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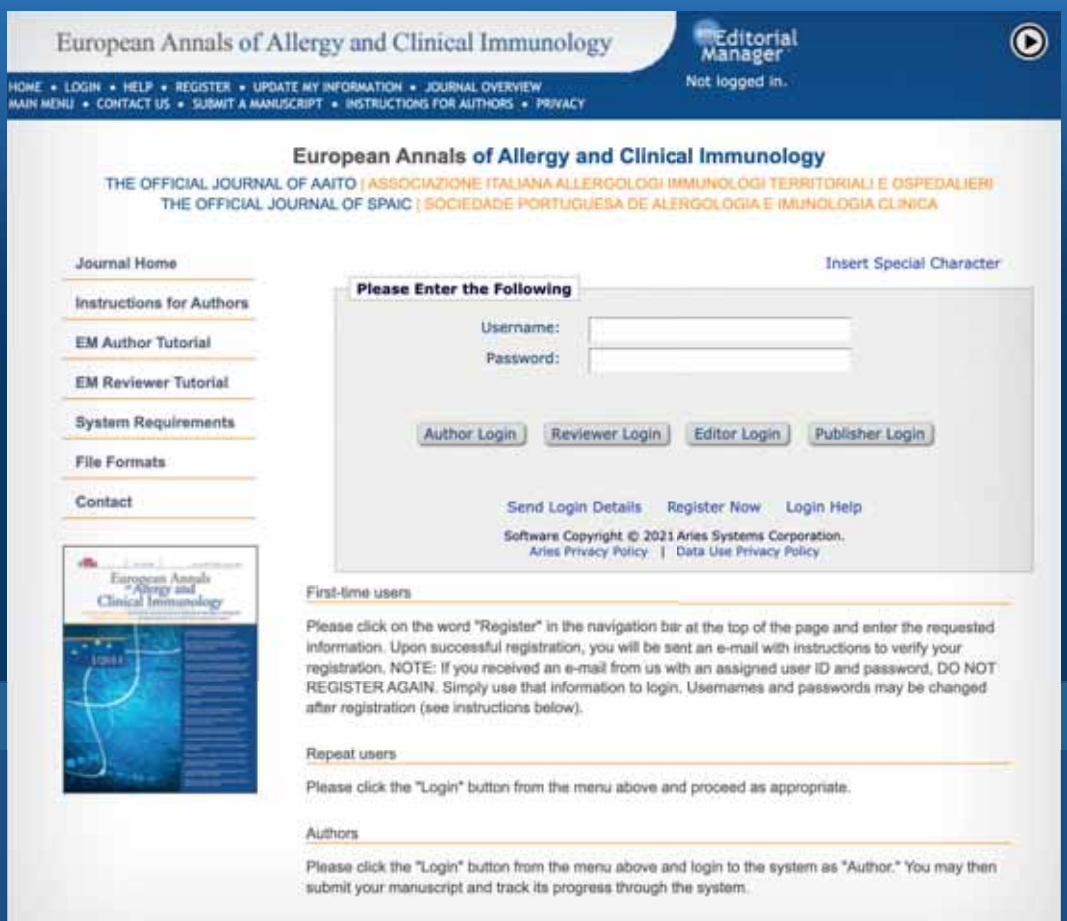

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Errata corrige

In the printed version of European Annals of Allergy and Clinical Immunology Volume 53 n. 2/2021 of March 2021, figure 2 mentioned at page 76 misses (Corresponding author: D. Çağdaş).

Editorial Office apologizes to the readers for the inconvenience.

Figure 2 *- Bullous skin eruption in the distal part of right upper extremity of P6, occured one time before extremity angioedema.*

S. Maio[1], S. Baldacci[1], L. Cecchi[2], G. Viegi[1,3]

The severe asthma registries: a way to better know and fight the disease

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Asthma is a heterogeneous disease, generally characterized by chronic inflammation. Severe asthma (SA) affects 5-10% of the asthma population, with the highest values in adolescents (1). The Global Burden of Disease study reported 495,000 asthma deaths worldwide in 2017 (2). In Europe, the mortality rate from asthma can vary according to the disease severity, reaching values of 11.3-14.8/1000 person-years in severe asthmatics (3). According to the European Lung White Book, annual direct costs for asthma (*i.e.*, due to treatment) and indirect costs (*i.e.*, due to the worsening of the quality of life and work disability) were 19.5 billion and 14.4 billion euros at European level in 2011. Individual total annual cost for asthma was € 3400 (4), with about 50% due to SA or difficult to treat asthma (1, 5).

The interest of the international scientific community towards SA has increased in the last decades due to its high burden in terms of direct and indirect costs; moreover, there is the need to better understand the mechanisms underlying the severity of asthma and resistance to therapy through a better characterization of the disease itself (6).

Many countries have developed regional and/or national disease registries providing valuable information on country-specific epidemiological patterns, natural history, progression, impact and therapeutic risks and benefits (1).

In 2020, the International Severe Asthma Registry (ISAR) was established, as the first global registry for SA in adults. It is a joint initiative where national registries retain ownership of the data, sharing data in ISAR for research purposes. Its strength comes from the collection of patient-level, anonymous, longitudinal, real, standardized and high-quality data from countries around the world, with the aim of assessing existing knowledge, generating new knowledge and identifying gaps to be filled, thus promoting new lines of research (1). ISAR currently contains data on more than 10000 patients from over 24 countries, including Italy.

In Italy, since 2010 registries and initiatives for the monitoring of SA have been developed such as the Italian Registry for severe/uncontrolled asthma (RitA) (7), the Italian network for severe asthma (SANI) (8), the Italian Registry on Severe Asthma (IRSA) (9) and the Italian Network on Pediatric Severe Asthma (IPSAN) (10).

Briefly, the RitA Registry was implemented within the AGAVE ("Severe Asthma: epidemiological and clinical cohorts follow up by registry and questionnaires; therapeutic appropriateness and outcome assessment, according to GINA guidelines") project (2010-2014), funded by AIFA (Agenzia Italiana del Farmaco, the Italian drugs agency) in order to assess the feasibility and usefulness of a SA registry in Italy. It is to point out that the study was carried out when Omalizumab was the only available biological drug for asthma treatment. The aim was to evaluate the appropriateness of different therapeutic strategies and to obtain longitudinal information on subjects with SA and uncontrolled asthma, selected from general and clinical populations (children and adults) at national level. The first published results regarded 493 clinical patients (7). Data about follow-up will be soon available. More recently, results about the longitudinal asthma patterns in 452 subjects from the general population sample were published (12).

SANI was established in 2017 and it currently consists of 64 clinical centers spread throughout the country. This project is supported by the Global Initiative for Asthma (GINA), the Italian Society of Allergy, Asthma and Clinical Immunology (SIAAIC), the Italian Society of Pneumology (SIP / IRS) and the FederAsma e Allergie Onlus - Italian Patients Federation. SANI also collaborates with the European SHARP and global ISAR projects. The goal of the network is to create an observatory for the monitoring of SA in patients over 12 years of age. Particular attention is paid to the follow-up of patients in order to assess the natural history of the disease, the cost/benefit of new biological products, the adherence to therapy and the presence of particular disease biomarkers (8). 698 patients were enrolled by March 2019 (11).

Bilò *et al*. published in this issue of the Journal the findings about the most recent and currently largest registry in Italy, the IRSA (9). IRSA was implemented in 2017 and supported by the Italian Association of Hospital Pulmonologists (AIPO) and by the Italian Association of Hospital and Territorial Allergists and Immunologists (AAIITO). IRSA aimed to collect data in SA patients (≥ 14 years of age) during a 5-years period in a real-life setting. It involves 71 Units of Allergy and Pulmonology all over the country. In particular, the registry arose from the need to understand the phenotypes of asthma refractory to standard therapies, collecting information on the epidemiological, clinical and therapeutic aspects relating to the natural course of the disease, bridging the gap between adolescents and adults (13).

Comparing the data coming from these 3 Italian initiatives (**table I**), it clearly emerges that the descriptive characteristics of the patients are widely comparable, even if in RiTA there are more obese subjects and in IRSA more smokers. Comorbidities data are also comparable, with the exception of allergic rhinitis and atopy, more frequent in RiTA and of nasal polyps and sinusitis in IRSA. The RiTA patients appear to have less exacerbations and health services access, a lower obstructive pattern, lower FENO and IgE values, but higher eosinophilia values. Finally, as regard drugs, in SANI there is a higher use of oral corticosteroids and a lower use of anti-leukotrienes. The highest percentage of patients using Omalizumab was found in RiTA (64.1%) (due to the inclusion criteria), then SANI (57%) and IRSA (32.2%). SANI patients were treated with Mepolizumab (11.2%), IRSA patients with Mepolizumab (28.2%) and Benralizumab (4.1%). Thus, overall, the enrolled SA patients are characterized by similar features, with some variability that may be due to the different peculiarities of the involved clinical centers.

As reported by Bilò *et al*. (9), the presence of multiple registries at national level might be interpreted as an overlap and a limitation; on the contrary, it may represent an opportunity to increase the number of cases, widen the spectrum of information, and check their homogeneity.

Only a joint effort, also in line with what has already been done in the context of ISAR worldwide, can allow pooling the data thus covering a larger part of the national territory, with more cases, more comprehensive information and more precise estimates. Such a national collaboration would strengthen the monitoring of SA patients, leading to a better comprehension of the epidemiological, clinical, inflammatory and functional characteristics of these patients, and of the treatment efficacy (including the new biological drugs), in order to effectively counteract SA with its elevated socio-economic burden.

