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Blood eosinophils as a marker of likely corticosteroid response in children with preschool wheeze: time for an eosinophil guided clinical trial?

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Answers to reviewers comments:

We would like to thank the reviewers for their helpful comments on this manuscript and these are addressed 'in bold' below each comment.

We have also added a paragraph to include the findings of a study by Wagener *et al* published in the February 2015 issue of *Thorax* that we felt is highly relevant to this review.

Changes in the manuscript are shown as 'underlined'.

Reviewer: 1

Comments for Authors:
The Manuscript written by Gaillard et al dealing with the role of blood eosinophils as a biomarker of corticosteroid responsiveness in pre-school children with wheezing is overall reasonably well written, appropriately referenced, and a worthwhile contribution to the asthma literature. The following comments are provided to the authors for their consideration:

1. Vuillermin et al's article is appropriately cited as evidence that oral corticosteroids have limited efficacy in asthma exacerbations in school aged children. An additional reference to consider is the article by Biegelman A. et al. J Allergy Clin Immunol 131(6):1518-25, 2013. This article specifically addresses this same issue in preschool children.

This *post hoc* analysis study by Beigelman *et al* has been added as a reference to the manuscript.

2. Although not published yet at the time of this manuscript submission, another reference to consider mentioning in the introduction section regarding asthma phenotypes in children is an article by Guilbert TW et al. J Allergy Clin Immunol Pract 2(6):664-70, 2014.

A sentence relating to epidemiological phenotypes has been added to the introduction and the Guilbert *et al* reference included.

3. The modified asthma predictive index is currently an accepted tool in assessing risk of developing persistent asthma. One of the minor criteria of the modified asthma predictive index is level of peripheral blood eosinophilia greater than or equal to 4%. It would be pertinent for the authors to reference this predictive tool in their manuscript and their interpretation of its usefulness.

We have added the reference, Castro-Rodriguez *et al* AJRCCM 2000. The API has a positive predictive value (i.e., the proportion of subjects with a positive index who develop the outcome) of 47.5% for the development of asthma (physician diagnosed asthma or greater than three wheezing episodes during the prior year) at age 6 years. Eosinophils constitute only a minor criteria. In the study by Guilbert et al 2006, NEJM only children with a positive asthma predictive index were randomised to either ICS or placebo.

4. Since one of the possible consequences of this review is to provide impetus for the design of clinical trials to prospectively evaluate the utility of peripheral blood eosinophils either as a predictor or efficacy measure of response to ICS, the manuscript could be enhanced if the authors proposed a framework for a clinical trial to study the issues they are raising.

We propose a blood eosinophil stratified randomised controlled trial to establish if children with higher eosinophil counts are more likely to respond to inhaled corticosteroids compared to children with low blood eosinophil counts and a sentence has been added just before the conclusions.

5. One aspect of the use of peripheral blood eosinophil counts as a biomarker that is not well discussed is the developmental aspects regarding its age of onset and its fluctuations over time. That is, at what age and threshold level does peripheral blood eosinophilia occur/begin during early childhood? Also, is the development of blood eosinophilia associated temporally with the development of allergic sensitization? Also, are these two factors independently associated with asthma risk and/or ICS response or are they linked developmentally?

This is an important point raised. There is a lack of longitudinal data reported in the literature in adults and children. We are not aware of any studies reporting blood eosinophils in children (or adults) with asthma longitudinally therefore we cannot comment on fluctuation. In the study reported by Karakoc *et al*, 2002 the serial estimation of blood eosinophils was years apart (reference included). There are important ethical issues with repeat blood taking in children that is an important limitation to such studies. There are however data to show that blood eosinophils >0.4 x 10^9/L are detectable in many young children. This information has been added to the manuscript under the heading 'eosinophils and asthma'.

6. On page 2, lines 7-8, the author's state that there is "diagnostic and therapeutic uncertainty in children aged 5 and younger". Could the authors elaborate for what the diagnostic and therapeutic uncertainty is for (e.g., asthma)?

We have expanded this paragraph to elaborate more on the diagnostic and therapeutic difficulties.

7. On page 3, line 21, the authors state that "children with human rhinovirus induced wheeze are also more likely to be atopic". We recommend changing the word "atopic", which is defined as an increased genetic propensity of developing IgE antibody formation, to "atopic disease". The sentence would read "more likely to have atopic diseases". Similarily, throughout the entire manuscript, when the authors want to refer to children who have demonstrable allergic sensitization, the term "allergic sensitization" should be used and not "atopy" or "atopic".

We have made changes throughout the text and changed 'atopy' to 'allergic disease' or 'allergic sensitisation'.

8. On page 6, line 39: We advise that the authors include the age (age range) of children referenced in the statement: "Children with symptomatic, but not elevated concentrations of eosinophil mediator…"

Mean ages of symptomatic and asymptomatic children were 13 and 15 years respectively. We added the word 'teenage' to this sentence to indicate the ages of the children studied.

9. The authors should consider revising the sentence on page 8, lines 28-37 that reads "Eosinophil cationic protein (ECP), eosinophil protein X (EPX), eosinophil peroxidase (EPO) and major basic protein (MBP) are capable of inducing tissue damage and dysfunction and are toxic to a variety of tissues including the heart, brain, and bronchial epithelium causing airways hyperresponsiveness."

The effects of these mediators on the heart and brain do not contribute to airway hyperresponsiveness.

The paragraph has been revised as suggested.

10. On page 10, line 10, please change the word "that" to "than".

Done

Reviewer: 2

Comments for Authors:
The authors set out to examine the value of blood eosinophils as a biomarker to predict corticosteroid responsive disease in pre-school children presenting with wheeze. I have a few specific comments.

1. The authors nicely framed the problem within the introductory section.

2. There are well over 100 references; some of them are around 2 decades old with some of the older ones being editorials or reviews. It would be good to reduce the number of references by deleting any older ones that really aren't needed.

We reviewed all the references older than 20 years and removed 4 editorials and reviews (Frette, Durham, Dahl, Kirby). Many studies of blood eosinophils date back more than 20 years. More recently sputum eosinophils have been the preferred target for adult asthma studies and blood eosinophils have been relatively neglected. However the interest in blood eosinophils is increasing again due to the difficulties in obtaining regular sputum samples particularly in children. For the purpose of the review we feel it is important to include the earlier studies that have answered some important questions.

3. Page 5, line 39: "1st" should be spelt out in full.

Done

4. The authors consider a number of other possible biomarkers of corticosteroid responsiveness. The one they fail to mention is exhaled nitric oxide. There at least ought to be some reference of it in the review, if only to explain why it is not appropriate.

We added a paragraph to discuss the role of eNO as a marker of steroid sensitive asthma and its relationship with blood and sputum eosinophils.

Reviewer: 3

Comments for Authors:
Summary
This review makes a case for identifying biomarkers to determine which children with preschool wheeze or asthma will respond best to steroid treatment. Although the title implies a focus on preschool wheeze that actual content mainly includes data from studies in adults, school-aged asthmatic children and some data on preschoolers, and includes a variety of disease severity.

Comments
The paragraph that discusses current treatment recommendations for preschool wheeze confuses prediction of asthma development and treatment according to current symptom pattern. The implication is that clinical predictive indices need to be used to decide treatment, but the episodic viral wheeze and multiple trigger wheeze phenotypes that have been recommended by the ERS are for prospective use and do not rely on retrospective allocation of phenotypes. This requires some clarification.

We have provided clarification on these issues. Few RCTs in children with preschool wheeze have distinguished between episodic viral wheeze and multiple trigger wheezing when defining inclusion criteria. To confuse matters further the asthma prediction indices used in many larger North American studies in children with preschool wheeze are very similar to the criteria used by investigators to define inclusion criteria for RCTs. The implication is that preschool children with troublesome wheezing are more likely to remain symptomatic as older children, which has been shown to be correct. We have clarified these issues in more detail.

In a similar manner, it needs to be made clear that determining which patients are steroid responsive will not help to prevent development of asthma by school-age as we know steroids are not disease modifying. The main advantage would be to avoid unnecessary side effects in steroid unresponsive patients and to more effectively treat those children that are responsive.

We agree with this statement and have added a sentence to say that corticosteroids are not disease modifying.

One aspect that is missing in this review is a discussion about what is known about the inflammatory pathology of preschool wheeze. Several studies have reported airway inflammation in preschoolers, although they have looked bronchoscopically in BAL samples, it would be good to have a discussion – as many preschoolers have evidence of neutrophilic inflammation, not eosinophilic inflammation. This would support the case for identifying a group that are likely to be corticosteroid unresponsive. The authors imply all bronchoscopic studies are done only in severe wheezers, but this is not true, there are several publications that include mil-moderate wheezers as well (Barbato A AJRCCM, Baraldo S AJRCCM).

A paragraph was added to discuss in more detail the inflammatory airway pathology in children with preschool wheeze. There are relatively few studies reporting airway inflammation in young children and particularly in those 3 years and younger airway eosinophilic inflammation is detectable but generally at a level below that which is considered eosinophilic inflammation in older children and adults. A classification into eosinophilic and neutrophilic asthma/wheeze is therefore difficult and has not been reported in studies for that age group.
We have discussed in the review the studies suggested that included children with mild to moderate wheeze severity.

Also, the limitations of the current proposed clinical phenotypes of episodic and multiple trigger wheeze to determine management, including phenotype switching, should be included.

We added a sentence on phenotype switching to the description of the preschool wheeze phenotypes.

