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Asthma similarities across ProAR (Brazil) and U-BIOPRED (Europe) adult cohorts of contrasting locations, ethnicity and socioeconomic status

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ABSTRACT

Background: Asthma prevalence is 339 million globally. 'Severe asthma' (SA) comprises subjects with uncontrolled asthma despite proper management.
Objectives: To compare asthma from diverse ethnicities and environments.
Methods: A cross-sectional analysis of two adult cohorts, a Brazilian (ProAR) and a European (U-BIOPRED). U-BIOPRED comprised of 311 non-smoking with Severe Asthma (SAn), 110 smokers or ex-smokers with SA (SAs) and 88 mild to moderate asthmatics (MMA) while ProAR included 544 SA and 452 MMA. Although these projects were independent, there were similarities in objectives and methodology, with ProAR adopting operating procedures of U-BIOPRED.
Results: Among SA subjects, age, weight, proportion of former smokers and FEV_1 pre-bronchodilator were similar. The proportion of SA with a positive skin prick tests (SPT) to aeroallergens, the scores of sino-nasal symptoms and quality of life were comparable. In addition, blood eosinophil counts (EOS) and the % of subjects with EOS > 300 cells/μl were not different. The Europeans with SA however, were more severe with a greater proportion of continuous oral corticosteroids (OCS), worse symptoms and more frequent exacerbations. FEV_1/FVC pre- and post-bronchodilator were lower among the Europeans. The MMA cohorts were less comparable in control and treatment, but similar in the proportion of allergic rhinitis, gastroesophageal reflux disease and EOS >3%.
Conclusions: ProAR and U-BIOPRED cohorts, with varying severity, ethnicity and environment have similarities, which provide the basis for global external validation of asthma phenotypes. This should stimulate collaboration between asthma consortia with the aim of understanding SA, which will lead to better management.

1. Introduction

In 2016, the world-wide asthma prevalence has been estimated to be 339 million individuals, with approximately 420,000 people dying prematurely from asthma each year[1]. Its prevalence varies geographically, with its incidence plateauing in high income countries while in low to middle income countries, it has been increasing [1]. Under-diagnosis and under-treatment of asthma present a serious health problem particularly in low and middle-income countries [2] where access to preventive healthcare facilities may be challenging resulting in patients treating their asthma when it worsens with hospital admissions and emergency room visits. In Salvador City, for example, the Programme for Control of Asthma in Bahia (ProAR) was established in 2003 to provide care for patients with severe asthma (SA) who previously had limited preventive healthcare support – an intervention that resulted in

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Abbreviations

ACQ	Asthma Control Questionnaire
AQLQ	Asthma Quality of Life Questionnaire
BIOAIR	The study of longitudinal assessment of clinical course and BIOmarkers in Severe Chronic AIRway disease
BMI	Body Mass Index
COREA	The Cohort for Reality and Evolution of adult Asthma in Korea
CT scan	Computerized tomography
ENFUMOSA	The European Network for Understanding Mechanisms of Severe Asthma
ESS	Epworth Sleepiness Scale
FeNO	Exhaled fraction of nitric oxide
GORD	Gastroesophageal Reflux Disease
HADS	Hospital Anxiety and Depression Scale
ICU	Intensive care unit
IQR	Interquartile range
MARS	Medication Adherence Report Scale
MMA	Mild to moderate asthma
U-BIOPRED	Unbiased Biomarkers for the Prediction of Respiratory Disease Outcomes
ProAR	Programme for Control of Asthma in Bahia (Brazil)
SA	Severe asthma
SAn	Severe asthma non-smoking
SAs	Smokers or ex-smokers with severe asthma
SARP	The Severe Asthma Research Programme (USA)
SNOT20	Sino-nasal symptom score
SPT	Skin prick tests
WHO	World Health Organization

a citywide rapid reduction in hospitalisations [3].

Severe asthma comprises of several disease endotypes with different clinical and pathophysiological characteristics that result in symptoms of cough, wheeze, breathlessness and tightness in the chest, often recurrent and severe enough to characterize asthma attacks or exacerbations [4]. While several options for treatments are available for asthma, there is an unmet need for more effective treatments, to improve symptoms, avoid exacerbations and prevent lung function decline for SA [5]. SA cohorts have now been followed up in different locations worldwide, with the aim of improving our understanding of asthma and in particular of severe asthma. The more recent cohorts include the ProAR [3] Cohort from Salvador in Brazil and the European U-BIOPRED Cohort (Unbiased Biomarkers for the Prediction of Respiratory Disease Outcomes) [6], which were preceded by others such as ENFUMOSA [7] (The European Network for Understanding Mechanisms of Severe Asthma), BIOAIR [8], The Severe Asthma Research Programme (SARP) in the United States of America [9], The Severe Asthma Cohort of WESSEX, in the United Kingdom [10], and The Cohort for Reality and Evolution of adult Asthma in Korea [11] (COREA) to name but a few. Bringing them together to determine similarities and differences in symptoms, lung function and inflammatory patterns may enable validation of previous findings from bio-clinical phenotyping of individual cohorts and may also indicate differences that will inform our understanding of risk factors, mechanisms of disease, treatments, environment and ultimately leading to improvements in management.

To address the issue of phenotypic variability and heterogeneity, the U-BIOPRED project was set up in 2009 as a public–private partnership within the framework of the Innovative Medicines Initiative, with engagement from academia, the pharmaceutical industry and patient groups. U-BIOPRED represents a European Union consortium of 20 academic institutions, 11 pharmaceutical companies and six patient organisations, working together with the objective of improving the understanding of asthma mechanisms. The adult patients with SA, were compared with patients with mild to moderate asthma (MMA) and healthy controls from 11 European countries, in terms of patient-reported outcomes, lung function, blood and airway inflammatory measurements [6]. The aim of U-BIOPRED was to identify multi-dimensional phenotypes of severe asthma and new treatment targets.

From 2003, a cohort with previously-untreated severe asthma, as defined by the NIH EPR-2 in 1997 [12] and subsequently reiterated by an expert panel to The World Health Organization (WHO) in 2009 [13], was enrolled by the Programme for Control of Asthma in Bahia (ProAR) [3], in Brazil, with continuing follow-up. Good clinical management and access to proper treatment was unavailable in the public health system in the City of Salvador (3 million inhabitants), prior to 2002. Four reference clinics were subsequently made available, offering specialized secondary care and treatment free of charge to patients with severe asthma. From 2013, a sample of subjects of the ProAR SA cohort was compared, in a case control study, to subjects with mild to moderate (MMA) and controls with no asthma, from the same community, to investigate risk factors, phenotypes and biomarkers of severity.

The purpose of this report is to compare baseline demographic, clinical and spirometric characteristics of the participants of U-BIOPRED (European) and ProAR (Brazilian) SA and MMA cohorts, looking for similarities that might allow validation of research findings, and differences that could help to build an understanding of specific phenotypes associated with diverse genetic and environmental factors.

