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

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Editorial comment in: *Eur Respir J* 2015; 46: 1227–1231 [DOI: 10.1183/13993003.01414-2015].

This article has supplementary material available from erj.ersjournals.com

A press release for this article is available from erj.ersjournals.com/press

Received: May 18 2015 | Accepted after revision: July 03 2015 | First published online: Sept 10 2015

ABSTRACT U-BIOPRED is a European Union consortium of 20 academic institutions, 11 pharmaceutical companies and six 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 (nonsmokers, n=311; 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-BIOPRED is characterised by poor symptom control, increased comorbidity 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.

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Severe asthma results in more airway inflammation, worse symptoms and lower lung function, despite increased therapy http://ow.ly/QznR3

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, 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 mild/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 that are at the core of this programme.

Methods

Participants

This was a multicentre prospective cohort study recruiting from 16 clinical centres in 11 European countries. Details of the participating centres, assessments and standard operating procedures are available

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This article has been amended according to the correction published in the June 2017 issue of the *European Respiratory Journal*.

This study is registered on ClinicalTrials.gov (identifier: NCT01982162).

Support statement: 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 would also like to acknowledge help from the IMI funded eTRIKS project (EU Grant Code No.115446). Funding information for this article has been deposited with FundRef.

Conflict of interest: Disclosures can be found alongside the online version of this article at erj.ersjournals.com

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 6 months, during which time assessments had been undertaken to optimise asthma control and 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 forced expiratory volume in 1 s (FEV_1) >12% predicted or 200 mL following inhalation of 400 µg salbutamol), airway hyperresponsiveness (methacholine provocative concentration causing a 20% fall in FEV_1 <8 $mg \cdot mL^{-1}$, or diurnal peak expiratory flow amplitude >8% of mean), or a decrease in FEV_1 of 12% predicted or 200 mL within 4 weeks after tapering maintenance treatment. Four groups were recruited, as follows.

Group A: severe nonsmoking asthma (SAn)

Participants in this group were nonsmokers for at least the past 12 months, with a less than 5 pack-year smoking history, with asthma and uncontrolled symptoms defined according to Global Initiative for Asthma (GINA) guidelines [10] and/or frequent exacerbations (more than two per year) despite high-dose inhaled corticosteroids (⩾1000 µg fluticasone propionate per day or equivalent dose).

Group 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.

Group C: mild/moderate nonsmoking asthmatics (MMA)

Participants in this group were nonsmokers for at least the past 12 months, with a less than 5 pack-year smoking history and had controlled or partially controlled asthma symptoms, as defined by GINA, whilst receiving a dose of <500 µg fluticasone propionate/day or equivalent.

Group D: healthy nonsmoking controls (HC)

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

Protocol and assessments

Participants attended a screening visit to assess eligibility for the study (figure 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 (F_{ENO}) at 50 $mL \cdot s^{-1}$ 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 immunoglobulin (Ig)E 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 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).

 DOI: 10.1183/13993003.00779-2015

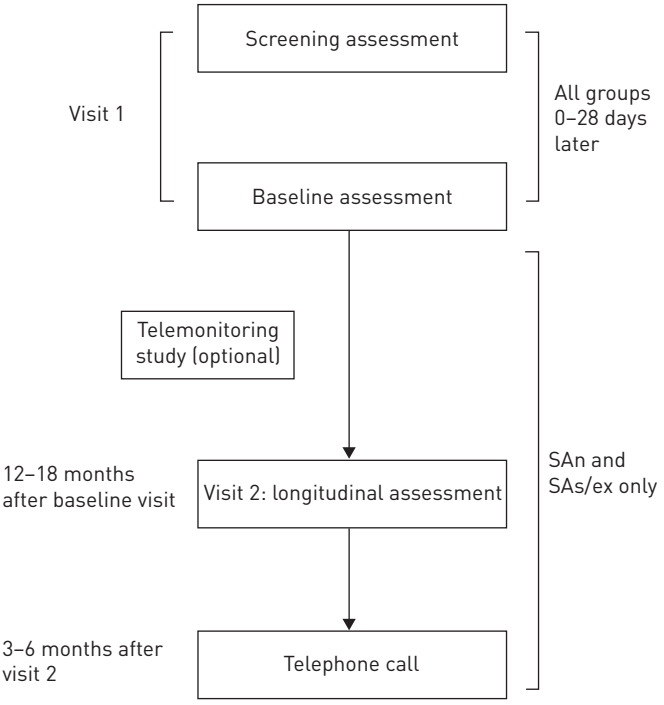

FIGURE 1 Visit schedule. SAn: severe nonsmoking asthma group; SAs/ex: smokers and ex-smokers with severe asthma.