The only other aspect that warrants a little discussion is normal values for blood eosinophils in young children. Counts of up to 0.7x109/L can be considered normal in preschool children, as the normal

range reduces with age. So, it is worth emphasising that in the context of clinical symptoms what is considered an elevated eosinophil count (for example >0.4x109/L) as implied by the authors, may be different to what can be considered normal in an asymptomatic child.

We have included a short paragraph on normal blood eosinophil levels in preschool children and a reference. We added the sentence that blood eosinophils need to be interpreted in the context of clinical symptoms.

The authors state at least twice that sputum induction requires bronchoscopy and general anaesthetic, this is not true. Numerous data have been published on the performance of sputum induction in preschool children, and cytology can be obtained – this is mainly in the context of TB and CF – but certainly GA is not required. This needs to be amended. GA is only required for BAL collection.

We are aware of studies reporting sputum collection in young children particularly for the diagnosis of TB. Most reported studies in children that we are aware of obtain sputum as BAL during a GA. Induction of sputum for leukocyte differential is difficult and time consuming and this technique is impossible for widespread clinical practice. We have however included a sentence to say that sputum induction in children with preschool wheeze is possible without the need for general anaesthesia.

Overall, this review would be much easier to follow if the data from adults, school-aged children and preschool children were discussed separately, thus in the end, based on dtat available, making a case for a trial in preschool children. A table summarising the data that makes a case for a trial would also be very helpful. At the moment, it is a little hard to follow and for key messages to come across.

We have grouped the subjects within the subheadings as much as possible into data from 'adults', followed by 'older children' followed by 'children with preschool wheeze'.

We have also created a table summarising the data that makes a case for a trial. To our knowledge there are no trials in either adults or children based on blood eosinophils.

I think the paragraph labelled "anti-interleukin 5" should either say Interleukin 5 or anti-interleukin 5 antibody – does not make sense as is.

This mistake has been corrected to 'interleukin-5'.

Title:

Blood eosinophils as a marker of likely corticosteroid response in children with preschool wheeze: time for an eosinophil guided clinical trial?

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Abstract:

Childhood wheezing is common particularly in children under the age of six years and in this age-group is generally referred to as preschool wheezing. Particular diagnostic and treatment uncertainties exist in these young children due to the difficulty in obtaining objective evidence of reversible airways narrowing and inflammation. A diagnosis of asthma depends on the presence of relevant clinical signs and symptoms and the demonstration of reversible airways narrowing on lung function testing, which is difficult to perform in young children. Few treatments are available and inhaled corticosteroids are the recommended preventer treatment in most international asthma guidelines. There is however considerable controversy about its effectiveness in children with preschool wheeze and a corticosteroid responder phenotype has not been established. These diagnostic and treatment uncertainties in conjunction with the knowledge of corticosteroid side-effects, in particular the reduction of growth velocity, has resulted in a variable approach to inhaled corticosteroid prescribing by medical practitioners and a reluctance in carers to regularly administer the treatment. Identifying children who are likely responders to corticosteroid therapy would be a major benefit in the management of this condition. Eosinophils have emerged as a promising biomarker of corticosteroid responsive airways disease and evaluation of this biomarker in sputum has successfully been employed to direct management in adults with asthma. Obtaining sputum from young children is time-consuming and difficult and it is hard to justify more invasive procedures such as a bronchoscopy in young children routinely. Recently, in children, interest has shifted to assessing the value of less invasive biomarkers of likely corticosteroid response and the biomarker 'blood eosinophils' has emerged as an attractive candidate. The aim of this review is to summarise the evidence for blood

eosinophils as a predictive biomarker for corticosteroid responsive disease with a particular focus on the difficult area of preschool wheeze.

The preschool wheeze epidemic

It is estimated that 1.1 million children in the UK have asthma, which is considered severe in over ten percent of children using data from the International Study of Asthma and Allergies in Childhood (1). Wheezing in young children aged six months to five years is particularly common and affects approximately one in three children growing up in the UK (2,3). Exacerbations, unscheduled healthcare visits and hospital admissions in this age band are the highest for any age group (4,5) and result in considerable family stress (6) and a significant healthcare burden.

The vast majority of acute exacerbations in young children with recurrent wheezing are associated with viral respiratory tract infections, particularly the human rhinovirus (7,8). Children with human rhinovirus induced wheeze are also more likely to have allergic sensitization (9). In addition, recent studies have suggested that acute rhinovirus bronchiolitis in infancy is linked with genetic variation at the asthma susceptibility 17q21 locus (10) and to be a strong predictor of later asthma (11,12). Absolute blood eosinophil counts are higher in young children with human rhinovirus associated bronchiolitis compared to bronchiolitis caused by other respiratory viruses (8,12) and a recent study of infants hospitalised with bronchiolitis found that absolute blood eosinophil counts > 0.4 x 10^9/L in conjunction with human rhinovirus infection are highly predictive of recurrent wheezing three years later (12).

For the medical professional faced with a child with preschool wheezing two broad patterns of presentation are recognised: “episodic viral wheeze”, comprising children who experience exacerbations with colds but are asymptomatic between episodes; and “multiple trigger wheeze” where children have interval symptoms including day and night time wheezing and wheezing with exercise as well as viral triggered episodes (13,14). It is widely thought that the latter group is more likely to respond to regular treatment with inhaled corticosteroids

(ICS) although only few studies have specifically recruited from a clinical preschool wheeze phenotype. Moreover, several problems exist with these phenotypes including phenotype switching (15) which means that the clinical pattern of preschool wheeze is more easily recognised retrospectively than prospectively. In addition, "episodic viral wheeze" and "multiple trigger wheeze" may represent a marker of disease severity rather than pathophysiologically different disease entities (16). Moreover, severity and frequency of acute wheezing episodes are not taken into account when assigning the clinical phenotype.

One other difficulty in practice is to predict which children with preschool wheeze will continue to have persistent asthma as older children or teenagers. Children with 'multiple trigger wheezing' are more likely to have persistent asthma at school-age (2) and several asthma predictive tools and indices have been described based on data obtained from prospective cohort studies (17,18). The key characteristic features associated with persistence of wheezing in young children have been recently reviewed (19). This is important because medical practitioners are more likely to prescribe regular preventer medication to children they believe will continue to wheeze into older childhood.

In summary, despite the common nature of preschool wheezing there is uncertainty and controversy about which children should be treated with regular preventer medication and we do not know which children will respond to regular ICS treatment, currently the most effective medication available to treat preschool wheezing with an estimated 40 percent responders based on available pooled data (4,20). The validation of phenotypic and biological markers capable of identifying children with preschool wheeze who respond to treatment with corticosteroids would be an important advance.

Current treatment recommendations particularly for preschool wheeze are controversial

Treatment algorithms for preschool wheeze are based on existing treatment strategies largely derived from data obtained in adults with asthma. These recommendations do not distinguish between clinical preschool wheeze patterns despite some limited evidence suggesting differences in treatment responses between the clinical phenotypes (21). Inhaled corticosteroids are recommended as first line controller treatment by the British Thoracic Society (BTS) asthma guideline (22) and the European Preschool Wheeze Task Force (13). The BTS asthma guideline states that: "In children aged 0-4 years with a high probability of asthma: start a trial of treatment". It goes on to say that: "the choice of treatment (for example, inhaled short acting bronchodilators or corticosteroids) depends on the severity and frequency of symptoms". There is no test for asthma in preschool children who generally cannot perform lung function testing and the decision to start treatment and to measure improvements is therefore subjective based upon an assessment of perceived severity of symptoms and number of exacerbations. If ICS fail to control the symptoms an oral leukotriene receptor antagonist can be prescribed as add-on treatment.

Previous research in preschool children has shown that oral corticosteroids have no benefit over placebo when given during an acute exacerbation (23,24) and only modest efficacy in school-age children. Vuillermin *et al* reported a recent randomised controlled trial and calculated that 20 children aged 5-12 years with acute asthma needed to be treated with oral corticosteroids for benefit in one (25).

There are more than 20 published randomised controlled trials involving regular ICS in children with preschool wheeze. A frequently quoted systematic review and meta-analysis found moderate benefit of regular ICS treatment on day-time and night-time symptoms and

the reduction of acute exacerbations requiring unscheduled healthcare visits when pooling all the data (20). These findings have been confirmed again by a more recent study (26). Patient numbers recruited have frequently been relatively small and selection criteria have varied from study to study but they all included some degree of symptomatic or troublesome wheezing with frequent short acting bronchodilator use and usually a history of exacerbations requiring unscheduled healthcare visits. None of the studies stratified on the basis of one or more biomarkers and a corticosteroid responsive phenotype has not been identified. What the pooled data shows is that patient stratification based on clinical criteria alone is inadequate to identify those children that are likely corticosteroid responders.

Identifying responders however is important because children on regular long-term ICS experience a significant reduction in growth velocity (27,28). Moreover, it has been shown that regular use of ICS in children with preschool wheeze does not alter the natural history of asthma or persistent wheeze in later childhood (27,28). Both these studies with long-term follow up found similar numbers of children with persistent older childhood wheeze irrespective as to whether they were prescribed regular long-term ICS in the preschool period or not. Both studies selected patients on the basis of current wheeze and a history of exacerbations requiring unscheduled healthcare visits and a history of atopic disease in either parent. Patient selection in the large RCT reported by Guilbert *et al* (28) was based on the well described 'Asthma Predictive Index' (API). The API has been derived from epidemiological studies and takes into consideration wheeze frequency, parental history of asthma and physician diagnosed atopic dermatitis as major criteria and wheezing away from colds, allergic sensitisation to milk, egg or peanuts and blood eosinophils above 4% as minor criteria (17). The hypothesis in the development of the API was that the index would identify preschool children with early onset atopic asthma and that these children were more likely to respond to corticosteroid therapy. However the proportion of subjects with a positive index

who develop the outcome, defined as physician diagnosed asthma or more than three wheezing episodes during the year prior to age six years, is less than 50% (29). Moreover when children with a positive API were randomised to either long-term ICS or placebo there was no significant difference in unscheduled healthcare visits for wheeze exacerbations between the groups during the treatment period suggesting that a positive API does not predict a corticosteroid response. Therefore, whilst an estimated 40 percent of children with troublesome preschool wheeze may benefit from regular ICS treatment the lack of a responder phenotype paired with the knowledge of known side-effects on growth velocity have resulted in hesitant medical prescribing, poor parental adherence and unabated high rates of exacerbations in these children. We urgently need to assess simple and currently widely available biological markers for their ability to predict treatment responses, particularly to ICS, as validated personalised treatments are likely to be the approach that is most likely to succeed. Recently blood eosinophils have emerged as a potentially promising biomarker of corticosteroid sensitive asthma (30).

