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Clinical characteristics and possible phenotypes of an adult severe asthma population

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Summary
Background: Currently, there are no studies of well-characterized severe asthmatics in Brazil. We aimed to study a population of severe treated asthmatics still uncontrolled to characterize them and define possible phenotypes.
Methods: Descriptive cross-sectional outpatient study of severe asthmatics, evaluating functional and inflammatory markers, health-related quality of life, anxiety and depression symptoms, clinical control status, and characteristics related to atopy, age of asthma onset, induced sputum eosinophil levels, and airflow limitation. We also grouped the subgroups characteristics to identify phenotypes. The study is registered on ClinicalTrial.gov NCT 01089322.
Results: From 128 eligible patients with severe/uncontrolled asthma, 74 fulfilled the inclusion criteria. The cohort was comprised of 85% women, frequently with a body mass index higher than 31 kg m^{-2}, atopy (60%), early-onset disease (50%), sputum eosinophilia (80%), comorbidities, and reduced quality of life. Nonatopics had significant higher asthma onset (19 y.a.) and twice level of induced sputum eosinophil. Late-onset patients had significantly less atopy (57%) and higher levels of induced sputum eosinophils. Non-eosinophilics had lower levels of inflammatory markers. Patients with airflow limitation had more intensive care unit admissions (56%) and 1.5 times more airway resistance. Subgroups characteristics identified *a priori* four well-characterized phenotypes, with 55% presenting sputum eosinophilia.

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Conclusion: Our data emphasize the high burden of disease, the persistence of inflammation and the existence of clinical possible phenotypes population sharing common features with published cohorts. Despite the necessity of further investigation into pathogenic mechanisms, this study with clinically difficult patient group may help to improve future asthma care.

Background

Many clinical studies have demonstrated the efficacy of adequate asthma treatment in reducing symptoms and exacerbations, improving health-related quality of life (HRQoL) and lung function, achieving asthma control,[1] controlling airway inflammation,[2] and reducing mortality rates.[3] However, 5%—10% of asthmatics do not achieve clinical control despite having an accurate diagnosis, receiving proper medication, and adhering to treatment plans. Within this group, severe asthmatics clearly experience the greatest HRQoL impairment[4] and represent a significant proportion of health care costs.[5]

Brazil has the sixth highest asthma prevalence in the world,[6] but the prevalence of severe asthma in this population is unknown. The lack of national, large-scale asthma management programs and limited access to medication for many years are probably related to this information gap.

In 2005, the Brazilian government implemented a nationwide asthma program that provides free access to medication for patients with doctor-diagnosed severe asthma. This program greatly impacted asthma control and treatment adherence,[7] improved HRQoL,[8] decreased health care utilization (HCU),[9] reduced morbidity,[8] and lowered overall health care system and family costs.[8,10,11]

Over the last decade, several international cohort studies[4,5,12] have demonstrated severe asthmatics' characteristics and shown that a group of factors, including atopy, age of disease onset, duration of disease, and comorbidities,[13] are possible determinants of the different phenotypes observed in persistent severe asthma patients. The current research thus aims to improve phenotype characterization[12] to increase our understanding of disease pathogenesis and help to tailor effective therapies.

Brazilians have varied genetic and environmental backgrounds, and asthma proper regular treatment has historically been different from treatment in the so far published American and European cohorts. Because of the current lack of studies characterizing the clinical features, risk factors, and phenotype distribution of severe asthma in Brazil, we present herein the clinical, functional and inflammatory characteristics of a severe asthmatic population and describe comorbidities and aggravating factors. We also attempt to identify clinical subgroups and possible phenotypes that could be shared with similar populations around the world.

Methods

The local institutional review board approved this study, and all patients provided written informed consent. The study is registered on ClinicalTrial.gov as NCT 01089322. This is a cross-sectional descriptive study. The participants were recruited from 2500 asthma patients from several regions of São Paulo, who registered at the outpatient asthma clinic of a university hospital. This population was 75% female, 75% Caucasian and 15% of African descent. The average monthly family income of 55% of the patients was less than US$380. At our asthma center, patients diagnosed with asthma have free access to anti-inflammatory therapies and educational programs.

Inclusion criteria

Based on previous studies,[4,5] the inclusion criteria for severe asthma patients were (a) 18—65 years old; (b) diagnosed with asthma by a specialist and treated for more than 1 year, according to Global Initiative for Asthma (GINA) guidelines[14]; (c) airway reversibility documented within 5 years before the start of the study using at least one of the following criteria: an FEV_1 increase $\geq$ 12% of predicted (and a 200-ml increase) after 400 μg of salbutamol, an FEV_1 increase of at least 400 ml after prednisolone 0.5 mg/kg/day for 14 days or, in patients with FEV_1 $\geq$ 70% of predicted, a methacholine-positive hyper-responsiveness challenge with a cut-off value of PD20 $<$ 2490 μg; (d) treatment with $\geq$1000 μg/day of beclomethasone or equivalent; (e) daily use of a long-acting $beta_2$ agonist (LABA); (f) at least one asthma exacerbation in the last year that required an oral corticosteroid burst; and (g) smokers, non-smokers or ex-smokers of $\leq$30 pack-years. If the patient was a smoker, asthma symptoms must have been present before the onset smoking, and cigarette use could not exceed 10 per day.

