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Heterogeneity of phenotypes in severe asthmatics. The Belgian Severe Asthma Registry (BSAR)

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Summary

The Belgian severe asthma registry is a web-based registry encompassing demographic, clinical, functional and inflammatory data of severe asthmatics (SA), aiming at improving awareness, knowledge on its natural history and subphenotypes, and offering tools to optimize care of this asthma population.
Methods: The cross-sectional analyses of this registry included 350 SA as defined by the ATS (2000) from 9 Belgian centres, with at least one year follow up.
Results: Mean age was 55 ± 14 yrs. SA were more frequently female (57%) and atopic (70%). Late-onset asthma (≥40 yr) was observed in 31% of SA. Current smokers represented 12% while 31% were ex-smokers. In addition to high doses ICS + LABA, 65% of patients were receiving LTRA, 27% anti-Ige and 24% maintenance oral corticosteroids (8 mg (Interquartile range-IQR:4-8) methylprednisolone). Despite impaired airflow (median FEV,:67%; IQR: 52-81) only 65% had a post-bronchodilator FEV,/FVC ratio <70%. The median blood eosinophil count was 240/mm^3. The median FENO was 26 ppb (IQR: 15-43) and 22% of SA had FENO ≥ 50 ppb. Induced sputum was successful in 86 patients. Eosinophilic asthma (sputum Eos ≥ 3%) was the predominant phenotype (55%) while neutrophilic (sputum Neu > 76%) and paucigranulocy-tic asthma accounted for 22% and 17% respectively. Comorbidities included rhinitis and chronic rhinosinusitis (49%), nasal polyposis (19%), oesophageal reflux (36%), overweight and obesity (47%) and depression (19%). In addition, 8% had aspirin-induced asthma and 3% ABPA. Asthma was not well-controlled in 83% according to ACT < 20 and 77% with ACQ > 1.5.
Conclusion: In this cohort of patients with severe asthma, the majority displayed indices of persistent airflow limitation and eosinophilic inflammation despite high-dose corticosteroids, suggesting potential for eosinophil-targeted biotherapies.

KEYWORDS : Comorbidities ; Inflammation ; Phenotype ; Severe asthma

Introduction

It is recognized that the majority of asthmatics may be controlled by regular treatment with ICS/LABA. However there remains a small proportion of patients who do not respond to this treatment [1,2]. Severe asthma accounts for a major part of financial burden to health care system posed by asthma [3]. Refractory asthmatics are patients in whom alternative diagnoses have been excluded, comorbidities have been treated, trigger factors have been removed and adherence with treatment has been checked. Patients with severe asthma still have poor asthma control or frequent severe exacerbations despite intake of high-intensity treatment or can only maintain adequate control when taking systemic corticosteroids [4,5]. Severe asthma is not a single disease but can be divided into several phenotypes according to inflammatory, clinical and functional characteristics [6]. Those phenotypes may have prognostic value and therapeutic implications. However, severe asthma phenotypes have not yet been fully characterized.

Severe asthma is a poorly understood patho-physiologically condition and is therefore difficult to treat. Several severe asthma cohorts and registries reported in the literature aimed to collect data and information on severe asthma. The Belgian Severe Asthma Registry (SAR) is a national Belgian secured web database for severe asthma, collecting relevant demographic, clinical and social data of severe asthmatics. Here we report the data collected in the severe asthma population in Belgium and compare the findings to that of ENFUMOSA [7] and BIOAIR European study [8], the TENOR study [9], the SARP study [10] and the UK multicentre registry on refractory asthma [11].

The aim of the registry was to collect epidemiological and clinical data in order to raise awareness on severe asthma, to identify several phenotypes, to promote optimal care for these patients and to be a valuable platform of patients for testing new drugs in severe asthma.

Methods Selection criteria

After detailed assessment, 350 severe asthmatics (SA) that fulfilled the American Thoracic Society definition of refractory asthma [12] were recruited between March 2009 and January 2014 from 9 Belgian centres.

The Belgian Registry is a secured web database and admits password protected anonymised data, after fully informed written consent. Individual centre data can be downloaded locally by registered users.

Asthma was diagnosed based on symptoms of cough, breathlessness or dyspnoea together with the demonstration of airflow variability. The latter was defined by one or more of the following: increase in forced expiratory volume in 1s (FEV_1) of 12% or greater following inhalation of 400 µg of salbutamol or inhaled concentration of methacholine provoking a 20% fall in FEV_1 of less than 16 mg/ml. Methacholine challenges were performed according to a standardized methodology as previously described [13].

SA was defined according to ATS criteria [12]. The definition requires one major criterion either treatment with continuous or near continuous (>50% of year) oral corticosteroids or requirement for combination high dose ICS (Beclomethasone or Budesonide > 1000 µg/d, Fluticasone > 500 µg/d) and Long acting β2 agonists (LABA). The major criterion has to be associated with at least two minor criteria: need for additional daily controller medication in addition to ICS-LABA combinations (Leucotrienes antagonists (LTRA), theophylline), persistent airway obstruction (FEV_1, < 80% pred, PEF variability > 20%), asthma symptoms needing short acting β2 agonist on a daily or near daily basis, one or more urgent care visits for asthma per year, three or more oral corticosteroids bursts per year, prompt deterioration with <25% reduction in oral or ICS use or near fatal asthma event in the past.