Eosinophils and asthma:

Allergic asthma in adults and older children is characterised by increased numbers of circulating eosinophils (31,32) thought to be the result of an inappropriate immune response to common aero-allergens in genetically susceptible individuals (33). In animal models of asthma, aerosol challenge with ovalbumin induces an influx of eosinophils into the blood and the lung (34). Eosinophils are bone marrow derived inflammatory effector cells that differentiate from myeloid precursor cells in response to interleukin (IL)-3 and granulocyte macrophage-colony stimulating factor (33). Mediators such as IL-4, IL-5, and IL-13 released

by CD4 positive T-helper (Th) 2 cells are central to the pathogenesis of asthma, orchestrating the recruitment and activation of mast cells and eosinophils, the principal effector cells of allergic asthma (33). IL-5 is the key mediator necessary for the development, differentiation, recruitment, activation, and survival of circulating eosinophils (35,36). Blood and sputum IL-5, eosinophil numbers and their secreted products correlate with the severity and frequency of asthma exacerbations (34,37-39). Moreover, in a prospective study involving more than 1000 subjects with asthma, an absolute peripheral blood eosinophil count >0.45 x 10^9 /L was associated with a more than 7-fold increase in the relative risk of asthma-related death (40).

Blood eosinophils: Blood eosinophils rise during the late allergic response occurring 24 hours after inhalation allergen challenge (41) and peripheral blood eosinophilia has long been known to be a characteristic feature of asthma and is considered an indirect marker of airway eosinophilic inflammation (31,42). Teenage children with symptomatic, but not acute asthma have significantly greater numbers of activated blood eosinophils but not elevated concentrations of the eosinophil mediator eosinophil cationic protein (ECP) in serum compared to children with well-controlled asthma (43). Geometric mean blood eosinophils >0.40 x 10^9/L were reported in children with uncontrolled asthma in that study. Peripheral blood eosinophil numbers correlate with the severity of symptoms (44,45), the degree of airflow limitation (31,42) and airways responsiveness to direct (46) and indirect bronchial challenge testing (47). In a study of young adults with doctor-diagnosed asthma, the presence of an absolute blood eosinophil count >0.35 x 10^9/L was the best predictor of significant exercise induced bronchoconstriction (≥15% reduction in FEV_1) (47). Ulrik *et al* studying school-age children with allergic and non-allergic asthma found that numbers of blood eosinophils correlated with the asthma symptom score, diurnal peak expiratory flow

variation and airway histamine responsiveness. An inverse correlation was reported with FEV_1 (31).

Moreover, two recent, large cross-sectional studies link peripheral blood eosinophils with asthma exacerbations. Malinovschi *et al* reviewing the laboratory markers of more than 12,000 individuals with asthma aged 6-80 years found that peripheral blood eosinophils of more than 3% are independently associated with emergency healthcare visits due to exacerbations (48). This finding has since been confirmed by a separate study (49). Reviewing data from 3,162 subjects with asthma from the National Health and Nutrition Examination Survey, an annual cross-sectional survey of the US general population, the authors found that the presence of absolute blood eosinophil counts $\geq 0.3 \times 10^9$/L was associated with an increased frequency of acute asthma attacks in respondents, particularly in children.

In preschool children, systemic eosinophil activation is present in those experiencing an acute exacerbation (50) and in several longitudinal epidemiological studies the presence of elevated blood eosinophils in children with preschool wheeze was associated with the persistence of asthma at school-age (51-53) which was independent of allergic sensitisation (54).

Values for blood eosinophils in young children need to be interpreted in the context of clinical presentation. The blood eosinophil range in children five years and younger is wide and has been reported between $0.04\text{-}1.28 \times 10^9$/L in a study of >1200 apparently healthy children aged 0-16 years (55). Increased numbers of blood eosinophils are present in individuals with atopic diseases such as rhinitis (56) and eczema (57,58).

Recent data presented as part of a small study in preschool children showed that absolute blood eosinophil counts $>0.5 \times 10^9$/L are present in about half the children with preschool wheeze (59) and levels of blood eosinophils correlated with airway eosinophils obtained at bronchoscopy.

There is a lack of longitudinal blood eosinophil data reported in the literature in adults and children. We are not aware of any studies reporting serially measured blood eosinophils in children or adults with asthma to study fluctuation. It is also not known if the numbers of blood eosinophils are higher during exacerbations. In children particularly there are important ethical issues with repeat blood taking that is an important limitation to perform such studies.

Sputum eosinophils: Following early observations of peripheral blood eosinophilia in subjects with asthma further studies established the presence of eosinophils in the sputum and airways as a characteristic, albeit not universal, feature of asthma in adults (60-63) and older children (64-67). Elevated sputum eosinophils are also an important feature in subjects with poorly controlled asthma (68) and children experiencing an exacerbation (66,67). Treatment strategies in adults with asthma, based on regular monitoring and titrating of corticosteroid medication based on sputum eosinophils have been shown to reduce exacerbations and lower sputum eosinophils (68-70).

There are relatively few studies with often small numbers that have investigated airway inflammation in young children with mild, moderate and severe wheezing. Most studies in preschool children suggest that eosinophilic airway inflammation is detectable in bronchoalveolar lavage fluid (71) and in subepithelial bronchial biopsy tissue (72-75) obtained from children with recurrent wheezing with greater numbers of eosinophils present in children with concomitant atopic diseases. Overall however, the level of eosinophilic airway inflammation particularly in children three years and younger is low (75,76), compared to that found in older children (64,77,78) and adults (68).

One large study in young children with virus associated wheeze, with and without coexisting atopic disease, reported airway eosinophils well below 2.5%, a frequently quoted cut-off point for a diagnosis of eosinophilic asthma in children (71). The median percentage of airway eosinophils in this study was 0.8%, well below the threshold for eosinophilic asthma. It is of note that the study was not limited to preschool children and that more than half of the recruits were prescribed regular ICS. Age stratification suggested that more eosinophils were present in children older than five years. Similar findings have been reported by two other studies (77,78).

In young children with wheezing, in particular those under six years old, the sampling of sputum usually involve a bronchoscopy and a general anaesthetic. Such techniques are invasive, and cannot repeatedly be performed in the same patient. Sputum induction in young children is possible and has been employed in infants to obtain sputum samples in the investigation of tuberculosis (79). The procedure is time-consuming and requires specialist laboratory staff trained to work with children, and this test is unlikely to be performed in large numbers to guide treatment (80).

Eosinophil cationic protein: Activated eosinophils release mediators that induce changes in the airways and produce the symptoms of the disease. Eosinophil granules contain four major cationic proteins released upon activation. Eosinophil cationic protein (ECP), eosinophil protein X (EPX), eosinophil peroxidase (EPO) and major basic protein (MBP) are capable of causing bronchial epithelial tissue damage (81-83) and dysfunction ~~and are toxic to a variety of tissues, including heart, brain, and bronchial epithelium causing~~ resulting in airways hyperresponsiveness (84,85).

ECP is the most widely studied biomarker of eosinophil activity in asthma and it has been suggested that serum ECP may be a useful indirect and more accurate marker of airway

inflammation in asthma (86). ECP is synthesized in eosinophil progenitors in human bone marrow and stored in specific granules in mature peripheral blood eosinophils (87). Serum ECP levels are increased in adults and older children with asthma and correlate with disease activity and adherence with inhaled corticosteroid therapy (88-90). Serum ECP levels are significantly raised in children during an asthma exacerbation (91). In infants (all <12 months old) with wheezing but free from other allergic disease, serum ECP concentrations were found to be significantly higher when compared to infants with respiratory tract infection without wheezing or healthy controls. Infants with levels >20 μg/L were more likely to still wheeze one year later (92).

However, the relationship between bronchial hyperresponsiveness and serum ECP is less clear (93). In a study by Rao *et al* involving 48 children with asthma aged 5-10 years, serum markers of eosinophil activation were negatively correlated with FEV_1, FEF_{25-75} and the PC20 for histamine (94). This was not confirmed by a separate study involving nearly 200 children with asthma (95). Here, the authors also reported higher levels of serum ECP in children with asthma and the highest levels in children with severe asthma, however serum ECP was not associated with the response to direct bronchial challenge testing. Similarly, a large study in children aged 12-30 months found no association between airway hyperresponsiveness to direct challenge and serum ECP concentration (96).

The measurement of mediators such as ECP may add little to the simple cell counts (97). Moreover, there are important limitations in the use of eosinophil markers such as the need to collect and process blood under tightly controlled and standardized conditions. Immediately after collection the blood needs to be clotted in a water bath at 24^{o}C for exactly 90 minutes followed by centrifugation at 1300 g for 10 min at room temperature making this test impractical for widespread clinical use.

