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Impact and Associations of Eosinophilic Inflammation in COPD: Analysis of the AERIS Cohort

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Key Words:	COPD, eosinophils, Inflammation, exacerbations of COPD, AERIS

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To: Professor Ian Pavord

European Respiratory Journal

Professor M. Humbert

European Respiratory Journal Chief Editor

Professor A.T. Dinh-Xuan

Deputy Chief Editor

Dear Professor Pavord, Professor Humbert and Professor Dinh-Xuan,

Re: Submission of the revised manuscript "Impact and Associations of Eosinophilic Inflammation in COPD: Analysis of the AERIS Cohort" (reference ERJ-00853-2017)

Thank you for the opportunity to revise our manuscript in the light of the reviewers' comments. We have carefully reviewed all the comments and addressed them in the manuscript accordingly. All the comments are addressed in a point by point manner. We believe that our manuscript has substantially improved following the suggested edits.

We appreciate your kind consideration of our original research article.

Yours Sincerely,

Professor Tom Wilkinson

ERJ-00853-2017

Response To Reviewers

Reviewer: 1

Comments to the Author

This is an excellent paper and validates the eosinophilic hypothesis and goes a long way to teasing out underlying patterns of exacerbations and previous observations.

1.1. The only weakness of the paper is that it is post hoc, I would prefer to see the analysis plan (dated prior to analysis) included in the appendix or at least a statement about the fact a plan was generated and followed rather than an exploratory approach of which we only see some analysis. In any event this should be clarified.

- We fully acknowledge that the analyses conducted for the manuscript were post-hoc (page 7 lines 2-4). Post-hoc analyses of the AERIS data were broadly outlined in a statistical analysis plan for Viktoriya Kim's PhD thesis, which was submitted on the 16th September 2015, approved by the GSK-Southampton scientific steering committee, and followed. The manuscript resulted from the analyses conducted as part of the thesis."

1.2 The data seems to allow for an assessment of which has the better validity(sens v spec) for predicting eo and non eo exacerbations, sputum or blood in stable disease, this is a major area of interest and the assumption is often made that sputum is better (with not much data). there is already a lot of data in the paper so I could understand if the authors did not want to include this, but in my view it would add to an already very good manuscript.

- The reviewer raises a very important point and we can confirm that we have explored this. Sputum eosinophils (%) at stable state are not associated with sputum eosinophilia at exacerbations (AUC 0.585 CI 0.485; 0.684, n=187 included all patients with valid enrolment and exacerbation sputum data). However, blood eosinophils at stable state can predict blood eosinophils≥2% at exacerbations (AUC 0.760 CI 0.709; 0.811, n=336). Given the current word limit of the journal, we are unable to add these results to the manuscript.

1.3 Minor point table 2 in the low EO group sputum neuts are quoted as 9.7% is this a typo?
- We have reviewed our data and confirm that RE median(IQR) sputum neutrophils % = 9.77(63.96). This variable is positively skewed and has mean(SD) of 30.09(35.84).

Reviewer: 2

Comments to the Author

Overall this is a very interesting manuscript with important new data that supports blood eosinophil count as a biomarker in COPD patients to predict exacerbation phenotype, and could be highly relevant to stratify and select patients in future trials of novel COPD treatments targeting a reduction in exacerbations. The data on seasonal changes in exacerbation phenotype are especially unique. However, improvements are recommended prior to publication.

General comments

- Overall this is a very interesting manuscript with important new data that supports blood eosinophil count as a biomarker in COPD patients to predict exacerbation phenotype, and could be highly relevant to stratify and select patients in future trials of novel COPD treatments targeting a reduction in exacerbations. The data on seasonal changes in exacerbation phenotype are especially unique. However, improvements are recommended prior to publication.

2.1 Use of the term “eosinophilia” is not accurate based on cut-points employed in this study; suggest an alternative term to describe patients with levels >2% e.g. patients with higher blood eosinophil count

- We agree fully with this point and apologise for this lack of specificity. Where necessary we have replaced the term “eosinophilia” with “raised eosinophils” “eosinophils≥2%” or “eosinophil predominance”.

Methods

2.2 Can the authors please describe in the manuscript how the patients diagnosis of COPD was confirmed? Were patients with a prior history of concurrent history of asthma permitted to enrol?

- Only patients with a confirmed diagnosis of COPD based on postbronchodilator spirometry with FEV1 ≤80% of predicted normal and FEV1/FVC <0.7, ex-smokers or current smokers with significant smoking history (≥10 pack year history) were included into the cohort.[1] Only 8.7% of patients had a prior diagnosis of asthma, with no patient having diagnosis of asthma at the time of recruitment.

- The sentence below was added to the Methods section (page 5 lines 10-15)

“Only patients with a confirmed diagnosis of COPD based on postbronchodilator spirometry with FEV1 ≤80% of predicted normal and FEV1/FVC <0.7, ex-smokers or current smokers with significant smoking history (≥10 pack year history) were included into the cohort, with no patients having diagnosis of asthma at the time of recruitment[1]”

2.3 Needs to be made clear the eos count cut-points explored e.g. 200 cell/ul

- This is now detailed in the manuscript.

Page 8, lines 17-18 “Using absolute cell numbers (≥200cells/uL) rather than percentage gave similar results (Table E1).”

Page 14 lines 7-13 “In our study the blood eosinophil count was reported up to one decimal, therefore it was not possible to apply the cut off of 150cells/uL. 200cells/uL cutoff offered a similar sensitivity (95.8%) but inferior specificity (27.3%). We conducted a sensitivity analysis with the ≥200cells/uL cut off and found that there was a significant difference in age, smoking history, exacerbation rate with raised eosinophils in the first year and presence of sputum eosinophils>3% at enrolment.”

Results

2.4 The results were difficult to follow and an alternative structure is proposed below to add clarity for the reader

- We thank the reviewer for this suggestion and have made the majority of the changes they have suggested by restructuring the results section.

General characteristics of the cohort

- Description of Baseline characteristics of patients including average eos levels and by eos category at enrolment

Clinical and Inflammatory characterisation at enrolment

- Correlation between blood eos (% and absolute) and sputum at enrolment
- Ability of blood eos to predict airway eosinophilia at enrolment
  - ROC curve
  - Sensitivity/specificity with percentage and absolute count

Stability of eos phenotype over time

- % PE, IE, RE and characteristics of these patients
- Stability over time (ICC)
- Ability of blood eos at enrolment to predict PE status during study period

Exacerbation phenotypes

Eosinophil associated exacerbations

- Correlation between blood eos and sputum at point of exacerbation
- Ability of blood eos at enrolment to predict airway eosinophilia at point of exacerbation
  - ROC curve
  - Sensitivity/specificity
  - Odds of eos associated exacerbation in patients with enrolment eos >2% or >200 cells/ul
- Ability of blood eos status during study period (PE) to predict airway eosinophilia at point of exacerbation

- o ROC curve
- o Sensitivity/specificity
- o Odds of eos associated exacerbation in patients with PE vs RE

Seasonal changes

Bacterial associated exacerbations

2.5 The Tables and Figures were not always correctly labelled in the text; please check

- Thank you for pointing out - amended

2.6 Could the proportion of patients with prior history of frequent exacerbations (>=2) be included in baseline characteristics?

-This result has now been added to the Table 1 as suggested.

2.7 Further, are patients with a prior history of FE more likely to have eos associated exacerbations?

- There was no propensity for patients with a prior history of frequent exacerbations to have higher eosinophils in exacerbations in the following year (OR 0.610 CI 0.208; 1.784 p= 0.366, subjects multiple measurements accounted for).

2.8 Can the authors please provide information on asthma features for this cohort e.g. IgE, % with atopy, % with significant bronchial reactivity, % with prior history of asthma

- This is always a possible confounder in any observational study investigating eosinophils, however patients with a current diagnosis of asthma were not included into the study. Out of 127 subjects, only 8.7% (n=11) had any prior history of asthma - often a primary care diagnosis prior to COPD diagnosis being confirmed, substantially less than the proportion presenting with raised blood eosinophils. Furthermore, whilst we did not test for IgE and bronchial reactivity in our study, no patients with atopy were enrolled in the cohort. Furthermore all patients had a significant smoking history and CT findings were consistent with COPD diagnoses across the cohort.

2.9 It would be helpful to include a venn diagram of exacerbation phenotypes to allow the reader to see the number and relative proportions and overlap of exacerbation phenotypes

- We agree that Venn diagrams are helpful to illustrate the number and relative proportions of different exacerbation phenotypes and have utilised these diagrams in our previous publication reporting the exacerbation phenotypes based on the viral, bacterial and viral/bacterial etiology.[2] As the focus of this manuscript is solely on the eosinophils at stable state and exacerbation, we would not want to duplicate any of this previously published work.

2.10 It could be mentioned in the discussion that PE vs RE patients and >2% vs <2% were slightly older, more likely to be male, less likely to be a current smoker and this is consistent with other cohorts

-This is now added to the manuscript.

Page 14, lines 19-21: “These PE subjects were slightly older than RE patients (p=0.034) and there were trends towards a lower prevalence of current smokers but with a greater smoking history in the PE group.”

2.11 The exacerbation frequency during the study appears to have increased vs prior to enrolment – is there a reason for this? Is it due to hypervigilance and being involved in an exacerbation study? Or deterioration in disease status? Or differences in the way exacerbations are captured? In other studies the opposite trend is observed e.g. Salford Lung Study.

- Median exacerbation frequency in Y0 (12 months prior to the study) and Y1 appear to be increased however, these was no statistical difference between Y0 and Y1 and so we are wary of over-interpreting this finding. If we express these data as means rather than medians, then there is near equivalence between Y0 and Y1 as we report previously (Wilkinson,2017).

Table X1

Total exacerbations frequency	**N**	399	355	**p-value**
Total exacerbation rate	**Mean**	3.14	3.09	0.402
	Median(IQR)	2.00(2.00)	2.94(3.91)	
	Min	1.00	0.00	
	Max	14.00	13.77	

Data is non-parametric, mean reported along with median as median is not informative.
*To test for significance - Wilcoxon test applied.

2.12 What is the relative proportion of mild, moderate and severe events?

- During the first year of follow up, the majority (85.6%) of events were moderate exacerbations (i.e. requiring treatment). Mild events not requiring treatment comprised 8.7%, whilst severe events necessitating hospitalisation comprised 5.6% of exacerbations.

2.13 Does exacerbation phenotype change according to severity of exacerbation e.g. mild, moderate, severe?

- Severe exacerbations were far less likely to be associated with raised blood eosinophils than mild or moderate exacerbations. (Table X2)

Table X2

Severity	**Blood eosinophils ≥2%**
Mild	16(9.5%)
Moderate	150(89.3%)
Severe	2(1.2%)
Total	168

2.14 Are patients with higher eos count at enrolment more likely to have moderate/severe vs mild exacerbations than those with lower eos count at enrolment?

-There were n=31 mild exacerbations and n=324 moderate+severe exacerbations. The odds of having moderate/severe exacerbations in patients with higher blood eosinophil compared to those with lower eosinophil % was not significant (OR 0.72 CI 0.268; 1.924 p=0.511, multiple measurement were taken into account). However, we recognise that statistically this analysis is not well powered, as only 8.7% of exacerbations were mild.

2.15 Table 1 –include categorical variable of blood eos count > and < 200 cells/ul

-The results have now been added to the Table under the categorical variables.

2.16 Table 2 – it is not clear why FU in first year of study or EXACT score are significantly different across the groups because their median values are almost identical; please explain

We thank the reviewer for asking this question and triggering us to re-analyse the data. Upon re-analysis the median values are correct but some of the p values we report were incorrect and thus the significance of some of these p values has changed. This has resulted in the following changes:

Table 2 - blood neutrophils now being significant; WBC, EXACT score, Exacerbation rate in 1st year and follow up in the first year losing significance;

Table E1 - WBC, EXACT score, exacerbation rate in the first year and follow up in the first year of study losing significance.

We have subsequently re-checked all the data in our analysis and can confirm all other values are now correct. These changes do not affect the main argument of our paper.

We thank the reviewer for prompting us to perform this re-analysis.

2.17 Table 3 – add rows for eos associated exacerbations as defined by blood and sputum eos
-These have been added to the table.

2.18 Table E3 – include a row on "overall" population for comparison with each longitudinal phenotype

-These have been added to the table.

2.19 Table E4 – need to include the number of eos associated exacerbations in the summer and in the winter as well as total number overall

-These have been added to the table.

2.20 Discussion

• A particular limitation of this study is that the majority of patients were on ICS containing regimens; this may shift the underlying phenotype of exacerbations

- 78% of our patients were frequent exacerbators and in severe-very severe airflow obstruction category at enrolment therefore were already on ICS as per guidelines. We feel the fact that the EI was present despite widespread ICS use (89%) may be viewed as strength of this study as this represents a real world scenario. Still we recognise this point in the discussion section of the revised paper (page 15 lines 24-25 and page 16 lines 1-3)

"It is important to recognise that these results are representative of a cohort of moderate to very severe COPD patients with frequent exacerbations receiving a high level of clinical intervention, including ICS, as part of an intensive study. This might have had an impact on the severity of exacerbations and recovery, as previous reports indicate that early therapy improves exacerbation outcomes."

2.21 Since both enrolment blood eos and persistence of higher blood eos appear to predict eos associated exacerbations, and levels are stable over time, it may be valid to conclude that a single blood eos measure during stable state is sufficient to categorise patients; can the authors discuss?

-It is difficult to ascertain given this study is of an observational design and the EI analysis is post-hoc. In our opinion, further prospective studies are required to assess if we can use only a one-point measurement to predict associations with exacerbations or the longitudinal phenotype.

2.22

Suggested structure of the results section:
General characteristics of the cohort
- Description of Baseline characteristics of patients including average eos levels and by eos category at enrolment

Clinical and Inflammatory characterisation at enrolment
- Correlation between blood eos (% and absolute) and sputum at enrolment
- Ability of blood eos to predict airway eosinophilia at enrolment
  - ROC curve
  - Sensitivity/specificity with percentage and absolute count

Stability of eos phenotype over time
- % PE, IE, RE and characteristics of these patients
- Stability over time (ICC)
- Ability of blood eos at enrolment to predict PE status during study period

Exacerbation phenotypes
- To include description of exacerbation phenotypes in overall population with reference to a Venn diagram

Eosinophil associated exacerbations
- Correlation between blood eos and sputum at point of exacerbation
- Ability of blood eos at enrolment to predict airway eosinophilia at point of exacerbation
  - ROC curve
  - Sensitivity/specificity
  - Odds of eos associated exacerbation in patients with enrolment eos >2% or >200 cells/ul
- Ability of blood eos status during study period (PE) to predict airway eosinophilia at point of exacerbation
  - ROC curve
  - Sensitivity/specificity
  - Odds of eos associated exacerbation in patients with PE vs RE

Bacterial associated exacerbations
Seasonal changes
- Venn diagram in winter and summer would be helpful to see the overlap and how this changes by season

- The results have been restructured largely as suggested by the reviewer please see our detailed response above (comment 2.4)

Reviewer: 3

Comments to the Author

Thank you for the opportunity to review this paper and understand the work that went into it. There is a paucity of prospective data in the world literature which makes the work valuable. There are significant issues with the data which may preclude acceptance and if addressed may add to the utility of the data.

General Comments:

3.1.Could all eosinophil data be examined by using the eosinophil level as a continuous variable rather than having a discrete cut off. This would allow the data to lead the results rather than having a pre-specified cut (2%) driving the findings. Perhaps there would be a greater effect at 2.5% or 3% etc.

- We agree that this approach may be informative, however, the aim of this manuscript was to compare this work with established literature which suggests 2% cut off based on the cross sectional analyses/single point measurement [3-6] and to expand this cut off to the longitudinal cohort. A cut point needs to be applied the data to enable longitudinal analysis. Longitudinal groups may become a basis for the future management pending further studies.

3.2. In each exacerbation event there is no comment or data on any possible treatment effect. The samples were collected "within 72" hours, but were the patient on oral corticosteroids already, which may affect both the clinical outcome and the blood or sputum eosinophil count.

- There were no patients on long term oral steroids at enrolment. 71% of our exacerbations were collected within 2 days of the onset of exacerbations and 91.4% of our patients had no acute oral therapy administered prior to the point of exacerbation assessment and sample collections.[2] Hence no effects of OCS were discernible.

3.3. There are no comments on outcomes of the exacerbation events or if treatment varied. It could be postulated that if your eos were high then it might be more likely that you will be treated and respond to oral steroids. Also there is no comment on the potential of those with low eosinophil counts having an increased treatment failure rate when treated with oral corticosteroids.

AERIS was an observational study and all patients with suspected exacerbations were reviewed by the respiratory clinicians to confirm an exacerbation event and commence the AECOPD treatment and did not use eosinophil counts to stratify treatment as this is not standard. Treatment of AECOPD was as per local hospital policy. To answer the reviewer's question, further interventional studies are required, thus, we feel that ana analysis of the response to AECOPD treatment from an observational study is beyond the scope of this manuscript.

3.4. I am concerned that really this work is a piece of tautology as in defining the group of eosinophils >2% all that is being demonstrated is that they behave as a group with high eosinophils, thus the analysis is really driven by the first definition rather than a novel finding. Of course the finding that each type of patients (Pe, RE, and IE) is relatively stable is a useful observation.

- Exacerbations and stable COPD are different clinical states with different types of inflammatory responses. thus what we demonstrate here is the persistence of this phenotype in both clinical states and the possibility that we may be able to predict the longitudinal phenotype from a single blood measurement. To the best of our knowledge this is the first study presenting relationships between eosinophils at a stable state, exacerbations and longitudinal phenotypes. The clinical utility of this observation is of real potential in guiding future treatment.

3.5. Once the patients have been defined the numbers in each group becomes rather small to draw definite conclusions.
-Yes we agree this is a limitation that we acknowledge in the manuscript.

Page 16, line 4-9 "Secondly, the AERIS study was not originally designed, and therefore was not powered, to specifically investigate longitudinal EI and hence the numbers that were included in each eosinophilic group were limited. Larger prospective studies are required to further understand the impact of eosinophilic inflammatory status on a range of clinical outcomes."