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; and 6) the Medication Adherence Report Scale (MARS) [18] to measure adherence.

Statistical analysis

Continuously distributed data were either summarised using mean±SE if symmetrical, or median (interquartile range) values. Nonsymmetrical 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; www.r-project.org).

Results

A total of 610 adults were recruited over an 18-month period: 311, 110, 88 and 101 into the SAn, SAs/ex, MMA and HC groups, respectively (table 1 and figure 2).

There were more females in the SAn group (66%) compared to the other asthma groups (50%), with the age of onset of asthma 18 years later in the SAs/ex compared with SAn group. Participants with severe asthma had a higher body mass index (BMI) than those in the MMA and HC groups and were older (table 1). 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 intensive care unit 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

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. Forced vital capacity (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 (table 1).

TABLE 1 Group demographics

	Severe nonsmoking asthma	Smokers and ex-smokers with severe asthma[#]	Mild/moderate nonsmoking asthma	Healthy nonsmoking controls	p-value
Subjects n	311	110	88	101	
Age years	51.01±0.8 (n=311)	54.51±1.08 (n=110)	41.66±1.65 (n=88)	38.85±1.34 (n=101)	<0.001
Age at diagnosis years	20 (7–38) (n=302)	38 (20–48) (n=109)	14 (6–32) (n=83)	NA	<0.001
Females	205/311 (66%)	56/110 (51%)	44/88 (50%)	39/101 (39%)	<0.001
BMI kg·m^{-2}	29.11±0.36 (n=311)	29.59±0.6 (n=110)	25.73±0.47 (n=88)	25.31±0.36 (n=101)	<0.001
BMI >30 kg·m^{-2}	120/311 (38.6%)	44/110 (40%)	16/88 (18.18%)	12/101 (11.88%)	<0.001
Serum IgE IU·mL^{-1}	119.5 (45–342) (n=302)	126 (63–328) (n=104)	89.4 (49–244) (n=85)	23.45 (9–65) (n=98)	<0.001
FEV_1 % pred	67.5±1.26 (n=308)	67.2±1.84 (n=110)	89.5±1.86 (n=87)	101.76±1.29 (n=101)	<0.001
FVC % pred	87.2±1.12 (n=308)	89.7±1.74 (n=110)	104.5±2.02 (n=87)	107.8±1.3 (n=101)	<0.001
FEV_1/FVC ratio	0.64±0.01 (n=308)	0.61±0.01 (n=110)	0.72±0.01 (n=87)	0.79±0.01 (n=101)	<0.001
Exacerbations in previous year n	2.48±0.13 (n=310)	2.55±0.26 (n=110)	0.38±0.08 (n=88)	NA	<0.001
Smoking history pack-years	2 (1–4) (n=47)	17.38 (10–26) (n=110)	4 (1–4) (n=13)	0.9 (0–3) (n=20)	<0.001
Intubation (ever)	35/307 (11%)	6/109 (6%)	0/87 (0%)	NA	0.083
ICU admission (ever)	80/307 (26%)	18/109 (17%)	1/86 (1%)	NA	<0.001
Atopy test positive	213/272 (78.3%)	62/87 (71.3%)	72/78 (92.3%)	36/78 (46.2%)	<0.001

Data are presented as mean±SE, median (interquartile range) or n/N (%), unless otherwise stated. BMI: body mass index; FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; ICU: intensive care unit; NA: not applicable. [#]: 42 current smokers and 68 ex-smokers.

