Clinical and inflammatory characteristics of the European U-BIOPRED adult severe asthma cohort

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Short title: U-BIOPRED adult severe asthma

Word count: 3248

Figures: 5

Tables: 5

Key Words: severe asthma, lung function, quality of life, asthma control, sputum.

Message: Patients with severe asthma have more airway inflammation, worse symptoms and lower lung function, despite higher doses of treatment.

Abbreviations

BMI; Body mass index

FeNO; Fraction of exhaled nitric oxide

$FEV_{1;}$ Forced Expiratory Volume in one second

FVC; Forced Vital Capacity

HC; Healthy non-smoking controls

ICS; Inhaled Corticosteroids

MMA; Mild/moderate non-smoking asthma

OCS; Oral corticosteroids

SAn; Severe non-smoking asthma

SAs/ex; Smokers and ex-smokers with severe asthma

U-BIOPRED: Unbiased Biomarkers for the Prediction of Respiratory Disease Outcome

Abstract (196)

U-BIOPRED is an EU consortium of 20 academic institutions, 11 pharmaceutical companies and 6 patient organisations with the objective of improving the understanding of asthma disease mechanisms using a systems biology approach.

This cross-sectional assessment of adults with severe asthma, mild/moderate asthma and healthy controls from 11 European countries consisted of analyses of patient-reported outcomes, lung function, blood and airway inflammatory measurements.

Patients with severe asthma (non-smokers n=311 and smokers/ex-smokers n=110) had more symptoms and exacerbations compared to patients with mild-moderate disease (n=88) (2.5 exacerbations versus 0.4 in the preceding 12 months, $p<0.001$), with worse quality of life, and higher levels of anxiety and depression. They also had a higher incidence of nasal polyps and gastro-oesophageal reflux with lower lung function. Sputum eosinophil count was higher in severe asthma compared to mild-moderate asthma (median count 2.99% versus 1.05%, $p=0.004$) despite treatment with higher doses of inhaled and/or oral corticosteroids.

Consistent with other severe asthma cohorts, U-BIOPPRED is characterised by poor symptom control, increased co-morbidity and airway inflammation, despite high levels of treatment. It is well suited to identify asthma phenotypes using the array of 'omic' datasets that are at the core of this systems medicine approach.

Introduction

A substantial number of patients with asthma require systemic corticosteroids to control symptoms and/or suffer from poor control and frequent severe exacerbations despite currently available treatment (1, 2). Although recently-developed biologic compounds targeting cytokines of the Type 2 pathways show promise (3, 4), identification of new treatment targets and the selection of patients best suited to respond to individual biologics is still hampered by a poor understanding of the physiological, pathological, and molecular heterogeneity of severe asthma (5, 6).

Severe asthma is a collection of disease entities with varying pathophysiological characteristics (7) that result in symptoms of cough, wheeze and breathlessness, with frequent exacerbations. To address the problem of phenotypic difference and heterogeneity, the Unbiased Biomarkers for the Prediction of Respiratory Disease Outcomes (U-BIOPRED) project was set up in 2009 as a public-private partnership within the framework of the Innovative Medicines Initiative (IMI), engaging academia, the pharmaceutical industry and patient groups. The aim of U-BIOPRED is to identify multi-dimensional phenotypes of severe asthma and new treatment targets using a combination of state of the art 'omics' (transcriptomic, proteomic, lipidomic and metabolomic) technologies applying a systems biology approach (8) thereby driving unbiased discovery in both adult and paediatric severe asthma (9). This novel approach is designed to make drug development more effective and efficient.

We present the baseline characteristics of the adult participants with severe asthma who form the majority of the U-BIOPRED cohort and compare these participants with those suffering from with mild-to-moderate disease, in terms of their clinical, symptomatic, functional and biomarker features. In a parallel paper the characteristics of the paediatric cohort are reported. These first publications of U-BIOPRED will serve as the reference documents for all subsequent publications using the 'omics technologies which are at the core of this programme.

Methods

Participants

This was a multi-centre prospective cohort study recruiting from 16 clinical centres in 11 European countries. Details of the participating centres, assessments, and standard operating procedures are available in the online supplement. Prior to enrolment, participants with severe asthma were required to have been under follow-up by a respiratory physician for at least six months during which time assessments had been undertaken to optimise asthma control and assess medication

adherence (2). The study was approved by the ethics committee for each participating clinical institution, and adhered to the standards set by International Conference on Harmonisation and Good Clinical Practice. It is registered on *ClinicalTrials.gov*, (Identifier: NCT01982162). All participants gave written and signed informed consent.

Adult Groups

The definition of severe asthma used in this study was agreed at a U-BIOPRED consensus meeting (2). Participants with asthma had either airflow reversibility (increase in FEV_1 >12% predicted or 200ml following inhalation of 400µg salbutamol), airway hyper-responsiveness (methacholine PC_{20} < 8mg/ml, or diurnal PEF amplitude % mean >8%), or a decrease in FEV_1 of 12% predicted or 200ml within 4 weeks after tapering maintenance treatment. Four groups were recruited:

A) Severe non-smoking asthma (SAn):

Participants in this group were non-smokers for at least the past 12 months, with a less than five pack-year smoking history, with asthma and uncontrolled symptoms defined according to GINA guidelines (10) and/or frequent exacerbations (more than two per year) despite high-dose inhaled corticosteroids (ICS) (ICS ≥ 1000µg fluticasone propionate/day or equivalent dose).

B) Smokers and ex-smokers with severe asthma (SAs/ex):

This group was defined as for the SAn group except that they were either current smokers or ex-smokers with a smoking history of at least 5 pack years.

C) Mild/Moderate non-smoking asthmatics (MMA):

Participants in this group were non-smokers for at least the past 12 months, with a less than five pack-year smoking history and had controlled or partially controlled asthma symptoms, as defined by the Global Initiative for Asthma (GINA), whilst receiving a dose of less than 500µg fluticasone propionate/day or equivalent.

D) Healthy non-smoking controls (HC):

These participants had no history of asthma or wheeze, had no other chronic respiratory disease, were non-smokers for at least the past 12 months with a smoking history of ≤ 5 pack years and their pre-bronchodilator FEV_1 was ≥ 80% predicted.