Table I *- Comparison among patients' characteristics of IRSA (9) registry, SANI network (8) and RiTA registry (7).*

	IRSA	**SANI**	**RiTA**
N	851	437	493
Females (%)	61.1	57.2	60.6
Age (mean ± SD), yrs	54.8 ± 13.8	54.1 ± 13.7	53.8 ± 13.4
Age at symptoms' onset (mean ± SD), yrs	29.0 ± 16.7	32.4 ± 17.1	30.2 ± 16.8
Late asthma diagnosis (> 40 yrs) (%)	---	38.2	37.6
Late asthma symptoms (> 40 yrs) (%)	25.0	---	29.8
BMI (mean ± SD), Kg/m^2	26.6 ± 5.0	26.2 ± 5.0	27.3 ± 5.0
BMI groups (%):			
underweight/normal	39.5	45.1	35.4
overweight	40.9	35.0	38.2
obese	19.6	19.9	26.4
Smoking habits (%):			
smoker	6.3	2.7	2.8
ex-smoker	21.4	20.1	33.2
no smoker	72.3	77.2	64.0
Allergic rhinitis (%)	---	44.6	62.4
Bronchiectasis (%)	---	16.0	13.9
Nasal polyps (%)	42.7	---	30.2
Sinusitis (%)	51.8	---	37.9

→

	IRSA	SANI	RiTA
CRSwNP (%)	---	42.6	---
GERD (%)	43.5	---	42.1
Aspirin intolerance (%)	16.1	---	22.0
Psychic disorders (%)	8.9	---	9.2
Atopy (%)	73.1	70.7	81.9
Occupational related asthma (%)	6.0	---	4.8
At least one exacerbation last 12 months (%)	83.1	82.6	55.7
At least one hospitalization last 12 months (%)	17.9	---	7.3
At least one ED visit last 12 months (%)	23.6	---	9.7
ACT score (mean ± SD), pts	17.2 ± 4.9	17.2 ± 5.4	19.4 ± 4.6
FENO level > 25 ppb (%)	---	59.1	48.3
FEV_1% pred (mean ± SD)	70.8 ± 19.9	71.4 ± 20.2	75.1 ± 20.5
FVC% pred (mean ± SD)	86.4 ± 18.3	85.7 ± 21.1	91.3 ± 19.8
FEV_1/FVC% (mean ± SD)	69.5 ± 15.3	65.3 ± 14.2	70.0 ± 31.0
Blood Eosinophils level ≥ 300 (mm^3) (%)	53.7	58.8	71.2
Total IgE (median, IQR) (kU/l)	---	---	323.0 (152.5-598.5)
Total IgE (mean ± SD) (kU/l)	448.2 ± 930.6	470.3 ± 812.9	---
ICS + LABA (%)	94.2	100	93.6
Omalizumab (%)	32.2	57.0	64.1
Mepolizumab (%)	28.2	11.2	---
Benralizumab (%)	4.1	---	---
Oral corticosteroids (%)	31.8	64.1	16.0
LTRAs (%)	51.9	46.4	53.3
LAMA (%)	39.1	35.7	---

SD: standard deviation; BMI: body mass index; CRSwNP: chronic rhinosinusitis with nasal polyps; GERD: gastroesophageal reflux disease; ED: emergency department; ACT: asthma control test; FENO: fractional exhaled nitric oxide; FEV1% pred percentage of predicted values of forced expiratory volume in the first second; FVC% pred: percentage of predicted values of forced vital capacity; IgE: immunoglobulin E; IQR: interquartile range; ICS: inhaled corticosteroids; LABA: long-acting beta agonists; LTRAs: leukotriene receptor antagonists; LAMA: long-acting muscarinic antagonist.

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Severe asthma management in the era of biologics: insights of the Italian Registry on Severe Asthma (IRSA)

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Key words
Asthma; biologics; eosinophils; IgE; registry.

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Summary
Background. *The Italian Registry on Severe Asthma (IRSA) is the most recent and largest registry in Italy.* ***Objective.*** *To improve the knowledge on the clinical and biological features of severe asthma (SA), and to monitor its treatments.* ***Methods.*** *To analyze clinical, functional, inflammatory, and treatment characteristics of severe asthmatics from the IRSA registry.* ***Results.*** *851 subjects were enrolled. 31.8% and 64.5% of patients were submitted to oral corticosteroids (OCS), and monoclonal antibodies (MABs), respectively. At least two comorbidities affected 77.4% patients. Asthma was uncontrolled in 62.2% patients. Uncontrolled patients had a higher frequency of exacerbations, and hospitalization, showing a higher eosinophilic phenotype, a greater use of OCS, and being treated with MAB less frequently. However, uncontrolled patients treated with MAB had a lower use of OCS and a lower rate of hospitalization. Comparing SA patients with atopy and without atopy, the latter showed a greater use of OCS, and more frequent nasal polyposis and osteoporosis. Among SA patients with atopy treated with MAB, 36% were on a treatment targeting the IL-5 pathway.* ***Conclusions and clinical relevance.*** *This study shows the features of the greatest Italian registry of SA patients, revealing at the time of enrollment a poor disease control, and the use of OCS and MABs in about one third and two thirds of patients, respectively. SA is a complex disease that requires a more precise phenotyping and a greater disease control.*

Introduction

Asthma is a chronic and heterogeneous respiratory disease affecting 1-21% of the population in different countries (1). Even though most of the asthmatic patients are successfully managed according to the acknowledged model of steps therapy, the subset of them affected by severe asthma (SA) can represent a challenge in the medical practice (2, 3).

Asthma represents a major economic issue worldwide (4, 5). Direct medical expenditures (DMEs), represented by pharmacological treatment, account for 37.5% of total cost per patient, being the indirect non-medical costs (INMCs) the remaining 62.5% (6). SA patients can be held accountable for most of both INMCs and DMEs expenditure. With the introduction of the new biologics, their benefit must be weighed against their costs, not just for individual patients but also for the society (7, 8). Severe, uncontrolled asthma is related to a large proportion of the burden of the disease (9-11).

Moreover, although a wide range of therapeutic options is available, the management of SA frequently remains complex because of the well-known differences in phenotypes and clinical outcome (3, 12, 13).

Several European and International registries on severe asthma, as a source of real-world data for asthma management, have tried to address these issues (14-24).

In 2017 the Italian Association of Hospital Allergists and Immunologists (AAIITO) and the Italian Thoracic Society (ITS–AIPO) proposed the institution of the Italian Registry on Severe Asthma (IRSA), aimed to collect data in SA patients during a 5-year period in a real life setting (25).

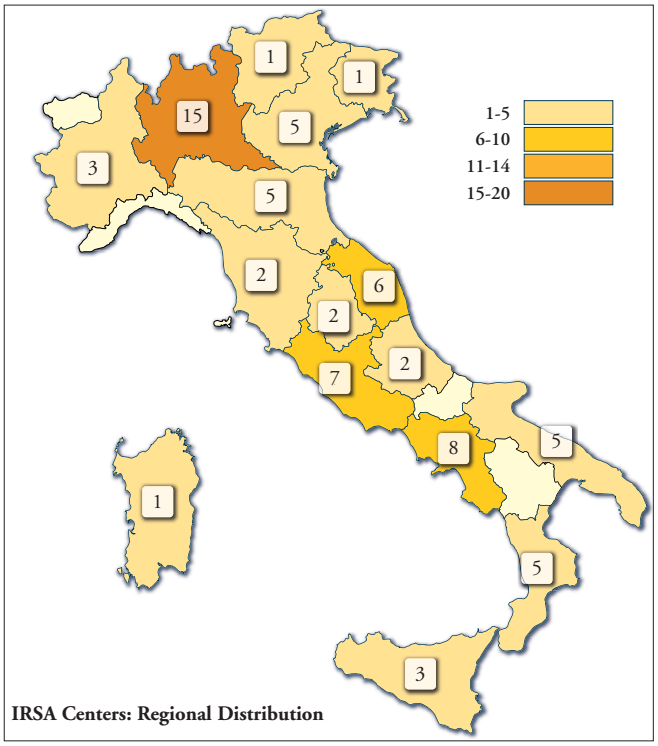
The present analysis of the IRSA data focuses on patients' characteristics in general (lung function, inflammatory and allergic indices, co-morbidities, treatment choices, and asthma control) as well as on specific subgroups of patients at the time of enrollment.

Methods

In this cross-sectional study, eligible patients were consecutively enrolled to the registry by 71 Units of Allergy and Pulmonology well distributed all over the country (**figure 1**) with expertise in managing SA, from March 2018 to July 2019. The planned length of follow-up is 5 years; patients attend the Units several times during the year, with a scheduled study visit every 12 months.

As in Italy no national accreditation system for SA Centers does exist, self-referenced accreditation criteria are at the moment only arbitrary; however, the prescription of biologics is accredited by AIFA (the Italian Medicines Agency of the National Health Care System) and applied at regional level. For these reasons, only centers authorized to prescribe biologics were included in the IRSA.

Figure 1 *- IRSA Centers: regional distribution.*



Enrolled patients were male or female ≥ 14 years of age, with a diagnosis of SA according to the Global Initiative for Asthma (GINA) guidelines (3).

The specialists collected information for each patient on demographics, risk factors, comorbidities, pharmacological treatments, and other functional and clinical data (25). Data were collected on the electronic Case Report Form (eCRF) and registered in the electronic database developed by CINECA (Bologna, Italy, www.cineca.it), a no-profit Consortium made up of 70 Italian Universities, 8 Italian Research Institutions and the Italian Ministry of Education, operating in the management and development of web-based services. All the eCFR were stored online in the central database for data processing and analysis performed on aggregated data.

The study was approved by the Ethical Committee of each centre participating to the registry (positive evaluation of the Central Committee nr. 568-112017 – November 10, 2017).

Statistical analyses

We conducted descriptive data analyses by tabulating frequencies and percentages (for categorical variables) and mean values, median values, and standard deviations (SD, for continuous variables). For the analysis of comorbidities, descriptive data were also examined graphically through histograms. With reference to comparison between groups (*i.e.*, patients with controlled *vs* non-controlled asthma; patients with atopic SA *versus*

SA without atopy; different treatment groups), categorical data were analyzed using the contingency table analysis with the Chi-square or Fisher's exact test, as appropriate, whereas continuous data were analyzed using a Student's t test, after checking whether data were normally distributed (based on the Shapiro-Wilk statistic), or a Wilcoxon rank-sum test otherwise. All tests were two-sided and a p-value of less than 0.05 was reported as significant. Data analyses were conducted using SAS version 9.4 (SAS Institute, Cary, NC, USA) statistical software.