Specific points:

3.6. KCO and TCO would be more usefully expressed as % predicted rather than as values

- Thank you for your suggestion. These have now been amended as per the reviewer's suggestions. Please note when TLCO re-calculated for %, TLCO% is no longer significant between groups included and excluded from the cohort (Table E2)

3.7. Intro line 3, suggest to add "global" to define where the mortality will be seen.

- This has been amended

3.8. Analysis p6 line 14. This reviewer believes that mutable events may be significant and there may be a difference between raised eosinophil patients and low eosinophil patients from the number of recurrent exacerbations.

- In order to not have our analysis skewed towards those subjects with a large number of exacerbations in the descriptive analysis, we have only used first exacerbations. However, our conditional logistic regression analysis has included all exacerbations and the Odds ratio therefore does include those patients with recurrent exacerbations and thus we do show the predictive ability of blood eosinophils across the spectrum of patients.

3.9.Results p8 line 3. There should be some comment on the other significant differences found for CRP, TLC, 6MWT and length of follow up. Why have these results be found. The differences in exacerbation rate (3 Vs. 2) and 6MWT (229 Vs. 324) Table E2 are quite large and merit comment.

- We have elaborated on the findings as suggested

- Table E2 (characteristics of the group included and excluded from the longitudinal analyses). The group of patients was excluded from the analysis (n=28) due to a lack of available stable samples to enable longitudinal classification. This is likely due to them suffering more exacerbations (Ex rate is 3.99 vs 1.99), and leaving the study earlier (FU rate 0.58). Whether this high baseline CRP level and decreased 6MWT was associated with or predictive of the increased number of exacerbations or contributed to this loss to follow up is a question for a future manuscript.

3.10. Page 9, line 3. The finding that the PE group have more eosinophilic exacerbations, as defined by a blood eos >2% is hardly surprising. This is tautological. all that is perhaps being demonstrated is that the high eos type is stable. Were there many patients who had low eos levels at steady state but who had high levels at exacerbation?

- Yes, we do recognise your point, however, the main message of this paper is to highlight the stability of this phenotype. In further support of this, there were only 5 subjects (out of 40) who had low blood eosinophils at enrolment that went on to have high (≥2%) eosinophils upon 1st exacerbation.

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Impact and Associations of Eosinophilic Inflammation in COPD: Analysis of the AERIS Cohort

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Take Home Message: Blood eosinophil levels in COPD predicts the nature of inflammation at future exacerbations and may guide therapy

MANUSCRIPT WORD COUNT – 3180

ABSTRACT WORD COUNT – 195

Abstract

Eosinophilic inflammation in COPD predicts response to treatment especially corticosteroids. We studied the nature of eosinophilic inflammation in COPD prospectively to examine the stability of this phenotype and its dynamics across exacerbations and its associations with clinical phenotype, exacerbations and infection.

127 patients aged 40–85 with moderate-severe COPD underwent repeated blood and sputum sampling at stable visits and within 72 h of exacerbation for one year.

Blood eosinophils ≥2% was prevalent at baseline and predicted both predominantly raised stable state eosinophils across the year (AUC 0.841, 95%CI 0.755; 0.928), and increased risk of eosinophilic inflammation at exacerbation (OR 9.16 p<0.001). Eosinophils≥2% at exacerbation and eosinophil predominance at stable visits were associated with a lower risk of bacterial presence at exacerbation (OR 0.49, p=0.049 and OR 0.25, p=0.065 respectively). Bacterial infection at exacerbation was highly seasonal (Winter vs Summer OR 4.74, p=0.011) in predominantly eosinophilic patients.

Eosinophilic inflammation is a common and stable phenotype in COPD. Blood eosinophil counts in the stable state can predict the nature of inflammation at future exacerbations which when combined with an understanding of seasonal variation provides the basis for the development of new treatment paradigms for this important condition.

Key Words: COPD EOSINOPHILS INFLAMMATION EXACERBATIONS AERIS

Introduction

Chronic obstructive airway disease (COPD) is an established cause of global mortality and morbidity, predicted to be the third leading cause of death by 2030.[1] Current guidelines for COPD management are based on airway obstruction, symptoms and exacerbation frequency.[2] The guidelines advise on step-wise management but do not fully account for the biological heterogeneity of this debilitating condition.

Eosinophilic inflammation (EI) was historically thought to be a feature of asthma with neutrophilic inflammation being a classical hallmark of COPD. However, recent studies have demonstrated that EI is present in a subset of COPD patients both at exacerbations[3] and during clinical stability.[4] A sputum eosinophil differential count of >3% is an accepted marker of airway EI and is derived from the reported enhanced response of this group of patients to corticosteroids.[5, 6] Moreover, a good relationship has been demonstrated between airway and systemic eosinophil counts.[3, 4, 7] It was previously reported that a >2% blood eosinophil cut off had a high sensitivity in identifying >3% airway eosinophils during acute exacerbations of COPD (AECOPD)[3], suggesting that peripheral eosinophils are a clinically accessible marker to predict inflammatory phenotype.

Using 2% blood eosinophils as a cut off in the longitudinal ECLIPSE cohort, persistently elevated blood eosinophils was reported as a common finding,[4] especially in milder disease, but exacerbations were not sampled. Reports of relationships between eosinophils and FEV1 and exacerbation frequency vary [4 8 9, 10 11, 12], but emerging evidence suggests measurement of eosinophils has clinical relevance. For example, severe AECOPD with EI are associated with a shorter length of stay in hospital.[13] Furthermore, there is an association between EI with a

greater response to steroid therapy at both stable COPD and during exacerbations.[5, 6, 14, 15 16]

Management of asthma stratified by airway EI has led to a reduced rate of exacerbations.[17] Siva et al demonstrated that COPD treatments targeting airway eosinophils was associated with a significant reduction of severe exacerbations[14].

Hence EI is an important COPD endotype but little is known about its stability over time, or its relationships to the inflammatory nature and aetiology of exacerbations. To improve our understanding, we investigated these factors in a secondary analysis of the AERIS cohort, a prospective, longitudinal study of patients with COPD.[18]

Methods

Study design and study population

The Acute Exacerbation and Respiratory InfectionS in COPD (AERIS) study is a prospective, observational cohort study based at University Hospital Southampton (UHS), registered with ClinicalTrials.gov (NCT01360398). The study protocol and full inclusion and exclusion criteria have been published previously.[18] The protocol summary is available at www.gsk-clinicalstudyregister.com (study identifier, 114378). AERIS was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice, and was approved by the Southampton and South West Hampshire Research Ethics Committee. All participants provided written informed consent. Only patients with a confirmed diagnosis of COPD based on postbronchodilator spirometry with FEV1 ≤80% of predicted normal and FEV1/FVC <0.7, ex-smokers or current smokers with significant smoking history (≥10 pack year history) were included into the cohort, with no patients having diagnosis of asthma at the time of recruitment[18]

Patients were followed monthly in the stable state and reviewed within 72 hours of onset of AECOPD symptoms. Exacerbations were detected using daily electronic diary cards. Definition of AECOPD and severity, was described previously[18, 19].

Procedures

Blood and sputum analyses were performed as previously described[18, 19]. Further methodological detail is provided in the online supplement.

Criteria for eosinophilic groups and seasonality

EI was defined as sputum eosinophils >3% and blood eosinophils ≥2% in line with previous studies.[3, 5, 6, 14, 20] To investigate the stability of blood EI over time we divided subjects into three groups: predominantly (PE), intermittent (IE) and rarely (RE) eosinophilic. Only those subjects who had at least 3 (out of 5 potential) stable visits with valid blood results over 12 months were included in the group analyses (n=99). The PE group was defined as blood eosinophils ≥2% at either all visits, or all but 1 visits where the blood eosinophils were <2%; the RE group was defined as blood eosinophils <2% at all visits, or all but 1 visit where the blood eosinophils were ≥2%; the IE group was defined when none of the abovementioned criteria were met.

To investigate an impact of seasonality on exacerbations we divided the year into 2 seasons; Winter (October-March) and Summer (April – September).

Statistical analysis

Bivariate analyses testing for differences between eosinophilic groups were conducted using two-tailed, Kruskall-Wallis, ANOVA, Chi-Square, or Fisher's Exact test, as appropriate. Receiver Operator Curves (ROC) were used to assess the predictive ability of different cut offs to correctly identify presence of sputum EI. Intra-class correlations were used to assess the reliability of measures within individuals over time. Because some subjects were represented multiple times in exacerbation analysis, descriptive analyses were conducted only for the first exacerbation occurring to each subject, and multivariate analyses with binary outcomes (presence/absence of different conditions at exacerbation) were conducted using conditional logistic regression, including the subject number as a random effect. SPSS (version 22) was used for all analyses with the exception of intra-class correlation coefficients (ICC) and conditional logistic regression, which were

conducted using STATA (version 14). All of these analyses should be considered post hoc as they were not pre-specified in the AERIS statistical analysis plan. All of these analyses should be considered post hoc as they were not pre-specified in the AERIS statistical analysis plan.

Results

General characteristics of the cohort

152 patients were screened but only 127 patients were included in the AERIS cohort (Figure 1). The cohort consisted of patients with moderate to very severe airway obstruction, the majority (87.3%) were receiving inhaled corticosteroids and had a substantial smoking history (mean pack years 50.3, SD 28.2 - Table 1). Only patients who experienced at least 1 moderate to severe exacerbation in the last 12 months were included in the cohort (median rate 2.00, IQR 2.00).

Clinical and Inflammatory Characterisation at Enrolment

The prevalence of EI ≥3% in sputum in our cohort at enrolment was 33% (n=27) and in blood ≥2% was 68% (n=86). Blood eosinophils (% and count) displayed moderately strong positive correlations with sputum eosinophil % (rho 0.463 and 0.581 respectively at enrolment, $p<0.001$) and were predictive of sputum eosinophils>3% (AUC 0.851, 95%CI 0.750; 0.951, and AUC 0.768, 95%CI 0.651; 0.884 respectively) (Figure 2). This ≥2% blood eosinophil cut-point was 95.8% sensitive and 31.8% specific. Using absolute cell numbers (≥200cells/uL) rather than percentage gave similar results (Table E1).

Stability of Eosinophilic Phenotype over Time

We categorised our patients into three longitudinal EI phenotypic groups based on blood eosinophils ≥2% and found that, out of the 99 patients with sufficient data, 57 (58%) were predominantly (PE), 16 (16%) intermittently (IE) and 26 (26%) rarely

eosinophilic (RE) over the 12 months (Table 2). Between the three longitudinal groups, the only significant differences observed at enrolment were age, blood neutrophils, and presence of sputum eosinophils>3%. (Table 2) There were also significant differences in the eosinophil predominant exacerbation rate over the first year. Significant differences between patients included and excluded from this analysis were seen for CRP, 6MWT and the length of follow-up (Table E2). In order to assess the longitudinal stability of blood EI, we analysed the reliability of the marker in defining a longitudinal phenotype and found blood eosinophils to be relatively stable within individuals across the 12 months examined, including enrolment (ICC 0.66, CI 0.58; 0.74).

We next examined whether blood eosinophils at a single time point (enrolment) was a useful predictor of being PE over time (categorisation for the EI groups excluding enrolment, n=78). Blood eosinophils (% and count) at enrolment were predictive of being in the PE group over the next 12 months (AUC 0.841, 95%CI 0.755; 0.928 and 0.806, 0.710; 0.901 respectively, Figure 3). A blood eosinophil cut off of ≥2% was 84.3% sensitive and 48.1% specific in identifying those in the PE group over the next 12 months.

Blood EI groups at exacerbation and exacerbation rates

Across all exacerbations with valid data the prevalence of EI in sputum and in blood was 23.9% (n=52/218) and 49.7% (n=168/338) respectively. Blood eosinophils (% and count) were again predictive of sputum eosinophils≥3% at exacerbation (AUC 0.735, 95%CI 0.654; 0.817 and 0.729, 0.650; 0.809 respectively - Figure E1).

The total exacerbation rate (median(IQR)) in the first year of the study was similar in the RE and the PE groups, but lower in the intermittent group (PE 2.04(3.95), IE

1.02(2.00) and RE 2.47(3.22)). There was a significant difference in the rate of exacerbations with blood eosinophils ≥2%, with the predominant group having a higher eosinophilic exacerbation rate (PE(1.38(2.99)), IE(0.00(0.99)), and RE(0.00(0.99)), p<0.001).

Longitudinal eosinophilic groups did not differ symptomatically at first exacerbation (CAT and EXACT-PRO) but there was a significant difference in blood neutrophils at exacerbation between these groups (p=0.045), and the predominantly eosinophilic group was less likely to have bacteria present (p=0.044 - Table 3). There was an association between longitudinal eosinophilic phenotype and eosinophils at exacerbation, with the odds of eosinophils≥2% at exacerbation much higher in the PE group compared to the RE group (OR 11.16, 95%CI 5.26; 23.68, p<0.001). Similarly the odds of an exacerbation being eosinophilic was 9 times higher in those who had blood eosinophils≥2% than in subjects with blood eosinophils<2% at enrolment (OR 9.16, 95%CI 4.10; 20.47, p<0.001).

Seasonality of Eosinophilic Exacerbations

Exacerbations were more common in the Winter than in the Summer season (217 and 138 respectively). The proportion of exacerbations with eosinophils≥2% was higher in the Summer than Winter, however the number of eosinophilic exacerbations per season was similar (86/217 in Winter and 82/138 in Summer). (Figure 4). The PE group had a similar exacerbation rate in Summer and Winter seasons while the IE and RE groups had higher exacerbation rates in Winter (Table E3).

After adjusting for the contribution of multiple exacerbations by some subjects, the odds of an exacerbation having blood eosinophils≥2% were 2.65 times higher in the

Summer season than in Winter. (Table E4)

Both being in the PE compared to the RE group, and Summer compared to Winter were independently associated with the odds of an exacerbation having blood eosinophils≥2% (OR 11.39, 95%CI 4.68; 27.72, p<0.001 and 2.57, 1.37; 4.85, p=0.003 respectively).

We then repeated these analyses, stratifying by EI longitudinal phenotypes. The odds of an exacerbation having eosinophils≥2% in the Summer compared to the Winter season tended to be stronger in the PE group (OR 3.73, 95%CI 1.56; 8.91, p=0.003) than in the IE and RE groups (OR 2.00, 95%CI 0.32; 12.59, p=0.460 and 1.36, 0.42; 4.44, p=0.606 respectively).

Airway infection and EI

The increase in the number of non-eosinophilic exacerbations during Winter may indicate underlying changes in the lung microbiome as a result of the known seasonal effects of viral and bacterial infections[19]. We therefore examined whether the association between seasonality and prevalence of blood eosinophils≥2% at exacerbation persisted when accounting for PPM presence at exacerbation, and found that this association did not diminish when accounting for presence of PPMs (OR 2.39, 95%CI 1.29; 4.41, p=0.005).

PPMs were present in 59% of all exacerbations with valid data (n=320) and 61% of 1st exacerbations (n=98). We studied PPM prevalence at 1st exacerbation in the longitudinal eosinophilic groups and found a significant difference in PPM prevalence; PPMs were more prevalent in RE subjects (86%) than in the other two groups (predominant and intermittent, 57% and 55% respectively, p=0.044) (Table 3). There was no such association in microbiology found between the three groups

at enrolment (n=99, p=0.952) (Table 2). Respiratory viruses were detected at 41% of all exacerbations with valid data (n=305) and 43.3% of 1st exacerbations (n=90) (Table 3). We found no significant difference between the eosinophilic groups in the prevalence of the respiratory viruses either at enrolment or 1st exacerbations (Tables 2 and 3).

The odds of having a PPM present at an exacerbation was 75% lower in the PE group compared to the RE group but this association was of borderline significance (OR 0.25, 95%CI 0.06; 1.09; p=0.065). The odds of PPM presence were 55% lower in eosinophilic AECOPD compared to exacerbations with blood eosinophils<2%, however this was also of borderline significance (OR 0.450, 95%CI 0.240; 0.998, p=0.049).

The odds of having PPM present at exacerbation were higher in Winter than Summer (OR 2.51, 95%CI 1.27; 4.96, p=0.008). This seasonal effect was apparent in the PE group (OR 4.74, 95%CI 1.43; 15.71, p=0.011) but in the IE and RE groups, no statistically significant seasonal variation in PPM at exacerbation was detected (OR 4.42, 95%CI 0.01; 3476.74, p=0.662, and OR 1.15, 95%CI 0.29; 4.56, p=0.838).

Discussion

Eosinophilic inflammation is a stable longitudinal phenotype in a substantial proportion of COPD patients, which can be predicted over 12 months by an initial blood level measurement. We report for the first time that EI was more prevalent at exacerbation in those with predominantly raised eosinophils at stable state. In the Summer a larger proportion of exacerbations were eosinophilic, although this was driven by fewer non-eosinophilic exacerbations in Summer compared to Winter. We also report evidence that eosinophilic exacerbations are less frequently associated with airway bacterial infection, with the prevalence of airway bacterial infection at exacerbations greater among the group who rarely had raised eosinophils over time. These findings have a potential implication for future therapeutic clinical trials and eosinophil targeted treatment with a view to stratifying patients' care.

To our knowledge this is the most detailed description of inflammatory phenotype at COPD exacerbations, seasonality and infectious aetiology in longitudinal groups stratified by eosinophil levels. The seasonality of exacerbations has been previously described.[21-24] In our analysis we saw a clear seasonal pattern for all exacerbations but when focused on eosinophil-associated exacerbations we report that these did not appear to show much seasonal variation, resulting in a larger proportion of exacerbations being associated with EI in the summer. The aetiology of EI in COPD would appear to be driven by factors other than atopy as, although not formally tested, our patients had no recorded manifestations of atopic disease. Furthermore, the stable incidence of eosinophil-associated AECOPD throughout the year may suggest an intrinsic factor in triggering their incidence, and requires further study.

