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Airway IL-1 β and systemic inflammation as predictors of future exacerbation risk in asthma and COPD

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Airway IL-1 β and systemic inflammation as predictors of future exacerbation risk in asthma and COPD

RUNNING TITLE: Inflammatory predictors of exacerbation

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CONFLICT OF INTERESTS

Dr Juan-juan Fu and Dr Katherine J Baines have no competing interest to declare. A/Prof Vanessa M McDonald is supported by the Lung Foundation of

Australia and Boehringer Ingelheim's COPD research fellowship and has participated in educational symposia funded by GlaxoSmithKline, AstraZeneca, Menarini, Boehringer Ingelheim and Novartis. She has participated in studies funded by GlaxoSmithKline and advisory boards for Novartis, AstraZeneca and Menarini. Professor Peter G Gibson holds an NHMRC Practitioner Fellowship. He has participated in educational symposia funded by AstraZeneca, Boehringer Ingelheim, GlaxoSmithKline and Novartis, and has participated in studies funded by Pharmaxis and GlaxoSmithKline.

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This article has supplemental material.

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ABBREVIATIONS LIST

ACQ	asthma control questionnaire
CCI	Charlson comorbidity index
COPD	chronic obstructive pulmonary disease
CRP	C-reactive protein
ER	emergency room
GOLD	global initiative for chronic obstructive lung disease
GP	general practice
ICS	inhaled corticosteroid
IL	interleukin
OAD	obstructive airway disease
OCS	oral corticosteroid
qPCR	quantitative real-time polymerase chain reaction
SD	standard deviation
VAS	visual analogue scale

ABSTRACT

Background: The innate inflammatory pathways involved in the frequent exacerbator phenotypes of asthma and COPD are not well understood. This study aimed to investigate airway innate immune activation and systemic inflammation as predictors of exacerbations in asthma and COPD.

Methods: In this prospective cohort study, baseline airway interleukin (IL)-1 β , serum C-reactive protein and IL-6 were assessed in 152 participants with stable asthma (n=63) or COPD (n=89), and related to exacerbations over the following 12 months. Clinical characteristics and inflammatory biomarkers were compared between the frequent (≥ 2 exacerbations in the follow-up) and infrequent exacerbators. The frequent exacerbation phenotype and exacerbation frequency were analyzed with multivariable modeling. The relationships between airway inflammation, systemic inflammation and future exacerbations were examined using path analysis.

Results: Ninety-four participants experienced a total of 201 exacerbations, and 36.4% had ≥ 2 exacerbations. Serum IL-6 ($P < 0.001$) and sputum gene expression and protein levels of IL-1 β at baseline were higher in the frequent exacerbators with COPD. Significant pathways initiated by previous exacerbations were identified to occur through activation of the IL-1 β -systemic inflammatory axis leading to future exacerbations in COPD. Systemic inflammation was also associated with increased exacerbation risk in asthma.

Conclusion: Airway IL-1 β and systemic inflammation are associated with frequent exacerbations and may mediate a vicious cycle between previous and future exacerbations in COPD. Treatment strategies aimed at attenuating

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these inflammatory pathways to reduce COPD exacerbations deserve further investigation.

This study was approved by the Hunter New England Local Health District Human Research Ethics Committee, Australia (Reference number: 10/08/18/5.03).

Privileged Communication

INTRODUCTION

Asthma and chronic obstructive pulmonary disease (COPD) are common diseases that cause exacerbations of deteriorating symptoms and lung function that can result in hospitalization, increased health care utilization and death.^{1,2} Exacerbations impose a substantial economic burden and result in a faster decline in lung function,^{3,4} and poorer quality of life.^{5,6} A subset of patients experience frequent exacerbations^{4,7,8} and require more effective management strategies. While there are some known clinical factors that are associated with frequent exacerbations of asthma^{2,9} and COPD,¹⁰ the underlying mechanisms, including the role of inflammation and innate immunity in frequent exacerbations, are not well established. If inflammation were a determinant of exacerbations, this would permit both disease monitoring using biomarkers as well as novel approaches to treatment.

Inflammation in COPD and asthma has airway and systemic components.^{11,12} Elevated circulating levels of C-reactive protein (CRP) and interleukin (IL)-6 are indicators of a systemic inflammation. In COPD, a subset of patients experience chronic low-grade systemic inflammation during stable disease. This is associated with a rapid decline in lung function,¹³ increased mortality,¹⁴ and a higher exacerbation rate,^{15,16} however mechanisms behind the observed association between systemic inflammation and exacerbations in COPD¹⁶ remain unclear. While systemic inflammation is increased in asthma,¹² the effect on future exacerbation risk is not known.

IL-1 β is a typical innate immune cytokine involved in the initiation and persistence of inflammation.^{17,18} IL-1 β secretion is increased in stable and exacerbating COPD^{19,20} and asthma.^{18,21} We previously reported that airway

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IL-1 β was associated with systemic inflammation in asthma,^{12,18,21} and hypothesize that the airway innate immune activation and/or systemic inflammation are important determinants of exacerbation risk in asthma and COPD. We therefore conducted a prospective cohort study of patients with asthma and COPD to investigate the determinants of future exacerbation risk and focused on innate immune responses including airway IL-1 β and systemic inflammation (CRP, IL-6). Additionally, we conducted a statistical pathway analysis to explore the relationships between clinical risk factors, airway inflammation, systemic inflammation and future exacerbations.

METHODS

Study design and protocol

Participants (n=152) with doctor diagnosed asthma (n=63) or COPD (n=89) were recruited from research registers and enrolled in a prospective cohort study (details in the Online Supplements). Participants gave written informed consent and the study was approved by the Hunter New England Research Ethics Committee.

Participants attended a baseline visit to assess demographics, smoking status, exacerbation history prior to the study entry, medical history, medication use, symptoms (Visual analogue scale (VAS)), comorbidity (Charlson Comorbidity Index CCI),²² and for the asthma group, the asthma control questionnaire (ACQ).²³ Pre and post bronchodilator spirometry, sputum induction²⁴ and venepuncture were performed (details in the Online Supplement).

After baseline clinical assessment, clinical interviews were conducted by telephone to assess medication use and exacerbation frequency and severity every 3 months for 12 months (Figure 1). Respiratory hospitalization, emergency room (ER) visits, unscheduled primary care doctor visits, and medication use were recorded at each assessment. An exacerbation of COPD was defined as a COPD-related episode that led to a) hospitalization; or b) ER visit; or c) the need for oral corticosteroids (OCS) and/or antibiotics for at least 3 days.⁸ An exacerbation of asthma was defined using the severe asthma exacerbation criteria of the ATS/ERS Task Force,²⁵ or an asthma exacerbation with lower respiratory tract infection requiring antibiotics.²⁶ A frequent exacerbator was defined as a participant who had ≥ 2 exacerbations in the 12 months follow-up.^{8,27}

Details of biomarker measurements are provided in the online supplement. Sputum gene expression was measured using quantitative real-time polymerase chain reaction (qPCR) as previously described.¹⁸ Sputum IL-1 β , IL-1 receptor antagonist (IL-1Ra) proteins, serum high sensitivity CRP and IL-6 were measured using enzyme linked immunosorbent assay. Elevated CRP and IL-6 levels were defined as CRP ≥ 4.12 mg/L and IL-6 ≥ 1.55 pg/mL based on prior data.¹²

Statistical analysis

Data were analyzed using STATA 13 (Stata Corp, Texas, USA) and were reported as mean (SD) or median (quartile 1-3) depending on the distribution. Comparisons between two independent groups were performed using student's t-test or the two-sample Wilcoxon Rank Sum test. Fisher's exact test

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was used to test categorical data. Differences in exacerbation rates (number of events per participant) were analyzed by Poisson regression adjusted for follow-up time and corrected by robust variance estimation. Multivariable Logistic and Poisson regression analyses were performed to identify predictors of frequent exacerbations and the number of exacerbations in asthma and COPD groups separately. Variables that were tested in univariable regression with $P<0.1$ were selected for inclusion in the multivariable regression model followed by a stepwise approach always adjusting for age and sex. A $P<0.05$ was considered significant.

Path analysis using generalized structural equation modelling (SEM) was performed to explore the relationships between exacerbation history, airway inflammation (sputum IL-1 β gene expression), systemic inflammation (serum IL-6 level) and exacerbation frequency in the following year. Each variable was modeled and an overall model of future exacerbations was constructed. Δ Ct of IL-1 β gene expression assay (change in cycle threshold compared to β -actin) and ln(IL-6) were used in this analysis due to the normality of the data.

RESULTS

Baseline characteristics of the participants

Of 152 participants who underwent the baseline assessments, 13 withdrew and 1 died during the follow-up period, leaving 138 participants that completed the 12-month follow-up. Exacerbation data were available prior to withdrawal for a further 2 participants, and data from 140 participants were analyzed (Figure 1).

Participants with COPD were older (68.9 years) than those with asthma (60.5 years), and had greater tobacco smoke exposure and airflow limitation. Inhaled corticosteroid use was similar between the asthma and COPD, but there was greater comorbidity in the COPD group (e-Table 1). Systemic inflammatory markers tended to be similar between groups, but airway neutrophils were greater in COPD (e-Table 2).

Exacerbations by asthma and COPD groups

There were 1.10 exacerbations/participant in the asthma group and 1.67 exacerbations/participant in the COPD group over the 12-month follow-up period ($P=0.056$, Table 1). The distribution of total exacerbation number (Figure 2) and the exacerbation types (Table 1) were similar between asthma and COPD. Overall from this cohort, 94 participants (67.1%) experienced a total of 201 exacerbations during a median follow-up period of 53.4 (52.4-57.0) weeks. Forty-three (30.7%) participants experienced one exacerbation, and 51 (36.4%) of participants reported ≥ 2 exacerbations.

Clinical characteristics of frequent exacerbators

The frequent versus infrequent exacerbators in the asthma and COPD groups were compared (Table 2). Prior year exacerbation frequency and symptom scores were higher in frequent exacerbators. Frequent exacerbators with COPD were older, and had greater lung function impairment and more comorbidities compared to the infrequent exacerbators with COPD. In asthma, the use of long-acting anticholinergics was greater in the frequent exacerbators than infrequent exacerbators.

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Airway IL-1 β , systemic inflammation and frequent exacerbators

Sputum cell counts were not different between the frequent and infrequent exacerbators in either asthma or COPD group (Table 3). Sputum IL-1 β gene expression was significantly up-regulated in frequent exacerbators with COPD (Figure 3C). IL-1 β (Figure 3) or IL-1Ra (Table 3) protein levels were not different between the frequent exacerbators and infrequent exacerbators in either the asthma and COPD groups.

At baseline, serum IL-6 was significantly higher in the frequent exacerbators with asthma (Figure 4A) and COPD (Figure 4C) compared to infrequent exacerbators in each group. Baseline CRP was also elevated in frequent asthma exacerbators (Figure 4B), but not in COPD (Figure 4D). Participants were categorized into three groups based on the number of elevated systemic inflammatory markers at baseline. An increasing number of elevated systemic markers was associated with the frequent exacerbators phenotype in both asthma and COPD (Table 3). Systemic inflammatory markers were significantly correlated with airway IL-1 β gene expression and protein levels (Table 4).

Predictors of future exacerbations

Univariable regression analyses of baseline characteristics that predict exacerbations in asthma (e-Table 3) and COPD (e-Table 4) were performed (Online Supplement). After stepwise selection and adjustment for significant clinical parameters in the regression models, baseline sputum IL-1 β gene expression and protein level significantly predicted the frequent exacerbation

phenotype and the number of exacerbations in COPD (Table 5). Sputum IL-1 β gene and protein expression did not predict frequent exacerbation phenotype and exacerbation number in asthma.

Systemic inflammation, represented by the level of IL-6, was an independent predictor of exacerbation frequency in the following year in both asthma and COPD (Table 6).

Path analysis of exacerbation predictors

In COPD, the number of exacerbations experienced in prior year, airway IL-1 β and serum IL-6, each independently predicted future exacerbation frequency (Table 5 and Table 6). e-Table 5 shows the modelling of each variable, and an overall model of future exacerbations was constructed in which exacerbation history and serum IL-6 levels were significant baseline variables associated with future exacerbations. The path diagrams (Figure 5) indicate that the number of exacerbations in the past year directly and significantly predicted exacerbation frequency in the following year in COPD. In addition to this, there was an indirect association between previous and future exacerbations, via an up-regulation of airway IL-1 β and subsequent increase in the level of systemic inflammation measured by IL-6.

DISCUSSION

This is the first study to record comprehensive types of exacerbations resulting in hospitalizations, ER visits and community exacerbations in both asthma and COPD, and to link these to airway and systemic inflammation.

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This prospective cohort study identifies that airway IL-1 β and increased systemic inflammation are associated with frequent future exacerbations in COPD, and that systemic inflammation is associated with exacerbation risk in asthma for the first time. Importantly, we identified a potential causal pathway that shows how airway innate immune activation through IL-1 β results in systemic inflammation and exacerbations in COPD. This pathway could contribute to a vicious cycle between previous and future exacerbations and may identify potential molecular treatment targets that could reduce exacerbations in COPD.

There is some controversy over the origin of systemic inflammation in COPD. It is hypothesized that there is a “spill-over” of local airway inflammation into the systemic circulation. Others propose that COPD is a systemic multi-organ disease with multiple sources of systemic inflammation.³⁰ SEM is a collection of statistical techniques that allow a set of relations between one or more independent and dependent variables to be examined, and takes a hypothesis testing approach to the multivariate analysis of a structural theory that stipulates causal relations. Path analysis is a type of SEM in which hypotheses of causal relations among multiple variables are modeled.³¹ We used this approach of novel path analysis to test the hypotheses relating to causal relations between airway and inflammation and, future exacerbations. Our findings may further support the ‘spill-over’ theory as airway IL-1 β significantly predicted systemic inflammation in COPD. The path diagram indicates a vicious cycle of exacerbations in patients with COPD, with frequent exacerbations aggravating airway inflammation and leading to increased systemic inflammation, linking previous and future exacerbations.

Therefore, the measurement of airway IL-1 β and systemic IL-6 might provide additional benefit for the prediction of exacerbation beyond clinical assessments. Anti-inflammatory therapies targeting the airway IL-1 β -systemic inflammatory pathway, which break the vicious cycle of inflammation leading to exacerbations, show promise as a strategy for preventing exacerbations. In addition, further studies investigating the role of IL-1 β including its relationship to the inflammasome activation²⁸ and bacterial colonization²⁰ are warrants.

While the importance of systemic inflammation is well established in COPD, our data shows a similar prevalence of systemic inflammation in older asthmatics, and identifies that elevated systemic inflammation is associated with increased future exacerbations in asthma. A greater number of elevated systemic inflammatory markers is a known predictor of COPD exacerbation.¹⁶ Our data extend these findings from COPD to asthma. To our knowledge this is the first report of the prognostic role of systemic inflammation in asthma, indicating that the presence of systemic inflammation is not limited to COPD, rather than a feature of OAD and predicts future exacerbation risk.

The dissociation between sputum eosinophil counts and asthma exacerbation risk conflicts with some previous studies.^{32,33} The primary reason for this would be the small sample size of asthma group and only 16 asthmatics identified as frequent exacerbators. Additionally, asthmatics in our study were recruited from the community, had preserved lung function and well controlled asthma based on the ACQ score which may be due to high dose ICS therapy, resulting in less asthmatics with eosinophilic bronchitis compared with some other studies recruiting severe asthma and participants with increased eosinophils.^{33,34} This may indicate that sputum eosinophil count is not

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associated with exacerbations in well-controlled and non-eosinophilic asthma. The sample size is also a likely reason why we only observed a trend towards an increased IL-1 β gene and protein expression in asthma. There is an urgent need for larger studies in asthma to clarify the IL-1 β -systemic inflammatory pathway activation and its role in asthma.

It is well known that a history of exacerbation is the single best clinical predictor of future exacerbations in COPD.⁸ Our study extends this finding from COPD to asthma. Interestingly, the severity of respiratory symptoms measured by VAS symptom score also predicted the exacerbation frequency in both asthma and COPD. This finding supports the concepts of the combined assessment which integrates both symptoms and risk assessments as recommended in the 2014 GOLD document,³⁵ and promotes the clinical use of combined assessment and individualized management in asthma and COPD.

Several limitations of the study should be noted. We only examined two systemic inflammatory markers and other mediators may also be involved.¹⁶ Agusti et al defined persistent systemic inflammation as stability of elevated systemic inflammatory markers over one year,¹⁵ Therefore future studies that examine more biomarkers and at different time points are desired. Due to the limitations imposed by phone interviews we were not able to identify the aetiology of exacerbations nor their duration and this warrants further investigation. Although SEM allows for the testing of causal hypotheses, the statistical technique does not prove causal relationships without satisfying the necessary conditions for causal inference.³¹ While we proved the causal hypotheses using path analysis, we cannot make warranted causal claims

based this dataset, therefore replication of the findings is needed. A weak to modest correlation between airway and systemic inflammation may suggest the complexity of the cross-talking and that other confounders can also affect each of the two dimensions. Therefore, the hypotheses established in this study need to be evaluated and may be further proved by interventional studies blocking specific components of the pathway.

CONCLUSIONS

In summary, we have identified that an increase in systemic inflammation predicted more frequent exacerbations in asthma, and demonstrated that frequent exacerbations in COPD are associated with both increased airway IL-1 β and systemic inflammation. While an exacerbation history predicted future exacerbations, an indirect association existed between previous and future exacerbation through the airway IL-1 β -systemic inflammatory pathway, contributing to a vicious cycle of exacerbations in COPD. These are important observations that identify a specific subset of patients with COPD who are at high risk of future exacerbations, and identified specific inflammatory pathway and molecules that related to future exacerbations. Treatment strategies aimed at attenuating airway IL-1 β and systemic inflammation to reduce COPD exacerbation deserve further investigation.