The prerequisite for inclusion was age > 18 years, asthma follow-up by a respiratory physician for at least 12 months, education on the disease provided to the patient and compliance thought to be satisfactory. All the data presented were collected at the timepoint of recruitment into the registry.

Demographic, comorbidity and control criteria

Patients were characterised as atopic if they had at least one positive specific IgE (>0.35 kU/l; Phadia) for at least one common aeroallergen (cat, dog, house dust mites, grass pollen, tree pollen and a mixture of moulds) or positive skin prick tests. Exacerbation in the previous year was defined by a course of oral corticosteroids for at least 3 days in case of asthma worsening. Nasal polyps and sinusitis was diagnosed by Ear Nose and Throat specialist either by endoscopy or Sinus CT scanner. Gastrooesophageal reflux was diagnosed either by symptoms of heartburn at history taking or the presence of oesophagitis demonstrated by gastroscopy. Quality of life was assessed using the self-administered Asthma Quality of Life Questionnaire (AQLQ) [14] and asthma control by Juniper Asthma Control Questionnaire (ACQ) [15] and Asthma Control Test (ACT) [16].

Criteria for inflammatory phenotyping

Patients underwent FENO measurement at a flow rate of 50 ml/s according to the ERS/ATS recommendations [17] (NIOX, Aerocrine, Sweden). Sputum was only induced at CHU of Liege and processed as previously reported [18,19] and was successful in 77% of the patients. Cell counts were carried out on 500 cells after centrifugation (Cytospin) and staining with Diff Quick (Dade, Brussels, Belgium). We defined an abnormally high sputum neutrophil count as a percentage >76% (>mean + 1.7SD of our reference value found in healthy subjects matched for age) [20].

In a subanalysis we identified patients with type 2-high and type 2-low inflammation according to the following criteria: sputum eosinophil count ≥ or <3% [21], exhaled nitric oxide ≥ or <27 ppb [22] and blood eosinophil count ≥ or <188/mm^3 [20,23].

Table 1 *Demographic, functional, clinical and inflammatory characteristics of severe asthmatics in Belgium.*

N.	350
Female (%)	57%
Age	55 ± 0.8
Age at onset	
<12 years	32%
12-40 years	36%
>40 years	31%
Height, m	167 ± 0.5
Weight, kg	75 ± 0.9
BMI	26 (16-43)
Smoking status	
Never	200 (57%)
Ex-smoker	108 (31%)
(pack-years median IQR)	(15 (11-24))
Current smokers	40 (12%)
(pack-years median IQR)	(11 (10-15))
Atopy, %	70
Current house environment (%)	
Country side	39
Suburban area	29
City	31
Unknown	1
FEV_1, % pred	68 ± 1.2
FVC, % pred	89 ± 1.1
FEV_1/FVC, %	63 ± 0.7
FEV_1 reversibility (% from baseline)	11 ± 0.8
FRC (%) (*n* = 271)	120 ± 2
RV(%) (*n* = 311)	140 ± 2.8
TLC (%) (*n* = 305)	102 ± 1.1
DLCO (%) (*n* = 273)	78 ± 1.2
KCO (%) (*n* = 273)	97 ± 1.3
Airway inflammatory indices	
$FENO_{50}$ (ppb) (*n* = 271)	26 (4-250)
Sputum eosinophil count	7 (0-92)
(%) (*n* = 86)	
Sputum neutrophil count	51 (0-99)
(%) (*n* = 86)	
Sputum inflammatory	
subphenotype (n = 86)	
Paucigranulocytic	17%
Eosinophilic (≥3%)	55%
Neutrophilic (≥76%)	22%
Mixed granulocytic	6%
Serum IgE (kU/l) (*n* = 295)	207 (2-10,000)
Blood eosinophils (%) (*n* = 272)	3 (0-50)
Blood eosinophils (/mm^3) (*n* = 272)	240 (0-3144)
ACT (*n* = 207)	13 ± 0.4

ACQ (*n* = 213)	2.57 ± 0.09
AQLQ (*n* = 244)	4.14 ± 0.09
ICS dose (BDP µg equivalent/d)	2000 (190-6000)
LABA	91
Anti-histamines, %	26
LTRA, %	65
Anti-cholinergics, %	28
Anti-Ige, %	27
Theophylline, %	22
Maintenance oral corticosteroids, %	24
Specific immunotherapy, %	0.6
Comorbidities (%)	
Rhinosinusitis % (Y/N/Ukn)	49% (167/151/32)
Gastrooesophageal reflux (Y/N/Ukn)	36% (124/205/21)
Nasal polyps (Y/N/Ukn)	19% (167/151/32)
Overweight (Y/N/Ukn)	47% (162/173/15)
Psychopathology (Y/N/Ukn)	19% (65/266/19)
Catamenial asthma (Y/N/Ukn)	0.9% (3/340/7)
Aspirin sensitive asthma (Y/N/Ukn)	8% (28/315/7)
Occupational asthma (Y/N/Ukn)	4% (15/328/7)
Churg Strauss syndrom (Y/N/Ukn)	3% (10/333/7)
ABPA (Y/N/Ukn)	3% (11/332/7)
Bronchiectasis (Y/N/Ukn)	16% (54/289/7)
Emphysema (Y/N/Ukn)	7% (24/319/7)
Treatment of comorbidities	
Proton pump inhibitors	39%
Anti-depressive/anxiolytics	17%/14%
Intranasal steroids	39%
Oral steroids courses during previous yr	2.03 (0-7)
Number of hospitalisations during previous yr	0.95 (0-7) (*n* = 113)
Number of hospitalization during the last three years	1.7 (0-8) (*n* = 103)