Exhaled nitric oxide: Several inflammatory cells in the lung produce and secrete nitric oxide (NO) including eosinophils. However the inflamed airway epithelium, not confined to eosinophilic inflammation, contributes to the amount of exhaled NO measured (98). There is a moderate correlation only between eosinophil percentages in sputum and the level of exhaled NO in adults (99,100) and children (64) with asthma. A link between blood eosinophils and eNO has also been reported (101,102). It has been suggested that eNO and blood eosinophils relate to different inflammatory pathways. Whilst eNO is considered a marker of corticosteroid responsive asthma (103), the tailoring of the dose of ICS prescribed to the value of eNO is controversial. A Cochrane systematic review of studies concluded that this approach resulted in only small reductions of acute exacerbations and children in the eNO study arms tended to be on higher doses of ICS by the end of the study compared to controls (104).

Relationship between blood and sputum eosinophils

Only a small number of studies systematically studied the association between sputum and blood eosinophils. Pizzichini *et al* compared blood and sputum eosinophils and eosinophil markers obtained at the same visit from 19 adults with symptomatic asthma (105). The median sputum eosinophils were 5.2% and the median absolute blood eosinophil count 0.35 x 10^9/L. When analysing the data using the area under receiver operator curves (ROC) the authors found that sputum eosinophils were more sensitive and specific (0.9) compared to blood eosinophils (0.72) at distinguishing patients with asthma from controls however both, sputum and blood eosinophils showed a good correlation with clinical and physiological markers of asthma severity. Blood eosinophils were a better marker that serum ECP. The

usefulness of blood eosinophils as a surrogate marker of airway eosinophilia has been confirmed by two recent studies (106,107). Wagener *et al.* prospectively studied over 100 patients with mild to moderate asthma and found that an absolute blood eosinophil cut-point of 0.27 x 10^9/L had a sensitivity of 78% and specificity of 91% in distinguishing between airway eosinophilic (defined as 3% or more sputum eosinophils) and non-eosinophilic airway inflammation. The addition of eNO into the ROC analysis did not improve the prediction model. The findings were replicated in a separate cohort of patients with moderate to severe asthma (106) and similar results have been reported in a large but retrospective study of over 500 patients with asthma (107).

This association has also been found in preschool children with viral induced wheeze and allergic asthma where a close relationship between blood and sputum eosinophilic inflammation has been reported (71). Further support is provided by a bronchial biopsy study reporting that numbers of blood eosinophils mirrored eosinophilic inflammation in bronchial biopsies of young children with recurrent wheeze. In particular, of all children considered non-eosinophilic based on bronchial tissue analysis, none had peripheral blood eosinophilia and nearly half the children considered eosinophilic by tissue analysis, had peripheral blood eosinophilia >0.45 x 10^9/L (72).

Overall, the evidence suggests that sputum eosinophils are more closely and accurately associated with asthma symptoms and severity than blood eosinophils. However, although an asthma management and treatment strategy for adults with asthma based upon numbers of sputum eosinophils is feasible and potentially cost effective in specialist secondary and tertiary care settings, it has proved difficult to implement nationally even in this setting (68). It is therefore unrealistic to expect that a strategy based on sputum eosinophils would be suitable for young children and that this could be implemented routinely in primary or secondary care. Blood eosinophils in contrast are a relatively easy biomarker to measure in

children that has been shown to be highly predictive of sputum eosinophilia in patients with asthma.

Eosinophils and eosinophil products as markers of corticosteroid responsive asthma

Treatment with oral corticosteroids results in a decrease in sputum and blood eosinophils and a drop in the blood ECP concentration in adults with asthma (108). Moreover, the reduction in the numbers of blood and sputum eosinophils is mirrored by the clinical and lung function improvement following an acute exacerbation of asthma in response to treatment with corticosteroids as shown by serial testing (109-111). Following oral corticosteroids, absolute blood eosinophil counts reach their lowest reading after three days of treatment and sputum eosinophils after seven days (111).

Blood eosinophils: There is less reported data of associations between asthma severity, the response to corticosteroids and blood eosinophils. There is however, good evidence that blood eosinophils are associated with corticosteroid responsive asthma (30). In an early study Horn *et al* reported absolute blood eosinophil counts of $>0.35 \times 10^9$/L in a group of adult patients with poorly controlled asthma. Blood eosinophils dropped significantly after adjusting corticosteroid treatment doses and asthma control improved (42). In a separate adult study, lung function was significantly negatively correlated to both blood eosinophil counts and serum ECP. Blood eosinophil numbers were more closely associated with respiratory function than eosinophil markers (110).

In children there is a reluctance to perform blood tests; hence few data exist describing the relationship between blood eosinophilia and corticosteroid responsive asthma. Nonetheless,

in a corticosteroid reduction study conducted in children, blood eosinophils increased significantly in the withdrawal group but not in the continuous treatment group (112).

Sputum eosinophils: The presence of airway eosinophils predicts a response to corticosteroid therapy in adult patients with asthma (113,114). In adult subjects with eosinophilic asthma, defined as sputum eosinophils ≥ 3%, ICS treatment leads to a reduction in airway eosinophils (115,116) and a reduction in airway hyperresponsiveness (116). Several studies also reported a rise in sputum eosinophils that was associated with a loss of asthma control following the withdrawal of ICS (117,118). In support of these findings several other studies have shown that non-eosinophilic asthma responds poorly to ICS therapy (119-121). In a rare paediatric study the absence of sputum eosinophils has been shown to be a predictor for successful ICS dose reduction in children with asthma (122).

Some of the best evidence for corticosteroid responsiveness of eosinophilic asthma comes from randomised controlled trials. In a landmark study involving adults with moderate to severe asthma adjustments of the corticosteroid dose based on sputum eosinophil counts resulted not only in a significant reduction in sputum eosinophils in the sputum management group over a 12-month period compared to patients where treatment was based on symptoms and lung function alone, but the reduction in sputum eosinophils was associated with a significant reduction in severe asthma exacerbations requiring unscheduled healthcare visits or admission to hospital (68). The findings from this study suggest that eosinophils are an indicator of corticosteroid responsive asthma in adults and anti-inflammatory treatments directed at reducing elevated numbers result in better asthma control. Blood eosinophils were not reported. The findings from this study have been replicated in two other studies involving adult patients (69,70).

The effectiveness of a management strategy based on sputum eosinophils has not been confirmed in children. One small study in older children with severe asthma found little benefit in titrating corticosteroid dose in accordance with the sputum eosinophil count (123) at three monthly reviews. The annual rate of exacerbations was similar between the clinical and the sputum management group, but significantly fewer subjects in the sputum management group experienced an exacerbation within 28 days of a study visit, perhaps suggesting that more frequent measures would be needed for a clinically useful effect. Also, there was no run-in period therefore the results could be confounded by improved adherence in the clinical group as described previously in a study involving children with severe asthma (124). However it is of note that the sputum management group was on lower doses of ICS at the end of the study compared to the clinical group and in both groups of children the median percentage sputum eosinophils fell to below 2.5%, a level considered within normal limits.

Serum and sputum ECP: The investigators of a 12-month prospective intervention pilot study in school-age children monitoring and adapting corticosteroid dose according to the serum ECP concentration found that raised levels of serum ECP denoted active disease better than lung function parameters (125). The blood ECP concentration fell after initiation of ICS treatment and the authors suggested that ECP may be a useful marker of adherence to corticosteroid treatment (125). However a study by Wolthers *et al* showed that blood ECP is not sensitive to ICS dose changes (126) and in a study involving adults with chronic persistent asthma ICS caused a significant reduction in sputum and blood eosinophils but not sputum or blood ECP (127). Review of the evidence suggests that sputum and blood ECP concentrations are not a sensitive or reliable means of evaluating airway inflammation.

Interleukin-5: This mediator has a critical role in the expansion of the eosinophil pool in the bone marrow and in the induction of blood eosinophilia in response to allergic stimulation (128). Two recent randomized, double-blind, placebo-controlled clinical trials using a monoclonal anti-IL-5 antibody (mepolizumab) showed a significant reduction in the exacerbation frequency in a group of patients with refractory eosinophilic asthma. Mepolizumab treatment also led to a significant reduction in blood and sputum eosinophil counts (129,130). These findings have been confirmed by two further large multicentre clinical trials each involving more than 500 patients. The MENSA study enrolled patients with exacerbation prone asthma on high dose corticosteroid maintenance treatment who had evidence of blood eosinophilic inflammation defined as an absolute blood eosinophil count of 0.15 x 10^9/L or more (131). The exacerbation frequency in the mepolizumab group was approximately halved at the end of the study. In the DREAM study higher blood eosinophil counts were associated with a greater treatment response to mepolizumab (132). There are no reported data in children < 12 years old.

Towards a personalised approach to treatment of preschool and childhood wheeze

Current treatment algorithms based on clinical predictive indices are not working in young children with troublesome wheeze. They have not led to reduced morbidity or indeed a reduction in severe exacerbations. Furthermore, expert reports agree that the benefit of ICS in an unselected cohort of children with troublesome preschool wheeze is modest and recommend more research into identifying corticosteroid responsive disease (13,14). The controversy surrounding the efficacy of anti-inflammatory treatments particularly but not

exclusively in preschool wheeze combined with the concerns about side-effects of corticosteroids has resulted in inconsistent medical prescribing and parental adherence (4). There is an urgent clinical need to identify a reliable and widely available biomarker with the ability to predict which children are likely to have corticosteroid responsive disease. Eosinophils are strongly associated with corticosteroid responsive allergic asthma and exacerbations in older children and adults. Blood eosinophils are an easily measurable and widely available indirect marker of eosinophilic airway inflammation and blood testing is more likely to succeed in young children. This biomarker merits further study and the best way to answer the question as to whether blood eosinophils predict a corticosteroid response in children with troublesome preschool wheeze is to conduct a blood eosinophil stratified randomised controlled trial.