ACKNOWLEDGEMENT

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Author contributions: All authors had full access to all of the data in the study and can take responsibility for the integrity of the data and the accuracy of the data analysis. JJF participated in the study design and clinical data collection, carried out laboratory gene and protein assays, performed statistical analysis, and drafted the manuscript. VMM contributed to the data collection, participated in data analysis and interpretation, and critically revised the manuscript. KJB participated in the novel biomarkers identification, supervised the laboratory analysis, and contributed to the data explanation and reviewed the manuscript. PGG contributed to the study design and interpretation of the data, supervised the clinical recruitment, and reviewed the manuscript. All authors read and approved the final manuscript.

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REFERENCES

1. Connors AFJ, Dawson NV, Thomas C, et al. Outcomes following acute exacerbation of severe chronic obstructive lung disease. The SUPPORT investigators (Study to Understand Prognoses and Preferences for Outcomes and Risks of Treatments). *Am J Respir Crit Care Med.* 1996;154(4 Pt 1):959-967.
2. McDonald VM, Gibson PG. Exacerbations of severe asthma. *Clin Exp Allergy.* 2012;42(5):670-677.
3. Bai TR, Vonk JM, Postma DS, Boezen HM. Severe exacerbations predict excess lung function decline in asthma. *European Respiratory Journal.* 2007;30(3):452-456.
4. Donaldson GC, Seemungal TA, Bhowmik A, Wedzicha JA. Relationship between exacerbation frequency and lung function decline in chronic obstructive pulmonary disease. *Thorax.* 2002;57(10):847-852.

5. Kupczyk M, ten Brinke A, Sterk PJ, et al. Frequent exacerbators-a distinct phenotype of severe asthma. *Clin Exp Allergy*. 2014;44(2):212-221.

6. Spencer S, Calverley PM, Burge PS, Jones PW. Impact of preventing exacerbations on deterioration of health status in COPD. *Eur Respir J*. 2004;23(5):698-702.

7. Dougherty RH, Fahy JV. Acute exacerbations of asthma: epidemiology, biology and the exacerbation-prone phenotype. *Clin Exp Allergy*. 2009;39(2):193-202.

8. Hurst JR, Vestbo J, Anzueto A, et al. Susceptibility to exacerbation in chronic obstructive pulmonary disease. *N Engl J Med*. 2010;363(12):1128-1138.

9. ten Brinke A, Sterk PJ, Masclee AA, et al. Risk factors of frequent exacerbations in difficult-to-treat asthma. *Eur Respir J*. 2005;26(5):812-818.

10. Miravittles M, Calle M, Soler-Cataluna JJ. Clinical phenotypes of COPD: identification, definition and implications for guidelines. *Arch Bronconeumol*. 2012;48(3):86-98.

11. Simpson JL, Grissell TV, Douwes J, Scott RJ, Boyle MJ, Gibson PG. Innate immune activation in neutrophilic asthma and bronchiectasis. *Thorax*. 2007;62(3):211-218.

12. Wood LG, Baines KJ, Fu J, Scott HA, Gibson PG. The Neutrophilic Inflammatory Phenotype Is Associated With Systemic Inflammation in Asthma. *CHEST*. 2012;142(1):86-93.

13. Donaldson GC, Seemungal TA, Patel IS, et al. Airway and systemic inflammation and decline in lung function in patients with COPD. *Chest*. 2005;128(4):1995-2004.
14. Dahl M, Vestbo J, Lange P, Bojesen SE, Tybjaerg-Hansen A, Nordestgaard BG. C-reactive protein as a predictor of prognosis in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 2007;175(3):250-255.
15. Agusti A, Edwards LD, Rennard SI, et al. Persistent systemic inflammation is associated with poor clinical outcomes in COPD: a novel phenotype. *PLoS One*. 2012;7(5):e37483.
16. Thomsen M, Ingebrigtsen TS, Marott JL, et al. Inflammatory biomarkers and exacerbations in chronic obstructive pulmonary disease. *JAMA*. 2013;309(22):2353-2361.
17. Slack J, McMahan CJ, Waugh S, et al. Independent binding of interleukin-1 alpha and interleukin-1 beta to type I and type II interleukin-1 receptors. *J Biol Chem*. 1993;268(4):2513-2524.
18. Baines KJ, Simpson JL, Wood LG, Scott RJ, Gibson PG. Transcriptional phenotypes of asthma defined by gene expression profiling of induced sputum samples. *J Allergy Clin Immunol*. 2011;127(1):153-160, 160 e151-159.
19. Pauwels NS, Bracke KR, Dupont LL, et al. Role of IL-1alpha and the Nlrp3/caspase-1/IL-1beta axis in cigarette smoke-induced pulmonary inflammation and COPD. *Eur Respir J*. 2011;38(5):1019-1028.

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20. Bafadhel M, McKenna S, Terry S, et al. Acute exacerbations of chronic obstructive pulmonary disease: identification of biologic clusters and their biomarkers. *Am J Respir Crit Care Med*. 2011;184(6):662-671.

21. Fu JJ, Baines KJ, Wood LG, Gibson PG. Systemic inflammation is associated with differential gene expression and airway neutrophilia in asthma. *OMICS*. 2013;17(4):187-199.

22. Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. *J Chronic Dis*. 1987;40(5):373-383.

23. Juniper EF, O'Byrne PM, Guyatt GH, Ferrie PJ, King DR. Development and validation of a questionnaire to measure asthma control. *Eur Respir J*. 1999;14(4):902-907.

24. Gibson PG, Wlodarczyk JW, Hensley MJ, et al. Epidemiological association of airway inflammation with asthma symptoms and airway hyperresponsiveness in childhood. *Am J Respir Crit Care Med*. 1998;158(1):36-41.

25. Reddel HK, Taylor DR, Bateman ED, et al. An official American Thoracic Society/European Respiratory Society statement: asthma control and exacerbations: standardizing endpoints for clinical asthma trials and clinical practice. *Am J Respir Crit Care Med*. 2009;180(1):59-99.

26. Brusselle GG, Vanderstichele C, Jordens P, et al. Azithromycin for prevention of exacerbations in severe asthma (AZISAST): a multicentre randomised double-blind placebo-controlled trial. *Thorax*. 2013;68(4):322-329.

27. Kupczyk M, ten Brinke A, Sterk PJ, et al. Frequent exacerbators--a distinct phenotype of severe asthma. *Clin Exp Allergy*. 2014;44(2):212-221.
28. Dolinay T, Kim YS, Howrylak J, et al. Inflammasome-regulated cytokines are critical mediators of acute lung injury. *Am J Respir Crit Care Med*. 2012;185(11):1225-1234.
29. Dinarello CA, Simon A, van der Meer JW. Treating inflammation by blocking interleukin-1 in a broad spectrum of diseases. *Nat Rev Drug Discov*. 2012;11(8):633-652.
30. Agustí A. Systemic effects of chronic obstructive pulmonary disease: what we know and what we don't know (but should). *Proc Am Thorac Soc*. 2007;4(7):522-525.
31. Lei PW, Wu Q. Introduction to Structural Equation Modeling: Issues and Practical Considerations. *Educational Measurement: Issues and Practice*. 2007;26(3):33-43.
32. Green RH, Brightling CE, McKenna S, et al. Asthma exacerbations and sputum eosinophil counts: a randomised controlled trial. *Lancet*. 2002;360(9347):1715-1721.
33. Jayaram L, Pizzichini MM, Cook RJ, et al. Determining asthma treatment by monitoring sputum cell counts: effect on exacerbations. *Eur Respir J*. 2006;27(3):483-494.
34. Gibson PG, Fujimura M, Niimi A. Eosinophilic bronchitis: clinical manifestations and implications for treatment. *Thorax*. 2002;57(2):178-182.

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35. Global Initiative for Chronic Obstructive Lung Disease (GOLD). Global strategy for the diagnosis, management and prevention of COPD. Updated 2014. Available from: <http://www.goldcopd.com>.

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Table 1. Exacerbations during the 12-month observation period by asthma and COPD groups

Rate (number per participant)	Asthma	COPD	IRR *	P value †	Number (%)	Asthma	COPD	P value
Hospitalization	0.10	0.25	2.44	0.130	≥1 hospitalization	8.6%	15.8%	0.217
ER visits	0.02	0.09	4.49	0.224	≥1 ER visits	1.7%	5.3%	0.389
Unscheduled doctor visits	0.95	0.91	0.93	0.750	≥2 unscheduled doctor visits	22.4%	19.7%	0.706
OCS courses	0.69	0.94	1.28	0.411	≥2 OCS courses	19.0%	21.1%	0.765
Antibiotic courses	1.14	1.51	1.28	0.224	≥2 Antibiotic courses	31.0%	38.2%	0.392
Number of exacerbations	1.10	1.67	1.45	0.056	≥2 Exacerbations	27.6%	42.1%	0.082

Definition of abbreviations: IRR = incidence rate ratio; OCS = oral corticosteroid.

* COPD versus asthma; † Analyzed by Poisson regression.

Table 2. Baseline characteristics of frequent VS infrequent exacerbators with asthma and COPD (mean(SD) unless stated)

	Asthma			COPD		
	Frequent exacerbators	Infrequent exacerbators	P value	Frequent exacerbators	Infrequent exacerbators	P value
Number, n(%)	16(27.1)	43(72.9)	N/A	35(43.2)	46(56.8)	N/A
Age	62.6(11.4)	59.7(14.4)	0.475	71.3(9.0)	67.2(8.7)	0.046
Sex, male female (n)	4 12	16 27	0.539	14 21	21 25	0.611
Body Mass Index	30.6(6.5)	30.6(8.2)	0.987	29.4(9.4)	28.2(6.0)	0.488
Smoking, never ex current smokers (n)	12 4 0	22 19 2	0.241	9 25 1	20 22 4	0.101
Smoking pack-years *	21.5(4.1-42.0)	7.0(2.0-32.0)	0.711	28.2(15.3-44.0)	26.5(10.8-47.0)	0.798
FEV ₁ %predicted	82.1(15.0)	87.6(15.6)	0.234	50.9(20.6)	62.9(14.7)	0.003
FVC %predicted	83.9(15.5)	90.8(16.5)	0.157	75.9(21.6)	83.4(15.9)	0.081
FEV ₁ /FVC %	74.1(5.0)	74.8(6.7)	0.729	50.8(14.8)	56.9(8.2)	0.020
AHR or BDR at visit, n(%)	8(50.0)	16(37.2)	0.374	18(51.4)	28(60.9)	0.395
VAS symptom score *	26.4(10.2-32.8)	13.4(7.2-26.1)	0.068	24.1(16.8-34.4)	16.2(7.2-25.7)	0.004
ICS use, n(%)	16(100)	34(79.1)	0.097	33(94.3)	38(82.6)	0.114
ICS dose, BDP equivalent, mcg/day *	2000(1000-2000)	1000(500-2000)	0.074	2000(1000-2000)	1000(500-2000)	0.034

Long-acting anticholinergic use, n(%)	7(43.8)	7(16.3)	0.027	22(62.9)	23(50.0)	0.249
Number of exacerbations in the year prior to baseline visit *	2(0.5-4)	0(0-0)	<0.001	3(1-4)	0.5(0-1)	<0.001
≥2 exacerbations in the prior year, n(%)	9(56.3)	3(7.0)	<0.001	23(65.7)	11(23.9)	<0.001
CCI *	3(2-4)	3(2-4)	0.675	4(3-5)	4(3-4)	0.039
Cardiovascular disease, n(%)	7(43.8)	23(53.5)	0.506	24(68.6)	28(60.9)	0.474
HMGCoA reductase inhibitor (Statin) use, n(%)	6(37.5)	12(27.9)	0.477	10(28.6)	10(21.7)	0.480

Definition of abbreviations: ACQ = asthma control questionnaire; AHR = airway Hyper-responsiveness; BDR = bronchodilator response; ICS = inhaled corticosteroids; BDP = beclometasone dipropionate; CCI = Charlson comorbidity index.

* Median(quartile 1-3)

Table 3. Airway and systemic inflammation of frequent VS infrequent exacerbators with asthma and COPD (median (quartile 1-3))

	Asthma			COPD		
	Frequent exacerbators	Infrequent exacerbators	<i>P</i> value	Frequent exacerbators	Infrequent exacerbators	<i>P</i> value
Sputum total cell count, ×10 ⁶ /mL	3.5(2.6-7.0)	4.0(2.3-6.2)	0.844	6.6(2.3-12.0)	4.3(2.9-8.6)	0.510
Sputum neutrophil%	38.8(30.5-69.0)	44.8(23.5-60.3)	0.786	60.8(39.4-76.6)	61.0(30.8-73.9)	0.766
Sputum eosinophil%	3.0(1.0-15.5)	1.0(0.3-2.5)	0.065	1.6(0.9-2.6)	1.8(0.5-5.0)	0.870
Sputum macrophage%	47.8(24.3-58.5)	49.3(32.3-72.8)	0.310	29.8(17.0-51.5)	27.5(13.8-52.5)	0.989
Sputum lymphocyte%	1.0(0.5-1.5)	1.0(0.5-2.3)	0.527	0.6(0.3-1.8)	0.8(0.3-1.5)	0.955
Sputum IL-1β gene expression	0.8(0.5-2.3)	0.4(0.3-1.8)	0.073	6.2(0.7-10.5)	0.6(0.2-2.1)	0.018
Sputum IL-1β protein level, ng/mL	0.6(0.2-1.9)	0.3(0.1-0.6)	0.141	0.8(0.1-3.7)	0.2(0.1-0.9)	0.065
Sputum IL-1Ra, ng/mL	150.9(129.1-294.2)	214.5(134.6-275.6)	0.674	141.8(92.4-323.5)	199.9(111.7-260.2)	0.553
Serum CRP, mg/L	6.3(1.7-9.6)	2.5(1.0)	0.040	4.4(1.4-7.6)	2.7(1.1-6.2)	0.535
Serum CRP ≥4.12 mg/L, n(%)	9(56.3)	11(26.2)	0.031	17(53.1)	18(40.9)	0.291
Serum IL-6, pg/mL	2.7(1.8-4.3)	1.5(1.1-2.2)	0.008	3.2(1.9-4.5)	1.9(1.2-3.4)	0.004
Serum IL-6 ≥1.55 pg/mL, n(%)	13(81.3)	21(50.0)	0.039	29(90.6)	27(61.4)	0.007
Number of elevated systemic	3 4 9	19 14 9	0.031	3 12 17	14 15 15	0.050

inflammatory mediators (n) *						
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* None of CRP and IL-6 elevated | either CRP or IL-6 elevated | both CRP and IL-6 elevated

Table 4. Spearman rank correlations of airway IL-1β with systemic inflammation

Systemic inflammation Airway inflammation	Serum CRP	Serum IL-6	Number of elevated markers *
IL-1β gene expression	r=0.291 P=0.010	r=0.394 P<0.001	r=0.326 P=0.004
IL-1β protein level	r=0.228 P=0.030	r=0.276 P=0.008	r=0.327 P=0.002
IL-1Ra protein level	r=0.023 P=0.829	r=-0.124 P=0.239	r=-0.187 P=0.073

* Three groups: with normal CRP and IL-6 levels; with either CRP or IL-6 elevated; and with both CRP and IL-6 elevated

Table 5. Sputum IL-1 β gene and protein expression predicted the frequent exacerbation phenotype (Logistic regression) and exacerbation frequency (number, Poisson regression) in COPD

Frequent VS Infrequent exacerbators				Number of exacerbations			
Baseline variables	OR	95% CI	P value	Baseline variables	IRR	95% CI	P value
Age	1.07	0.98-1.17	0.136	Age	1.02	0.99-1.05	0.123
Sex	1.36	0.29-6.50	0.696	Sex	1.06	0.66-1.71	0.794
Sputum IL-1 β gene expression	1.26	1.06-1.51	0.011	The number of exacerbations in prior year	1.09	1.02-1.16	0.015
VAS symptom score	1.07	1.00-1.13	0.042	Sputum IL-1 β gene expression	1.06	1.02-1.11	0.001
				VAS symptom score	1.04	1.02-1.06	<0.001
Frequent VS Infrequent exacerbators				Number of exacerbations			
Baseline variables	OR	95% CI	P value	Baseline variables	IRR	95% CI	P value
Age	1.09	1.00-1.20	0.045	Age	1.03	1.01-1.06	0.017
Sex	1.89	0.44-8.13	0.395	Sex	1.06	0.67-1.65	0.813
Sputum IL-1 β protein level, ng/mL	1.32	1.07-1.62	0.008	The number of exacerbations in prior year	1.13	1.06-1.19	<0.001
VAS symptom score	1.07	1.01-1.12	0.019	Sputum IL-1 β protein level, ng/mL	1.06	1.01-1.13	0.040
				VAS symptom score	1.03	1.02-1.05	<0.001

Definition of abbreviations: OR = odds ratio; CI = confidence interval; IRR = incidence rate ratio

Table 6. Systemic inflammation predicted exacerbation frequency (number, Poisson regression) in asthma and COPD

Asthma				COPD			
	Number of exacerbations				Number of exacerbations		
Baseline variables	IRR	95% CI	P value	Baseline variables	IRR	95% CI	P value
Age	1.01	0.99-1.03	0.344	Age	1.03	1.01-1.06	0.006
Sex	1.22	0.68-2.19	0.514	Sex	1.16	0.77-1.74	0.481
The number of exacerbations in prior year	1.17	1.03-1.32	0.014	The number of exacerbations in prior year	1.12	1.06-1.19	<0.001
Serum IL-6, pg/mL	1.10	1.02-1.17	0.007	Serum IL-6, pg/mL	1.01	1.00-1.02	0.044
VAS symptom score	1.02	1.00-1.05	0.071	VAS symptom score	1.02	1.01-1.04	0.004
				FEV ₁ %predicted	0.99	0.98-1.00	0.066

Definition of abbreviations: OR = odds ratio; CI = confidence interval; IRR = incidence rate ratio

FIGURE LEGEND

Figure 1. Study flow chart.