2. Patients and methods

This was a cross sectional comparative analysis of patients with asthma from two distinct cohorts of adults. Details of the cohorts can be found in the Online supplement. Briefly, the sample of European subjects with asthma from U-BIOPRED comprised 311 adults with severe asthma, non-smoking (SAn), 110 adult smokers or ex-smokers with severe asthma (SAs) and 88 adult non-smokers with MMA. Subjects $\geq$18 years old were recruited from 16 clinical centres in 11 European countries. Prior to enrolment participants were required to have been under follow-up by a respiratory physician for at least 6 months, in which assessments had been undertaken to optimise asthma control and medication adherence. In short, severe asthma was defined in U-BIOPRED as a disease requiring high doses of inhaled corticosteroids (>1,000 μg of Fluticasone Propionate) combined with other controller medication (LABA, LAMA, Theophyline, Leucotriene Antagonists or oral corticosteroids) to achieve control, or that remains uncontrolled in spite of optimal therapy, after issues of adherence, inhaler technique and treatment of comorbidities have all been addressed. Subjects with MMA were non-smokers for at least 12 months, with less than 5 pack-year smoking history and controlled or partially controlled asthma symptoms, as defined by GINA, whilst receiving a dose of <500 μg fluticasone propionate/day or equivalent [6]. The sample of Brazilian subjects from ProAR included 544 adults from a cohort with previously-untreated SA (upon enrolment in the cohort) together with 452 adults, invited from the same communities by public advertisement and classified as having MMA by a doctor's interview at the time of the present study [14]. The classification of MMA was based on current concepts of severity, including subjects with intermittent symptoms and no regular treatment or low dose of controller medications, upon evaluation by a specialist. The Brazilian subjects with SA were a sample of the ProAR outpatient asthma cohort constituted from 2003 as a reference centre and maintained with provision of free medication and multidisciplinary care in a public clinic, selected by a double specialist audit to validate their diagnosis, exclude other relevant respiratory or systemic condition that

could interfere with the assessment of asthma control (eg. lung scars, cancer, psychiatric and neurological disorders). The definition of severe asthma for the Brazilian sample was based on much older criteria, as its follow up started from 2003. It is a cohort of subjects with previously-untreated severe asthma, as defined by the NIH EPR-2 in 1997 [12]. These subjects had uncontrolled asthma upon their enrolment, in the past. The classification did not take into consideration the previous treatment. For the purpose of the present study, the sample of patients with severe asthma from ProAR was evaluated from 2013 to 2015, from 6 months to 12 years after the enrolment in the cohort. The projects were independent in their conception, proposal and funding, but shared many similarities in terms of execution, and the study of ProAR has in general adopted the standard operating procedures of U-BIOPRED. Both samples of severe asthma, the European and the Brazilian, were picked up from centres of reference for severe asthma assessible to all, being the best representation of the communities they are located one can get.

All investigations in U-BIOPRED were performed according to standardised operating procedures available in the online supplement of the initial manuscript [6], which were shared and adopted by the ProAR Study group for the common procedures to strengthen cross cohort comparability. For ProAR, the information included in this comparative analysis comprised of demography, age of onset of asthma, history of smoking and exposure to smoke, history of exacerbations and hospital admissions in the last 12 months, intensive care unit (ICU) admissions due to asthma ever, weight, medication use, spirometry, skin prick test (SPT), white blood cell counts, serum total IgE, depression, gastroesophageal-reflux disease (GORD), chronic rhinitis, scores of sleepiness, sino-nasal symptoms, asthma control and quality of life. The instruments and procedures for these data collection are described in the online supplementary information appendix of this manuscript. Information on diet and nutrition, oral health, stress, resilience, domestic and community violence, induced sputum and nasal lavage fluids, genetics and immunological phenotyping were collected and are under analysis but have not been included in this report.

The studies were 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. All participants gave written and signed informed consent.

2.1. Analysis

Continuously-distributed data were either summarized using mean ± SE if symmetrical, or median (interquartile range) values if they were not. Nonsymmetrical variables exhibiting a positive skew were log-transformed prior to association testing. Missing data were not imputed. Cross-cohort comparisons were made using the Kruskall-Wallis Test for continuous variables. Discrete variables were summarized using percentages, and cross-cohort comparisons were made using the Chi-square Test. 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) or STATA® (College Station, USA).

3. Results

3.1. Criteria used for severe asthma

The classification of asthma severity of the 544 individuals recruited to ProAR SA cohort, corresponds to a proposal presented to WHO by experts in 2009 [13], which divides SA into 3 categories: (i) untreated severe asthma, (ii) difficult-to-treat severe asthma, and (iii) treatment-resistant severe asthma. The majority of subjects from ProAR SA Cohort (89%) are in the difficult-to-treat category, while in the U-BIOPRED cohort using the WHO definition all subjects were in the treatment-resistant category.

3.2. General characteristics

Some of the general characteristics of the two groups were remarkably similar notwithstanding the aforementioned distinct categorization of severity. For the SA subjects, the age was similar across the two cohorts, as were the Body Mass Index (BMI), proportion of former smokers (Table 1) and FEV_1% pre-bronchodilator (Table 2). Suplementary Online Table 2 shows also other comparable observations across the U-BIOPRED and ProAR SA Cohorts: the sino-nasal symptom (SNOT20) score was similar, so were the Asthma Related Quality of Life (AQLQ) scores. Table 3 shows that the proportion of SA subjects with allergic rhinitis was similar between the cohorts, as was the proportion of atopics defined by a positive SPT. The average number of blood eosinophils and the % of subjects with a blood eosinophil counts >300 cells/μl across cohorts were also similar. Subjects of the MMA samples from Brazil and Europe are less comparable in age (Table 1) and treatment (Table 1 online supplement), yet they present notable similarities: the rate of ever-smokers was similar, and BMI was not different (Table 1). The proportion of subjects with MMA presenting allergic rhinitis was comparable between groups, as was the percentage of subjects with GORD and with a blood count >3% eosinophils (Table 3). The most relevant differences between the South American and the European cohorts were as follows: (i) the age of onset was lower in Brazil in both cohorts, (ii) the burden of smoking (pack-years) was higher among the U-BIOPRED SA cohort than the Brazilian SA Cohort (Table 1), (iii) FEV_1% post-BD and FVC % Pre-BD and post-BD were higher among the Europeans, whereas FEV_1/FVC pre and post-BD were lower in the comparisons of SA and MMA across cohorts (Table 2), and (iv) the proportion of females was higher in the Brazilian cohorts of any severity (Table 1). The proportion of Afro-descendants among the Brazilian cohorts was above 90%.

The age of onset, which was higher among the Europeans, also differed by severity and gender, with a trend of being higher in SA and, among subjects with SA, in females of both cohorts (Fig. 1). Nevertheless, the shape of the curves in both study groups and severities was unimodal, decreasing until about 38 years of age where U-BIOPRED displays late onset disease representing about 35% or 22% of the population in the SA and MMA respectively (Fig. 1 online supplement). This is in contrast to 17% of the ProAR SA population with such late onset, and 10% or the ProAR MMA group.