Exclusion criteria

Patients were excluded if they had any respiratory tract infection within 4 weeks prior to inclusion in the study, had another active or chronic pulmonary disorder, were pregnant or had any clinically significant disease that could interfere with the results.

Clinical data and questionnaires

The Asthma Control Questionnaire (ACQ)[15] and Asthma Control Test (ACT)[16] were used to evaluate the level of asthma control. HRQoL was measured using St. George's Respiratory Questionnaire (SGRQ)[17] and the SF-36 Questionnaire, Medical Outcomes Study 36-Item Short Form Health Survey (SF-36).

Depression symptoms were evaluated according to the Beck Depression Inventory (BDI),[18] and anxiety symptoms

were evaluated using the State-Trait Anxiety Inventory (STAI).[19]

Patients were divided according to age of asthma onset (either early onset (<12 years old) or late onset (≥12 years old)) as described earlier.[13]

Pulmonary function

Spirometry was performed according to ATS/ERS guidelines.[20] European Coal and Steel Community (ECSC) prediction equations were used.[21] Lung volumes were measured with a body plethysmograph.[22] Reversibility was assessed 20 min after the inhalation of 400 μg of salbutamol. Persistent airflow obstruction was expressed as post-bronchodilator FEV_1 or $FEV_1/FVC < 75\%$ predicted. Carbon monoxide-diffusing capacity (D_{LCO}) was measured using a single-breath technique (Elite DX® — Medical Graphics Corporation, St. Paul, MN; USA), and exhaled carbon monoxide concentration (COex) was measured using a Micro CO Meter device (Micro Medical Ltd., Rochester, RU). The cut-off value of COex for non-smokers was <6 ppm.[23]

Atopy

Prick tests for common aeroallergens (mites, fungi, pollen, and dog and cat fur) were performed. The test was considered positive when at least one antigen-induced reaction was 3 mm in diameter greater than the negative control (saline solution) as measured 15 min after the puncture.[24] Patients with at least one positive prick test were categorized as atopic. Total serum IgE was determined using a fluorescence enzyme immunoassay.

Inflammatory markers

Sputum induction (IS) was performed as previously described.[25] Eosinophil-positive (EOS+) patients were defined as those with induced sputum eosinophils ≥3%, and eosinophil-negative (EOS−) patients had eosinophil levels <3%.[26] Exhaled nitric oxide (FeNO) was collected using an offline system following published guidelines,[27] and its concentration was determined using chemiluminescence with a properly calibrated Siervers® analyzer (Model NOA 280, Siervers Instruments, Inc.).

Statistical analysis

Continuous variables are presented as means ± SD or medians (CI: 25%—75%), and categorical variables are presented as both a number and percentage. Mann—Whitney and *t* tests were used to compare continuous variables between groups: atopic vs. nonatopic, early vs. late onset, eosinophilic vs. non-eosinophilic, and persistent vs. non-persistent airflow limitation. Categorical variables were compared using chi-square (χ^2) or Fisher tests. Pearson's or Spearman's correlation tests were used to analyze variable correlations. $p < 0.05$ was considered statistically significant. All analyses were performed using the SPSS v. 16.0 statistical package for Windows (SPSS Inc., Chicago, IL, USA) and Sigma Stat v. 3.5 (Systat Inc., AL).

Results

One hundred twenty-eight eligible patients with severe asthma that was non-controlled despite proper treatment (approximately 5.1% of the outpatient asthma clinic database) were referred for the study. Of these, 74 (57.8%) patients fulfilled our study's inclusion criteria (Fig. 1).

The patients' demographic and clinical characteristics are shown in Table 1. There was a higher prevalence of women than men. Forty-one (55%) patients were obese (BMI ≥ 30 kg m^{-2}), and 33 (80%) were female. All of the patients had been using inhaled corticosteroids plus LABA for at least 4 years (CI 3.34—4.17). The COex values were low, meaning that none of the patients were current smokers. A skin prick test was performed for 69 patients and was positive for 44 (63.8%) patients: 30 (68.2%) reacted positively only to mites, 2 (4.5%) only to fungi and 12 (27.3%) to both mites and fungi. None of our patients was using antihistamines. IgE levels were 509.6 ± 539 UI/mL.

Sixty-seven (90.5%) patients had been hospitalized at least once for an asthma exacerbation, and 47 (63%) had reported ≥5 hospitalizations. Twenty-five (34%) had been hospitalized at least once in the year prior to our study, while 37 (50%) had required ICU (intensive care unit) admission due to an exacerbation and 28 (38%) reported an episode of intubation. Spirometry, body plethysmography, and FeNO data are shown in Table 2.