Protocol and assessments

Participants attended a screening visit to assess eligibility for the study **(Fig 1)**. They underwent a baseline visit (up to 28 days later) and were invited to attend for an optional bronchoscopy, high resolution lung computed tomography and telemonitoring sessions. Spirometry, haematological profiles, and fraction of exhaled nitric oxide level (FeNO) at 50ml/sec were performed. Induced sputum was obtained (11) and differential sputum eosinophil and neutrophil counts measured following a standardised operating procedure. Sputum supernatants and cell pellets were collected. Allergic status was obtained by either skin prick testing or measurement of specific IgE to six common aeroallergens. Blood and urine samples were taken for lipidomic, proteomic and transcriptomic analyses for later assessment. An optional sample was taken for genetic analysis. Subsets of participants underwent plethysmographic measurements, high-resolution computed tomography (HRCT) and collection of exhaled breath for measurement of metabolites including volatile organic compounds, all for future analyses. All investigations were performed according to standardised operating procedures (online supplement).

Participants with severe asthma were reviewed at 12-18 months after enrolment and were also invited to attend if they experienced an exacerbation. At 12-24 months, they were contacted by phone or by post to obtain information on asthma control.

Data were entered on an electronic case report form. The study was run and monitored by Cromsource (www.cromsource.com). Samples were sent to the Centre for Integrated Genomic Medical Research Biobank in Manchester, UK. Datasets were uploaded on to the tranSMART system, an open-source knowledge management platform for sharing research data (12) supported by the European Translational Information and Knowledge Management Services (eTRIKS) project.

The study aims are published on the U-BIOPRED home page (www.europeanlung.org/en/projects-and-research/projects/u-biopred/home).

Questionnaires

The following were administered at baseline:

1. The Asthma Control Questionnaire (ACQ5) (13) to assess current asthma control.
2. The Asthma Quality of Life Questionnaire (AQLQ) (14) to assess quality of life and psychological morbidity.
3. The Hospital Anxiety and Depression Scale (HADS) (15).
4. Sino-Nasal Outcomes Test (SNOT20) (16) to measure upper airway symptoms.

5. The Epworth Sleepiness Scale (ESS) (17) to measure sleep and daytime drowsiness.
6. The Medicines Adherence Response Scale (MARS) (18) to measure adherence.

Statistical Analysis

Continuously distributed data were either summarised using mean (standard error; SE) if symmetrical, or median (inter-quartile range; IQR) values. Non-symmetrical variables all exhibited positive skew and were log-transformed prior to association testing. Missing data were not imputed. P-values were calculated using a general linear model for continuous variables or a general logistic model for categorical variables. No adjustment for multiple testing was applied as the analyses were considered exploratory. Analyses were performed using R version 2.15.2 (R Core Team, 2012).

Results

A total of 610 adults were recruited over an 18-month period: 311, 110, 88 and 101 into the SAn, SAs/ex, MMA, HC groups respectively **(Table i).**

	N	severe non-smoking asthma n=311	smokers and ex-smokers with severe asthma n=110*	mild/moderate non-smoking asthma n=88	healthy non-smoking controls n=101	P-value
Age (yr)	Mean (SE) [N]	51.01 (0.8) [311]	54.51 (1.08) [110]	41.66 (1.65) [88]	38.85 (1.34) [101]	p<0.001
Age at Diagnosis(yr)	Median (IQR) [N]	20 (7_38) [302]	38 (20_48) [109]	14 (6_32) [83]	NA	p<0.001
Female	n/N (%)	205/311 (66)	56/110 (51)	44/88 (50)	39/101 (39)	p<0.001
BMI (kg/m^2)	Mean (SE) [N]	29.11 (0.36) [311]	29.59 (0.6) [110]	25.73 (0.47) [88]	25.31 (0.36) [101]	p<0.001
BMI>30 (kg/m^2)	n/N %	120/311 (38.6)	44/110 (40)	16/88 (18.18)	12/101 (11.88)	<0.001
Serum IgE (IU/ml)	Median (IQR) [N]	119.5 (45_342) [302]	126 (63_328) [104]	89.4 (49_244) [85]	23.45 (9_65) [98]	p<0.001
FEV_1 (%)	Mean (SE) [N]	67.5 (1.26) [308]	67.2 (1.84) [110]	89.5 (1.86) [87]	101.76 (1.29) [101]	p<0.001
FVC (%)	Mean (SE) [N]	87.2 (1.12) [308]	89.7 (1.74) [110]	104.5 (2.02) [87]	107.8 (1.3) [101]	p<0.001
FEV_1/FVC ratio	Mean (SE) [N]	0.64 (0.01) [308]	0.61 (0.01) [110]	0.72 (0.01) [87]	0.79 (0.01) [101]	p<0.001
Exacerbations in Previous Year	Mean (SE) [N]	2.48 (0.13) [310]	2.55 (0.26) [110]	0.38 (0.08) [88]	NA	p<0.001
Pack Years	Median (IQR) [N]	2 (1_4) [47]	17.38 (10_26) [110]	4 (1_4) [13]	0.9 (0_3) [20]	<0.001
Intubation (Ever)	n/N (%)	35/307 (11)	6/109 (6)	0/87 (0)	NA	0.083
ICU Admission (Ever)	n/N (%)	80/307 (26)	18/109 (17)	1/86 (1)	NA	p<0.001
Atopy test positive	n/N (%)	213/272 (78.3)	62/87 (71.3)	72/78 (92.3)	36/78 (46.2)	p<0.001

Table i

Group Demographics

FEV_1: Forced Expiratory Volume in one second

FVC: Forced Vital Capacity

IQR: Inter quartile range

ICU: Intensive care unit

NA: Not applicable

SE: Standard error

* 42 current smokers and 68 ex-smokers

There were more females in the SAn group (66%) compared to the other asthma groups (50%), with the age of onset of asthma 18yrs later in SAs/ex compared with SAn. Participants with severe asthma had a higher BMI than those in MMA and HC groups and were older **(Table i)**. Both severe asthma groups experienced 2.5 exacerbations in the preceding 12 months as compared with 0.4 in the MMA group ($p< 0.001$). There was a higher rate of ICU admissions in the SAn participants compared to the SAs/ex group ($p<0.05$). Further split of the severe asthma groups based on current and ex-smoking is presented in the online supplement (Table S5).

Spirometry **(Table i)**

FEV_1 (% predicted or actual) was lower in the three asthma groups compared to the HC group ($p<0.001$), with the severe asthma groups having the lowest FEV_1. FVC (% predicted or actual) was also lower in both the SAn and SAs/ex groups when compared to the MMA ($p<0.001$) and HC groups ($p<0.001$). The mean FEV_1/FVC ratio was lower in those with severe asthma (0.64 and 0.61, respectively) compared to the MMA (ratio 0.72, $p<0.001$) and HC groups (ratio 0.79, $p<0.001$) respectively).