Interestingly, the proportion of ProAR patients reporting previous exposure to household air pollution from wood stoves, was 64.3% for SA, but only 36% for MMA, suggesting this is a relevant risk factor for severe asthma in Brazil, but likely uncommon in Europe although this was not enquired in U-BIOPRED.

Lung function parameters were, as expected, worse in the SA versus the MMA for U-BIOPRED and ProAR (Table 2). Reversibility was greater in the U-BIOPRED cohort whether subjects had SA or MMA. However, the lung function characteristics commonly used to define Asthma COPD Overlap (ACO) namely a post-bronchodilator $FEV_1/FVC < 70\%$ and $FEV_1 < 80\%$, showed similar high proportions in the SA Brazilian and European cohorts, and similar but lower proportions in the MMA cohorts.

3.3. History of exacerbations

Table 4 presents data on the history of severe exacerbations and hospitalizations due to asthma in the last 12 months, and ICU admission due to asthma ever. The U-BIOPRED SA cohort consistently looked worse on any exacerbation parameters studied. A comparison in the frequency distribution of severe exacerbations indicated that less subjects had experienced an exacerbation in the ProAR SA cohort (Fig. 2 online supplement). However, a small minority (5%) of ProAR subjects experienced over 9 severe asthma exacerbations per year.

Table 1
General characteristics of the subjects of the severe and mild to moderate asthma study groups from the European and the Brazilian samples.

Severity	Severe Asthma			Mild-Moderate Asthma		
Cohort	European U-BIOPRED (n = 421)	Brazilian ProAR (n = 544)	P-value	European U-BIOPRED (n = 88)	Brazilian ProAR (n = 452)	P-value
Age (years) mean (SE) [N]	51.9 (0.7) [421]	51.9 (0.6) [544]	0.4099	41.7 (1.7), [88]	36.8 (0.6), [452]	0.0109
Age of onset (years)* median (IQR) [N]	26 (9–42) [411]	10 (2–25) [543]	p < 0.001	14 (6–32), [83]	7 (1–18), [439]	<0.001
Females n/N (%)	261/421 (62.0)	444/544 (81.6)	p < 0.001	44/88 (50%)	350/452 (77.4%)	<0.001
BMI (Kg/m^2) mean (SE) [N]	29.2 (0.3) [421]	29.0 (0.2) [544]	0.9137	25.7 (0.5), [88]	27.1 (0.3), [451]	0.097
Smoking ever n/N (%)	157/421 (37.3)	150/544 (27.6)	0.0017	13/88 (14.8%)	83/452 (18.4%)	0.5134
Current smoking n/N (%)	42/421 (10.0)	5/544 (0.9)	p < 0.001	0/88	16/452 (3.5%)	0.1476
Number of pack/years median (IQR) [N]	12.5 (4–21) [157]	5 (2–18) [149]	p < 0.001	4 (1–4) [13]	1.2 (1–8) [81]	1

SE – standard error of the mean; N or n – number of observations; IQR – interquartile range; BMI – body mass index. *In U-BIOPRED the age of onset considered was the age of the initial diagnosis. In ProAR the age of onset was considered the age the symptoms of asthma had started.

Table 2
Spirometric measurements of subjects of the severe and mild to moderate asthma cohorts from the European and the Brazilian samples.

Severity	Severe Asthma			Mild-Moderate Asthma		
Cohort	European U-BIOPRED (n = 421)	Brazilian ProAR (n = 544)	P-value	European U-BIOPRED (n = 88)	Brazilian ProAR (n = 452)	P-value
FEV_1% predicted Pre-BD, mean (SE) [N]	66.2 (1.0) [417]	63.5 (0.8) [537]	0.075	89.01 (1.91) [85]	80.95 (0.71) [447]	p < 0.001
FVC % predicted Pre-BD, mean (SE) [N]	87.3 (0.9) [417]	78.8 (0.7) [537]	<0.001	104.63 (2.07) [85]	84.3 (0.63) [447]	p < 0.001
FEV_1/FVC % Pre-BD mean (SE) [N]	63.0 (1.0) [415]	65.0 (0.0) [537]	0.001	72.0 (1.0) [85]	80.0 (0.0) [447]	p < 0.001
FEV_1/FVC % Post-BD mean (SE) [N]	66.0 (1.0) [417]	68.0 (0.0) [535]	0.052	77.0 (0.01) [85]	84.0 (0) [445]	p < 0.001
FEV_1 Increase ≥ 200 ml and ≥12% after BD. n/N (%)	209/415 (50.4)	162/535 (30.3)	<0.001	33/85 (38.82)	101/445 (22.7)	0.0027
FEV_1 Post-BD/FVC <70%, FEV_1% predicted <80%. n/N (%)	190/417 (45.6)	267/535 (49.9)	0.206	4/85 (4.71)	27/445 (6.07)	0.8119

SE – standard error of the mean; N or n – number of observations.

Table 3
Common comorbidities, biomarkers of atopy and blood cell counts of individuals with asthma from the European and Brazilian samples.

Severity	Severe Asthma			Mild-Moderate Asthma		
Cohort	European U-BIOPRED (n = 421)	Brazilian ProAR (n = 544)	P-value	European U-BIOPRED (n = 88)	Brazilian ProAR (n = 452)	P-value
Allergic Rhinitis, n/N (%)	208/378 (55.0)	299/491 (60.9)	0.095	42/70 (60)	264/413 (63.9)	0.6201
Nonallergic Rhinitis, n/N (%)	59/385 (15.32)	168/491 (34.22)	<0.001	8/72 (11.11)	111/414 (26.81)	0.0067
SPT positive to any aeroallergen, n/N (%)	273/419 (65.2)	313/491 (63.8)	0.659	NA	287/414 (69.32)	NA
GORD diagnosis[a], n/N (%)	161/421 (38.2)	264/544 (48.5)	0.002	5/88 (5.68)	36/452 (7.96)	0.6033
Total IgE (IU/mL), mean (SE) [N]	321.57 (31.5) [406]	533.38 (29.8) [533]	0.001	289.8 (64.13) [85]	444.89 (29.05) [447]	p < 0.001
Blood neutrophils %, mean (SE) [N]	63.1 (0.6) [408]	55.1 (0.5) [540]	<0.001	57.54 (0.86) [88]	54.54 (0.54) [449]	0.0131
Blood eosinophils %, mean (SE) [N]	3.9 (0.2) [408]	5.0 (0.2) [540]	<0.001	3.84 (0.3) [88]	4.65 (0.16) [449]	0.0172
Blood eosinophils N. Mean (SE) [N]	322.87 (21.35) [406]	323.55 (12.44) [540]	0.070	233.48 (18.07) [88]	297.15 (11.36) [449]	0.0169
Proportion with eosinophils > 300/μl, n/N (%)	157/406 (38.67)	206/540 (38.15)	0.9237	23/88 (26.14)	179/449 (39.87)	0.0208

[a] GORD (Gastroesophageal reflux diseases) diagnosed by a physician and/or current prescription of a proton pump inhibitor.