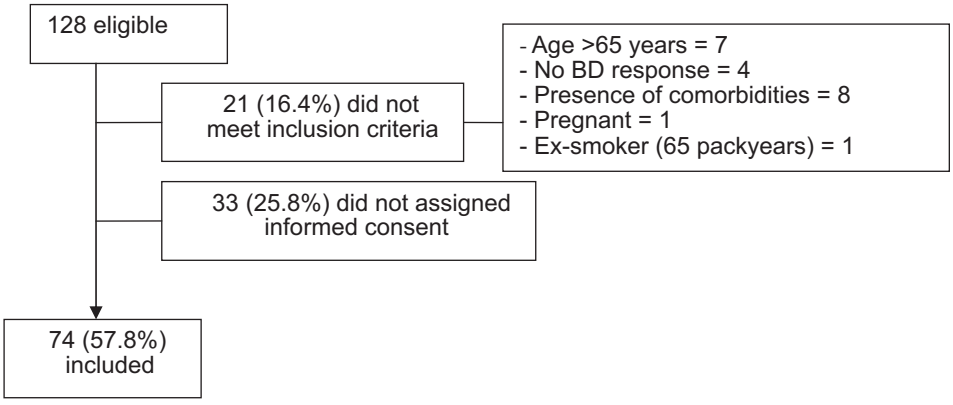


Figure 1 Flow chart showing the number of patients in each category.

Table 1 Demographic and clinical characteristics.

Variable	$n = 74$
Age, yr[a]	44.5 ± 10.7
Gender (% female)	77
BMI kg/m^{-2} [a]	30.0 ± 6.2
Age of asthma onset, yr; [b]	9 (1–22)
Asthma duration, yr[a]	31.5 ± 15.8
Time without adequate treatment, yr[a]	27.7 ± 15.9
Smoking status (never/ex) *n* (%)	53 (71.6)/21 (28.4)
Pack year,[b]	2.8 (3.7–10)
ICs dose, mcg/d[a]	1394 ± 337
OCs use, *n* (%)	20 (27)
SABA, puff.day[a]	5 ± 3
Leukotriene receptor antagonist, *n* (%)	31(41.9)
exCO, ppm[a]	4.9 ± 1.9

Data are reported as means ± SD unless otherwise indicated; BMI: body mass index; ICs: Inhaled corticosteroid; OCs: Oral corticosteroid; SABA: Short acting beta agonist: exCO: Exhaled carbon monoxide concentration.

[a] Data are represented as mean ± SD.

[b] Data are represented as median (CI 25%–75%).

Sixty-seven (90.5%) patients were able to produce an IS sample (Table 3). There was a correlation between FeNO and IS eosinophils (Pearson's $r = 0.279$, $p = 0.022$).

Table 4 lists the scores for asthma control, HRQoL, and the anxiety and depression questionnaires. The asthma control evaluation showed that 93.2% of the patients were non-controlled based on ACQ (>1.57) and ACT (≤19) values, and we observed a significant correlation between ACQ and ACT scores (Pearson's $r = -0.777$, $p < 0.001$). There was also a significant association between FEV_1% and ACQ (Pearson's $r = -0.390$, $p = 0.001$) and ACT scores ($r = 0.303$, $p = 0.009$). All SGRQ domains, especially the total score, showed a correlation with both ACQ ($r = 0.566$, $p < 0.001$) and ACT scores ($r = -0.541$, $p < 0.001$). The physical function ($r = -0.462$ and $r = 0.470$, $p < 0.001$), physical role ($r = -0.330$ and $r = 0.448$, $p < 0.001$) and vitality ($r = -0.380$ and $r = 0.410$, $p < 0.001$) domains of the SF-36 also correlated with ACQ and ACT scores, respectively. The majority of patients displayed moderate anxiety, and one third showed signs of moderate depression.

There was a high frequency of aggravating factors and comorbidities with 72% of patients reporting rhinosinusitis

Table 2 Pulmonary function.

Parameters	
FVC (L) ($n = 74$)[a]	2.68 ± 0.72
FVC, % predicted ($n = 74$)[a]	74 ± 15
FEV_1 (L) ($n = 74$)[a]	1.58 ± 0.52
FEV_1, % predicted ($n = 74$)[a]	54 ± 18
FEV_1/FVC ($n = 74$)[a]	59 ± 13
BD % ($n = 74$)[a]	16 ± 16
TLC, % predicted ($n = 60$)[b]	109 ± 13
RV/TLC, % predicted ($n = 60$)[b]	159 ± 31
Raw, % predicted ($n = 55$)[b]	282 ± 126
Gaw, % predicted ($n = 55$)[b]	24 ± 12
D_{LCO}, % predicted ($n = 57$)	103 ± 24
FeNO, ppb ($n = 74$)	33.4 ± 17.6

Data are reported as means ± SD. FVC: Forced Vital Capacity; FEV_1: Forced Expiratory Volume in first second; BD: Bronchodilator response; TLC: Total Lung Capacity; RV: Residual Volume; Raw: Airway Resistance; Gaw: Airway complacence; D_{LCO}: Carbon Monoxide-Diffusing Capacity; FeNO: Exhaled nitric oxide.