It was previously reported that ≥2% blood eosinophils identified sputum EI at exacerbation (>3%) and was 90% sensitive and 60% specific.[3] Using the same cut

off for blood EI at enrolment in our analysis, it corresponded to the >3% sputum cut off with similar sensitivity but lower specificity (sensitivity 95.8% and specificity 31.8%). One reason for this discrepancy in specificity might be due to the difference in clinical states at the time of analysis; Bafadhel et al conduced the analysis at exacerbations when sputum capture is greater whereas in our study we conducted the analysis during clinical stability. We found that at exacerbation ≥2% blood eosinophils were 79.6% sensitive and 53.9% specific. In our study the blood eosinophil count was reported up to one decimal, therefore it was not possible to apply the cut off of 150cells/uL. Using the 200cells/uL cutoff offered a similar sensitivity (95.8%) but inferior specificity (27.3%). We conducted a sensitivity analysis with the ≥200cells/uL cut off and found that there was a significant difference in age, smoking history, exacerbation rate with raised eosinophils in the first year and presence of sputum eosinophils>3% at enrolment.

The rationale of our method to describe the longitudinal eosinophilic phenotype was to focus the analysis on subjects who were PE (allowance of one non-eosinophilic event) as opposed to persistently (all visits were eosinophilic). This rule, we feel, represents a more pragmatic and “real world” approach. Applying this rule, we demonstrated that 58% of subjects in our cohort had predominantly raised eosinophils over the period of 12 months. These PE subjects were slightly older than RE patients (p=0.034) and there were trends towards a lower prevalence of current smokers but with a greater smoking history in the PE group. Singh et al studied longitudinal eosinophilic phenotype and reported that 37.4% of subjects had persistently elevated blood eosinophils ≥2% at all visits over the period of 3 years.[4] In a previous asthma study a 90% rule was applied to identify persistent eosinophilic inflammation in sputum.[25] However, due to the limited number of samples available

(maximum 5 samples) in our cohort this rule was not applied. A limitation of the longitudinal phenotype method was that individuals with 3 visits could not be classified as IE (n=20/99 for blood, and 14/80 for sputum).

Bacteria play an important role in exacerbations of COPD.[26-30] Bacterial exacerbation had been previously reported to be rarely associated with sputum eosinophilic exacerbation.[3] We investigated the prevalence of PPMs at exacerbation in the prospective eosinophilic groups and found that PPMs were far less common in the eosinophilic group. When we analysed PPM presence at exacerbation in combination with blood EI, we found eosinophilic inflammation to be associated with reduced odds of PPM presence, and again while the magnitude of the difference was large (55% less likely) this was again of borderline significance (p=0.049). Therefore understanding the clinical phenotype of stable inflammation may be a useful tool to stratify bacterial aetiology of exacerbations and hence antibiotic use.

Whilst our detection of respiratory viruses at AECOPD corresponded with previously reported findings,[30, 31] we observed no significant difference in the prevalence of respiratory viruses between the three groups at enrolment and at 1st exacerbations. Previously Papi et al reported airway eosinophilic inflammation to be a good predictor of viral infections[30]. However in their study only severe exacerbations were included [30] whereas in our study we captured mild, moderate and severe events. Further studies across the disease spectrum are required to ascertain the mechanisms linking infection and inflammatory patterns of disease. However, it is noteworthy, that there was no significant difference in use of inhaled corticosteroids and bronchial reversibility across groups, similar to previously reported studies.[3, 15]

It is important to recognise that these results are representative of a cohort of moderate to very severe COPD patients with frequent exacerbations receiving a high

level of clinical intervention, including ICS, as part of an intensive study. This might have had an impact on the severity of exacerbations and recovery, as previous reports indicate that early therapy improves exacerbation outcomes.[32] Thus the number of potential severe exacerbations might be smaller. Secondly, the AERIS study was not originally designed, and therefore was not powered, to specifically investigate longitudinal EI and hence the numbers that were included in each eosinophilic group were limited. Larger prospective studies are required to further understand the impact of eosinophilic inflammatory status on a range of clinical outcomes.

In summary, this is the first study to report that EI is a stable phenotype in COPD and predictive of eosinophilic exacerbations. These events are seasonal in nature and relate to bacterial aetiology. Our data suggest that stratifying COPD patients into eosinophilic groups to potentially aid management is clinically relevant and potentially important, as is the consideration of season in management of exacerbations. Whether oral corticosteroids should be administered during exacerbations of COPD to PE patients, in particular, outside the Winter season, requires further investigation along with other stratification paradigms through well designed intervention studies.

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Authors' contributions:

JMD, EA, SCB, SAW, ACT, SCC, VLK and TMAW were involved in the study conception and design

JMD, EA, SCB, SAW, ACT, NPW, KKO, KJS, SCC, VLK, and TMAW were involved in acquisition and generation of data

JMD, EA, SCB, SAW, NPW, KKO, KJS, SCC, NAC, VLK and TMAW were involved in data analysis and data interpretation

All authors contributed substantially to the development of the manuscript and approved the final version.

Conflict of interest: TMAW has received reimbursement for travel and meeting attendance from Boehringer Ingelheim and AstraZeneca, outside of the submitted work. KJS received grants from Asthma UK (08/026) and BMA HC Roscoe Award outside of the submitted work, and he has a patent PCT/GB2010/050821 "Ex Vivo Modelling of Therapeutic Interventions" pending. EA, JMD are employees of the GSK group of companies. SCC received grants from Pfizer outside of the submitted work. EA, JMD hold shares/restricted shares in the GSK group of companies. KJS, VK, NPW, KKO, SAW, SCC, and TMAW received an institutional grant from the GSK group of companies to conduct this study. ACT, NAC, and SCB declare no conflict of interest.

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Figure legends

Figure 1.

Flow diagram of the subjects screened and included in the full cohort at the start of Year 1 and the number of exacerbations in Year 1.

Figure 2.

Receiver operating characteristic curve with area under the curve for blood eosinophil (count & %) at enrolment predicting sputum eosinophilia >3% at enrolment (n=68).

Figure 3.

Receiver operating characteristic curve for blood eosinophils (count and %) at enrolment predicting the predominantly eosinophilic group over 12 months following enrolment) (n=78).

Figure 4.

Seasonal distribution of total and eosinophil-associated exacerbations. **A** -Number of total and exacerbations with blood eosinophils≥2%. **B** - Proportion of exacerbations with blood eosinophils≥2% defined as exacerbations with blood eosinophils≥2% to total exacerbation rates in the predominantly, intermittent and rarely groups.

Table 1. Baseline characteristics of the total COPD cohort (n=127).

Continuous variables	N	Median	(IQR)
Age α	127	66.8	(8.61)
Smoking history (pack/years) α	127	50.3	(28.2)
BMI	127	27.0	(6.69)
FFBM	125	48.9	(21.5)
WBC	126	7.60	(2.20)
Eosinophils (count)	126	0.20	(0.20)
Eosinophils (%)	126	2.94	(3.08)
Neutrophils (count)	126	4.80	(1.70)
Fibrinogen	114	4.80	(1.02)
CRP	127	5.00	(8.00)
Eosinophils (%)≠	83	1.93	(5.05)
Neutrophils (%)≠	83	47.0	(70.4)
FEV1 (%)	126	46.7	(25.3)
ΔFEV1(% of baseline)ξ	85	4.93	(21.1)
FEV1 reversibility (% of preBDFEV1)€	105	11.2	(17.0)
KCO (%)	122	69.2	(30.7)
TLCO(%)	122	57.9	(29.5)
CAT	126	16.0	(10.0)
6MWT (distance in meters)	125	300	(170)
Exact score	101	37.0	(12.0)
Exacerbation rate in year before study	127	2.00	(2.00)
Exacerbation rate in first year of study	127	2.94	(3.91)
Eosinophilic exacerbation rate in first year of study	127	0.99	(2.02)
Follow up (in years) in first year of study	127	1.00	(0.02)

Categorical variables		N	(%)
Sex	Male	68	(54%)
	Female	59	(46%)
Current smoker γ	Yes	54	(43%)
	No	73	(57%)
Frequent Exacerbators in Yr0	Yes	99	(78%)
	No	28	(22%)
Use of ICS at enrolment β ¥	Yes	113	(89%)
	No	14	(11%)
Sputum eosinophilia (>3%)≠	Yes	27	(33%)
	No	56	(67%)
Blood eosinophilia (>=2%)	Yes	86	(68%)
	No	40	(32%)
Bacteria present*	Yes	57	(52%)
	No	53	(48%)
Virus present**	Yes	18	(17%)
	No	85	(83%)
Blood eosinophils ≥200 cells/uL	Yes	90	(71.4%)
	No	36	(28 .6%)

α reported as Mean(±SD)

γ smoking status report based derived from ATS Q7A4

¥ICS use were coded as "Yes" if one of the following medications/inhalers was on the list (SYMBICORT, SERETIDE, QVAR, FOSTAIR, BECLOMETHASONE, BECLAMETHOSONE/FORMOTEROL, BECLOMETHASONE dipropiionate, CLENIL, FLUTICASONE/SALMETEROL, BUDESONIDE/FORMOTEROL)

≠Sputum eosinophil and neutrophil % at baseline is reported. "Baseline" is equal to enrolment if good quality data (SQC<30) is present at enrolment, or the next (pre-exacerbation) stable visit with quality data within four months of enrolment.

ξ calculated as FEV1 at month 12 * 100 / FEV1 at enrolment

€ calculated as (post broncho dilator BDFEV1 - preBD broncho dilator FEV1) / pre BDbroncho dilator FEV1 * 100

* Sputum sampling, measured by culture. Includes Haemophilus influenzae, Moraxella catarrhalis, Streptococcus pneumoniae, Staphylococcus aureus and Pseudomonas aeruginosa.

** Sputum sampling, measured by PCR. Includes adenovirus, enterovirus, influenza, coronavirus, metapneumovirus, bocavirus, parainfluenza, RSV, and rhinovirus.

Table 2. Baseline characteristics by longitudinal blood eosinophil group over 12 months (n=99).

		Rarely eosinophilic (n=26)			Intermittently eosinophilic (n=19)			Predominantly eosinophilic (n=59)			P valueΩ
Continuous variables		N	Median	(IQR)	N	Median	(IQR)	N	Median	(IQR)	
Age α		26	62.7	(8.63)	16	67.9	(6.87)	57	68.0	(9.05)	0.034
Smoking history (pack/years) α		26	46.3	(22.8)	16	64.9	(43.2)	57	51.0	(28.6)	0.098
BMI		26	28.2	(8.15)	16	27.9	(5.69)	57	25.9	(4.73)	0.280
FFBM		25	53.5	(21.5)	16	49.3	(18.3)	56	48.8	(22.4)	0.886
WBC		25	8.30	(3.30)	16	7.65	(1.65)	57	7.30	(2.00)	0.060
Blood eosinophils (count)		25	0.10	(0.10)	16	0.20	(0.10)	57	0.30	(0.20)	N/A
Blood eosinophils (%)		25	1.35	(1.45)	16	2.12	(1.09)	57	4.08	(3.05)	N/A
Blood neutrophils (count)		25	5.20	(2.55)	16	4.80	(0.50)	57	4.30	(1.75)	0.030
Fibrinogen		23	4.70	(1.40)	14	4.75	(0.97)	51	4.80	(0.90)	0.905
CRP		26	4.00	(7.50)	16	5.00	(5.75)	57	5.00	(7.00)	0.966
Sputum eosinophils (%)≠		18	0.43	(2.89)	7	0.82	(1.78)	40	2.81	(7.40)	0.070
Sputum neutrophils (%)≠		18	9.77	(64.0)	7	62.3	(63.4)	40	38.0	(71.6)	0.223
FEV1 (%)		26	47.7	(25.6)	16	43.9	(22.4)	57	47.5	(26.0)	0.661
ΔFEV1(% of baseline)ξ		19	3.79	(27.3)	15	10.8	(23.2)	45	5.03	(21.4)	0.375
FEV1 reversibility (% of preBDFEV1)€		24	7.69	(15.0)	13	17.9	(16.6)	46	12.6	(12.5)	0.355
KCO (%)		25	74.2	(31.0)	16	68.3	(26.7)	53	69.3	(30.3)	0.526
TLCO(%)		25	62.0	(41.1)	16	57.8	(28.8)	53	56.3	(27.7)	0.407
CAT		26	15.5	(10.0)	16	20.5	(13.0)	57	16.0	(9.00)	0.101
6MWT (distance in meters)		25	300	(199)	16	323	(172)	56	327	(164)	0.914
Exact score		23	36.0	(14.0)	14	36.5	(7.00)	46	36.0	(15.0)	0.607
Exacerbation rate in year before study		26	2.00	(3.00)	16	2.00	(3.00)	57	2.00	(2.00)	0.941
Exacerbation rate in first year of study		26	2.47	(3.22)	16	1.02	(2.00)	57	2.04	(3.95)	0.197
Eosinophil predominant exacerbation rate in first year of study		26	0.00	(0.99)	16	0.00	(0.99)	57	1.38	(2.99)	<0.001
Follow up (years) in first year of study		26	1.01	(0.02)	16	1.01	(0.01)	57	1.01	(0.02)	0.448
Categorical variables		N	(%)		N	(%)		N	(%)		
Sex	Male	12	(46%)		9	(56%)		34	(60%)		0.547
	Female	14	(54%)		7	(44%)		23	(40%)		
Current smokerγ μ	Yes	15	(58%)		4	(25%)		24	(42%)		0.125
	No	11	(42%)		12	(75%)		33	(58%)		
Use of ICS at enrolment β ¥	Yes	21	(81%)		15	(94%)		51	(89%)		0.468
	No	5	(19%)		1	(6%)		6	(11%)		
Sputum eosinophils (>3%)≠	Yes	2	(11%)		0	(0%)		19	(48%)		0.003
	No	16	(89%)		7	(100%)		21	(53%)		
Blood eosinophils (>=2%)	Yes	7	(28%)		9	(56%)		55	(96%)		N/A
	No	18	(72%)		7	(44%)		2	(4%)		
Bacteria present*	Yes	12	(55%)		6	(50%)		26	(50%)		0.952
	No	10	(45%)		6	(50%)		26	(50%)		
Virus present**	Yes	4	(19%)		0	(0%)		10	(20%)		0.288

No	17 (81%)	12 (100%)	40 (80%)	

Ω Test for differences between the three longitudinal eosinophil groups. Kruskall-Wallis test for continuous variables, Chi-Square test for categorical variables

α reported as Mean(±SD)

γ smoking status report based derived from ATS Q7A4

¥ICS use were coded as "Yes" if one of the following medications/inhalers was on the list (SYMBICORT, SERETIDE, QVAR, FOSTAIR, BECLOMETHASONE, BECLAMETHOSONE/FORMOTEROL, BECLOMETHASONE dipropiionate, CLENIL, FLUTICASONE/SALMETEROL, BUDESONIDE/FORMOTEROL)

≠Sputum eosinophil and neutrophil % at baseline. "Baseline" is equal to enrolment if good quality data (SQC<30) is present, or the next (pre-exacerbation) stable visit with quality data within four months of enrolment.

ξ calculated as FEV1 at month 12 * 100 / FEV1 at enrolment

Є calculated as (post broncho dilator BDFEV1 - preBD broncho dilator FEV1) / pre BDbroncho dilator FEV1 * 100

* Sputum sampling, measured by culture. Includes Haemophilus influenzae, Moraxella catarrhalis, Streptococcus pneumoniae, Staphylococcus aureus and Pseudomonas aeruginosa.

** Sputum sampling, measured by PCR. Includes adenovirus, enterovirus, influenza, coronavirus, metapneumovirus, bocavirus, parainfluenza, RSV, and rhinovirus.

Table 3. Characteristics at 1st exacerbation overall (n=108), and by longitudinal blood eosinophil group over 12 months (n=87)

		Overall cohort (n=108)			Rarely eosinophilic (n=26)			Intermittently eosinophilic (n=13)			Predominantly eosinophilic (n=48)			P value Ω
Continuous variables		**N**	**Median**	**(IQR)**	N	Median	(IQR)	N	Median	(IQR)	N	Median	(IQR)	
WBC		**102**	**8.00**	**(3.53)**	25	8.70	(4.25)	12	8.95	(2.55)	48	7.30	(3.23)	0.349
Blood eosinophils (count)		**102**	**0.20**	**(0.20)**	25	0.10	(0.15)	12	0.15	(0.10)	48	0.30	(0.27)	<0.001
Blood eosinophils (%)		**102**	**2.07**	**(2.78)**	25	1.19	(1.74)	12	1.92	(0.88)	48	3.39	(4.40)	<0.001
Blood neutrophils (count)		**102**	**5.30**	**(3.20)**	25	5.60	(4.05)	12	5.55	(2.57)	48	4.45	(2.98)	0.045
Fibrinogen		**103**	**5.10**	**(1.40)**	25	4.80	(1.80)	12	5.50	(2.25)	46	5.10	(1.60)	0.135
CRP		**103**	**8.00**	**(14.0)**	25	7.00	(12.5)	12	26.5	(49.8)	47	7.00	(13.0)	0.087
Sputum eosinophils (%)		**61**	**1.20**	**(2.38)**	15	0.62	(2.29)	9	1.40	(3.23)	28	1.48	(3.56)	0.273
Sputum neutrophils (%)		**61**	**74.0**	**(47.1)**	15	76.8	(51.6)	9	86.6	(40.3)	28	74.1	(52.3)	0.616
FEV1 (%)		**97**	**42.7**	**(21.2)**	22	47.3	(30.0)	12	41.5	(17.5)	44	41.7	(20.0)	0.395
CAT		**106**	**21.0**	**(11.0)**	26	22.0	(12.0)	13	22.0	(17.0)	47	19.0	(8.00)	0.915
EXACT-PRO		**97**	**41.0**	**(11.0)**	23	43.0	(13.0)	12	40.0	(10.0)	44	39.5	(7.00)	0.142
Categorical variables		**N**	**(%)**		N	(%)		N	(%)		N	(%)		
Bacteria present*	Yes	**60**	**(61%)**		19	(86%)		6	(55%)		27	(57%)		0.044
	No	**38**	**(39%)**		3	(14%)		5	(45%)		20	(43%)		
Virus present**	Yes	**40**	**(43%)**		12	(55%)		4	(40%)		14	(33%)		0.235
	No	**53**	**(57%)**		10	(45%)		6	(60%)		28	(67%)		
Sputum eosinophils>3%	Yes	**23**	**(30%)**		2	(11%)		0	(0%)		17	(47%)		0.004
	No	**54**	**(70%)**		16	(89%)		7	(100%)		19	(53%)		
Blood eosinophils≥2%	Yes	**73**	**(68%)**		7	(28%)		7	(54%)		47	(98%)		<0.001
	No	**34**	**(32%)**		18	(72%)		6	(46%)		1	(2%)		

Ω Test for differences between the three longitudinal eosinophil groups. Kruskall-Wallis test for continuous variables, Chi-Square test for categorical variables
* Sputum sampling, measured by culture. Includes Haemophilus influenzae, Moraxella catarrhalis, Streptococcus pneumoniae, Staphylococcus aureus and Pseudomonas aeruginosa.
** Sputum sampling, measured by PCR. Includes adenovirus, enterovirus, influenza, coronavirus, metapneumovirus, bocavirus, parainfluenza, RSV, and rhinovirus

Impact and Associations of Eosinophilic Inflammation in COPD: Analysis of the AERIS Cohort

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Take Home Message: Blood eosinophil levels in COPD predicts the nature of inflammation at future exacerbations and may guide therapy

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Abstract

Eosinophilic inflammation in COPD predicts response to treatment especially corticosteroids. We studied the nature of eosinophilic inflammation~~eosinophilia~~ in COPD prospectively to examine the stability of this phenotype and its dynamics across exacerbations and its associations with clinical phenotype, exacerbations and infection.