Identifying those children who are corticosteroid responsive would allow promotion of this treatment in this group to reduce exacerbations, improve quality of life and reduce healthcare costs whilst avoiding unnecessary side effects in those likely to be unresponsive.

References:

[1] Lai CK, Beasley R, Crane J, Foliaki S, Shah J, Weiland S. Global variation in the prevalence and severity of asthma symptoms: phase three of the International Study of Asthma and Allergies in Childhood [ISAAC]. *Thorax* 2009;**64**:476-483.

[2] Spycher BD, Silverman M, Brooke AM, Minder CE, Kuehni CE. Distinguishing phenotypes of childhood wheeze and cough using latent class analysis. *Eur Respir J* 2008;**31**:974-981.

[3] Henderson J, Granell R, Heron J, Sherriff A, Simpson A, Woodcock A, Strachan DP, Shaheen SO, Sterne JA. Associations of wheezing phenotypes in the first 6 years of life with atopy, lung function and airway responsiveness in mid-childhood. *Thorax* 2008;**63**:974-980.

[4] Ducharme FM, Tse SM, Chauhan B. Diagnosis, management, and prognosis of preschool wheeze. *Lancet* 2014;**383**:1593-1604.

[5] Garner R, Kohen D. Changes in the prevalence of asthma among Canadian children. *Health Rep* 2008;**19**:45-50.

[6] Staley KG, Herzallah R, Pandya H, Humphreys E, Gaillard EA. A novel evaluation of the impact of severe allergic asthma in children: The family perspective. *Eur.Respir.J.* 2010;**36**[supplement].

[7] Costa LD, Costa PS, Camargos PA. Exacerbation of asthma and airway infection: is the virus the villain? *J Pediatr [Rio J]* 2014;**90**:542-555.

[8] Rakes GP, Arruda E, Ingram JM, Hoover GE, Zambrano JC, Hayden FG, Platts-Mills TA, Heymann PW. Rhinovirus and respiratory syncytial virus in wheezing children requiring emergency care. IgE and eosinophil analyses. *Am J Respir Crit Care Med* 1999;**159**:785-790.

[9] Jartti T, Kuusipalo H, Vuorinen T, Soderlund-Venermo M, Allander T, Waris M, Hartiala J, Ruuskanen O. Allergic sensitization is associated with rhinovirus-, but not other virus-, induced wheezing in children. *Pediatr Allergy Immunol* 2010;**21**:1008-1014.

[10] Caliskan M, Bochkov YA, Kreiner-Moller E, Bonnelykke K, Stein MM, Du G, Bisgaard H, Jackson DJ, Gern JE, Lemanske RF, Nicolae DL, Ober C. Rhinovirus wheezing illness and genetic risk of childhood-onset asthma. *N Engl J Med* 2013;**368**:1398-1407.

[11] Jackson DJ, Gangnon RE, Evans MD, Roberg KA, Anderson EL, Pappas TE, Printz MC, Lee WM, Shult PA, Reisdorf E, Carlson-Dakes KT, Salazar LP, DaSilva DF, Tisler CJ, Gern JE, Lemanske RF, Jr. Wheezing rhinovirus illnesses in early life predict asthma development in high-risk children. *Am J Respir Crit Care Med* 2008;**178**:667-672.

[12] Midulla F, Nicolai A, Ferrara M, Gentile F, Pierangeli A, Bonci E, Scagnolari C, Moretti C, Antonelli G, Papoff P. Recurrent wheezing 36 months after bronchiolitis is associated with rhinovirus infections and blood eosinophilia. *Acta Paediatr* 2014;**103**:1094-1099.

[13] Brand PL, Caudri D, Eber E, Gaillard EA, Garcia-Marcos L, Hedlin G, Henderson J, Kuehni CE, Merkus PJ, Pedersen S, Valiulis A, Wennergren G, Bush,A. Classification and pharmacological treatment of preschool wheezing: changes since 2008. *Eur Respir J* 2014;**43**:1172-1177.

[14] Bush A, Grigg J, Saglani S. Managing wheeze in preschool children. *BMJ* 2014;**348**:g15.

[15] Schultz A, Devadason SG, Savenije OE, Sly PD, Le Souef PN, Brand PL. The transient value of classifying preschool wheeze into episodic viral wheeze and multiple trigger wheeze. *Acta Paediatr* 2010;**99**:56-60.

[16] Garcia-Marcos L, Martinez FD. Multitrigger versus episodic wheeze in toddlers: new phenotypes or severity markers? *J Allergy Clin Immunol* 2010;**126**:489-490.

[17] Castro-Rodriguez JA, Holberg CJ, Wright AL, Martinez FD. A clinical index to define risk of asthma in young children with recurrent wheezing. *Am J Respir Crit Care Med* 2000;**162**:1403-1406.

[18] Pescatore AM, Dogaru CM, Duembgen L, Silverman M, Gaillard EA, Spycher BD, Kuehni CE. A simple asthma prediction tool for preschool children with wheeze or cough. *J Allergy Clin Immunol* 2014;**133**:111-118.

[19] Guilbert TW, Mauger DT, Lemanske RF,Jr. Childhood asthma-predictive phenotype. *J Allergy Clin Immunol Pract* 2014;**2**:664-670.

[20] Castro-Rodriguez JA, Rodrigo GJ. Efficacy of inhaled corticosteroids in infants and preschoolers with recurrent wheezing and asthma: a systematic review with meta-analysis. *Pediatrics* 2009;**123**:e519-25.

[21] Wilson N, Sloper K, Silverman M. Effect of continuous treatment with topical corticosteroid on episodic viral wheeze in preschool children. *Arch Dis Child* 1995;**72**:317-320.

[22] https://www.brit-thoracic.org.uk/document-library/clinical-information/asthma/btssign-asthma-guideline-2014/.

[23] Panickar J, Lakhanpaul M, Lambert PC, Kenia P, Stephenson T, Smyth A, Grigg J. Oral prednisolone for preschool children with acute virus-induced wheezing. *N Engl J Med* 2009;**360**:329-338.

[24] Beigelman A, King TS, Mauger D, Zeiger RS, Strunk RC, Kelly HW, Martinez FD, Lemanske RF Jr, Rivera-Spoljaric K, Jackson DJ, Guilbert T, Covar R, Bacharier LB. Do oral corticosteroids reduce the severity of acute lower respiratory tract illnesses in preschool children with recurrent wheezing? *J Allergy Clin Immunol* 2013;**131**:1518-1525.

[25] Vuillermin PJ, Robertson CF, Carlin JB, Brennan SL, Biscan MI, South M. Parent initiated prednisolone for acute asthma in children of school age: randomised controlled crossover trial. *BMJ* 2010;**340**:c843.

[26] Brand PL, Luz Garcia-Garcia M, Morison A, Vermeulen JH, Weber HC. Ciclesonide in wheezy preschool children with a positive asthma predictive index or atopy. *Respir Med* 2011;**105**:1588-1595.

[27] Murray CS, Woodcock A, Langley SJ, Morris J, Custovic A, IFWIN study team. Secondary prevention of asthma by the use of Inhaled Fluticasone propionate in Wheezy INfants (IFWIN): double-blind, randomised, controlled study. *Lancet* 2006;**368**:754-762.

[28] Guilbert TW, Morgan WJ, Zeiger RS, Mauger DT, Boehmer SJ, Szefler SJ, Bacharier,L.B.; Lemanske,R.F.,Jr; Strunk RC, Allen DB, Bloomberg GR, Heldt G, Krawiec M, Larsen G, Liu AH, Chinchilli VM, Sorkness CA, Taussig LM, Martinez FD. Long-term inhaled corticosteroids in preschool children at high risk for asthma. *N Engl J Med* 2006;**354**:1985-1997.

[29] Guilbert TW, Morgan WJ, Krawiec M, Lemanske RF,Jr, Sorkness C, Szefler SJ, Larsen G, Spahn JD, Zeiger RS, Heldt G, Strunk RC, Bacharier LB, Bloomberg GR, Chinchilli VM, Boehmer SJ, Mauger EA, Mauger DT, Taussig LM, Martinez FD. The Prevention of Early Asthma in Kids study: design, rationale and methods for the Childhood Asthma Research and Education network. *Control Clin Trials* 2004;**25**:286-310.

[30] Kupczyk M, Haque S, Middelveld RJ, Dahlen B, Dahlen SE, BIOAIR Investigators. Phenotypic predictors of response to oral glucocorticosteroids in severe asthma. *Respir Med* 2013;**107**:1521-1530.

[31] Ulrik CS. Peripheral eosinophil counts as a marker of disease activity in intrinsic and extrinsic asthma. *Clin Exp Allergy* 1995;**25**:820-827.

[32] Grol MH, Postma DS, Vonk JM, Schouten JP, Rijcken B, Koeter GH, Gerritsen J. Risk factors from childhood to adulthood for bronchial responsiveness at age 32-42 yr. *Am J Respir Crit Care Med* 1999;**160**:150-156.

[33] Wills-Karp M. Immunologic basis of antigen-induced airway hyperresponsiveness. *Annu Rev Immunol* 1999;**17**:255-281.

[34] Foster PS, Hogan SP, Ramsay AJ, Matthaei KI, Young IG. Interleukin 5 deficiency abolishes eosinophilia, airways hyperreactivity, and lung damage in a mouse asthma model. *J Exp Med* 1996;**183**:195-201.

