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Clinical and biological factors associated with irreversible airway obstruction in adult asthma

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ABSTRACT

Background and objective: Airway remodeling, as many other factors, may lead to lung function decline and irreversible airflow obstruction (IRAO) in asthma. This study was undertaken in order to highlight predictors of incomplete reversibility of airflow obstruction in adult asthmatics to identify patients with poorer prognosis and improve their care, and decrease morbidity.
Methods: A retrospective study was conducted in 973 asthmatics recruited from the University Asthma Clinic of Liege. Patients with IRAO (post-BD $FEV_1/FVC < 0.7$ & $FEV_1 < 80\%$ predicted) were compared to patients with reversible airway obstruction (RAO) (post-BD $FEV_1/FVC \geq 0.7$ & $FEV_1 \geq 80\%$ predicted). TGF-β was measured in sputum supernatant of 85 patients.
Results: Seventeen percent of asthmatics presented with IRAO. These patients were significantly older, more smokers, with a lower proportion of female, a longer disease duration, were more poorly controlled with a lower quality of life. This sub-population of asthmatics also showed more often elevated blood and sputum eosinophils and neutrophils, and higher exacerbation and hospitalisation rates in the previous year. The multivariable analysis revealed male gender, longer disease duration, cigarette smoking, ACQ score, sputum eosinophils and neutrophils, ICS dose and OCS maintenance, BMI, and asthma onset as variables independently linked to IRAO. Total TGF-β levels appeared higher in patients with IRAO (n = 38) compared to patients with RAO (n = 47).
Conclusion: These data show that risk factors for IRAO are male gender, smoking, a longer disease duration, uncontrolled asthma, eosinophilic or neutrophilic airway inflammation, lower BMI, and later asthma onset. Moreover, TGF-β levels are higher in IRAO.

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1. Introduction

Asthma is a chronic inflammatory airway disease commonly associated with reversible airway obstruction. On average, asthma patients have lower lung function than healthy individuals [1] and their lung function (FEV_1) decline can be greater over time [2,3]. Most of patients with mild to moderate asthma can be controlled with regular medication. However, asthma patients are at risk of developing structural changes resulting in persistent airflow limitations [1,2,4] despite anti-inflammatory therapies. The features of remodeling include sub-epithelial reticular basement membrane (RBM) thickening, hypertrophy and hyperplasia of airway smooth muscle (ASM) cells, angiogenesis and

goblet cell hyperplasia [5], responsible for airway narrowing. A small proportion of non-smoking asthmatics present with irreversible airway obstruction (IRAO) which can be considered another form of lung function decline in asthma [6]. IRAO is defined as a significantly reduced ratio between FEV_1 and FVC after bronchodilation [7,8].

Studies on risk factors and prevalence of IRAO in asthma are limited. Moreover, no consensus is reached on a definition of IRAO [9]. Yet, predictors for IRAO including smoking [10], longer disease duration [10,11], male gender [10,12], aspirin sensitivity [13], greater airway hyperresponsiveness (AHR) [14], less chronic rhinitis [15], adult onset [14], and FeNO [9,10,14] have been reported.

Persistent airway obstruction partly relates to airway remodeling the histological substrate of which is a sub-epithelial fibrosis [16] [–] [18]. TGF-β is known to be the prominent mediator in airway fibrosis but has been poorly investigated in the context of asthma with fixed airway

Abreviation list		FEV$_1$	forced expiratory volume in 1 s
		FVC	functional vital capacity
ACQ	asthma control questionnaire	ICS	inhaled corticosteroids
ACT	asthma control test	IgE	immunoglobulin E
AQLQ	asthma quality of life questionnaire	IRAO	irreversible airway obstruction
ATS	American Thoracic Society	LABA	long-acting ß2 agonists
BD	bronchodilation	LAMA	long-acting muscarinic antagonists
BEC	blood eosinophil count	LTRA	antileukotrienes
BNC	blood neutrophil count	OCS	oral corticosteroids
CRP	C-reactive protein	RAO	reversible airway obstruction
ERS	European Respiratory Society	TGF-β	transforming growth factor beta
FeNO	fractional exhaled nitric oxide		

obstruction [19]. The active TGF-β present at steady state is the biologically active form while total TGF-β1 is the active form plus the latent TGF-β liberated by acidification. Assessing both the active and latent TGF-β simultaneously is useful to assess how TGF-β is involved in the pathogenesis of the disease.

This study was undertaken in order to compare patients with irreversible airway obstruction to patients with reversible airway obstruction, and highlight predictors of incomplete reversibility of airflow obstruction in adult asthmatics to identify patients with poorer prognosis and improve their care, and decrease morbidity.

2. Methods

A retrospective study was conducted on adult asthmatics at stable state with post-bronchodilation (BD) spirometry measurements and successful sputum induction recruited from the University Asthma Clinic of Liege, Belgium.