Results

General, functional and biological characteristics

Eight hundred fifty-one patients were enrolled to the registry. Shortly, most were female (61.1%), being the mean age and the mean Body Mass Index (BMI) 54.8 years and 26.6 kg/m^2, respectively (**table I**). Most have never smoked, while passive smoking was reported in more than 20% of the subject (**online supplements table IS**).

The mean age for asthma symptoms onset was 29 years, the age being > 40 years in 25% of subjects. Patients were frequently atopic (73.1%), meaning at least one sensitization towards the most common Italian triggers of respiratory allergy.

At pre-bronchodilator assessment, the ratio between forced expiratory volume in the first second (FEV_1) and forced vital capacity (FVC) (FEV_1/FVC), the FEV_1 percentage predicted (FEV_1% pred.), and the FVC% pred. showed a mean value of 69.5%, 70.8%, and 86.4%, respectively (**table I**). FEV_1% pred. ≤ 70% was registered in 53% of the patients (**online supplements table IS**). The mean FEV_1 at baseline were 1.98 L (SD ± 0.83) and after short-acting bronchodilator 2.24 L (SD ± 0.90) (78.6 ± 20.6%).

A peripheral blood eosinophil count > 300 mm^3 was reported for 53.7% of cases, with a mean of 563.4 (SD ± 1983.4) (**table I**). A mean value of 448.2 Ul/ml (SD ± 930.6) of total immunoglobulin E (IgE) was found.

Treatments

At the time of enrollment, 802 patients (94.2%) were on treatments with a combination of inhaled corticosteroids (ICS) and long-acting β2-Agonists (LABA) (**table II**); in remaining patients, high dose of ICS plus other controllers or, in few cases, high dose of ICS plus LABA in two different devices were used. In addition to ICS ± LABA, montelukast, tiotropium, and theophylline were used in 51.9%, 39.1% and 4.9% of cases, respectively. Oral corticosteroids (OCS) were administered to 31.8% of the patients (in 62.1% of them for more than three months). Monoclonal antibodies (MAB) were administered in 64.5% of patients (omalizumab, mepolizumab and benralizumab). Other treatments are listed in **table II**.

According to patients treatment association four groups were identified: 1) high dose of ICS + LABA (single or combined): 4.2%; 2) ICS and LABA + other drugs excluding long term (> 3 months)

***Table I** - Characteristics of IRSA 851 patients.*

General characteristics	**Values**
Female (%)	61.1
Age, years (mean ± SD)	54.8 ± 13.8
BMI, kg/m^2 (mean ± SD)	26.6 ± 5.0
BMI groups (%) - Overweight - Obese	 40.9 19.6
Active smokers (%)	6.3
Former smokers (%)	21.4
Age at symptoms' onset (mean ± SD) y	29.0 ± 16.7
Age groups at symptoms' onset y (%) - ≤ 40 - > 40	 75.0 25.0
Atopy (%)	73.1
Occupational exposure at risk (%)	22.2
Occupational related asthma (%)	6.0
Biological and functional characteristics	**Values**
Total IgE (kU/L), mean ± SD	448.2 ± 930.6
Eosinophils (mm^3), mean ± SD - ≤ 150 - 151-300 - > 300	563.4 ± 1983.4 26.5 19.8 53.7
ACT score (mean ± SD) - < 20 (%) - 20-24 (%) - 25 (%)	17.2 ± 4.9 62.2 32.0 5.8
FEV_1 bronchodilator withhold % (mean ± SD)	70.8 ± 19.9
FEV_1 post bronchodilator % (mean ± SD)	78.6 ± 20.6
FVC bronchodilator withhold % (mean ± SD)	86.4 ± 18.3
FVC post bronchodilator % (mean ± SD)	91.8 ± 17.6
FEV_1/FVC bronchodilator withhold % (mean ± SD)	69.5 ± 15.3
FEV_1/FVC post bronchodilator % (mean ± SD)	72.8 ± 15.7
Exacerbations	**Values**
Exacerbation* (mean ± SD) - 1 or more (%) **In the previous 12 months*	3.3 ± 4.3 83.1
Access to an Emergency Department (mean ± SD) - 1 or more (%)	1.8 ± 2.1 23.6
Hospitalization (mean ± SD) - 1 or more (%)	1.6 ± 1.5 17.9
Access to Intensive Care Department (mean ± SD) - 1 or more (%)	1.6 ± 1.5 2.7

ACT, asthma control test; BMI, body mass index; FEV_1, forced expiratory volume in the first second; FVC, forced vital capacity; IgE, immunoglobulin E; SD, standard deviation.

Table II *- Drugs use in 851 patients with severe asthma.*

Patients (all treatments during the period observed) %	
Combination of ICS and LABA	94.2
Formoterol + beclometasone	38.0
Formoterol + budesonide	19.6
Salmeterol + fluticasone	18.3
Vilanterol + fluticasone	13.3
Formoterol + fluticasone	5.7
Oral corticosteroids (OCS)	31.8
Duration of OCS	
< 1 months	1.1
1-3 months	36.8
> 3 months	62.1
Monoclonal antibodies	64.5
Montelukast	51.9
Tiotropium	39.1
Theophylline	4.9
Immunotherapy ongoing	0
Immunotherapy in the past	18.3
Thermoplastic treatment ongoing	1.8
Thermoplastic treatment in the past	2.9
Treatments groups (%)	
A-ICS and LABA (single or combined)	4.2
B-ICS and LABA (single or combined) + others excluding systemic steroids > 3 months and monoclonal antibodies	22.7
C-ICS and LABA (single or combined) + monoclonal antibodies	53.5
D-Systemic steroids > 3 months + any other drugs	19.6

ICS, inhaled corticosteroids; LABA, long-acting β2-Agonists.

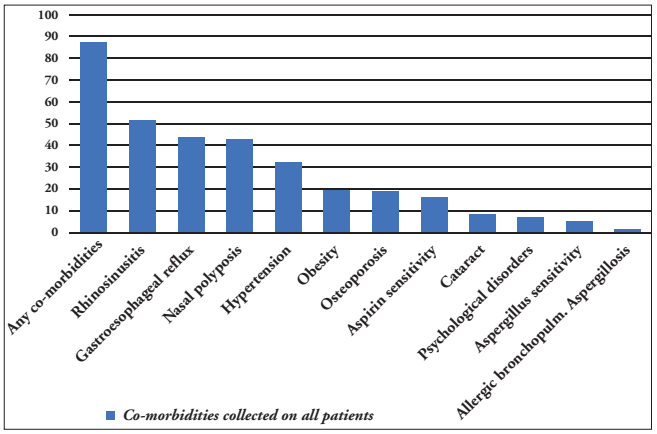
OCS and MAB: 22.7%; 3) ICS and LABA + Monoclonal antibodies (excluding long term OCS): 53.5%; 4) OCS > 3 months (+ other drugs) 19.6% (**table II**).

Comorbidities and association with treatment

Several comorbidities were reported affecting up to 745 patients (87.5%); two or more comorbidities were present in 77.4% of patients. The most observed were chronic rhinosinusitis (51.8%), gastroesophageal reflux disease (GERD) (43.5%), nasal polyposis (NP) (42.7%), hypertension (32.3%), osteoporosis (19.1%), and Aspirin intolerance (16.1%) (**figure 2**).

The distribution of all the comorbidities in the four treatment groups was different (p = 0.02), with statistically significance for osteoporosis and cataract (p < 0.001), which were significantly more prevalent in patients treated with long-term OCS than in three other groups of treatment (p < 0.001). Chronic rhinosinusitis frequency was higher in more severe patients (*e.g.*, those treated with ICS, LABA, MAB, and or OCS), than in patients treated with ICS, LABA and other combinations (**online supplements table IIS**).

Figure 2 *- Co-morbidities in 851 patients with severe asthma.*



Asthma control and risk factors

Asthma was defined uncontrolled in 62.2% of IRSA patients, according to Asthma Control Test (ACT) scoring, with a mean value of 17.2. The mean number of asthma exacerbations in the previous 12 months was 3.3 with 23.6% of patients having one or more accesses to the Emergency Department and 17.9% being hospitalized, while access to Intensive Care Department was uncommon (2.7%) (**table I**).

Comparing patients with controlled and uncontrolled asthma, some associations (p < 0.05) emerged (**table III**). Uncontrolled asthmatic patients were more frequent females (p=0.02), with a BMI ≥ 30 (p = 0.04.); moreover, they had a higher number of exacerbations in the previous 12 months with a mean value of 4.3 (p < 0.001), as well as the use of OCS (p < 0.001) and hospitalization (p < 0.001) were greater.

Among the comorbidities, obesity and psychological conditions were significantly higher in patients with uncontrolled asthma (22.4% *vs* 13.3%: p = 0.002 and 8.5% *vs* 4.4%; p = 0.03, respectively).

Patients with uncontrolled asthma had more frequently an eosinophil count > 300 mm^3 (p < 0.001), with a mean of 563.6 (SD ± 1017.4) compared to 391.1 (SD ± 452.0) of controlled patients (p < 0.001). The latter were treated with MAB less frequently than controlled patients (58.4% and 75.8%, respectively; p < 0.001).

Comparing uncontrolled patients treated with or without MAB, a significantly lower ACT mean score, a higher hospitalization rate, as well as a higher percentage of patients using of OCS were observed in the latter subgroup (**table IV**).

