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

2 **cohort**

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101   **Short title:** U-BIOPRED adult severe asthma

102   **Word count:** 3248

103   **Figures:** 5

104   **Tables:** 5

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

106

107   **Message:** Patients with severe asthma have more airway inflammation, worse symptoms and lower

108   lung function, despite higher doses of treatment.

109

110

111   **Abbreviations**

112   BMI; Body mass index

113   FeNO; Fraction of exhaled nitric oxide

114   FEV_1; Forced Expiratory Volume in one second

115   FVC; Forced Vital Capacity

116   HC; Healthy non-smoking controls

117   ICS; Inhaled Corticosteroids

118   MMA; Mild/moderate non-smoking asthma

119   OCS; Oral corticosteroids

120   SAn; Severe non-smoking asthma

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

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

123

124   **Abstract** (196)

125   U-BIOPRED is an EU consortium of 20 academic institutions, 11 pharmaceutical companies and 6

126   patient organisations with the objective of improving the understanding of asthma disease

127   mechanisms using a systems biology approach.

128

129   This cross-sectional assessment of adults with severe asthma, mild/moderate asthma and healthy

130   controls from 11 European countries consisted of analyses of patient-reported outcomes, lung

131   function, blood and airway inflammatory measurements.

132

133   Patients with severe asthma (non-smokers n=311 and smokers/ex-smokers n=110) had more

134   symptoms and exacerbations compared to patients with mild-moderate disease (n=88) (2.5

135   exacerbations versus 0.4 in the preceding 12 months, p<0.001), with worse quality of life, and higher

136   levels of anxiety and depression. They also had a higher incidence of nasal polyps and gastro-

137   oesophageal reflux with lower lung function. Sputum eosinophil count was higher in severe asthma

138   compared to mild-moderate asthma (median count 2.99% versus 1.05%, p=0.004) despite treatment

139   with higher doses of inhaled and/or oral corticosteroids.

140

141   Consistent with other severe asthma cohorts, U-BIOPPRED is characterised by poor symptom

142   control, increased co-morbidity and airway inflammation, despite high levels of treatment. It is well

143   suited to identify asthma phenotypes using the array of 'omic' datasets that are at the core of this

144   systems medicine approach.

145

146

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

181 adherence (2). The study was approved by the ethics committee for each participating clinical

182 institution, and adhered to the standards set by International Conference on Harmonisation and

183 Good Clinical Practice. It is registered on *ClinicalTrials.gov*, (Identifier: NCT01982162). All participants

184 gave written and signed informed consent.

185

186 ***Adult Groups***

187 The definition of severe asthma used in this study was agreed at a U-BIOPRED consensus meeting

188 (2). Participants with asthma had either airflow reversibility (increase in FEV_1 >12% predicted or

189 200ml following inhalation of 400µg salbutamol), airway hyper-responsiveness (methacholine PC_{20} <

190 8mg/ml, or diurnal PEF amplitude % mean >8%), or a decrease in FEV_1 of 12% predicted or 200ml

191 within 4 weeks after tapering maintenance treatment. Four groups were recruited:

192

193 A) Severe non-smoking asthma (SAn):

194 Participants in this group were non-smokers for at least the past 12 months, with a less than five

195 pack-year smoking history, with asthma and uncontrolled symptoms defined according to GINA

196 guidelines (10) and/or frequent exacerbations (more than two per year) despite high-dose inhaled

197 corticosteroids (ICS) (ICS ≥ 1000µg fluticasone propionate/day or equivalent dose).

198

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

200 This group was defined as for the SAn group except that they were either current smokers or ex-

201 smokers with a smoking history of at least 5 pack years.

202

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

204 Participants in this group were non-smokers for at least the past 12 months, with a less than five

205 pack-year smoking history and had controlled or partially controlled asthma symptoms, as defined

206 by the Global Initiative for Asthma (GINA), whilst receiving a dose of less than 500µg fluticasone

207 propionate/day or equivalent.

208

209 D) Healthy non-smoking controls (HC):

210 These participants had no history of asthma or wheeze, had no other chronic respiratory disease,

211 were non-smokers for at least the past 12 months with a smoking history of ≤ 5 pack years and their

212 pre-bronchodilator FEV_1 was ≥ 80% predicted.

213

214 ***Protocol and assessments***

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

228

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

232

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

238

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

241

242 ***Questionnaires***
243 The following were administered at baseline:
244 1.   The Asthma Control Questionnaire (ACQ5) (13) to assess current asthma control.
245 2.   The Asthma Quality of Life Questionnaire (AQLQ) (14) to assess quality of life and
246 psychological morbidity.
247 3.   The Hospital Anxiety and Depression Scale (HADS) (15).
248 4.   Sino-Nasal Outcomes Test (SNOT20) (16) to measure upper airway symptoms.

249   5.   The Epworth Sleepiness Scale (ESS) (17) to measure sleep and daytime drowsiness.

250   6.   The Medicines Adherence Response Scale (MARS) (18) to measure adherence.

251

252 **Statistical Analysis**

253 Continuously distributed data were either summarised using mean (standard error; SE) if

254 symmetrical, or median (inter-quartile range; IQR) values. Non-symmetrical variables all exhibited

255 positive skew and were log-transformed prior to association testing. Missing data were not imputed.

256 P-values were calculated using a general linear model for continuous variables or a general logistic

257 model for categorical variables. No adjustment for multiple testing was applied as the analyses were

258 considered exploratory. Analyses were performed using R version 2.15.2 (R Core Team, 2012).