127 patients aged 40–85 with moderate-severe COPD underwent repeated blood and sputum sampling at stable visits and within 72 h of exacerbation for one year.

Blood eosinophils ≥2% was prevalent at baseline and predicted both predominantly raised stable state eosinophils across the year (AUC 0.841, 95%CI 0.755; 0.928), and increased risk of eosinophilic inflammation at exacerbation (OR 9.16 p<0.001). ~~Higher e~~Eosinophils≥2% at exacerbation and ~~predominant eosinophilia predominantly raised eosinophils~~eosinophil predominance at stable visits were associated with a lower risk of bacterial presence at exacerbation (OR 0.49, p=0.049 and OR 0.25, p=0.065 respectively). Bacterial infection at exacerbation was highly seasonal (Winter vs Summer OR 4.74, p=0.011) in predominantly eosinophilic patients.

Eosinophilic inflammation is a common and stable phenotype in COPD. Blood eosinophil counts in the stable state can predict the nature of inflammation at future exacerbations which when combined with an understanding of seasonal variation provides the basis for the development of new treatment paradigms for this important condition.

Key Words: COPD EOSINOPHILS INFLAMMATION EXACERBATIONS AERIS

1

3

Introduction

Chronic obstructive airway disease (COPD) is an established cause of global mortality and morbidity, predicted to be the third leading cause of death by 2030.[1] Current guidelines for COPD management are based on airway obstruction, symptoms and exacerbation frequency.[2] The guidelines advise on step-wise management but do not fully account for the biological heterogeneity of this debilitating condition.

Eosinophilic inflammation (EI) was historically thought to be a feature of asthma with neutrophilic inflammation being a classical hallmark of COPD. However, recent studies have demonstrated that EI is present in a subset of COPD patients both at exacerbations[3] and during clinical stability.[4] A sputum eosinophil differential count of >3% is an accepted marker of airway EI and is derived from the reported enhanced response of this group of patients to corticosteroids.[5, 6] Moreover, a good relationship has been demonstrated between airway and systemic eosinophil counts.[3, 4, 7] It was previously reported that a >2% blood eosinophil cut off had a high sensitivity in identifying >3% airway eosinophils during acute exacerbations of COPD (AECOPD)[3], suggesting that peripheral eosinophils are a clinically accessible marker to predict inflammatory phenotype.

Using 2% blood eosinophils as a cut off in the longitudinal ECLIPSE cohort, persistently elevated blood eosinophils was reported as a common finding,[4] especially in milder disease, but exacerbations were not sampled. Reports of relationships between eosinophils and FEV1 and exacerbation frequency vary [4 8 9, 10] [11, 12], but emerging evidence suggests measurement of eosinophils has clinical relevance. For example, severe AECOPD with EI are associated with a shorter length of stay in hospital.[13] Furthermore, there is an association between EI with a

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greater response to steroid therapy at both stable COPD and during exacerbations.[5, 6, 14, 15, 16]

Management of asthma stratified by airway EI has led to a reduced rate of exacerbations.[17] Siva et al demonstrated that COPD treatments targeting airway eosinophils was associated with a significant reduction of severe exacerbations[14].

Hence EI is an important COPD endotype but little is known about its stability over time, or its relationships to the inflammatory nature and aetiology of exacerbations. To improve our understanding, we investigated these factors in a secondary analysis of the AERIS cohort, a prospective, longitudinal study of patients with COPD.[18]

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Methods

Study design and study population

The Acute Exacerbation and Respiratory InfectionS in COPD (AERIS) study is a prospective, observational cohort study based at University Hospital Southampton (UHS), registered with ClinicalTrials.gov (NCT01360398). The study protocol and full inclusion and exclusion criteria have been published previously.[18] The protocol summary is available at www.gsk-clinicalstudyregister.com (study identifier, 114378). AERIS was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice, and was approved by the Southampton and South West Hampshire Research Ethics Committee. All participants provided written informed consent. Only patients with a confirmed diagnosis of COPD based on postbronchodilator spirometry with FEV1 ≤80% of predicted normal and FEV1/FVC <0.7, ex-smokers or current smokers with significant smoking history (≥10 pack year history) were included into the cohort, with no patients having diagnosis of asthma at the time of recruitment.[18]

Patients were followed monthly in the stable state and reviewed within 72 hours of onset of AECOPD symptoms. Exacerbations were detected using daily electronic diary cards. Definition of AECOPD and severity, was described previously[18, 19].

Procedures

Blood and sputum analyses were performed as previously described[18, 19]. Further methodological detail is provided in the online supplement.

Criteria for eosinophilic groups and seasonality

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EI was defined as sputum eosinophils >3% and blood eosinophils ≥2% in line with previous studies.[3, 5, 6, 14, 20] To investigate the stability of blood EI over time we divided subjects into three groups: predominantly (PE), intermittent (IE) and rarely (RE) eosinophilic. Only those subjects who had at least 3 (out of 5 potential) stable visits with valid blood results over 12 months were included in the group analyses (n=99). The PE group was defined as blood eosinophils ≥2% at either all visits, or all but 1 visits where the blood eosinophils were <2%; the RE group was defined as blood eosinophils <2% at all visits, or all but 1 visit where the blood eosinophils were ≥2%; the IE group was defined when none of the abovementioned criteria were met.

To investigate an impact of seasonality on exacerbations we divided the year into 2 seasons; Winter (October-March) and Summer (April – September).

Statistical analysis

Bivariate analyses testing for differences between eosinophilic groups were conducted using two-tailed, Kruskall-Wallis, ANOVA, Chi-Square, or Fisher's Exact test, as appropriate. Receiver Operator Curves (ROC) were used to assess the predictive ability of different cut offs to correctly identify presence of sputum EI. Intra-class correlations were used to assess the reliability of measures within individuals over time. Because some subjects were represented multiple times in exacerbation analysis, descriptive analyses were conducted only for the first exacerbation occurring to each subject, and multivariate analyses with binary outcomes (presence/absence of different conditions at exacerbation) were conducted using conditional logistic regression, including the subject number as a random effect. SPSS (version 22) was used for all analyses with the exception of intra-class correlation coefficients (ICC) and conditional logistic regression, which were

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conducted using STATA (version 14). All of these analyses should be considered post hoc as they were not pre-specified in the AERIS statistical analysis plan. All of these analyses should be considered post hoc as they were not pre-specified in the AERIS statistical analysis plan.

Results

General characteristics of the cohort

152 patients were screened but only 127 patients were included in the AERIS cohort (Figure 1). The cohort consisted of patients with moderate to very severe airway obstruction, the majority (87.3%) were receiving inhaled corticosteroids and had a substantial smoking history (mean pack years 50.3, SD 28.2 - Table 1). Only patients who experienced at least 1 moderate to severe exacerbation in the last 12 months were included in the cohort (median rate 2.00, IQR 2.00).

Clinical and Inflammatory Characterisation at Enrolment

~~Applying the previously published cut-offs, t~~The prevalence of EI ≥3% in sputum in our cohort at enrolment was 33% (n=27) and in blood ≥2% was 68% (n=86). Blood eosinophils (% and count) displayed moderately strong positive correlations with sputum eosinophil % (rho 0.463 and 0.581 respectively at enrolment, p<0.001) and were predictive of ~~raised~~ sputum eosinophils≥3% (AUC 0.851, 95%CI 0.750; 0.951, and AUC 0.768, 95%CI 0.651; 0.884 respectively) (Figure 2)~~, with a~~. This ≥2% blood eosinophil cut-point ~~being~~ was 95.8% sensitive and 31.8% specific. ~~Similar associations were observed at exacerbation (Figure E1).~~ Using absolute cell numbers (≥200cells/uL) rather than percentage gave similar results (Table E1).

~~In order to assess the longitudinal stability of blood EI, we analysed the reliability of the marker in defining a longitudinal phenotype and found blood eosinophils to be relatively stable within individuals across the 12 months examined, including enrolment (ICC 0.66, CI 0.58; 0.74). We categorised our patients into the three longitudinal EI phenotypic groups and found that, out of the 99 patients with sufficient~~

~~data, 57 (58%) were predominantly (PE), 16 (16%) intermittently (IE) and 26 (26%) rarely eosinophilic (RE) over the 12 months (Table 1). Significant differences between patients included and excluded from this analysis were seen for CRP, TLCO, 6MWT and the length of follow-up (Table E2)~~

~~Between the three longitudinal groups, the only significant differences observed at enrolment were age, WBC, EXACT score, and sputum eosinophils~~blood neutrophils, presence of sputum eosinophils>3% and trending towards significance in WBC and sputum eosinophils. ~~(Table 2) There was also a significant difference at exacerbation rate with blood eosinophils≥2% over the first year.~~

Stability of Eosinophilic Phenotype over Time

We categorised our patients into three longitudinal EI phenotypic groups based on blood eosinophils ≥2% and found that, out of the 99 patients with sufficient data, 57 (58%) were predominantly (PE), 16 (16%) intermittently (IE) and 26 (26%) rarely eosinophilic (RE) over the 12 months (Table 2). Between the three longitudinal groups, the only significant differences observed at enrolment were age, blood neutrophils, and presence of sputum eosinophils>3%. (Table 2) There were also significant differences in the eosinophil predominant exacerbation rate over the first year. Significant differences between patients included and excluded from this analysis were seen for CRP, 6MWT and the length of follow-up (Table E2). In order to assess the longitudinal stability of blood EI, we analysed the reliability of the marker in defining a longitudinal phenotype and found blood eosinophils to be relatively stable within individuals across the 12 months examined, including enrolment (ICC 0.66, CI 0.58; 0.74).

We next examined whether blood eosinophils at a single time point (enrolment) was a useful predictor of being PE over time (categorisation for the EI groups excluding enrolment, n=78). Blood eosinophils (% and count) at enrolment were predictive of being in the PE group over the next 12 months (AUC 0.841, 95%CI 0.755; 0.928 and 0.806, 0.710; 0.901 respectively, Figure 3). A blood eosinophil cut off of ≥2% was 84.3% sensitive and 48.1% specific in identifying those in the PE group over the next 12 months.

Blood EI groups at exacerbation and exacerbation rates

Across all exacerbations with valid data the prevalence of EI in sputum and in blood was 23.9% (n=52/218) and 49.7% (n=168/338) respectively. ~~Similar associations~~Blood eosinophils ~~≥2~~(% and count) were again predictive of sputum eosinophils≥3% ~~were observed~~ at exacerbation (AUC 0.735 95%CI 0.654; 0.817 and and 0.729, 0.650; 0.809 respectively - Figure E1). ~~Longitudinal eosinophilic groups did not differ symptomatically at first exacerbation (CAT and EXACT-PRO) but there was a significant difference in blood neutrophils at exacerbation between these groups (p=0.045), and the predominantly eosinophilic group was less likely to have bacteria present (p=0.044). (Table 3)~~

The total exacerbation rate (median(IQR)) in the first year of the study was similar in the RE and the PE groups, but lower in the intermittent group (PE 2.04(3.95), IE 1.02(2.00) and RE 2.47(3.22)). There was a significant difference in the rate of exacerbations with blood eosinophils ≥2%, with the predominant group having a higher eosinophilic exacerbation rate (PE(1.38(2.99)), IE(0.00(0.99)), and RE(0.00(0.99)), p<0.001).

Longitudinal eosinophilic groups did not differ symptomatically at first exacerbation

(CAT and EXACT-PRO) but there was a significant difference in blood neutrophils at exacerbation between these groups (p=0.045), and the predominantly eosinophilic group was less likely to have bacteria present (p=0.044 -). (Table 3).

There was an association between longitudinal eosinophilic phenotype and eosinophils at exacerbation, with the odds of raised eosinophils≥2% at exacerbation much higher in the PE group compared to the RE group (OR 11.16, 95%CI 5.26; 23.68, p<0.001). Similarly the odds of an exacerbation being eosinophilic was 9 times higher in those who had raised blood eosinophils≥2% than in subjects without raisedwith blood eosinophils (<2%) at enrolment (OR 9.16, 95%CI 4.10; 20.47, p<0.001).

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Seasonality of Eosinophilic Exacerbations

Exacerbations were more common in the Winter than in the Summer season (217 and 138 respectively). The proportion of exacerbations with raised eosinophils≥2% was higher in the Summer than Winter, however the number of eosinophilic exacerbations per season was similar (86/217 in Winter and 82/138 in Summer). (Figure 4). The PE group had a similar exacerbation rate in Summer and Winter seasons while the IE and RE groups had higher exacerbation rates in Winter (Table E3).

After adjusting for the contribution of multiple exacerbations by some subjects, the odds of an exacerbation having blood eosinophils≥2% were 2.292.65 times higher in the Summer season than in Winter (95%CI 1.46; 3.58). (Table E4)

Both being in the PE compared to the RE group, and Summer compared to Winter

were independently associated with the odds of an exacerbation having blood eosinophils ~~–~~≥2% (OR 11.39, 95%CI 4.68; 27.72, p<0.001 and 2.57, 1.37; 4.85, p=0.003 respectively).

We then repeated these analyses, stratifying by EI longitudinal phenotypes. The odds of an exacerbation having ~~raised~~ eosinophils≥2% in the Summer compared to the Winter season tended to be stronger in the PE group (OR 3.73, 95%CI 1.56; 8.91, p=0.003) than in the IE and RE groups (OR 2.00, 95%CI 0.32; 12.59, p=0.460 and 1.36, 0.42; 4.44, p=0.606 respectively).

Airway infection and EI

The increase in the number of non-eosinophilic exacerbations during Winter may indicate underlying changes in the lung microbiome as a result of the known seasonal effects of viral and bacterial infections[19]. We therefore examined whether the association between seasonality and prevalence of ~~raised~~ blood eosinophils≥2% at exacerbation persisted when accounting for PPM presence at exacerbation, and found that this association did not diminish when accounting for presence of PPMs (OR 2.39, 95%CI 1.29; 4.41, p=0.005).

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PPMs were present in 59% of all exacerbations with valid data (n=320) and 61% of 1st exacerbations (n=98). We studied PPM prevalence at 1st exacerbation in the longitudinal eosinophilic groups and found a significant difference in PPM prevalence; PPMs were more prevalent in RE subjects (86%) than in the other two groups (predominant and intermittent, 57% and 55% respectively, p=0.044) (Table 3). There was no such association in microbiology found between the three groups at enrolment (n=99, p=0.952) (Table 2). Respiratory viruses were detected at 41% of all exacerbations with valid data (n=305) and 43.3% of 1st exacerbations (n=90)

(Table 3). We found no significant difference between the eosinophilic groups in the prevalence of the respiratory viruses either at enrolment or 1st exacerbations (Tables 2 and 3).

The odds of having a PPM present at an exacerbation was 75% lower in the PE group compared to the RE group but this association was of borderline significance (OR 0.25, 95%CI 0.06; 1.09; p=0.065). The odds of PPM presence were 55% lower in eosinophilic AECOPD compared to exacerbations with ~~out raised~~ blood eosinophils<2%, however this was also of borderline significance (OR 0.450, 95%CI 0.240; 0.998, p=0.049).

The odds of having PPM present at exacerbation were higher in Winter than Summer (OR 2.51, 95%CI 1.27; 4.96, p=0.008). This seasonal effect was apparent in the PE group (OR 4.74, 95%CI 1.43; 15.71, p=0.011) but in the IE and RE groups, no statistically significant seasonal variation in PPM at exacerbation was detected (OR 4.42, 95%CI 0.01; 3476.74, p=0.662, and OR 1.15, 95%CI 0.29; 4.56, p=0.838).

Discussion

Eosinophilic inflammation is a stable longitudinal phenotype in a substantial proportion of COPD patients, which can be predicted over 12 months by an initial blood level measurement. We report for the first time that EI was more prevalent at exacerbation in those with predominantly raised eosinophils at stable state. In the Summer a larger proportion of exacerbations were eosinophilic, although this was driven by fewer non-eosinophilic exacerbations in Summer compared to Winter. We also report evidence that eosinophilic exacerbations are less frequently associated with airway bacterial infection, with the prevalence of airway bacterial infection at exacerbations greater among the group who rarely had raised eosinophils over time. These findings have a potential implication for future therapeutic clinical trials and eosinophil targeted treatment with a view to stratifying patients' care.

To our knowledge this is the most detailed description of inflammatory phenotype at COPD exacerbations, seasonality and infectious aetiology in longitudinal groups stratified by eosinophil levels. The seasonality of exacerbations has been previously described.[21-24] In our analysis we saw a clear seasonal pattern for all exacerbations but when focused on eosinophil-associated exacerbations we report that these did not appear to show much seasonal variation, resulting in a larger proportion of exacerbations being associated with EI in the summer. The aetiology of EI in COPD would appear to be driven by factors other than atopy as, although not formally tested, our patients had no recorded manifestations of atopic disease. Furthermore, the stable incidence of ~~eosinophilic~~ eosinophil-associated AECOPD throughout the year may suggest an intrinsic factor in triggering their incidence, and requires further study.