Medications **(Table ii)**

Within the SAn and SAs/ex groups 46% and 45% respectively received daily OCS, and 17% and 16% respectively received anti-IgE therapy. Use of nebulised β-agonist was higher in the SAn and SAs/ex groups. Other classes of therapy were also used.

		severe non-smoking asthma N=311	smokers and ex-smokers with severe asthma N=110	mild/moderate non-smoking asthma N=88
Oral corticosteroid	n/N (%)	135/295 (45.8)	46/103 (44.7)	0/88 (0)
Prednisolone Equ. (mg)*	Mean (SE) [N]	13.2 (0.85) [122]	14.8 (1.81) [36]	NA (NA)
Inhaled corticosteroids	n/N (%)	310/311 (99.7)	110/110 (100)	87/88 (98.9)
Long acting beta agonist	n/N (%)	305/309(98.7)	109/110 (99.1)	2/88 (2.3)
Short acting beta agonist	n/N (%)	260/301 (86.3)	82/105 (78.1)	68/88 (77.3)
Injected corticosteroids	n/N (%)	19/284 (6.7)	1/97 (1.0)	0/88 (0)
Mucolytic	n/N (%)	31/286 (10.8)	18/100 (18.0)	0/88 (0)
Anti-histamine	n/N (%)	75/311 (24.1)	16/110 (14.6)	4/88 (4.5)
Antibiotic (excluding macrolide)	n/N (%)	11/288 (3.8)	4/98 (4.1)	0/88 (0)
Macrolide	n/N (%)	32/311 (10.3)	13/110 (11.8)	0/88 (0)
Long-acting muscarinic antagonist	n/N (%)	65/284 (22.9)	27/97 (27.9)	0/88 (0)
Short acting muscarinic antagonist	n/N (%)	127/292 (43.5)	48/104 (46.2)	0/88 (0)
Omalizumab	n/N (%)	50/287 (17.4)	16/98 (16.3)	0/88 (0)
Immunosuppressant	n/N (%)	9/311 (2.9)	4/110 (3.6)	0/88 (0)
Leukotriene modifier	n/N (%)	139/298 (46.6)	45/106 (42.5)	0/88 (0)
Cromones	n/N (%)	10/284 (3.5)	2/97 (2.1)	0/88 (0)
Anti-fungal agent	n/N (%)	5/311 (1.6)	1/110 (1.0)	0/88 (0)
Xanthine	n/N (%)	59/289 (20.4)	21/100 (21.0)	0/88 (0)
Nebulised beta-agonist	n/N (%)	82/284 (28.9)	24/97 (24.7)	2/88 (2.3)

Table ii

Medications

* Hydrocortisone and Triamcinolone doses were converted to equivalent prednisolone dose

(4 healthy control participants took as required antihistamines)

Questionnaires **(Table iii)**

ACQ and AQLQ scores reflected worse asthma control and increased morbidity in both severe asthma groups with minimal impairment in the MMA group. A similar pattern was seen with anxiety and depression. There were more upper airway symptoms measured using the SNOT20 in both severe asthma groups compared with the MMA group. Similarly the ESS scores indicated that there was an increase in sleepiness in the severe asthma groups compared to only a very mild impairment in the MMA group.

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		severe non-smoking asthma N=311	smokers and ex-smokers with severe asthma N=110	mild/moderate non-smoking asthma N=88	Unadjusted P-value SA* vs. MMA
Asthma control questionnaire (ACQ)					
Mean ACQ5	Mean (SE) [N]	2.28 (0.07) [277]	2.23 (0.12) [96]	0.86 (0.07) [85]	p<0.001
Mean ACQ7	Mean (SE) [N]	2.67 (0.08) [277]	2.62 (0.12) [96]	1.01 (0.07) [85]	p<0.001
Asthma quality of life questionnaire (AQLQ)					
Total	Mean (SE) [N]	4.48 (0.07) [276]	4.44 (0.13) [92]	5.84 (0.1) [84]	p<0.001
Symptoms	Mean (SE) [N]	4.46 (0.08) [276]	4.36 (0.14) [92]	5.87 (0.1) [84]	p<0.001
Emotional	Mean (SE) [N]	4.63 (0.1) [276]	4.52 (0.16) [92]	5.98 (0.13) [84]	p<0.001
Environmental stimuli	Mean (SE) [N]	4.69 (0.09) [276]	4.57 (0.16) [92]	5.63 (0.14) [84]	p<0.001
Activity limitation	Mean (SE) [N]	4.35 (0.07) [276]	4.45 (0.13) [92]	5.81 (0.11) [84]	p<0.001
Hospital and anxiety and depression score (HADS)					
Total	Mean (SE) [N]	12.33 (0.54) [223]	13.64 (1.01) [72]	7.01 (0.7) [70]	p<0.001
Anxiety	Mean (SE) [N]	6.94 (0.3) [223]	7.71 (0.54) [72]	4.24 (0.41) [70]	p<0.001
Depression	Mean (SE) [N]	5.39 (0.28) [223]	5.93 (0.56) [72]	2.77 (0.39) [70]	p<0.001
Sino-nasal outcome test 20 (SNOT 20)					
Total	Mean (SE) [N]	31.76 (1.01) [283]	32.12 (1.92) [97]	15.42 (1.42) [83]	p<0.001
Epworth sleepiness scale (ESS)					
Total	Mean (SE) [N]	7.48 (0.26) [277]	7.95 (0.47) [95]	5.49 (0.41) [85]	p<0.001
Medication adherence rating scale (MARS)					
Total	Mean (SE) [N]	22.44 (0.14) [278]	22.17 (0.29) [94]	21.35 (0.4) [84]	0.002

310 **Table iii**

311 Questionnaires

312 *SA represents SAn and SAs/ex groups combined

313 The MARS questionnaire scores for adherence to treatment recorded by the three asthma groups
314 were in the range of 21 to 22, with the severe asthma groups recording higher scores ($p<0.005$)
315 indicating better adherence. The AQLQ score was correlated to several variables, including FEV_1 (95%
316 CI 0.5_0.7, $p<0.001$), FEV_1/FVC (95% CI 1.14_2.8, $p<0.001$), exacerbations in the previous year (95%
317 CI -0.8_-0.2, $p<0.001$), BMI (95% CI -000.6_-0.002, $p<0.001$) and pack years smoked (95% CI -0.003_-
318 0.001, $p<0.001$) **(Figure 3)**.
319
320 Atopy and co-morbidities **(Table iv)**
321 There was a high incidence of atopy in the 4 groups, at 70% in the asthma groups and 46% in the HC
322 group. The incidence of allergic rhinitis, hay fever and non-allergic rhinitis were highest in the
323 asthma groups. The HC group were much less allergic with only a third reporting hay fever and only a
324 sixth, rhinitis or eczema.
325