Statistical analysis

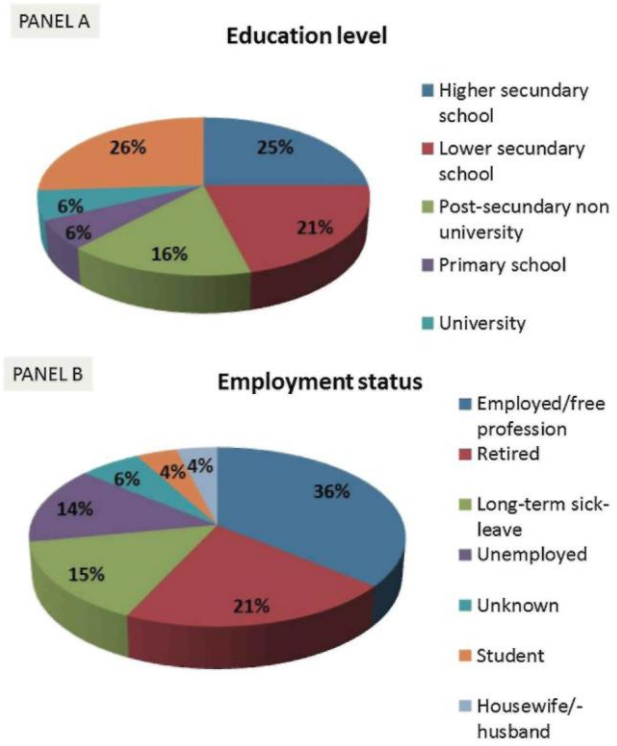
Anonymised data were expressed as mean ± SEM for continuous variables; median and ranges were preferred for skewed distributions. For categorical variables, the number of observations and percentages were given in each category. Comparisons between different subgroups were performed using one-way analysis of variance (ANOVA) and Kruskal-Wallis testing. The Spearman correlation coefficient was used to measure the association between clinical parameters. The receiver-operating characteristic (ROC) curve was constructed to determine the cut-off of blood eosinophil count which best identified a sputum eosinophil count >3% in severe refractory asthma. Statistical significance was taken as $p < 0.05$.

Results

We recruited 350 SA as defined by the ATS (2000) criteria from 9 Belgian centres. From those patients, 333 are still defined as severe asthmatics according to ERS/ATS guidelines on severe asthma (2014).

The demographic, functional, clinical and inflammatory characteristics of severe asthmatics are summarized in Table 1.

Figure 1 *Panel A. Education level in severe asthma in Belgium. Panel B. Employment status in severe asthmatics in Belgium.*



Demographics and treatment characteristics

Female was the predominant gender (57%) and mean age was 55 yrs. 31% of the severe asthmatics had late-onset asthma (starting after the age of 40). BMI was slightly increased (26 kg/m^2) and one quarter of the patients had a BMI > 30. 31% were ex-smokers (64% with at least 10 pack-yrs) while 12% were current smokers. The prevalence of atopy was 70%.

One third of Belgian SA lived in cities, one third in suburban area while 39% lived in country side environment.

In addition to high doses of ICS + LABA, 65% of the patients received anti-leukotrienes. 24% of severe asthmatics were treated with systemic corticosteroids on a daily basis, 26% with anti-histamines and 27% with anti-Ige. Theophylline was administered to 22% of the patients and 0.6% received specific immunotherapy.

Educational level was low in the majority of patients with 6% quitting school after primary school, 21% after lower secondary school and 25% after higher secondary school. Only 16% of severe asthmatics were graduated from non-university post-secondary school and 6% from university (Fig. 1A). Data are unknown in one quarter of the patients. 36% of SA were employed while 21% were retired (Fig. 1B).

Table 2 *Number of hospitalization and steroid bursts in Belgian severe asthmatics during the previous year.*

Number during the last year	% of patients with hospitalization (*n* = 106)	% of patients with systemic corticosteroid courses (*n* = 344)
0	40 (*n* = 42)	26 (*n* = 90)
1	35 (*n* = 37)	15 (*n* = 53)
2	19 (*n* = 20)	16 (*n* = 56)
3	5 (*n* = 5)	14 (*n* = 48)
>3	2 (*n* = 2)	28 (*n* = 97)

Comorbidities

Comorbidities were highly prevalent and included chronic rhinosinusitis (49%), nasal polyposis (19%), oesophageal reflux (36%), overweight and obesity (47%) and depression (19%). Bronchiectases diagnosed based on classical CT criteria were reported in 16% and aspirin sensitive asthma in 8% of SA while occupational asthma (4%), Churg Strauss syndrome (3%), ABPA (3%) and catamenial asthma (0.9%) were less frequent. Emphysema was present in 24 SA (7%) with 42% of those patients being current smokers, 42% ex-smokers and 16% non-smokers.