[a] According to references 20,21.

[b] According to reference 22.

Table 3 Induced sputum.

Total count cell (× 10^6 cells/mL)	0.88 ± 1.08
Neutrophils %	51.4 ± 19.6
Eosinophils %	16.5 ± 16.4
Macrophages %	13.4 ± 10.5
Lymphocytes %	3.3 ± 3.6
Bronchial Epithelial cells %	15.3 ± 15.9
Squamous Cell Contamination %	5 ± 7.8
Viability %	72.4 ± 16.2

Data are reported as means ± SD.

Table 4 Asthma Control Test, Asthma Control Questionnaire, SGRQ, SF-36, IDATE and BECK questionnaires.

ACT, points ($n = 74$)	11.7 ± 4.5
ACQ, points ($n = 69$)	3.2 ± 1.1
SGRQ scores	
Symptoms	63 ± 18
Activity	80 ± 17
Impact	64 ± 14
Total	67 ± 14
SF-36 scores	
Physical function	34.9 ± 22.9
Physical role	25.4 ± 31.4
Body pain	39.4 ± 22.5
General health	40.5 ± 19.8
Vitality	40.8 ± 20.2
Social function	54.4 ± 26.8
Emotional aspects	32.4 ± 42.2
Mental health	50.1 ± 22.9
IDATE ($n = 70$)	36.6 ± 9.2
IDATE score ≥ 30, *n* (%)	57 (81)
IDATE score ≥ 50, *n* (%)	6 (8.5)
BecK ($n = 70$)	16.2 ± 11.7
BecK score ≥19, *n* (%)	22 (31)
BecK score ≥30, *n* (%)	8 (11)

ACT: Asthma Control Test; ACQ: Asthma Control Questionnaire; SGRQ: St. George Respiratory Questionnaire; SF-36: SF-36 Questionnaire, Medical Outcomes Study 36 – Item Short Form Health Survey; IDATE: State-Trait Anxiety Inventory; Beck: Beck Depression Inventory; ACT ≤ 19 points: non-controlled asthma; ACQ > 1.57: non-controlled asthma; SGRQ score ranges from 0 to 100: a lower score represents a better HRQoL; SF-36: a higher score represents a better HRQoL; IDATE score: ≥30 points = moderate anxiety; ≥50 points = severe anxiety; Beck maximum score is 63 points: 10–18 points = mild depression; 19–29 points = moderate; ≥30 points = severe.

and 60% reporting symptoms of gastroesophageal reflux disease (GERD). The patients were already being treated for these conditions. Asthma symptoms with aspirin intake were present in 19%, and symptoms worsened during menstruation in 22% of females.

Baseline characteristics divided by subgroups are shown in Tables 5 and 6. Nonatopic patients tended to be older than atopics and had a higher asthma onset age and a higher level of IS eosinophils. The late-onset subgroup had significantly fewer ICU admissions and lower percentages of positive prick tests than the early-onset group (Table 5). We also observed a statistically significant inverse correlation between ACT and ACQ scores ($r = -0.78$, $p < 0.01$) in the late-onset subgroup. There was a higher prevalence of patients in the EOS+ subgroup, but no differences were observed compared with the EOS− group except for significantly higher FeNO values in EOS+ patients. There was a correlation between IS eosinophils and FeNO in the EOS+ subgroup ($r = 0.318$, $p < 0.01$). Patients with persistent airflow limitation had significantly more ICU hospitalizations and higher airway resistance (Table 6). There were no significant differences reported among the comparison groups for the presence of rhinosinusitis, GERD, anxiety and depression symptoms, or for BDI and STAI scores.

Next, we produced an arbitrary hierarchy of the interrelations of genotypic (atopy), clinical (asthma onset age), inflammatory (IS eosinophils) and functional (persistent or non persistent airflow limitation) characteristics. Sixty-three (91.3%) patients had all data and could be grouped into four possible phenotypes as demonstrated in Fig. 2. The major group (24.6% of patients) included atopic patients with early-onset asthma, EOS+ and persistent airflow limitation. The second group (15.9%) included nonatopics, late-onset asthma, EOS+ and persistent airflow limitation. The third group (14.5%) consisted of patients with atopic characteristics, late-onset asthma, EOS+ and persistent airflow limitation, and the fourth group (7.2%) included nonatopics, early-onset asthma, EOS− and persistent airflow limitation. We also identified a miscellaneous group (1−3 patients per group) with mixed characteristics, including classic asthmatics (3 [4.3%] of patients) with atopy, early-onset asthma, EOS+ and non-persistent airflow limitation.