3.4. Patient reported outcomes

Patient reported outcomes (PRO's) are presented in Suplementary Online Table 2. Overall there were worse PROs among the Brazilians for both SA and MMA cohorts. There were some similarities in scores in SA but not in MMA. The Asthma Control Questionnaire (ACQ) with 5 or 6 questions indicated that the status of control of asthma was significantly worse in the SA and the MMA groups of U-BIOPRED compared to ProAR with clinically meaningful differences of >0.5 for the SA comparison. As shown in Fig. 2, there was a higher proportion of moderate to severe depression in both ProAR cohorts than in their U-BIOPRED comparators. The frequency distribution of sleepiness of both study groups, according to the Epworth Sleepiness Scale (ESS), is presented in (Supplementary online Fig. 3). Subjects from the Brazilian group more often had moderate and severe sleepiness than those from U-BIOPRED in both SA and MMA cohorts.

Table 3 depicts common comorbidities, biomarkers of atopy and blood cell counts of individuals with asthma from U-BIOPRED and ProAR. Allergic rhinitis was equally frequent across U-BIOPRED and ProAR, in SA and MMA, affecting the majority of subjects in all study groups. Positive skin prick tests (SPT) to any aeroallergen were remarkably similar in SA across the continents, affecting some two thirds of subjects (65.2% in U-BIOPRED and 63.8% in ProAR). For MMA, the proportion of SPT-positive (69.3%) in ProAR was comparable to those of the SA groups. This was not conducted for U-BIOPRED in the MMA group. Symptoms of GORD were more common among the SA in U-BIOPRED, but a diagnosis of gastroesophageal reflux disease was more frequent in ProAR; GORD diagnosis were lower in the MMA compared to the SA in both cohorts. Patients in Brazil had significantly higher T2-inflammation biomarkers, (total serum IgE, absolute numbers and % of blood eosinophils) whether SA or MMA and the values for SA trend to be higher than for MMA. Absolute numbers and proportion of neutrophils were different between the SA cohorts, being higher in U-BIOPRED subjects, but for the MMA only the proportion was higher in Europe. The proportion of individuals with eosinophils above 3% differed between the SA cohorts, being somewhat higher in Brazil, but not in the MMA cohorts. Applying the threshold for blood eosinophil counts of 300 cells/ul, ProAR MMA had a higher proportion of subjects above the cut-off than the U-BIOPRED MMA; however, the proportions in the SA cohorts were almost equal.

A markedly different pattern was observed in the use of medication, as presented in Supplementary online Table 1. Whereas many of subjects

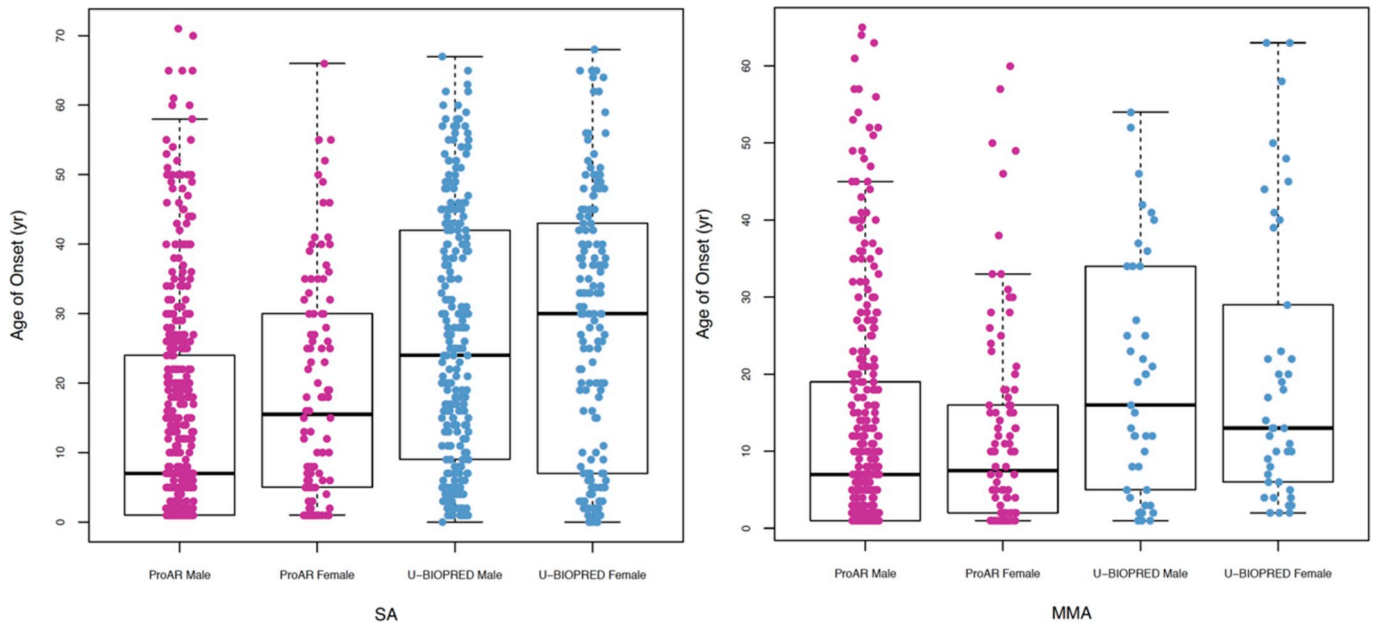

Fig. 1. Jitter plots showing a comparison of the distribution of the age of onset of asthma in the subjects of a Brazilian (ProAR) and a European (U-BIOPRED) severe asthma cohort (SA) and their respective mild to moderate asthma control cohorts (MMA). In U-BIOPRED the age of onset considered was the age of the initial diagnosis, whereas In ProAR, the age of onset was the age the symptoms of asthma had started.

Table 4
History of severe exacerbations, hospitalizations in the last 12 months, intensive Care Unit admission due to asthma ever and regular use of oral corticosteroids, of individuals of the European and Brazilian samples of severe asthma.

Severity	Severe Asthma		
Cohort	European U-BIOPRED (n = 421)	Brazilian ProAR (n = 544)	P-value
Total number of severe asthma exacerbations[a] in the last 12 months [n]	1050	1106	NA
Number of severe exacerbations per patient/year [Median (IQR)]	2(1_3) [420]	1(0_3) [544]	<0.001
Proportion of subjects with ≥2 severe exacerbation in the last 12 months	264/421 (62.71)	227/544 (41.73)	<0.001
Proportion of subjects with no severe exacerbation in the last 12 months	79/421 (18.76)	212/544 (38.97)	<0.001
Proportion of subjects hospitalized due to asthma in the last 12 months	69/337 (20.47)	27/544 (4.96)	<0.001
Proportion of subjects with a history of ICU admission due to asthma ever	98/416 (23.56)	94/544 (17.28)	0.0199

[a] Severe exacerbations defined as exacerbations requiring a course of systemic corticosteroids ≥ 3 days and/or emergency room visit and/or hospital admission.

in U-BIOPRED SA used anti-muscarinics, either short or long acting, patients from ProAR were treated mainly with a combination of long acting beta 2-agonist and inhaled corticosteroid with short acting beta 2 agonists as needed for asthma control. Furthermore, 45.5% of the U-BIOPRED SA cohort used oral corticosteroid regularly, but none of the patients from ProAR cohort did at the time of the evaluation. Even more substantial treatment differences were observed in the MMA patients where treatment with inhaled corticosteroids was used by the majority of U-BIOPRED (99%) but less than 1% in ProAR and short acting beta-agonists were used by 77% in U-BIOPRED compared to 7.3% in ProAR. Fig. 3 presents the frequency distribution of asthma control according the ACQ-5 scores and indicates a higher proportion of patients from U-BIOPRED SA were uncontrolled despite using oral corticosteroids. Again, for the MMA, a higher proportion of U-BIOPRED patients were not well-controlled, despite using more medication.