Patients were allocated in two distinct groups based on post-BD FEV$_1$/FVC and post-BD FEV$_1$ measurements. Patients with IRAO (FEV$_1$/FVC<0.7 and FEV$_1$<80% predicted) were compared with patients with reversible airway obstruction (RAO) (post-BD FEV$_1$/FVC ≥0.7 and FEV$_1$≥80% predicted). Patients that did not fit into one of these categories (i.e. FEV$_1$/FVC <0.7 and FEV$_1$ ≥ 80% predicted or FEV$_1$/FVC ≥0.7 and FEV$_1$<80% predicted) were not included (Fig. 1).

All procedures were performed in the context of clinical practice and the retrospective data collection was conducted with approval from the ethics committee of CHU Liège (2005/181) in accordance to the Helsinki Declaration.

Quality of Life was assessed using self-administered Asthma Quality of Life Questionnaire (AQLQ) [20] and Asthma control by the Juniper Asthma Control Questionnaire (ACQ7) [21] and an Asthma Control Test (ACT) [22]. Subjects were characterized as atopic if they had at least one
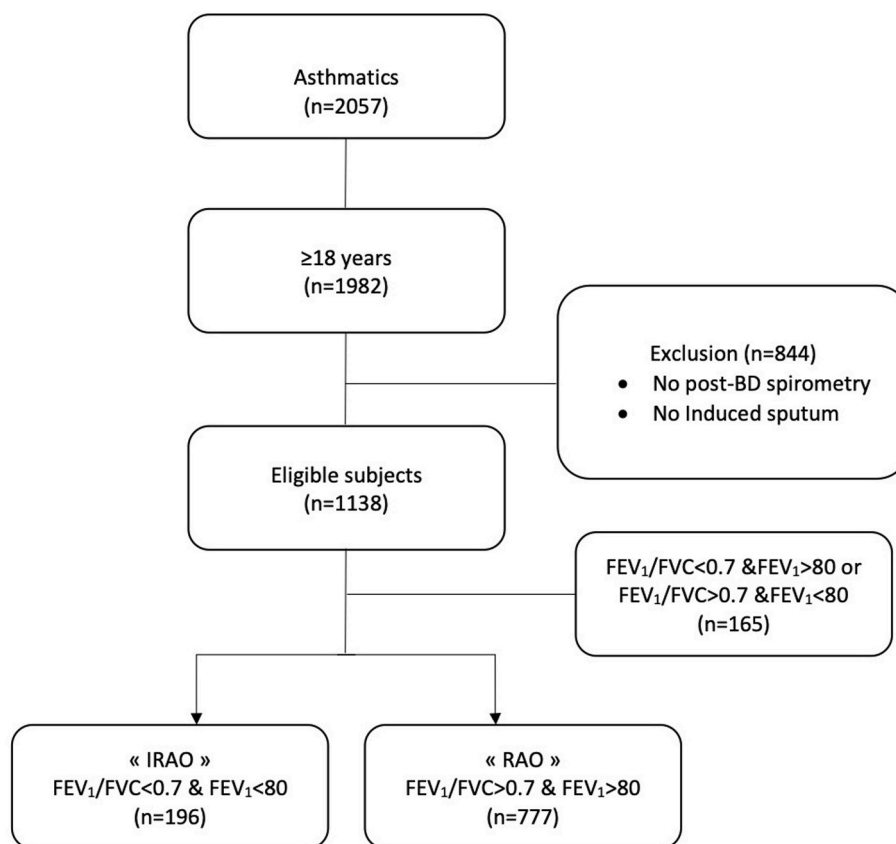

Fig. 1. Study design: FEV$_1$, forced expiratory volume in 1 s (% predicted); FVC, Forced vital capacity; post-BD, post-bronchodilation; RAO, Reversible airway obstruction; IRAO, irreversible airway obstruction.

positive specific Immunoglobulin E (IgE) test (0.35 kU.L-1; Phadia, Groot-Bijgaarden, Belgium) for at least one common aeroallergen.

Patients underwent Fractional exhaled Nitric Oxide (FeNO) measurements at flow rate of 50 mL/s according to the ERS/ATS recommendations [23] (NIOX, Aerocrine, Sweden) followed by spirometry with bronchodilation, sputum induction on the same day. Sputum induction and processing were performed as previously described [24] using the whole expectorate.

Cell counts were estimated on samples centrifuged (Cytospin) and stained with Hemacolor® Staining set after counting 500 non-squamous cells (Merck chemical, Overijste, Belgium). Sputum cytology was analyzed and 4 phenotypes were defined: the eosinophilic phenotype with 3% sputum eosinophil count (and < 76% neutrophil count), the neutrophilic phenotype with 76% sputum neutrophil count (and <3% eosinophil count), and the mixed granulocytic phenotype being a combination of the above [25]. The paucigranulocytic phenotype was defined as an inflammatory cell count below these thresholds.

Routine laboratory of the University Hospital of Liege performed blood cell count and analysis of C-reactive protein (CRP), fibrinogen, and total IgE levels (ImmunoCAP system (Phadia AB, Uppsala; Sweden)).