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It was previously reported that ≥2% blood eosinophils identified sputum EI at

exacerbation (>3%) and was 90% sensitive and 60% specific.[3] Using the same cut off for blood EI at enrolment in our analysis, it corresponded to the >3% sputum cut off with similar sensitivity but lower specificity (sensitivity 95.8% and specificity 31.8%). One reason for this discrepancy in specificity might be due to the difference in clinical states at the time of analysis; Bafadhel et al conduced the analysis at exacerbations when sputum capture is greater whereas in our study we conducted the analysis during clinical stability. We found that at exacerbation ≥2% blood eosinophils were 79.6% sensitive and 53.9% specific. In our study the blood eosinophil count was reported up to one decimal, therefore it was not possible to apply the cut off of 150cells/uL. Using the 200cells/uL cutoff offered a similar sensitivity (95.8%) but inferior specificity (27.3%). We conducted a sensitivity analysis with the ≥200cells/uL cut off and found that there was a significant difference in age, smoking history, exacerbation rate with raised eosinophils in the first year and presence of sputum eosinophils>3% at enrolment. ~~There were more males and less active smokers in PE compared to RE, however, these results were not clinically significant.~~

The rationale of our method to describe the longitudinal eosinophilic phenotype was to focus the analysis on subjects who were PE (allowance of one non-eosinophilic event) as opposed to persistently (all visits were eosinophilic). This rule, we feel, represents a more pragmatic and “real world” approach. Applying this rule, we demonstrated that 58% of subjects in our cohort had predominantly raised eosinophils over the period of 12 months. These PE subjects were slightly older than RE patients (p=0.034) and there were trends towards a lower prevalence of current smokers but with a greater smoking history in the PE group. Singh et al studied longitudinal eosinophilic phenotype and reported that 37.4% of subjects had

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persistently elevated blood eosinophils ≥2% at all visits over the period of 3 years.[4] In a previous asthma study a 90% rule was applied to identify persistent eosinophilic inflammation in sputum.[25] However, due to the limited number of samples available (maximum 5 samples) in our cohort this rule was not applied. A limitation of the longitudinal phenotype method was that individuals with 3 visits could not be classified as IE (n=20/99 for blood, and 14/80 for sputum).

Bacteria play an important role in exacerbations of COPD.[26-30] Bacterial exacerbation had been previously reported to be rarely associated with sputum eosinophilic exacerbation.[3] We investigated the prevalence of PPMs at exacerbation in the prospective eosinophilic groups and found that PPMs were far less common in the eosinophilic group. When we analysed PPM presence at exacerbation in combination with blood EI, we found eosinophilic inflammation to be associated with reduced odds of PPM presence, and again while the magnitude of the difference was large (55% less likely) this was again of borderline significance (p=0.049). Therefore understanding the clinical phenotype of stable inflammation may be a useful tool to stratify bacterial aetiology of exacerbations and hence antibiotic use.

Whilst our detection of respiratory viruses at AECOPD corresponded with previously reported findings,[30, 31] we observed no significant difference in the prevalence of respiratory viruses between the three groups at enrolment and at 1st exacerbations. Previously Papi et al reported airway eosinophilic inflammation to be a good predictor of viral infections[30]. However in their study only severe exacerbations were included [30] whereas in our study we captured mild, moderate and severe events. Further studies across the disease spectrum are required to ascertain the mechanisms linking infection and inflammatory patterns of disease. However, it is noteworthy, that there was no significant difference in use of inhaled corticosteroids

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and bronchial reversibility across groups, similar to previously reported studies.[3, 15] It is important to recognise that these results are representative of a cohort of moderate to very severe COPD patients with frequent exacerbations receiving a high level of clinical intervention, including ICS, as part of an intensive study. This might have had an impact on the severity of exacerbations and recovery, as previous reports indicate that early therapy improves exacerbation outcomes.[32] Thus the number of potential severe exacerbations might be smaller. Secondly, our study was not originally designed, and therefore was not powered, to specifically investigate longitudinal EI and hence ~~this limited~~ the numbers that were included in each eosinophilic group. Larger prospective studies are required to further understand the impact of eosinophilic inflammatory status on a range of clinical outcomes. ~~This is reflected in the fact that the results are post hoc, that multiplicity of analyses has not been taken into account, and in the presence of wide confidence intervals, which result in borderline statistical significance despite sizeable differences.~~

In summary, this is the first study to report that EI is a stable phenotype in COPD and predictive of eosinophilic exacerbations. These events are seasonal in nature and relate to bacterial aetiology. Our data suggest that stratifying COPD patients into eosinophilic groups to potentially aid management is clinically relevant and potentially important, as is the consideration of season in management of exacerbations. Whether oral corticosteroids should be administered during exacerbations of COPD to PE patients, in particular, outside the Winter season, requires further investigation along with other stratification paradigms through well designed intervention studies.

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The AERIS Study Group

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Authors' contributions:

JMD, EA, SCB, SAW, ACT, SCC, VLK and TMAW were involved in the study conception and design

JMD, EA, SCB, SAW, ACT, NPW, KKO, KJS, SCC, VLK, and TMAW were involved in acquisition and generation of data

JMD, EA, SCB, SAW, NPW, KKO, KJS, SCC, NAC, VLK and TMAW were involved in data analysis and data interpretation

All authors contributed substantially to the development of the manuscript and approved the final version.

Conflict of interest: TMAW has received reimbursement for travel and meeting attendance from Boehringer Ingelheim and AstraZeneca, outside of the submitted work. KJS received grants from Asthma UK (08/026) and BMA HC Roscoe Award outside of the submitted work, and he has a patent PCT/GB2010/050821 "Ex Vivo Modelling of Therapeutic Interventions" pending. EA, JMD are employees of the GSK group of companies. SCC received grants from Pfizer outside of the submitted work. EA, JMD hold shares/restricted shares in the GSK group of companies. KJS, VK, NPW, KKO, SAW, SCC, and TMAW received an institutional grant from the GSK group of companies to conduct this study. ACT, NAC, and SCB declare no conflict of interest.

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Figure legends

Figure 1.

Flow diagram of the subjects screened and included in the full cohort at the start of Year 1 and the number of exacerbations in Year 1.

Figure 2.

Receiver operating characteristic curve with area under the curve for blood eosinophil (count & %) at enrolment predicting sputum eosinophilia >3% at enrolment (n=68).

Figure 3.

Receiver operating characteristic curve for blood eosinophils (count and %) at enrolment predicting the predominantly eosinophilic group over 12 months following enrolment) (n=78).

Figure 4.

Seasonal distribution of total and eosinophil-associated exacerbations. **A** -Number of total and exacerbations with blood eosinophils≥2%. **B** - Proportion of exacerbations with blood eosinophils≥2% defined as exacerbations with blood eosinophils≥2% to total exacerbation rates in the predominantly, intermittent and rarely groups.

Table 1. Baseline characteristics of the total COPD cohort (n=127).

Continuous variables				Categorical variables			
	N	Median	(IQR)			N	(%)
Age α	127	66.8	(8.61)	Sex	Male	68	(54%)
Smoking history (pack/years) α	127	50.3	(28.2)		Female	59	(46%)
BMI	127	27.0	(6.69)	Current smoker γ	Yes	54	(43%)
FFBM	125	48.9	(21.5)		No	73	(57%)
WBC	126	7.60	(2.20)	Frequent Exacerbators in Yr0Use of ICS at enrolment β ¥	YesYes	9911 3	(78%)(89%)
Eosinophils (count)	126	0.20	(0.20)		NoNo	2814	(22%)(11%)
Eosinophils (%)	126	2.94	(3.08)	Use of ICS at enrolment β ¥Sputum eosinophilia (>3%)≠	YesYes	1132 7	(89%)(33%)
Neutrophils (count)	126	4.80	(1.70)		NoNo	1456	(11%)(67%)
Fibrinogen	114	4.80	(1.02)	Sputum eosinophilia (>3%)≠Blood eosinophilia (>=2%)	YesYes	2786	(33%)(68%)
CRP	127	5.00	(8.00)		NoNo	5640	(67%)(32%)
Eosinophils (%)≠	83	1.93	(5.05)	Blood eosinophilia (>=2%)Bacteria present*	YesYes	8657	(68%)(52%)
Neutrophils (%)≠	83	47.0	(70.4)		NoNo	4053	(32%)(48%)
FEV1 (%)	126	46.7	(25.3)	Bacteria present*Virus present**	YesYes	5718	(52%)(17%)
ΔFEV1(% of baseline)ξ	85	4.93	(21.1)		NoNo	5385	(48%)(83%)
FEV1 reversibility (% of preBDFEV1)€	105	11.2	(17.0)	Virus present** Blood eosinophils ≥200 cells/uL	YesYes	1890	(17%)(71.4%)
KCO (mmol/kPa/min%)	122	0.9469.2	(30.70.48)		NoNo	8536	(83%)(28 .6%)
TLCO(mmol/kPa/min%)	122	57.94.48	(29.52.36)	Blood eosinophils ≥200 cells/uL	Yes	90	(71.4%)
CAT	126	16.0	(10.0)		No	36	(28 .6%)
6MWT (distance in meters)	125	300	(170)				
Exact score	101	37.0	(12.0)				
Exacerbation rate in year before study	127	2.00	(2.00)				
Exacerbation rate in first year of study	127	2.94	(3.91)				
Eosinophilic exacerbation rate in first year of study	127	0.99	(2.02)				
Follow up (in years) in first year of study	127	1.00	(0.02)				

α reported as Mean(±SD)
γ smoking status report based derived from ATS Q7A4
¥ICS use were coded as "Yes" if one of the following medications/inhalers was on the list (SYMBICORT, SERETIDE, QVAR, FOSTAIR, BECLOMETHASONE, BECLAMETHOSONE/FORMOTEROL, BECLOMETHASONE dipropiionate, CLENIL, FLUTICASONE/SALMETEROL, BUDESONIDE/FORMOTEROL)
≠ Sputum eosinophil and neutrophil % at baseline is reported. "Baseline" is equal to enrolment if good quality data (SQC<30) is present at enrolment, or the next (pre-exacerbation) stable visit with quality data within four months of enrolment.
ξ calculated as FEV1 at month 12 * 100 / FEV1 at enrolment
€ calculated as (post broncho dilator BDFEV1 - preBD broncho dilator FEV1) / pre BDbroncho dilator FEV1 * 100
* Sputum sampling, measured by culture. Includes Haemophilus influenzae, Moraxella catarrhalis, Streptococcus pneumoniae, Staphylococcus aureus and Pseudomonas aeruginosa.
** Sputum sampling, measured by PCR. Includes adenovirus, enterovirus, influenza, coronavirus, metapneumovirus, bocavirus, parainfluenza, RSV, and rhinovirus.

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Table 2. Baseline characteristics by longitudinal blood eosinophil group over 12 months (n=99).

		Rarely eosinophilic (n=27)			Intermittently eosinophilic (n=19)			Predominantly eosinophilic (n=59)			p valueΩ
Continuous variables		N	Median	(IQR)	N	Median	(IQR)	N	Median	(IQR)	
Age α		26	62.7	(8.63)	16	67.9	(6.87)	57	68.0	(9.05)	0.034
Smoking history (pack/years) α		26	46.3	(22.8)	16	64.9	(43.2)	57	51.0	(28.6)	0.098
BMI		26	28.2	(8.15)	16	27.9	(5.69)	57	25.9	(4.73)	0.280
FFBM		25	53.5	(21.5)	16	49.3	(18.3)	56	48.8	(22.4)	0.886
WBC		25	8.30	(3.30)	16	7.65	(1.65)	57	7.30	(2.00)	<0.001 0.060
Blood eosinophils (count)		25	0.10	(0.10)	16	0.20	(0.10)	57	0.30	(0.20)	N/A
Blood eosinophils (%)		25	1.35	(1.45)	16	2.12	(1.09)	57	4.08	(3.05)	N/A
Blood neutrophils (count)		25	5.20	(2.55)	16	4.80	(0.50)	57	4.30	(1.75)	0.9050 30
Fibrinogen		23	4.70	(1.40)	14	4.75	(0.97)	51	4.80	(0.90)	0.9059 66
CRP		26	4.00	(7.50)	16	5.00	(5.75)	57	5.00	(7.00)	0.0709 66
Sputum eosinophils (%)≠		18	0.43	(2.89)	7	0.82	(1.78)	40	2.81	(7.40)	0.2230 70
Sputum neutrophils (%)≠		18	9.77	(64.0)	7	62.3	(63.4)	40	38.0	(71.6)	0.6612 23
FEV1 (%)		26	47.7	(25.6)	16	43.9	(22.4)	57	47.5	(26.0)	0.3756 61
ΔFEV1(% of baseline)ξ		19	3.79	(27.3)	15	10.8	(23.2)	45	5.03	(21.4)	0.3553 75
FEV1 reversibility (% of preBDFEV1)€		24	7.69	(15.0)	13	17.9	(16.6)	46	12.6	(12.5)	0.1673 55
KCO (%mmol/kPa/min)		25	74.21.11	(31.00. 51)	16	0.8768.3	(0.3826 .7)	53	69.30.92	(0.4630 .3)	0.5435 26
TLCO(mmol/kPa/min%)		25	4.8662.0	(3.4141 .1)	16	4.9457.8	(2.2028 .8)	53	4.4556.3	(2.67)2 7.7)	0.1014 07
CAT		26	15.5	(10.0)	16	20.5	(13.0)	57	16.0	(9.00)	0.9411 01
6MWT (distance in meters)		25	300	(199)	16	323	(172)	56	327	(164)	0.1979 14
Exact score		23	36.0	(14.0)	14	36.5	(7.00)	46	36.0	(15.0)	<0.001 0.607
Exacerbation rate in year before study		26	2.00	(3.00)	16	2.00	(3.00)	57	2.00	(2.00)	0.4480. 941
Exacerbation rate in first year of study		26	2.47	(3.22)	16	1.02	(2.00)	57	2.04	(3.95)	<0.001 0.197
Eosinophil predominantic exacerbation rate in first year of study		26	0.00	(0.99)	16	0.00	(0.99)	57	1.38	(2.99)	<0.001
Follow up (years) in first year of study		26	1.01	(0.02)	16	1.01	(0.01)	57	1.01	(0.02)	<0.001 0.448
Categorical variables		N	(%)		N	(%)		N	(%)		
Sex	Male	12	(46%)		9	(56%)		34	(60%)		0.547
	Female	14	(54%)		7	(44%)		23	(40%)		
Current smokerγ μ	Yes	15	(58%)		4	(25%)		24	(42%)		0.125
	No	11	(42%)		12	(75%)		33	(58%)		
Use of ICS at enrolment β ¥	Yes	21	(81%)		15	(94%)		51	(89%)		0.468
	No	5	(19%)		1	(6%)		6	(11%)		

Sputum eosinophil~~slia~~ (>3%)≠	Yes	2	(11%)	0	(0%)	19	(48%)	0.003
	No	16	(89%)	7	(100%)	21	(53%)	
Blood ~~eosinophilia~~ eosinophils (>=2%)	Yes	7	(28%)	9	(56%)	55	(96%)	N/A
	No	18	(72%)	7	(44%)	2	(4%)	
Bacteria present*	Yes	12	(55%)	6	(50%)	26	(50%)	0.952
	No	10	(45%)	6	(50%)	26	(50%)	
Virus present**	Yes	4	(19%)	0	(0%)	10	(20%)	0.288
	No	17	(81%)	12	(100%)	40	(80%)	

Ω Test for differences between the three longitudinal eosinophil groups. Kruskall-Wallis test for continuous variables, Chi-Square test for categorical variables

α reported as Mean(±SD)

γ smoking status report based derived from ATS Q7A4

¥ICS use were coded as "Yes" if one of the following medications/inhalers was on the list (SYMBICORT, SERETIDE, QVAR, FOSTAIR, BECLOMETHASONE, BECLAMETHOSONE/FORMOTEROL, BECLOMETHASONE dipropiionate, CLENIL, FLUTICASONE/SALMETEROL, BUDESONIDE/FORMOTEROL)

≠ Sputum eosinophil and neutrophil % at baseline. "Baseline" is equal to enrolment if good quality data (SQC<30) is present, or the next (pre-exacerbation) stable visit with quality data within four months of enrolment.

ξ calculated as FEV1 at month 12 * 100 / FEV1 at enrolment

€ calculated as (post broncho dilator BDFEV1 - preBD broncho dilator FEV1) / pre BDbroncho dilator FEV1 * 100

* Sputum sampling, measured by culture. Includes Haemophilus influenzae, Moraxella catarrhalis, Streptococcus pneumoniae, Staphylococcus aureus and Pseudomonas aeruginosa.

** Sputum sampling, measured by PCR. Includes adenovirus, enterovirus, influenza, coronavirus, metapneumovirus, bocavirus, parainfluenza, RSV, and rhinovirus.