Definition of abbreviations: OAD = Obstructive airway disease, ELISA = enzyme-linked immunosorbent assay, qPCR = quantitative real-time polymerase chain reaction

Figure 2. Distribution of the number of exacerbations in asthma and COPD group

Figure 3. Baseline levels of sputum IL-1 β gene and protein expression in asthma (A and B) and COPD (C and D) between the frequent and infrequent exacerbators. Lines in B) and D) represent median values

Figure 4. Baseline levels of serum IL-6 and CRP between frequent VS infrequent exacerbators in asthma (A and B) and COPD (C and D). Lines represent median values

Figure 5. A) Path diagram of Generalized Structural Equation Modelling for the predictors of future exacerbation frequency generated by STATA 13: Arrows represent paths between variables, and a path points from one variable to another represents the first variable affects the second. Numbers under variables represent overall coefficient of different models, and numbers near the circled ϵ are the variance of the models. Small number along with the arrow connecting two variables means variable specified coefficient. Red lines

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represent statistically significant paths. B) A simplified pathway diagram of number of exacerbations in prior year, airway inflammation measured by sputum IL-1 β gene expression, systemic inflammation measured by serum IL-6 and number of future exacerbations based on the modelling

* Variable that predicted by age

Privileged Communication

**Airway IL-1 β and systemic inflammation as predictors of future
exacerbation risk in asthma and COPD**

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Katherine J Baines, PhD

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SUPPLEMENTAL MATERIAL

METHODS

Subject

Asthma was confirmed by current (past 12 months) episodic respiratory symptoms, doctor’s diagnosis and prior airway hyperresponsiveness and/or bronchodilator response.¹ COPD was confirmed according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria.² Participants gave written informed consent. The Hunter New England Local Health District Human Research Ethics Committee approved this study. Exclusion criteria included respiratory disorders other than asthma and COPD, or current haematologic or solid organ malignancy. An exacerbation in the past month was cause to delay visits until recovery (4 weeks of stability).

Baseline assessment

Questionnaires

A respiratory symptom questionnaire assessing subjective symptom severity was administered, in which each symptom score including nasal symptoms, breathlessness, wheeze, cough and sputum production were recorded on a 10cm visual analogue scale (VAS), and a total cumulative score was calculated by adding up each individual score, with a range from 0 to 50cm. The Charlson Comorbidity Index (CCI) was calculated using information collected in the medical history.³ GINA severity for asthmatics was assessed according to the classification criteria for those who are on treatment,⁴ and GOLD stage for patients with COPD was classified.²

Pulmonary function tests

Participants withheld bronchodilators for their duration of action before testing. Three reproducible measurements of FEV₁ and force vital capacity (FVC) were obtained (KoKo PD Instrumentation Louisville CO USA) before and after inhalation of 400mcg salbutamol via a metered dose inhaler with valved holding chamber (Volumatic, Allen and Hanbury's, Melbourne Victoria, Australia). Predicted values of lung function were calculated according to Hankinson et al.⁵

Sputum induction and analysis

Sputum induction with hypertonic saline (4.5%) was performed as previously described.⁶ Airway inflammation was assessed using induced sputum. Lower respiratory sputum portions were selected from saliva, dispersed using dithiothreitol (DTT), cytopins were prepared and stained, and differential cell counts obtained.⁶ For gene expression assays, 100µl of selected sputum was immediately placed into Buffer RLT (Qiagen, Hilden, Germany) and stored at -80°C until RNA extraction. Eosinophilic bronchitis is defined as sputum eosinophil percentage >3%,^{7,8} and neutrophilic bronchitis is defined as sputum neutrophil percentage >61%.⁹

Measurement of inflammatory biomarkers

Sputum IL-1β genes and proteins

RNA was prepared and reverse-transcribed to cDNA as previously described.¹⁰ Primers and probes for target genes were purchased in kit form (Applied Biosystems). Gene expression of IL-1β was analysed using Taqman quantitative real-time polymerase chain reaction (qPCR) on a 7500 Fast Real Time PCR system (Applied Biosystems) according to standard Taqman methods and samples were tested in duplicate as previously described,¹⁰ and the expression value of each gene

was normalized to housekeeping gene (β -actin) and the mean of all samples to calculate the relative amount of RNA present in each sample according to the $2^{-\Delta\Delta C_t}$ method. The concentrations of IL-1 β and IL-1Ra proteins (R&D Systems, Minneapolis, MN, USA) in sputum supernatant were determined in duplicate by ELISA according to manufacturers' instructions.

Systemic inflammatory markers

Peripheral venous blood was collected into Vacutainer® tubes (BD Worldwide, North Ryde, NSW, Australia), and serum was obtained by centrifugation at 3000rpm, 4°C for 10 minutes. High sensitivity CRP (MP Biomedicals, Australiasia, Seven Hills, NSW, Australia) and IL-6 (R&D Systems, Minneapolis, MN, USA) were determined in duplicate using enzyme-linked immunosorbent assay (ELISA) according to the manufacturers' instructions.

REFERENCES

1. Global Initiative for Asthma (GINA). Global strategy for asthma management and prevention. Updated 2012. Available at: <http://www.ginasthma.com>.
2. Global Initiative for Chronic Obstructive Lung Disease (GOLD). Global strategy for the diagnosis, management and prevention of COPD. Updated 2014. Available from: <http://www.goldcopd.com>.
3. Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. *J Chronic Dis*. 1987;40(5):373-383.

4. Global Initiative for Asthma (GINA). Global strategy for asthma management and prevention. Updated 2002. Available at: <http://www.ginasthma.com>.
5. Hankinson JL, Odencrantz JR, Fedan KB. Spirometric reference values from a sample of the general U.S. population. *Am J Respir Crit Care Med*. 1999;159(1):179-187.
6. Gibson PG, Wlodarczyk JW, Hensley MJ, et al. Epidemiological association of airway inflammation with asthma symptoms and airway hyperresponsiveness in childhood. *Am J Respir Crit Care Med*. 1998;158(1):36-41.
7. Green RH, Brightling CE, McKenna S, et al. Asthma exacerbations and sputum eosinophil counts: a randomised controlled trial. *Lancet*. 2002;360(9347):1715-1721.
8. Siva R, Green RH, Brightling CE, et al. Eosinophilic airway inflammation and exacerbations of COPD: a randomised controlled trial. *European Respiratory Journal*. 2007;29(5):906-913.
9. Simpson JL, Scott R, Boyle MJ, Gibson PG. Inflammatory subtypes in asthma: assessment and identification using induced sputum. *Respirology*. 2006;11(1):54-61.
10. Baines KJ, Simpson JL, Wood LG, Scott RJ, Gibson PG. Transcriptional phenotypes of asthma defined by gene expression profiling of induced sputum samples. *J Allergy Clin Immunol*. 2011;127(1):153-160, 160 e151-159.

e-Table 1. Clinical characteristics of participants at baseline (mean(SD) unless stated)

Baseline variables	Asthma	COPD	P value
Number, n(%)	59(42.1)	81(57.9)	
Age	60.5(13.6)	68.9(9.0)	<0.001
Sex, male female (n)	20 39	35 46	0.265
BMI	30.6(7.7)	28.8(7.7)	0.162
Smoking, never ex current smokers (n)	34 23 2	29 47 5	0.038
Smoking pack-years *	8(2-35)	28(13-45.5)	0.030
FEV ₁ %predicted	86.0(15.5)	57.7(18.4)	<0.001
FVC %predicted	88.8(16.4)	80.2(18.7)	0.006
FEV ₁ /FVC %	74.6(6.3)	54.3(11.8)	<0.001
AHR or BDR at visit, n(%)	24(40.7)	46(56.8)	0.060
VAS symptom score *	15.8(7.4-27.5)	19.3(11.6-29.0)	0.210
ACQ/6 *	0.50(0.17-0.83)	N/A	N/A
GINA severity (n), step 1 2 3 4	3 11 22 23	N/A	N/A
GOLD stage (n), stage 1 2 3 4	N/A	11 42 22 6	N/A
ICS use, n(%)	50(84.8)	71(87.7)	0.620
ICS dose, BDP equivalent, mcg/day *	1000(500-2000)	1000(500-2000)	0.956
Long-acting anticholinergic use, n(%)	14(23.7)	45(55.6)	<0.001
Number of exacerbations in prior year *	0(0-1)	1(0-3)	0.001
Frequent exacerbators in prior year, n(%)	12(20.3)	34(42.0)	0.007
CCI *	3(2-4)	4(3-5)	0.002
Cardiovascular disease, n(%)	30(50.9)	52(64.2)	0.113
HMGC α reductase inhibitor (Statin) use, n(%)	18(30.5)	20(24.7)	0.445

Definition of abbreviations: ACQ = asthma control questionnaire; ICS = inhaled corticosteroids; BDP = beclometasone dipropionate; CCI = Charlson comorbidity index.

* Median (quartile 1-3)

e-Table 2. Systemic and airway inflammatory characteristics of participants at baseline
(mean(SD) unless stated)

Baseline variables	Asthma	COPD	P value
Sputum total cell count, $\times 10^6/\text{mL}$ *	3.7(2.4-6.3)	4.6(2.7-10.2)	0.248
Sputum eosinophil% *	1.3(0.3-3.0)	1.8(0.8-3.8)	0.214
Sputum eosinophil% >3%, n(%)	9(21.4)	18(28.6)	0.412
Sputum neutrophil% *	42.8(24.0-60.3)	61.0(36.5-76.0)	0.006
Sputum neutrophil% >61%, n(%)	10(23.8)	32(50.8)	0.006
Sputum macrophage% *	49.0(32.3-66.3)	28.0(14.8-52.5)	0.001
Sputum lymphocyte% *	1.0(0.5-2.2)	0.8(0.3-1.5)	0.098
Sputum IL-1 β gene expression	0.5(0.3-1.2)	1.1(0.3-7.6)	0.132
Sputum IL-1 β protein level, ng/mL	0.3(0.1-1.3)	0.3(0.1-2.0)	0.843
Sputum IL-1Ra, ng/mL	213.3(131.5-284.9)	174.2(100.1-269.4)	0.273
Serum CRP, mg/L *	2.7(1.2-7.0)	3.0(1.2-6.8)	0.676
Serum CRP $\geq 4.12\text{mg/L}$, n(%)	20(34.5)	35(46.1)	0.177
Serum IL-6, pg/mL *	1.9(1.2-3.0)	2.6(1.5-4.1)	0.013
Serum IL-6 $\geq 1.55\text{ pg/mL}$, n(%)	34(58.6)	56(73.7)	0.066
Number of elevated systemic inflammatory mediators (n) [†]	22 18 18	17 27 32	0.134

* Median (quartile 1-3)

[†] None of CRP and IL-6 elevated | either CRP or IL-6 elevated | both CRP and IL-6 elevated

e-Table 3. Univariate regression analysis of baseline characteristics with the frequent exacerbation phenotype (logistic regression) and exacerbation frequency (number, Poisson regression) in asthma

Frequent VS Infrequent exacerbators				Number of exacerbations			
Variables	OR	95% CI	P value	Variables	IRR	95% CI	P value
Age	1.02	0.97-1.06	0.469	Age	1.01	0.99-1.03	0.400
Sex	1.78	0.49-6.46	0.382	Sex	1.28	0.75-2.18	0.366
BMI	1.00	0.93-1.08	0.986	BMI	1.01	0.98-1.05	0.335
Smoking	0.35	0.10-1.26	0.107	Smoking	0.71	0.43-1.19	0.199
FEV ₁ %predicted	0.98	0.94-1.02	0.233	FEV ₁ %predicted	0.99	0.97-1.00	0.124
VAS symptom score	1.05	1.00-1.11	0.055	VAS symptom score	1.03	1.01-1.06	0.002
ICS use	0.14	0.26-0.85	0.013	ICS use	3.47	0.69-17.52	0.131
Long-acting anticholinergic use	4.00	1.11-14.35	0.033	Long-acting anticholinergic use	2.11	1.28-3.46	0.003
Number of exacerbations in prior year	2.17	1.34-3.52	0.002	Number of exacerbations in prior year	1.26	1.15-1.38	<0.001
CCI	1.01	0.71-1.45	0.945	CCI	1.02	0.88-1.19	0.763
The number of elevated systemic inflammatory markers *	2.60	1.20-5.66	0.016	The number of elevated systemic inflammatory markers *	1.79	1.31-2.45	<0.001
Serum IL-6, pg/mL	1.79	1.10-2.92	0.020	Serum IL-6, pg/mL	1.13	1.07-1.19	<0.001
Serum CRP, mg/L	1.12	1.01-1.25	0.038	Serum CRP, mg/L	1.05	1.02-1.09	0.003
Sputum total cell	1.00	0.95-1.04	0.933	Sputum total cell	1.00	0.98-1.02	0.910

count, $\times 10^6/\text{mL}$				count, $\times 10^6/\text{mL}$			
Sputum eosinophil%	1.04	0.98-1.10	0.234	Sputum eosinophil%	1.01	0.99-1.03	0.166
Sputum neutrophil%	1.01	0.98-1.04	0.640	Sputum neutrophil%	1.00	0.99-1.01	0.763
Sputum IL-1 β gene expression	1.29	0.76-2.10	0.372	Sputum IL-1 β gene expression	1.15	0.95-1.39	0.149
Sputum IL-1 β protein level, ng/mL	1.29	0.93-1.80	0.131	Sputum IL-1 β protein level, ng/mL	1.10	0.97-1.25	0.141

Definition of abbreviations: OR = odds ratio; IRR = incidence rate ratio; OCS = oral corticosteroid; ICS

= inhaled corticosteroids; CCI = Charlson comorbidity index.

* None of CRP and IL-6 elevated | either CRP or IL-6 elevated | both CRP and IL-6 elevated

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e-Table 4. Univariate regression analysis of baseline characteristics with the frequent exacerbation phenotype (logistic regression) and exacerbation frequency (number, Poisson regression) in COPD

Frequent VS Infrequent exacerbators				Number of exacerbations			
Variables	OR	95% CI	P value	Variables	IRR	95% CI	P value
Age	1.06	1.00-1.11	0.051	Age	1.02	1.00-1.04	0.021
Sex	1.26	0.52-3.07	0.611	Sex	1.14	0.81-1.60	0.469
BMI	1.02	0.96-1.08	0.485	BMI	1.02	1.00-1.04	0.124
Smoking	2.22	0.85-5.78	0.102	Smoking	1.40	0.96-2.03	0.079
FEV ₁ %predicted	0.96	0.94-0.99	0.005	FEV ₁ %predicted	0.98	0.97-0.99	<0.001
VAS symptom score	1.06	1.02-1.10	0.005	VAS symptom score	1.03	1.02-1.05	<0.001
ICS use	3.47	0.69-17.52	0.131	ICS use	2.62	1.23-5.61	0.013
Long-acting anticholinergic use	1.69	0.69-4.15	0.250	Long-acting anticholinergic use	1.39	0.98-1.96	0.065
Number of exacerbations in prior year	1.61	1.22-2.12	0.001	Number of exacerbations in prior year	1.15	1.10-1.20	<0.001
CCI	1.33	0.94-1.88	0.105	CCI	1.12	0.99-1.27	0.065
The number of elevated systemic inflammatory markers *	2.07	1.10-3.91	0.025	The number of elevated systemic inflammatory markers *	1.59	1.24-2.04	<0.001
Serum IL-6, pg/mL	1.14	0.97-1.34	0.109	Serum IL-6, pg/mL	1.01	1.00-1.02	0.009
Serum CRP, mg/L	1.04	0.98-1.11	0.190	Serum CRP, mg/L	1.02	1.01-1.03	<0.001
Sputum total cell count, ×10 ⁶ /mL	1.03	0.98-1.08	0.236	Sputum total cell count, ×10 ⁶ /mL	1.00	0.99-1.02	0.575
Sputum eosinophil%	1.00	0.95-1.04	0.838	Sputum eosinophil%	1.00	0.98-1.02	0.918

Sputum neutrophil%	1.00	0.98-1.02	0.791	Sputum neutrophil%	1.00	0.99-1.01	0.823
Sputum IL-1 β gene expression	1.26	1.08-1.48	0.004	Sputum IL-1 β gene expression	1.11	1.07-1.16	<0.001
Sputum IL-1 β protein level, ng/mL	1.24	1.03-1.50	0.024	Sputum IL-1 β protein level, ng/mL	1.05	1.00-1.11	0.063

Definition of abbreviations: OR = odds ratio; IRR = incidence rate ratio; OCS = oral corticosteroid; ICS

= inhaled corticosteroids; CCI = Charlson comorbidity index.

* None of CRP and IL-6 elevated | either CRP or IL-6 elevated | both CRP and IL-6 elevated

e-Table 5. Generalized structural equation modeling (SEM) for different variables of path analyses in COPD

Model 1: The number of exacerbation in prior year

Variable	Coefficient	95% CI	P value
Age	0.013	-0.052-0.079	0.691

Model 2: Sputum IL-1 β gene expression

Variable	Coefficient	95% CI	P value
Age	-0.001	-0.071-0.070	0.990
The number of exacerbations in prior year	-0.233	-0.454-(-0.011)	0.039

Model 3: Serum In(IL-6)

Variable	Coefficient	95% CI	P value
Age	0.028	0.011-0.045	0.001
The number of exacerbations in prior year	-0.020	-0.075-0.035	0.471
Sputum IL-1 β gene expression	-0.141	-0.206-(-0.076)	<0.001

Model 4: The number of exacerbations in the following year

Variable	Coefficient	95% CI	P value
Age	0.018	-0.012-0.049	0.241
The number of exacerbations in prior year	0.130	0.067-0.192	<0.001
Sputum IL-1 β gene expression	-0.040	-0.150-0.069	0.470
Serum In(IL-6)	0.430	0.037-0.822	0.032

Figure 1. Study flow chart.