259

260 **Results**

261 A total of 610 adults were recruited over an 18-month period: 311, 110, 88 and 101 into the SAn,

262 SAs/ex, MMA, HC groups respectively **(Table i).**

263

	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

264

265 **Table i**

266 Group Demographics

267 FEV$_1$: Forced Expiratory Volume in one second

268 FVC: Forced Vital Capacity

269   IQR: Inter quartile range

270   ICU: Intensive care unit

271   NA: Not applicable

272   SE: Standard error

273   * 42 current smokers and 68 ex-smokers

274

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

282

283 Spirometry **(Table i)**

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

290

291 Medications **(Table ii)**

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

295

		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)

296

297 **Table ii**

298 Medications

299 * Hydrocortisone and Triamcinolone doses were converted to equivalent prednisolone dose

300 (4 healthy control participants took as required antihistamines)

301   Questionnaires **(Table iii)**

302   ACQ and AQLQ scores reflected worse asthma control and increased morbidity in both severe

303   asthma groups with minimal impairment in the MMA group. A similar pattern was seen with anxiety

304   and depression. There were more upper airway symptoms measured using the SNOT20 in both

305   severe asthma groups compared with the MMA group. Similarly the ESS scores indicated that there

306   was an increase in sleepiness in the severe asthma groups compared to only a very mild impairment

307   in the MMA group.

308

309

		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

331

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

338

339   Blood and sputum biomarkers **(Table v)**
340   Blood eosinophil counts were similar in all three asthma groups. Each group had a significantly
341   higher blood eosinophil count than the HC group (SAn vs. HC p=0.002, SAs/ex vs. HC p=0.005, MMA
342   vs. HC p<0.001). Blood neutrophil counts were significantly higher in the severe asthma groups
343   compared to the MMA group.

344

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

	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
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

	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
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

351   Sputum samples were provided and met criteria for analysis in 44.2% of the asthma participants and

352   40.6% of the HC group. Median sputum eosinophil counts for the SAn, SAs/ex, MMA, and HC groups

353   were 2.75%, 4.13%, 1.05% and 0% respectively **(Table v)**. The sputum eosinophil count was higher in

354   the two severe asthma groups combined compared to the mild/moderate asthma group **(Table v,**

355   **Fig 4)**.

356   There were no significant differences in differential sputum neutrophil counts between the two

357   severe asthma groups, which when combined were significantly higher compared to the MMA group

358   **(Table v)**.

359   There was a significant negative association between log sputum eosinophils (Absolute or %) and

360   FEV_1 (% predicted or actual value) when all cohorts were considered and an adjustment for age, sex

361   and smoking was applied. There were significant negative associations between log blood

362   eosinophils (%) and FEV_1/FVC ratio (p=0.002) and between blood neutrophils (%) and actual FEV_1

363   (p=0.002) and FEV_1/FVC ratio (p=0.026).

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

365   FeNO levels in all asthma groups were higher than those in the HC group, but the FeNO levels in the

366   severe asthma groups were not different from the levels in the MMA group. The presence of nasal

367   polyps was associated with a higher FeNO (mean increase 2.1ppb, 95% CI 1.5_2.9, p<0.001).

368

369   **Discussion**

370   In this large European cohort, patients with severe asthma experienced more symptoms, more

371   exacerbations, higher levels of anxiety and depression, and a higher incidence of nasal polyps,

372   gastro-oesophageal reflux symptoms and airflow obstruction than patients with milder disease. The

373   clinical characteristics of asthma were present despite higher doses of treatment that included doses

374   of inhaled corticosteroids equal or more than 1,000µg of fluticasone (or equivalent), with 45% of the

375   combined severe asthma group receiving a daily dose of prednisolone. The characteristic features of

376   the severe asthma U-BIOPRED cohort are similar to those reported in previous cohort studies (6, 19-

377   21). While the entry criteria for severe asthma were comparable for most of these cohort studies,

378   the ENFUMOSA study required a lower threshold with an ICS dose of ≥ 1,200µg of budesonide or

379   beclomethasone with at least one exacerbation in the past year. Of these 5 cohorts, the current U-

380   BIOPRED severe asthma cohort appears to be the most severe with a higher reported exacerbation

381   rate of 2.5 per year, a reduced mean FEV_1 of 67.5% of predicted and a higher proportion of patients

382   on oral corticosteroid therapy taking a mean dose of 14 mg/day.

383

384 One of the novel features of the U-BIOPRED cohort is the inclusion of a smoking and ex-smoking

385 severe asthma group. Patients with asthma who smoke have been reported to have poorer disease

386 control and a reduced therapeutic response to ICS (22), possibly through the induction of

387 corticosteroid insensitivity (23, 24). However our analyses of the non-smoking and the smoking/ex

388 smoking severe asthma groups identified few differences in demographics, airway physiology,

389 inflammatory markers and asthma symptoms between these groups. In both groups, a similar

390 percentage received oral corticosteroid therapy; they also had similar degrees of airflow

391 obstruction. The slightly lower level of FeNO in the smoking/ex smoking group might be explained

392 by an effect of current smoking (26). One notable difference is that asthma onset occurred on

393 average 18 years later in the smokers and ex-smokers than in the non-smokers, and yet the degree

394 of airflow obstruction measured was similar. One interpretation is that there may be a more rapid

395 rate of loss of lung function in the patients with asthma who smoke. The significant correlation

396 between AQLQ scores and the number of pack-years of smoking exposure would also support a

397 contribution of cigarette smoke to impaired quality of life in this group. We also split the

398 demographic data of the groups by smoking status rather than severity (see Table S5) in the online

399 supplement. This revealed that current smokers had a lower BMI compared with ex and never

400 smokers.

401

402 In agreement with the SARP study (20), patients with severe asthma (especially smokers) were less

403 frequently atopic than those with mild/moderate disease. There was also a clear association of both

404 nasal polyps and gastro-oesophageal reflux disease with disease severity, with approximately one-

405 third and one half reporting polyps and reflux respectively, a finding that is in keeping with previous

406 reports (5). Nasal polyps are commonly found in severe asthma, and are associated with a

407 particularly severe phenotype. There is evidence that treating nasal polyps with anti-IgE therapy

408 results in better asthma outcomes (25), however whether this is due to an effect on the underlying

409 asthma or the polyps is unknown. The link with higher FeNO levels is in keeping with work showing

410 that nasal polypectomy leads to a fall in FeNO (26).