The number of oral steroid courses during the previous year was 2.03. The number of hospitalisations during the previous year and the last three years was 0.95 and 1.7 respectively. The detailed description of the number of hospitalisations and steroid course is descried in Table 2.

Patients having a history of hospitalization due to asthma did not have predominant sputum inflammation feature or more severe airway obstruction.

Lung function

Despite impaired flow rates (mean FEV_1, 68%pred; FEV_1/ FVC ratio, 63%), 65% of SA had post-bronchodilator FEV,/ FVC ratio < 70%. We found that 60% of SA had FEV_1, < 80% and FEV_1/FVC < 70%. The mean reversibility was still 11% despite patients were on long-acting β2 agonists. 36% of SA exhibited ≥12% FEV_1 reversibility to 400 µg Salbutamol and 16% of SA had a reversibility >20%.

Severe asthma was associated with significant air trapping. Despite normal total lung capacity (102% pred) there were signs of air trapping suggested by raised FRC (120% of predicted values) and RV (140% of predicted). DLCO was slightly impaired (78%) but KCO was well preserved (97% pred).

Figure 2 *Panel A. Distribution of FENO in the population of severe asthmatics. 49% of SA had FENO levels higher than 27 ppb suggestive of persistant sputum eosinophilic inflammation. Panel B. Distribution of blood eosinophils in severe asthmatics. 58% had blood eosinophil count ≥188/mm^3.*

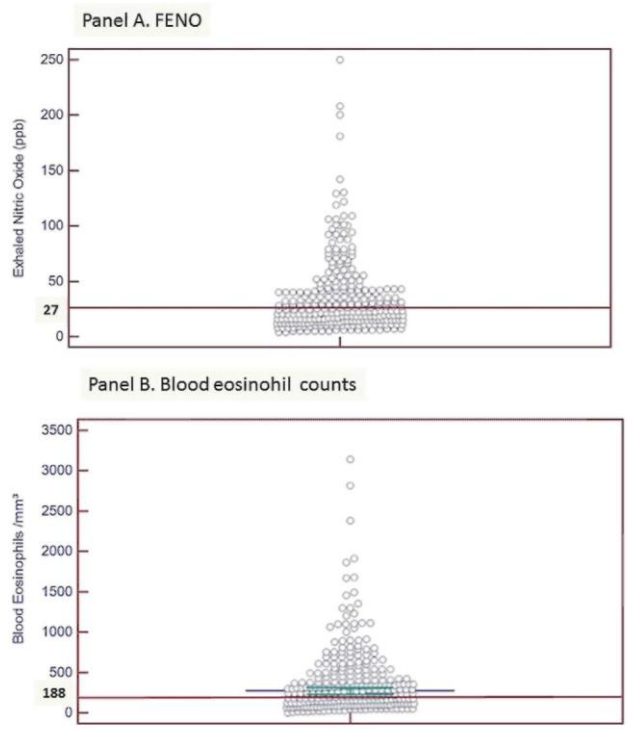


Figure 3 *Correlation between blood eosinophil count (/mm^3) and sputum eosinophil count (%) ($p < 0.0001$; $r = 0.53$).*

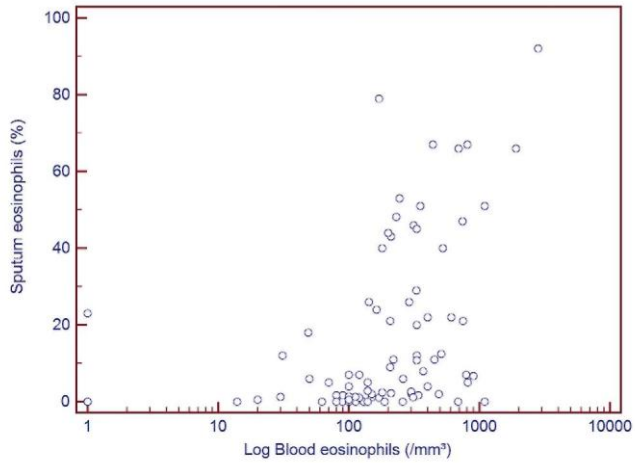
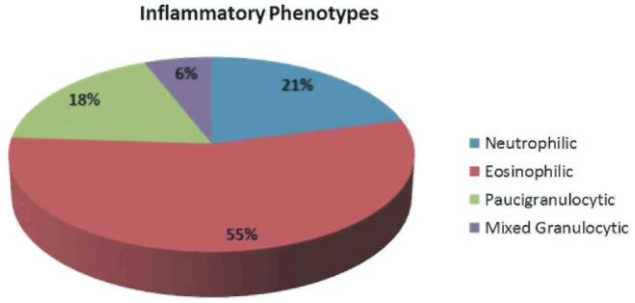


Figure 4 *Distribution of sputum cellular phenotypes in severe asthma (n = 88). Eosinophilic asthma (≥3% sputum eosinophils, <76% sputum neutrophils); Neutrophilic asthma (<3% sputum eosinophils, ≥76% sputum neutrophils); Pauci-granulocytic asthma (<3% eosinophils and <76% neutrophils in induced sputum); Mixed granulocytic asthma (≥3% eosinophils and ≥76% neutrophils in induced sputum).*



Inflammatory characteristics

The median FENO value was 26 ppb (4-250 ppb). The fraction of patients with FENO > 50 ppb was 22% (Fig. 2).