Definition of abbreviations: OAD = Obstructive airway disease, ELISA = enzyme-linked immunosorbent assay, qPCR = quantitative real-time polymerase chain reaction

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Figure 2. Distribution of the number of exacerbations in asthma and COPD group
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Figure 3. Baseline levels of sputum IL-1 β gene and protein expression in asthma (A and B) and COPD (C and D) between the frequent and infrequent exacerbators. Lines in B) and D) represent median values
68x94mm (600 x 600 DPI)

Figure 4. Baseline levels of serum IL-6 and CRP between frequent VS infrequent exacerbators in asthma (A and B) and COPD (C and D). Lines represent median values
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Figure 5. A) Path diagram of Generalized Structural Equation Modelling for the predictors of future exacerbation frequency generated by STATA 13: Arrows represent paths between variables, and a path points from one variable to another represents the first variable affects the second. Numbers under variables represent overall coefficient of different models, and numbers near the circled ϵ are the variance of the models. Small number along with the arrow connecting two variables means variable specified coefficient. Red lines represent statistically significant paths. B) A simplified pathway diagram of number of exacerbations in prior year, airway inflammation measured by sputum IL-1 β gene expression, systemic inflammation measured by serum IL-6 and number of future exacerbations based on the modelling

* Variable that predicted by age

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**Airway IL-1 β and systemic inflammation as predictors of
future exacerbation risk in ~~older patients with~~ asthma and
COPD**

RUNNING TITLE: Inflammation predictors of asthma and COPD
exacerbation

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CONFLICT OF INTERESTS

Dr Juan-juan Fu and Dr Katherine J Baines have no competing interest to declare. A/Prof Vanessa M McDonald is supported by the Lung Foundation of Australia and Boehringer Ingelhiem's COPD research fellowship and has participated in educational symposia funded by GlaxoSmithKline, AstraZeneca, Menarini, Boehringer Ingelhiem and Novartis. She has participated in studies funded by GlaxoSmithKline and advisory boards for Novartis, AstraZeneca and Menarini. Professor Peter G Gibson holds an NHMRC Practitioner Fellowship. He has participated in educational symposia funded by AstraZeneca, Boehringer Ingelheim, GlaxoSmithKline and Novartis, and has participated in studies funded by Pharmaxis and GlaxoSmithKline.

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NOTATION OF PRIOR ABSTRACT PUBLICATION

A subset of the data in study was published as an abstract and present as an oral presentation in 2014 ATS conference at San Diego, US on 21st May
~~2014.oral presentation in 2014 ATS conference at San Diego, US on 21st May,~~
~~2014.~~

This article has supplemental material.

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Privileged Communication

ABBREVIATIONS LIST

ACQ	asthma control questionnaire
CCI	Charlson comorbidity index
COPD	chronic obstructive pulmonary disease
CRP	C-reactive protein
ER	emergency room
GOLD	global initiative for chronic obstructive lung disease
GP	general practice
ICS	inhaled corticosteroid
IL	interleukin
OAD	obstructive airway disease
OCS	oral corticosteroid
qPCR	quantitative real-time polymerase chain reaction
SD	standard deviation
VAS	visual analogue scale

ABSTRACT

Background: The innate inflammatory pathways involved in the frequent exacerbator phenotypes of asthma and COPD are not well understood. This study aimed to investigate airway innate immune activation and systemic inflammation as predictors of exacerbations in asthma and COPD.

Methods: In this prospective cohort study, baseline airway interleukin (IL)-1 β , ~~and~~ serum C-reactive protein and IL-6 were assessed ~~at baseline~~ in 152 participants with stable asthma (n=63) or COPD (n=89), and related to exacerbations over the following 12 months. Clinical characteristics and inflammatory biomarkers were compared between the frequent (≥ 2 exacerbations in the follow-up) and infrequent exacerbators. The frequent exacerbation phenotype and exacerbation frequency were analyzed with multivariable modeling. The ~~causal~~ relationships between airway inflammation, systemic inflammation and future exacerbations were examined using path analysis.

Results: Ninety-four participants experienced a total of 201 exacerbations, and 36.4% had ≥ 2 exacerbations. Serum IL-6 ($P<0.001$) and sputum gene expression ~~of IL1B along with~~ and protein levels of IL-1 β at baseline were higher in the frequent exacerbators with COPD. ~~IL-1 receptor antagonist levels were not different between the frequent and infrequent exacerbators (P=0.438).~~ Significant pathways initiated by previous exacerbations were identified to occur through activation of the IL-1 β -systemic inflammatory axis leading to future exacerbations in COPD. Systemic inflammation was also associated with increased exacerbation risk in asthma.

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6 *Conclusion:* Airway IL-1 β and systemic inflammation are associated with
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8 frequent exacerbations and may mediate a vicious cycle between previous
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10 and future exacerbations ~~in asthma and in~~ COPD. Treatment strategies aimed
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12 at attenuating these inflammatory pathways to reduce COPD exacerbations
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14 deserve further investigation.
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18 ~~Targeting these inflammatory pathways may reduce exacerbations in asthma~~
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20 ~~and COPD.~~
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24 This study was approved by the Hunter New England Local Health District
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26 Human Research Ethics Committee, Australia (Reference number:
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28 10/08/18/5.03).
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INTRODUCTION

Asthma and chronic obstructive pulmonary disease (COPD) are common diseases that cause exacerbations of deteriorating symptoms and lung function that can result in hospitalization, increased health care utilization and death.^{1,2} Exacerbations impose a substantial economic burden and result in a faster decline in lung function,^{3,4} and poorer quality of life.^{5,6} A subset of patients experience frequent exacerbations^{4,7,8} and require more effective management strategies. While there are some known clinical factors that have been identified to be associated with frequent exacerbations of asthma^{2,9} and COPD,¹⁰ the underlying mechanisms, including the role of inflammation and innate immunity activation in frequent exacerbations, are not well established. If inflammation were a determinant of exacerbations, this would permit both disease monitoring using biomarkers as well as novel approaches to treatment.

Inflammation associated with innate immune activation in COPD and asthma has airway and systemic components.^{11,12} Elevated circulating levels of C-reactive protein (CRP) and interleukin (IL)-6 are indicators of a systemic response to innate immune activation inflammation. In COPD, a subset of COPD patients also experience chronic low-low-grade systemic inflammation during stable disease. This is associated with a rapid decline in lung function,¹³ increased mortality,¹⁴ and a higher exacerbation rate.^{15,16} however mechanisms behind the observed association between systemic inflammation and exacerbations in COPD¹⁶ remain unclear. While systemic inflammation is increased in asthma,¹² the effect on future exacerbation risk is not known.

Moreover, the mechanisms behind the observed association between systemic inflammation and exacerbations in COPD¹⁶ remain unclear.

IL-1 β is a typical innate immune cytokine involved in the initiation and persistence of inflammation.^{17,18} IL-1 β secretion is increased in stable and exacerbating COPD^{19,20} and asthma.^{18,21} We previously reported that airway

IL-1 β was associated with systemic inflammation in asthma,^{12,18,21} and hypothesize that the airway innate immune activation and/or the resulting systemic inflammatory response may be important determinants of exacerbation risk in asthma and COPD. We therefore conducted a prospective cohort study of older people with asthma and COPD to investigate the innate immune pathway determinants of future exacerbation risk and focused on innate immune responses including airway IL-1 β and systemic inflammation (CRP, IL-6). Additionally, we conducted a statistical pathway analysis to explore the relationships between clinical risk factors, airway inflammation, systemic inflammation and future exacerbation risk.

METHODS

Study design and protocol

Participants (n=152) with previously doctor diagnosed asthma (n=63) or COPD (n=89) were recruited from research registers and enrolled in a prospective cohort study (details of study subjects see in the Online Supplements). Participants gave written informed consent and the study was approved by the Hunter New England Research Ethics Committee.

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Participants attended a baseline visit to assess demographics, smoking status, exacerbation history prior to the study entry, medical history, medication use, symptoms (Visual analogue scale (VAS)), comorbidity (Charlson Comorbidity Index CCI),²² and for the asthma group, the asthma control questionnaire (ACQ).²³ Pre and post bronchodilator spirometry, sputum induction²⁴ and venepuncture ~~with blood collection~~ were performed ~~(details in the Online Supplement). Additional details are provided in the Online Supplement.~~

After baseline clinical assessment, clinical interviews were conducted by telephone to assess medication use and exacerbation frequency and severity every 3 months for 12 months (Figure 1). Respiratory hospitalization, emergency room (ER) visits, unscheduled primary care doctor ~~(Dr)~~ visits, and medication use ~~including antibiotics and systemic corticosteroids~~ were recorded at each assessment. An exacerbation of COPD was defined as a COPD-related episode that led to a) hospitalization; or b) ER visit; or c) the need for oral corticosteroids (OCS) and/or antibiotics for at least 3 days.⁸ An exacerbation of asthma was defined using the severe asthma exacerbation criteria of the ATS/ERS Task Force,²⁵ or an asthma exacerbation with lower respiratory tract infection ~~(LRTI)~~ requiring antibiotics.²⁶ A frequent exacerbator was defined as a participant who had ≥2 exacerbations in the 12 months follow-up.^{8,27}

Details of biomarker measurements are provided in the online supplement. Sputum gene expression was measured using quantitative real-time polymerase chain reaction (qPCR) as previously described.¹⁸ Sputum IL-1β, IL-1 receptor antagonist (IL-1Ra) proteins, serum high sensitivity CRP and IL-

6 were measured using enzyme linked immunosorbent assay. Elevated CRP and IL-6 levels were defined as CRP ≥ 4.12 mg/L and IL-6 ≥ 1.55 pg/mL based on prior data.¹²

Statistical analysis

Data were analyzed using STATA 13 (Stata Corp, Texas, USA) and were reported as mean (SD) or median (~~interquartile range (25-75%)~~¹⁻³, IQR) depending on the distribution. Comparisons between two independent groups were performed using student's t-test or the two-sample Wilcoxon Rank Sum test ~~as appropriate~~. Fisher's exact test was used to test categorical data. Differences in exacerbation rates (number of events per participant) were analyzed by Poisson regression adjusted for follow-up time and corrected by robust variance estimation. Multivariable ~~logistic~~ Logistic and Poisson regression analyses were performed to identify predictors of frequent exacerbations and the number of exacerbations in asthma and COPD groups separately. Variables that were tested in univariable regression with $P < 0.1$ were selected for inclusion in the multivariable regression model followed by a stepwise approach always adjusting for: Age ~~age~~ and sex ~~were forced into the regression analyses~~. A $P < 0.05$ was ~~defined~~ considered ~~as statistically~~ significant.

Path analysis using generalized structural equation modelling (SEM) ~~in STATA 13~~ was performed to explore the ~~causal~~ relationships between exacerbation history, airway inflammation (sputum ~~IL-1 β~~ B gene expression), systemic inflammation (serum IL-6 level) and ~~future exacerbations (frequent exacerbation~~ frequency in the following year phenotype). Each variable was

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modeled and an overall model of future exacerbations was constructed. ΔC_t of ~~IL-1 β~~ ~~IL-1 β~~ gene expression assay (change in cycle threshold compared to β -actin) and $\ln(\text{IL-6})$ were used in this analysis due to the normality of the data.

RESULTS

Baseline characteristics of the participants

Of 152 participants who underwent the baseline assessments, 13 withdrew and 1 died during the follow-up period, leaving 138 participants that completed the 12-month follow-up. Exacerbation data were available prior to withdrawal for a further 2 participants, and data from 140 participants were analyzed (Figure 1).

~~The mean age of participants was 64.7 years, and those~~Participants with COPD were older (68.9 years) than those with asthma (60.5 years), and had greater tobacco smoke exposure and airflow limitation. Inhaled corticosteroid use was similar between the asthma and COPD~~groups~~, but there was greater comorbidity in the COPD group (e-Table 41). Systemic inflammatory markers tended to be similar between groups, but airway neutrophils were greater in COPD (e-Table 22).

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Exacerbations by asthma and COPD groups

There were 1.10 exacerbations/participant in the asthma group and 1.67 exacerbations/participant in the COPD group over the 12-month follow-up period ($P=0.056$) (Table 31). The distribution of total exacerbation number (Figure 2) and the exacerbation types (Table 31) were similar between the

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asthma and COPD ~~groups~~. Overall from this cohort, 94 participants (67.1%) experienced a total of 201 exacerbations during a median follow-up period of 53.4 (52.4-57.0) weeks. Forty-three (30.7%) participants experienced one exacerbation, and 51 (36.4%) of participants ~~had reported~~ ≥ 2 exacerbations.

Clinical characteristics of Frequent frequent versus infrequent exacerbators

The frequent versus infrequent exacerbators in the asthma and COPD groups were compared (Table 2). Prior year exacerbation frequency and symptom scores were higher in frequent exacerbators. Frequent exacerbators with COPD were older, and had greater lung function impairment and more comorbidities compared to the infrequent exacerbators with COPD. In asthma, the use of long-acting anticholinergics was greater in the frequent exacerbators than infrequent exacerbators. and were similar for most characteristics (e-Table 1). The differences between the frequent exacerbators with asthma and COPD were related to prior diagnosis, i.e. FEV₁, FEV₁/FVC and therefore the frequent exacerbators were combined for further analysis.

At baseline, the frequent exacerbators were older, had significantly greater lung function impairment and higher VAS symptom score, more comorbidity, more inhaled corticosteroid (ICS) and long acting anticholinergic use ($P < 0.05$). They also experienced more exacerbations in the year prior to study entry ($P < 0.001$). Sputum cell counts were not different between the frequent and infrequent exacerbators (Table 4).

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Airway IL-1 β , systemic inflammation and frequent exacerbations

Sputum cell counts were not different between the frequent and infrequent exacerbators in either asthma or COPD group (Table 3). Sputum IL-1 β /IL-1B gene expression was significantly up-regulated in frequent exacerbators with COPD ($P=0.018$) and a trend was observed in the frequent exacerbators with asthma (Figure 3C) ($P=0.073$). IL-1 β (Figure 3) or IL-1B gene and protein expression were neither different between asthma and COPD groups (gene $P=0.132$, protein $P=0.843$) nor different between the frequent exacerbators with asthma and those with COPD (gene $P=0.116$, protein $P=0.695$). When combined, sputum IL-1 β gene (Figure 3A) and protein (Figure 3B) expression was significantly higher at baseline in frequent exacerbators than infrequent exacerbators. IL-1Ra (Table 3) protein levels were not different between the frequent exacerbators and infrequent exacerbators in either the asthma and COPD groups (Figure 3C), however there was a trend towards a decreased IL-1Ra/IL-1 β ratio in the frequent exacerbators (Figure 3D). At baseline, Baseline levels of serum IL-6 were significantly higher in the frequent exacerbators with asthma (Figure 4B4A) and the frequent exacerbators with COPD (Figure 4D4C) compared to infrequent exacerbators with asthma or COPD in each group, respectively. Baseline CRP was also elevated in frequent asthma exacerbators (Figure 4B4A), but not in COPD (Figure 4D4C). Levels of CRP and IL-6 were not different between frequent exacerbators with asthma and COPD (e-Table 1). When the asthma and COPD groups were combined, baseline systemic inflammatory

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mediators (IL-6, CRP) were also elevated in the frequent exacerbators (Figure 4E and 4F).

Participants were categorized into three groups based on the number of elevated systemic inflammatory markers at baseline. An increasing number of elevated systemic markers was associated with the frequent exacerbators phenotype in both asthma and COPD (Table 3). Systemic inflammatory markers were significantly correlated with airway IL-1 β gene expression and protein levels (Table 4).

~~The exacerbation rate increased with the number of elevated inflammatory mediators, and the proportion of frequent exacerbators was significantly higher in participants who had both elevated CRP and IL-6 than those with normal levels of both markers (Figure 5, details see Online Supplement). Systemic inflammatory markers CRP and IL-6 were significantly correlated with airway IL-1 β gene expression and IL-1 β protein levels (Table 5).~~

Predictors of future ~~Factors associated with~~ exacerbations~~ss~~

Univariable regression analyses of baseline characteristics that predict exacerbations in asthma (e-Table 3) and COPD (e-Table 4) were performed ~~as in e-Table 2~~ (Online Supplement). ~~Variables such as asthma or COPD diagnosis were removed by stepwise selection.~~ After stepwise selection and adjustment for significant clinical parameters in the regression models, baseline sputum IL-1 β ~~IL-1 β~~ gene expression and ~~IL-1 β~~ protein level significantly predicted the frequent exacerbation phenotype and the number of exacerbations in COPD (Table 56). Sputum IL-1 β gene and protein

expression did not predict frequent exacerbation phenotype and exacerbation number in asthma.

Systemic inflammation, ~~represented by the level of IL-6 assessed, as the number of elevated systemic inflammatory mediators, was an independent predictor of exacerbation frequency in the following year in both asthma and COPD (Table 6).~~
~~also independently associated with the frequent exacerbation phenotype and with exacerbation frequency (Table 7).~~

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Path analysis of exacerbation predictors

In COPD, the number of exacerbations experienced in prior year, airway IL-1 β and serum IL-6, each independently predicted future exacerbation frequency (Table 5 and Table 6). e-Table 3-5 shows the modelling of each variable, and an overall model of ~~future future~~ exacerbations ~~was constructed~~ in which exacerbation history and serum IL-6 levels were significant baseline variables associated with future exacerbations. The path diagrams (Figure 65) indicate that ~~the number of a history of frequent~~ exacerbations ~~in the past year~~ directly and significantly predicted ~~future~~ exacerbation ~~frequency in the following year in COPDs. Additionally~~ ~~In addition to this~~, there was an indirect association between previous ~~frequent exacerbations~~ and future exacerbations, via an up-regulation of airway ~~IL-1 β IL1 β gene expression~~ and subsequent increase in the level of systemic inflammation measured by IL-6.