Table III - *Characteristics of controlled vs uncontrolled asthma patients.*

	Controlled Asthma N 301	**Not Controlled Asthma N 550**	**P-value**
Sex, female (%)	55.8	64.0	0.02
Age at onset of symptoms, years (mean ± SD)	29.5 ± 17.2	28.8 ± 16.4	0.72
Body Mass Index (kg/m^2) (mean ± SD) - ≥ 30	26.0 ± 4.6 14.9	26.9 ± 5.2 22.2	0.04 0.01
Exacerbation* (%) - Mean ± SD **In the previous 12 months*	61.5 1.3 ± 1.8	94.9 4.3 ± 4.8	< 0.001 < 0.001
Hospitalization	9.3	22.6	< 0.001
ACT Score mean ± SD	21.2 ± 3.6	15.1 ± 4.2	< 0.001
Eosinophils (mm^3) - > 300 (%)	391.1 ± 452.0 45.6	563.6 ± 1017.4 57.9	< 0.001 < 0.001
Presence of co-morbidities (%) - Obesity - Psychological disorders	86.1 13.3 4.4	88.4 22.4 8.5	0.33 0.002 0.03
Use of OCS (%) - Duration of use (users only), mean ± SD (months)	16.0 15.0 ± 19.3	40.5 11.1 ± 24.7	< 0.001 0.04
Use of monoclonal antibodies n (%) - Omalizumab (n) - Mepolizumab (n) - Benralizumab (n)	228 (75.8) 130 85 13	321 (58.4) 144 155 22	< 0.001 < 0.001 0.99 0.60
Duration of use of monoclonal antibodies (users only) mean ± SD (months)	21.4 ± 22.3	18.6 ± 25.4	< 0.001
Treatment group (%) - Group D - Systemic steroids > 3 months (independent of other drugs)	10.0	25.0	< 0.001

ACT, asthma control test; OCS, oral corticosteroids; SD, standard deviation.

Severe asthma with or without atopy

Patients with severe asthma without atopy (SAsA) experienced more exacerbations (p < 0.001), and showed a higher number of eosinophils (p < 0.001) than those with atopy (SAwA); moreover, they suffered from nasal polyposis (p = 0.008) and osteoporosis (p = 0.02) more frequently (**table V**).

As expected, the mean value of total IgE was significantly greater in patients with SAwA (535 IU/ml) than in those with SAsA (224 IU/ml) (p < 0.001).

Subjects with SAsA reported a higher use of OCS (41.9% *vs* 28.1%; p < 0.001), with a longer duration of therapy (17.0 ± 32.5 months *vs* 8.9 ± 16.7 months; p < 0.001), with a higher Mepolizumab use (84.1% *vs* 32.4%; p < 0.001) compared to subjects with SAwA (**table V**).

Discussion

Data from several national and one international registries on asthmatic patients have been published at the present time (14-24). IRSA study describes the characteristics of the largest population of Italian SA patients.

Relevant characteristics of this study are: the wide sample size (851 subjects), compared to other Italian and European registries; a wide synoptic view of some clinical, patho-physiologic and hematic values, with the chance to study their correlation and their evolution during the follow up period of 5 years; the use of the three MAB currently available in the market.

Some general, functional, and biological characteristics of IRSA patients were consistent with other European registries (*e.g.*, sex, BMI, smoking *habitus*, obstructive airway pattern, eosinophilic inflammation) (16-21, 24). Passive smoke exposure was present in 22.2% of SA patients, highlighting the persistent relevance of smoke as social problem.

An occupational risk was reported by more than 20% of IRSA patients, even though only 6% of them received a diagnosis of occupational-related asthma, probably due to the change of the workplace before the progression of the disease.

Table IV - *Characteristics of 550 uncontrolled asthma patients, according to the use of monoclonal antibodies.*

	No use of monoclonal antibodies N 229	**Use of monoclonal antibodies N 321**	**P-value**
Sex, female (%)	62.9	64.8	0.64
Age at onset of symptoms (mean ± SD)	28.9 ± 17.2	28.7 ± 15.8	0.91
Body Mass Index (kg/m^2) (mean ± SD) - ≥ 30	27.2 ± 5.6 25.8	26.6 ± 4.8 19.6	0.24 0.09
Exacerbation* (%) - Mean ± SD **In the previous 12 months*	96.9 4.7 ± 5.1	93.5 4.1 ± 4.6	0.07 0.12
Hospitalization	27.1	19.3	0.03
ACT Score mean ± SD	14.4 ± 4.1	15.6 ± 4.1	0.001
Eosinophils (mm^3) - > 300 (%)	467.0 ± 451.3 57.6	632.9 ± 1273.9 58.0	0.33 0.60
Presence of co-morbidities (%) - Obesity - Psychological disorders	87.8 27.4 9.6	88.8 18.6 7.7	0.72 0.02 0.48
Use of OCS (%) - Duration of use (users only) mean ± SD (months)	47.6 9.1 ± 25.9	35.5 13.0 ± 23.5	0.004 0.04
Treatment group (%) - Group D - Systemic steroids > 3 months (independent of other drugs)	28.2	22.7	0.15

ACT, asthma control test; OCS, oral corticosteroids; SD, standard deviation.

In seventy-five per cent of IRSA patients asthmatic symptoms started at age ≤ 40 years, a value higher compared to other registries (16, 17, 19, 20, 24); that is not surprising considering that a high percentage of IRSA patients were atopic (73%).

Comorbidities were reported by 87.5% of IRSA patients, most of them being affected by two or more diseases. To go further into the complexity of the management of SA patients, unlike other registers (16-21, 24) we included among the comorbidities not only other type-2 diseases (*i.e.*, NP and asthma-related diseases (*i.e.*, GERD)) but also pathologies related to the OCS chronic use (*i.e.*, osteoporosis, cataract). Not surprisingly, osteoporosis and cataract were significantly more prevalent in patients treated with long term OCS.

Almost all IRSA patients were treated with a combination of ICS and LABA. As observed in other registries (17, 19-21), they received montelukast quite frequently (51.9%), due at least in part to the high percentage of IRSA patients with NP.

Tiotropium was only used in 39% of the patients, even though guidelines recommend adding it to ICS and LABA in uncontrolled SA in order to reduce the risk of exacerbations before to start the MAB therapy (3).

About 18% of patients were treated with allergen specific immunotherapy (AIT) in the past, consistent with both the role of allergy in some IRSA patients, and the contribution of allergists to the registry.

Consistent with the continuing development of the therapeutic options and with the more recent institution of IRSA compared to the other registries, more than 60% of IRSA patients were treated with biologics, the cost of which in Italy is covered by the national health care system.

In Italy two registries of severe asthma, SANI (21) and IRSA (25), do exist, as in other fields of medicine. Although this can be interpreted as an overlap and a limitation, on the contrary it may represent an opportunity to increase the number of cases, widen the spectrum of information, and check their homogeneity.

In this regard it is noteworthy that the use of OCS in our registry (31.8%) was comparable to that reported by most European registries (18, 20, 24) as well as by an Italian pharmacoeconomic study (26), whose range is between 11% and 45%. The highest percentage of oral steroids use underlined by SANI registry may be due, according to the authors, to the inclusion of more severe asthmatic patients who chronically took OCS in 64% of cases (21). Howev-

Table V - *Characteristics of patients with or without atopy.*

	Severe asthma with atopy 622 Pts	**Severe asthma without atopy 229 Pts**	**P-value**
Sex, female (%)	59.8	64.6	0.20
Age mean, years (mean ± SD)	53.7 ± 14.3	57.9 ± 12.1	< 0.001
Body Mass Index (kg/m^2), mean ± SD - ≥ 30	26.6 ± 5.0 19.4	26.4 ± 5.0 20.1	0.44 0.84
Active smoker (%)	7.5	3.1	0.055
Age at onset of symptoms (years)	27.3 ± 16.5	33.8 ± 16.4	< 0.001
Controlled asthma (%)	36.3	32.8	0.33
Exacerbation* (%) - Mean ± SD **In the previous 12 months*	81.2 3.0 ± 4.1	88.2 3.9 ± 4.6	0.02 0.001
Hospitalization (%)	17.9	17.9	0.98
ACT score mean ± SD	17.3 ± 4.9	17.1 ± 4.8	0.41
Eosinophils (mm^3) - ≤ 150 - 151-300 - > 300	422.7 ± 528.4 28.4 22.9 48.7	725.9 ± 1409.8 21.4 11.6 67.0	< 0.001 < 0.001
Total IgE (kU/L), mean ± SD	535 ± 1060.9	224.3 ± 362.2	< 0.001
Co-morbidities (%) - Nasal polyposis - Osteoporosis	86.7 39.9 17.4	90.0 50.2 24.9	0.20 0.008 0.02
Use of systemic steroids (%) - Duration: mean ± SD (months)	28.1 8.9 ± 16.7	41.9 17.0 ± 32.5	< 0.001 < 0.001
Use of monoclonal antibodies n (%) - Omalizumab (n) - Mepolizumab (n) - Benralizumab (n)	423 (68.0) 271 133 19	126 (55.0) 2 106 18	< 0.001 < 0.001 < 0.001 0.03
Duration of use of MAB, mean ± SD (months)	23.0 ± 26.0	7.9 ± 6.3	< 0.001
Treatment group (%) - Group D - Systemic steroids >3 months (independent of other drugs)	15.2	31.4	< 0.001

ACT, asthma control test; OCS, oral corticosteroids; SD, standard deviation.

er, without information on the average duration of OCS therapy and/or number of OCS courses, data is difficult to interpret.

At the time of enrollment, asthma was uncontrolled in 62.2% of IRSA patients, confirmed by ACT scores. The subgroup of uncontrolled SA patients identified a more severe phenotype, in terms of eosinophil count and exacerbation, obesity and psychological conditions. Moreover, they were treated with MAB less frequently and for shorter periods compared to the controlled SA group. This finding was confirmed by the results of another analysis showing that IRSA patients without exacerbations were using MAB more frequently than those with exacerbations (81% *vs* 61%) (data not shown). Other registries and studies showed that patients treated with MAB have a significantly lower risk of exacerbations (20, 27-30).

Moreover, the study showed that more than 50% of uncontrolled patients were treated with MAB. However, a further analysis of the uncontrolled subgroup indicates that uncontrolled patients not treated with MAB had a greater rate of hospitalization and a lower mean ACT score, as well as a statistically significant higher percentage of these patients were treated with

OCS than those using MAB. This finding shows that among uncontrolled patients those treated with MAB are more likely to achieve asthma control and to reduce OCS use in clinical practice, as demonstrated by clinical trials (30).