4. Discussion

The goal of this analysis was to investigate the degrees of similarity and diversity in clinical phenotypes in two asthma populations of very different ethnic and socio-environmental characteristics, one Brazilian and the other European.

Most subjects in the ProAR cohorts are Afro-Brazilians, whereas the U-BIOPRED cohorts are largely composed of Caucasians. The climate in Salvador da Bahia, Brazil, is tropical, warm and humid all year around, as opposed to the temperate climatic zone of Europe. The socioeconomic conditions are also very different. The sample of Brazilians comprise exclusively users of the public health system, which belong to the lower socioeconomic strata. These subjects live in overcrowded poor neighbourhoods in a large urban centre of 3 million inhabitants. The treatment history also differs between the two continents.

In spite of all these differences and of disparities in medical care, as well as in the definition of severe asthma applied to these cohorts, remarkable similarities were reported herein in major clinical characteristics of the SA cohorts, namely age, BMI, $FEV_{1\ pre\text{-}BD}$, proportion of atopy, scores of nasal symptoms, quality of life and blood eosinophil counts. In the SA cohorts, these observations indicate there is truly a disease named asthma, which expresses itself in a certain syndromic pattern, no matter how diverse the combination of genetics and environment might be. This empirical evidence argues against the recent proposition of classifying asthma into treatable traits for better management [4]. We do not propose that asthma is a homogeneous disease. We recognize it as being heterogeneous and comprising of different phenotypes but there are sufficient characteristics that are common between phenotypes and populations to support its status as a disease entity.

Differences in age of onset between the Europeans and Brazilian may be explained, at least in part, by the varied criteria used to define asthma onset. In ProAR, it was the onset of symptoms while in U-BIOPRED it was the time the diagnosis was made. Among patients with SA in both continents, we found a trend among females for a later onset of severe asthma than males, that was not observed in the MMA cohorts.

The proportion of female participants was greater in both ProAR Cohorts, which may be due to the women attributing more time for their health concerns in Brazil. The use of daily oral corticosteroid therapy

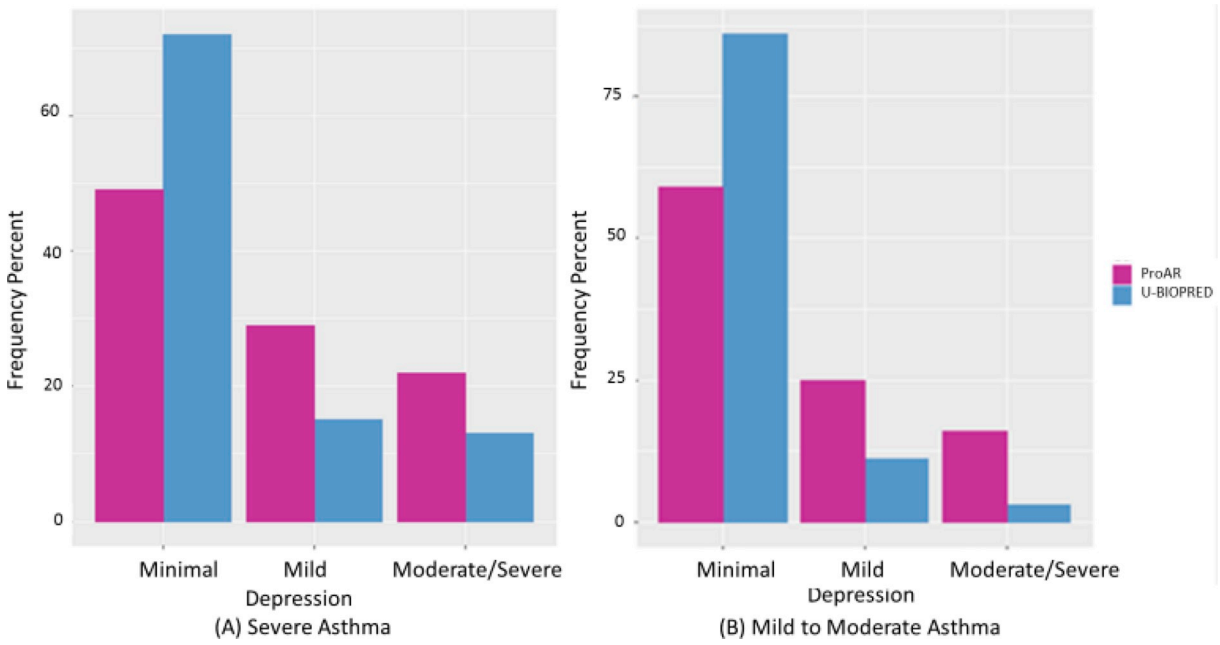

Fig. 2. Frequency distribution of minimal, mild and moderate to severe depression, according to the Hospital Anxiety and Depression Scale [19] among subjects with asthma of the European samples, and according to the Beck inventory scores[29] of those with asthma from the Brazilian samples.