Inhaled corticosteroids (ICS) dosages and treatment with anti-leukotrienes (LTRA), Long-acting muscarinic antagonists (LAMA), Long-acting beta-2 agonists (LABA), Anti-IgE, anti-IL5 and oral corticosteroids (OCS) were recorded and used in the analyses.

In a subpopulation of 85 non-smoking, OCS naïve patients, with either an eosinophilic or a neutrophilic inflammatory phenotype, both active (present at steady state) and total (present at steady state & latent form liberated after acidification) Transforming growth factor beta (TGF-β) were measured in sputum supernatant [19]. Briefly, transformed mink lung cells (TMLC, gift of Daniel Rifkin, New York University medical center, NY) stably transfected with plasminogen activator inhibitor-1 (PAI-1) promoter fused to the firefly luciferase reporter gene, were cultured with sputum supernatant. To activate the latent TGF-β, the supernatants were incubated with 1 N H Cl for 10 min and neutralized by 1.2 N NaOH/0.5 M HEPES. The TMLC were cultured in DMEM with 10% of FBS and were plated at the density of 15,000 cells/well in a 96-well plate. The supernatants were then added (final dilution 4X) and incubated 16–20 h. Recombinant human TGF-β 1 (4 ng/ml, R&D Systems, Minneapolis, USA) were used as positive controls. Each condition was done with and without anti-hTGF-β 1 antibody (1 µg/ml, R&D Systems, Minneapolis, USA). Each sample was done in triplicate and results were expressed as relative light units.

2.1. Statistical methods

Variables independently associated to IRAO were identified by logistic regression. Independent variables such as atopy, gender, cigarette smoking (Pack Year), Body Mass Index, asthma onset, disease duration, FeNO, ACQ score, ICS dose, OCS maintenance, hospitalizations and exacerbations during the last 12 months, blood eosinophil (BEC) and blood neutrophil (BNC) counts (/mm^3), sputum eosinophils and neutrophils counts (%) were included in the univariate model. FEV_1/FVC <70 and FEV_1<80% predicted was used as the dependent variable. A multivariable analysis was done including all independent variables. In order to test the robustness of the analysis, the same logistic regression analysis was performed with the never-smoking patients. Factors affecting FEV_1/FVC ratio were evaluated with a conventional linear regression using the same independent variables as in the logistic regression. FEV_1/FVC ratio was used as the dependent variable.

We constructed receiver-operating characteristic (ROC) curves for all continuous variables independently associated with IRAO to determine the cut-off which best identified IRAO (FEV_1/FVC <0.7 and FEV_1<80% predicted) in asthma. Optimal cutoff points were determined by the method of the nearest point to (0,1). A p value < 0.05 was considered statistically significant. Statistical analysis was done using STATA

version 14.0 (Statistical Software, College Station, TX: StataCorp LP).

3. Results

3.1. IRAO patients' characteristics

In our database, 1138 patients recruited between January 2005 and March 2019 had post-BD spirometry measurement and successful sputum induction. Out of these, 973 of these patients were allocated in two distinct groups. A hundred and ninety-six asthmatics (17% of total population, 196/1138) presented with IRAO (FEV_1/FVC<0.7 & FEV_1<80% predicted) and 777 patients (68% of the total population 777/1138) with RAO (FEV_1/FVC ≥0.7 & FEV_1>80% predicted). Demographic, clinical and inflammatory characteristics of these patients are presented in Table.1. Patients with IRAO were significantly more often male (p = 0.034), older (p < 0.0001), more (ex)smokers (p < 0.0001), with a longer disease duration (p < 0.0001), were more poorly controlled (ACT and ACQ scores) (p < 0.0001) with a lower quality of life (AQLQ score) (p < 0.0001), treated with higher ICS daily dose (p < 0.0001) and more often with OCS maintenance (p < 0.0001), LABA (p < 0.0001), LAMA (p < 0.0001), and LTRA (p = 0.001). This subpopulation of asthmatics also presented more often with diffuse (blood and sputum) eosinophilic and neutrophilic inflammation (p < 0.0001), and significantly higher IgE (p = 0.007) and markers of inflammation (fibrinogen (p = 0.008), CRP (p = 0.02) and blood leukocytes (p < 0.0001)). Exacerbation and hospitalisation rates in the previous year (p < 0.001) were also higher in this sub-population.

3.2. Factors associated with IRAO

The univariate model of the logistic regression (Table.2) showed a positive association between FEV_1/FVC<0.7 & FEV_1<80% predicted and male gender (OR:0.71 (95%CI 0.52–0.97)), Pack-Year (1.77 (1.44–2.16)), disease duration (1.02 (1.02–1.04)), ACQ score (3.25 (2.71–3.91)), ICS dose (2.07 (1.78–2.42)), OCS maintenance (4.75 (3.12–7.22)), exacerbation (1.39; (1.24–1.56)) and hospitalisation rate (2.35 (1.70–3.25)) in the previous year, BNC (/mm^3) (1.0 (1.0–1.0)), BEC (1.41 (1.23–1.63)), and sputum eosinophil count (/mm^3) (1.36 (1.18–1.56)).