Discussion

In the present study, we describe the clinical, functional, and inflammatory characteristics of a severe asthmatic adult population in Brazil. The group was predominately female and displayed frequent atopy, high average BMI, early disease onset, presence of several comorbidities (particularly anxiety), and elevated burden of disease as evidenced by the frequency of hospitalization and a reduced HRQoL. The patients displayed elevated sputum eosinophils despite using high doses of inhaled corticosteroids. Although these patients had been receiving regular treatment for at least four years, they failed to achieve full clinical asthma control and continued to have persistent airflow limitation. Non-adherence to treatment is always a problem to consider, even when patients are followed for an extended period. Subgroup analyses showed that nonatopic patients had higher levels of sputum eosinophils than atopics. Patients with early-onset asthma tended to be more atopic and had more ICU admissions than did the late-onset group. In addition, EOS+ patients had higher FeNO values, and the persistent airflow limitation subgroup had more ICU admissions and greater airway resistance. Based on the subgroups' characteristics, we identified 4 *a priori* phenotypes that included 62.2% of well-described patients. In contrast to published European[4] and American[12] studies on severe asthma, in which patients were recruited from various centers, our severe asthma patients were followed by pulmonary specialists in one tertiary university hospital where they received treatment and medication free of charge based on asthma management guidelines. Interestingly, the 5.1% prevalence of severe asthmatics in our population is similar to those reported in other studies.[5]

Our population of severe asthma patients came from various sociocultural backgrounds and lived in environments different from those described in previous studies. Most importantly, these patients had not received regular treatment for their asthma for extended periods since the disease's onset. Nevertheless, this population shares several clinical characteristics with previously reported cohorts of severe asthma patients in the developed world.[4,12]

The female predominance observed in our patient group was reported in the European cohort[4] but not in the American cohort,[12] suggesting that severe asthma could be a gender-related disease.[4] We do, however, concede that this observation could result from a selection bias[4] because, on average, women attended medical visits more regularly than men.

Obesity affects approximately 7.4% of the adult female population in São Paulo. A high average BMI has been associated with severe asthma[4] and was observed both in our population and in the Enfumosa study.[4] Although mechanisms linking both conditions are still poorly understood, there is evidence that obesity impairs clinical control.[5] However, we did not find any significant BMI differences in the subgroups analyzed.

Although atopy has previously been considered a less important predisposing disease severity factor when compared with infections,[28] the percentage of positive prick tests in our patients was similar to the positive percentage reported in international studies[4,12] and represented fewer positive tests than those from mild and moderate asthmatics in other cohorts.[12]

Hospitalizations account for the largest share of asthma-related health care costs,[5] and programs based on providing no-cost medication to severe asthmatics have been shown to lead to a reduction in overall hospital admissions.[9,29] In our study, hospitalization rates were still high and were similar to those seen in other severe asthma cohorts. We did find that our patients had fewer hospitalizations during the year prior to our study, which may be attributed to their access to free medication. We also found a high percentage of ICU admissions and the need for intubation in our patient group. Prior studies found that more than 40% of severe asthmatics had been admitted to the ICU, and approximately 10%[5]—20% required intubation.[12]

Table 5 Demographic and clinical characteristics divided by subgroups.

	Atopic (n = 44) (64%)	Non-atopic (n = 25) (36%)[b]	Early-onset (n = 39) (53%)	Late-onset (n = 35) (47%)
Age, y[c, g]	42.5 ± 11.2	47.6 ± 9.6	43.8 ± 10.8	45.3 ± 10.6
Gender (F/M) (%)[d]	84/16	68/32	72/28	83/17
BMI, kg/m^{-2}[c, g]	29.2 ± 5.9	31.8 ± 6.5	30 ± 6.7	30.3 ± 5.8
Asthma onset age, y[e, h]	4.5 (1–17)	19 (1–33)[a]	1 (1–2)	25 (17–33)[b]
Asthma duration, y[c, g]	32.5 ± 13.5	29.8 ± 19.3	42.1 ± 10.9	19.6 ± 11.4[b]
ACQ score[c, g]	3.19 ± 1.5	3.21 ± 1.0	3.27 ± 1.15	3.13 ± 1.05
ACT score[c, g]	12 ± 4.5	11.5 ± 4.5	11.5 ± 4.7	12 ± 4.3
Hospitalization last year, n (%)[d]	15 (34)	10 (40)	36 (92.3)	32 (91.4)
ICU hospitalization, n (%)[f]	14 (32)	9 (36)	27 (54)	16 (46)[b]
FEV_1, % predicted[c, g]	54 ± 19	51 ± 16	54 ± 19	54 ± 17
FEV_1/FVC[c, g]	67 ± 13	66 ± 14	65 ± 13	68 ± 12
BD %[c, g]	16.7 ± 16.0	14.2 ± 15.9	13.4 ± 13.8	18.5 ± 17.7
RV/TLC, % predicted[c, g]	159 ± 35 (n = 33)	160 ± 26 (n = 23)	161 ± 32 (n = 27)	155 ± 30 (n = 30)
Raw, % predicted[c, g]	279 ± 122 (n = 31)	280 ± 139 (n = 21)	293 ± 135 (n = 27)	272 ± 117 (n = 28)
ICs dose (mg day^{-1})[c, g]	1395 ± 354	1400 ± 316	1432 ± 335	1321 ± 306
OCs, n (%)[d]	9 (28)	4 (20)	13 (33)	7 (20)
IgE (UI/mL)[e, h]	405 (164–741)	220 (65–643)	369 (135–738)	295 (167–696)
Sputum eosinophils, %[e, h]	9 (3.5–22)	18 (5–32)[a]	7 (2–27)	12 (5.3–28.7)
Sputum neutrophils, %[c, g]	55 ± 19	46 ± 19	54 ± 20	49 ± 18
FeNO (ppb)[c, g]	34.1 ± 18.8	31.9 ± 15.6	31.3 ± 18.7	35.2 ± 16.2
Prick test positive, n (%)[d]	44 (100)	0 (0)	27 (69)	20 (57)[b]