411

412 Our findings are also similar to other studies published from severe asthma registries. In agreement

413 with both the British Thoracic Society's (27) and Belgium's (28) severe asthma registries our patients

414 are predominantly female, with a high BMI and evidence of fixed airflow obstruction. Moreover

415 there are similarly high levels of reflux, nasal polyps and exacerbations despite greater levels of

416 medication.

417

418   We found a greater degree of sputum eosinophilia in the two severe asthma groups compared to

419   the mild-moderate asthma group. Up to 60% of patients in the two severe asthma groups had a

420   differential sputum eosinophil of >1.9% (the established upper limit of normal for differential

421   sputum eosinophil counts (29)). This percentage is similar to previous reports in severe asthma (21).

422   The level of sputum eosinophilia observed in the mild/moderate asthma group are also similar to

423   those reported previously (30).

424

425   The higher blood neutrophil count in participants with severe asthma may represent the effect of

426   systemic corticosteroids which can increase blood neutrophil numbers. Sputum neutrophil counts

427   were similar in the three asthma groups and were significantly higher than the in healthy control

428   group. This similarly could represent the effect of corticosteroids although severe asthma has been

429   linked to a higher level of sputum neutrophils (31, 32).

430

431   The impact and burden on our participants' health was noticeable with measures of symptoms and

432   quality of life being far worse in severe asthma as compared to mild/moderate asthma, despite the

433   use of higher doses and more classes of asthma treatment. Levels of anxiety and depression were

434   also higher with severe asthma. There were significant relationships between quality of life

435   measures and airflow obstruction, smoking history and BMI, supporting the contribution of these

436   factors to an impairment of quality of life however the scatter of data reveals that these parameters

437   are not closely related. The number of exacerbations experienced was greater than 2.5

438   exacerbations per participant in both severe asthma groups in the preceding year. These findings

439   highlight the need for an integrative assessment of clinical and physiological disease markers but

440   additionally biological markers of disease in the assessment of severe asthma. For example, the

441   finding that bariatric surgery has an effect on measures of airway hyper-responsiveness (33) and is

442   associated with a lower all-cause mortality at 5 years particularly in younger, predominantly female

443   populations (34) may point towards the need for specific and targeted intervention in people with

444   severe asthma and obesity.

445

446   There are several limitations to our study. Firstly, there is no perfect way to assess treatment

447   adherence; however, we only approached patients managed in a specialist respiratory clinic and only

448   those who had been assessed to be adherent were eligible for the study. Furthermore MARS scores

449   were high indicating good levels of self-reported adherence. Secondly, subjective or historical data

450   were assessed by questionnaire which may be prone to recall bias. Thirdly, the success rate in

451   obtaining adequate quality sputum for analysis was in the 42-50% range and the number of

452 bronchoscopies was relatively lower in the SA and SAs/ex groups. Thirdly due to the numerous

453 formulations and inhaler devices used across Europe it was not possible to calculate the precise daily

454 equivalent ICS dose for each participant and therefore these data are not shown, however high

455 (>1000mcg FP) or low (<500mcg FP) dose was a study entry requirement for the severe and

456 moderate groups respectively.

457

458 We have been successful in recruiting a substantial cohort of patients with the most severe asthma

459 that has similar characteristics to previously-reported cohorts. This gives confidence that the U-

460 BIOPRED consortium will define distinct phenotypes and endotypes of severe asthma. Matching

461 these data to the 'omics' information with future unsupervised analyses will help identify new

462 treatments for patients with severe asthma who currently have limited treatment options, and will

463 improve our understanding of this important chronic disease.

464

465 **Acknowledgements**

475

476

477

478   **Fig 1**

479   **Visit Schedule**

480

481

482   Visit 1

All groups

0-28 days later

```
┌─────────────────────────────────┐
│       Screening assessment      │
└─────────────────────────────────┘

┌─────────────────────────────────┐
│        Baseline assessment      │
└─────────────────────────────────┘

┌──────────────────────┐
│    Telemonitoring     │
│   study (optional)    │
└──────────────────────┘

┌─────────────────────────────────┐
│  Visit 2: longitudinal assessment │
└─────────────────────────────────┘

┌─────────────────────────────────┐
│         Telephone call          │
└─────────────────────────────────┘
```