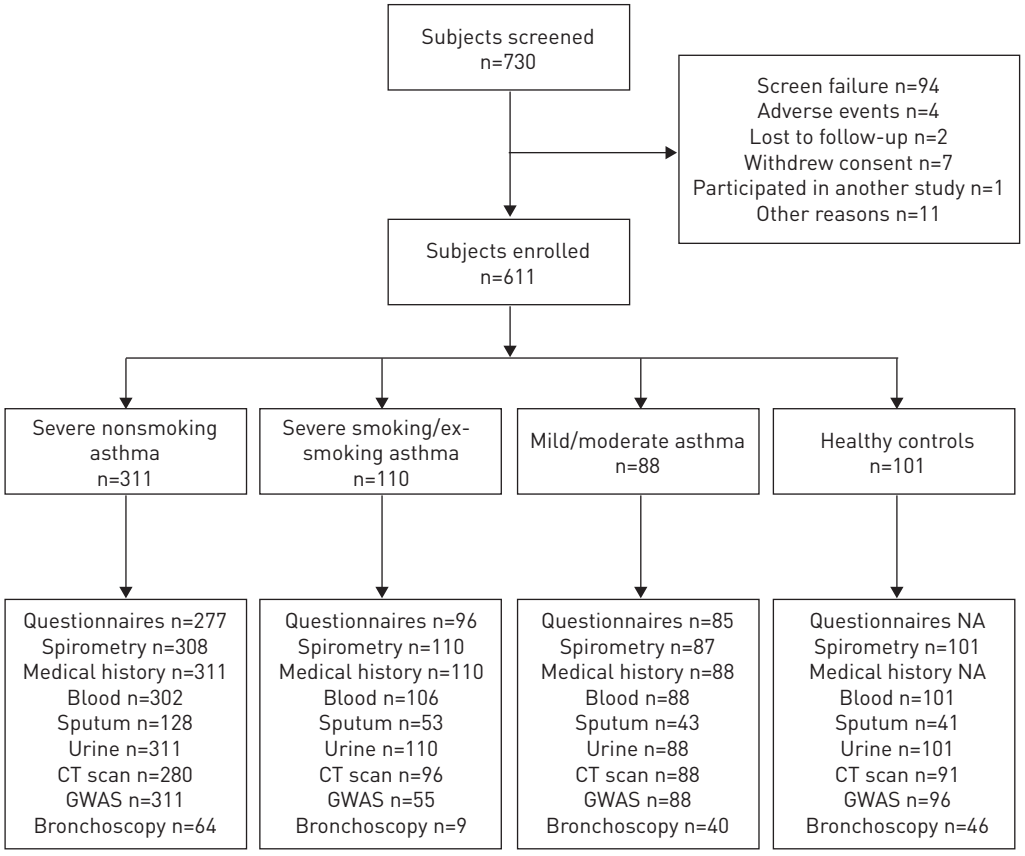

FIGURE 2 Consort diagram. CT: computed tomography; GWAS: genome-wide association study; NA: not applicable.

DOI: 10.1183/13993003.00779-2015

Medications

Within the SAn and SAs/ex groups 46% and 45% respectively received daily oral corticosteroids, 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 (table 2).

Questionnaires

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 (table 3).

The MARS questionnaire scores for adherence to treatment recorded by the three asthma groups were in the range of 21 to 22, with the severe asthma groups recording higher scores ($p<0.005$), indicating better adherence. The AQLQ score was correlated to several variables, including FEV_1 (95% CI 0.5 to 0.7; $p<0.001$), FEV_1/FVC (95% CI 1.14 to 2.8; $p<0.001$), exacerbations in the previous year (95% CI −0.8 to −0.2; $p<0.001$), BMI (95% CI −0.006 to −0.002; $p<0.001$) and pack-years smoked (95% CI −0.003 to −0.001; $p<0.001$) (figure 3).

Atopy and comorbidities

There was a high incidence of atopy in the four groups, at 70% in the asthma groups and 46% in the HC group. The incidence of allergic rhinitis, hay fever and nonallergic rhinitis were highest in the asthma groups. Participants in the HC group were much less allergic with only a third reporting hay fever and only a sixth, rhinitis or eczema.

The presence of nasal polyps was associated with severe asthma, regardless of smoking status (four-fold increased incidence in SAn and SAs/ex groups *versus* MMA group; $p<0.001$) (table 4). No such association was seen with allergic or nonallergic 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

Blood eosinophil counts were similar in all three asthma groups. Each group had a significantly higher blood eosinophil count than the HC group (SAn *versus* HC, $p=0.002$; SAs/ex *versus* HC, $p=0.005$; MMA

TABLE 2 Medications

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

Data are presented as n/N (%) or mean±SE, unless otherwise stated. NA: not applicable. [#]: hydrocortisone and triamcinolone doses were converted to equivalent prednisolone dose (four healthy control participants took as required antihistamines).