ICU: Intensive Care Unit; FEV_1: Forced expiratory volume in 1 s; FEV_1/FVC: Forced expiratory volume in 1 s/Forced vital capacity ratio; RV/TLC: Residual volume/total lung capacity ratio; Raw: Airway resistance; ICs: Inhaled corticosteroid; OCs: Oral corticosteroid; IgE: Imunoglobulin E; FeNO: Exhaled nitric oxide. BD: Bronchodilator response.

[a] $p < 0.05$.
[b] $p < 0.001$.
[c] t Test.
[d] Fisher test.
[e] Mann–Whitney test.
[f] χ^2 test.
[g] Are presented as mean ± SD.
[h] Are presented as median (CI 25%–75%).

Severe asthma patients often display a high number of comorbidities,[12,30] and it has been shown that treating these accompanying disorders can improve clinical asthma control.[31,32] Rhinosinusitis and GERD contribute to uncontrolled asthma, but, because our patients were receiving regular treatments for both these conditions, they may not be directly linked to the lack of asthma control.

In contrast to previous Brazilian cohorts,[7–9,11,30,33] this is the first study of a well-characterized severe population treated regularly and sufficiently enough to control their asthma. Of note in this context is the fact that these patients had a long disease history with irregular treatment periods with a maximum treatment time of 50% of their asthma lives.

Currently, there is little information on the natural progression of severe asthma. One possibility, as least in our patients, is that the absence of routine asthma treatments for extended periods contributed to the development of severe asthma by remodeling the patient's airway, which was suggested by abnormal functional values. To validate this hypothesis, further and more invasive studies of disease pathology in these individuals is necessary to determine the extent of airway structural changes.

The patients' inability to take physical and emotional control of the disease clearly impacts their lives and probably causes increased levels of anxiety and depression. The values obtained on the SGRQ were comparable to those of our severe COPD patients[34] and were almost double those of mild to moderate asthma patients and elderly COPD patients.[35] Low HRQoL was not associated with poor lung function (FEV_1 %), but was correlated with poor asthma control (ACT and ACQ scores) and depression symptoms. The evaluation of depression and anxiety symptoms is recommended in asthmatics, as these symptoms heavily influence HRQoL scores.[36] HRQoL can be used as an objective measurement of the personal burden of asthma, independent of pulmonary function changes. Most of our patients exhibited mood disorders, such as anxiety and depression, which are associated with noncompliance, poor asthma control, increased HCU, near-fatal asthma attacks, and mortality.[37] Thus, mood evaluation represents one area of potential intervention in patients with severe asthma.

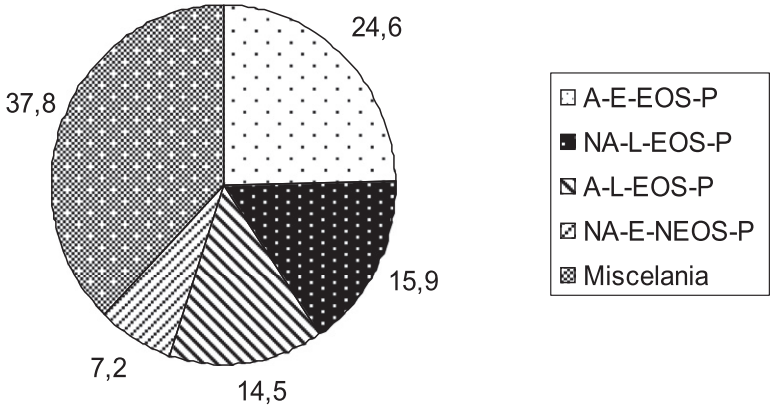
Although the patients had severe asthma, their lung function abnormalities were moderate. However, their high residual volume/total lung capacity ratio values suggest an air-trapping component that is associated with the most severe forms of asthma.[38] The higher rate of ICU hospitalization among the persistent airflow limitation subgroup of

Table 6 Demographic and clinical characteristics divided by subgroups.