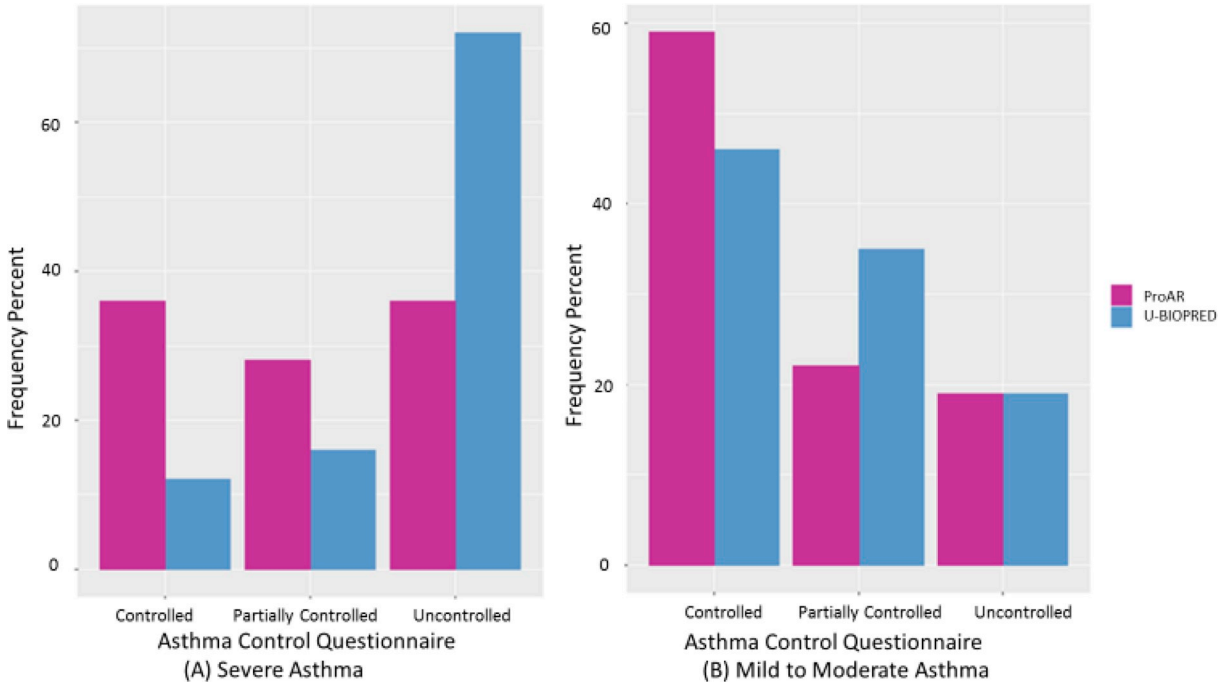

Fig. 3. Frequency distribution of asthma control according the Asthma Control Questionnaire (ACQ-5) scores among subjects of the severe asthmatics and mild to moderate asthmatics, European samples, and of those from the Brazilian samples.

was frequent in U-BIOPRED SA, but virtually non-existent in ProAR SA at the time of this evaluation. Doctors and patients in ProAR are constantly trying to avoid prolonged regular use of oral corticosteroids, even when the disease is not fully controlled. In comparison with the U-BIOPRED severe asthma cohort, the Brazilians had a less severe disease, which explains in part the lower usage of oral corticosteroids. Anyway, the observations reported herein warrant a revision of their management strategies to include oral corticosteroids and immunobiologics in those at risk of frequent exacerbations. Despite taking adequate treatment, U-BIOPRED participants still had more exacerbations and higher ACQ scores suggesting that overall the U-BIOPRED cohort had progressive, more active and severe disease. Lung function parameters (FEV_1 and FVC_{preBD}) were lower in ProAR SA and MMA cohorts, as compared to U-BIOPRED, but FEV_1/FVC was lower in U-BIOPRED. The lower values among the Brazilians may be due to a technical artefact, in that the reference equations used in Brazil assume a similar ethnic mix of participants across the country, whereas in Salvador da Bahia, the proportion of Afro-descendance is much higher than the rest of Brazil. It is well established Afro-descendants have lower lung volumes as compared to Caucasians of the same gender, age and height [16].

Indeed, we have confirmed this observation in a healthy non-asthmatic control group (N = 452) from the same community. Furthermore, a novel equation for lung function prediction of Afro-Brazilians confirms the values are lower than a previous equation derived from a sample of white Brazilians [17]. The lower FEV_1/FVC among the Europeans likely reflects more severe airflow limitation, which is congruent with other observations from the U-BIOPRED groups.

Among the Brazilians, 64% of those with SA and 36% with MMA have been exposed to household pollution of wood burning for cooking, which is a problem of larger dimension than tobacco smoking in this sample and has just been recently reported [18]. Within the SA patients, 11% U-BIOPRED and 24% ProAR show signs of moderate to severe depression compared to 0% U-BIOPRED and 16% ProAR MMA samples. We have no data to inform an interpretation of these discordant observations, which could be explained in part by the different instruments used for assessment of depression. We speculate this may also be related to poverty among the Brazilians, as 9% of our control group with no asthma had moderate to severe depression.

Among the T2 biomarkers: total IgE levels were higher in both ProAR cohorts, but eosinophil blood counts were similar between the cohorts despite the occurrence of helminths in Brazil, particularly among underserved communities, which have been reported to raise blood eosinophil numbers [19]. In the ProAR cohort only about 3% of participants had helminths detected in their stools. In the U-BIOPRED cohorts raised eosinophils persist despite the use of OCS and/or ICS, which are reported to reduce blood eosinophils and increase neutrophils [20,21]. The neutrophil numbers are higher in U-BIOPRED SA, perhaps due to the number of participants taking oral steroids daily. The blood cell counts may also differ between the cohorts due to the ethnic diversity. White blood counts are reportedly lower in Afro-descendants [22]. Medication use for ProAR control cohort of MMA was minimal, with few patients on any treatment. These individuals were not recruited from asthma clinics, but directly from the community, many of which did not know their symptoms were caused by asthma. The majority of subjects with MMA from Brazil had intermittent symptoms. As for the use of short acting beta 2 agonist bronchodilators, much lower in ProAR Cohorts, the reason for the difference may be that it was noted only for individuals taking these medications regularly. In Salvador – Brazil, there is no re-imbursement for long-acting anti-muscarinic compounds, so no participants took them, unlike in U-BIOPRED. In SA, ICS/LABA use is similar between the cohorts due to re-imbursement in Brazil.

This study has the advantage that these two large severe asthma populations have been well characterised using standard clinical definitions and instruments. As there are differences in the ethnicities and the environment which cannot be controlled, every effort was made to ensure the data was collected and reported as harmonized as possible. By studying the characteristics of patients of all levels of the severity spectrum from two diverse settings, we hope to have contributed to the understanding of the boundaries of heterogeneity of the disease and to inform decisions on innovative approaches to improve early diagnosis and phenotyping, control symptoms and lung function, and reduce asthma exacerbations, the major cause of asthma morbidity and mortality. The strenght of the study is the comparison of two different asthma populations with findings of many similarities, despite different ethnic and socioeconomic characteristics.

Population based studies of severe asthma according to current criteria are extremely difficult as the definition requires a series of assessments, step up of treatment and reassessments, including investigation of co-morbidities, adherence, proper inhaler technique and environmental exposure. Nevertheless, a retrospective study using a Dutch pharmacy database, estimated 3.6% of asthmatic adults qualified for a diagnosis of severe refractory asthma, representing 10.4 patients per 10,000 inhabitants [23]. No such studies have been reported in Brazil to date. There are multiple reports of reduced rates of hospital admissions due to asthma throughout the world, which suggests better control. However, as the estimates of prevalence are scarce, it is even more inaccurate to estimate trends on prevalence of severe asthma. A decline in hospital admissions can be a result of earlier diagnosis, better treatment and control, but not necessarily a reduced proportion of severe asthma.