326

		severe non-smoking asthma N=311	smokers and ex-smokers with severe asthma N=110	Mild and moderate non-smoking asthma N=88	healthy non-smoking controls N=101	P-value	P-value SA* vs. MMA
Allergic rhinitis diagnosed	n/N (%)	164/277 (59.2)	44/101 (43.6)	42/70 (60)	5/30 (16.7)	p<0.001	0.442
Hayfever diagnosed	n/N (%)	135/284 (47.5)	51/100 (51)	46/73 (63.0)	10/33 (30.3)	0.019	0.024
Non-allergic rhinitis diagnosed	n/N (%)	42/284 (14.8)	17/101 (16.8)	8/72 (11.1)	1/34 (2.9)	0.090	0.356
Nasal polyps diagnosed	n/N (%)	103/291 (35.4)	34/101 (33.7)	7/76 (9.2)	3/34 (8.8)	p<0.001	p<0.001
Eczema diagnosed	n/N (%)	107/294 (36.4)	31/101 (30.7)	25/75 (33.3)	5/35 (14.3)	0.013	0.789
GORD diagnosed	n/N (%)	135/289 (46.7)	63/99 (63.6)	16/75 (21.3)	4/35 (11.4)	p<0.001	p<0.001

327

328 **Table iv.** Co-morbidities.

329 GORD: Gastro-oesophageal reflux disease

330 *SA represents SAn and SAs/ex groups combined

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The presence of nasal polyps was associated with severe asthma, regardless of smoking status (4-fold increased incidence in SAn and SAs/ex groups versus MMA group, $p < 0.001$) **(Table iv)**. No such association was seen with allergic or non-allergic rhinitis, hay fever or reported eczema. Gastro-oesophageal reflux disease was more common in severe asthma (46% SAn, 63% SAs/ex) than in MMA (21%) and HC (11%), with a greater incidence reported in the SAs/ex group versus the SAns group ($p=0.004$).

Blood and sputum biomarkers **(Table v)**

Blood eosinophil counts were similar in all three asthma groups. Each group had a significantly higher blood eosinophil count than the HC group (SAn vs. HC $p=0.002$, SAs/ex vs. HC $p=0.005$, MMA vs. HC $p<0.001$). Blood neutrophil counts were significantly higher in the severe asthma groups compared to the MMA group.

345

	N	severe non-smoking asthma N=311	smokers and ex-smokers with severe asthma N=110	Mild and moderate non-smoking asthma N=88	healthy non-smoking controls N=101	P-value	P-value SA* vs. MMA
Exhaled NO ppb	Median (IQR) [N]	26.5 (16_47) [290]	23.5 (12_42) [104]	25 (18_54) [87]	19.25 (13_29) [96]	<0.001	0.438
Sputum							
Sputum eosinophils (%)	Median (IQR) [N]	2.75 (0_19) [128]	4.13 (1_14) [53]	1.05 (0_3) [43]	0 (0_0) [41]	p<0.001	0.004
Sputum neutrophils (%)	Median (IQR) [N]	53.69 (34_75) [128]	55.15 (35_65) [53]	44.5 (26_62) [43]	39.56 (21_56) [41]	0.002	0.042
Sputum differential eosinophil count >1.9%	n (%) [N]	74 (57.81) [128]	32 (60.38) [53]	17 (39.53) [43]	1 (2.44) [41]	<0.001	0.026
Blood							
Blood eosinophils (%)	Median (IQR) [N]	2.94 (1_6) [302]	2.88 (1_5) [106]	3.00 (2_5) [88]	2.10 (1_3) [101]	0.001	0.295
Blood Eosinophils (absolute)	Median (IQR) [N]	0.2 (0.3) 302	0.22 (0.29) 106	0.23 (0.2) 88	0.1 (0.11) 101	0.001	0.295
Blood neutrophils (%)	Median (IQR) [N]	62 (55_70) [302]	61.75 (55_69) [106]	56.83 (52_63) [88]	57.34 (51_64) [101]	p<0.001	p<0.001
Blood neutrophils (absolute)	Median (IQR) [N]	4.73 (3.1) 302	4.97 (2.87) 106	3.64 (1.75) 88	3.03 (1.6) 101	p<0.001	p<0.001

346

347 **Table v**

348 Biomarkers in blood, sputum and exhaled air

349 *SA represents SAn and SAs/ex groups combined

350

Sputum samples were provided and met criteria for analysis in 44.2% of the asthma participants and 40.6% of the HC group. Median sputum eosinophil counts for the SAn, SAs/ex, MMA, and HC groups were 2.75%, 4.13%, 1.05% and 0% respectively **(Table v)**. The sputum eosinophil count was higher in the two severe asthma groups combined compared to the mild/moderate asthma group **(Table v, Fig 4)**.

There were no significant differences in differential sputum neutrophil counts between the two severe asthma groups, which when combined were significantly higher compared to the MMA group **(Table v)**.

There was a significant negative association between log sputum eosinophils (Absolute or %) and FEV_1 (% predicted or actual value) when all cohorts were considered and an adjustment for age, sex and smoking was applied. There were significant negative associations between log blood eosinophils (%) and FEV_1/FVC ratio (p=0.002) and between blood neutrophils (%) and actual FEV_1 (p=0.002) and FEV_1/FVC ratio (p=0.026).

Exhaled Nitric Oxide (FeNO) **(Table v)**

FeNO levels in all asthma groups were higher than those in the HC group, but the FeNO levels in the severe asthma groups were not different from the levels in the MMA group. The presence of nasal polyps was associated with a higher FeNO (mean increase 2.1ppb, 95% CI 1.5_2.9, p<0.001).

Discussion

In this large European cohort, patients with severe asthma experienced more symptoms, more exacerbations, higher levels of anxiety and depression, and a higher incidence of nasal polyps, gastro-oesophageal reflux symptoms and airflow obstruction than patients with milder disease. The clinical characteristics of asthma were present despite higher doses of treatment that included doses of inhaled corticosteroids equal or more than 1,000µg of fluticasone (or equivalent), with 45% of the combined severe asthma group receiving a daily dose of prednisolone. The characteristic features of the severe asthma U-BIOPRED cohort are similar to those reported in previous cohort studies (6, 19-21). While the entry criteria for severe asthma were comparable for most of these cohort studies, the ENFUMOSA study required a lower threshold with an ICS dose of ≥ 1,200µg of budesonide or beclomethasone with at least one exacerbation in the past year. Of these 5 cohorts, the current U-BIOPRED severe asthma cohort appears to be the most severe with a higher reported exacerbation rate of 2.5 per year, a reduced mean FEV_1 of 67.5% of predicted and a higher proportion of patients on oral corticosteroid therapy taking a mean dose of 14 mg/day.