	EOS+ (n = 53) (79%)	EOS− (n = 14) (21%)[b]	No persistent airflow limitation (n = 13) (18%)	Persistent airflow limitation (n = 61) (82%)[b]
Age, y[c, g]	43.9 ± 10.7	44.3 ± 11.3	39.5 ± 8.8	45.6 ± 10.8
Gender (F/M) (%)[d]	79/21	57/43	9/4	47/14
BMI, kg/m^{-2}[c, g]	29.5 ± 6.3	31.9 ± 6.7	28.7 ± 5.4	30.5 ± 6.4
Asthma onset age, y[e, h]	12 (1–26)	1.5 (1–10)	8 (1–25)	10 (1–23)
Asthma duration, y[c, g]	29.8 ± 16.2	35.9 ± 14.8	25.3 ± 14.1	32.8 ± 16.0
ACQ score[c, g]	3.22 ± 1.11	3.04 ± 1.22	2.75 ± 1.00	3.30 ± 1.10
ACT score[c, g]	11.8 ± 4.8	12 ± 3.8	12.2 ± 3.7	11.6 ± 4.7
Hospitalization last year, n (%)[d]	49 (92.5)	13 (92.8)	4 (31)	20 (33)
ICU hospitalization, n (%)[f]	26 (49)	7 (50)	3 (23)	34 (56)[a]
FEV_1, % predicted[c, g]	53 ± 17	59 ± 18	75 ± 10	49 ± 15[b]
FEV_1/FVC[c, g]	67 ± 13	66 ± 14	75 ± 7	65 ± 13[b]
BD %[c, g]	15.5 ± 15.1	14.2 ± 18.6	14.1 ± 14.3	16.2 ± 16.3
RV/TLC, % predicted[c, g]	159 ± 33 (n = 44)	157 ± 33 (n = 10)	141 ± 37 (n = 10)	163 ± 29 (n = 50)[a]
Raw, % predicted[c, g]	277 ± 134 (n = 42)	268 ± 71 (n = 9)	193 ± 78 (n = 9)	299 ± 126 (n = 46)[a]
ICs dose (mg day^{-1})[c, g]	1393 ± 343	1440 ± 337	1477 ± 413	1377 ± 321
OCs, n (%)[d]	16 (30)	4 (28)	2 (15)	18 (30)
IgE (UI/mL)[e, h]	304 (159–686)	459 (131–745)	537 (259–903)	292 (143–668)
Sputum eosinophils, %[e, h]	15 (6.7–31.2)	1 (1–2)[b]	19 (6–40)	9 (4–25)
Sputum neutrophils, %[c, g]	50 ± 18	56 ± 26	44 ± 17	53 ± 20
FeNO (ppb)[c, g]	36.2 ± 18.8	22.9 ± 7.5[a]	34.1 ± 18.3	33.6 ± 17.7
Prick test positive, n (%)[d]	35 (66)	8 (57)	10 (77)	37 (61)

ICU: Intensive Care Unit; FEV_1: Forced expiratory volume in 1 s; FEV_1/FVC: Forced expiratory volume in 1 s/Forced vital capacity ratio; RV/TLC: Residual volume/total lung capacity ratio; Raw: Airway resistance; ICs: Inhaled corticosteroid; OCs: Oral corticosteroid; IgE: Immunoglobulin E; FeNO: Exhaled nitric oxide. BD: Bronchodilator response; EOS+: Eosinophil-positive; EOS−: Eosinophil-negative.

[a] $p < 0.05$.
[b] $p < 0.001$.
[c] t Test.
[d] Fisher test.
[e] Mann–Whitney test.
[f] χ^2 test.
[g] Are presented as mean ± SD.
[h] Are presented as median (CI 25%–75%).



A = atopic; NA = non atopic; E= early; L= late; EOS = Eosinophil-positive; NEOS: Eosinophil-negative; P = persistent airflow limitation

Figure 2 Patients (%) subdivided by possible phenotypic characteristics according to atopy, asthma onset age, induced sputum eosinophils and airflow limitation.

our cohort reinforces this notion. Our functional results indicated fixed changes in the peripheral airway and possible airway remodeling. Persistent airflow limitation despite effective asthma treatment has been associated with adult-onset asthma, increased airway hyperresponsiveness, and sputum eosinophilia.[39] These findings highlight the need to evaluate the degree of small airway alterations in severe asthma patients.

Most of the patients in this relatively homogeneous cohort of severe asthmatics presented sputum eosinophilia despite receiving steroid treatments. Persistent eosinophilia is a characteristic of some severe asthma subgroups[40] but, in our study, was not related to any specific clinical characteristic or to the ICS dose used. The higher prevalence of chronic oral corticosteroid use among atopic patients could explain the lower eosinophilic inflammation in this group, although both variants of the disease (atopic and nonatopic) may be associated with bronchial mucosal eosinophilic inflammation. The normalization of sputum eosinophilia after an intramuscular corticosteroid trial suggests that some severe asthmatics required additional anti-inflammatory treatment to improve airway inflammation.[41] Our data suggest the need to follow these patients with a maximum anti-inflammatory treatment and objective measures of adherence and response.