Patients with SAsA had a more severe disease, in terms of exacerbations, use and duration of OCS, compared to patients with SAwA and they showed an eosinophilic phenotype, and comorbidities like nasal polyposis and osteoporosis. It is noteworthy that among SAwA patients treated with MAB, 64.3% of them were on a biologic treatment targeting the IgE pathway, while the remaining patients were on a treatment targeting the IL-5 pathway. The presence of overlapping phenotypes of severe asthma and/or comorbidities may explain these findings (27, 31). Moreover, it can be assumed that some of these patients are atopic, but they do not have an allergic asthma. In both registries and in clinical studies "atopy concept" is often confused with "allergy concept". Asthmatic patients with atopy will not necessarily have an allergic aetiology to their asthma (32, 33). The hypothesis that in atopic patients with blood eosinophilia the association between allergen exposure and asthma symptoms/exacerbations as well as age at asthma onset, and presence of fixed airflow obstruction and/or upper airway comorbidities could help to differentiate between severe allergic and severe eosinophilic asthma need to be further investigated (32). However, a recent study in real world confirmed that the overlap between asthma with or without atopy is resolved by doctors, taking into account comorbidities rather than biomarkers (7).

Conclusions

This study underlines demographic, clinical, functional, and inflammatory features of the greatest number of Italian patients with SA enrolled to a specific clinical registry. Most of severe asthmatic patients in Italy were suffering from more than one comorbidity and had poor asthma control at the time of enrollment, giving a real-world representation of SA. Uncontrolled patients had a higher frequency of exacerbations and hospitalization, indirectly confirming the increased consumption of economic resources. Moreover, they showed a prevalent eosinophilic phenotype, frequently used OCS and were treated with MAB less frequently than controlled subjects. However, among uncontrolled patients, those treated with MAB are more prone to achieve asthma control and to reduce OCS use in real life. Among SA patients with atopy treated with MAB, 36% were on a treatment targeting the IL-5 pathway. All these findings suggest potential for a more targeted use of biotherapies after proper phenotyping SA patients.

Their annual follow-up for five years will monitor the changes that will occur in terms of treatment in relation to a better definition of their phenotype characteristics and of disease control in the era of biologics.

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Table IS - *General, functional and biological characteristics of IRSA 851 patients.*

Characteristics	Values
Ethnicity (%)	
- Caucasian	98.2
- Other	1.8
Educational Level (%)	
- Primary or secondary school	35.4
- High school	47.8
- University	16.8
Area of Italy (%)	
- North	44.2
- Centre	16.8
- South and Isles	39.0
Passive smoking (%)	22.2
- At home	50.8
- At work	39.2
- Both	10.0
Occupational exposure at risk (%)	22.2
- Cleaner	25.9
- Agricultural worker	25.9
- Chemical worker	25.3
- Others	22.9
Biological and functional characteristics	**Values**
FEV_1 Bronchodilator withhold groups (%)	
- ≤ 70	52.9
- > 70	47.1
FEV_1 Post Bronchodilator groups (%)	
- ≤ 70	34.6
- > 70	65.4
Total IgE values (%)	
- < 100	30.8
- 100-300	31.5
- > 300	37.7

FEV_1, forced expiratory volume in the first second; IgE, immunoglobulin E.

Table IIS - *Co-morbidities and their relations with treatments.*

	Group A	Group B	Group C	Group D	p-value All groups	p-value A vs B	p-value A vs C	p- value A vs D	p-value B vs C	p-value B vs D	p-value C vs D
%	4.2	22.7	53.5	19.6							
Co-morbidities (%)											
Rhinosinusitis	55.6	43.3	52.9	57.4	0.06	0.23	0.79	0.86	**0.03**	**0.009**	0.32
Nasal polyposis	41.9	39.8	42.8	43.2	0.91	0.82	0.93	0.89	0.50	0.53	0.92
Hypertension	37.1	30.8	29.6	39.5	0.12	0.46	0.35	0.80	0.76	0.09	**0.02**
Osteoporosis	7.1	17.1	16.0	34.5	**< 0.001**	0.26	0.28	**0.004**	0.74	**< 0.001**	**< 0.001**
Cataract	6.2	5.6	6.5	17.8	**< 0.001**	0.99	0.99	0.10	0.66	**< 0.001**	**< 0.001**
Diabetes	2.9	8.6	5.2	8.0	0.28	0.48	0.99	0.47	0.12	0.84	0.21
Obesity	21.2	21.2	16.9	22.0	0.42	0.99	0.53	0.93	0.20	0.86	0.15
Aspirin sensitivity	3.6	13.0	17.1	19.9	0.09	0.21	0.06	0.054	0.21	0.09	0.44
Gastroesophageal reflux	44.8	41.7	40.9	51.3	0.16	0.75	0.68	0.52	0.85	0.08	**0.03**
Psychological disorders	3.4	7.4	6.2	9.4	0.51	0.70	0.99	0.47	0.60	0.52	0.19
Aspergillus sensitivity	5.6	4.9	5.7	4.4	0.94	0.99	0.99	0.59	0.70	0.85	0.55
Any comorbidity	74.3	83.7	88.8	90.8	**0.02**	0.18	**0.03**	**0.02**	0.08	**0.045**	0.47

The percentages keep into account the presence of some missing values.

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Immune signature of CCR7$^+$ central memory T cells associates with disease severity and Immunoglobulin E in bronchial asthma

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Summary

Objective. *CD4$^+$ T cell subtypes are the central orchestrators of airway inflammation in bronchial asthma (BA); however, the mechanisms that regulate their accumulation in asthmatic airways are still a challenging subject. In addition, neutrophils play a significant role in the development of airway remodeling and their presence may influence clinical presentation of BA being linked to the development of severe BA. Neutrophils have also been found to acquire antigen presenting functions, enabling them to directly activate T cells. The study aimed to evaluate the possible association of chemokine receptor 7 (CCR7)$^+$ memory CD4$^+$ T cells and CCR4$^+$ effector T cells with disease severity and immunoglobulin E (IgE) production as well as to explore the relationship between these cells and neutrophil function in both allergic and non-allergic asthmatic patients.* ***Methods.*** *Flow cytometry was used to determine the expression of different T cell subset phenotypes (CCR7 memory CD4$^+$ and CCR4$^+$ T cells using anti-human CD3, CD4, CD45RO, CCR4 and CCR7 monoclonal antibodies) utilizing peripheral blood mononuclear cells (PBMCs) isolated from 78 allergic asthmatic patients, 41 non-allergic asthmatic patients, and 40 healthy individuals. Moreover, neutrophils' phagocytic activity was assessed by ingestion of candida particles.* ***Results.*** *We demonstrated increased percentages of CCR7$^+$ memory CD4$^+$ T cells and CCR4$^+$ CD4$^+$ T cells in patients compared to control, where this upregulation was significantly higher in allergic than non-allergic asthmatic patients. Additionally, these cells were negatively correlated with improved pulmonary tests and significantly associated with disease severity scores and IgE levels. The neutrophil phagocytic activity was markedly increased in patients compared to control, showing a significant positive correlation with disease severity.* ***Conclusions.*** *These findings suggest that increased CCR4$^+$ CD4$^+$ T cells and CCR7$^+$ memory CD4$^+$ T cells (Tcm) may be associated with BA severity, especially in allergic BA patients and can potentially contribute to the rational design of new therapeutic approaches for asthma in the future.*

Introduction

Bronchial asthma (BA) is considered as a globally major public health issue that has a negative impact on quality of life, and is associated with high levels of co-morbid diseases (1). It is estimated that numbers of BA patients worldwide may be as high as 334 million with a suggested steady increase (2). The prevalence among adults was estimated to be 6.7% of the general population in Egypt (3) and about 8.2% in children aged 3-15 years (4).

BA is a heterogeneous disease with different phenotypes, being one of the main obstacles to successful management (5). The clinical phenotype of allergic BA is the most recognizable one, since it is associated with history of allergic diseases and reversible lung obstruction. It is characterized by eosinophilic airway inflammation, which is associated with immunoglobulin E (IgE) antibodies to various allergens, as evidenced by serology or skin prick test (6). It has been characterized that the pathogenesis of asthma is classically defined as a T helper (Th2) -type inflammatory response. These elevated Th2-type lymphocytes have been characterized in the blood of BA patients, indicating that these immune cells responsible for chronic inflammation in the lung circulate in the blood (7). The accumulation of Th2 cells in lungs is essential for both the initiation and persistence of airway inflammation being attributed to a number of candidates, including the chemokine receptor CCR4 because of its preferential expression on this type of cells (8). Mucosal $CD14^+$ mononuclear phagocytes are major producers of four chemokines (Chemokine C-C motif ligand 13 (CCL13), CCL17, CCL18, and CCL24), which are recognized as ligands for chemokine receptors that are typically expressed on differentiated Th2 cells including CCR4 that is involved in Th2 responses (9). Though, roles of $CCR4^+CD4^+$T cells in the pathogenesis of asthma are still controversial in both humans and murine model of asthma.

However, it has been suggested that the pathogenesis of asthma must not be solely driven by Th2-type immune responses, owing to the high level of clinical heterogeneity of asthma (10). Memory T cells have been previously reported to be associated with chronic inflammatory conditions and autoimmune diseases (11, 12). They can be categorized into central memory T cells (TCM) that circulate among secondary lymphoid organs, and effector memory T cells (TEM) that search for their cognate antigen in the non-lymphoid organs. These two subsets also show differential chemokine receptor expression, where TCMs express high levels of CCR7, can migrate from peripheral tissues to the lymph nodes via the afferent lymph, and can quickly proliferate in response to infiltrating antigen-presenting cells (APCs) (13). In response to this, memory $CD4^+$ T cells can acquire an effector-like phenotype with the secretion of cytokines and chemokines being considered as reactive memory cells (14). The exit of cytokine-producing $CCR7^+$ cells from peripheral tissues and entry into the draining lymph node might amplify and polarize the developing lymph node immune response and may contribute to the maintenance and distribution of the T cell memory pool (15), linking the lymphoid and peripheral T cell compartments with an important implication for the generation and maintenance of immune responses.