The median blood eosinophil count was 240/mm^3 (Fig. 2). We found an elevated blood eosinophil counts (>220/mm^3) in 53% of SA. Importantly we demonstrated a significant correlation between blood eosinophil count (/mm^3) and sputum eosinophil count (%) (*p*-value < 0.0001; $r = 0.53$, Fig. 3).

Sputum was induced in 86 out of 111 patients at CHU of Liege (success rate of 77%). The median sputum eosinophil count and sputum neutrophil count was 7% and 51% respectively. Eosinophilic asthma (sputum Eos ≥ 3%) was the predominant phenotype (55%), while neutrophilic (sputum Neu ≥ 76%) and paucigranulocytic asthma accounted for 22% and 17% respectively (Fig. 4).

By constructing an ROC curve, we found that the blood eosinophil count was able to identify sputum eosinophil count ≥3% with the best cutoff point of 188/mm^3 providing a 72% sensitivity and 73% specificity ($n = 80$, AUC = 0.745, Fig. 5).

We found a modest but significant correlation between FENO and sputum eosinophil count ($r = 0.37$, $p < 0.001$) and between FENO and blood eosinophils ($r = 0.29$, $p < 0.0001$). By constructing an ROC curve, we found that the best cutoff of FENO was 28 ppb to identify sputum eosinophil counts ≥3% in severe asthmatics.

We assessed the proportion of patients exhibiting concordant and discordant blood and sputum eosinophilia. Blood eosinophilia ≥400/mm^3 [24] and sputum eosinophil count ≥3% was found in 23% of the patients while exhibiting elevated sputum eosinophil count without increased blood eosinophil count was found in 35%. Normal blood and sputum eosinophil count was common (38%) while isolated elevation of blood eosinophil count was rare (4%). If we chose the threshold value of ≥300/mm^3 as recommended by some authors [21], diffuse eosinophilic inflammation was found in 36% of the patients, isolated sputum eosinophilic inflammation was found in 24%, Normal blood and sputum eosinophil count was common (33%) while isolated blood eosinophilia was rare (7%).

Figure 5 *ROC curve showing the best cut-off of blood eosinophil count to identify sputum eosinophil count ≥3% in SRA. Sensitivity 72.3%, specificity 72.7%, cut-off: 188/mm^3, p < 0.0001, AUC: 0.745, n = 80.*

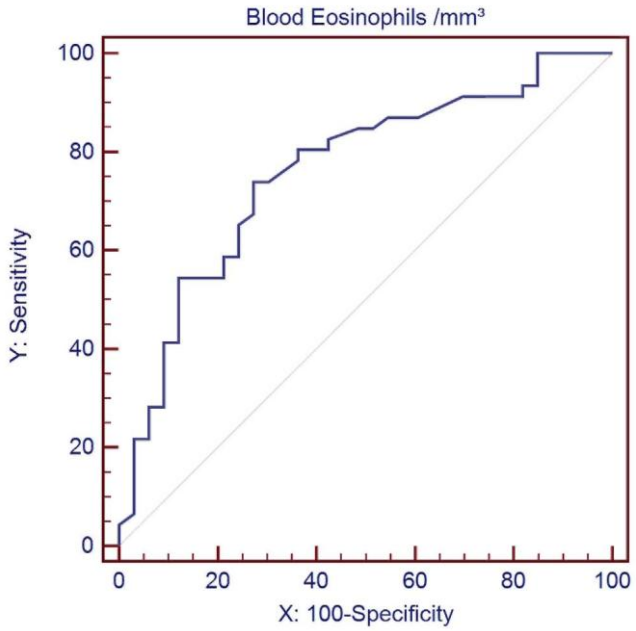


Table 3 *Comparison of characteristics of smoking versus nonsmoking severe asthmatics.*

	Nonsmoking SA (including ex-smokers)	Smoking SA
n	308	40
BMI	26.1	24.7
ACQ	2.32 (0-5.86)	3.57 (1.29-5.71)**
ACT	13 (5-25)	11 (5-24)*
AQLQ	4.16 (1.2-7)	3.37 (1.5-6.45)*
Emergency visits	1 ± 0.64	2.17 ±0.65*
Rescue courses of oral CS	1.89/patient/yr	1.81/patient/yr
FEV_1, % pred	68 ±21	63 ± 18
Sputum eosinophils	5.5 (0-79) (*n* = 66)	7 (0-92) (*n* = 20)
Sputum neutrophils	52 (0-99) (*n* = 66)	47 (0-97) (*n* = 20)
Blood eosinophils	280 (0-3144)	167 (0-1677)*

*$p < 0.05$, **$p < 0.01$.

Asthma control and quality of life

Asthma was uncontrolled in 77% of severe asthmatics as defined by ACQ score >1.5 and in 83% as defined by ACT <20.