SAn and
SAs/ex only

12 - 18 months
after baseline visit

3 – 6 months after
Visit 2
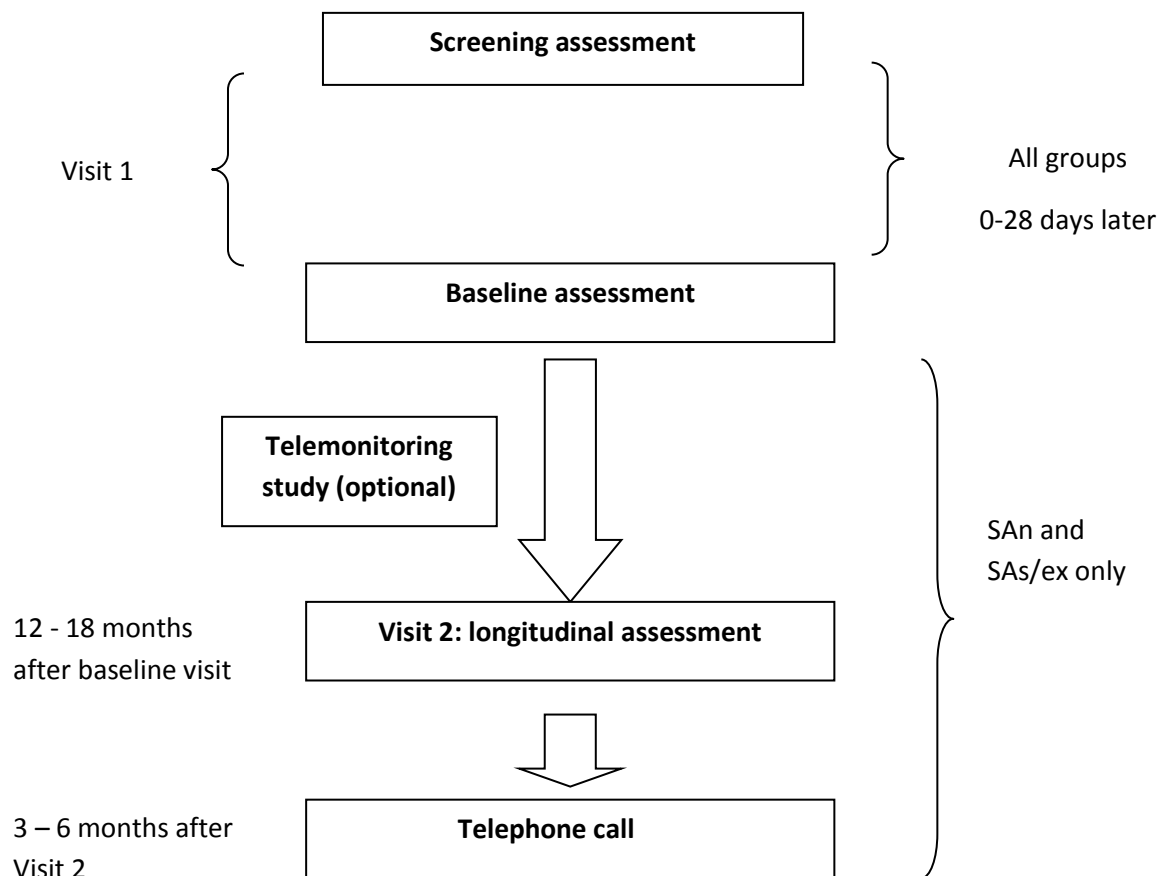
483

Figure 2

Consort diagram


```
                          ┌─────────────────────┐
                          │  Subjects screened  │
                          │      N= 730         │
                          └─────────────────────┘
                                    │
                                    │          ┌──────────────────────────────────┐
                                    │          │ Screen Failure n=94              │
                                    │          │ Adverse events n=4               │
                                    │          │ Lost to follow up n=2            │
                                    │          │ Withdrew consent n= 7            │
                                    │          │ Participated in another study n=1│
                                    │          │ Other reasons n=11               │
                                    ▼          └──────────────────────────────────┘
                          ┌─────────────────────┐
                          │  Subjects enrolled  │
                          │      N= 611         │
                          └─────────────────────┘
```