In addition, it has been demonstrated that neutrophils may play an important role in the development of airway remodeling and fibrosis in severe asthmatic airways being an important source of transforming growth factor beta (TGF-β1) and inducer of Epithelial-Mesenchymal transition (16). In addition, freshly isolated human neutrophils can function as APCs to memory $CD4^+$ T cells (17) with an evidence of the antigen-presenting capacity of human neutrophils for local allergen specific effector T cells in patients with allergic late phase reactions (18, 19).

Alternatively, reports have classified granulocytes as the main effector cells in inflammation, which migrate to inflammatory sites along the chemotactic gradient of inflammatory mediators. The migration of neutrophils to lymphoid organs has been linked to upregulation of the chemokine receptor CCR7 (20). In addition, it has been shown that the severity of asthma affects the functioning of peripheral blood cells where in severe forms, the numbers of neutrophils and eosinophils are significantly increased in the blood (21) with altered expression profile of proinflammatory cytokines (22).

Although memory T cells have been intensively characterized in response to infections and autoimmunity, the importance of these cells in allergic diseases remains to be elucidated. Herein, we investigated the interactions of CCR7 expressing memory $CD4^+$ cells and $CCR4^+$ T cells in mediating severity and clinical outcomes of BA as well as the production of IgE, which is considered as a characteristic feature of allergic bronchial asthma and is thought to be critical for pathology. In addition, we explored the relation between these cells and neutrophil function in asthmatic patients.

Subjects and methods

Study population

The current study was conducted on 119 patients with bronchial asthma who were recruited from Chest Department, Main Alexandria University Hospital, Egypt. The diagnosis was based on the criteria of the Global Initiative on Asthma (GINA; http://www.ginaasthma.org) (23). Forty age and sex matched healthy controls with no history of asthma or any allergic disease, currently non-smokers and not receiving any drug at their inclusion in this study were included too.

Patients were further categorized into 78 allergic asthmatic patients and 41 non-allergic asthmatics. The allergic status of patients was determined by patient history, clinical examination, a positive specific IgE (ImmunoCAP test: ≥ 0.7 kUA/L; ThermoFisher) correlated with the clinical history or the allergen challenge, and a positive skin prick allergen test (wheal-a raised white bump surrounded by a small circle of itchy red skin to allergens ≥ 3 mm diameter above background) (24). A positive family his-

tory of asthma and/or other allergic diseases, particularly allergic rhinitis, was also recorded in 100% of allergic BA patients.
All subjects had no change of asthma medications 4 weeks prior to recruitment to the study. All subjects were non-smokers and free from upper respiratory tract infection for at least 4 weeks preceding the study. Pulmonary flow rates were measured using DATOSPIR-120 spirometer with automatic dosimeter (FG0304-Datospir 120; Spain - the DATOSPIR-120 spirometer). Interpretation of common test values: the forced vital capacity (FVC), forced expiratory volume in one second (FEV1), FEV-1/FVC was done (25). Bronchial hyperresponsiveness was assessed with methacoline challenge test. The dose of methacoline that results in 20% reduction of FEV-1 was determined (26). At entry to the study, patients were taking inhaled glucocorticoids at dosages up to 400 µg/day. All patients were taking inhaled β2-agonists "as required". Patients receiving increased inhaled glucocorticoid therapy were followed longitudinally for the purpose of the present study.
Disease severity was measured by different ways: all patients were assessed for their control of asthma by using asthma control test (ACT) scoring (total-score ranging from 5 to 25) and GINA guideline, Medical Research Council (MRC) dyspnea scale (27); symptoms of asthma including: cough and wheezing which were scored from 0 to 3 according to GINA (28, 29); and clinical severity score GINA (1995). C-reactive protein (CRP) was determined using BN ProSpecNephlometry (Siemenes, USA) (30) and erythrocyte sedimentation rate (ESR) was determined using Westergren tube (31). The collection of blood samples and the related assays were approved by Ethical guidelines of Medical Research Institute, Alexandria University.

Total IgE assay

Total serum IgE was measured in duplicates using an enzyme linked immunosorbent assay (ELISA) kit (RIDASCREEN®; R-Biopharm, Darmstadt, Germany - RIDASCREEN® Total IgE A0141; R-Biopharm AG). Venous peripheral blood samples were collected from all subjects. Serum was used for total ELISA IgE assay (RIDASCREEN® Total IgE; R-Biopharm, Darmstadt, Germany) according to the manufacturer's instructions (RIDASCREEN® Total IgE A0141; R-Biopharm AG). Using the mean absorbance value for each sample, the corresponding concentration of IgE in IU/ml was determined from the standard curve, and patients were divided into three categories (< 20 IU/mL; 20-100 IU/mL; > 100 IU/ml).

Isolation of peripheral blood mononuclear cells and lymphocytes

Peripheral blood mononuclear cells (PBMCs) were isolated from sodium heparin-treated blood obtained from healthy donors or BA patients by Ficoll-Hypaque 1077 (Sigma-Aldrich) gradient centrifugation (32). Erythrocytes were lysed using an ammonium chloride solution. Suspension was centrifuged at 524/g for 10 min at RT. The pellets were washed with PBS and then resuspended in complete RPMI 1640 medium (Invitrogen, Grand Island, NY, USA, cat. 11875093) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Invitrogen, Grand Island, NY, USA), 100 U/mL penicillin (cat. 15071163), 100 mg/mL streptomycin (15071163), 2 mM L-glutamine (cat. 25030081), and 50 mM 2-mercaptoethanol (cat. 21985023; Invitrogen, Grand Island, NY, USA).
Freshly isolated peripheral blood mononuclear cells (PBMCs) were labeled with the selected combination of cell surface antibodies including: anti-CD4-Percep, anti-CCR4 (CD194)-PE and anti-CD45RO-FITC, anti-CCR7 (CD197)-PE respectively.

Phenotypic characterization

The pooled PBMCs from the healthy donors and the BA patients were stained for flow cytometry. The following panel of mouse anti-human mAbs, all purchased from BD Biosciences (San Jose, CA, USA) or eBioscience (San Diego, CA, USA), was used: anti-human CD3-APC.cy7 (BD, 557832, SK7), anti-human CD4-Percp.cy5.5 (BD, 560650, RPA-T4), anti-human CD45RO-FITC (eBioscience, 11-0458-42, HI100), anti-human CCR4-PE.cy7 (BD, 557864) and anti-human CCR7-PE. cy7 (BD, 557648, 150503). The cell data were acquired using a 10-laser Gallios (Beckman Coulter Inc., Brea, CA, USA) analytical flow cytometer. Unstained and single fluorochrome-stained cells were used as controls to provide accurate compensation and data analysis. The results were analyzed with BD FACS Calibur flow cytometer using Cell Quest software (Becton-Dickinson).

Assessment of phagocytic activity

This test relies on the uptake of heat killed candida albicans (yeast) by neutrophils over a brief period of time where stained intracellular candida can be identified and counted (33). Heat-killed candida suspended in phosphate-buffered saline was adjusted to 2×10^7 cells/mL where 250 µL of pooled serum and 250 µL of heat-killed candida were added to 250 µL of buffy coat obtained by using polymorph prep and incubated at 37 °C for 1 hour with occasional mixing. Number of candida-engulfed neutrophils was counted as positive cells and phagocytic activity was calculated as follows: *Number of positive phagocytic cells / total number of cells × 100.*

Statistical analysis

Data were expressed as mean ± standard deviations (SD) (standard deviation of mean) and were compared with the tabulated probability value (P value) that was considered significant if it was 0.05 or less using SPSS statistical package (SPSS Inc., Chicago, IL). Student t-test was used for normally distributed data while Mann-Whitney U test was applied for non-normally distributed data. A Pearson chi-square test was applied for categorical variables. Multiple comparisons were performed using one-way ANOVA and Kruskal-Wallis tests. The correlation between two quantitative variables was evaluated using Pearson correlation coefficient (r).

Results

Subjects' demographic and laboratory data

There was no statistical significant difference as regards age between allergic (mean ± SD = 48.2 ± 10.3), non-allergic patients (52.2 ± 8) and control subjects (48 ± 9.4; P = 0.063), as well as sex distribution between groups (P = 0.731). Females represented 64.1% of allergic asthmatic patients, 58.5% and 57.5% of non-allergic and control subjects, respectively. Disease duration (years) showed a significant difference between the two patients' groups (P < 0.001).

CBC data showed that total lymphocyte and eosinophil counts were significantly increased in allergic asthma patients compared to non-allergic BA patients and control. On the other hand, non-allergic asthma patients had significantly more circulating neutrophils and monocytes compared to allergic BA patients and control (**table I**). There was also a notable marked elevation in ESR (mm/hr) (median = 38 (14.3-52)) and CRP (mg/L) (median = 4.5 (2.2-10.8)) in non-allergic BA patients' group relative to allergic patients and control groups.

Clinical indicators of respiratory function

Clinical indicators of pulmonary function were measured on the day of sample acquisition. As shown in **table I**, FEV1, FVC, FVC% pred., FEV1%/FVC, forced expiratory flow between the 25% and 75% of the FVC (FEF25-75%) (L/min), and FEF25-75% pred. were significantly lower in allergic than in non-allergic patients' groups (P < 0.001), whereas, PD20 of the methacholine challenge test was significantly decreased in non-allergic BA patients (P < 0.001).