We found however that 8% had well-controlled severe asthma (ACQ <0.75) and that 71% of those patients were women with a high proportion being employed (65%). None of these patients were current smokers (88%

nonsmokers, 12% former smokers). BMI was slightly lower (24 kg/m^2) than in the general SA population and atopy was more frequent (77%). Quality of life assessed by AQLQ was also better in this sub-population. They had a better lung function (FEV_1, mean: 92% ± 1.2, FEV_1/FVC: 70 ± 0.9) and fewer signs of air trapping (RV: 120 ± 2.5, FRC: 113 ± 2). Only 12% were treated with oral corticosteroids. They exhibited lower FENO levels (17 (5-76)) and lower blood eosinophil counts (120 (0-1200)).

Characteristics of smoking versus nonsmoking severe asthmatics

Current smokers with SA had poorer asthma control assessed by ACQ and ACT as compared to ex- or never smoking SA. Smoking severe asthmatics had more frequent unscheduled health care visits than exsmokers or never smokers with SA and exhibited lower levels of blood eosinophils (Table 3).

Relationship between lung function, inflammation, asthma control and quality of life

Average AQLQ was 4.14 (1.2-7). We found a positive correlation between FEV*1*, and AQLQ ($r = 0.21$, $p < 0.0001$) and an inverse correlation between FEV-*1* and ACQ ($r = -0.49$, $p < 0.0001$). We did not find any significant correlation between blood eosinophils or sputum eosinophil count and AQLQ ($r = -0.01$, $p = 0.8$; $r = -0.06$, $p = 0.58$ respectively) or between blood eosinophils or sputum eosinophils and ACQ ($r = -0.02$, $p = 0.8$; $r = 0.06$, $p = 0.57$). We did not find any significant correlation between BMI and ACQ or AQLQ.

Type 2-high versus type 2-low criteria for targeted therapy

The median total serum IgE level was 207 kU/l and 58% of SA with atopic status exhibited IgE levels between 76 and 700 kU/l, the range to possibly consider treatment with omalizumab in Belgium [25]. In nonatopic SA, 48% had IgE levels between 76 and 700 kU/l. Detailed data on sensitization were available in the Liege cohort ($n = 111$) where 45% of SA were atopic to house dust mite, 39% to cat, 31% to dog, 29% to grass pollen, 23% to birch pollen, 21% to moulds and 12% to horse. According to Belgian's reimbursement criteria, treatment with omalizumab could be proposed in 27% of SA in Liège.

In the DREAM study [21], asthmatics had a history of 2 or more exacerbations requiring systemic corticosteroids in the previous year. Additionally, they had evidence of eosinophilic inflammation as shown by one or more criteria: a sputum eosinophil count of ≥3%, FENO ≥ 50 ppb, blood eosinophils ≥300/mm^3 or prompt deterioration of asthma control after a 25% or less reduction in regular maintenance ICS or OCS. According to these criteria [21], 106 patients (30%) of this cohort could reveal eligible for anti-IL5 therapy.

We further classified our SA into inflammatory pheno-types according to evidence of either sputum eosinophil count ≥3% or the presence of both exhaled nitric oxide ≥27 ppb and blood eosinophil count ≥188/mm^3. We found that 57% of SA fulfilled those criteria (Fig. 6). Eosinophilic SA was more frequently associated with chronic rhinosinusitis (65%) and nasal polyps (24%) in case of late onset asthma compared to 52% and 18% in early onset asthma.

Discussion

Our data confirm that the majority of severe asthmatics are female and atopic. Moreover we have shown that asthma started after 12 yrs in two third of SA and that SA exhibited increased airway eosinophilic inflammation compared to a general population of asthmatics [20]. Dominant comorbidities were chronic rhinosinusitis, overweight and gastrooesophageal reflux. Type 2-high and Type 2-low SA were diagnosed based on induced sputum, blood eosinophil count, IgE and FENO levels. A trait suggesting type 2-high was identified in the majority of SA despite treatment with high doses of ICS (and oral corticoids in a subgroup).

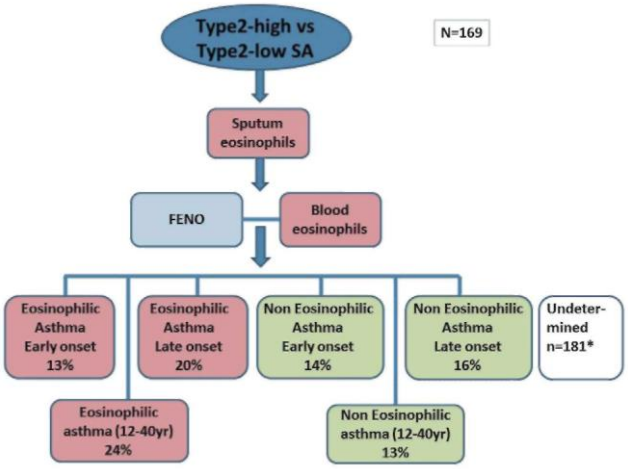
Demographic and general clinical data

Our data confirm the usual female preponderance [10,11,26,27]. In comparison with a general population of asthmatics [28], SA was rather similar in age (55 vs 52). Late onset asthma, starting after the age of 40, was observed in 31% of patients. The proportion of current smokers was higher than that observed in UK registry [11] (12% vs 6%) while 31% of our population recognised past smoking history (similar to UK registry). The study of Thomson [29] showed similar data with 9% of British severe asthmatics being current smokers, while 28% were ex-smokers and 62% never smokers (for detailed comparison with other registries, see online supplement). In this study, current smokers with SA had poorer asthma control and more unscheduled health care visits than exsmokers or never smokers. BMI was slightly increased but remained in the non-obese range in

the majority of patients (only one quarter had BMI > 30) and was similar to that observed in the general population of asthmatics [28], in UK registry [11], ENFUMOSA [7] and BIOAIR studies [8]. Severe asthmatics in Belgium have rather low level of education with only 22% graduated from post-secondary school. Our interpretation is that asthmatics with low level of education are more prone to be exposed to noxious particles, massive allergen amount in their daily life because being less cautious in taking care of their health.