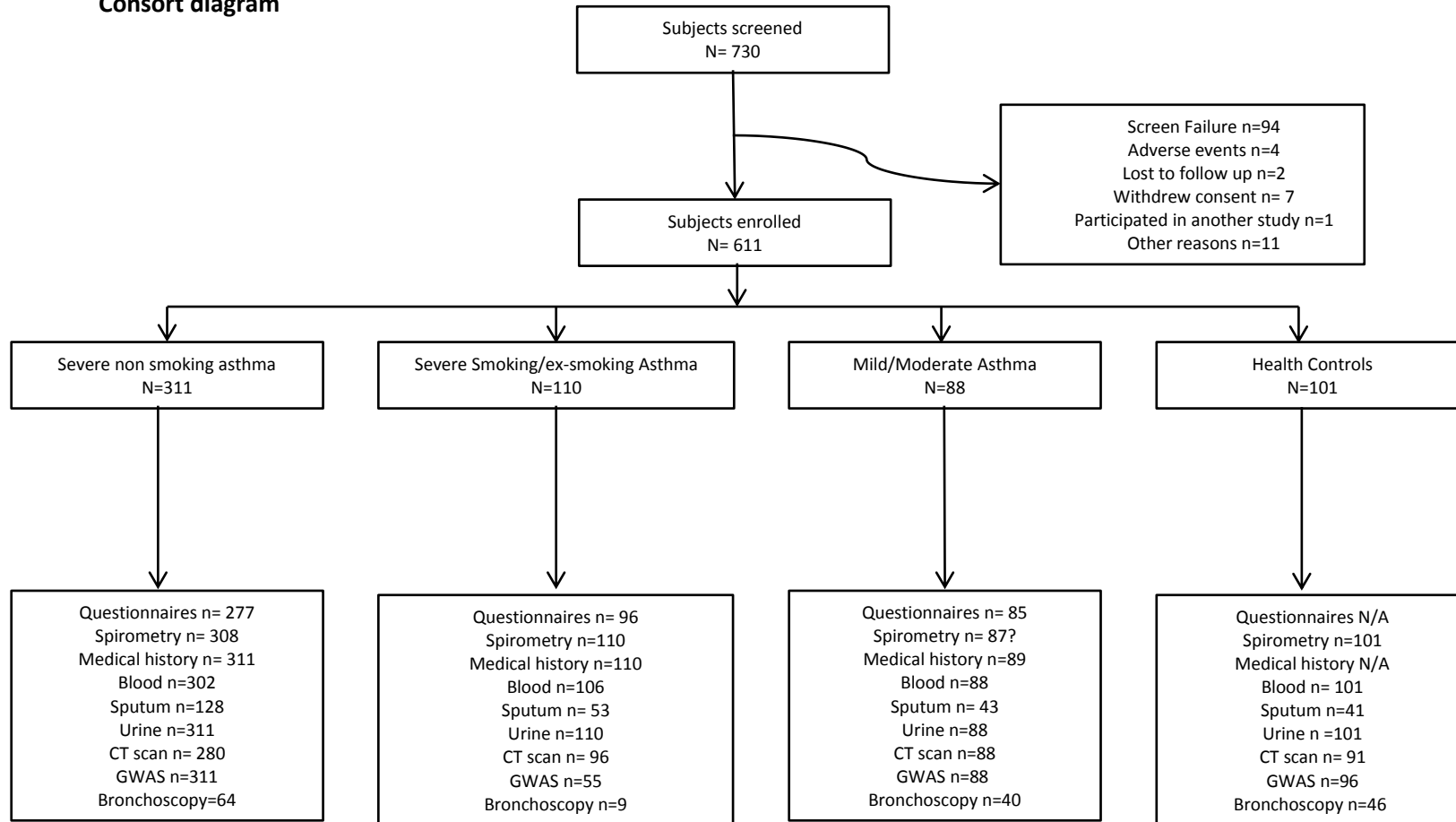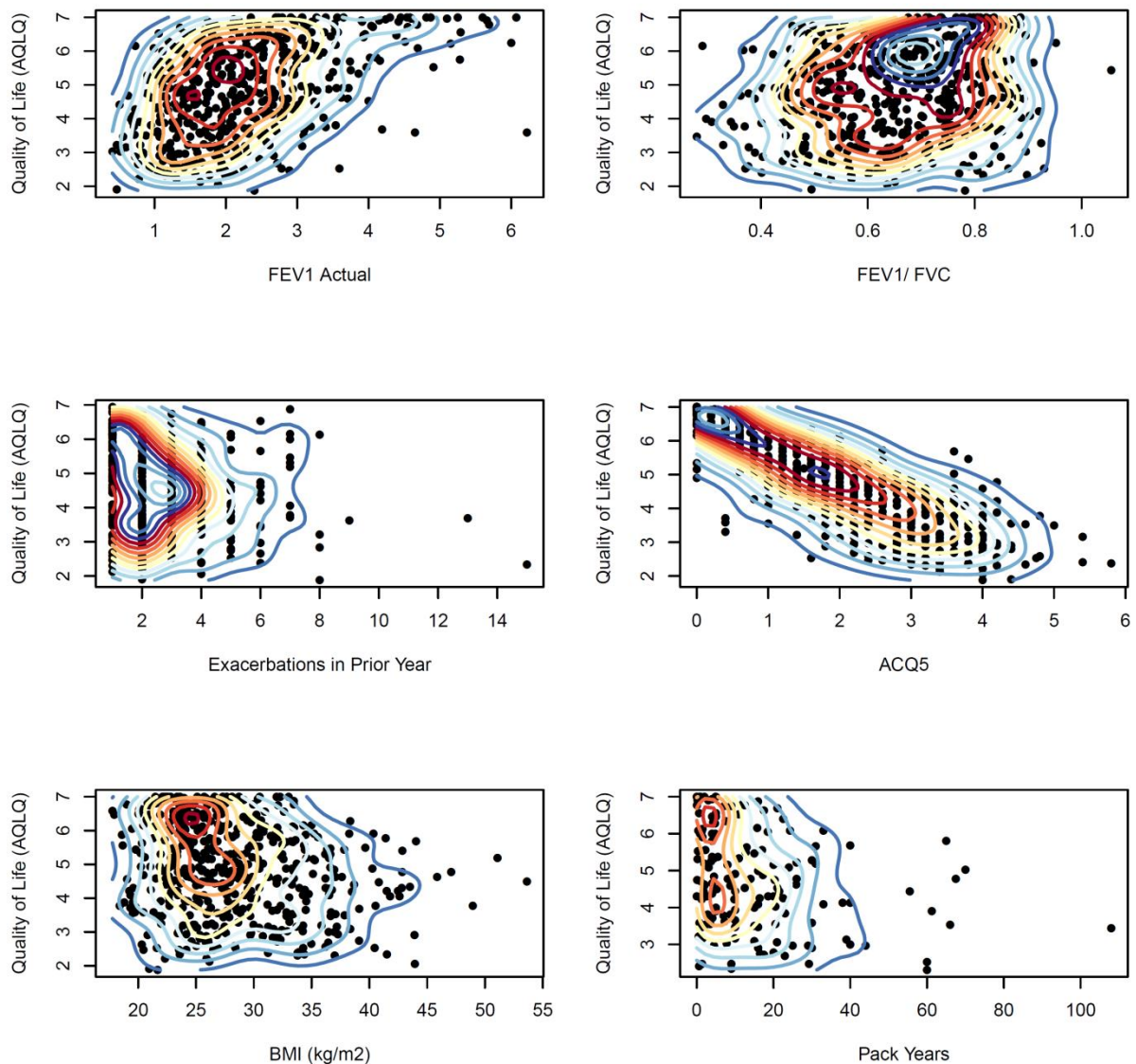| Severe non smoking asthma N=311	Severe Smoking/ex-smoking Asthma N=110	Mild/Moderate Asthma N=88	Health Controls N=101
Questionnaires n= 277	Questionnaires n= 96	Questionnaires n= 85	Questionnaires N/A
Spirometry n= 308	Spirometry n=110	Spirometry n= 87?	Spirometry n=101
Medical history n= 311	Medical history n=110	Medical history n=89	Medical history N/A
Blood n=302	Blood n=106	Blood n=88	Blood n= 101
Sputum n=128	Sputum n= 53	Sputum n= 43	Sputum n=41
Urine n=311	Urine n=110	Urine n=88	Urine n =101
CT scan n= 280	CT scan n= 96	CT scan n=88	CT scan n= 91
GWAS n=311	GWAS n=55	GWAS n=88	GWAS n=96
Bronchoscopy=64	Bronchoscopy n=9	Bronchoscopy n=40	Bronchoscopy n=46