Figure 1 - *The percentages of phagocytic activity in BA patients:* ***(a)*** *representative dot plots were shown from allergic asthmatic patients, non-allergic asthmatic patients and healthy individuals,* ***(b)*** *representative dot plots were shown from BA patients who were classified into well controlled, not well controlled and poorly controlled patients based on asthma control test,* ***(c)*** *representative dot plots were shown from BA patients who were classified according to their IgE serum levels into categories (< 20, 20-100, > 100 IU/ml).*

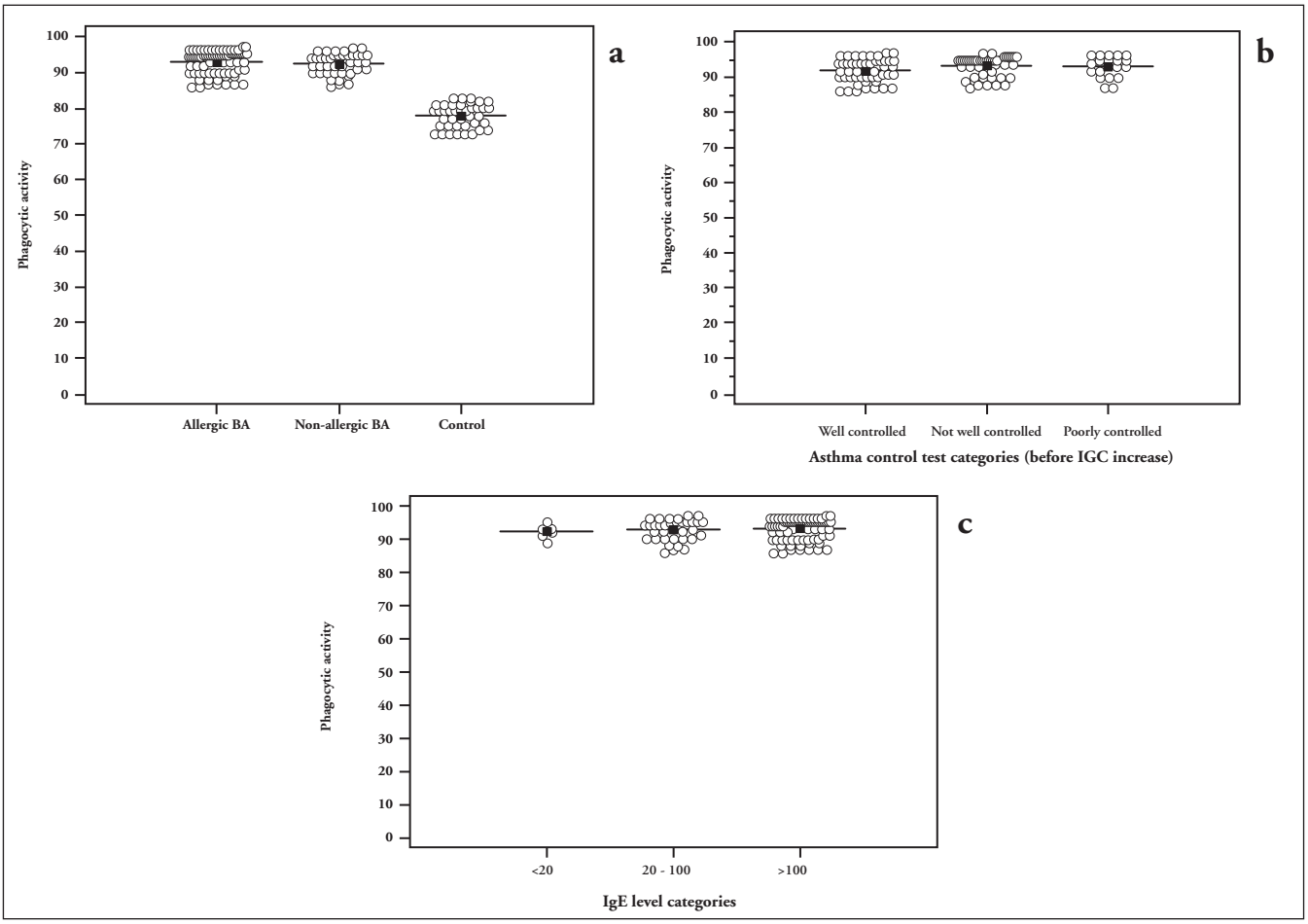


Table I *- Comparison between the studied groups according to demographic and clinical characteristics.*

	Allergic asthmatic patients (n = 78)	Non-allergic asthmatic patients (n = 41)	Control (n = 40)	P
Age	48.2 ± 10.3	52.2 ± 8	48 ± 9.4	0.063
Sex				
Male	28 (35.9%)	17 (41.5)	17 (42.5%)	0.731
Female	50 (64.1%)	24 (58.5%)	23 (57.5%)	
Family history of an allergic disease	78 (100.0%)	5 (12.2%)	–	< 0.001*
Disease duration (years)	22.3 ± 2.2	15.7 ± 4.6	-	< 0.001*
WBCs (x 10^3/mm^3)	10.9 (4.6 – 25.6)	10.7 (8.1 – 17.8)	5.4 (4.2 – 7.4)	< 0.001*
Sig. bet. groups		$P_1 = 0.861$, $P_2 < 0.001$*, $P_3 < 0.001$*		
Lymphocytes	2.8 (1.7 – 5.3)	1.8 (1.3 – 3.1)	2.1 (1.8 – 2.4)	< 0.001*
Sig. bet. groups		$P_1 < 0.001$*, $P_2 < 0.001$*, $P_3 = 0.005$*		
Basophils	0.03 (0.01 – 0.2)	0.03 (0.01 – 0.07)	0.03 (0.01 – 3.0)	0.850
Monocytes	0.8 (0.3 – 1.9)	0.97 (0.5 – 7.9)	0.4 (0.2 – 0.6)	< 0.001*
Sig. bet. groups		$P_1 = 0.001$*, $P_2 < 0.001$*, $P_3 < 0.001$*		
Eosinophil's	0.2 (0 – 1.1)	0.1 (0 – 0.2)	0.1 (0 – 0.2)	< 0.001*
Sig. bet. groups		$P_1 < 0.001$*, $P_2 = 0.003$*, $P_3 = 0.029$*		
Neutrophils	6.8 (1.8 – 21.1)	7.4 (5.8 – 15.5)	2.3 (1.3 – 4.8)	< 0.001*
Sig. bet. groups		$P_1 = 0.041$*, $P_2 < 0.001$*, $P_3 < 0.001$*		
ESR 1st hr. (mm/hr.)	17.0 (4 – 53)	38 (14.3–52)	9 (1 – 19)	< 0.001*
Sig. bet. groups		$P_1 < 0.001$*, $P_2 < 0.001$*, $P_3 < 0.001$*		
CRP	3.6 (1.7 – 6)	4.5 (2.2 – 10.8)	0.8 (0.2 – 2.6)	< 0.001*
Sig. bet. groups		$P_1 = 0.010$*, $P_2 < 0.001$*, $P_3 < 0.001$*		
FVC(L)	2.7 ± 0.7	3.5 ± 0.3	-	< 0.001*
FVC% pred.	78.9 ± 11.1	87.9 ± 7.2	-	< 0.001*
FEV1(L)	2.4 ± 0.7	2.8 ± 0.6	-	< 0.001*
FEV1%/FVC	84.7 ± 7.1	89.2 ± 4.6	-	< 0.001*
FEF25-75%(L/min)	2.5 ± 0.9	3.7 ± 0.7	-	< 0.001*
PD20 of Methacholine challenge (mg/ml)	0.025 (0.002 – 0.164)	0.014 (0.002 – 0.032)	-	< 0.001*

Data were assessed using: Chi square test (c2), student t-test (t), Mann Whitney test (U), ANOVA test (F), and Kruskal Wallis test (H). Family history of an allergic disease including: allergic asthma or/ and allergic rhinitis; Sig. bet. groups: significance between groups; ESR: Erythrocyte sedimentation rate; CRP: C-reactive protein; FVC: forced vital capacity; pred.: predicted; FEV1: forced expiratory volume in one second; FEF25-75%: mean forced expiratory flow between the 25% and 75% of the FVC.

P: P value for comparing between the studied groups; P1: P value for comparing between allergic asthmatic patients and non-allergic asthmatic patients; P2: P value for comparing between allergic asthmatic patients and control; P3: P value for comparing between non-allergic asthmatic patients and control; *: statistically significant at $p \leq 0.05$.

Patients starting inhaled glucocorticoid therapy were given fluticasone propionate Diskus at a starting mean dosage of 350 µg/day. Those patients with increasing inhaled glucocorticoid therapy were followed up for the purposes of the present study, for a mean of 6.8 months (allergic patients) or 7.2 months (non-allergic patients). During this period, dosages of inhaled glucocorticoid were increased by a mean of 418.7 µg/day (95% confidence interval: 278-533) in the allergic asthmatics and 298 µg/day (95% confidence interval: 139-473) in the non-allergic BA patients. This was associated with significant improvements in FEV1% pred., asthma control test, coughs, and wheezing score and along with significant reduction in inhaled β2-agonist usage in both groups (**table II**). **Table III** showed the comparison between allergic and non-allergic patients with regard to different disease severity scores.

Table II *- Comparison between allergic and non-allergic asthmatic patients according to clinical measurements and symptoms before and after IGC.*

	Allergic asthmatic patients		Test of Sig. (P)	Non-allergic asthmatic patients		Test of Sig. (P)
	Before IGC increase	After IGC increase"		Before IGC increase	After IGC increase"	
FEV1% pred.	74.7 ± 11.1	107.4 ± 14.3	< 0.001*	83.5 ± 6.7	124.5 ± 16	< 0.001*
Asthma control test	17 ± 4	19.5 ± 3.7	< 0.001*	18.6 ± 3	21.5 ± 3	< 0.001*
Cough score	2 (0 – 3)	1 (0 – 1)	< 0.001*	1 (0 – 3)	0 (0 – 1)	< 0.001*
Wheezing score	2 (0 – 3)	0 (0 – 1)	< 0.001*	1 (0 – 3)	0 (0 – 1)	< 0.001*
Inhaled β2 agonists doses/day	3 (2 – 5)	2 (1 – 3)	< 0.001*	2 (1 – 3)	0 (0 – 2)	< 0.001*

IGC indicates inhaled glucocorticoid therapy; FEV1: forced expiratory volume in one second. Cough score and Wheezing score (GINA 2020): 0 is well controlled, 1-2 is partly controlled, and 3-4 is uncontrolled. P: P value for comparing between before IGC and after IGC using: Paired t-test or Wilcoxon signed ranks test. *: statistically significant at P ≤ 0.05.