TABLE 3 Questionnaires

	Severe nonsmoking asthma	Smokers and ex-smokers with severe asthma	Mild/moderate nonsmoking asthma	Unadjusted p-value[#]
Subjects n	311	110	88	
ACQ				
Mean ACQ5	2.28±0.07 (n=277)	2.23±0.12 (n=96)	0.86±0.07 (n=85)	<0.001
Mean ACQ7	2.67±0.08 (n=277)	2.62±0.12 (n=96)	1.01±0.07 (n=85)	<0.001
AQLQ				
Total	4.48±0.07 (n=276)	4.44±0.13 (n=92)	5.84±0.1 (n=84)	<0.001
Symptoms	4.46±0.08 (n=276)	4.36±0.14 (n=92)	5.87±0.1 (n=84)	<0.001
Emotional	4.63±0.1 (n=276)	4.52±0.16 (n=92)	5.98±0.13 (n=84)	<0.001
Environmental stimuli	4.69±0.09 (n=276)	4.57±0.16 (n=92)	5.63±0.14 (n=84)	<0.001
Activity limitation	4.35±0.07 (n=276)	4.45±0.13 (n=92)	5.81±0.11 (n=84)	<0.001
HADS				
Total	12.33±0.54 (n=223)	13.64±1.01 (n=72)	7.01±0.7 (n=70)	<0.001
Anxiety	6.94±0.3 (n=223)	7.71±0.54 (n=72)	4.24±0.41 (n=70)	<0.001
Depression	5.39±0.28 (n=223)	5.93±0.56 (n=72)	2.77±0.39 (n=70)	<0.001
SNOT20 (total)	31.76±1.01 (n=283)	32.12±1.92 (n=97)	15.42±1.42 (n=83)	<0.001
ESS (total)	7.48±0.26 (n=277)	7.95±0.47 (n=95)	5.49±0.41 (n=85)	<0.001
MARS (total)	22.44±0.14 (n=278)	22.17±0.29 (n=94)	21.35±0.4 (n=84)	0.002

Data are presented as mean±SE, unless otherwise stated. ACQ: Asthma Control Questionnaire; AQLQ: Asthma Quality of Life Questionnaire; HADS: Hospital Anxiety and Depression Score; SNOT20: Sino-nasal Outcome Test 20; ESS: Epworth Sleepiness Scale; MARS: Medication Adherence Report Scale. [#]: severe asthma (nonsmoking asthma group and smokers and ex-smokers with severe asthma group combined) *versus* mild/moderate nonsmoking asthma group.

versus HC, p<0.001). Blood neutrophil counts were significantly higher in the severe asthma groups compared to the MMA group.

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 5). The sputum eosinophil count was higher in the two severe asthma groups combined compared to the mild/moderate asthma group (table 5; figure 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 5).

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

F_{eNO} levels in all asthma groups were higher than those in the HC group, but the F_{eNO} 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 F_{eNO} (mean increase 2.1 ppb, 95% CI 1.5 to 2.9 ppb; p<0.001) (table 5).

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 1000 µg 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 inhaled corticosteroid dose of ⩾1200 µg of budesonide or beclomethasone with at least one exacerbation in the past year. Of these five 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 per day.

DOI: 10.1183/13993003.00779-2015

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 inhaled corticosteroids [22], possibly through the induction of corticosteroid insensitivity [23, 24]. However, our analyses of the nonsmoking 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 F_{ENO} in the smoking/ex-smoking group might be explained by an effect of current smoking [25]. One notable difference is that asthma onset occurred on average 18 years later in the smokers and ex-smokers than in the nonsmokers, and