[35] O'Byrne PM, Inman MD, Parameswaran K. The trials and tribulations of IL-5, eosinophils, and allergic asthma. *J Allergy Clin Immunol* 2001;**108**:503-508.

[36] Collins PD, Marleau S, Griffiths-Johnson DA, Jose PJ, Williams TJ. Cooperation between interleukin-5 and the chemokine eotaxin to induce eosinophil accumulation in vivo. *J Exp Med* 1995;**182**:1169-1174.

[37] Sur S, Gleich GJ, Offord KP, Swanson MC, Ohnishi T, Martin LB, Wagner JM, Weiler DA, Hunt LW, Allergen challenge in asthma: association of eosinophils and lymphocytes with interleukin-5. *Allergy* 1995;**50**:891-898.

[38] Sur S, Gleich GJ, Swanson MC, Bartemes KR, Broide DH. Eosinophilic inflammation is associated with elevation of interleukin-5 in the airways of patients with spontaneous symptomatic asthma. *J Allergy Clin Immunol* 1995;**96**:661-668.

[39] Foster PS, Hogan SP, Yang M, Mattes J, Young IG, Matthaei KI, Kumar RK, Mahalingam S, Webb DC. Interleukin-5 and eosinophils as therapeutic targets for asthma. *Trends Mol Med* 2002;**8**:162-167.

[40] Ulrik CS, Frederiksen J. Mortality and markers of risk of asthma death among 1,075 outpatients with asthma. *Chest* 1995;**108**:10-15.

[41] Cookson WO, Craddock CF, Benson MK, Durham SR. Falls in peripheral eosinophil counts parallel the late asthmatic response. *Am Rev Respir Dis* 1989;**139**:458-462.

[42] Horn BR, Robin ED, Theodore J, Van Kessel A. Total eosinophil counts in the management of bronchial asthma. *N Engl J Med* 1975;**292**:1152-1155.

[43] Ferguson AC, Vaughan R, Brown H, Curtis C. Evaluation of serum eosinophilic cationic protein as a marker of disease activity in chronic asthma. *J Allergy Clin Immunol* 1995;**95**:23-28.

[44] Gibson PG, Dolovich J, Girgis-Gabardo A, Morris MM, Anderson M, Hargreave FE, Denburg JA. The inflammatory response in asthma exacerbation: changes in circulating eosinophils, basophils and their progenitors. *Clin Exp Allergy* 1990;**20**:661-668.

[45] Nadif R, Siroux V, Oryszczyn MP, Ravault C, Pison C, Pin I, Kauffmann F. Heterogeneity of asthma according to blood inflammatory patterns. *Thorax* 2009;**64**:374-380.

[46] Taylor KJ, Luksza AR. Peripheral blood eosinophil counts and bronchial responsiveness. *Thorax* 1987;**42**:452-456.

[47] Koh YI, Choi S. Blood eosinophil counts for the prediction of the severity of exercise-induced bronchospasm in asthma. *Respir Med* 2002;**96**:120-125.

[48] Malinovschi A, Fonseca JA, Jacinto T, Alving K, Janson C. Exhaled nitric oxide levels and blood eosinophil counts independently associate with wheeze and asthma events in National Health and Nutrition Examination Survey subjects. *J Allergy Clin Immunol* 2013;**132**:821-7.

[49] Tran TN, Khatry DB, Ke X, Ward CK, Gossage D. High blood eosinophil count is associated with more frequent asthma attacks in asthma patients. *Ann Allergy Asthma Immunol* 2014;**113**:19-24.

[50] Oommen A, McNally T, Grigg J. Eosinophil activation and preschool viral wheeze. *Thorax* 2003;**58**:876-879.

[51] Just J, Nicoloyanis N, Chauvin M, Pribil C, Grimfeld A, Duru G. Lack of eosinophilia can predict remission in wheezy infants? *Clin Exp Allergy* 2008;**38**:767-773.

[52] Hyvarinen MK, Kotaniemi-Syrjanen A, Reijonen TM, Piippo-Savolainen E, Korppi M. Eosinophil activity in infants hospitalized for wheezing and risk of persistent childhood asthma. *Pediatr Allergy Immunol* 2010;**21**:96-103.

[53] Kotaniemi-Syrjanen A, Reijonen TM, Korhonen K, Korppi M. Wheezing requiring hospitalization in early childhood: predictive factors for asthma in a six-year follow-up. *Pediatr Allergy Immunol* 2002;**13**:418-425.

[54] Karakoc F, Remes ST, Martinez FD, Wright AL. The association between persistent eosinophilia and asthma in childhood is independent of atopic status. *Clin Exp Allergy* 2002;**32**:51-56.

[55] Cranendonk E, van Gennip AH, Abeling NG, Behrendt H, Hart AA. Reference values for automated cytochemical differential count of leukocytes in children 0-16 years old: comparison with manually obtained counts from Wright-stained smears. *J Clin Chem Clin Biochem* 1985;**23**:663-667.

[56] Bradding P, Feather IH, Wilson S, Bardin PG, Heusser CH, Holgate ST, Howarth PH. Immunolocalization of cytokines in the nasal mucosa of normal and perennial rhinitic subjects. The mast cell as a source of IL-4, IL-5, and IL-6 in human allergic mucosal inflammation. *J Immunol* 1993;**151**:3853-3865.

[57] Schauer U, Trube M, Jager R, Gieler U, Rieger CH. Blood eosinophils, eosinophil-derived proteins, and leukotriene C4 generation in relation to bronchial hyperreactivity in children with atopic dermatitis. *Allergy* 1995;**50**:126-132.

[58] Sugai T, Sakiyama Y, Matumoto S. Eosinophil cationic protein in peripheral blood of pediatric patients with allergic diseases. *Clin Exp Allergy* 1992;22:275-281.

[59] Tupker F, Blok F, Irving S, Bush A, Fleming L, Saglani S. Non-invasive markers of airway eosinophilic inflammation in pre-school children. *Eur Respir J* 2014;**44** (Suppl 58):P4201.

[60] Brightling CE. Eosinophils, bronchitis and asthma: pathogenesis of cough and airflow obstruction. *Pulm Pharmacol Ther* 2011;**24**:324-327.

[61] Wardlaw AJ, Dunnette S, Gleich GJ, Collins JV, Kay AB. Eosinophils and mast cells in bronchoalveolar lavage in subjects with mild asthma. Relationship to bronchial hyperreactivity. *Am Rev Respir Dis* 1988;**137**:62-69.

[62] Djukanovic R, Wilson JW, Britten KM, Wilson SJ, Walls AF, Roche WR, Howarth PH. Quantitation of mast cells and eosinophils in the bronchial mucosa of symptomatic atopic asthmatics and healthy control subjects using immunohistochemistry. *Am Rev Respir Dis* 1990;**142**:863-871.

[63] Pizzichini E, Pizzichini MM, Kidney JC, Efthimiadis A, Hussack P, Popov T, Cox G, Dolovich J, O'Byrne P, Hargreave FE. Induced sputum, bronchoalveolar lavage and blood from mild asthmatics: inflammatory cells, lymphocyte subsets and soluble markers compared. *Eur Respir J* 1998;**11**:828-834.

[64] Fleming L, Tsartsali L, Wilson N, Regamey N, Bush A. Sputum inflammatory phenotypes are not stable in children with asthma. *Thorax* 2012;**67**:675-681.

[65] Gibson PG, Simpson JL, Chalmers AC, Toneguzzi RC, Wark PA, Wilson AJ, Hensley MJ. Airway eosinophilia is associated with wheeze but is uncommon in children with persistent cough and frequent chest colds. *Am J Respir Crit Care Med* 2001;**164**:977-981.

[66] Twaddell SH, Gibson PG, Carty K, Woolley KL, Henry RL. Assessment of airway inflammation in children with acute asthma using induced sputum. *Eur Respir J* 1996;**9**:2104-2108.

[67] Norzila MZ, Fakes K, Henry RL, Simpson J, Gibson PG. Interleukin-8 secretion and neutrophil recruitment accompanies induced sputum eosinophil activation in children with acute asthma. *Am J Respir Crit Care Med* 2000;**161**:769-774.

[68] Green RH, Brightling CE, McKenna S, Hargadon B, Parker D, Bradding P, Wardlaw AJ, Pavord ID. Asthma exacerbations and sputum eosinophil counts: a randomised controlled trial. *Lancet* 2002;**360**:1715-1721.

[69] Chlumsky J, Striz I, Terl M, Vondracek J. Strategy aimed at reduction of sputum eosinophils decreases exacerbation rate in patients with asthma. *J Int Med Res* 2006;**34**:129-139.

[70] Jayaram L, Pizzichini MM, Cook RJ, Boulet LP, Lemiere C, Pizzichini E, Cartier A, Hussack P, Goldsmith CH, Laviolette M, Parameswaran K, Hargreave FE. Determining asthma treatment by monitoring sputum cell counts: effect on exacerbations. *Eur Respir J* 2006;**27**:483-494.

[71] Shields MD, Brown V, Stevenson EC, Fitch PS, Schock BC, Turner G, Taylor R, Ennis M. Serum eosinophilic cationic protein and blood eosinophil counts for the prediction of the presence of airways inflammation in children with wheezing. *Clin Exp Allergy* 1999;**29**:1382-1389.

[72] Baraldo S, Turato G, Bazzan E, Ballarin A, Damin M, Balestro E Lokar Oliani K, Calabrese F, Maestrelli P, Snijders D, Barbato A, Saetta M.. Noneosinophilic asthma in children: relation with airway remodelling. *Eur Respir J* 2011;**38**:575-583.