One of the novel features of the U-BIOPRED cohort is the inclusion of a smoking and ex-smoking severe asthma group. Patients with asthma who smoke have been reported to have poorer disease control and a reduced therapeutic response to ICS (22), possibly through the induction of corticosteroid insensitivity (23, 24). However our analyses of the non-smoking and the smoking/ex smoking severe asthma groups identified few differences in demographics, airway physiology, inflammatory markers and asthma symptoms between these groups. In both groups, a similar percentage received oral corticosteroid therapy; they also had similar degrees of airflow obstruction. The slightly lower level of FeNO in the smoking/ex smoking group might be explained by an effect of current smoking (26). One notable difference is that asthma onset occurred on average 18 years later in the smokers and ex-smokers than in the non-smokers, and yet the degree of airflow obstruction measured was similar. One interpretation is that there may be a more rapid rate of loss of lung function in the patients with asthma who smoke. The significant correlation between AQLQ scores and the number of pack-years of smoking exposure would also support a contribution of cigarette smoke to impaired quality of life in this group. We also split the demographic data of the groups by smoking status rather than severity (see Table S5) in the online supplement. This revealed that current smokers had a lower BMI compared with ex and never smokers.

In agreement with the SARP study (20), patients with severe asthma (especially smokers) were less frequently atopic than those with mild/moderate disease. There was also a clear association of both nasal polyps and gastro-oesophageal reflux disease with disease severity, with approximately one-third and one half reporting polyps and reflux respectively, a finding that is in keeping with previous reports (5). Nasal polyps are commonly found in severe asthma, and are associated with a particularly severe phenotype. There is evidence that treating nasal polyps with anti-IgE therapy results in better asthma outcomes (25), however whether this is due to an effect on the underlying asthma or the polyps is unknown. The link with higher FeNO levels is in keeping with work showing that nasal polypectomy leads to a fall in FeNO (26).

Our findings are also similar to other studies published from severe asthma registries. In agreement with both the British Thoracic Society's (27) and Belgium's (28) severe asthma registries our patients are predominantly female, with a high BMI and evidence of fixed airflow obstruction. Moreover there are similarly high levels of reflux, nasal polyps and exacerbations despite greater levels of medication.

We found a greater degree of sputum eosinophilia in the two severe asthma groups compared to the mild-moderate asthma group. Up to 60% of patients in the two severe asthma groups had a differential sputum eosinophil of >1.9% (the established upper limit of normal for differential sputum eosinophil counts (29)). This percentage is similar to previous reports in severe asthma (21). The level of sputum eosinophilia observed in the mild/moderate asthma group are also similar to those reported previously (30).

The higher blood neutrophil count in participants with severe asthma may represent the effect of systemic corticosteroids which can increase blood neutrophil numbers. Sputum neutrophil counts were similar in the three asthma groups and were significantly higher than the in healthy control group. This similarly could represent the effect of corticosteroids although severe asthma has been linked to a higher level of sputum neutrophils (31, 32).

The impact and burden on our participants' health was noticeable with measures of symptoms and quality of life being far worse in severe asthma as compared to mild/moderate asthma, despite the use of higher doses and more classes of asthma treatment. Levels of anxiety and depression were also higher with severe asthma. There were significant relationships between quality of life measures and airflow obstruction, smoking history and BMI, supporting the contribution of these factors to an impairment of quality of life however the scatter of data reveals that these parameters are not closely related. The number of exacerbations experienced was greater than 2.5 exacerbations per participant in both severe asthma groups in the preceding year. These findings highlight the need for an integrative assessment of clinical and physiological disease markers but additionally biological markers of disease in the assessment of severe asthma. For example, the finding that bariatric surgery has an effect on measures of airway hyper-responsiveness (33) and is associated with a lower all-cause mortality at 5 years particularly in younger, predominantly female populations (34) may point towards the need for specific and targeted intervention in people with severe asthma and obesity.

There are several limitations to our study. Firstly, there is no perfect way to assess treatment adherence; however, we only approached patients managed in a specialist respiratory clinic and only those who had been assessed to be adherent were eligible for the study. Furthermore MARS scores were high indicating good levels of self-reported adherence. Secondly, subjective or historical data were assessed by questionnaire which may be prone to recall bias. Thirdly, the success rate in obtaining adequate quality sputum for analysis was in the 42-50% range and the number of

bronchoscopies was relatively lower in the SA and SAs/ex groups. Thirdly due to the numerous formulations and inhaler devices used across Europe it was not possible to calculate the precise daily equivalent ICS dose for each participant and therefore these data are not shown, however high (>1000mcg FP) or low (<500mcg FP) dose was a study entry requirement for the severe and moderate groups respectively.

We have been successful in recruiting a substantial cohort of patients with the most severe asthma that has similar characteristics to previously-reported cohorts. This gives confidence that the U-BIOPRED consortium will define distinct phenotypes and endotypes of severe asthma. Matching these data to the 'omics' information with future unsupervised analyses will help identify new treatments for patients with severe asthma who currently have limited treatment options, and will improve our understanding of this important chronic disease.

Acknowledgements

This paper is presented on behalf of the U-BIOPRED Study Group with input from the U-BIOPRED Patient Input Platform, Ethics Board and Safety Management Board. U-BIOPRED is supported through an Innovative Medicines Initiative Joint Undertaking under grant agreement no. 115010, resources of which are composed of financial contribution from the European Union's Seventh Framework Programme (FP7/2007-2013) and EFPIA companies' in kind contribution (www.imi.europa.eu). We thank all the members of each recruiting Centre (Appendix) for their dedicated effort, devotion, promptness and care in the recruitment and assessment of the participants in this study. We would also like to acknowledge help from the IMI funded eTRIKS project (EU Grant Code No.115446).