Less than 40% of severe asthmatics are still professionally active. This was similar to what was observed in UK where 53.4% were not working [11]; ENFUMOSA study [30] concluded that fewer severe asthma patients than mild asthma patients were currently employed.

Figure 6 *Classification of SA in Th2-high versus Th2-low phenotype according to sputum eosinophil count (≥or <3%), FENO levels (≥ or <27 ppb), and blood eosinophil count (≥or <188/mm^3). SA were classified as Th2-high phenotype if induced sputum showed >3% eosinophils or FENO levels ≥27 ppb and blood eosinophil count ≥188/mm^3 while Th2-low was chosen if induced sputum eosinophil count was <3% or FENO and Blood eosinophil count were <27 ppb and <188/mm^3 respectively. 169 patients were classified in eosinophilic and non eosinophilic asthma according to this definition and further characterized as early (<12 yrs), intermediate (12-40 yrs) or late onset (>40 yr). *181 patients were unclassified due to the lack of information on induced sputum, FENO or blood eosinophil count or discordant FENO and blood eosinophils information.*



Treatment and comorbidities

In addition to high doses ICS-LABA, SA in Belgium received more frequently LTRA and omalizumab than in UK [11] while theophylline and oral corticosteroids were less commonly encountered than in UK registry and ENFUMOSA. The higher number of anti-Ige treated patients in our registry is probably due to the inclusion of a high number of Belgian severe refractory asthmatics in the PERSIST study [31]. For the management of comorbidities, intranasal corticosteroids and proton pump inhibitors (PPI) were more frequently prescribed in Belgium than in UK [11].

The number of oral steroids courses during the year preceding inclusion in the SA was lower than that observed in the UK register [11] while the number of hospitalisations was higher.

The dominant comorbidities encountered in the Belgian severe asthma population were rhinosinusitis, overweight and gastrooesophageal reflux. The proportions were similar to previously reported [11] except for chronic rhinosinusitis that was more frequently encountered in Belgium. The treatment of rhinosinusitis by nasal corticosteroids is in accordance with the high proportion of rhinitis observed in a large population of asthmatics in Belgium [32]. We found a similar proportion of occupational asthma and aspirin-sensitive asthma in our cohort as compared to UK cohort [11].

Lung function

Overall there was a moderate obstructive airway pattern. The obstruction level was similar to ENFUMOSA [7] and UK register [11] but lower than in SARP study [10]. Surprisingly, several patients with normal FEV_1 were included in the registry due to high respiratory symptoms despite high-dose ICS. Those are patients who still report symptoms and bronchodilator use despite good airway function. Although we can not exclude that these patients exhibit persistent bronchial hyperresponsiveness, they might have "discordant disease" [33] as their indices of airway inflammation were low (median FENO 22 ppb, median sputum eosinophils 2.4%). 59% of these SA with normal FEV-, had obesity and late onset disease (>40 yrs) with similar characteristics as "obese non-eosinophilic" asthma reported by Haldar et al. As in UK registry [11] and ENFUMOSA study [7], the coefficient transfer was well preserved in our population which can be considered as a sign that our asthmatics are well different from COPD patients even if the majority of our patients displayed fixed airway obstruction. The normal KCO suggests a preserved alveolocapillary membrane. We confirmed that severe asthma is associated with significant increase in air trapping with normal total lung capacity suggesting involvement of small airways [1,10,34].

Inflammatory subphenotypes

The frequency of atopy remained high despite two thirds of SA having onset of asthma after the age of 12 yrs. Atopy was similar to that observed in SARP study [10] but higher than in BIOAIR study [8], ENFUMOSA [7] and UK registry [11]. SARP data and ours are based on skin prick tests and/or specific IgE level while in the UK registry it was based on the history, which may have led to underestimation.

The median FENO value is in the lower part of the grey zone (25-50 ppb [35]) in severe asthma. This is probably due to higher doses of inhaled corticosteroids in this sub-population. This is suggesting the persistence of an airway inflammatory process since we were able to show that in patients receiving high dose ICS the FENO threshold predicting sputum eosinophil count ≥3% was shown to be 27 ppb [22]. 49% of severe asthmatics exhibited FENO levels >27 ppb suggesting an eosinophilic phenotype. As compared to UK register [11], SARP [10] and BIOAIR [8], FENO levels were lower in our register.

Our median blood eosinophil count was slightly lower than that observed in the UK register [11]. The blood eosinophil count threshold that best predicts the presence of uncontrolled airway eosinophilia in SA was found to be 188/mm^3. According to this threshold, 58% exhibited eosinophilic asthma.