[73] Barbato A, Turato G, Baraldo S, Bazzan E, Calabrese F, Tura M, Zuin R, Beghe B, Maestrelli P, Fabbri LM, Saetta M.. Airway inflammation in childhood asthma. *Am J Respir Crit Care Med* 2003;**168**:798-803.

[74] Saglani S, Malmstrom K, Pelkonen AS, Malmberg LP, Lindahl H, Kajosaari M, Turpeinen M, Rogers AV, Payne DN, Bush A, Haahtela T, Mäkelä MJ, Jeffery PK. Airway remodeling and inflammation in symptomatic infants with reversible airflow obstruction. *Am J Respir Crit Care Med* 2005;**171**:722-727.

[75] Saglani S, Payne DN, Zhu J, Wang Z, Nicholson AG, Bush A, Jeffery PK. Early detection of airway wall remodeling and eosinophilic inflammation in preschool wheezers. *Am J Respir Crit Care Med* 2007;**176**:858-864.

[76] Krawiec ME, Westcott JY, Chu HW, Balzar S, Trudeau JB, Schwartz LB, Wenzel SE. Persistent wheezing in very young children is associated with lower respiratory inflammation. *Am J Respir Crit Care Med* 2001;**163**:1338-1343.

[77] Marguet C, Jouen-Boedes F, Dean TP, Warner JO. Bronchoalveolar cell profiles in children with asthma, infantile wheeze, chronic cough, or cystic fibrosis. *Am J Respir Crit Care Med* 1999;**159**:1533-1540.

[78] Gibson PG. Asthma phenotypes in childhood. *Paediatr Respir Rev* 2011;**12**:151.

[79] Zar HJ, Tannenbaum E, Apolles P, Roux P, Hanslo D, Hussey G. Sputum induction for the diagnosis of pulmonary tuberculosis in infants and young children in an urban setting in South Africa. *Arch Dis Child* 2000;**82**:305-308.

[80] Gaillard EA, Grigg J, Tellabati A, McNally T, Whittaker A, Beardsmore CS. Isolation of cells from the lower airways in infants with wheeze by sputum induction. *Eur Respir J* 2013;**41**:483-485.

[81] Gleich GJ, Adolphson CR. The eosinophilic leukocyte: structure and function. *Adv Immunol* 1986;**39**:177-253.

[82] Gleich GJ, Frigas E, Loegering DA, Wassom DL, Steinmuller D. Cytotoxic properties of the eosinophil major basic protein. *J Immunol* 1979;**123**:2925-2927.

[83] Frigas E, Loegering DA, Gleich GJ. Cytotoxic effects of the guinea pig eosinophil major basic protein on tracheal epithelium. *Lab Invest* 1980;**42**:35-43.

[84] Flavahan NA, Slifman NR, Gleich GJ, Vanhoutte PM. Human eosinophil major basic protein causes hyperreactivity of respiratory smooth muscle. Role of the epithelium. *Am Rev Respir Dis* 1988;**138**:685-688.

[85] Gleich GJ, Flavahan NA, Fujisawa T, Vanhoutte PM. The eosinophil as a mediator of damage to respiratory epithelium: a model for bronchial hyperreactivity. *J Allergy Clin Immunol* 1988;**81**:776-781.

[86] Venge P. Eosinophil activity in bronchial asthma. *Allergy Proc* 1994;**15**:139-141.

[87] Venge P, Bystrom J, Carlson M, Hakansson L, Karawacjzyk M, Peterson C, Seveus L, Trulson A. Eosinophil cationic protein [ECP]: molecular and biological properties and the use of ECP as a marker of eosinophil activation in disease. *Clin Exp Allergy* 1999;**29**:1172-1186.

[88] Zimmerman B, Lanner A, Enander I, Zimmerman RS, Peterson CG, Ahlstedt S. Total blood eosinophils, serum eosinophil cationic protein and eosinophil protein X in childhood asthma: relation to disease status and therapy. *Clin Exp Allergy* 1993;**23**:564-570.

[89] Juntunen-Backman K, Jarvinen P, Sorva R. Serum eosinophil cationic protein during treatment of asthma in children. *J Allergy Clin Immunol* 1993;**92**:34-38.

[90] Villa JR, Garcia G, Rueda S, Nogales A. Serum eosinophilic cationic protein may predict clinical course of wheezing in young children. *Arch Dis Child* 1998;**78**:448-452.

[91] Koh YY, Kang H, Kim CK. Ratio of serum eosinophil cationic protein/blood eosinophil counts in children with asthma: comparison between acute exacerbation and clinical remission. *Allergy Asthma Proc* 2003;**24**:269-274.

[92] Koller DY, Wojnarowski C, Herkner KR, Weinlander G, Raderer M, Eichler I, Frischer T. High levels of eosinophil cationic protein in wheezing infants predict the development of asthma. *J Allergy Clin Immunol* 1997;**99**:752-756.

[93] Koh YY, Kang H, Nah KM, Kim CK. Absence of association of peripheral blood eosinophilia or increased eosinophil cationic protein with bronchial hyperresponsiveness during asthma remission. *Ann Allergy Asthma Immunol* 2003;**91**:297-302.

[94] Rao R, Frederick JM, Enander I, Gregson RK, Warner JA, Warner JO. Airway function correlates with circulating eosinophil, but not mast cell, markers of inflammation in childhood asthma. *Clin Exp Allergy* 1996;**26**:789-793.

[95] Joseph-Bowen J, de Klerk N, Holt PG, Sly PD. Relationship of asthma, atopy, and bronchial responsiveness to serum eosinophil cationic proteins in early childhood. *J Allergy Clin Immunol* 2004;**114**:1040-1045.

[96] Reichenbach J, Jarisch A, Khan S, Homberg M, Bez C, Zielen S. Serum ECP levels and methacholine challenge in infants with recurrent wheezing. *Ann Allergy Asthma Immunol* 2002;**89**:498-502.

[97] Holgate ST. Biomarkers of asthma. *Lancet* 1998;**351**:1300-1301.

[98] Lane C, Knight D, Burgess S, Franklin P, Horak F, Legg J, Moeller A, Stick S. Epithelial inducible nitric oxide synthase activity is the major determinant of nitric oxide concentration in exhaled breath. *Thorax* 2004;**59**:757-760.

[99] Berry MA, Shaw DE, Green RH, Brightling CE, Wardlaw AJ, Pavord ID. The use of exhaled nitric oxide concentration to identify eosinophilic airway inflammation: an observational study in adults with asthma. *Clin Exp Allergy* 2005;**35**:1175-1179.

[100] Nair P, Kjarsgaard M, Armstrong S, Efthimiadis A, O'Byrne PM, Hargreave FE. Nitric oxide in exhaled breath is poorly correlated to sputum eosinophils in patients with prednisone-dependent asthma. *J Allergy Clin Immunol* 2010;**126**:404-406.

[101] Strunk RC, Szefler SJ, Phillips BR, Zeiger RS, Chinchilli VM, Larsen G, Hodgdon K, Morgan W, Sorkness CA, Lemanske RF Jr;. Relationship of exhaled nitric oxide to clinical and inflammatory markers of persistent asthma in children. *J Allergy Clin Immunol* 2003;**112**:883-892.

[102] van den Toorn LM, Overbeek SE, de Jongste JC, Leman K, Hoogsteden HC, Prins JB. Airway inflammation is present during clinical remission of atopic asthma. *Am J Respir Crit Care Med* 2001;**164**:2107-2113.

[103] Dweik RA, Boggs PB, Erzurum SC, Irvin CG, Leigh MW, Lundberg JO, Olin AC, Plummer AL, Taylor DR. An official ATS clinical practice guideline: interpretation of exhaled nitric oxide levels (FENO) for clinical applications. *Am J Respir Crit Care Med* 2011;**184**:602-615.

[104] Petsky HL, Cates CJ, Li A, Kynaston JA, Turner C, Chang AB. Tailored interventions based on exhaled nitric oxide versus clinical symptoms for asthma in children and adults. *Cochrane Database Syst Rev* 2009;(4):CD006340.

[105] Pizzichini E, Pizzichini MM, Efthimiadis A, Dolovich J, Hargreave FE. Measuring airway inflammation in asthma: eosinophils and eosinophilic cationic protein in induced sputum compared with peripheral blood. *J Allergy Clin Immunol* 1997;**99**:539-544.

[106] Wagener AH, de Nijs SB, Lutter R, Sousa AR, Weersink EJ, Bel EH, Sterk PJ. External validation of blood eosinophils, FENO and serum periostin as surrogates for sputum eosinophils in asthma. *Thorax* 2015;**70**:115-120.

[107] Schleich FN, Manise M, Sele J, Henket M, Seidel L, Louis R. Distribution of sputum cellular phenotype in a large asthma cohort: predicting factors for eosinophilic vs neutrophilic inflammation. *BMC Pulm Med* 2013;**13**:11.

[108] Claman DM, Boushey HA, Liu J, Wong H, Fahy JV. Analysis of induced sputum to examine the effects of prednisone on airway inflammation in asthmatic subjects. *J Allergy Clin Immunol* 1994;**94**:861-869.

[109] Baigelman W, Chodosh S, Pizzuto D, Cupples LA. Sputum and blood eosinophils during corticosteroid treatment of acute exacerbations of asthma. *Am J Med* 1983;**75**:929-936.

[110] Griffin E, Hakansson L, Formgren H, Jorgensen K, Peterson C, Venge P. Blood eosinophil number and activity in relation to lung function in patients with asthma and with eosinophilia. *J Allergy Clin Immunol* 1991;**87**:548-557.