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DISCUSSION

This is the first study to record comprehensive types of exacerbations resulting in hospitalizations, ER visits and community exacerbations in both asthma and COPD, and to link these to airway and systemic inflammation.

This prospective cohort study identifies that airway IL-1 β and increased systemic inflammation are associated with frequent future exacerbations in ~~the COPD~~, and that systemic inflammation is associated with exacerbation risk in asthma for the first time ~~obstructive airways disease (OAD) of asthma and COPD~~. This is the first study to record comprehensive types of exacerbations resulting in hospitalizations, ER visits and community exacerbations in both asthma and COPD, and to link these to airway and systemic inflammation. Importantly, we identified a potential causal pathway that shows how airway innate immune activation through IL-1 β results in systemic inflammation and exacerbations in COPD. This pathway could contribute to a vicious cycle between previous and future exacerbations ~~in asthma and COPD~~, and may identify identifies potential molecular treatment targets that could reduce exacerbations in ~~asthma and~~ COPD.

~~While the importance of systemic inflammation is well established in COPD, our data shows a similar prevalence of systemic inflammation in older asthmatics compared with COPD, and identifies that systemic inflammation is associated with frequent exacerbations of asthma. To our knowledge this is the first report of the prognostic role of systemic inflammation in asthma, indicating that the presence of systemic inflammation is not limited to COPD, but a feature of OAD and predicts future exacerbations. The assessment of systemic inflammation is useful in identifying patients who are at high risk of~~

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~~exacerbation in the clinic based on the wide availability of blood samples and assays.~~

~~IL-1 β is a typical innate immune cytokine involved in the initiation and persistence of inflammation.^{17,18} IL-1 β secretion is increased in stable and exacerbating COPD,^{19,20} and also in asthma.^{18,21} The elevation of IL-1 β in the frequent exacerbators may implicate inflammasome activation²⁸ and bacterial colonization²⁰ which warrants evaluation in future studies. Additionally, a trend towards a decreased IL-1Ra/IL-1 β ratio in the frequent exacerbators may also indicate an imbalance of anti/pro-inflammatory mediators in those patients. Recombinant human IL-1Ra blocks the biological activity of IL-1²⁹ and could be a potential therapeutic strategy in COPD with frequent exacerbations.~~

~~There are is some controversy overies about the origin of systemic inflammation in COPD. It is hypothesized that there is a “spill-over” of local airway inflammation into the systemic circulation. Others propose that COPD is a systemic multi-organ disease with multiple sources of systemic inflammationthat affects many organs, and that the source of systemic inflammation in COPD is multifactorial resulting from smoking, tissue hypoxia and skeletal muscle dysfunction.³⁰ SEM is a collection of statistical techniques that allow a set of relations between one or more independent and dependent variables to be examined, and takes a hypothesis testing approach to the multivariate analysis of a structural theory that stipulates causal relations. Path analysis is a type of SEM in which hypotheses of causal relations among multiple variables are modeled.³¹ We used this approach of We used a novel path analysis to test the hypotheses relating toexplore the causal relationsships between airway and inflammation, systemic~~

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inflammation, and ~~importantly, their associations with~~ future exacerbations.

Our findings may further support the 'spill-over' theory as airway ~~inflammation measured by IL-1 β IL1B gene expression~~ significantly predicted systemic inflammation ~~in COPD~~. The path diagram indicates a vicious cycle of exacerbations in patients with COPD ~~and asthma; with frequent exacerbations aggravating airway inflammation and leading to increased systemic inflammation, linking previous and future exacerbations. a history of exacerbations predict future exacerbations directly, while frequent exacerbations may aggravate airway inflammation measured by IL1B gene expression, then lead to an increase in systemic inflammation and form an indirect loop between previous and future exacerbations.~~ Therefore, the measurement of airway IL-1 β and systemic ~~inflammation-IL-6~~ might provide additional benefit for the prediction of exacerbation beyond clinical assessments. Anti-inflammatory therapies targeting the airway IL-1 β -systemic inflammatory pathway, which break the vicious cycle of inflammation leading to exacerbations, ~~might be a show promise as a strategy for the prevention of exacerbations of OAD.~~ In addition, further studies ~~investigating the role of IL-1 β including its relationship to the inflammasome activation²⁸ and bacterial colonization²⁰ are warrants.~~

~~While the importance of systemic inflammation is well established in COPD, our data shows a similar prevalence of systemic inflammation in older asthmatics, and identifies that elevated systemic inflammation is associated with increased future exacerbations in asthma. A greater number of elevated systemic inflammatory markers is a known predictor of COPD exacerbation.¹⁶~~ Our data extend these findings from COPD to asthma. To our knowledge this

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is the first report of the prognostic role of systemic inflammation in asthma, indicating that the presence of systemic inflammation is not limited to COPD, rather than a feature of OAD and predicts future exacerbation risk.

The dissociation between sputum eosinophil counts and asthma exacerbation risk conflicts with some previous studies.^{32,33} The primary reason for this would be the small sample size of asthma group and only 16 asthmatics identified as frequent exacerbators. Additionally, asthmatics in our study were recruited from the community, had preserved lung function and well controlled asthma based on the ACQ score which may be due to high dose ICS therapy, resulting in less asthmatics with eosinophilic bronchitis compared with some other studies recruiting severe asthma and participants with increased eosinophils.^{33,34} This may indicate that sputum eosinophil count is not associated with exacerbations in well-controlled and non-eosinophilic asthma. The sample size is also a likely reason why we only observed a trend towards an increased IL-1 β gene and protein expression in asthma. There is an urgent need for larger studies in asthma to clarify the IL-1 β -systemic inflammatory pathway activation and its role in asthma.

~~A greater number of elevated systemic inflammatory markers is a known predictor of COPD exacerbation.¹⁶ Our data extend these findings from COPD to asthma. Additionally,~~ It is well known that a history of exacerbation is the single best clinical predictor of future exacerbations in COPD.⁸ Our study extends this finding from COPD to asthma. Interestingly, the severity of respiratory symptoms measured by VAS symptom score also predicted the exacerbation frequency in both asthma and COPD, which This finding

supports the concepts of the combined assessment which integrates both symptoms and risk assessments as recommended in the 2014 updated GOLD document,³⁵ and promotes the clinical use of combined assessment and individualized management in asthma and COPD.

Several limitations of the study should be noted. We only examined a ~~limited two~~ number of systemic inflammatory markers ~~and as such~~ other mediators may also be involved, ~~for example fibrinogen and leucocyte count~~.¹⁶ ~~However, our approach of analysis by examining the number of elevated markers revealed significant differences in the exacerbation rates.~~

Agusti et al defined persistent systemic inflammation as stability of elevated systemic inflammatory markers over one year,¹⁵ ~~but the long time interval required to define this phenotype may limit its clinical application.~~ Therefore future studies that examine more biomarkers and at different time points are desired ~~to further investigate the pattern of systemic inflammation with clinical outcomes of OAD.~~ Due to the limitations imposed by phone interviews we were not able to Our findings need to be confirmed in other cohorts with a larger sample size with an enrichment of asthma or COPD population alone and a range of disease severity. We did not identify bacteria colonization at baseline and the aetiology of exacerbations nor their duration and this the aetiology of the exacerbations captured, linking the up-regulation of the IL-1 β and systemic inflammation, which warrants further investigation. Although SEM allows for the testing of causal hypotheses, the statistical technique does not prove causal relationships without satisfying the necessary conditions for causal inference.³¹ While we proved the causal hypotheses using path analysis, we cannot make warranted causal claims based this

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dataset, therefore replication of the findings is needed. A weak to modest correlation between airway and systemic inflammation may suggest the complexity of the cross-talking and that other confounders can also affect each of the two dimensions. Therefore, the hypotheses established in this study need to be evaluated and may be further proved by interventional studies blocking specific components of the pathway.

CONCLUSIONS

In summary, we have identified that an increase in systemic inflammation predicted more frequent exacerbations in asthma, and demonstrated that the frequent exacerbator-exacerbations phenotype in asthma and COPD is associated with both increased airway IL-1 β and systemic inflammation, and an imbalance in the secretion of anti/pro-inflammatory mediators. While an exacerbation history predicted future exacerbations, an indirect association existed between previous and future exacerbation through the airway IL-1 β -systemic inflammatory pathway, contributing to a vicious cycle of exacerbations in asthma and COPD. These are important observations that identify a specific subset of patients with OAD-COPD who are at high risk of future exacerbations, and identified specific inflammatory pathway and molecules that related to future exacerbations. Treatment strategies aimed at attenuating airway IL-1 β and systemic inflammation to reduce COPD exacerbation deserve further investigation. Prevention or optimal treatment of exacerbations by targeting IL-1 β and systemic inflammation are potential strategies for the management of OAD.

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Author contributions: All authors had full access to all of the data in the study and can take responsibility for the integrity of the data and the accuracy of the data analysis. JJF participated in the study design and clinical data collection, carried out laboratory gene and protein assays, performed statistical analysis, and drafted the manuscript. VMM contributed to the data collection, participated in data analysis and interpretation, and critically revised the manuscript. KJB participated in the novel biomarkers identification, supervised the laboratory analysis, and contributed to the data explanation and reviewed the manuscript. PGG contributed to the study design and interpretation of the data, supervised the clinical recruitment, and reviewed the manuscript. All authors read and approved the final manuscript.

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REFERENCES

1. Connors AFJ, Dawson NV, Thomas C, et al. Outcomes following acute exacerbation of severe chronic obstructive lung disease. The SUPPORT investigators (Study to Understand Prognoses and Preferences for Outcomes and Risks of Treatments). *Am J Respir Crit Care Med*. 1996;154(4 Pt 1):959-967.
2. McDonald VM, Gibson PG. Exacerbations of severe asthma. *Clin Exp Allergy*. 2012;42(5):670-677.
3. Bai TR, Vonk JM, Postma DS, Boezen HM. Severe exacerbations predict excess lung function decline in asthma. *European Respiratory Journal*. 2007;30(3):452-456.
4. Donaldson GC, Seemungal TA, Bhowmik A, Wedzicha JA. Relationship between exacerbation frequency and lung function decline in chronic obstructive pulmonary disease. *Thorax*. 2002;57(10):847-852.

5. Kupczyk M, ten Brinke A, Sterk PJ, et al. Frequent exacerbators-a distinct phenotype of severe asthma. *Clin Exp Allergy*. 2014;44(2):212-221.
6. Spencer S, Calverley PM, Burge PS, Jones PW. Impact of preventing exacerbations on deterioration of health status in COPD. *Eur Respir J*. 2004;23(5):698-702.
7. Dougherty RH, Fahy JV. Acute exacerbations of asthma: epidemiology, biology and the exacerbation-prone phenotype. *Clin Exp Allergy*. 2009;39(2):193-202.
8. Hurst JR, Vestbo J, Anzueto A, et al. Susceptibility to exacerbation in chronic obstructive pulmonary disease. *N Engl J Med*. 2010;363(12):1128-1138.
9. ten Brinke A, Sterk PJ, Masclee AA, et al. Risk factors of frequent exacerbations in difficult-to-treat asthma. *Eur Respir J*. 2005;26(5):812-818.
10. Miravittles M, Calle M, Soler-Cataluna JJ. Clinical phenotypes of COPD: identification, definition and implications for guidelines. *Arch Bronconeumol*. 2012;48(3):86-98.
11. Simpson JL, Grissell TV, Douwes J, Scott RJ, Boyle MJ, Gibson PG. Innate immune activation in neutrophilic asthma and bronchiectasis. *Thorax*. 2007;62(3):211-218.
12. Wood LG, Baines KJ, Fu J, Scott HA, Gibson PG. The Neutrophilic Inflammatory Phenotype Is Associated With Systemic Inflammation in Asthma. *CHEST*. 2012;142(1):86-93.

1
2
3
4
5
6 13. Donaldson GC, Seemungal TA, Patel IS, et al. Airway and systemic
7 inflammation and decline in lung function in patients with COPD. *Chest*.
8 2005;128(4):1995-2004.
9
10
11 14. Dahl M, Vestbo J, Lange P, Bojesen SE, Tybjaerg-Hansen A,
12 Nordestgaard BG. C-reactive protein as a predictor of prognosis in
13 chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*.
14 2007;175(3):250-255.
15
16 15. Agusti A, Edwards LD, Rennard SI, et al. Persistent systemic
17 inflammation is associated with poor clinical outcomes in COPD: a
18 novel phenotype. *PLoS One*. 2012;7(5):e37483.
19
20 16. Thomsen M, Ingebrigtsen TS, Marott JL, et al. Inflammatory
21 biomarkers and exacerbations in chronic obstructive pulmonary
22 disease. *JAMA*. 2013;309(22):2353-2361.
23
24 17. Slack J, McMahan CJ, Waugh S, et al. Independent binding of
25 interleukin-1 alpha and interleukin-1 beta to type I and type II
26 interleukin-1 receptors. *J Biol Chem*. 1993;268(4):2513-2524.
27
28 18. Baines KJ, Simpson JL, Wood LG, Scott RJ, Gibson PG.
29 Transcriptional phenotypes of asthma defined by gene expression
30 profiling of induced sputum samples. *J Allergy Clin Immunol*.
31 2011;127(1):153-160, 160 e151-159.
32
33 19. Pauwels NS, Bracke KR, Dupont LL, et al. Role of IL-1alpha and the
34 Nlrp3/caspase-1/IL-1beta axis in cigarette smoke-induced pulmonary
35 inflammation and COPD. *Eur Respir J*. 2011;38(5):1019-1028.
36
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42
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20. Bafadhel M, McKenna S, Terry S, et al. Acute exacerbations of chronic obstructive pulmonary disease: identification of biologic clusters and their biomarkers. *Am J Respir Crit Care Med*. 2011;184(6):662-671.
21. Fu JJ, Baines KJ, Wood LG, Gibson PG. Systemic inflammation is associated with differential gene expression and airway neutrophilia in asthma. *OMICS*. 2013;17(4):187-199.
22. Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. *J Chronic Dis*. 1987;40(5):373-383.
23. Juniper EF, O'Byrne PM, Guyatt GH, Ferrie PJ, King DR. Development and validation of a questionnaire to measure asthma control. *Eur Respir J*. 1999;14(4):902-907.
24. Gibson PG, Wlodarczyk JW, Hensley MJ, et al. Epidemiological association of airway inflammation with asthma symptoms and airway hyperresponsiveness in childhood. *Am J Respir Crit Care Med*. 1998;158(1):36-41.
25. Reddel HK, Taylor DR, Bateman ED, et al. An official American Thoracic Society/European Respiratory Society statement: asthma control and exacerbations: standardizing endpoints for clinical asthma trials and clinical practice. *Am J Respir Crit Care Med*. 2009;180(1):59-99.
26. Brusselle GG, Vanderstichele C, Jordens P, et al. Azithromycin for prevention of exacerbations in severe asthma (AZISAST): a multicentre randomised double-blind placebo-controlled trial. *Thorax*. 2013;68(4):322-329.

27. Kupczyk M, ten Brinke A, Sterk PJ, et al. Frequent exacerbators--a distinct phenotype of severe asthma. *Clin Exp Allergy*. 2014;44(2):212-221.

28. Dolinay T, Kim YS, Howrylak J, et al. Inflammasome-regulated cytokines are critical mediators of acute lung injury. *Am J Respir Crit Care Med*. 2012;185(11):1225-1234.

29. Dinarello CA, Simon A, van der Meer JW. Treating inflammation by blocking interleukin-1 in a broad spectrum of diseases. *Nat Rev Drug Discov*. 2012;11(8):633-652.

30. Agusti A. Systemic effects of chronic obstructive pulmonary disease: what we know and what we don't know (but should). *Proc Am Thorac Soc*. 2007;4(7):522-525.

31. Lei P-W, Wu Q. Introduction to Structural Equation Modeling: Issues and Practical Considerations. *Educational Measurement: Issues and Practice*. 2007;26(3):33-43.

32. Green RH, Brightling CE, McKenna S, et al. Asthma exacerbations and sputum eosinophil counts: a randomised controlled trial. *Lancet*. 2002;360(9347):1715-1721.

33. Jayaram L, Pizzichini MM, Cook RJ, et al. Determining asthma treatment by monitoring sputum cell counts: effect on exacerbations. *Eur Respir J*. 2006;27(3):483-494.

34. Gibson PG, Fujimura M, Niimi A. Eosinophilic bronchitis: clinical manifestations and implications for treatment. *Thorax*. 2002;57(2):178-182.

35. Global Initiative for Chronic Obstructive Lung Disease (GOLD). Global strategy for the diagnosis, management and prevention of COPD.

Updated ~~2011~~2014. Available from: <http://www.goldcopd.com>.