The multivariable analysis revealed male gender (OR female: 0.38 (95%CI 0.20–0.72)), pack-year (1.76 (1.19–2.61)), disease duration (1.06 (1.04–1.09)), ACQ score (2.63 (1.94–3.56)), sputum eosinophils (1.73 (1.22–2.47)), sputum neutrophils (1.53 (1.15–2.04)), ICS daily dose (1.60 (1.21–2.13)), BMI (0.70 (0.54–0.92)), age of onset (2.20 (1.27–3.83), and OCS therapy (2.67 (1.11–6.39)) (Table.2).

3.3. IRAO in never-smokers

In the never-smoking patients (n = 521) (Supplementary Table S1), seventy-six (15%) presented with IRAO compared to 445 (85%) with RAO. Results of the multivariable logistic regression showed ACQ (OR:2.27 (95%CI:1.47–3.48)), sputum eosinophils (1.85 (1.08–3.14)), disease duration (1.06 (1.02–1.09)) were independently associated with the outcome.

3.4. Factors affecting FEV_1/FVC ratio

The univariate analysis of the linear regression analysis (Table.3) revealed a negative association between FEV_1/FVC ratio and cigarette smoking (Pack Year) (Reg. coeff:-0.17 (95%CI:-0.21 to −0.13)), ACQ score (−4.26 (−4.80 to −3.71), BEC (−0.0017(-0.0031 to −0.0002), BNC (−0.0004 (−0.0006 to −0.0002)), sputum eosinophil count (%) (−0.13(-0.17 to −0.09)), ICS daily dose (−3.04(-3.63 to −2.43)), OCS maintenance therapy (−8.30(-10.6 to −5.99), exacerbation (−1.54 (-2.07 to −0.99)), and hospitalisation (−4.35(-5.74 to −2.96)) rates over the last 12 months, and disease duration (−0.15 (−0.19 to −0.096)). A

Table 1

Demographic, functional, inflammatory, and treatment characteristics.

Characteristics	RAO	IRAO	p-value
N. (%)	777/1138 (68)	196/1138 (17)	
Women (%)	478 (62)	104 (53)	0.034
Age (yr)	48 ± 16	56 ± 13	<0.0001
Height (cm)	168 ± 9	169 ± 10	0.6043
Weight (Kg)	75 ± 16	75 ± 16	0.9189
BMI (kg/m^2)	27 ± 5	26 ± 5	0.6017
Atopy (Y) (%)	416 (57) (n=730)	95 (52) (n=182)	0.278
Smoking status: (%)	(n=775)	(n=192)	<0.0001
Never Smokers	443 (57)	72 (38)	
Current Smokers	140 (18)	40 (21)	
Ex-Smokers	192 (25)	80 (42)	
Pack-years:			<0.0001
Current smokers	14 (7-26)	27 (11-41)	
Ex-smokers	14 (6-26)	19 (8-36)	
Age of onset (yr)	32 ± 22 (n=585)	32 ± 21 (n=158)	0.9756
Disease duration (yr)	16 ± 16 (n=585)	24 ± 19 (n=158)	<0.0001
Pre-BD FEV$_1$			
(L)	2.854 ± 0.809	1.559 ± 0.588	<0.0001
(% pred)	94 ± 14	54 ± 14	<0.0001
Post-BD FEV$_1$			
(L)	3.04 ± 0.84	1.69 ± 0.60	<0.0001
(% pred)	100 ± 12	58 ± 14	<0.0001
Post-BD FVC			
(% pred)	103 ± 13	81 ± 16	<0.0001
Post-BD FEV$_1$/FVC			
(% pred)	81 ± 7	58 ± 8	<0.0001
PC20M (mg/mL)	1.54 (0.18-13.13) (n=549)	0.64 (0.44-0.93) (n=17)	0.4120
Reversibility (%)	7.00 ± 8.41	9.53 ± 14.07	0.0013
ACT score	16.4 ± 5.1 (n=767)	12.8 ± 5.2 (n=188)	<0.0001
ACQ score	1.62 ± 1.02 (n=749)	3.08 ± 1.14 (n=185)	<0.0001
AQLQ score	4.73 ± 1.29 (n=766)	3.81 ± 1.29 (n=195)	<0.0001
FeNO (ppb)	23 (13-44) (n=762)	22 (13-44) (n=187)	0.6642
Sputum eosinophil count			
(% of non-squamous cells)	1.2 (0.2-7.8)	3.7 (0.5-26.5)	<0.0001
(/μL)	20.94 (0.68-150.53)	77.34 (8.96-788.76)	<0.0001
Sputum neutrophil count			
(% of non-squamous cells)	57.0 (35.4-77.2)	63.1 (35.4-83.0)	0.0698
(/μL)	711.5 (260.0-2060.0)	1297.0 (377.0-3626.0)	0.0002
Inflammatory phenotypes:			<0.0001
Paucigranulocytic	315 (41%)	34 (17%)	
Eosinophilic	252 (32%)	97 (50%)	
Neutrophilic	167 (21%)	55 (28%)	
Mixed granulocytic	43 (6%)	10 (5%)	
Total serum IgE (kU/L)	104 (34-295) (n=733)	139 (64-384) (n=177)	0.0072
Blood leukocytes (x 10^3/μL)	7.10 (5.88-8.39) (n=761)	8.43 (6.76-10.13) (n=186)	<0.0001
Fibrinogen (g/L)	3.23 (2.71-3.72) (n=734)	3.38 (2.87-3.93) (n=167)	0.0080
CRP (mg/L)	1.90 (0.84-4.54) (n=734)	2.44 (1.15-6.26) (n=167)	0.0190
Blood eosinophils	(n=761)	(n=185)	
(%)	2.4 (1.4-3.9)	3.0 (1.4-5.6)	0.0031
(/μL)	168 (92-283) (n=761)	249 (119-433) (n=185)	<0.0001
Blood neutrophils			
(%)	53.9 (47.8-60.8)	59.1 (51.7-65.9)	<0.0001
(/μL)	3761 (2935-4910)	4766 (3641-6374)	<0.0001
ICS dose (μg/d)	4 (0-1000)	1500 (800-2000)	<0.0001
ICS category: (%)			<0.0001
Steroid naïve	347 (47)	29 (11)	
Low dose	99 (13)	14 (7)	
Medium dose	135 (18)	40 (21)	
High dose	162 (22)	107 (56)	
LABA, N (%)	403 (52) (n=774)	167 (86) (n=195)	<0.0001
LTRA, N (%)	175 (23)	67 (34)	0.001
LAMA, N (%)	13 (2) (n=773)	53 (27) (n=195)	<0.0001