[111] Pizzichini MM, Pizzichini E, Clelland L, Efthimiadis A, Mahony J, Dolovich J, Hargreave FE. Sputum in severe exacerbations of asthma: kinetics of inflammatory indices after prednisone treatment. *Am J Respir Crit Care Med* 1997;**155**:1501-1508.

[112] Lonnkvist K, Anderson M, Hedlin G, Svartengren M. Exhaled NO and eosinophil markers in blood, nasal lavage and sputum in children with asthma after withdrawal of budesonide. *Pediatr Allergy Immunol* 2004;**15**:351-358.

[113] Petsky HL, Cates CJ, Lasserson TJ, Li AM, Turner C, Kynaston JA, Chang AB. A systematic review and meta-analysis: tailoring asthma treatment on eosinophilic markers [exhaled nitric oxide or sputum eosinophils]. *Thorax* 2012;**67**:199-208.

[114] Little SA, Chalmers GW, MacLeod KJ, McSharry C, Thomson NC. Non-invasive markers of airway inflammation as predictors of oral steroid responsiveness in asthma. *Thorax* 2000;**55**:232-234.

[115] Di Franco A, Bacci E, Bartoli ML, Cianchetti S, Dente FL, Taccola M, Vagaggini B, Zingoni M, Paggiaro PL. Inhaled fluticasone propionate is effective as well as oral prednisone in reducing sputum eosinophilia during exacerbations of asthma which do not require hospitalization. *Pulm Pharmacol Ther* 2006;**19**:353-360.

[116] Duddridge M, Ward C, Hendrick DJ, Walters EH. Changes in bronchoalveolar lavage inflammatory cells in asthmatic patients treated with high dose inhaled beclomethasone dipropionate. *Eur Respir J* 1993;**6**:489-497.

[117] Deykin A, Lazarus SC, Fahy JV, Wechsler ME, Boushey HA, Chinchilli VM, Craig TJ, Dimango E, Kraft M, Leone F, Lemanske RF, Martin RJ, Pesola GR, Peters SP, Sorkness

CA, Szefler SJ, Israel E. Sputum eosinophil counts predict asthma control after discontinuation of inhaled corticosteroids. *J Allergy Clin Immunol* 2005;**115**:720-727.

[118] Cowan DC, Cowan JO, Palmay R, Williamson A, Taylor DR. Effects of steroid therapy on inflammatory cell subtypes in asthma. *Thorax* 2010;**65**:384-390.

[119] Green RH, Brightling CE, Woltmann G, Parker D, Wardlaw AJ, Pavord ID. Analysis of induced sputum in adults with asthma: identification of subgroup with isolated sputum neutrophilia and poor response to inhaled corticosteroids. *Thorax* 2002;**57**:875-879.

[120] Berry M, Morgan A, Shaw DE, Parker D, Green R, Brightling C, Bradding P, Wardlaw AJ, Pavord ID. Pathological features and inhaled corticosteroid response of eosinophilic and non-eosinophilic asthma. *Thorax* 2007;**62**:1043-1049.

[121] Bacci E, Cianchetti S, Bartoli M, Dente FL, Di Franco A, Vagaggini B, Paggiaro P. Low sputum eosinophils predict the lack of response to beclomethasone in symptomatic asthmatic patients. *Chest* 2006;**129**:565-572.

[122] Zacharasiewicz A, Wilson N, Lex C, Erin EM, Li AM, Hansel T, Khan M Bush A. Clinical use of noninvasive measurements of airway inflammation in steroid reduction in children. *Am J Respir Crit Care Med* 2005;**171**:1077-1082.

[123] Fleming L, Wilson N, Regamey N, Bush A. Use of sputum eosinophil counts to guide management in children with severe asthma. *Thorax* 2012;**67**:193-8.

[124] Strunk RC, Bacharier LB, Phillips BR, Szefler SJ, Zeiger RS, Chinchilli VM, Martinez FD, Lemanske RF Jr, Taussig LM, Mauger DT, Morgan WJ, Sorkness CA, Paul IM, Guilbert T, Krawiec M, Covar R, Larsen G. Azithromycin or montelukast as inhaled corticosteroid-sparing agents in moderate-to-severe childhood asthma study. *J Allergy Clin Immunol* 2008;**122**:1138-1144.

[125] Prehn A, Seger RA, Torresani T, Molinari L, Sennhauser FH. Evaluation of a clinical algorithm involving serum eosinophil cationic protein for guiding the anti-inflammatory treatment of bronchial asthma in childhood. *Pediatr Allergy Immunol* 2000;**11**:87-94.

[126] Wolthers O, Heuck C. Impact of Age and Administration Regimens on the Suppressive Effect of Inhaled Glucocorticoids on Eosinophil Markers in Children with Asthma. *Pediatric Asthma, Allergy & Immunology* 2004;**17**:45-51.

[127] Aldridge RE, Hancox RJ, Cowant JO, Frampton CM, Town GI, Taylor DR. Eosinophils and eosinophilic cationic protein in induced sputum and blood: effects of budesonide and terbutaline treatment. *Ann Allergy Asthma Immunol* 2002;**89**:492-497.

[128] Leckie MJ, ten Brinke A, Khan J, Diamant Z, O'Connor BJ, Walls CM, Mathur AK, Cowley HC, Chung KF, Djukanovic R, Hansel TT, Holgate ST, Sterk PJ, Barnes PJ. Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airway hyper-responsiveness, and the late asthmatic response. *Lancet* 2000;**356**:2144-2148.

[129] Haldar P, Brightling CE, Hargadon B, Gupta S, Monteiro W, Sousa A, Marshall RP, Bradding P, Green RH, Wardlaw AJ, Pavord ID. Mepolizumab and exacerbations of refractory eosinophilic asthma. *N Engl J Med* 2009;**360**:973-984.

[130] Nair P, Pizzichini MM, Kjarsgaard M, Inman MD, Efthimiadis A, Pizzichini E, Hargreave FE, O'Byrne PM. Mepolizumab for prednisone-dependent asthma with sputum eosinophilia. *N Engl J Med* 2009;**360**:985-993.

[131] Ortega HG, Liu MC, Pavord ID, Brusselle GG, FitzGerald JM, Chetta A, Humbert M, Katz LE, Keene ON, Yancey SW, Chanez P. Mepolizumab treatment in patients with severe eosinophilic asthma. *N Engl J Med* 2014;**371**:1198-1207.

[132] Pavord ID, Korn S, Howarth P, Bleecker ER, Buhl R, Keene ON, Ortega H, Chanez P. Mepolizumab for severe eosinophilic asthma [DREAM]: a multicentre, double-blind, placebo-controlled trial. *Lancet* 2012;**380**:651-659.

Table: Studies that make a case for a clinical trial based on blood eosinophils in preschool children with recurrent wheezing

AUTHORS	SUBJECTS	METHODS	OUTCOME
Castro-Rodriguez, JA & Rodrigo,GJ. Pediatrics 123:e519	n=3592 children with preschool wheeze.	Systematic review and meta-analysis of RCTs.	Reduction in exacerbations in just fewer than 40 percent of children in the inhaled corticosteroid group compared to controls in the meta-analysis.
Malinovschi A *et al*. J Allergy Clin Immunol;132:821	n=12,408 aged 6 to 80 years.	Cohort study of blood eosinophils and questionnaire survey on current wheeze, unscheduled healthcare visits due to acute exacerbations of wheeze.	Blood eosinophils >0.3 x 10^9/l were independently associated with asthma related emergency department visits.
Green RH *et al.* Lancet 2002; 360: 1715	n=74 adults with moderate to severe asthma.	RCT; patients randomly assigned to management either by standard British Thoracic Society asthma guidelines OR by normalisation of the induced sputum eosinophil count.	Significantly fewer severe asthma exacerbations in the sputum management group over the 12-months study period.
Jayaram L *et al*. Eur Respir J 2006;27:483	n=117 adults with asthma and variable airflow limitation.	Multicentre RCT; patients randomly assigned to clinical management (symptoms and spirometry) OR sputum eosinophil strategy.	Significantly fewer asthma exacerbations in the sputum management group over the 24-months study period.
Chlumsky J *et al.* J Int Med Res 2006; 34:129	n=55 adults with moderate to severe asthma.	RCT; patients randomly assigned to management either by standard Global Initiative for Asthma guidelines OR sputum eosinophil strategy.	Significantly fewer asthma exacerbations in the sputum management group over the 18-months study period.
Pavord ID *et al.* Lancet 2012; 380: 651	n=621 aged 12 to 74 years with a clinical diagnosis of asthma and variable airflow limitation.	Multicentre RCT; patients randomly assigned to 3 different dosing regimens of monoclonal anti-IL-5 antibody mepolizumab (75mg, 250mg; 750mg intravenous, 4-weekly) OR placebo.	Significant reduction (between 39 and 52%) in exacerbations requiring admission or visits to an emergency department in all groups given mepolizumab compared to placebo. Mepolizumab reduced blood and sputum eosinophil counts. The rate of clinically significant exacerbations with mepolizumab varied according to blood eosinophil count.
Ortega HG *et al.* N Engl J Med 2014; 371: 1198	n=576 aged 12 to 82 years with severe eosinophilic asthma (defined as an absolute blood eosinophil count of ≥0.15 x 10^9/L).	Multicentre RCT; patients randomly assigned to 2 different dosing regimens and administration routes of monoclonal anti-IL-5 antibody mepolizumab (75mg intravenous OR 100mg subcutaneous) OR placebo, 4-weekly for 32 weeks).	Compared to placebo, the exacerbation rate was reduced by 47% in participants receiving intravenous mepolizumab and by 53% in patients receiving subcutaneous mepolizumab.