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Table 1. Clinical characteristics of participants at baseline (mean (SD) unless stated)

Baseline variables	Asthma	COPD	P-value
Number, n (%)	59(42.1)	81(57.9)	
Age	60.5(13.6)	68.9(9.0)	<0.004
Sex, male female (n)	20 39	35 46	0.265
BMI	30.6(7.7)	28.8(7.7)	0.162
Smoking, never ex current smokers (n)	34 23 2	29 47 5	0.038
Smoking pack-years *	8(2-35)	28(13-45.5)	0.038
FEV ₁ %predicted	86.0(15.5)	57.7(18.4)	<0.004
FVC %predicted	88.8(16.4)	80.2(18.7)	0.006
FEV ₁ /FVC %	74.6(6.3)	54.3(11.8)	<0.004
AHR or BDR at visit, n (%)	24(40.7)	46(56.8)	0.068
VAS symptom score *	15.8(7.4-27.5)	19.3(11.6-29.0)	0.218
ACQ/6 *	0.50(0.17-0.83)	N/A	N/A*
GINA severity (n), step 1 2 3 4	3 11 22 23	N/A	N/A*
GOLD stage (n), stage 1 2 3 4	N/A	11 42 22 6	N/A*
ICS use, n (%)	50(84.8)	71(87.7)	0.628
ICS dose, BDP equivalent, mcg/day *	1000(500-2000)	1000(500-2000)	0.956
Long-acting anticholinergic use, n (%)	14(23.7)	45(55.6)	<0.004
Number of exacerbations in prior year *	0(0-1)	1(0-3)	0.004
Frequent exacerbators in prior year, n (%)	12(20.3)	34(42.0)	0.007
CCI *	3(2-4)	4(3-5)	0.002
Cardiovascular disease, n (%)	30(50.9)	52(64.2)	0.113
MGCoA reductase inhibitor (Statin) use, n (%)	18(30.5)	20(24.7)	0.445

Definition of abbreviations: ACQ = asthma control questionnaire; ICS = inhaled corticosteroids; BDP = beclomethasone dipropionate; CCI = Charlson comorbidity index.

* Median (IQR)

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Table 2. Systemic and airway inflammatory characteristics of participants at baseline (mean (SD) unless stated)

Baseline variables	Asthma	COPD	P value
Number of samples analyzed, blood/sputum (n)	58/42	76/63	N/A
Serum CRP, mg/L *	2.7(1.2-7.0)	3.0(1.2-6.8)	0.676
Serum CRP ≥ 4.12 mg/L, n (%)	20(34.5)	35(46.1)	0.177
Serum IL-6, pg/mL *	1.9(1.2-3.0)	2.6(1.5-4.1)	0.013
Serum IL-6 ≥ 1.55 pg/mL, n (%)	34(58.6)	56(73.7)	0.066
Number of elevated systemic inflammatory mediators (n) [†]	22/18/18	17/27/32	0.134
Sputum total cell count, $\times 10^6$ /mL *	3.7(2.4-6.3)	4.6(2.7-10.2)	0.249
Sputum eosinophil% *	1.3(0.3-3.0)	1.8(0.8-3.8)	0.214
Sputum eosinophil% $> 3\%$, n (%)	9(21.4)	18(28.6)	0.412
Sputum neutrophil% *	42.8(24.0-60.3)	61.0(36.5-76.0)	0.006
Sputum neutrophil% $> 61\%$, n (%)	10(23.8)	32(50.8)	0.006
Sputum macrophage% *	49.0(32.3-66.3)	28.0(14.8-52.5)	0.001
Sputum lymphocyte% *	1.0(0.5-2.2)	0.8(0.3-1.5)	0.099

* Median (IQR)

[†] None of CRP and IL-6 elevated | either CRP or IL-6 elevated | both CRP and IL-6 elevated

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Table 13. Exacerbations during the 12-month observation period by asthma and COPD groups

Rate (number per participant)	Asthma	COPD	IRR *	P value †	Number (%)	Asthma	COPD	P value
Hospitalization	0.10	0.25	2.44	0.130	≥1 hospitalization	8.6%	15.8%	0.217
ER visits	0.02	0.09	4.49	0.224	≥1 ER visits	1.7%	5.3%	0.389
Unscheduled Dr doctor visits	0.95	0.91	0.93	0.750	≥2 unscheduled Dr doctor visits	22.4%	19.7%	0.706
OCS courses	0.69	0.94	1.28	0.411	≥2 OCS courses	19.0%	21.1%	0.765
Antibiotic courses	1.14	1.51	1.28	0.224	≥2 Antibiotic courses	31.0%	38.2%	0.392
Number of exacerbations	1.10	1.67	1.45	0.056	≥2 Exacerbations	27.6%	42.1%	0.082

Definition of abbreviations: IRR = incidence rate ratio; OCS = oral corticosteroid.

* COPD versus asthma; † Analyzed by Poisson regression.

Table 2. Baseline characteristics of frequent VS infrequent exacerbators with asthma and COPD (mean(SD) unless stated)

	<u>Asthma</u>			<u>COPD</u>		
	<u>Frequent exacerbators</u>	<u>Infrequent exacerbators</u>	<u>P value</u>	<u>Frequent exacerbators</u>	<u>Infrequent exacerbators</u>	<u>P value</u>
<u>Number, n(%)</u>	<u>16(27.1)</u>	<u>43(72.9)</u>	<u>N/A</u>	<u>35(43.2)</u>	<u>46(56.8)</u>	<u>N/A</u>
<u>Age</u>	<u>62.6(11.4)</u>	<u>59.7(14.4)</u>	<u>0.475</u>	<u>71.3(9.0)</u>	<u>67.2(8.7)</u>	<u>0.046</u>
<u>Sex, male female (n)</u>	<u>4 12</u>	<u>16 27</u>	<u>0.539</u>	<u>14 21</u>	<u>21 25</u>	<u>0.611</u>
<u>Body Mass Index</u>	<u>30.6(6.5)</u>	<u>30.6(8.2)</u>	<u>0.987</u>	<u>29.4(9.4)</u>	<u>28.2(6.0)</u>	<u>0.488</u>
<u>Smoking, never ex current smokers (n)</u>	<u>12 4 0</u>	<u>22 19 2</u>	<u>0.241</u>	<u>9 25 1</u>	<u>20 22 4</u>	<u>0.101</u>
<u>Smoking pack-years *</u>	<u>21.5(4.1-42.0)</u>	<u>7.0(2.0-32.0)</u>	<u>0.711</u>	<u>28.2(15.3-44.0)</u>	<u>26.5(10.8-47.0)</u>	<u>0.798</u>
<u>FEV₁ %predicted</u>	<u>82.1(15.0)</u>	<u>87.6(15.6)</u>	<u>0.234</u>	<u>50.9(20.6)</u>	<u>62.9(14.7)</u>	<u>0.003</u>
<u>FVC %predicted</u>	<u>83.9(15.5)</u>	<u>90.8(16.5)</u>	<u>0.157</u>	<u>75.9(21.6)</u>	<u>83.4(15.9)</u>	<u>0.081</u>
<u>FEV₁/FVC %</u>	<u>74.1(5.0)</u>	<u>74.8(6.7)</u>	<u>0.729</u>	<u>50.8(14.8)</u>	<u>56.9(8.2)</u>	<u>0.020</u>
<u>AHR or BDR at visit, n(%)</u>	<u>8(50.0)</u>	<u>16(37.2)</u>	<u>0.374</u>	<u>18(51.4)</u>	<u>28(60.9)</u>	<u>0.395</u>
<u>VAS symptom score *</u>	<u>26.4(10.2-32.8)</u>	<u>13.4(7.2-26.1)</u>	<u>0.068</u>	<u>24.1(16.8-34.4)</u>	<u>16.2(7.2-25.7)</u>	<u>0.004</u>
<u>ICS use, n(%)</u>	<u>16(100)</u>	<u>34(79.1)</u>	<u>0.097</u>	<u>33(94.3)</u>	<u>38(82.6)</u>	<u>0.114</u>
<u>ICS dose, BDP equivalent, mcg/day *</u>	<u>2000(1000-2000)</u>	<u>1000(500-2000)</u>	<u>0.074</u>	<u>2000(1000-2000)</u>	<u>1000(500-2000)</u>	<u>0.034</u>

<u>Long-acting anticholinergic use, n(%)</u>	<u>7(43.8)</u>	<u>7(16.3)</u>	<u>0.027</u>	<u>22(62.9)</u>	<u>23(50.0)</u>	<u>0.249</u>
<u>Number of exacerbations in the year prior to baseline visit *</u>	<u>2(0.5-4)</u>	<u>0(0-0)</u>	<u><0.001</u>	<u>3(1-4)</u>	<u>0.5(0-1)</u>	<u><0.001</u>
<u>≥2 exacerbations in the prior year, n(%)</u>	<u>9(56.3)</u>	<u>3(7.0)</u>	<u><0.001</u>	<u>23(65.7)</u>	<u>11(23.9)</u>	<u><0.001</u>
<u>CCI *</u>	<u>3(2-4)</u>	<u>3(2-4)</u>	<u>0.675</u>	<u>4(3-5)</u>	<u>4(3-4)</u>	<u>0.039</u>
<u>Cardiovascular disease, n(%)</u>	<u>7(43.8)</u>	<u>23(53.5)</u>	<u>0.506</u>	<u>24(68.6)</u>	<u>28(60.9)</u>	<u>0.474</u>
<u>HMGCoA reductase inhibitor (Statin) use, n(%)</u>	<u>6(37.5)</u>	<u>12(27.9)</u>	<u>0.477</u>	<u>10(28.6)</u>	<u>10(21.7)</u>	<u>0.480</u>

Definition of abbreviations: ACQ = asthma control questionnaire; AHR = airway Hyper-responsiveness; BDR = bronchodilator response; ICS = inhaled corticosteroids; BDP = beclometasone dipropionate; CCI = Charlson comorbidity index.

* Median(quantile 1-3)

Table 3. Airway and systemic inflammation of frequent VS infrequent exacerbators with asthma and COPD (median (quartile 1-3))

Table 4. Comparisons of baseline characteristics between frequent exacerbators VS infrequent exacerbators (mean (SD) unless stated)

	Overall	Frequent exacerbators	Infrequent exacerbators	P value *
Number, n (%)	140	51(36.4)	89(63.6)	
Age	65.4(11.9)	68.5(10.5)	63.6(12.3)	0.018
Sex, male female (n)	55 85	18 33	37 52	0.464
BMI	29.5(7.7)	29.8(8.6)	29.4(7.2)	0.764
Smoking, never ex current smokers (n)	63 70 7	21 26 4	42 44 3	0.451
Smoking pack-years [‡]	26.0(3.0-41)	28.2(14.0-44.0)	16.0(2.9-39.0)	0.280
FEV ₁ %predicted	69.4(22.2)	60.7(23.9)	74.6(19.5)	<0.001
FVC %predicted	83.9(18.2)	78.5(20.0)	86.9(16.5)	0.010
FEV ₁ /FVC %	62.7(14.1)	58.1(16.6)	65.3(11.7)	0.003
AHR or BDR at visit, n (%)	70(50.0)	26(51.0)	44(49.4)	0.861
VAS symptom score [‡]	18.8(9.9-28.0)	25.0(14.8-32.9)	15.3(7.2-26.1)	<0.001
ICS use, n (%)	121(86.4)	49(96.1)	72(80.9)	0.012
ICS dose, BDP equivalent, mcg/day	1000(500-2000)	2000(1000-2000)	1000(500-2000)	0.006

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Long-acting anticholinergic use, n (%)	59(42.1)	29(56.9)	30(33.7)	0.008
Number of exacerbations in the year prior to baseline visit†	1(0-2.5)	3(1-4)	0(0-1)	<0.001
≥2 exacerbations in the prior year, n (%)	46(32.9)	32(62.8)	14(15.7)	<0.001
GGL†	3(3-4)	4(3-5)	3(2.5-4)	0.035
Cardiovascular disease, n (%)	82(58.6)	31(60.8)	51(57.3)	0.687
HMGCoA reductase inhibitor (Statin) use, n (%)	38(27.1)	16(31.4)	22(24.7)	0.394
Diagnosis group, asthma COPD (n)	59 81	16 35	43 46	0.051
Number of samples analyzed, blood sputum (n)	134 105	48 35	86 70	N/A
Serum CRP, mg/L [†]	2.8(1.2-7.0)	4.6(1.4-8.7)	2.6(1.1-5.4)	0.064
Serum CRP ≥4.12 mg/L [†] , n (%)	61(43.6)	29(56.9)	32(36.0)	0.016
Serum IL-6, pg/mL [†]	2.1(1.4-3.5)	3.1(1.8-4.5)	1.7(1.1-3.0)	<0.001

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Serum IL-6 ≥1.55 pg/mL, n (%)	96(68.6)	45(88.2)	51(57.3)	<0.001
Number of elevated systemic inflammatory mediators (n) ^{†‡}	39 45 50	6 16 26	33 29 24	0.002
Had both CRP and IL-6 elevated, n (%)	50(35.7)	26(51.0)	24(27.0)	0.004
Sputum total cell count, ×10 ⁶ /mL [‡]	4.1(2.6-8.9)	4.0(2.6-11.7)	4.2(2.6-6.9)	0.667
Sputum neutrophil% [‡]	51.3(31.0-71.8)	49.3(35.0-76.0)	51.8(26.8-70.0)	0.469
Sputum eosinophil% [‡]	1.5(0.5-3.0)	1.8(1.0-3.8)	1.3(0.5-3.0)	0.149
Sputum macrophage% [‡]	37.8(20.7-57.5)	37.0(17.8-54.5)	38.5(20.7-61.0)	0.341
Sputum lymphocyte% [‡]	0.8(0.3-1.8)	0.8(0.3-1.5)	0.9(0.3-2.0)	0.570

	Asthma			COPD		
	Frequent exacerbators	Infrequent exacerbators	P value	Frequent exacerbators	Infrequent exacerbators	P value
Sputum total cell count, ×10 ⁶ /mL	3.5(2.6-7.0)	4.0(2.3-6.2)	0.844	6.6(2.3-12.0)	4.3(2.9-8.6)	0.510
Sputum neutrophil%	38.8(30.5-69.0)	44.8(23.5-60.3)	0.786	60.8(39.4-76.6)	61.0(30.8-73.9)	0.766
Sputum eosinophil%	3.0(1.0-15.5)	1.0(0.3-2.5)	0.065	1.6(0.9-2.6)	1.8(0.5-5.0)	0.870
Sputum macrophage%	47.8(24.3-58.5)	49.3(32.3-72.8)	0.310	29.8(17.0-51.5)	27.5(13.8-52.5)	0.989

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<u>Sputum lymphocyte%</u>	<u>1.0(0.5-1.5)</u>	<u>1.0(0.5-2.3)</u>	<u>0.527</u>	<u>0.6(0.3-1.8)</u>	<u>0.8(0.3-1.5)</u>	<u>0.955</u>
<u>Sputum IL-1β gene expression</u>	<u>0.8(0.5-2.3)</u>	<u>0.4(0.3-1.8)</u>	<u>0.073</u>	<u>6.2(0.7-10.5)</u>	<u>0.6(0.2-2.1)</u>	<u>0.018</u>
<u>Sputum IL-1β protein level, ng/mL</u>	<u>0.6(0.2-1.9)</u>	<u>0.3(0.1-0.6)</u>	<u>0.141</u>	<u>0.8(0.1-3.7)</u>	<u>0.2(0.1-0.9)</u>	<u>0.065</u>
<u>Sputum IL-1Ra, ng/mL</u>	<u>150.9(129.1-294.2)</u>	<u>214.5(134.6-275.6)</u>	<u>0.674</u>	<u>141.8(92.4-323.5)</u>	<u>199.9(111.7-260.2)</u>	<u>0.553</u>
<u>Serum CRP, mg/L</u>	<u>6.3(1.7-9.6)</u>	<u>2.5(1.0)</u>	<u>0.040</u>	<u>4.4(1.4-7.6)</u>	<u>2.7(1.1-6.2)</u>	<u>0.535</u>
<u>Serum CRP $\geq$4.12 mg/L, n(%)</u>	<u>9(56.3)</u>	<u>11(26.2)</u>	<u>0.031</u>	<u>17(53.1)</u>	<u>18(40.9)</u>	<u>0.291</u>
<u>Serum IL-6, pg/mL</u>	<u>2.7(1.8-4.3)</u>	<u>1.5(1.1-2.2)</u>	<u>0.008</u>	<u>3.2(1.9-4.5)</u>	<u>1.9(1.2-3.4)</u>	<u>0.004</u>
<u>Serum IL-6 $\geq$1.55 pg/mL, n(%)</u>	<u>13(81.3)</u>	<u>21(50.0)</u>	<u>0.039</u>	<u>29(90.6)</u>	<u>27(61.4)</u>	<u>0.007</u>
<u>Number of elevated systemic inflammatory mediators (n) *</u>	<u>3 4 9</u>	<u>19 14 9</u>	<u>0.031</u>	<u>3 12 17</u>	<u>14 15 15</u>	<u>0.050</u>

* None of CRP and IL-6 elevated | either CRP or IL-6 elevated | both CRP and IL-6 elevated

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Definition of abbreviations: ACQ = asthma control questionnaire; ICS = inhaled corticosteroids; BDP = beclometasone dipropionate; CCI = Charlson comorbidity index.