Table 1 (*continued*)

Characteristics	RAO	IRAO	p-value
OCS therapy, N (%)	55 (7) (n=773)	52 (27) (n=195)	<0.0001
Biotherapies:			0.055
Anti-IgE	8 (1)	5 (3)	
Anti-IL5	11 (1)	6 (3)	
Hospitalizations in previous year	0 (0-1) (n=664)	1 (0-2) (n=160)	<0.0001
Exacerbations in previous year	0 (0-0) (n=676)	0 (0-1) (n=170)	<0.0001

Comparison between RAO (Post-BD FEV$_1$/FVC≥0.7 & post-BD FEV$_1$≥80 predicted) and IRAO Post-BD FEV$_1$/FVC<0.7 & Post-BD FEV$_1$<80 predicted) asthmatics.

Data are presented as mean ± SD or median and IQR. PC20 M is presented as geometric mean (min-max). BMI, Body Mass Index; BD, bronchodilation; FEV$_1$, forced expiratory volume in 1s; FVC, Forced vital capacity; PC20 M, provocative concentration of metacholine causing a 20% fall in FEV$_1$; ACT, Asthma control test; ACQ, Asthma Control Questionnaire; AQLQ, Asthma Quality of Life Questionnaire; FeNO, fractional exhaled nitric oxide; ppb, parts per billion; CRP, C reactive protein; ICS, inhaled corticosteroid; LABA, Long-acting ß2-agonist; LTRA, Leucotriene receptor antagonist; LAMA, Long-acting muscarinic antagonist; OCS, oral corticosteroid; Low-dose ICS: <500 μg/d; moderate-dose ICS : >500–1000 μg/d; high-dose ICS : > 1000 μg/d beclomethasone dipropionate – chlorofluorocarbon.

positive association was observed for the female gender (2.48 (0.98–3.98)). The multivariable analysis (Table.3) showed that variables independently associated to FEV$_1$/FVC ratio were Body Mass Index (0.42 (0.26–0.58)), smoking (Pack-Year) (−0.11(-0.16 to −0.06)), female gender (3.26 (1.61–4.90)), ACQ (−2.57(-3.34 to −1.81)), BNC (−0.0002 (−0.001 to −0.00003)), ICS daily dose (−1.11 (−1.87 to −0.35)), hospitalisation rate over the last 12 months (−2.04 (−3.81 to −0.26)), disease duration (−0.23 (−0.29 to −0.17)), sputum eosinophil count (−0.08 (−0.14 to −0.02)), and age of onset (−3.39 (−4.88 to −1.90)).

3.5. Roc curves

Constructing a ROC curve, revealed that the ACQ score was able to identify IRAO with the best cut-off point of 2.36 providing a % 72 sensitivity and 77% specificity (AUC 0.8260, p < 0.0001, Fig. 2). We also tested other potential markers for IRAO, such as FeNO, disease duration, sputum neutrophil count, sputum eosinophil count, and BNC, but found AUC of 0.4898 (p = 0.6642), 0.6326 (p < 0.0001), 0.6133 (p = 0.0698), 0.5419 (p < 0.0001), and 0.6542 (p < 0.0001) respectively. These markers are not able to discriminate between IRAO and RAO.