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.
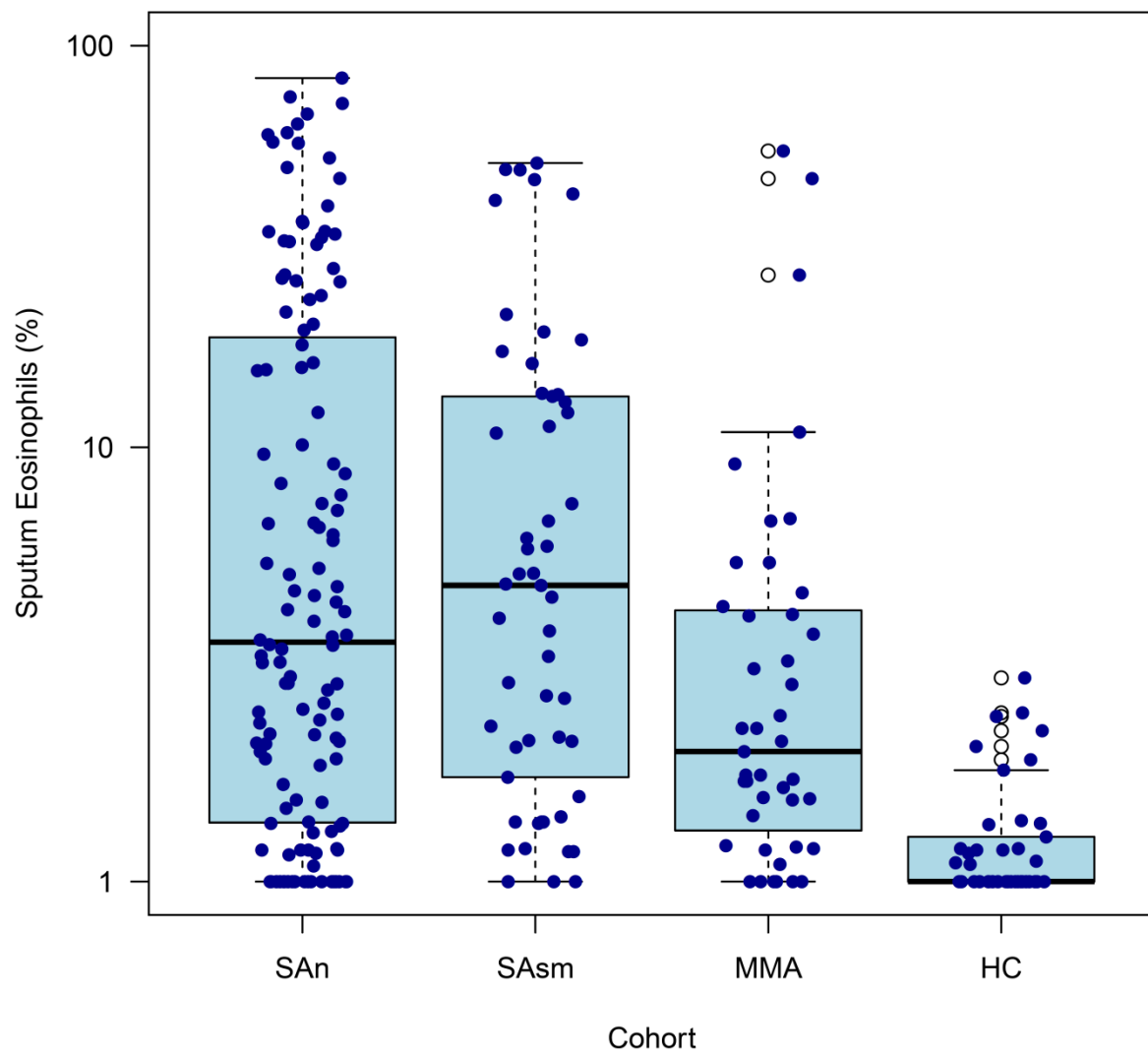
Fig 4A