*Frequent exacerbators versus infrequent exacerbators

†Median (IQR)

*None of CRP and IL-6 elevated | either CRP or IL-6 elevated | both CRP and IL-6 elevated

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Table 54. Spearman rank correlations of airway IL-1 β with systemic inflammation

Systemic inflammation	Serum CRP	Serum IL-6	Number of elevated markers *
Airway inflammation			
IL-1 β gene expression	$r=0.291$ $P=0.010$	$r=0.394$ $P<0.001$	$r=0.326$ $P=0.004$
IL-1 β protein level	$r=0.228$ $P=0.030$	$r=0.276$ $P=0.008$	$r=0.327$ $P=0.002$
IL-1Ra protein level	$r=0.023$ $P=0.829$	$r=-0.124$ $P=0.239$	$r=-0.187$ $P=0.073$

* Three groups: with normal CRP and IL-6 levels; with either CRP or IL-6 elevated; and with both CRP and IL-6 elevated

Table 5. Sputum IL-1 β gene and protein expression predicted the frequent exacerbation phenotype (Logistic regression) and exacerbation frequency (number, Poisson regression) in COPD

		Frequent VS Infrequent exacerbators			Number of exacerbations			
Baseline variables		OR	95% CI	P value	Baseline variables	IRR	95% CI	P value
Age		1.07	0.98-1.17	0.136	Age	1.02	0.99-1.05	0.123
Sex		1.36	0.29-6.50	0.696	Sex	1.06	0.66-1.71	0.794
Sputum IL-1 β gene expression		1.26	1.06-1.51	0.011	The number of exacerbations in prior year	1.09	1.02-1.16	0.015
VAS symptom score		1.07	1.00-1.13	0.042	Sputum IL-1 β gene expression	1.06	1.02-1.11	0.001
					VAS symptom score	1.04	1.02-1.06	<0.001

		Frequent VS Infrequent exacerbators			Number of exacerbations			
Baseline variables		OR	95% CI	P value	Baseline variables	IRR	95% CI	P value
Age		1.09	1.00-1.20	0.045	Age	1.03	1.01-1.06	0.017
Sex		1.89	0.44-8.13	0.395	Sex	1.06	0.67-1.65	0.813
Sputum IL-1 β protein level, ng/mL		1.32	1.07-1.62	0.008	The number of exacerbations in prior year	1.13	1.06-1.19	<0.001
VAS symptom score		1.07	1.01-1.12	0.019	Sputum IL-1 β protein level, ng/mL	1.06	1.01-1.13	0.040
					VAS symptom score	1.03	1.02-1.05	<0.001

Definition of abbreviations: OR = odds ratio; CI = confidence interval; IRR = incidence rate ratio

Table 6. Systemic inflammation predicted exacerbation frequency (number, Poisson regression) in asthma and COPD

<u>Asthma</u>					<u>COPD</u>			
<u>Number of exacerbations</u>					<u>Number of exacerbations</u>			
<u>Baseline variables</u>	<u>IRR</u>	<u>95% CI</u>	<u>P value</u>		<u>Baseline variables</u>	<u>IRR</u>	<u>95% CI</u>	<u>P value</u>
<u>Age</u>	<u>1.01</u>	<u>0.99-1.03</u>	<u>0.344</u>		<u>Age</u>	<u>1.03</u>	<u>1.01-1.06</u>	<u>0.006</u>
<u>Sex</u>	<u>1.22</u>	<u>0.68-2.19</u>	<u>0.514</u>		<u>Sex</u>	<u>1.16</u>	<u>0.77-1.74</u>	<u>0.481</u>
<u>The number of exacerbations in prior year</u>	<u>1.17</u>	<u>1.03-1.32</u>	<u>0.014</u>		<u>The number of exacerbations in prior year</u>	<u>1.12</u>	<u>1.06-1.19</u>	<u><0.001</u>
<u>Serum IL-6, pg/mL</u>	<u>1.10</u>	<u>1.02-1.17</u>	<u>0.007</u>		<u>Serum IL-6, pg/mL</u>	<u>1.01</u>	<u>1.00-1.02</u>	<u>0.044</u>
<u>VAS symptom score</u>	<u>1.02</u>	<u>1.00-1.05</u>	<u>0.071</u>		<u>VAS symptom score</u>	<u>1.02</u>	<u>1.01-1.04</u>	<u>0.004</u>
					<u>FEV₁%predicted</u>	<u>0.99</u>	<u>0.98-1.00</u>	<u>0.066</u>

Definition of abbreviations: OR = odds ratio; CI = confidence interval; IRR = incidence rate ratio

Table 6. Sputum *IL1B* gene and protein expression predicted the frequent exacerbation phenotype (Logistic regression) and exacerbation frequency (number, Poisson regression)

		Frequent VS Infrequent exacerbators				Number of exacerbations		
Baseline variables		OR	95% CI	P-value	Baseline variables	IRR	95% CI	P-value
Age		1.07	1.00-1.14	0.044	Age	1.01	0.99-1.03	0.171
Sex		1.46	0.45-4.71	0.529	Sex	1.07	0.69-1.66	0.754
Frequent exacerbations in prior year (yes VS no)		4.53	1.47-13.99	0.009	Frequent exacerbations in prior year (yes VS no)	1.91	1.17-3.11	0.009
Sputum <i>IL1B</i> gene expression		1.22	1.04-1.43	0.016	Sputum <i>IL1B</i> gene expression	1.08	1.03-1.13	0.003
					VAS symptom score	1.03	1.01-1.05	0.001

		Frequent VS Infrequent exacerbators				Number of exacerbations		
Baseline variables		OR	95% CI	P-value	Baseline variables	IRR	95% CI	P-value
Age		1.04	0.99-1.10	0.102	Age	1.02	1.00-1.03	0.065
Sex		1.06	0.40-2.82	0.901	Sex	1.01	0.65-1.58	0.932
Frequent exacerbations in prior year (yes VS no)		5.03	1.89-13.36	0.001	Frequent exacerbations in prior year (yes VS no)	2.08	1.36-3.18	0.001
Sputum IL-1β protein level, ng/mL		1.20	1.02-1.41	0.024	Sputum IL-1β protein level, ng/mL	1.06	1.01-1.11	0.012
					VAS symptom score	1.03	1.01-1.04	0.001

Definition of abbreviations: OR = odds ratio; CI = confidence interval; IRR = incidence rate ratio

Table 7. Systemic inflammation predicted the frequent exacerbation phenotype (Logistic regression) and exacerbation frequency (number, Poisson regression)

		Frequent VS Infrequent exacerbators				Number of exacerbations		
Baseline variables		OR	95% CI	P-value	Baseline variables	IRR	95% CI	P-value
Age		1.03	0.99-1.07	0.162	Age	1.01	1.00-1.03	0.062
Sex		1.34	0.55-3.31	0.520	Sex	1.16	0.84-1.61	0.362
Frequent exacerbations in prior year (yes VS no)		5.63	2.35-13.47	<0.001	Frequent exacerbations in prior year (yes VS no)	2.09	1.51-2.89	<0.001
Number of elevated systemic inflammatory markers *		2.05	1.20-3.53	0.009	Number of elevated systemic inflammatory markers *	1.39	1.12-1.71	0.002
					VAS symptom score	1.02	1.01-1.03	0.003

Definition of abbreviations: OR = odds ratio; CI = confidence interval; IRR = incidence rate ratio;

* Three groups: with normal CRP and IL-6 levels; with either CRP or IL-6 elevated; and with both CRP and IL-6 elevated.

FIGURE LEGEND

Figure 1. Study flow chart.

Definition of abbreviations: OAD = Obstructive airway disease, ELISA = enzyme-linked immunosorbent assay, qPCR = quantitative real-time polymerase chain reaction

Figure 2. Distribution of the number of exacerbations in asthma and COPD group

Figure 3. ~~Baseline Levels levels~~ of sputum ~~A)IL-1 β -IL1B~~ gene ~~and protein~~ expression ~~in asthma (A and B) and COPD (C and D), between the B) IL-1 β , C) IL-1Ra and D) In (IL-1Ra/IL-1 β) ratio proteins at baseline associated with frequent exacerbations and infrequent exacerbators in the following year.~~ Lines ~~in B) and D)~~ represent median ~~values in B) and C), and mean $\pm$ 95% confidence interval in D)~~

Figure 4. ~~Baseline Levels levels~~ of serum ~~IL-6 and CRP and IL-6 at baseline~~ between frequent VS infrequent exacerbators in asthma (A and B), ~~and~~ COPD (C and D). ~~Lines represent median values and OAD (complete cohort) (E and F)~~

~~Figure 5. Exacerbations were associated with the number of elevated systemic inflammatory markers at baseline. White bar represents participants who had normal levels of both CRP and IL6, grey bar represents participants~~

that had either CRP or IL-6 elevated, and the black bar represents participants that had elevated levels of both CRP and IL-6

Figure 65. A) Path diagram of Generalized Structural Equation Modelling for the predictors of frequent future exacerbation frequency generated by STATA 13: Arrows represent paths between variables, and a path points from one variable to another represents the first variable affects the second. Numbers under variables represent overall coefficient of different models, and numbers near the circled ϵ are the variance of the models. Small number along with the arrow connecting two variables means variable specified coefficient. Red lines represent statistically significant paths. B) A simplified pathway diagram of number of previous exacerbations in prior year, airway inflammation measured by sputum IL-1 β /IL-4B gene expression, systemic inflammation measured by serum IL-6 and number of future exacerbations based on the modelling

* Variable that predicted by age

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Airway IL-1 β and systemic inflammation as predictors of future
exacerbation risk in asthma and COPD

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SUPPLEMENTAL MATERIAL

METHODS

Subject

Asthma was confirmed by current (past 12 months) episodic respiratory symptoms, doctor's diagnosis and prior airway hyperresponsiveness and/or bronchodilator response.¹ COPD was confirmed according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria.² Participants gave written informed consent. The Hunter New England Local Health District Human Research Ethics Committee approved this study. Exclusion criteria included respiratory disorders other than asthma and COPD, or current haematologic or solid organ malignancy. An exacerbation in the past month was cause to delay visits until recovery (4 weeks of stability).

Baseline assessment

Questionnaires

A respiratory symptom questionnaire assessing subjective symptom severity was administered, in which each symptom score including nasal symptoms, breathlessness, wheeze, cough and sputum production were recorded on a 10cm visual analogue scale (VAS), and a total cumulative score was calculated by adding up each individual score, with a range from 0 to 50cm. The Charlson Comorbidity Index (CCI) was calculated using information collected in the medical history.³ GINA severity for asthmatics was assessed according to the classification criteria for those who are on treatment,⁴ and GOLD stage for patients with COPD was classified.²

Pulmonary function tests

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Participants withheld bronchodilators for their duration of action before testing. Three reproducible measurements of FEV₁ and force vital capacity (FVC) were obtained (KoKo PD Instrumentation Louisville CO USA) before and after inhalation of 400mcg salbutamol via a metered dose inhaler with valved holding chamber (Volumatic, Allen and Hanbury's, Melbourne Victoria, Australia). Predicted values of lung function were calculated according to Hankinson et al.⁵

Sputum induction and analysis

Sputum induction with hypertonic saline (4.5%) was performed as previously described.⁶ Airway inflammation was assessed using induced sputum. Lower respiratory sputum portions were selected from saliva, dispersed using dithiothreitol (DTT), cytopspins were prepared and stained, and differential cell counts obtained.⁶ For gene expression assays, 100µl of selected sputum was immediately placed into Buffer RLT (Qiagen, Hilden, Germany) and stored at -80°C until RNA extraction. Eosinophilic bronchitis is defined as sputum eosinophil percentage >3%,^{7,8} and neutrophilic bronchitis is defined as sputum neutrophil percentage >61%.⁹

Measurement of inflammatory biomarkers

Sputum IL-1β genes and proteins

RNA was prepared and reverse-transcribed to cDNA as previously described.¹⁰ Primers and probes for target genes were purchased in kit form (Applied Biosystems). Gene expression of ~~IL-1β~~^{IL-1B} was analysed using Taqman quantitative real-time polymerase chain reaction (qPCR) on a 7500 Fast Real Time PCR system (Applied Biosystems) according to standard Taqman methods and samples were tested in duplicate as previously described,¹⁰ and the expression value of each gene

was normalized to housekeeping gene (β -actin) and the mean of all samples to calculate the relative amount of RNA present in each sample according to the $2^{-\Delta\Delta Ct}$ method. The concentrations of IL-1 β and IL-1Ra proteins (R&D Systems, Minneapolis, MN, USA) in sputum supernatant were determined in duplicate by ELISA according to manufacturers' instructions.

Systemic inflammatory markers

Peripheral venous blood was collected into Vacutainer® tubes (BD Worldwide, North Ryde, NSW, Australia), and serum was obtained by centrifugation at 3000rpm, 4°C for 10 minutes. High sensitivity CRP (MP Biomedicals, Australasia, Seven Hills, NSW, Australia) and IL-6 (R&D Systems, Minneapolis, MN, USA) were determined in duplicate using enzyme-linked immunosorbent assay (ELISA) according to the manufacturers' instructions.

RESULTS

Systemic inflammation and exacerbations

There were 50 (37.3%) participants that had both high CRP and IL-6, 45 (33.6%) had either high CRP or high IL-6, and 39 (29.1%) had normal levels of both biomarkers. The proportion of frequent exacerbators were significantly different among the three groups ($P=0.002$) such that significantly more frequent exacerbators were found in those who had both CRP and IL-6 elevated (52.0%) than those who had normal levels of both biomarkers (15.4%) (Figure 5A). Moreover, the exacerbation rate was significantly higher in participants who had either CRP or IL-6 elevated (1.36 exacerbations; IRR 1.90, 95% confidence interval (CI) 1.18-3.08) and in participants

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~~that had both CRP and IL-6 elevated (2.02 exacerbations; IRR 2.97, 95%CI 1.85-4.77) than those with normal levels of both CRP and IL-6 (0.69 exacerbations) (Figure 5B).~~

REFERENCES

1. Global Initiative for Asthma (GINA). Global strategy for asthma management and prevention. Updated 2012. Available at: <http://www.ginasthma.com>.
2. Global Initiative for Chronic Obstructive Lung Disease (GOLD). Global strategy for the diagnosis, management and prevention of COPD. Updated ~~2011~~2014. Available from: <http://www.goldcopd.com>.
3. Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. *J Chronic Dis*. 1987;40(5):373-383.
4. Global Initiative for Asthma (GINA). Global strategy for asthma management and prevention. Updated 2002. Available at: <http://www.ginasthma.com>.
5. Hankinson JL, Odencrantz JR, Fedan KB. Spirometric reference values from a sample of the general U.S. population. *Am J Respir Crit Care Med*. 1999;159(1):179-187.
6. Gibson PG, Wlodarczyk JW, Hensley MJ, et al. Epidemiological association of airway inflammation with asthma symptoms and airway hyperresponsiveness in childhood. *Am J Respir Crit Care Med*. 1998;158(1):36-41.

7. Green RH, Brightling CE, McKenna S, et al. Asthma exacerbations and sputum eosinophil counts: a randomised controlled trial. *Lancet*. 2002;360(9347):1715-1721.
8. Siva R, Green RH, Brightling CE, et al. Eosinophilic airway inflammation and exacerbations of COPD: a randomised controlled trial. *European Respiratory Journal*. 2007;29(5):906-913.
9. Simpson JL, Scott R, Boyle MJ, Gibson PG. Inflammatory subtypes in asthma: assessment and identification using induced sputum. *Respirology*. 2006;11(1):54-61.
10. Baines KJ, Simpson JL, Wood LG, Scott RJ, Gibson PG. Transcriptional phenotypes of asthma defined by gene expression profiling of induced sputum samples. *J Allergy Clin Immunol*. 2011;127(1):153-160, 160 e151-159.

e-Table 1. Baseline characteristics of frequent asthma exacerbators VS frequent COPD exacerbators (mean (SD) unless stated)

	Frequent asthma exacerbators	Frequent COPD exacerbators	P-value*
Number, n (%)	46(27.1)	35(43.2)	0.054
Age	62.6(11.4)	71.3(9.0)	0.005
Sex, male female (n)	4 12	14 21	0.298
BMI	30.6(6.5)	29.4(9.4)	0.664
Smoking, never ex current smokers (n)	12 4 0	9 22 4	0.004
Smoking pack-years [†]	21.5(4.1-42.0)	28.2(15.3-44.0)	0.542
FEV ₁ %predicted	82.1(15.0)	50.9(20.6)	<0.001
FVC %predicted	83.9(15.5)	75.9(21.6)	0.192
FEV ₁ /FVC %	74.1(5.0)	50.8(14.8)	<0.001
AHR or BDR at visit, n (%)	8(50.0)	18(51.4)	0.925
VAS symptom score [‡]	26.4(10.2-32.8)	24.1(16.8-34.4)	0.540
ICS use, n (%)	16(100)	33(94.3)	1.000
ICS dose, BDP equivalent, mcg/day [‡]	2000(1000-2000)	2000(1000-2000)	0.667
Long-acting anticholinergic use, n (%)	7(43.8)	22(62.9)	0.204
Number of exacerbations in the year prior to baseline visit [‡]	2(0.5-4)	3(1-4)	0.285
≥2 exacerbations in the prior year, n(%)	9(56.3)	23(65.7)	0.517
CCI [‡]	3(2-4)	4(3-5)	0.024
Cardiovascular disease, n (%)	7(43.8)	24(68.6)	0.092
HMGCoA reductase inhibitor (Statin) use, n (%)	6(37.5)	10(28.6)	0.524
Number of samples analyzed, blood sputum (n)	46 11	32 22	N/A
Serum CRP, mg/L [‡]	6.3(1.7-9.6)	4.4(1.4-7.6)	0.314
Serum CRP ≥4.12 mg/L [‡] , n (%)	9(56.3)	20(62.5)	0.208

Serum IL-6, pg/mL [†]	2.7(1.8-4.3)	3.2(1.9-4.5)	0.370
Serum IL-6 ≥1.55 pg/mL, n (%)	13(81.3)	32(100)	0.032
Number of elevated systemic inflammatory mediators (n) ^{†‡}	3 4 9	3 12 17	0.573
Had both CRP and IL-6 elevated, n(%)	9(56.3)	17(53.1)	0.838
Sputum total cell count, ×10 ⁶ /mL [†]	3.5(2.6-7.0)	6.6(2.3-12.0)	0.468
Sputum neutrophil% [†]	38.8(30.5-69.0)	60.8(39.4-76.6)	0.201
Sputum eosinophil% [†]	3.0(1.0-15.5)	1.6(0.9-2.6)	0.374
Sputum macrophage% [†]	47.8(24.3-58.5)	29.8(17.0-51.5)	0.166
Sputum lymphocyte% [†]	1.0(0.5-1.5)	0.6(0.3-1.8)	0.567

Definition of abbreviations: ACQ = asthma control questionnaire; ICS = inhaled corticosteroids;

BDP = beclomethasone dipropionate; CCI = Charlson comorbidity index.