3.6. TGF-β activation levels

TGF-β levels were measured in 85 asthmatics. Thirty-eight patients with IRAO were compared to 47 patients with RAO. In the IRAO group, 21 patients presented with eosinophilic asthma and 17 were classified as having neutrophilic asthma. Twenty-four patients were eosinophilic and 23 were neutrophilic in the RAO group. These 85 patients presented with the same characteristics as the total population in this study (Supplementary Table S2).

Active TGF-β levels were not different in IRAO and RAO groups (p = 0.2775). Total TGF-β levels were significantly higher in IRAO compared to RAO group (p = 0.0363) (Fig. 3 & Supplementary Table S3).

4. Discussion

In a general population of asthmatics, we found that risk factors for fixed airway obstruction are: male gender, smoking (pack-year), longer disease duration, poor asthma control, sputum eosinophils and neutrophils, ICS daily dose, BMI, later onset, and OCS therapy. Focusing on non-smoking patients we confirmed that ACQ score, disease duration

Table 2
Factors associated with IRAO. Results of the logistic regression *N = 973.

IRAO	Total population					
	UNIVARIATE			MULTIVARIABLE		
	OR	95%CI	P-value	OR	95%CI	P-value
Gender (M)	0.71	0.52–0.97	0.031	0.38	0.20–0.72	0.003
BMI	1.05	0.91–1.21	0.505	0.70	0.54–0.92	0.010
Age of onset	0.99	0.81–1.21	0.911	2.20	1.27–3.83	0.005
Pack-Year	1.77	1.44–2.16	<0.0001	1.76	1.19–2.61	0.005
Atopy	0.82	0.60–1.14	0.245	0.84	0.43–1.64	0.617
ACQ	3.25	2.71–3.91	<0.0001	2.63	1.94–3.56	<0.0001
ICS	2.07	1.78–2.42	<0.0001	1.60	1.21–2.13	0.001
OCS	4.75	3.12–7.22	<0.0001	2.67	1.11–6.39	0.028
BEC	1.41	1.23–1.63	<0.0001	1.12	0.82–1.53	0.468
BNC	1.0	1.0–1.0	0.023	1.00	0.99–1.00	0.272
SEC	1.36	1.18–1.56	<0.0001	1.73	1.22–2.47	0.002
SNC	1.10	0.96–1.26	0.148	1.53	1.15–2.04	0.004
FeNO	0.97	0.83–1.13	0.681	0.74	0.54–1.02	0.067
Exacerbations	1.39	1.24–1.56	<0.0001	0.97	0.78–1.20	0.776
Disease duration	1.02	1.02–1.04	<0.0001	1.06	1.04–1.09	<0.0001
Hospitalizations	2.35	1.70–3.25	<0.0001	1.12	0.60–2.07	0.720

IRAO, irreversible airway obstruction, Post-BD FEV_1/FVC<0.7 & post-BD FEV_1<80 predicted. Number of observations = 571. OR, odds ratio; CI, confidence interval; BMI, Body Mass Index (categories: ≤23; >23 to ≤26; >26 to ≤30; >30); FeNO, fractional exhaled nitric oxide (categories: ≤ 12; >12 to ≤ 25; >25 to ≤50; >50); ACQ, Asthma Control Questionnaire; ICS, inhaled corticosteroids (categories: Naïve; 0 to≤ 500; >500 to ≤ 1600; >1600); OCS, oral corticosteroids; Pack-Year categories: ≤0; >0 to ≤20; >20); Age of onset (categories: ≤12; >12 to ≤40; >40); BEC, blood eosinophil count (categories: ≤150; >150 to ≤300; >300 to ≤400; >400); SEC, sputum eosinophil count (categories: ≤0.2; >0.2 to ≤3; >3 to ≤10; >10); SNC, sputum neutrophil count (categories: ≤38; >38 to ≤59; >59 to ≤76; >76).

Table 3
Results of the linear regression model *N = 973.