We acknowledge the limitations of our analysis. The criteria for the definition of a case with severe asthma were very different. While in Brazil the criterion was that proposed by NIH [12] some 20 year ago, which was current when the cohort was established, the European criterion, is much strict and aligned to more recent definitions proposed by the European Respiratory Society and American Thoracic Society. There is language and interpretation variability across observations. The health system, access to care and medication is very diverse and normality ranges may vary for blood counts and lung function, two of the most objective measures we had, in relation to ethnicity. In the samples of ProAR <10% of subjects are white, as opposed to the white majority in the European study. Poverty and low schooling are also factors affecting disproportionally the Brazilians. All of these discrepancies are likely to influence the differences observed between the ProAR and U-BIOPRED cohorts. Some other inequalities might be relevant. For example, exposure to household air pollution, warm humid weather, poor hygienic conditions and parasites in Brazil, and the frequent use of OCS in U-BIOPRED. The high proportion of reported previous exposure to household air pollution in the ProAR SA cohort, not seen in U-BIOPRED, pinpoints a specific risk factor for severe asthma in Brazil. Except for urban traffic, air pollution is not a major problem in Salvador, which is located in a peninsula (surrounded by the sea) and has no industry. Exposure to certain helminth infections and poor hygienic conditions have been associated with modulation of allergies, but not so clearly with a reduction in asthma frequency or severity [24]. On the contrary, we have demonstrated lack of hygiene and infections are associated with risk of nonatopic wheezing in children of underserved areas of Salvador [25]. Moreover, among the patients of this study, the frequency of a positive stool examination to helminths was <3%. Therefore, one cannot expect a significant effect of helminths in attenuating asthma in the Brazilian sample. The same would apply to blood eosinophil counts. A preliminary analysis of the no asthma control group indicates the median blood eosinophil count is 152 cells/μl, confirming current helminth infections is likely not relevant. There are also some differences in management of these cohorts due to variations in clinical practices worldwide, and some of the definitions used. To a large extent this was minimised by sharing standard operating procedures across the cohorts. Both of the cohorts were well characterised at the clinical level, with U-BIOPRED being further characterised at the molecular level [26].

Taking into consideration the obvious environmental, social and individual heterogeneity across the Brazilian and European asthma cohorts compared in this report, in addition to the differences in the severe asthma definition, we find the similarities described on major clinical characteristics, lung function and biomarkers, particularly in the SA groups, are remarkable and indicate no matter what are the underlying gene-environment processes, the disease phenotypes are similar.

5. Conclusion

In summary, two cohorts of asthma from different continents, of varied severity, ethnicities and socioeconomic status, present remarkable demographic, clinical, spirometric and phenotypic similarities, providing the basis for global external validation of severe and mild to moderate asthma phenotypes by scientific collaboration. It may also stimulate the development of worldwide consortia to work together to refine the comparisons to the molecular level aiming to better understand treatment resistant severe asthma and search for more effective treatment strategies.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.rmed.2019.105817.