The data related to sputum were only available for the series of patients from CHU of Liege. We found a success rate of sputum induction of 77%, as usually reported in asthma [20]. The raised airway granulocytic inflammation is a common finding [36]. The median sputum eosinophil count was higher than that observed in the UK cohort [11]. We found a lower proportion of paucigranulocytic asthma in the severe asthma population than the 40% observed in the general population of asthmatics [20]. Eosinophilic asthma was more frequent in severe asthma (55% vs 41%) and neutrophilic asthma followed the same picture (21% vs 16% in the general population of asthmatics). Duncan [37] has demonstrated that reduced eosinophil apoptosis and increased sputum eosinophilia both significantly correlate with asthma severity. Neutrophilic inflammation was found to be increased in severe asthma [38]. It has been suggested that this could be due to a protection of neutrophils from apoptosis by corticosteroids. The mixed granulocytic asthma was twice more frequent in SA. Eosinophilic asthma was more frequently encountered in late onset asthma in line with previous reports [39].

Asthma was uncontrolled in 77% of severe asthmatics according to ACO· The 8% well controlled severe asthmatics were nonsmoking patients with higher rate of atopy, receiving high doses corticosteroids and exhibiting less severe airway obstruction and fewer respiratory symptoms. They had less intense eosinophilic inflammation.

Correlation between clinical and inflammatory data

We found a correlation between asthma control and airway calibre, a fact that has already been observed in asthma in general [40,41]. There was a weak correlation between quality of life and airway calibre. A similar correlation between FEV_1 and AOLO has already been demonstrated in a large population of persistent asthmatics [42]. We did not find any significant correlation between systemic eosinophilic inflammation and asthma control or quality of life. In a previous study conducted in a large population of unselected asthmatics [28], we did not find any significant correlation between ACOand blood eosinophil count while there was a weak correlation between ACO and sputum eosinophil count. The fact that we did not find any correlation

between blood eosinophil count or sputum eosinophil count and AOLO is in line with DREAM study, as anti-IL5 therapy were able to decrease sputum and blood eosinophil count but were not associated with an improvement in AOLO [43] in severe eosinophilic asthmatics compared with placebo.

BMI was correlated neither with ACO nor with AOLO in our severe asthma population. Median BMI in our Belgian severe asthma population is rather similar to BMI found in a general population of asthmatics [20,28]. Lavoie et al. previously failed to find any association with asthma severity, which is consistent with the present findings [44]. Therefore BMI might not be a critical factor in severe asthmatics as there are several other factors influencing asthma control in this population.

Therapeutic implications

We found elevated levels of IgE in our population of severe asthmatics whatever the atopic status. For the management of severe asthma in Belgium, the only biological treatment already commercially available is anti-Ige, reimbursed in case of sensitization to perennial allergen and a level of IgE comprised between 76 and 700 kU/l. The median IgE was higher in our register than that observed in UK register [11], ENFUMOSA [7] and SARP [10].

The majority of patients have residual eosinophilic inflammation both at systemic and airway level and are therefore potential candidates for anti-IL5 treatment. In the DREAM study [21], asthmatics had a history of 2 or more exacerbations requiring systemic corticosteroids in the previous year. Additionally, they had evidence of eosinophilic inflammation as shown by one or more criteria: a sputum eosinophil count of ≥3%, FENO of 50 ppb or more, blood eosinophils of 300/mm^3 or more or prompt deterioration of asthma control after a 25% or less reduction in regular maintenance ICS or OCS. We identified 30% potential candidates for anti-IL5 therapy according to DREAM criteria [21]. However taking into account the adapted threshold (for FENO and blood eosinophil count) to identify eosinophilic inflammation in asthmatics treated with high doses ICS, 57% of patients had eosinophilic asthma.

Although the mechanism of action of bronchial thermoplasty is not currently completely understood, this bronchoscopy technique seems to act on smooth muscle mass. Thermoplasty could emerge as a therapeutic option in SA who exhibit high bronchodilatation after salbutamol (16% of SA in our registry). The role of macrolides in non-eosinophilic severe asthma [45] remains to be clarified in larger studies.

For the recruitment of Belgian SA, we followed 2000 ATS criteria [12] because the collection of data started in 2009. Our study limitations are the limited number of patients with detailed allergic characteristics and induced sputum analysis and the recruitment at tertiary care University hospitals with potential selection bias.

Conclusion

In this Belgian cohort of patients with SA, we show that atopic background and eosinophilic inflammation represent the predominant features. Findings are consistent with other European and American registries, and will help to identify candidates for upcoming targeted therapeutic approaches. Further studies are also needed to clarify whether particular endotypes could be identified in this heterogeneous group of asthma patients with severe disease that is refractory to current therapies.

Listing of the contributions

Conception, design of the study and data collection: R. Louis, G. Joos, F. Schleich, G. Brusselle, O. Vandenpias, A. Michils, C. Pilette, R. Peche, M Manise, G. Joos. Data analysis: F. Schleich, M Manise; Data interpretation and drafting the manuscript for important intellectual content: F. Schleich, G. Brusselle, R. Louis, O. Vandenpias, A. Michils, C. Pilette, R. Peche, M Manise, G. Joos.

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Conflicts of interest

None of the named authors have conflicts of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.rmed.2014.10.007.

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