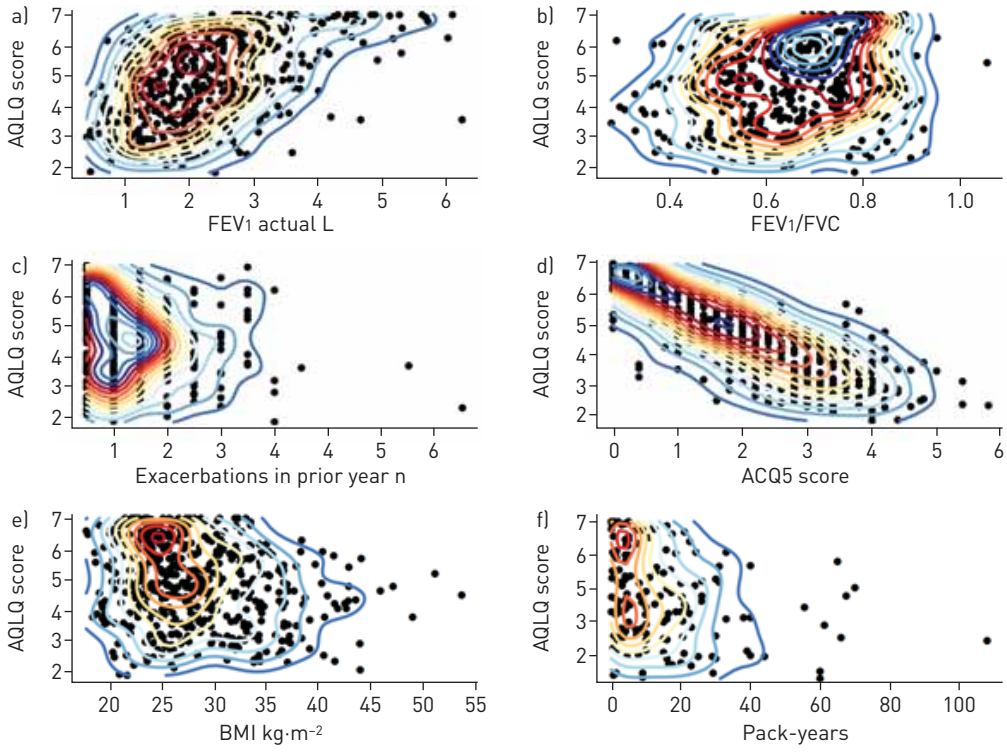

FIGURE 3 Contour plots of the Asthma Quality of Life Questionnaire (AQLQ) related to baseline demographics. Figures represent scatter plots describing the relationship between each factor and the asthma quality of life z-score. The contour lines are coloured blue to red, to indicate increasing density 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 c) exacerbations, e) body mass index (BMI) and f) smoking history (pack-years), a strong inverse relationship between quality of life and d) asthma control (ACQ5) and weak positive relationships between quality of life and a, b) measures of lung function (forced expiratory volume in 1 s (FEV_1) and FEV_1 to forced vital capacity (FVC) ratio).

TABLE 4 Comorbidities

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

GORD: gastro-oesophageal reflux disease. [#]: severe asthma (nonsmoking asthma group and smokers and ex-smokers with severe asthma group combined) *versus* mild/moderate nonsmoking asthma group.

TABLE 5 Biomarkers in blood, sputum and exhaled air

	Severe nonsmoking asthma	Smokers and ex-smokers with severe asthma	Mild/moderate nonsmoking asthma	Healthy nonsmoking controls	p-value	p-value[#]
Subjects n	311	110	88	101		
Exhaled NO ppb	26.5 (16–47) (n=290)	23.5 (12–42) (n=104)	25 (18–54) (n=87)	19.25 (13–29) (n=96)	<0.001	0.438
Sputum						
Sputum eosinophils %	2.75 (0–19) (n=128)	4.13 (1–14) (n=53)	1.05 (0–3) (n=43)	0 (0–0) (n=41)	<0.001	0.004
Sputum neutrophils %	53.69 (34–75) (n=128)	55.15 (35–65) (n=53)	44.5 (26–62) (n=43)	39.56 (21–56) (n=41)	0.002	0.042
Sputum differential eosinophil count >1.9%	74 (57.81%) (n=128)	32 (60.38%) (n=53)	17 (39.53%) (n=43)	1 (2.44%) (n=41)	<0.001	0.026
Blood						
Blood eosinophils %	2.94 (1–6) (n=302)	2.88 (1–5) (n=106)	3.00 (2–5) (n=88)	2.10 (1–3) (n=101)	0.001	0.295
Blood eosinophils absolute	0.2 (0.3) (n=302)	0.22 (0.29) (n=106)	0.23 (0.2) (n=88)	0.1 (0.11) (n=101)	0.001	0.295
Blood neutrophils %	62 (55–70) (n=302)	61.75 (55–69) (n=106)	56.83 (52–63) (n=88)	57.34 (51–64) (n=101)	<0.001	<0.001
Blood neutrophils absolute	4.73 (3.1) (n=302)	4.97 (2.87) (n=106)	3.64 (1.75) (n=88)	3.03 (1.6) (n=101)	<0.001	<0.001

Data are presented as median (interquartile range), unless otherwise stated. [#]: severe asthma (nonsmoking asthma group and smokers and ex-smokers with severe asthma group combined) *versus* mild/moderate nonsmoking asthma group.