Table 3. Characteristics at 1st exacerbation overall (n=108), and by longitudinal blood eosinophil group over 12 months (n=87)

		Overall cohort (n=108)			Rarely eosinophilic (n=26)			Intermittently eosinophilic (n=13)			Predominantly eosinophilic (n=48)			P value Ω
Continuous variables		**N**	**Median**	**(IQR)**	N	Median	(IQR)	N	Median	(IQR)	N	Median	(IQR)	
WBC		**102**	**8.00**	**(3.53)**	25	8.70	(4.25)	12	8.95	(2.55)	48	7.30	(3.23)	0.349
Blood eosinophils (count)		**102**	**0.20**	**(0.20)**	25	0.10	(0.15)	12	0.15	(0.10)	48	0.30	(0.27)	<0.001
Blood eosinophils (%)		**102**	**2.07**	**(2.78)**	25	1.19	(1.74)	12	1.92	(0.88)	48	3.39	(4.40)	<0.001
Blood neutrophils (count)		**102**	**5.30**	**(3.20)**	25	5.60	(4.05)	12	5.55	(2.57)	48	4.45	(2.98)	0.045
Fibrinogen		**103**	**5.10**	**(1.40)**	25	4.80	(1.80)	12	5.50	(2.25)	46	5.10	(1.60)	0.135
CRP		**103**	**8.00**	**(14.0)**	25	7.00	(12.5)	12	26.5	(49.8)	47	7.00	(13.0)	0.087
Sputum eosinophils (%)		**61**	**1.20**	**(2.38)**	15	0.62	(2.29)	9	1.40	(3.23)	28	1.48	(3.56)	0.273
Sputum neutrophils (%)		**61**	**74.0**	**(47.1)**	15	76.8	(51.6)	9	86.6	(40.3)	28	74.1	(52.3)	0.616
FEV1 (%)		**97**	**42.7**	**(21.2)**	22	47.3	(30.0)	12	41.5	(17.5)	44	41.7	(20.0)	0.395
CAT		**106**	**21.0**	**(11.0)**	26	22.0	(12.0)	13	22.0	(17.0)	47	19.0	(8.00)	0.915
EXACT-PRO		**97**	**41.0**	**(11.0)**	23	43.0	(13.0)	12	40.0	(10.0)	44	39.5	(7.00)	0.142
Categorical variables		**N**	**(%)**		N	(%)		N	(%)		N	(%)		
Bacteria present*	Yes	**60**	**(61%)**		19	(86%)		6	(55%)		27	(57%)		0.044
	No	**38**	**(39%)**		3	(14%)		5	(45%)		20	(43%)		
Virus present**	Yes	**40**	**(43%)**		12	(55%)		4	(40%)		14	(33%)		0.235
	No	**53**	**(57%)**		10	(45%)		6	(60%)		28	(67%)		
Sputum eosinophils>3%	Yes	**23**	**(30%)**		2	(11%)		0	(0%)		17	(47%)		0.004
	No	**54**	**(70%)**		16	(89%)		7	(100%)		19	(53%)		
Blood eosinophils≥2%	Yes	**73**	**(68%)**		7	(28%)		7	(54%)		47	(98%)		<0.001
	No	**34**	**(32%)**		18	(72%)		6	(46%)		1	(2%)		

Ω Test for differences between the three longitudinal eosinophil groups. Kruskall-Wallis test for continuous variables, Chi-Square test for categorical variables
* Sputum sampling, measured by culture. Includes Haemophilus influenzae, Moraxella catarrhalis, Streptococcus pneumoniae, Staphylococcus aureus and Pseudomonas aeruginosa.
** Sputum sampling, measured by PCR. Includes adenovirus, enterovirus, influenza, coronavirus, metapneumovirus, bocavirus, parainfluenza, RSV, and rhinovirus

Online supplement for:

Impact and Associations of Eosinophilic Inflammation in COPD: Analysis of the AERIS Cohort

Viktoriya L Kim[1,2,3], Ngaire A Coombs[4], Karl J Staples[1,2,5], Kristoffer K Ostridge[1,2], Nicholas P Williams[1,2], Stephen A Wootton[3], Jeanne-Marie Devaster[6], Emmanuel Aris[6], Stuart C Clarke[2,5], Andrew C Tuck[7], Simon C Bourne[2†], Tom MA Wilkinson[1,2,5] on behalf of the AERIS Study Group*.

Methods

Study design and study population

The Acute Exacerbation and Respiratory InfectionS in COPD (AERIS) study is a prospective, observational cohort study based at University Hospital Southampton (UHS), registered with ClinicalTrials.gov (NCT01360398). The study protocol has been published previously.[1] We describe an analysis of the first year of a two-year longitudinal epidemiological study which assessed the nature of infection and inflammation in the aetiology of AECOPD. Patients aged 40–85 years with a confirmed diagnosis of COPD, were recruited from UHS and referring practices from June 2011 to June 2012. AERIS was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice, and was approved by the Southampton and South West Hampshire Research Ethics Committee. All participants provided written informed consent. The protocol summary is available at www.gsk-clinicalstudyregister.com (study identifier, 114378). Full inclusion and exclusion criteria have been published previously.[1]

We report results of a secondary analysis focusing on eosinophilic inflammation for subjects followed over one year.

Procedures

Patients were followed monthly in the stable state and reviewed within 72 hours of onset of AECOPD symptoms. Exacerbations were detected using daily electronic diary cards. The definition of AECOPD, as described previously[1, 2], and definitions of severity categories are provided in the appendix.

Venous blood was taken for measurement of full blood count, serum C reactive

protein (CRP), serum fibrinogen and serum procalcitonin (PCT) at enrolment and then at quarterly visits over the following year. FBC, CRP and fibrinogen analyses were performed by the University Hospital Southampton Haematology laboratory. Sputum samples were obtained either by saline induction or spontaneous expectoration and were processed according to standard methods, as previously described [3].Briefly, sputum was solubilised with 0.1% dithiothreitol to liberate the cells from mucus. The resulting cells were resuspended in PBS and cytospin slides (Thermo Shandon Ltd, Runcorn, UK) were prepared. Differential cell counts were performed manually on cytospins stained with rapid Romanowsky stain (Raymond Lamb Ltd, Eastbourne, UK). Differential cell counts were obtained from a 400 cell count. Sputum samples were processed by conventional microbiology methods for identification of potentially pathogenic microorganisms (PPM) focusing on *H.influenzae* (HI), *M.catarrhais* (MC), *S.pneumoniae* (SP), *S.aureus* (SA) and *P.aeruginosa* (PA). Sputum samples were also processed for detection of respiratory viruses by PCR analysis. Only sputum samples with <30% squamous cells, and good quality spirometry samples (A or B) were considered in the analyses.

Criteria for eosinophilic groups and seasonality

Eosinophilic inflammation was defined as sputum eosinophils >3% and blood eosinophils ≥2% in line with previous studies.[4-8] To investigate the stability of blood eosinophilic inflammation over time we divided subjects into three groups: predominantly (PE), intermittent (IE) and rarely (RE) eosinophilic. Only those subjects who had at least 3 (out of 5 potential) stable visits with valid blood results over 12 months were included in the group analyses (n=99). The PE group was defined as blood eosinophils (≥2%) at either all visits, or all but 1 visits where the

blood eosinophils were <2%; the RE group was defined as blood eosinophils <2% at all visits, or all but 1 visit where the blood eosinophils were ≥2%; the IE group was defined when none of the abovementioned criteria were met.

To investigate an impact of seasonality on exacerbations we divided the year into 2 seasons, each containing six months: one containing exacerbation visits occurring in winter and the other summer months. For the simplicity we defined them as winter (October-March) and summer (April – September) seasons.

Statistical analysis

Bivariate analyses testing for differences between eosinophilic groups were conducted using Kruskall-Wallis, ANOVA, Chi-Square, or Fisher's Exact test, as appropriate. All tests were two-tailed. Receiver Operator Curves (ROC) were used to assess the predictive ability of different cut offs to correctly identify presence of sputum eosinophilic inflammation. Intra-class correlations were used to assess the reliability of measures within individuals over time. As subjects contributed differing numbers of exacerbations over the study period, some subjects would be represented multiple times in analyses exploring outcomes at exacerbation. To counter this, descriptive analyses were conducted for only the first exacerbation occurring to each subject, and multivariate analyses with binary outcomes (presence/absence of different conditions at exacerbation) were conducted using conditional logistic regression, including the subject number as a random effect. SPSS (version 22) was used for all analyses with the exception of intra-class correlation coefficients (ICC) and conditional logistic regression, which were conducted using STATA (version 14). All of these analyses should be considered post hoc as they were not pre-specified in the AERIS statistical analysis plan.

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Table E1. Baseline characteristics by longitudinal blood eosinophil group over 12 months using 200 cells/uL cutoff (n=99).

		Rarely eosinophilic (n=24)			Intermittently eosinophilic (n=12)			Predominantly eosinophilic (n=63)			P valueΩ
Continuous variables		N	Median	(IQR)	N	Median	(IQR)	N	Median	(IQR)	
Age α		24	61.4	(8.68)	12	67.3	(4.87)	63	68.4	(8.82)	0.002
Smoking history (pack/years) α		24	45.6	(23.4)	12	72.5	(47.7)	63	50.5	(27.4)	0.017
BMI		24	26.6	(8.69)	12	28.2	(10.6)	63	26.3	(4.44)	0.437
FFBM		23	43.6	(21.2)	12	53.7	(19.1)	62	48.6	(22.3)	0.467
WBC		23	7.60	(2.50)	12	7.35	(1.57)	63	7.40	(2.10)	0.788
Blood eosinophils (count)		23	0.10	(0.10)	12	0.15	(0.10)	63	0.30	(0.20)	NA
Blood eosinophils (%)		23	1.35	(1.44)	12	1.92	(1.08)	63	4.05	(3.14)	NA
Blood neutrophils (count)		23	5.00	(2.20)	12	4.80	(0.48)	63	4.70	(1.80)	0.330
Fibrinogen		21	4.70	(1.50)	11	4.60	(1.30)	56	4.80	(0.88)	0.624
CRP		24	4.00	(6.50)	12	5.00	(4.75)	63	5.00	(8.00)	0.699
Sputum eosinophils (%)≠		14	0.33	(2.24)	5	1.81	(1.78)	46	2.41	(7.32)	0.097
Sputum neutrophils (%)≠		14	9.77	(65.3)	5	45.6	(63.3)	46	45.1	(69.7)	0.500
FEV1 (%)		24	49.2	(26.9)	12	49.6	(25.1)	63	46.4	(23.7)	0.736
ΔFEV1(% of baseline)ξ		17	3.88	(23.9)	11	7.25	(31.3)	51	5.03	(21.6)	0.870
FEV1 reversibility (% of preBDFEV1)€		23	7.26	(15.0)	10	15.9	(21.3)	50	12.6	(13.3)	0.572
KCO (%)		22	75.1	(31.9)	12	73.0	(26.0)	60	69.2	(28.6)	0.410
TLCO(%)		22	65.0	(32.0)	12	61.9	(27.0)	60	56.2	(29.6)	0.312
CAT		24	15.5	(11.0)	12	19.0	(12.0)	63	16.0	(10.0)	0.371
6MWT (distance in meters)		23	326	(174)	12	321	(227)	62	326	(166)	0.769
Exact score		22	34.0	(16.0)	10	35.0	(11.0)	51	37.0	(14.0)	0.943
Exacerbation rate in year before study		24	2.50	(3.00)	12	2.00	(3.00)	63	2.00	(3.00)	0.705
Exacerbation rate in first year of study		24	1.98	(3.91)	12	1.52	(2.00)	63	2.96	(3.91)	0.480
Eosinophilic exacerbation rate in first year of study		24	0.00	(0.98)	12	0.00	(0.99)	63	1.01	(2.98)	<0.001
Follow up (years) in first year of study		24	1.01	(0.02)	12	1.00	(0.01)	63	1.00	(0.02)	0.072
Categorical variables		N	(%)		N	(%)		N	(%)		
Sex	Male	10	(42%)		8	(67%)		37	(59%)		0.297
	Female	14	(58%)		4	(33%)		26	(41%)		
Current smokerγ μ	Yes	14	(58%)		4	(33%)		25	(40%)		0.233
	No	10	(42%)		8	(67%)		38	(60%)		
Use of ICS at enrolment ¥	Yes	20	(83%)		11	(92%)		56	(89%)		0.733
	No	4	(17%)		1	(8%)		7	(11%)		
Sputum eosinophilia (>3%)≠	Yes	1	(7%)		0	(0%)		20	(43%)		0.011
	No	13	(93%)		5	(100%)		26	(57%)		
Blood eosinophilia (>=2%)	Yes	6	(26%)		6	(50%)		59	(94%)		NA
	No	17	(74%)		6	(50%)		4	(6%)		
Bacteria present*	Yes	10	(56%)		4	(44%)		30	(51%)		0.892
	No	8	(44%)		5	(56%)		29	(49%)		
Virus present**	Yes	4	(24%)		0	(0%)		10	(18%)		0.394
	No	13	(76%)		9	(100%)		47	(82%)		

Ω Kruskall-Wallis test used for continuous variables, Fisher's Exact test for categorical variables
α reported as Mean(±SD), p value calculated from ANOVA
γ smoking status report based derived from ATS Q7A4
¥IICS use were coded as "Yes" if one of the following medications/inhalers was on the list (SYMBICORT, SERETIDE, QVAR, FOSTAIR, BECLOMETHASONE, BECLAMETHOSONE/FORMOTEROL, BECLOMETHASONE dipropiionate, CLENIL, FLUTICASONE/SALMETEROL, BUDESONIDE/FORMOTEROL
≠Eosinophil% and Neutrophil% at baseline is reported. "Baseline" is equal to enrolment if good quality data (SQC<30) is present at enrolment, or the next (pre-exacerbation) stable visit with quality data within four months of enrolment.
ξ calculated as FEV1 at month 12 * 100 / FEV1 at enrolment
Є calculated as (post broncho dilator BDFEV1 - preBD broncho dilator FEV1) / pre BDbroncho dilator FEV1 * 100
* Sputum sampling, measured by culture. Includes Haemophilus influenzae, Moraxella catarrhalis, Streptococcus pneumoniae, Staphylococcus aureus and Pseudomonas aeruginosa.
** Sputum sampling, measured by PCR. Includes adenovirus, enterovirus, influenza, coronavirus, metapneumovirus, bocavirus, parainfluenza, RSV, and rhinovirus.

Table E2. Characteristic of those excluded and included in the longitudinal analyses at enrolment.

		Excluded			Included			P valueΩ
Continuous variables		N	Median	(IQR)	N	Median	(IQR)	
Age α		28	67.6	(7.80)	99	66.6	(8.85)	0.673
Smoking history (pack/years) α		28	44.2	(17.7)	99	52.0	(30.3)	0.263
BMI		28	28.4	(7.57)	99	26.7	(6.39)	0.080
FFBM		28	46.5	(20.0)	97	49.3	(21.7)	0.526
WBC		28	7.85	(2.60)	98	7.50	(2.13)	0.368
Blood eosinophils (count)		28	0.20	(0.20)	98	0.20	(0.15)	0.090
Blood eosinophils (%)		28	2.16	(3.11)	98	3.05	(3.01)	0.102
Blood neutrophils (count)		28	4.95	(1.75)	98	4.80	(1.60)	0.277
Fibrinogen		26	5.25	(1.13)	88	4.80	(1.08)	0.110
CRP		28	6.00	(11.3)	99	5.00	(7.00)	0.023
Sputum eosinophils (%)≠		18	1.97	(3.58)	65	1.81	(5.22)	0.665
Sputum neutrophils (%)≠		18	55.6	(52.7)	65	31.8	(69.7)	0.184
FEV1 (%)		27	42.4	(24.0)	99	47.0	(25.4)	0.746
ΔFEV1(% of baseline)ξ		6	5.74	N/A	79	4.93	(21.6)	0.864
FEV1 reversibility (% of preBDFEV1)€		22	8.56	(16.8)	83	11.3	(18.7)	0.431
KCO (%)		28	63.9	(40.3)	94	70.3	(28.9)	0.335
TLCO(%)		28	50.8	(26.1)	94	59.0	(29.1)	0.105
CAT		27	20.0	(13.0)	99	16.0	(10.0)	0.096
6MWT (distance in meters)		28	229	(130)	97	324	(169)	0.002
Exact score		18	38.5	(8.00)	83	36.0	(14.0)	0.149
Exacerbation rate in year before study		28	3.00	(3.00)	99	2.00	(2.00)	0.215
Exacerbation rate in first year of study		28	3.99	(6.70)	99	1.99	(3.02)	0.078
Eosinophilic exacerbation rate in first year of study		28	1.34	(3.32)	99	0.99	(1.99)	0.412
Follow up (years) in first year of study		28	0.58	(0.57)	99	1.01	(0.02)	<0.001
Categorical variables		N	(%)		N	(%)		
Sex	Male	13	(46%)		55	(56%)		0.401
	Female	15	(54%)		44	(44%)		
Current smokerγ μ	Yes	11	(39%)		43	(43%)		0.829
	No	17	(61%)		56	(57%)		
Use of ICS at enrolment ¥	Yes	26	(93%)		87	(88%)		0.733
	No	2	(7%)		12	(12%)		
Sputum eosinophilia (>3%)≠	Yes	6	(33%)		21	(32%)		1.000
	No	12	(67%)		44	(68%)		
Blood eosinophilia (>=2%)	Yes	15	(54%)		71	(72%)		0.068
	No	13	(46%)		27	(28%)		
Bacteria present*	Yes	13	(54%)		44	(51%)		0.821
	No	11	(46%)		42	(49%)		
Virus present**	Yes	4	(20%)		14	(17%)		0.747
	No	16	(80%)		69	(83%)		

α reported as Mean(±SD)
γ smoking status report based derived from ATS Q7A4

¥ICS use were coded as "Yes" if one of the following medications/inhalers was on the list (SYMBICORT, SERETIDE, QVAR, FOSTAIR, BECLOMETHASONE, BECLAMETHOSONE/FORMOTEROL, BECLOMETHASONE dipropiionate, CLENIL, FLUTICASONE/SALMETEROL, BUDESONIDE/FORMOTEROL)
≠Sputum eosinophil and neutrophil % at baseline is reported. "Baseline" is equal to enrolment if good quality data (SQC<30) is present at enrolment, or the next (pre-exacerbation) stable visit with quality data within four months of enrolment.
ξ calculated as FEV1 at month 12 * 100 / FEV1 at enrolment
€ calculated as (post broncho dilator BDFEV1 - preBD broncho dilatorFEV1) / pre BDbroncho dilator FEV1 * 100
* Sputum sampling, measured by culture. Includes Haemophilus influenzae, Moraxella catarrhalis, Streptococcus pneumoniae, Staphylococcus aureus and Pseudomonas aeruginosa.
** Sputum sampling, measured by PCR. Includes adenovirus, enterovirus, influenza, coronavirus, metapneumovirus, bocavirus, parainfluenza, RSV, and rhinovirus.

Table E3: Seasonality[μ] of eosinophilic exacerbations in longitudinal phenotypes

		Summer		Winter	
		Median	(IQR)	Median	(IQR)
Predominantly eosinophilic	Exacerbation rate	2.00	(4.00)	2.01	(3.03)
	Eosinophilic exacerbation rate	1.93	(3.93)	2.00	(2.02)
Intermittently eosinophilic	Exacerbation rate	0.00	(2.00)	2.00	(3.53)
	Eosinophilic exacerbation rate	0.00	(0.00)	0.00	(1.99)
Rarely eosinophilic	Exacerbation rate	1.98	(3.99)	3.73	(4.00)
	Eosinophilic exacerbation rate	0.00	(0.00)	0.00	(1.94)
OVERALL	Exacerbation rate	1.99	(3.99)	2.01	(2.05)
	Eosinophilic exacerbation rate	0.00	(2.00)	0.00	(2.00)

μ Summer season defined as April-September, Winter as October-March. 12 monthly rates are presented for all exacerbations, and for eosinophilic exacerbations.