Fig 1

Visit Schedule

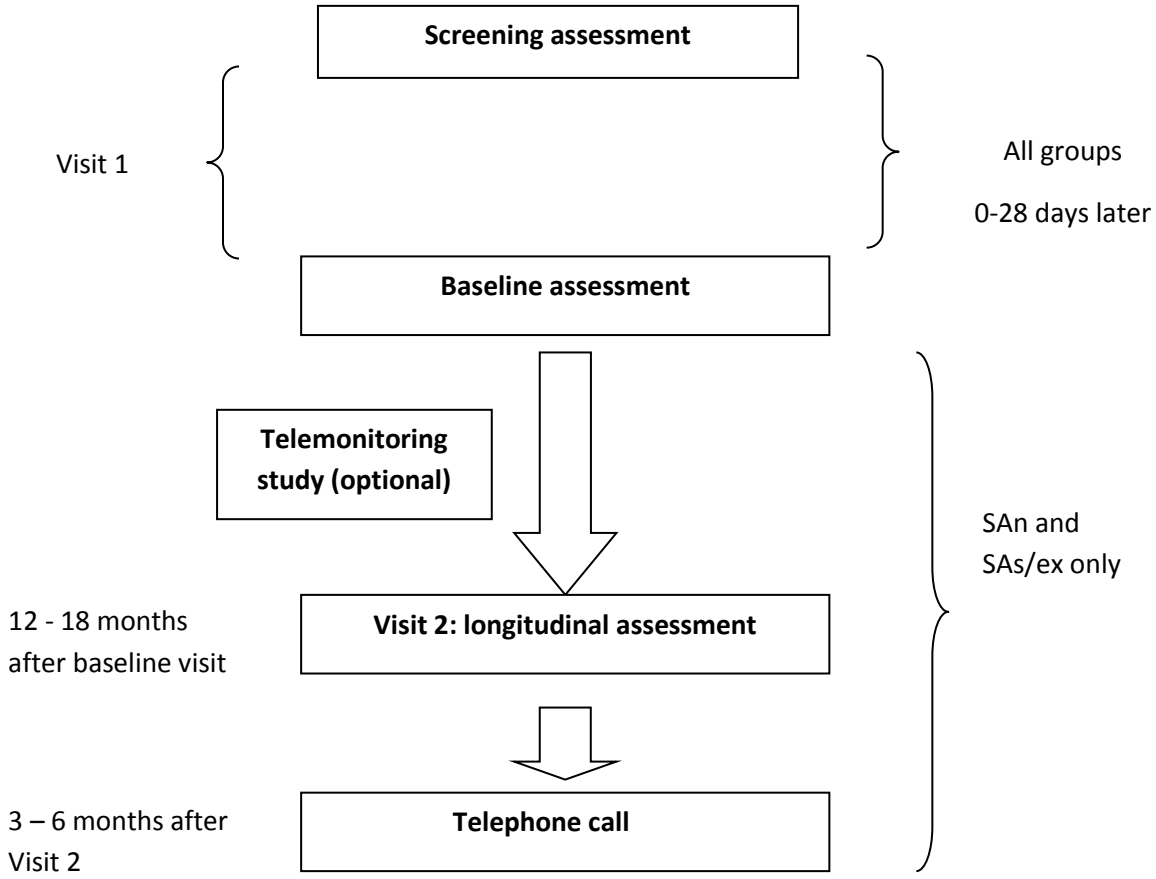


Figure 2

Consort diagram

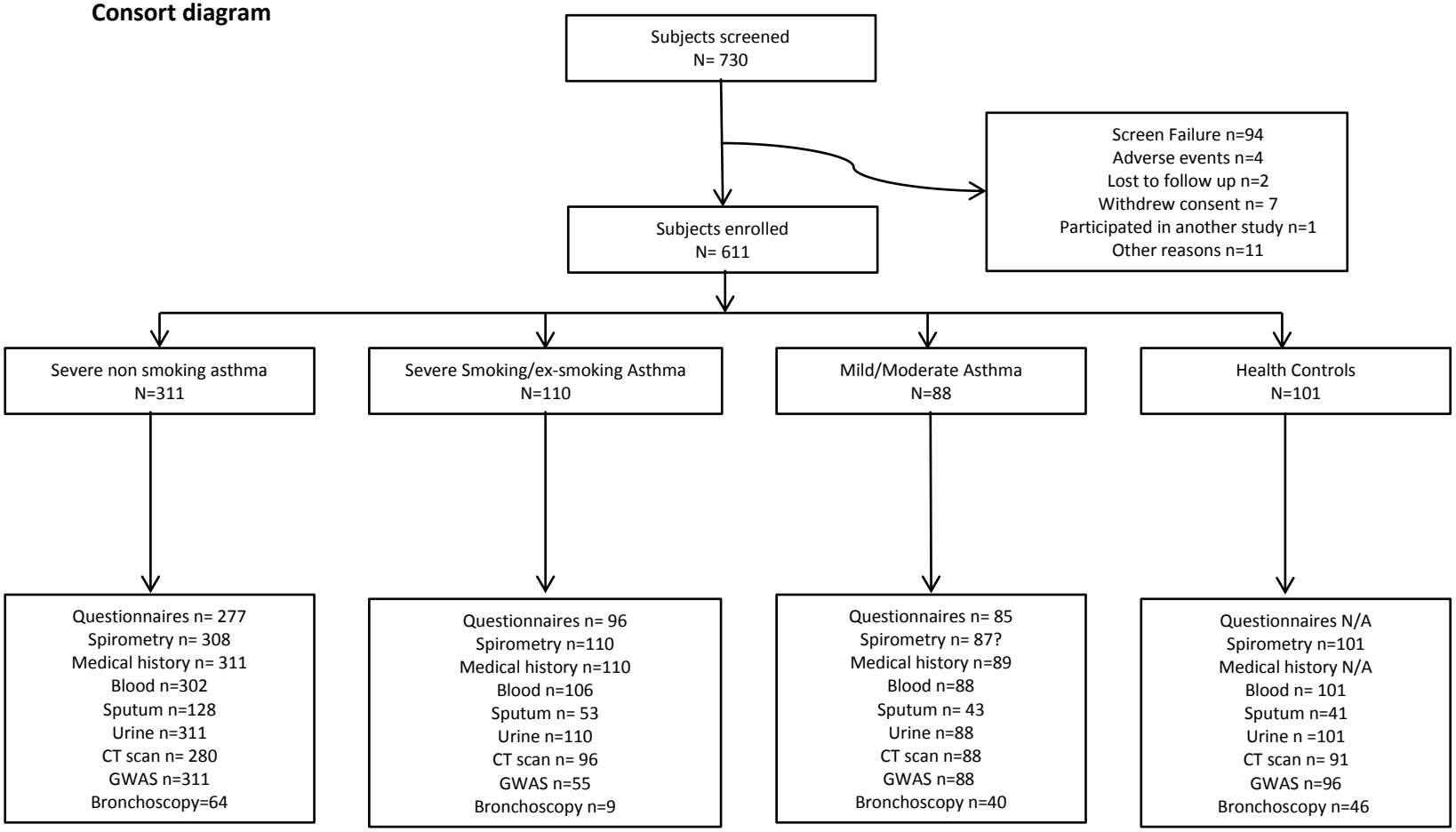
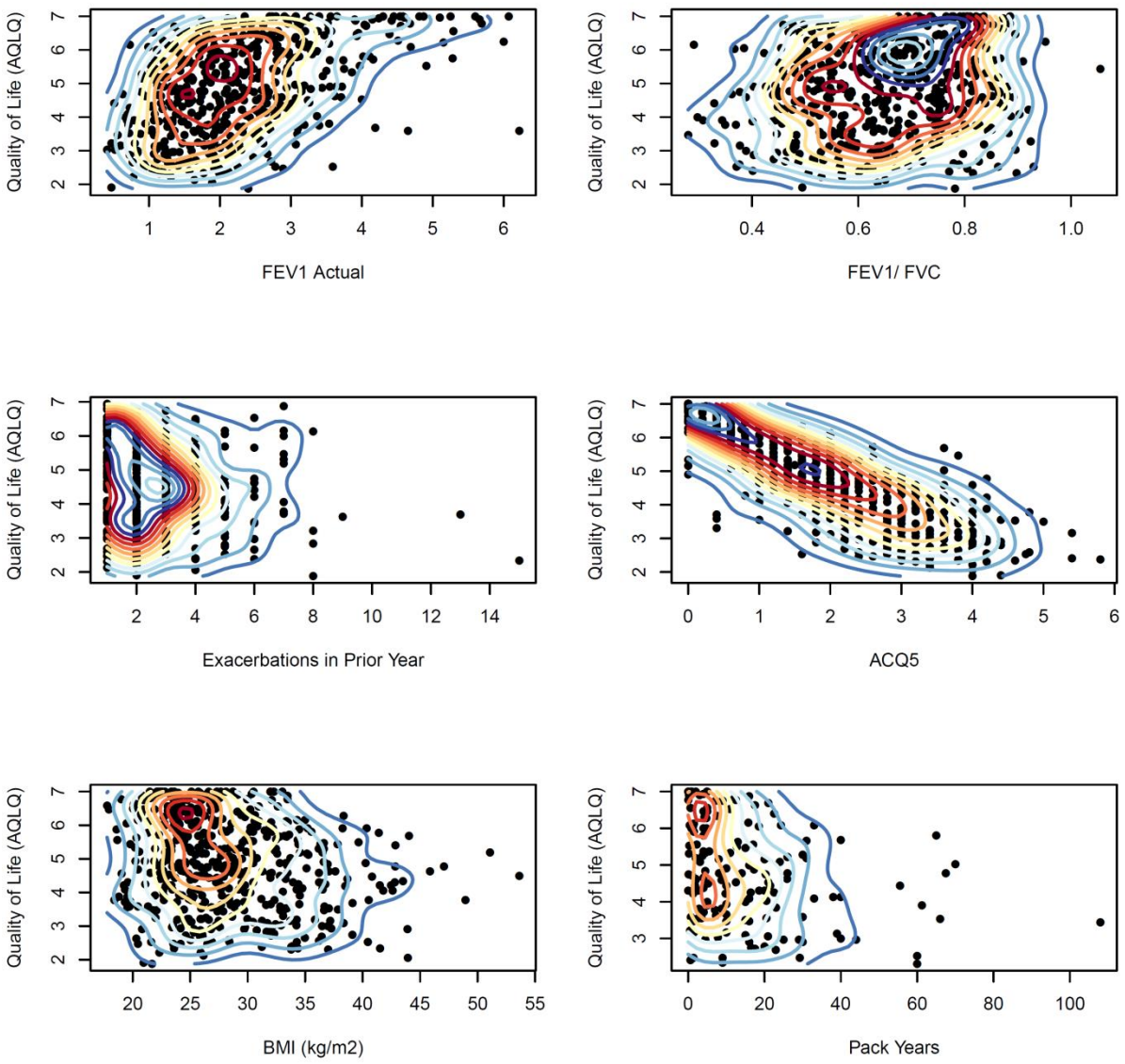


Figure 3

Contour plots of AQLQ related to baseline demographics



Figures represent scatter plots describing the relationship between each factor and the asthma quality of life z-score. The contour lines are coloured blue to red, to indicate increasing density of points in the graph. The density was modelled using two-dimensional kernel density estimation. The contour plots show weak inverse relationships and particularly the scatter between quality of life and exacerbations, BMI and pack years, a strong inverse relationship between quality of life and asthma control and weak positive relationships between quality of life and measures of lung function.