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 (table S5 of 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 [26]; however, whether this is due to an effect on the underlying asthma or the polyps is unknown. The link with higher F_{eNO} levels is in keeping with work showing that nasal polypectomy leads to a fall in F_{eNO} [25].

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.

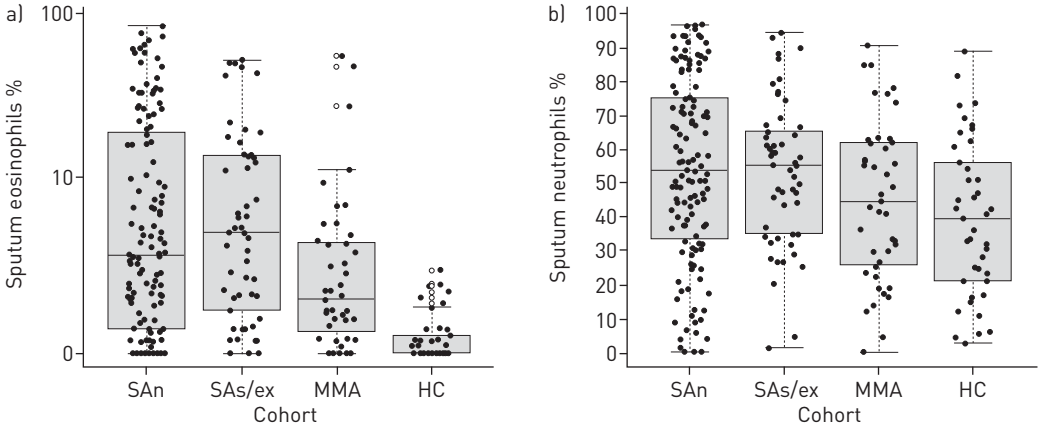

FIGURE 4 a) Sputum eosinophil count and b) sputum neutrophil count, by cohort. The boxes represent median and interquartile range values; whiskers extend to the extreme point, no more than 1.5 times the interquartile range from the box, with outliers denoted by open circles and raw data by black circles overlaid. SAn: severe nonsmoking asthma; SAs/ex: smokers and ex-smokers with severe asthma; MMA: mild/moderate nonsmoking asthma; HC: healthy nonsmoking controls.

DOI: 10.1183/13993003.00779-2015

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 in the 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 and, 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 hyperresponsiveness [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. First, 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. Finally, due to the numerous formulations and inhaler devices used across Europe it was not possible to calculate the precise daily equivalent inhaled corticosteroid dose for each participant and therefore these data are not shown; however, high (>1000 μg FP) or low (<500 μg fluticasone propionate per day) 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. We thank all the members of each recruiting centre for their dedicated effort, devotion, promptness and care in the recruitment and assessment of the participants in this study.

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The U-BIOPRED consortium wishes to acknowledge the help and expertise of the following individuals and groups without whom, the study would not have been possible.

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 DOI: 10.1183/13993003.00779-2015

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Third Parties to the project, contributing to the clinical trial: Academic Medical Centre (AMC), Amsterdam (In the U-BIOPRED consortium the legal entity is AMC Medical Research BV (AMR); AMR is a subsidiary of both AMC and the University of Amsterdam; AMC contribute across the U-BIOPRED project); University Hospital Southampton NHS Trust (third party of the University of Southampton and contributor to the U-BIOPRED clinical trial); South Manchester Healthcare Trust (third party to the University of Manchester, South Manchester Healthcare Trust,

contributor to the U-BIOPRED clinical trial and to the U-BIOPRED Biobank); Protisvalor Méditerranée SAS (third party to University of the Mediterranean; contributor to the U-BIOPRED clinical trial); Karolinska University Hospital (third party Karolinska Institutet (KI), contributor to the U-BIOPRED clinical trial); Nottingham University Hospital (third party to University of Nottingham, contributor to the U-BIOPRED clinical trial); NIHR-Wellcome Trust Clinical Research Facility.

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