Table E4: Odds* of eosinophilic inflammation at exacerbation in summer compared to winter[μ]

	N of total exacerbations	N of exacerbations in Summer	N of exacerbations in Winter	N of individuals	Odds Ratio	95% CI	p value
Exacerbations with blood eosinophilia (>=2%)	338	132	206	104	2.65	(1.50; 4.68)	0.001
Exacerbations with sputum eosinophilia (>3%)	218	88	130	91	1.94	(0.82; 4.52)	0.129

*Conditional logistic regression including subject as a random effect

μ Summer season defined as April-September, Winter as October-March

Figure E1

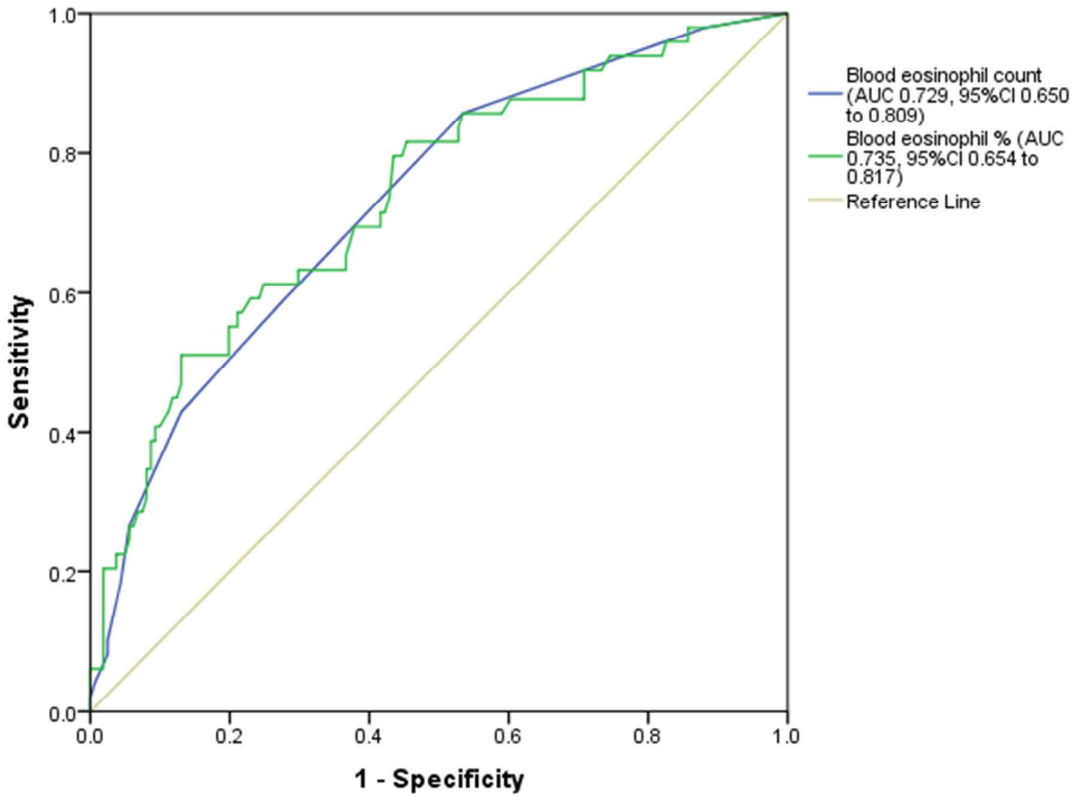

Figure E1 Receiver operating characteristic for blood eosinophil (count & %) at exacerbation predicting sputum eosinophilia >3% (n=210) at exacerbation. At exacerbations blood eosinophils ≥2% cut point was 79.6% sensitive and 55.3% specific in identifying sputum eosinophils (>3%).

Online supplement for:

Impact and Associations of Eosinophilic Inflammation in COPD: Analysis of the AERIS Cohort

Viktoriya L Kim[1,2,3], Ngaire A Coombs[4], Karl J Staples[1,2,5], Kristoffer K Ostridge[1,2], Nicholas P Williams[1,2], Stephen A Wootton[3], Jeanne-Marie Devaster[6], Emmanuel Aris[6], Stuart C Clarke[2,5], Andrew C Tuck[7], Simon C Bourne[2†], Tom MA Wilkinson[1,2,5] on behalf of the AERIS Study Group*.

Methods

Study design and study population

The Acute Exacerbation and Respiratory InfectionS in COPD (AERIS) study is a prospective, observational cohort study based at University Hospital Southampton (UHS), registered with ClinicalTrials.gov (NCT01360398). The study protocol has been published previously.[1] We describe an analysis of the first year of a two-year longitudinal epidemiological study which assessed the nature of infection and inflammation in the aetiology of AECOPD. Patients aged 40–85 years with a confirmed diagnosis of COPD, were recruited from UHS and referring practices from June 2011 to June 2012. AERIS was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice, and was approved by the Southampton and South West Hampshire Research Ethics Committee. All participants provided written informed consent. The protocol summary is available at www.gsk-clinicalstudyregister.com (study identifier, 114378). Full inclusion and exclusion criteria have been published previously.[1]

We report results of a secondary analysis focusing on eosinophilic inflammation for subjects followed over one year.

Procedures

Patients were followed monthly in the stable state and reviewed within 72 hours of onset of AECOPD symptoms. Exacerbations were detected using daily electronic diary cards. The definition of AECOPD, as described previously[1, 2], and definitions of severity categories are provided in the appendix.

Venous blood was taken for measurement of full blood count, serum C reactive

protein (CRP), serum fibrinogen and serum procalcitonin (PCT) at enrolment and then at quarterly visits over the following year. FBC, CRP and fibrinogen analyses were performed by the University Hospital Southampton Haematology laboratory. Sputum samples were obtained either by saline induction or spontaneous expectoration and were processed according to standard methods, as previously described [3].Briefly, sputum was solubilised with 0.1% dithiothreitol to liberate the cells from mucus. The resulting cells were resuspended in PBS and cytospin slides (Thermo Shandon Ltd, Runcorn, UK) were prepared. Differential cell counts were performed manually on cytospins stained with rapid Romanowsky stain (Raymond Lamb Ltd, Eastbourne, UK). Differential cell counts were obtained from a 400 cell count. Sputum samples were processed by conventional microbiology methods for identification of potentially pathogenic microorganisms (PPM) focusing on *H.influenzae* (HI), *M.catarrhais* (MC), *S.pneumoniae* (SP), *S.aureus* (SA) and *P.aeruginosa* (PA). Sputum samples were also processed for detection of respiratory viruses by PCR analysis. Only sputum samples with <30% squamous cells, and good quality spirometry samples (A or B) were considered in the analyses.

Criteria for eosinophilic groups and seasonality

Eosinophilic inflammation was defined as sputum eosinophils >3% and blood eosinophils ≥2% in line with previous studies.[4-8] To investigate the stability of blood eosinophilic inflammation over time we divided subjects into three groups: predominantly (PE), intermittent (IE) and rarely (RE) eosinophilic. Only those subjects who had at least 3 (out of 5 potential) stable visits with valid blood results over 12 months were included in the group analyses (n=99). The PE group was defined as blood eosinophils (≥2%) at either all visits, or all but 1 visits where the

blood eosinophils were <2%; the RE group was defined as blood eosinophils <2% at all visits, or all but 1 visit where the blood eosinophils were ≥2%; the IE group was defined when none of the abovementioned criteria were met.

To investigate an impact of seasonality on exacerbations we divided the year into 2 seasons, each containing six months: one containing exacerbation visits occurring in winter and the other summer months. For the simplicity we defined them as winter (October-March) and summer (April – September) seasons.

Statistical analysis

Bivariate analyses testing for differences between eosinophilic groups were conducted using Kruskall-Wallis, ANOVA, Chi-Square, or Fisher's Exact test, as appropriate. All tests were two-tailed. Receiver Operator Curves (ROC) were used to assess the predictive ability of different cut offs to correctly identify presence of sputum eosinophilic inflammation. Intra-class correlations were used to assess the reliability of measures within individuals over time. As subjects contributed differing numbers of exacerbations over the study period, some subjects would be represented multiple times in analyses exploring outcomes at exacerbation. To counter this, descriptive analyses were conducted for only the first exacerbation occurring to each subject, and multivariate analyses with binary outcomes (presence/absence of different conditions at exacerbation) were conducted using conditional logistic regression, including the subject number as a random effect. SPSS (version 22) was used for all analyses with the exception of intra-class correlation coefficients (ICC) and conditional logistic regression, which were conducted using STATA (version 14). All of these analyses should be considered post hoc as they were not pre-specified in the AERIS statistical analysis plan.

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Table E1. Baseline characteristics by longitudinal blood eosinophil group over 12 months using 200 cells/uL cutoff (n=99).

		Rarely eosinophilic (n=24)			Intermittently eosinophilic (n=12)			Predominantly eosinophilic (n=63)			p valueΩ
Continuous variables		N	Median	(IQR)	N	Median	(IQR)	N	Median	(IQR)	
Age α		24	61.4	(8.68)	12	67.3	(4.87)	63	68.4	(8.82)	0.002
Smoking history (pack/years) α		24	45.6	(23.4)	12	72.5	(47.7)	63	50.5	(27.4)	0.017
BMI		24	26.6	(8.69)	12	28.2	(10.6)	63	26.3	(4.44)	0.437
FFBM		23	43.6	(21.2)	12	53.7	(19.1)	62	48.6	(22.3)	0.467
WBC		23	7.60	(2.50)	12	7.35	(1.57)	63	7.40	(2.10)	0.788<0.001
Blood eosinophils (count)		23	0.10	(0.10)	12	0.15	(0.10)	63	0.30	(0.20)	<0.001NA
Blood eosinophils (%)		23	1.35	(1.44)	12	1.92	(1.08)	63	4.05	(3.14)	0.330NA
Blood neutrophils (count)		23	5.00	(2.20)	12	4.80	(0.48)	63	4.70	(1.80)	0.624
Fibrinogen		21	4.70	(1.50)	11	4.60	(1.30)	56	4.80	(0.88)	0.699
CRP		24	4.00	(6.50)	12	5.00	(4.75)	63	5.00	(8.00)	0.097
Sputum eosinophils (%)≠		14	0.33	(2.24)	5	1.81	(1.78)	46	2.41	(7.32)	0.500
Sputum neutrophils (%)≠		14	9.77	(65.3)	5	45.6	(63.3)	46	45.1	(69.7)	0.736
FEV1 (%)		24	49.2	(26.9)	12	49.6	(25.1)	63	46.4	(23.7)	0.572
ΔFEV1(% of baseline)ξ		17	3.88	(23.9)	11	7.25	(31.3)	51	5.03	(21.6)	0.870
FEV1 reversibility (% of preBDFEV1)€		23	7.26	(15.0)	10	15.9	(21.3)	50	12.6	(13.3)	0.098
KCO (~~mmol/kPa/min~~%)		22	~~1.14~~75.1	(~~31.90~~ ~~46~~)	12	~~0.95~~73.0	(26.00~~.~~ ~~44~~)	60	~~0.91~~69.2	(~~0.44~~28.6)	0.572
TLCO(~~mmol/kPa/min~~%)		22	~~4.87~~65.0	(~~3.00~~2.0)	12	~~5.26~~61.9	(~~2.00~~7.0)	60	~~4.40~~56.2	(~~2.64~~9.6)	0.295 0.410 0.371 0.312
CAT		24	15.5	(11.0)	12	19.0	(12.0)	63	16.0	(10.0)	0.705
6MWT (distance in meters)		23	326	(174)	12	321	(227)	62	326	(166)	0.371 0.480 0.769
Exact score		22	34.0	(16.0)	10	35.0	(11.0)	51	37.0	(14.0)	<0.001 <0.001 0.943
Exacerbation rate in year before study		24	2.50	(3.00)	12	2.00	(3.00)	63	2.00	(3.00)	0.072 0.705
Exacerbation rate in first year of study		24	1.98	(3.91)	12	1.52	(2.00)	63	2.96	(3.91)	<0.001 <0.001 0.480
Eosinophilic exacerbation rate in first year of study		24	0.00	(0.98)	12	0.00	(0.99)	63	1.01	(2.98)	<0.001
Follow up (years) in first year of study		24	1.01	(0.02)	12	1.00	(0.01)	63	1.00	(0.02)	<0.001 <0.001 0.072
Categorical variables		N	(%)		N	(%)		N	(%)		
Sex	Male	10	(42%)		8	(67%)		37	(59%)		0.297
	Female	14	(58%)		4	(33%)		26	(41%)		
Current smokerγ μ	Yes	14	(58%)		4	(33%)		25	(40%)		0.233
	No	10	(42%)		8	(67%)		38	(60%)		
Use of ICS at enrolment ¥	Yes	20	(83%)		11	(92%)		56	(89%)		0.733
	No	4	(17%)		1	(8%)		7	(11%)		
Sputum eosinophilia (>3%)≠	Yes	1	(7%)		0	(0%)		20	(43%)		0.011
	No	13	(93%)		5	(100%)		26	(57%)		
Blood eosinophilia (>=2%)	Yes	6	(26%)		6	(50%)		59	(94%)		<0.001 NA
	No	17	(74%)		6	(50%)		4	(6%)		

Bacteria present*	Yes	10	(56%)	4 (44%)	30 (51%)	0.892
	No	8	(44%)	5 (56%)	29 (49%)	
Virus present**	Yes	4	(24%)	0 (0%)	10 (18%)	0.394
	No	13	(76%)	9 (100%)	47 (82%)	

Ω Kruskall-Wallis test used for continuous variables, Fisher's Exact test for categorical variables
α reported as Mean(±SD), p value calculated from ANOVA
γ smoking status report based derived from ATS Q7A4
¥IICS use were coded as "Yes" if one of the following medications/inhalers was on the list (SYMBICORT, SERETIDE, QVAR, FOSTAIR, BECLOMETHASONE, BECLAMETHOSONE/FORMOTEROL, BECLOMETHASONE dipropiionate, CLENIL, FLUTICASONE/SALMETEROL, BUDESONIDE/FORMOTEROL
≠Eosinophil% and Neutrophil% at baseline is reported. "Baseline" is equal to enrolment if good quality data (SQC<30) is present at enrolment, or the next (pre-exacerbation) stable visit with quality data within four months of enrolment.
ξ calculated as FEV1 at month 12 * 100 / FEV1 at enrolment
€ calculated as (post broncho dilator BDFEV1 - preBD broncho dilator FEV1) / pre BDbroncho dilator FEV1 * 100
* Sputum sampling, measured by culture. Includes Haemophilus influenzae, Moraxella catarrhalis, Streptococcus pneumoniae, Staphylococcus aureus and Pseudomonas aeruginosa.
** Sputum sampling, measured by PCR. Includes adenovirus, enterovirus, influenza, coronavirus, metapneumovirus, bocavirus, parainfluenza, RSV, and rhinovirus.

Table E2. Characteristic of those excluded and included in the longitudinal analyses at enrolment.

		Excluded			Included			P valueΩ
Continuous variables		N	Median	(IQR)	N	Median	(IQR)	
Age α		28	67.6	(7.80)	99	66.6	(8.85)	0.673
Smoking history (pack/years) α		28	44.2	(17.7)	99	52.0	(30.3)	0.263
BMI		28	28.4	(7.57)	99	26.7	(6.39)	0.080
FFBM		28	46.5	(20.0)	97	49.3	(21.7)	0.526
WBC		28	7.85	(2.60)	98	7.50	(2.13)	0.368
Blood eosinophils (count)		28	0.20	(0.20)	98	0.20	(0.15)	0.090
Blood eosinophils (%)		28	2.16	(3.11)	98	3.05	(3.01)	0.102
Blood neutrophils (count)		28	4.95	(1.75)	98	4.80	(1.60)	0.277
Fibrinogen		26	5.25	(1.13)	88	4.80	(1.08)	0.110
CRP		28	6.00	(11.3)	99	5.00	(7.00)	0.023
Sputum eosinophils (%)≠		18	1.97	(3.58)	65	1.81	(5.22)	0.665
Sputum neutrophils (%)≠		18	55.6	(52.7)	65	31.8	(69.7)	0.184
FEV1 (%)		27	42.4	(24.0)	99	47.0	(25.4)	0.746
ΔFEV1(% of baseline)ξ		6	5.74	N/A	79	4.93	(21.6)	0.864
FEV1 reversibility (% of preBDFEV1)€		22	8.56	(16.8)	83	11.3	(18.7)	0.431
KCO (~~mmol/kPa/min~~%)		28	~~0.87~~63.9	(~~0.52~~40.3)	94	~~0.98~~70.3	(~~0.48~~28.9)	0~~.352~~.335
TLCO(~~mmol/kPa/min~~%)		28	~~3.85~~50.8	(~~2.25~~26.1)	94	~~4.62~~59.0	(2.~~55~~9.1)	~~0.045~~0.105
CAT		27	20.0	(13.0)	99	16.0	(10.0)	0.096
6MWT (distance in meters)		28	229	(130)	97	324	(169)	0.002
Exact score		18	38.5	(8.00)	83	36.0	(14.0)	0.149
Exacerbation rate in year before study		28	3.00	(3.00)	99	2.00	(2.00)	0.215
Exacerbation rate in first year of study		28	3.99	(6.70)	99	1.99	(3.02)	0.078
Eosinophilic exacerbation rate in first year of study		28	1.34	(3.32)	99	0.99	(1.99)	0.412
Follow up (years) in first year of study		28	0.58	(0.57)	99	1.01	(0.02)	<0.001
Categorical variables		N	(%)		N	(%)		
Sex	Male	13	(46%)		55	(56%)		0.401
	Female	15	(54%)		44	(44%)		
Current smokerγ μ	Yes	11	(39%)		43	(43%)		0.829
	No	17	(61%)		56	(57%)		
Use of ICS at enrolment ¥	Yes	26	(93%)		87	(88%)		0.733
	No	2	(7%)		12	(12%)		
Sputum eosinophilia (>3%)≠	Yes	6	(33%)		21	(32%)		1.000
	No	12	(67%)		44	(68%)		
Blood eosinophilia (>=2%)	Yes	15	(54%)		71	(72%)		0.068
	No	13	(46%)		27	(28%)		
Bacteria present*	Yes	13	(54%)		44	(51%)		0.821
	No	11	(46%)		42	(49%)		
Virus present**	Yes	4	(20%)		14	(17%)		0.747
	No	16	(80%)		69	(83%)		

α reported as Mean(±SD)
γ smoking status report based derived from ATS Q7A4

¥ICS use were coded as "Yes" if one of the following medications/inhalers was on the list (SYMBICORT, SERETIDE, QVAR, FOSTAIR, BECLOMETHASONE, BECLAMETHOSONE/FORMOTEROL, BECLOMETHASONE dipropiionate, CLENIL, FLUTICASONE/SALMETEROL, BUDESONIDE/FORMOTEROL)
≠Sputum eosinophil and neutrophil % at baseline is reported. "Baseline" is equal to enrolment if good quality data (SQC<30) is present at enrolment, or the next (pre-exacerbation) stable visit with quality data within four months of enrolment.
ξ calculated as FEV1 at month 12 * 100 / FEV1 at enrolment
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* Sputum sampling, measured by culture. Includes Haemophilus influenzae, Moraxella catarrhalis, Streptococcus pneumoniae, Staphylococcus aureus and Pseudomonas aeruginosa.
** Sputum sampling, measured by PCR. Includes adenovirus, enterovirus, influenza, coronavirus, metapneumovirus, bocavirus, parainfluenza, RSV, and rhinovirus.

