of blood for assessing markers of inflammation. By markers we mean substances that include molecules called proteins, lipids and messenger RNA. Messenger RNA can help us understand how genes function. These and other molecules can affect disease processes (in this case asthma) and how patients like you respond to medicines. In addition, we may need to use a small amount of the blood to look at a specific substance (IgE) that can be increased if you are allergic to certain things, if not already done in the 3 months before screening.
- Urine sample- we will ask you to provide a urine sample (about 40 ml) to look for substances that are indicators of asthma.
- Sputum induction - you will be asked to inhale a salty mist of different concentrations through a nebulizer and then asked to cough into a plastic container. This process will be repeated on the second day. Your lung function will be monitored. You may experience an unpleasant salty taste in your mouth, nausea or a sore throat. The salty mist may cause you to wheeze. You will be given salbutamol before the test to prevent the likelihood of wheezing and if necessary further salbutamol can be given if wheezing develops. This will be used to measure the inflammation in your airways.
- Exhaled Nitric Oxide -Nitric oxide is a gas present in everyone's breath. You will be asked to exhale into a mouthpiece, breathing out at different speeds. By doing this the nitrogen content of your breath will be measured by a computer. This will measure the inflammation in your airways.

- Exhaled Breath Condensate – you will be asked to breathe into a cold tube. Due to the warmth of your breath a liquid will be produced, called a ‘condensate’. This can then be analysed to determine the inflammation in your airways.
- Nasopharyngeal swabs – We will take a mild scrape of the back of the throat in order to look for bacteria.
- If any of the tests prove to be too uncomfortable for you, we will not proceed with the test.

Visit 3 – Recovery exacerbation visit – after 14-21 days after Visit 2. Most of the assessments will be over 3 hours. During this visit we would like to do the following:

- Questionnaires – these will include asthma questionnaires, to check for any changes since the Baseline Visit.
- Physical examination – the same as previous visits.
- Asthma and medication history- since the last visit.
- Lung function testing – the same tests as carried out at the Baseline Visit, including the ‘reversibility’ test.
- Diary card –we will review the information you have recorded.
- Pregnancy test- if appropriate

If you have attended the ‘Repeatability’ Visit you will not be required to attend this visit.

Visit 4 - Second Exacerbation visit (Similar to Visit 2)– This is optional as we are looking for half of the subjects undergoing this. The same tests will be performed as above. This visit will take place over not more than 3 hours). You will not be required to stay in hospital overnight.

Visit 5-Second recovery Exacerbation visit (Similar to Visit 3)- This is optional and will only be done if you have gone through Visit 4. The same tests will be performed again.