Most studies of severe asthma have identified the age of asthma onset and atopy as two of the most consistently reproducible clinical phenotypes.[12,13] Although we acknowledge that the number of patients included in this study is small, we identified *a priori* several clinically defined subgroups.

Remarkably significant differences were found when patients were divided according to atopy, with nonatopic patients presenting high sputum eosinophilia. Early-onset asthmatics were more atopic and had a higher number of ICU admissions than late-onset asthmatic patients despite having similar baseline FEV_1 and sputum eosinophil levels. The greater ICU rate in this group is probably related to the longer asthma duration. Patients with persistent airflow limitations also had higher ICU admission rates, which may be due to significant lung function impairment. In the EOS+ subgroup, the only distinct feature we observed was significantly higher FeNO levels. This finding may indicate that the use of a 3% cut-off to classify patients as eosinophil-positive or -negative was not ideal for this cohort, although it is widely used in the literature. And also indicate that eosinophils are not particularly relevant in this severe disease asthma cohort. Another point of note concerns the fact that the small number of patients in the EOS− subgroup may have affected these results. Other than the controversial findings regarding FeNO values and asthma severity,[12] FeNO measurements can generally be used to identify EOS+ individuals among patients whose severe asthma remains uncontrolled despite improved treatment.[42]

Recently, the aspects related to the burden of non-controlled severe asthma were discussed and some perspectives were proposed with a special focus on low- and middle-income countries.[43] The authors highlighted the need for researchers to characterize phenotypes and search for specific risk factors and associated comorbidities. We identified four possible phenotypes that included a substantial number of the patients in our cohort. The largest group included most typical asthma characteristics other than persistent airflow limitation, suggesting the possibility of airway remodeling, which is compatible with severe asthma. The second group included nonatopic and late-onset patients who nonetheless exhibited eosinophilic inflammatory characteristics (EOS+) and persistent airflow limitation. The smallest group differed from the others specifically in the absence of eosinophilic inflammation. Three quarters of the patients from the well-characterized (no miscellaneous) groups shared the same inflammatory pattern and persistent airflow limitation independent of atopic characteristic and asthma onset age, suggesting that corticosteroids should be the therapeutic management target in these patients to avoid future functional impairment. Only 62.2% of our patients could be classified into one of the four subgroups using the predefined patterns, suggesting that the miscellaneous group should be classified better using cluster analysis.

Our study does, however, contain some limitations. This research is a cross-sectional study with a small number of patients from a single center and a tertiary university hospital, and part of the comorbidity information and HCU relies on historical data. Tobacco use may be considered a confounding factor, as it is related to persistent airflow obstruction and lower corticosteroid response. However, less than one third of our patients were proven ex-smokers. Perhaps they represent an overlap between asthma and COPD/chronic bronchitis.

Conclusion

We have described a cohort of severe asthma patients derived from a large clinical database from an academic hospital in São Paulo, Brazil. These patients share a series of common features with other published cohorts from around the world and emphasize the high burden of disease, the persistence of inflammation, and the existence of clinical phenotypes. This cross-sectional analysis will be followed by controlled intervention trials and longitudinal observations. Further research into the specific characteristics of this cohort could uncover an understanding of the pathology and pathogenic mechanisms of severe asthma. This is the first study to characterize a clinically difficult patient group, and these findings may improve future asthma care in Brazil.

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Conflict of interest

All authors report no financial or otherwise conflict of interest regarding the subjects and the findings of this study.

Author's contribution

The contribution of each author for this document in context of the authorship criteria are listed below:

Regina Maria de Carvalho Pinto: Conception and design of the study, acquisition of data and analysis and interpretation of the data; Drafting the article and revising it critically; Final approval of the version submitted.

Alberto Cukier: Conception and design of the study, analysis and interpretation of the data; Revising the article critically for important intellectual content; Final approval of the version submitted.

Luciene Angelini: Acquisition of data and analysis and interpretation of the data; Drafting the article; Final approval of the version submitted.

Leila Antonangelo: Analysis and interpretation of the data; Drafting the article; Final approval of the version submitted.

Thais Mauad: Conception and design of the study, acquisition of data and analysis and interpretation of the data; Drafting the article and revising it critically for important intellectual content; Final approval of the version submitted.

Marisa Dolhnikoff: Conception and design of the study, acquisition of data and analysis and interpretation of the data; Drafting the article and revising it critically for important intellectual content; Final approval of the version submitted.

Klaus F. Rabe: Conception and design of the study and interpretation of the data; Revising the article critically for important intellectual content; Final approval of the version submitted.

Rafael Stelmach: Conception and design of the study and interpretation of the data; Drafting the article and revising it critically for important intellectual content; Final approval of the version submitted.

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