Table E3: Seasonality[μ] of eosinophilic exacerbations in longitudinal phenotypes

		Summer		Winter	
		Median	(IQR)	Median	(IQR)
~~Premodinantly~~Predominantly eosinophilic	Exacerbation rate	2.00	(4.00)	2.01	(3.03)
	Eosinophilic exacerbation rate	1.93	(3.93)	2.00	(2.02)
Intermittently eosinophilic	Exacerbation rate	0.00	(2.00)	2.00	(3.53)
	Eosinophilic exacerbation rate	0.00	(0.00)	0.00	(1.99)
Rarely eosinophilic	Exacerbation rate	1.98	(3.99)	3.73	(4.00)
	Eosinophilic exacerbation rate	0.00	(0.00)	0.00	(1.94)
OVERALL	Exacerbation rate	1.99	(3.99)	2.01	(2.05)
	Eosinophilic exacerbation rate	0.00	(2.00)	0.00	(2.00)

μ Summer season defined as April-September, Winter as October-March. 12 monthly rates are presented for all exacerbations, and for eosinophilic exacerbations.

Table E4: Odds* of eosinophilic inflammation at exacerbation in summer compared to winter[μ]

	N of total exacerbations	N of exacerbations in Summer	N of exacerbations in Winter	N of individuals	Odds Ratio	95% CI	p value
Exacerbations with blood eosinophilia (>=2%)	338	132	206	104	2.65	(1.50; 4.68)	0.001
Exacerbations with sputum eosinophilia (>3%)	218	88	130	91	1.94	(0.82; 4.52)	0.129

*Conditional logistic regression including subject as a random effect

μ Summer season defined as April-September, Winter as October-March

~~*Conditional logistic regression including subject as a random effect~~

~~μ Summer season defined as April-September, Winter as October-March~~

Formatted Table

Figure E1

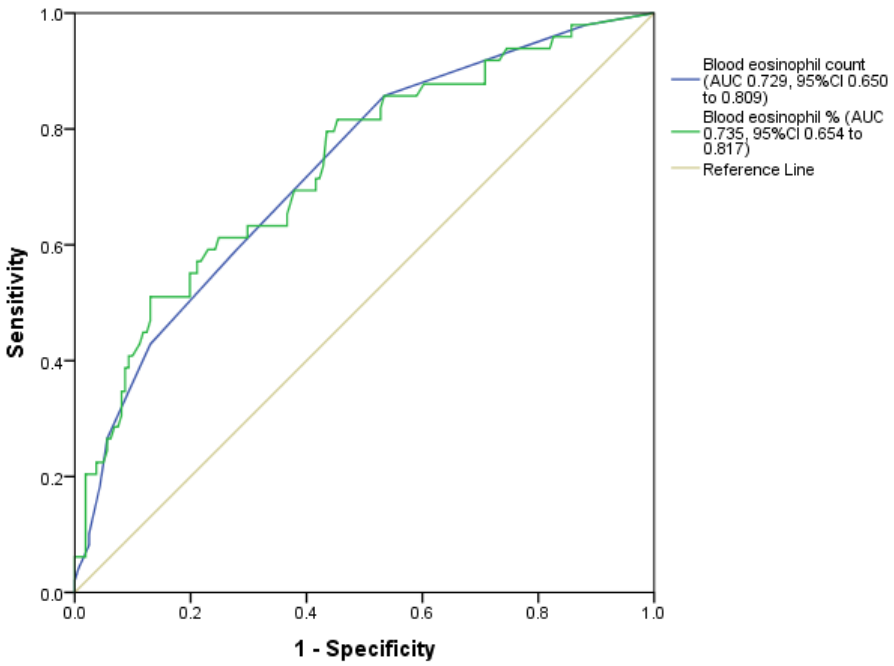
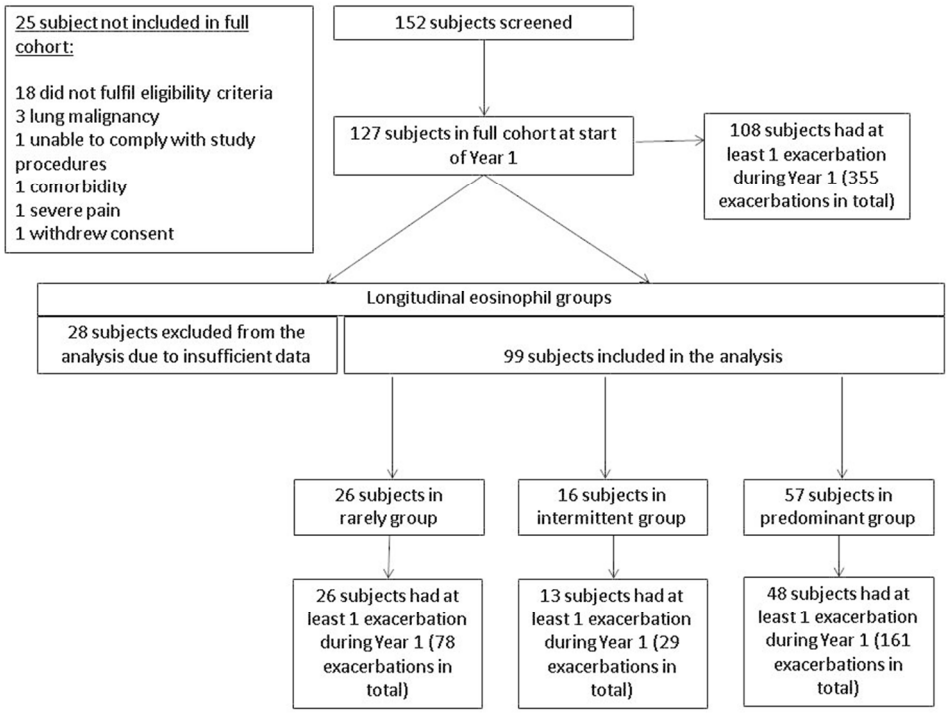


Figure E1 Receiver operating characteristic for blood eosinophil (count & %) at exacerbation predicting sputum eosinophilia >3% (n=210) at exacerbation. At exacerbations blood eosinophils ≥2% cut point was 79.6% sensitive and 55.3% specific in identifying sputum eosinophils (>3%).



Flow diagram of the subjects screened and included in the full cohort at the start of Year 1 and the number of exacerbations in Year 1.

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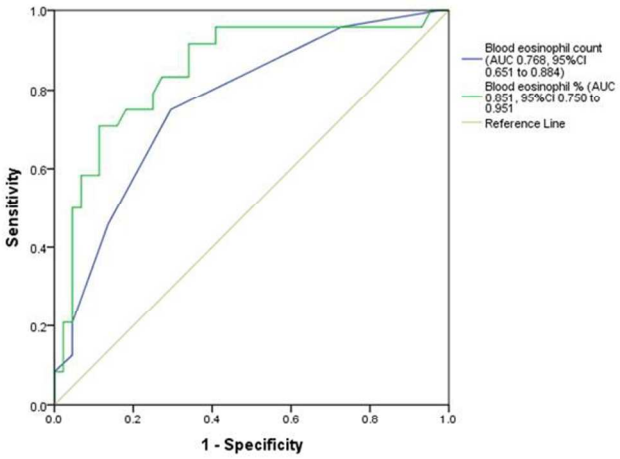
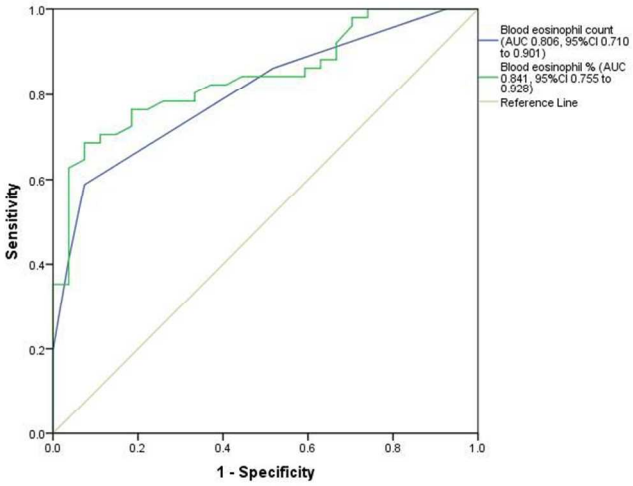


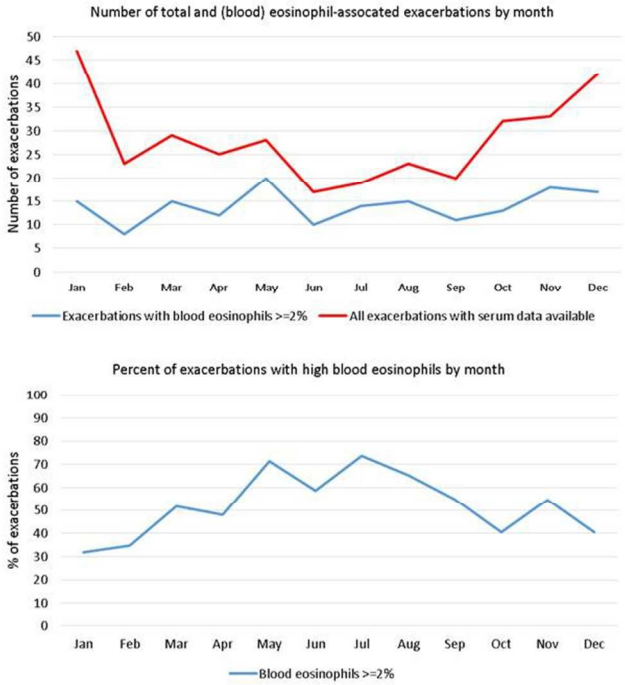
Receiver operating characteristic curve with area under the curve for blood eosinophil (count & %) at enrolment predicting sputum eosinophilia >3% at enrolment (n=68).

254x190mm (96 x 96 DPI)



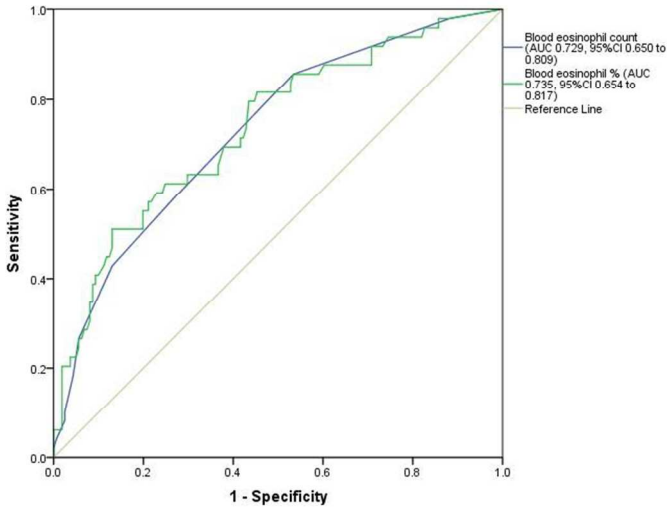
Receiver operating characteristic curve for blood eosinophils (count and %) at enrolment predicting the predominantly eosinophilic group over 12 months following enrolment) (n=78).

254x190mm (96 x 96 DPI)



Seasonal distribution of total and eosinophil-associated exacerbations. A -Number of total and exacerbations with blood eosinophils≥2%. B - Proportion of exacerbations with blood eosinophils≥2% defined as exacerbations with blood eosinophils≥2% to total exacerbation rates in the predominantly, intermittent and rarely groups.

254x190mm (96 x 96 DPI)



Receiver operating characteristic for blood eosinophil (count & %) at exacerbation predicting sputum eosinophilia >3% (n=210) at exacerbation.

254x190mm (96 x 96 DPI)

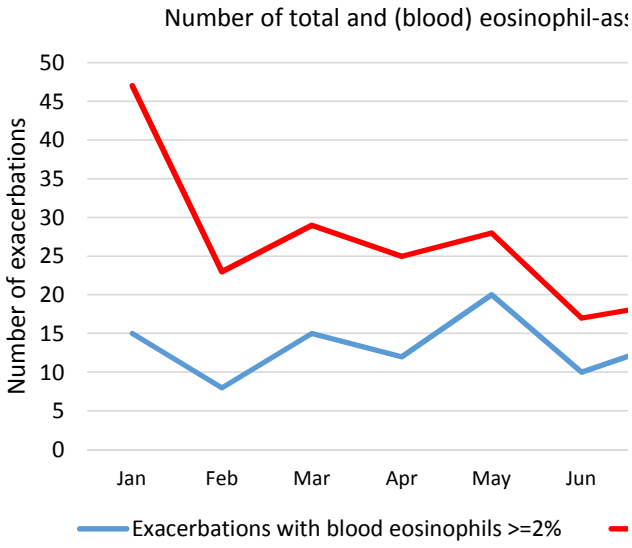
Month of exacerbation visit * Eosinophils categorical (>=2% in blood) [labo] Crosstabulation

Count

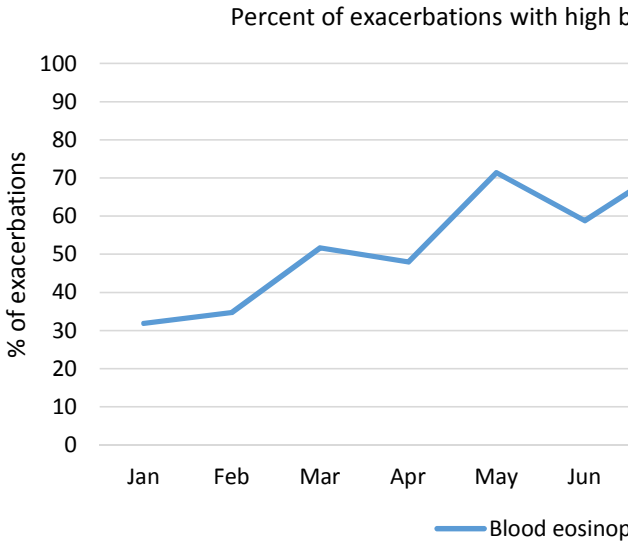
		Eosinophils categorical		Total	
		No (<2%)	Yes (>=2%)		
Month of e	Jan	32	15	47	Jan
	Feb	15	8	23	Feb
	Mar	14	15	29	Mar
	Apr	13	12	25	Apr
	May	8	20	28	May
	Jun	7	10	17	Jun
	Jul	5	14	19	Jul
	Aug	8	15	23	Aug
	Sep	9	11	20	Sep
	Oct	19	13	32	Oct
	Nov	15	18	33	Nov
	Dec	25	17	42	Dec
Total		170	168	338	

Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec

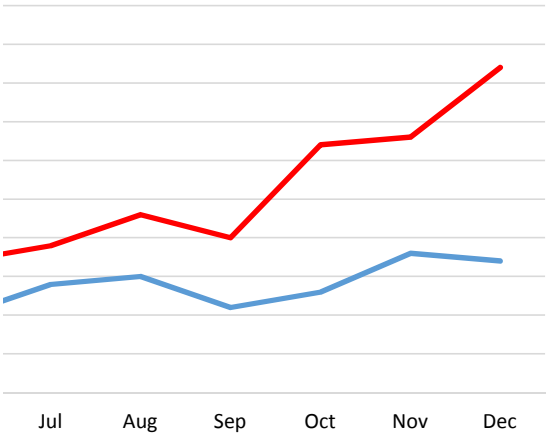
Exacerbatic	All exacerbations with blood data available
15	47
8	23
15	29
12	25
20	28
10	17
14	19
15	23
11	20
13	32
18	33
17	42



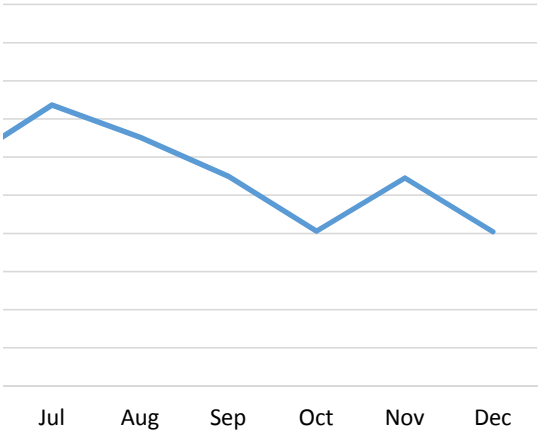
Blood eosinophils >=2%
31.91
34.78
51.72
48.00
71.43
58.82
73.68
65.22
55.00
40.63
54.55
40.48



socated exacerbations by month



lood eosinophils by month



hils >=2%