FEV_1/FVC	Total population*					
	UNIVARIATE			MULTIVARIABLE		
	Coeff.	95%CI	P-value	Coeff.	95%CI	P-value
Gender (M)	2.48	0.98 to 3.98	0.001	3.26	1.61 to 4.90	<0.0001
BMI	0.08	−0.07 to 0.23	0.308	0.42	0.26 to 0.58	<0.0001
Age of onset	0.02	−0.92 to 0.95	0.970	−3.39	−4.88 to −1.90	<0.0001
Pack-Year	−0.17	−0.21 to −0.13	<0.0001	−0.11	−0.16 to −0.06	<0.0001
Atopy	1.04	−0.49 to 2.57	0.182	0.52	−1.28 to 2.31	0.572
ACQ	−4.26	−4.80 to −3.71	<0.0001	−2.57	−3.34 to −1.81	<0.0001
ICS	−3.04	−3.63 to −2.43	<0.0001	−1.11	−1.87 to −0.35	0.004
OCS	−8.30	−10.6 to −5.99	<0.0001	−2.74	−5.59 to 0.11	0.060
BEC	−0.0017	−0.0031 to −0.0002	0.022	0.001	−0.003 to 0.004	0.756
BNC	−0.0004	−0.0006 to −0.0002	<0.0001	−0.0002	−0.001 to −0.00003	0.048
SEC	−0.13	−0.17 to −0.09	<0.0001	−0.08	−0.14 to −0.02	0.007
SNC	−0.02	−0.05 to 0.004	0.102	−0.03	−0.07 to 0.001	0.060
FeNO	−0.13	−0.84 to −0.57	0.705	0.31	−0.55 to 1.16	0.481
Exacerbations	−1.54	−2.07 to −0.99	<0.0001	0.03	−0.67 to 0.74	0.922
Disease duration	−0.15	−0.19 to −0.096	<0.0001	−0.23	−0.29 to −0.17	<0.0001
Hospitalizations	−4.35	−5.74 to −2.96	<0.0001	−2.04	−3.81 to −0.26	0.024

Number of observations = 319. OR, odds ratio; CI, confidence interval; BMI, Body Mass Index; FeNO, fractional exhaled nitric oxide (categories: ≤ 12; >12 to ≤ 25; >25 to ≤50; >50); ACQ, Asthma Control Questionnaire; ICS, inhaled corticosteroids (categories: Naïve; 0 to≤ 500; >500 to ≤ 1600; >1600); OCS, oral corticosteroids; BEC, blood eosinophil count; BNC, blood neutrophil count; SEC, sputum eosinophil count; SNC, sputum neutrophil count.

and sputum eosinophils are associated with IRAO. We also found that total TGF-β levels were significantly higher in IRAO.

There were significantly more ex-smokers in the IRAO group. Smoking was also a risk factor for IRAO and responsible for a decrease in the FEV_1/FVC ratio in the multivariable analyses, and seems to be a major risk factors for IRAO [9,26,27]. Cigarette exposure is responsible for neutrophilic inflammation and neutrophil apoptosis leading to damage-associated molecular pattern (DAMPs), which may amplify smoking induced airway inflammation [28,29]. Due to this neutrophilic inflammation in smokers or changes in glucocorticoid receptor sensitivity, treatment regimens for smoking asthma require higher doses of inhaled corticosteroids [30]. Cigarette smoking also contributes to severity and exacerbations. This might explain why sputum neutrophil count, ICS doses and OCS therapy were not independently associated to IRAO anymore in the never smoker population. One might be tempted to assume IRAO is simply due to smoking habit in our IRAO population.

However, the sub-analysis on never smokers with IRAO confirmed that ACQ score, sputum eosinophils and disease duration are also associated with irreversible airway obstruction. According to guidelines [31], it is required to treat comorbidities such as smoking habit in order to prevent lung function decline in asthma. Since therapy for remodeling is not on the market yet, this study adds evidence to the fact that quitting smoking is clearly a first step to help these patients.

Patients presenting with neutrophilic inflammation represent only one quarter of our study population while 50% had an eosinophilic phenotype. These rates are similar to what has been reported in severe asthma [32] that can be defined by FEV_1<80% predicted. In our study IRAO occurred almost twice more often in patients with increased percentages of sputum eosinophils. Eosinophilic airway inflammation has the potential to induce airway remodeling [33] and is present in 41% of a general population of asthmatics [34]. We previously found that patients exhibiting elevated blood and sputum eosinophils were
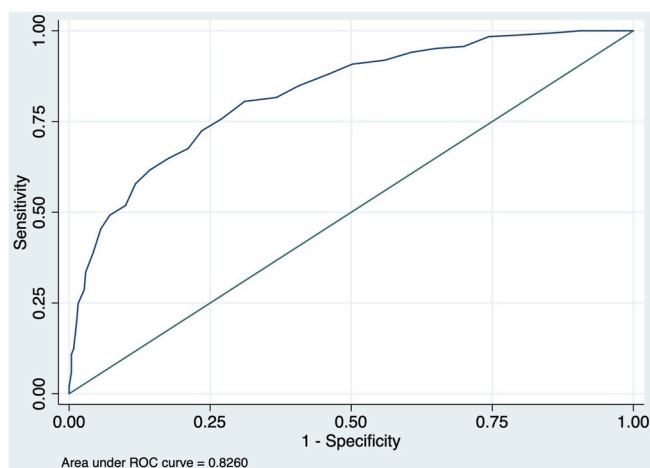
Fig. 2. ROC curve showing the best cut-off of ACQ score to identify IRAO in asthma. Sensitivity 72%, specificity 77%, cut-off: 2.36, p < 0.0001, AUC: 0.8260.
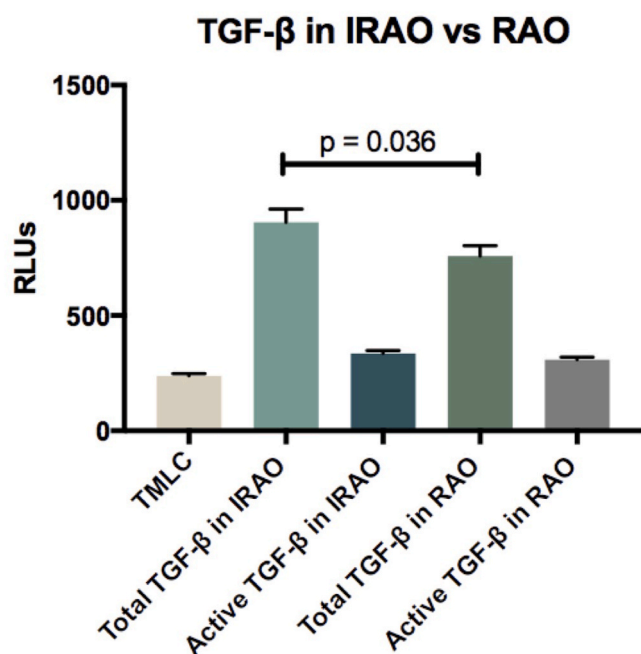