References

[1] Global Asthma Network, Global asthma report, Available at: http://globalasthmareport.org/, 2018. (Accessed 28 November 2018).
[2] A.A. Cruz, R. Stelmach, E.V. Ponte, Asthma prevalence and severity in low-resource communities, Curr. Opin. Allergy Clin. Immunol. 17 (3) (2017 Jun) 188–193, https://doi.org/10.1097/ACI.0000000000000360.
[3] C. Souza-Machado, A. Souza-Machado, R. Franco, E.V. Ponte, M.L. Barreto, L. C. Rodrigues, J. Bousquet, A.A. Cruz, Rapid reduction in hospitalisations after an intervention to manage severe asthma, Eur. Respir. J. 35 (3) (2010 Mar) 515–521, https://doi.org/10.1183/09031936.00101009.
[4] I.D. Pavord, R. Beasley, A. Agusti, G.P. Anderson, E. Bel, G. Brusselle, P. Cullinan, A. Custovic, F.M. Ducharme, J.V. Fahy, U. Frey, P. Gibson, L.G. Heaney, P.G. Holt, M. Humbert, C.M. Lloyd, G. Marks, F.D. Martinez, P.D. Sly, E. von Mutius, S. Wenzel, H.J. Zar, A. Bush, After asthma: redefining airways diseases, Lancet 391 (10118) (2018 Jan 27) 350–400, https://doi.org/10.1016/S0140-6736(17)30879-6.
[5] Global Initiative for Asthma (GINA), Global strategy for asthma management and prevention, Available at: http://www.ginasthma.org/, 2018. (Accessed 25 November 2018).
[6] D.E. Shaw, A.R. Sousa, S.J. Fowler, L.J. Fleming, G. Roberts, J. Corfield, I. Pandis, A.T. Bansal, E.H. Bel, C. Auffray, C.H. Compton, H. Bisgaard, E. Bucchioni, M. Caruso, P. Chanez, B. Dahlén, S.E. Dahlen, K. Dyson, U. Frey, T. Geiser, M. Gerhardsson de Verdier, D. Gibeon, Y.K. Guo, S. Hashimoto, G. Hedlin, E. Jeyasingham, P.P. Hekking, T. Higenbottam, I. Horváth, A.J. Knox, N. Krug, V. J. Erpenbeck, L.X. Larsson, N. Lazarinis, J.G. Matthews, R. Middelveld, P. Montuschi, J. Musial, D. Myles, L. Pahus, T. Sandström, W. Seibold, F. Singer, K. Strandberg, J. Vestbo, N. Vissing, C. von Garnier, I.M. Adcock, S. Wagers, A. Rowe, P. Howarth, A.H. Wagener, R. Djukanovic, P.J. Sterk, K.F. Chung, U-BIOPRED Study Group, Clinical and inflammatory characteristics of the European U-BIOPRED adult severe asthma cohort, Eur. Respir. J. 46 (5) (2015 Nov) 1308–1321, https://doi.org/10.1183/13993003.00779-2015.
[7] B. Abraham, J.M. Antó, E. Barreiro, E.H.D. Bel, G. Bonsignore, J. Bousquet, J. Castellsague, P. Chanez, F. Cibella, G. Cuttitta, B. Dahlén, S.-E. Dahlén, N. Drews, R. Djukanovic, L.M. Fabbri, G. Folkerts, M. Gaga, C. Gratziou, G. Guerrera, S. T. Holgate, P.H. Howarth, S.L. Johnston, F. Kanniess, J.C. Kips, H.A.M. Kerstjens, M. Kumlin, H. Magnussen, F.P. Nijkamp, N. Papageorgiou, A. Papi, D.S. Postma, R. A. Pauwels, K.F. Rabe, K. Richter, A.C. Roldaan, M. Romagnoli, A. Roquet, C. Sanjuas, N.M. Siafakas, W. Timens, N. Tzanakis, I. Vachier, A.M. Vignola, L. Watson, G. Yourgioti, European Network for understanding mechanisms of severe asthma, Eur. Respir. J. 22 (3) (2003 Sep) 470–477. The ENFUMOSA cross-sectional European multicentre study of the clinical phenotype of chronic severe asthma, PubMed PMID: 14516137.
[8] E. Delgado-Eckert, O. Fuchs, N. Kumar, J. Pekkanen, J.C. Dalphin, J. Riedler, R. Lauener, M. Kabesch, M. Kupczyk, S.E. Dahlen, E.V. Mutius, U. Frey, PASTURE and BIOAIR Study groups. Functional phenotypes determined by fluctuation-based clustering of lung function measurements in healthy and asthmatic cohort participants, Thorax 73 (107–115) (2018) 2.
[9] W.C. Moore, D.A. Meyers, S.E. Wenzel, W.G. Teague, H. Li, X. Li, R. D'Agostino Jr., M. Castro, D. Curran-Everett, A.M. Fitzpatrick, B. Gaston, N.N. Jarjour, R. Sorkness, W.J. Calhoun, K.F. Chung, S.A. Comhair, R.A. Dweik, E. Israel, S.P. Peters, W. W. Busse, S.C. Erzurum, E.R. Bleecker, Identification of asthma phenotypes using cluster analysis in the Severe Asthma Research Program, Am. J. Respir. Crit. Care Med. 181 (4) (2010 Feb 15) 315–323, https://doi.org/10.1164/rccm.200906-0896OC. National Heart, Lung, and Blood Institute's Severe Asthma Research Program.
[10] T.S. Hinks, T. Brown, L.C. Lau, H. Rupani, C. Barber, S. Elliott, J.A. Ward, J. Ono, S. Ohta, K. Izuhara, R. Djukanović, R.J. Kurukulaaratchy, A. Chauhan, P. H. Howarth, Multidimensional endotyping in patients with severe asthma reveals inflammatory heterogeneity in matrix metalloproteinases and chitinase 3-like protein 1, J. Allergy Clin. Immunol. 138 (1) (2016 Jul) 61–75, https://doi.org/10.1016/j.jaci.2015.11.020.
[11] T.B. Kim, C.S. Park, Y.J. Bae, Y.S. Cho, H.B. Moon, COREA Study Group, Factors associated with severity and exacerbation of asthma: a baseline analysis of the cohort for reality and evolution of adult asthma in Korea (COREA), Ann. Allergy Asthma Immunol. 103 (4) (2009 Oct) 311–317, https://doi.org/10.1016/S1081-1206(10)60530-3.
[12] NIH Publication No. 97-4051, Expert Panel Report 2: Guidelines for the Diagnosis and Management of Asthma (EPR-2 1997), U.S. Department of Health and Human Services; National Institutes of Health; National Heart, Lung, and Blood Institute; National Asthma Education and Prevention Program, Bethesda, MD, 1997.
[13] J. Bousquet, E. Mantzouranis, A.A. Cruz, N. Aït-Khaled, C.E. Baena-Cagnani, E. R. Bleecker, C.E. Brightling, P. Burney, A. Bush, W.W. Busse, T.B. Casale, M. Chan-Yeung, R. Chen, B. Chowdhury, K.F. Chung, R. Dahl, J.M. Drazen, L.M. Fabbri, S. T. Holgate, F. Kauffmann, T. Haahtela, N. Khaltaev, J.P. Kiley, M.R. Masjedi, Y. Mohammad, P. O'Byrne, M.R. Partridge, K.F. Rabe, A. Togias, C. van Weel, S. Wenzel, N. Zhong, T. Zuberbier, Uniform definition of asthma severity, control, and exacerbations: document presented for the World Health Organization Consultation on Severe Asthma, J. Allergy Clin. Immunol. 126 (5) (2010 Nov) 926–938, https://doi.org/10.1016/j.jaci.2010.07.019. EPR-2.
[14] A. Lima-Matos, E.V. Ponte, J.P.V. de Jesus, P.C.A. Almeida, V.B. Lima, N. Kwon, J. Riley, L.M. Mello, A.A. Cruz, Eosinophilic asthma, according to a blood eosinophil criterion, is associated with disease severity and lack of control among underprivileged urban Brazilians, Respir. Med. 145 (2018) 95–100.
[16] P.H. Quanjer, S. Stanojevic, T.J. Cole, X. Baur, G.L. Hall, B.H. Culver, P.L. Enright, J.L. Hankinson, M.S. Ip, J. Zheng, J. Stocks, ERS Global Lung Function Initiative, Multi-ethnic reference values for spirometry for the 3-95-yr age range: the global lung function 2012 equations, Eur. Respir. J. 40 (6) (2012 Dec) 1324–1343, https://doi.org/10.1183/09031936.00080312.
[17] T.A. Prata, E. Mancuzo, C.A.C. Pereira, S.S. Miranda, L.V. Sadigursky, C. Hirotsu, S. Tufik, Spirometry reference values for Black adults in Brazil, J. Bras. Pneumol. 44 (6) (2018 Nov-Dec) 449–455.
[18] A.G. Fernandes, C. Souza-Machado, G.P. Pinheiro, S.T. Oliva, R.C.L. Mota, V. B. Lima, C.S. Cruz, J.M. Chatkin, A.A. Cruz, Dual exposure to smoking and household air pollution is associated with na increased risk of severe asthma in adults in Brazil, Clin. Transl. Allergy 8 (2018 Dec 10) 48, https://doi.org/10.1186/s13601-018-0235-6.
[19] H.J.F. Salzer, T. Rolling, C.D. Vinnemeier, et al., Helminthic infections in returning travelers and migrants with eosinophilia: diagnostic value of medical history, eosinophil count and IgE, Trav. Med. Infect. Dis. 20 (2017) 49–55.
[20] V.M. Keatings, A. Jatakanon, Y.M. Worsdell, P.J. Barnes, Effects of inhaled and oral glucocorticoids on inflammatory indices in asthma and COPD, Am. J. Respir. Crit. Care Med. 155 (2) (1997 Feb) 542–548. PubMed PMID: 9032192.
[21] S.E. Wenzel, S.J. Szefler, D.Y. Leung, S.I. Sloan, M.D. Rex, R.J. Martin, Bronchoscopic evaluation of severe asthma. Persistent inflammation associated with high dose glucocorticoids, Am. J. Respir. Crit. Care Med. 156 (1997), 737±43.
[22] B.J. Bain, Ethnic and sex differences in the total and differential white cell count and platelet count, J. Clin. Pathol. 49 (8) (1996 Aug) 664–666.
[23] P.P. Hekking, R.R. Wener, M. Amelink, A.H. Zwinderman, M.L. Bouvy, E.H. Bel, The prevalence of severe refractory asthma, J. Allergy Clin. Immunol. 135 (4) (2015 Apr) 896–902.
[24] A.A. Cruz, P.J. Cooper, C.A. Figueiredo, N.M. Alcantara-Neves, L.C. Rodrigues, M. L. Barreto, Global issues in allergy and immunology: parasitic infections and allergy, J. Allergy Clin. Immunol. 140 (5) (2017 Nov) 1217–1228.
[25] N.M. Alcantara-Neves, R.V. Veiga, V.C. Dattoli, R.L. Fiaccone, R. Esquivel, A. A. Cruz, P.J. Cooper, L.C. Rodrigues, M.L. Barreto, The effect of single and multiple infections on atopy and wheezing in children, J. Allergy Clin. Immunol. 129 (2) (2012 Feb) 359–367, 367.e1-3.
[26] J. Bigler, M. Boedigheimer, J.P.R. Schofield, P.J. Skipp, J. Corfield, A. Rowe, et al., A severe asthma disease signature from gene expression profiling of peripheral blood from U-BIOPRED cohorts, Am. J. Respir. Crit. Care Med. 195 (10) (2017 May 15) 1311–1320, https://doi.org/10.1164/rccm.201604-0866OC.