*Median (IQR)

[†]None of CRP and IL-6 elevated | either CRP or IL-6 elevated | both CRP and IL-6 elevated

e-Table 1. Clinical characteristics of participants at baseline (mean(SD) unless stated)

<u>Baseline variables</u>	<u>Asthma</u>	<u>COPD</u>	<u>P value</u>
<u>Number, n(%)</u>	<u>59(42.1)</u>	<u>81(57.9)</u>	
<u>Age</u>	<u>60.5(13.6)</u>	<u>68.9(9.0)</u>	<u><0.001</u>
<u>Sex, male female (n)</u>	<u>20 39</u>	<u>35 46</u>	<u>0.265</u>
<u>BMI</u>	<u>30.6(7.7)</u>	<u>28.8(7.7)</u>	<u>0.162</u>
<u>Smoking, never ex current smokers (n)</u>	<u>34 23 2</u>	<u>29 47 5</u>	<u>0.038</u>
<u>Smoking pack-years *</u>	<u>8(2-35)</u>	<u>28(13-45.5)</u>	<u>0.030</u>
<u>FEV₁ %predicted</u>	<u>86.0(15.5)</u>	<u>57.7(18.4)</u>	<u><0.001</u>
<u>FVC %predicted</u>	<u>88.8(16.4)</u>	<u>80.2(18.7)</u>	<u>0.006</u>
<u>FEV₁/FVC %</u>	<u>74.6(6.3)</u>	<u>54.3(11.8)</u>	<u><0.001</u>
<u>AHR or BDR at visit, n(%)</u>	<u>24(40.7)</u>	<u>46(56.8)</u>	<u>0.060</u>
<u>VAS symptom score *</u>	<u>15.8(7.4-27.5)</u>	<u>19.3(11.6-29.0)</u>	<u>0.210</u>
<u>ACQ/6 *</u>	<u>0.50(0.17-0.83)</u>	<u>N/A</u>	<u>N/A</u>

<u>GINA severity (n), step 1 2 3 4</u>	<u>3 11 22 23</u>	<u>N/A</u>	<u>N/A</u>
<u>GOLD stage (n), stage 1 2 3 4</u>	<u>N/A</u>	<u>11 42 22 6</u>	<u>N/A</u>
<u>ICS use, n(%)</u>	<u>50(84.8)</u>	<u>71(87.7)</u>	<u>0.620</u>
<u>ICS dose, BDP equivalent, mcg/day *</u>	<u>1000(500-2000)</u>	<u>1000(500-2000)</u>	<u>0.956</u>
<u>Long-acting anticholinergic use, n(%)</u>	<u>14(23.7)</u>	<u>45(55.6)</u>	<u><0.001</u>
<u>Number of exacerbations in prior year *</u>	<u>0(0-1)</u>	<u>1(0-3)</u>	<u>0.001</u>
<u>Frequent exacerbators in prior year, n(%)</u>	<u>12(20.3)</u>	<u>34(42.0)</u>	<u>0.007</u>
<u>CCI *</u>	<u>3(2-4)</u>	<u>4(3-5)</u>	<u>0.002</u>
<u>Cardiovascular disease, n(%)</u>	<u>30(50.9)</u>	<u>52(64.2)</u>	<u>0.113</u>
<u>HMGCα reductase inhibitor (Statin) use, n(%)</u>	<u>18(30.5)</u>	<u>20(24.7)</u>	<u>0.445</u>

Definition of abbreviations: ACQ = asthma control questionnaire; ICS = inhaled corticosteroids;

BDP = beclometasone dipropionate; CCI = Charlson comorbidity index.

* Median (quartile 1-3)

Table 2. Univariable regression analysis of baseline characteristics with the frequent exacerbation phenotype (logistic regression) and exacerbation frequency (number, negative binomial regression)

Frequent VS Infrequent exacerbators					Number of exacerbations				
Variables		OR	95% CI	P-value	Variables		IRR	95% CI	P-value
Age		1.04	1.01-1.08	0.021	Age		1.02	1.00-1.03	0.012
Sex		1.30	0.64-2.66	0.465	Sex		1.14	0.75-1.73	0.542
BMI		1.01	0.96-1.05	0.762	BMI		1.01	0.99-1.03	0.224
Smoking		1.28	0.64-2.56	0.492	Smoking		1.20	0.84-1.74	0.320
FEV ₁ %predicted		0.97	0.95-0.99	0.001	FEV ₁ %predicted		0.98	0.98-0.99	<0.001
VAS symptom score		1.06	1.03-1.09	<0.001	VAS symptom score		1.03	1.02-1.05	<0.001
ICS use		5.78	1.28-26.17	0.023	ICS use		2.51	1.36-4.66	0.003
Long-acting anticholinergic use		2.59	1.28-5.26	0.008	Long-acting anticholinergic use		1.71	1.20-2.43	0.003
Frequent exacerbation in the prior year		9.02	4.03-20.18	<0.001	Frequent exacerbation in the prior year		2.95	2.16-4.04	<0.001
CCI		1.22	0.96-1.54	0.098	CCI		1.10	1.00-1.22	0.057
Asthma/COPD diagnosis		2.04	0.99-4.21	0.052	Asthma/COPD diagnosis		1.47	1.10-1.98	0.010
The number of elevated systemic inflammatory markers *		2.36	1.45-3.83	0.001	The number of elevated systemic inflammatory markers *		1.70	1.35-2.14	<0.001
Sputum total cell count		1.01	0.98-1.04	0.427	Sputum total cell count		1.00	0.99-1.01	0.750
Sputum eosinophil%		1.01	0.98-1.05	0.507	Sputum eosinophil%		1.00	0.99-1.02	0.695
Sputum neutrophil%		1.01	0.99-1.02	0.416	Sputum neutrophil%		1.00	0.99-1.01	0.931
Sputum IL1B gene expression		1.25	1.09-1.44	0.001	Sputum IL1B gene expression		1.13	1.05-1.18	<0.001

e-Definition of abbreviations: OR = odds ratio; IRR = incidence rate ratio; OCS = oral corticosteroid; ICS = inhaled corticosteroids; CCI = Charlson comorbidity index.

* None of CRP and IL-6 elevated | either CRP or IL-6 elevated | both CRP and IL-6 elevated

Table 2. Systemic and airway inflammatory characteristics of participants at baseline (mean(SD) unless stated)

<u>Baseline variables</u>	<u>Asthma</u>	<u>COPD</u>	<u>P value</u>
<u>Sputum total cell count, $\times 10^6$/mL *</u>	<u>3.7(2.4-6.3)</u>	<u>4.6(2.7-10.2)</u>	<u>0.248</u>
<u>Sputum eosinophil% *</u>	<u>1.3(0.3-3.0)</u>	<u>1.8(0.8-3.8)</u>	<u>0.214</u>
<u>Sputum eosinophil% >3%, n(%)</u>	<u>9(21.4)</u>	<u>18(28.6)</u>	<u>0.412</u>
<u>Sputum neutrophil% *</u>	<u>42.8(24.0-60.3)</u>	<u>61.0(36.5-76.0)</u>	<u>0.006</u>
<u>Sputum neutrophil% >61%, n(%)</u>	<u>10(23.8)</u>	<u>32(50.8)</u>	<u>0.006</u>
<u>Sputum macrophage% *</u>	<u>49.0(32.3-66.3)</u>	<u>28.0(14.8-52.5)</u>	<u>0.001</u>
<u>Sputum lymphocyte% *</u>	<u>1.0(0.5-2.2)</u>	<u>0.8(0.3-1.5)</u>	<u>0.098</u>
<u>Sputum IL-1β gene expression</u>	<u>0.5(0.3-1.2)</u>	<u>1.1(0.3-7.6)</u>	<u>0.132</u>
<u>Sputum IL-1β protein level, ng/mL</u>	<u>0.3(0.1-1.3)</u>	<u>0.3(0.1-2.0)</u>	<u>0.843</u>
<u>Sputum IL-1Ra, ng/mL</u>	<u>213.3(131.5-284.9)</u>	<u>174.2(100.1-269.4)</u>	<u>0.273</u>
<u>Serum CRP, mg/L *</u>	<u>2.7(1.2-7.0)</u>	<u>3.0(1.2-6.8)</u>	<u>0.676</u>
<u>Serum CRP ≥ 4.12mg/L, n(%)</u>	<u>20(34.5)</u>	<u>35(46.1)</u>	<u>0.177</u>
<u>Serum IL-6, pg/mL *</u>	<u>1.9(1.2-3.0)</u>	<u>2.6(1.5-4.1)</u>	<u>0.013</u>
<u>Serum IL-6 ≥ 1.55 pg/mL, n(%)</u>	<u>34(58.6)</u>	<u>56(73.7)</u>	<u>0.066</u>
<u>Number of elevated systemic inflammatory mediators (n) [†]</u>	<u>22 18 18</u>	<u>17 27 32</u>	<u>0.134</u>

* Median (quartile 1-3)

[†] None of CRP and IL-6 elevated | either CRP or IL-6 elevated | both CRP and IL-6 elevated

e-Table 3. Univariate regression analysis of baseline characteristics with the frequent exacerbation phenotype (logistic regression) and exacerbation frequency (number, Poisson regression) in asthma

Frequent VS Infrequent exacerbators				Number of exacerbations			
Variables	OR	95% CI	P value	Variables	IRR	95% CI	P value
Age	1.02	0.97-1.06	0.469	Age	1.01	0.99-1.03	0.400
Sex	1.78	0.49-6.46	0.382	Sex	1.28	0.75-2.18	0.366
BMI	1.00	0.93-1.08	0.986	BMI	1.01	0.98-1.05	0.335
Smoking	0.35	0.10-1.26	0.107	Smoking	0.71	0.43-1.19	0.199
FEV ₁ %predicted	0.98	0.94-1.02	0.233	FEV ₁ %predicted	0.99	0.97-1.00	0.124
VAS symptom score	1.05	1.00-1.11	0.055	VAS symptom score	1.03	1.01-1.06	0.002
ICS use	0.14	0.26-0.85	0.013	ICS use	3.47	0.69-17.52	0.131
Long-acting anticholinergic use	4.00	1.11-14.35	0.033	Long-acting anticholinergic use	2.11	1.28-3.46	0.003
Number of exacerbations in prior year	2.17	1.34-3.52	0.002	Number of exacerbations in prior year	1.26	1.15-1.38	<0.001
CCI	1.01	0.71-1.45	0.945	CCI	1.02	0.88-1.19	0.763
The number of elevated systemic inflammatory markers *	2.60	1.20-5.66	0.016	The number of elevated systemic inflammatory markers *	1.79	1.31-2.45	<0.001
Serum IL-6, pg/mL	1.79	1.10-2.92	0.020	Serum IL-6, pg/mL	1.13	1.07-1.19	<0.001
Serum CRP, mg/L	1.12	1.01-1.25	0.038	Serum CRP, mg/L	1.05	1.02-1.09	0.003
Sputum total cell	1.00	0.95-1.04	0.933	Sputum total cell	1.00	0.98-1.02	0.910

<u>count, ×10⁶/mL</u>				<u>count, ×10⁶/mL</u>			
<u>Sputum eosinophil%</u>	<u>1.04</u>	<u>0.98-1.10</u>	<u>0.234</u>	<u>Sputum eosinophil%</u>	<u>1.01</u>	<u>0.99-1.03</u>	<u>0.166</u>
<u>Sputum neutrophil%</u>	<u>1.01</u>	<u>0.98-1.04</u>	<u>0.640</u>	<u>Sputum neutrophil%</u>	<u>1.00</u>	<u>0.99-1.01</u>	<u>0.763</u>
<u>Sputum IL-1β gene expression</u>	<u>1.29</u>	<u>0.76-2.10</u>	<u>0.372</u>	<u>Sputum IL-1β gene expression</u>	<u>1.15</u>	<u>0.95-1.39</u>	<u>0.149</u>
<u>Sputum IL-1β protein level, ng/mL</u>	<u>1.29</u>	<u>0.93-1.80</u>	<u>0.131</u>	<u>Sputum IL-1β protein level, ng/mL</u>	<u>1.10</u>	<u>0.97-1.25</u>	<u>0.141</u>

Definition of abbreviations: OR = odds ratio; IRR = incidence rate ratio; OCS = oral corticosteroid; ICS = inhaled corticosteroids; CCI = Charlson comorbidity index.

* None of CRP and IL-6 elevated | either CRP or IL-6 elevated | both CRP and IL-6 elevated

Table 4. Univariate regression analysis of baseline characteristics with the frequent exacerbation phenotype (logistic regression) and exacerbation frequency (number, Poisson regression) in COPD

Frequent VS Infrequent exacerbators				Number of exacerbations			
Variables	OR	95% CI	P value	Variables	IRR	95% CI	P value
Age	1.06	1.00-1.11	0.051	Age	1.02	1.00-1.04	0.021
Sex	1.26	0.52-3.07	0.611	Sex	1.14	0.81-1.60	0.469
BMI	1.02	0.96-1.08	0.485	BMI	1.02	1.00-1.04	0.124
Smoking	2.22	0.85-5.78	0.102	Smoking	1.40	0.96-2.03	0.079
FEV ₁ %predicted	0.96	0.94-0.99	0.005	FEV ₁ %predicted	0.98	0.97-0.99	<0.001
VAS symptom score	1.06	1.02-1.10	0.005	VAS symptom score	1.03	1.02-1.05	<0.001
ICS use	3.47	0.69-17.52	0.131	ICS use	2.62	1.23-5.61	0.013
Long-acting anticholinergic use	1.69	0.69-4.15	0.250	Long-acting anticholinergic use	1.39	0.98-1.96	0.065
Number of exacerbations in prior year	1.61	1.22-2.12	0.001	Number of exacerbations in prior year	1.15	1.10-1.20	<0.001
CCI	1.33	0.94-1.88	0.105	CCI	1.12	0.99-1.27	0.065
The number of elevated systemic inflammatory markers *	2.07	1.10-3.91	0.025	The number of elevated systemic inflammatory markers *	1.59	1.24-2.04	<0.001
Serum IL-6, pg/mL	1.14	0.97-1.34	0.109	Serum IL-6, pg/mL	1.01	1.00-1.02	0.009
Serum CRP, mg/L	1.04	0.98-1.11	0.190	Serum CRP, mg/L	1.02	1.01-1.03	<0.001
Sputum total cell count, ×10 ⁶ /mL	1.03	0.98-1.08	0.236	Sputum total cell count, ×10 ⁶ /mL	1.00	0.99-1.02	0.575
Sputum eosinophil%	1.00	0.95-1.04	0.838	Sputum eosinophil%	1.00	0.98-1.02	0.918

<u>Sputum neutrophil%</u>	<u>1.00</u>	<u>0.98-1.02</u>	<u>0.791</u>	<u>Sputum neutrophil%</u>	<u>1.00</u>	<u>0.99-1.01</u>	<u>0.823</u>
<u>Sputum IL-1β gene expression</u>	<u>1.26</u>	<u>1.08-1.48</u>	<u>0.004</u>	<u>Sputum IL-1β gene expression</u>	<u>1.11</u>	<u>1.07-1.16</u>	<u><0.001</u>
<u>Sputum IL-1β protein level, ng/mL</u>	<u>1.24</u>	<u>1.03-1.50</u>	<u>0.024</u>	<u>Sputum IL-1β protein level, ng/mL</u>	<u>1.05</u>	<u>1.00-1.11</u>	<u>0.063</u>

Definition of abbreviations: OR = odds ratio; IRR = incidence rate ratio; OCS = oral corticosteroid; ICS = inhaled corticosteroids; CCI = Charlson comorbidity index.

* None of CRP and IL-6 elevated | either CRP or IL-6 elevated | both CRP and IL-6 elevated

e-Table 35. Generalized structural equation modeling (SEM) for different variables in-of path analyses in COPD

Model 1: Frequent exacerbations ~~The number of exacerbation~~ in prior year ~~(yes VS no)~~

Variable	Coefficient	95% CI	P value
Age	0.009013	-0.052-0.0790-0.002-0.015	0.009691

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Model 2: Sputum IL-1 β gene expression

Variable	Coefficient	95% CI	P value
Age	-0.001-0.013	-0.071-0.070-0.048-0.023	0.9900.486
The number of exacerbations in prior year Frequent exacerbations in prior year (yes VS no)	-0.233-1.121	-0.454-(-0.011)-2.040-(-0.201)	0.0390.017

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Model 3: Serum In(IL-6)

Variable	Coefficient	95% CI	P value
Age	0.0280-0.016	0.011-0.0450-0.006-0.026	0.0010.002
The number of exacerbations in prior year Frequent exacerbations in prior year (yes VS no)	-0.020-0.041	-0.075-0.035-0.326-0.244	0.4710.780
Sputum IL-1 β gene expression	-0.141-0.120	-0.206-(-0.076)-0.184-(-0.057)	<0.001<0.001

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Model 4: The number of exacerbations in the following year Frequent future exacerbation (Yes/No)

Variable	Coefficient	95% CI	P value
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Age	0.018 0.043	-0.012-0.049-0.022-	0.241 0.196
		0.109	
<u>The number of exacerbations</u>	<u>0.130</u> 1.941	<u>0.067-0.192</u> 0.720-	<u><0.001</u> 0.002
<u>in prior year</u> Frequent		3.161	
<u>exacerbations in prior year</u>			
<u>(yes VS no)</u>			
Sputum <u>IL-1β/IL-1β</u> gene	-0.040-0.070	-0.150-0.069-0.358-	<u>0.470</u> 0.634
expression		0.218	
Serum ln(IL-6)	<u>0.430</u> 1.425	<u>0.037-0.822</u> 0.304-	<u>0.032</u> 0.013
		2.547	