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 and definitions of severity categories were described previously[1, 2].

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.

References

1. Bourne S, Cohet C, Kim V, Barton A, Tuck A, Aris E, Mesia-Vela S, Devaster JM, Ballou WR, Clarke S, Wilkinson T. Acute Exacerbation and Respiratory InfectionS in COPD (AERIS): protocol for a prospective, observational cohort study. BMJ open. 2014;4(3):e004546.
2. Wilkinson TMA, Aris E, Bourne S, Clarke SC, Peeters M, Pascal TG, Schoonbroodt S, Tuck AC, Kim V, Ostridge K, Staples KJ, Williams N, Williams A, Wootton S, Devaster JM, Group AS. A prospective, observational cohort study of the seasonal dynamics of airway pathogens in the aetiology of exacerbations in COPD. Thorax. 2017; in press [http://dx.doi.org/10.1136/thoraxjnl-2016-209023].
3. Vignola AM, Rennar SI, Hargreave FE, Fah JV, Bonsignore MR, Djukanovic R, Sterk PJ. Standardised methodology of sputum induction and processing. Future directions. Eur Respir J Suppl. 2002;37:51s-5s.
4. Pizzichini E, Pizzichini MM, Gibson P, Parameswaran K, Gleich GJ, Berman L, Dolovich J, Hargreave FE. Sputum eosinophilia predicts benefit from prednisone in smokers with chronic obstructive bronchitis. American journal of respiratory and critical care medicine. 1998;158(5 Pt 1):1511-7.
5. Brightling CE, Monteiro W, Ward R, Parker D, Morgan MD, Wardlaw AJ, Pavord ID. Sputum eosinophilia and short-term response to prednisolone in chronic obstructive pulmonary disease: a randomised controlled trial. Lancet. 2000;356(9240):1480-5.
6. Bafadhel M, McKenna S, Terry S, Mistry V, Reid C, Haldar P, McCormick M, Haldar K, Kebadze T, Duvoix A, Lindblad K, Patel H, Rugman P, Dodson P, Jenkins M, Saunders M, Newbold P, Green RH, Venge P, Lomas DA, Barer MR, Johnston SL, Pavord ID, Brightling CE. Acute exacerbations of chronic obstructive pulmonary disease: identification of biologic clusters and their biomarkers. American journal of respiratory and critical care medicine. 2011;184(6):662-71.
7. Siva R, Green RH, Brightling CE, Shelley M, Hargadon B, McKenna S, Monteiro W, Berry M, Parker D, Wardlaw AJ, Pavord ID. Eosinophilic airway inflammation and exacerbations of COPD: a randomised controlled trial. Eur Respir J. 2007;29(5):906-13.
8. Pascoe S, Locantore N, Dransfield MT, Barnes NC, Pavord ID. Blood eosinophil counts, exacerbations, and response to the addition of inhaled fluticasone furoate to vilanterol in patients with chronic obstructive pulmonary disease: a secondary analysis of data from two parallel randomised controlled trials. The Lancet Respiratory medicine. 2015;3(6):435-42.

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
€ 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

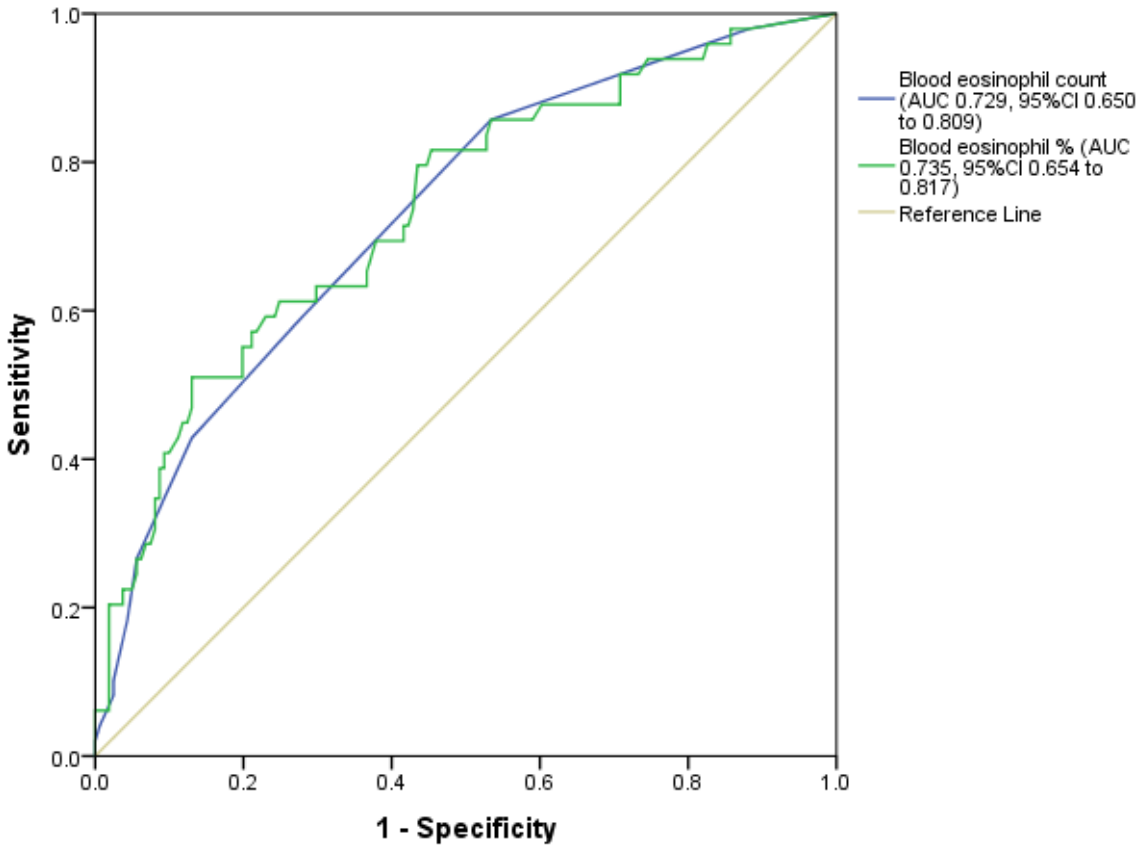

Figure E1 Receiver operating characteristic for blood eosinophil (count & %) at exacerbation predicting sputum eosinophilia >3% (n=210) at exacerbation. At exacerbations blood eosinophils ≥2% cut point was 79.6% sensitive and 55.3% specific in identifying sputum eosinophils (>3%).