Fig. 3. Comparison of (total and active) TGF-β levels in IRAO and RAO. RAO, Reversible airway obstruction; IRAO, irreversible airway obstruction.

characterized by poorer lung function [35]. In our study, blood eosinophil count was not associated with IRAO. Indeed, eosinophils must be attracted into the airways to induce remodeling [35]. Moreover, sputum eosinophil but not blood eosinophil counts were predictors of improvement of lung function with anti-IL5 therapy in severe eosinophilic asthmatics [36]. Eosinophils are the main source of TGF-β, a cytokine with profibrotic properties. We confirmed Cianchetti [37]'s results showing that TGF-ß identified a subgroup of severe asthmatic patients with IRAO. In studies evaluating efficacy of Mepolizumab (Anti-IL5), they found TGF-β expression in eosinophils and signs of airway remodeling are reduced in parallel [38]. TGF-β is one of the main factors implicated in airway remodeling in asthma. We found that total TGF-β levels were significantly higher in IRAO. Only a small fraction of TGF-β were biologically active in our samples and did not differ between groups. What differed was the pool of latent TGF-β as total TGF-β (which represent the active and latent TGF-β) was significantly different. The

airways may serve as a reservoir of latent TGF-β in the condition of IRAO representing a pool of "utilizable" TGF-β insuring a ready source for local activation. Indeed, TGF-β in the latent complex is the predominant form of the molecule and was shown to possess an extended plasma half-life compared with active TGF-β [39].

To our knowledge, this is the first report using this gene reporter assay in a context of IRAO and sputum samples.

Though a higher ACQ score is certainly a consequence of IRAO, we think it is interesting to mention that IRAO can be suspected in a patient with a poor asthma control. Indeed, remodeling induces reduction in airflow calibre, and more asthma symptoms [5]. ACQ score can be obtained easily and could be very useful for general practitioners to perform in order to find out if the patient is presenting with IRAO (cut-off point of 2.36) in which case, IRAO can be suspected and this patient must be sent to a pulmonologist to exclude residual type 2 inflammation in the absence of which overtreatment with ICS can be avoided [40]. As previously shown, men have a higher risk of being IRAO than women [14,27,41]. Indeed, males tend to present with fewer symptoms, are poorer perceivers, despite active eosinophilic inflammation, thus tend to be less compliant to their medication [42]. Not surprisingly, disease duration is associated with IRAO [9].

One limitation of this study is that we based our definition of IRAO on BTS [43]/NICE [44] guidelines. Using the lower limit of normal (LLN) FEV_1/FVC ratio reduces the misclassification of airway obstruction [45]. Unfortunately, visits prior 2018 did not include LLN values in our database. Since we purposely eliminated patients that did not fit in either IRAO nor RAO group, our patients in the IRAO group are truly asthmatics with persistent airway obstruction characteristics. As a tertiary care centre, the prevalence of IRAO might have been overestimated in our study.

5. Conclusion

We were able to show many risk factors independently associated to IRAO all together, previously mentioned separately in different studies. Risk factors for IRAO in a general population of asthmatics are male gender, smoking, a longer disease duration, uncontrolled asthma, eosinophilic or neutrophilic airway inflammation, lower BMI, and later asthma onset. Not surprisingly, sputum eosinophils are biomarkers for remodeling as they reflect local inflammation as opposed to blood eosinophils which reflect systemic inflammation. Moreover, TGF-β levels are higher in IRAO.

Disclosure

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.rmed.2020.106202.

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