Fig 4A

Eosinophil counts by group

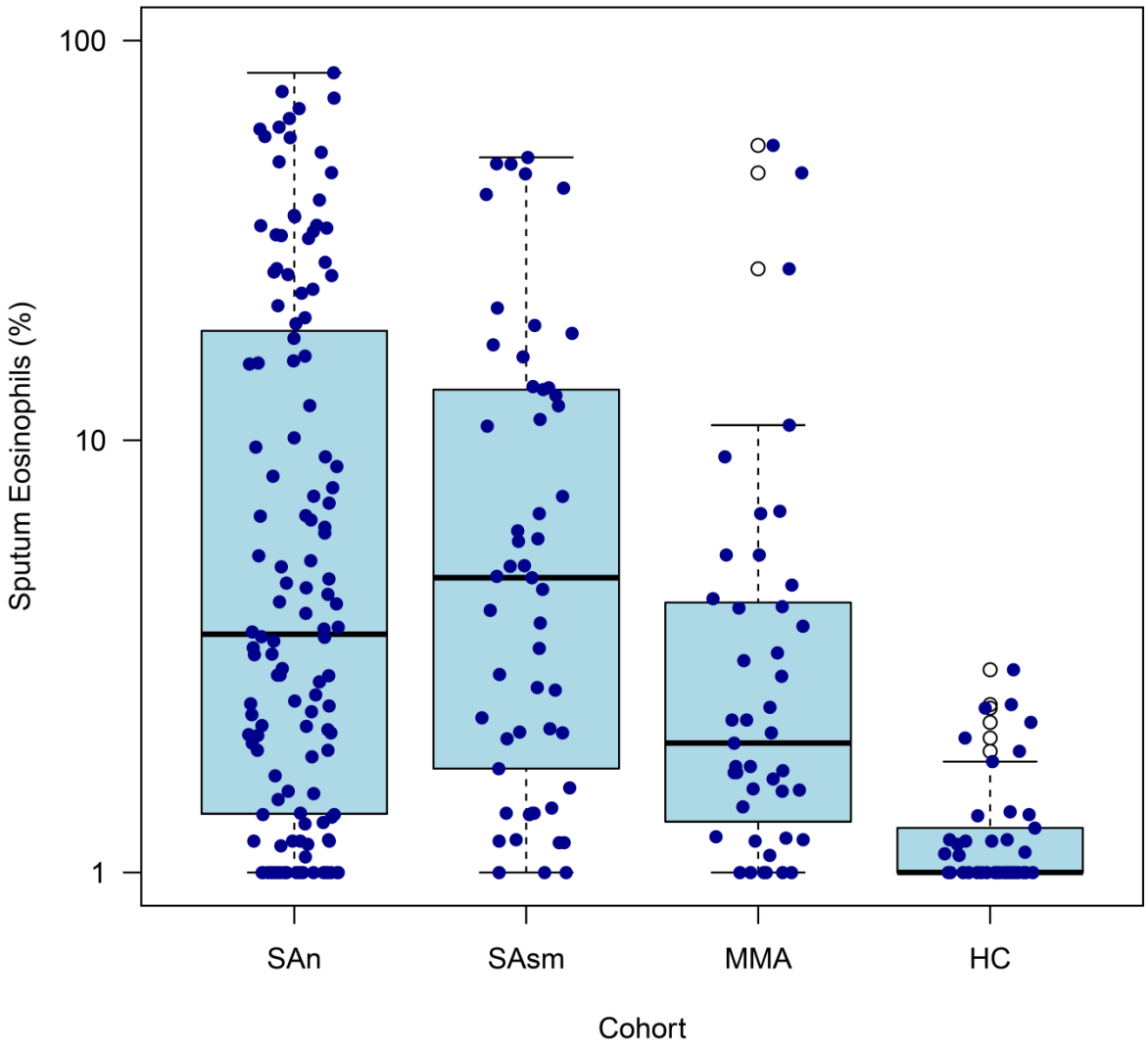


Fig 4B

Neutrophil counts by group

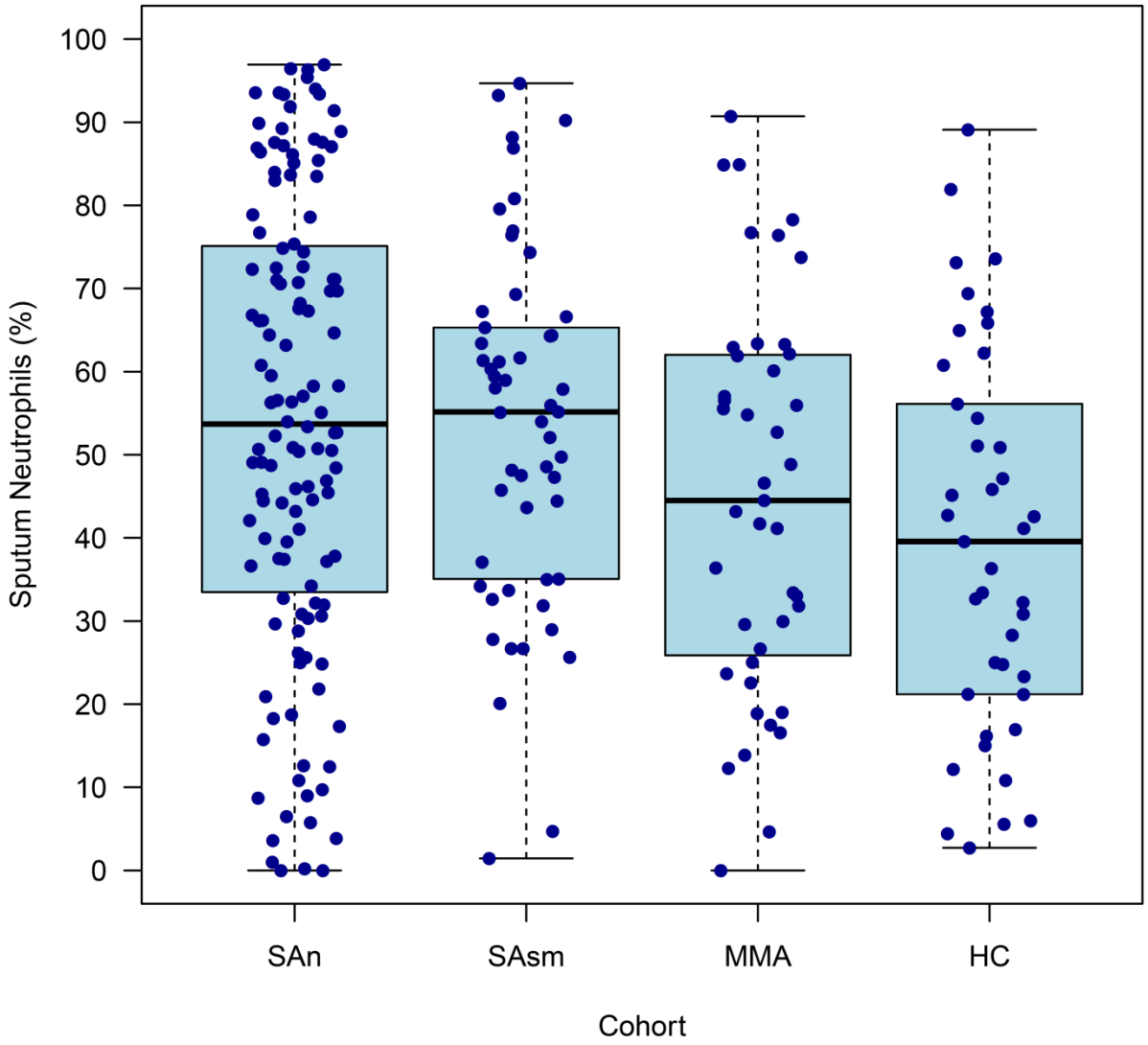


Fig 4

A) sputum eosinophil count and B) sputum neutrophil count, by cohort. The box and whisker plots are shaded in pale blue, with outliers denoted by open circles. The raw data are given by dark blue points overlaid.

SAn; Severe non-smoking asthma

SAs/ex; Smokers and ex-smokers with severe asthma

MMA; Mild/moderate non-smoking asthma

HC; Healthy non-smoking controls

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