Eosinophil counts by group
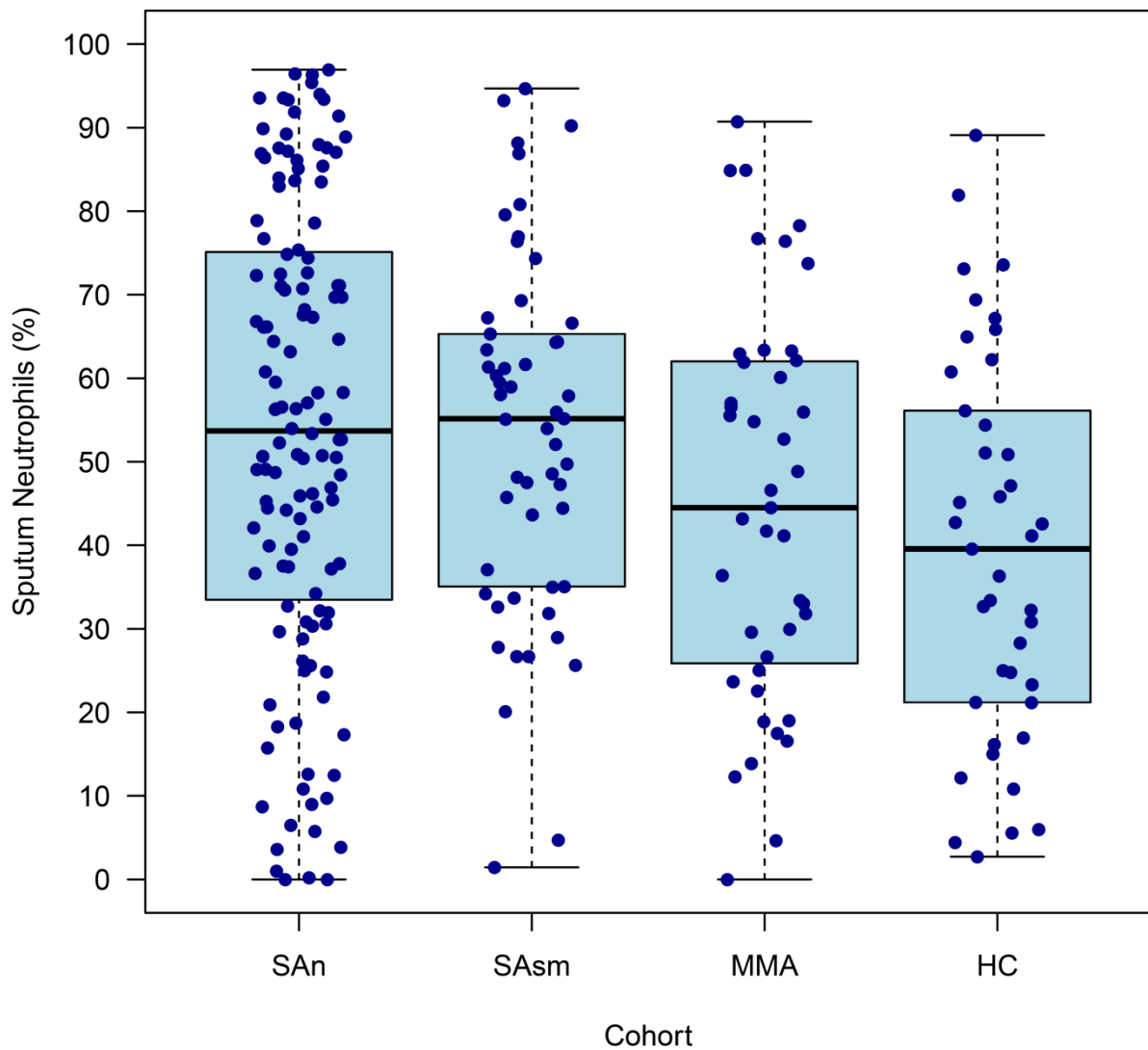
Fig 4B

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

REFERENCES

1.   Chung KF, Wenzel SE, Brozek JL, Bush A, Castro M, Sterk PJ, et al. International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. Eur Respir J. 2014 Feb;43(2):343-73. PubMed PMID: 24337046. Epub 2013/12/18. eng.

2.   Bel EH, Sousa A, Fleming L, Bush A, Chung KF, Versnel J, et al. Diagnosis and definition of severe refractory asthma: an international consensus statement from the Innovative Medicine Initiative (IMI). Thorax. 2011 Oct;66(10):910-7. PubMed PMID: 21106547. Epub 2010/11/26. Eng.

3.   Pavord ID, Korn S, Howarth P, Bleecker ER, Buhl R, Keene ON, et al. Mepolizumab for severe eosinophilic asthma (DREAM): a multicentre, double-blind, placebo-controlled trial. Lancet. 2012 Aug 18;380(9842):651-9. PubMed PMID: 22901886. Epub 2012/08/21. eng.

4.   Wenzel S, Ford L, Pearlman D, Spector S, Sher L, Skobieranda F, et al. Dupilumab in Persistent Asthma with Elevated Eosinophil Levels. NEnglJMed. 2013 5/21/2013.

5.   Wu W, Bleecker E, Moore W, Busse WW, Castro M, Chung KF, et al. Unsupervised phenotyping of Severe Asthma Research Program participants using expanded lung data. J Allergy Clin Immunol. 2014 May;133(5):1280-8. PubMed PMID: 24589344. Pubmed Central PMCID: 4038417. Epub 2014/03/05. eng.

6.   Haldar P, Pavord ID, Shaw DE, Berry MA, Thomas M, Brightling CE, et al. Cluster analysis and clinical asthma phenotypes. AmJ RespirCrit Care Med. 2008 08/01/;178(3):218-24. PubMed PMID: 8.

7.   Wenzel SE. Asthma phenotypes: the evolution from clinical to molecular approaches. Nature medicine. 2012;18(5):716-25.

8.   Wheelock CE, Goss VM, Balgoma D, Nicholas B, Brandsma J, Skipp PJ, et al. Application of 'omics technologies to biomarker discovery in inflammatory lung diseases. The European respiratory journal : official journal of the European Society for Clinical Respiratory Physiology. 2013 Sep;42(3):802-25. PubMed PMID: 23397306.

9.   Auffray C, Adcock IM, Chung KF, Djukanovic R, Pison C, Sterk PJ. An integrative systems biology approach to understanding pulmonary diseases. Chest. 2010 Jun;137(6):1410-6. PubMed PMID: 20525651.

10.   Bousquet J. Global initiative for asthma (GINA) and its objectives. Clin Exp Allergy. 2000 06;30 Suppl 1:2-5. PubMed PMID: 14.

11.   Pavord ID, Pizzichini MM, Pizzichini E, Hargreave FE. The use of induced sputum to investigate airway inflammation. Thorax. 1997 06/01/;52(6):498-501. PubMed PMID: 7.

12.   Szalma S, Koka V, Khasanova T, Perakslis ED. Effective knowledge management in translational medicine. Journal of translational medicine. 2010;8:68. PubMed PMID: 20642836. Pubmed Central PMCID: 2914663.

13.   Juniper EF, O'Byrne PM, Guyatt GH, Ferrie PJ, King DR. Development and validation of a questionnaire to measure asthma control. EurRespirJ. 1999 10;14(4):902-7. PubMed PMID: 42.

14.   Juniper EF, Guyatt GH, Ferrie PJ, Griffith LE. Measuring quality of life in asthma. The American review of respiratory disease. 1993 Apr;147(4):832-8. PubMed PMID: 8466117. Epub 1993/04/01. eng.

15.   Zigmond AS, Snaith RP. The hospital anxiety and depression scale. Acta PsychiatrScand. 1983 06;67(6):361-70. PubMed PMID: 137.

16.   Morley AD, Sharp HR. A review of sinonasal outcome scoring systems – which is best? Clinical Otolaryngology. 2006;31(2):103-9.

17.   Johns MW. A new method for measuring daytime sleepiness: the Epworth sleepiness scale. Sleep. 1991 Dec;14(6):540-5. PubMed PMID: 1798888. Epub 1991/12/01. eng.

18.   Cohen JL, Mann DM, Wisnivesky JP, Horne R, Leventhal H, Musumeci-Szabó TJ, et al. Assessing the validity of self-reported medication adherence among inner-city asthmatic adults: the Medication Adherence Report Scale for Asthma. Annals of Allergy, Asthma & Immunology. 2009;103(4):325-31.

19.   The ENFUMOSA cross-sectional European multicentre study of the clinical phenotype of chronic severe asthma. EurRespirJ. 2003 09;22(3):470-7. PubMed PMID: 25.

20.   Moore WC, Bleecker ER, Curran-Everett D, Erzurum SC, Ameredes BT, Bacharier L, et al. Characterization of the severe asthma phenotype by the National Heart, Lung, and Blood Institute's Severe Asthma Research Program. J Allergy Clin Immunol. 2007 Feb;119(2):405-13. PubMed PMID: 17291857. Pubmed Central PMCID: 2837934. Epub 2007/02/13. eng.

21.   Schleich FN, Manise M, Sele J, Henket M, Seidel L, Louis R. Distribution of sputum cellular phenotype in a large asthma cohort: predicting factors for eosinophilic vs neutrophilic inflammation. BMC Pulm Med. 2013;13:11. PubMed PMID: 23442497. Pubmed Central PMCID: 3657295. Epub 2013/02/28. eng.

22.   Chaudhuri R, Livingston E, McMahon AD, Thomson L, Borland W, Thomson NC. Cigarette Smoking Impairs the Therapeutic Response to Oral Corticosteroids in Chronic Asthma. American Journal of Respiratory and Critical Care Medicine. 2003 12/01/;168(11):1308-11. PubMed PMID: 17.

23.   Silva GE, Sherrill DL, Guerra S, Barbee RA. Asthma as a risk factor for COPD in a longitudinal study. Chest. 2004 07;126(1):59-65. PubMed PMID: 198.

24.   Bleecker ER. Similarities and differences in asthma and COPD. The Dutch hypothesis. Chest. 2004 08;126(2 Suppl):93S-5S. PubMed PMID: 196.

25.   Gevaert P, Calus L, Van Zele T, Blomme K, De Ruyck N, Bauters W, et al. Omalizumab is effective in allergic and nonallergic patients with nasal polyps and asthma. J Allergy Clin Immunol. 2013 Jan;131(1):110-6 e1. PubMed PMID: 23021878.

26.   Galli J, Montuschi P, Passàli GC, Laruffa M, Parrilla C, Paludetti G. Exhaled Nitric Oxide Measurement in Patients Affected by Nasal Polyposis. Otolaryngology -- Head and Neck Surgery. 2012 August 1, 2012;147(2):351-6.

27.   Heaney LG, Brightling CE, Menzies-Gow A, Stevenson M, Niven RM, Network obotBTSDA. Refractory asthma in the UK: cross-sectional findings from a UK multicentre registry. Thorax. 2010 September 1, 2010;65(9):787-94.

28.   Schleich F, Brusselle G, Louis R, Vandenplas O, Michils A, Pilette C, et al. Heterogeneity of phenotypes in severe asthmatics. The Belgian Severe Asthma Registry (BSAR). Respiratory medicine. 2014 12//;108(12):1723-32.

29.   Reddel HK, Taylor DR, Bateman ED, Boulet LP, Boushey HA, Busse WW, et al. An official American Thoracic Society/European Respiratory Society statement: asthma control and exacerbations: standardizing endpoints for clinical asthma trials and clinical practice. AmJ RespirCrit Care Med. 2009 07/01/;180(1):59-99. PubMed PMID: 306.

30.   McGrath KW, Icitovic N, Boushey HA, Lazarus SC, Sutherland ER, Chinchilli VM, et al. A Large Subgroup of Mild-to-Moderate Asthma Is Persistently Noneosinophilic. American Journal of Respiratory and Critical Care Medicine. 2012 March 15, 2012;185(6):612-9.

31.   Jatakanon A, Uasuf C, Maziak W, Lim S, Chung KF, Barnes PJ. Neutrophilic inflammation in severe persistent asthma. AmJ RespirCrit Care Med. 1999 11;160(5 Pt 1):1532-9. PubMed PMID: 20.

32.   Moore WC, Hastie AT, Li X, Li H, Busse WW, Jarjour NN, et al. Sputum neutrophil counts are associated with more severe asthma phenotypes using cluster analysis. J Allergy Clin Immunol. 2014 Jun;133(6):1557-63 e5. PubMed PMID: 24332216. Pubmed Central PMCID: 4040309.

33.   Dixon AE, Pratley RE, Forgione PM, Kaminsky DA, Whittaker-Leclair LA, Griffes LA, et al. Effects of obesity and bariatric surgery on airway hyperresponsiveness, asthma control, and inflammation. The Journal of allergy and clinical immunology. 2011;128(3):508-15.e2.

34.   Arterburn DE, Olsen MK, Smith VA, Livingston EH, Van Scoyoc L, Yancy WS, Jr., et al. Association Between Bariatric Surgery and Long-term Survival. JAMA. 2015 Jan 6;313(1):62-70. PubMed PMID: 25562267. Epub 2015/01/07. Eng.