Table III *- Comparison between allergic and non-allergic asthmatic patients according to disease severity scores.*

	Allergic asthmatic patients (n = 78)	Non-allergic asthmatic patients (n = 41)	P
Asthma control test	17.0 ± 4.0	18.6 ± 3.0	0.029*
FEV1% pred.	74.7 ± 11.1	83.5 ± 6.7	< 0.001*
Dyspnea scale	2 (0 – 4)	1 (0 – 2)	< 0.001*
Cough score	2.0 (0.0 – 3.0)	1.0 (0.0 – 3.0)	0.040*
Wheezing score	2.0 (0.0 – 3.0)	1.0 (0.0 – 3.0)	0.026*

The data were assessed using Mann Whitney test (U) and student t-test (t). Dyspnea scale: modified Medical Research Council scale (24). Data where relevant are expressed as the mean (range) and standard deviation values. P: P value for comparing between the studied groups. *: statistically significant at P ≤ 0.05.

Phagocytic activity

Comparing the phagocyte activity in the blood of BA patients and controls, there was a marked increase in BA patients compared to control (P < 0.001). On the other hand, no significant difference was observed between allergic and non-allergic patients regarding phagocytic activity (**figure 1 a**). We found a significant difference in phagocytic activity among patients based on asthma control test (P = 0.041) where well controlled patients had lower phagocytic activity (92.0 ± 3.2 %) compared to both poorly controlled (93.1 ± 2.9 %) and not well controlled patients (93.5 ± 2.7%) (**figure 1 b**), while no significant difference in phagocytic activity was observed among patients regarding their IgE levels (P = 0.734) (**figure 1 c**)

Total serum IgE

As regards total IgE level among study population, there was high significant difference between allergic (mean ± SD = 336.8 ± 171.9 IU/ml), non-allergic patients (mean ± SD = 31.6 ± 9.6 IU/ml) and the control group (mean ± SD = 11.3 ± 5.8 IU/ml; P < 0.001), as well as between allergic and non-allergic patients (P < 0.001). All allergic BA patients had the IgE level higher than 100 IU/ml whereas the IgE level in non-allergic patients ranged between (11-49 IU/ml).

$CCR4^+CD4^+$ T cells

Allergic asthma patients had a significant higher percentage of $CCR4^+$ $CD4^+$ T cells (mean = 24.2 ± 5.9) than non-allergic BA patients (mean ± SD = 17.8 ± 6.4) in comparison with control (mean ± SD =12.9 ± 2.5; P < 0.001) (**figure 2 a**). To elucidate the clinical implication of increased $CCR4^+CD4^+$ T cells in asthma, our results revealed that percentages of $CCR4^+CD4^+$T cells were positively correlated with disease duration, lymphocyte count, phagocytic activity, total IgE level and disease severity scores, whereas a negative correlation was observed with improved pulmonary tests (**table IV**, **figure 2 b**, **c**).

$CCR7^+$ memory $CD4^+$ T cells

We used the receptor CCR7 to define the subsets of $CD45RO^+$ T cells. We gated the $CD3^+CD4^+$ $CDRO^+CDRA^-CCR7^+$ T cells (TCM) in the healthy donors and BA patients. We found that the percentage of TCM cells was increased in patients compared

Table IV *- Correlation between percentages of CCR4+ in CD4+ T cell with different studied parameters.*

	% of CCR4+ in CD4+ T cell	
	r	P
Disease duration	0.333*	< 0.001
Lymphocytes	0.242*	0.008
FVC(L)	- 0.500*	< 0.001
FVC% pred	- 0.196*	0.032
FEV1(L)	- 0.228*	0.013
FEV1%/FVC	- 0.131	0.154
FEF25-75%(L/min)	- 0.428*	< 0.001
FEF25-75%pred.	- 0.358*	< 0.001
Methacoline challenge test	0.084	0.362
Asthma control test before IGC increase	- 0.206*	0.025
Asthma control test after IGC increase	- 0.247*	0.007
Cough score before IGC increase	0.196*	0.033
Cough score after IGC increase	0.225*	0.014
Wheezing score before IGC increase	0.186*	0.043
Wheezing score after IGC increase	0.093	0.313
Inhaled β2 agonists doses/day before IGC	0.473*	< 0.001
Inhaled β2 agonists doses/day after IGC	0.350*	< 0.001
FEV1% pred Before IGC increase	- 0.468*	< 0.001
FEV1% pred after IGC increase	- 0.273*	0.003
% of CCR7- CD45RO+ in CD4+ T cell	- 0.344*	< 0.001
% of CCR7+ CD45RO+ in CD4+ T cell	0.555*	< 0.001
Total serum IgE	0.622*	< 0.001
Phagocytic activity	0.143	0.121

r: Pearson coefficient. *: statistically significant at P ≤ 0.05.

Table V *- Correlation between percentages of CCR7+ CD45RO+ in CD4+ T cell with different studied parameters.*

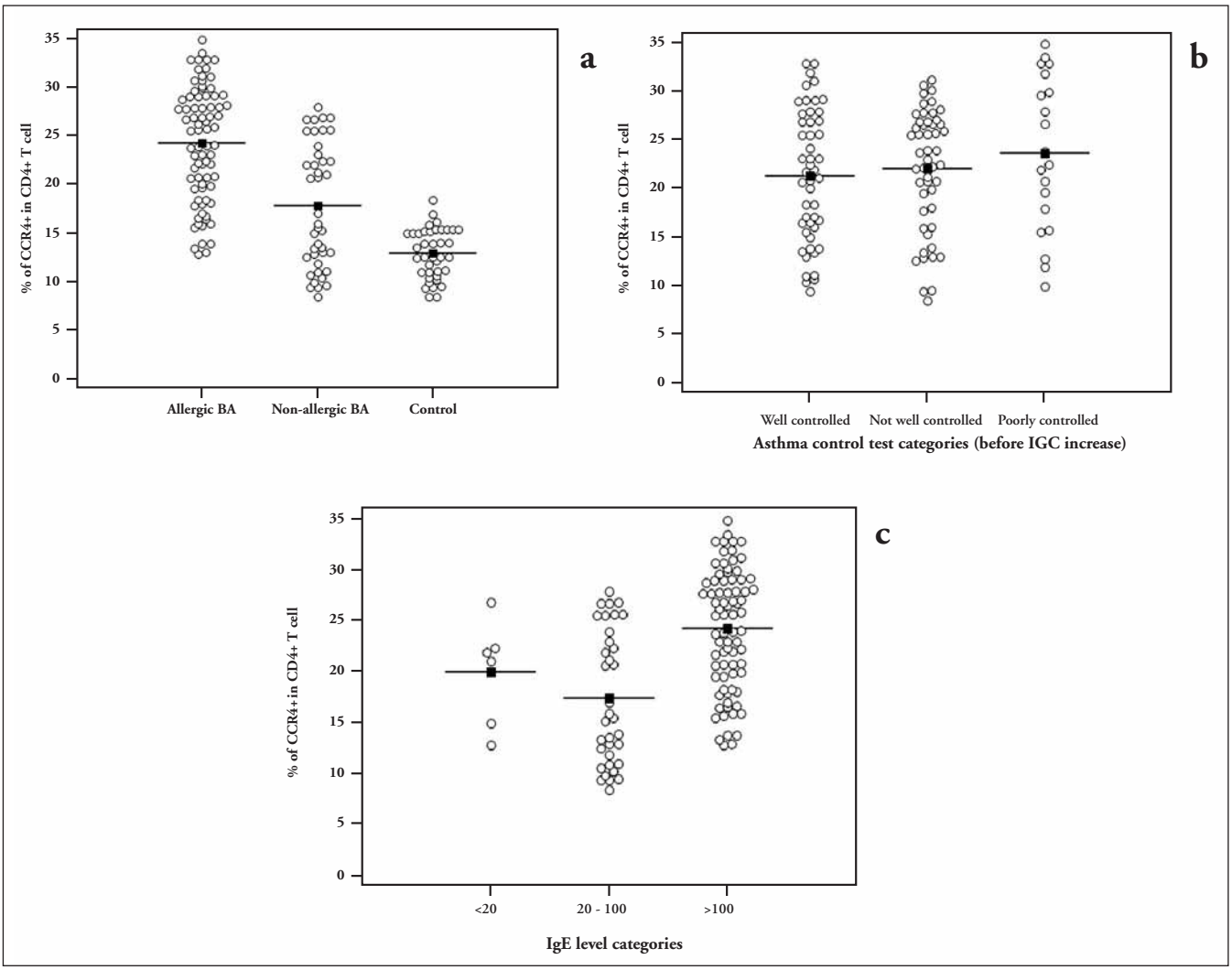
	% of CCR7+ CD45RO+ in CD4+ T cell	
	r	P
Disease duration	0.435*	< 0.001
Lymphocytes	0.481*	< 0.001
FVC(L)	- 0.622*	< 0.001
FVC% pred	- 0.417*	< 0.001
FEV1(L)	- 0.431*	< 0.001
FEV1%/FVC	- 0.353*	< 0.001
FEF25-75%(L/min)	- 0.613*	< 0.001
FEF25-75%pred.	- 0.314*	< 0.001
Methacoline challenge test	0.222*	0.015
Asthma control test before IGC increase	- 0.469*	< 0.001
Asthma control test after IGC increase	- 0.514*	< 0.001
Cough score before IGC increase	0.097	0.296
Cough score after IGC increase	0.014	0.882
Wheezing score before IGC increase	0.079	0.391
Wheezing score after IGC increase	0.067	0.468
Inhaled β2 agonists doses/day before IGC	0.386*	< 0.001
Inhaled β agonists doses/day after IGC	0.310*	0.001
FEV1% pred Before IGC increase	- 0.618*	< 0.001
FEV1% pred after IGC increase	- 0.393*	< 0.001
Total serum IgE	0.692*	< 0.001
Phagocytic activity	0.073	0.429
% of CCR7- CD45RO+ in CD4+ T cell	- 0.470*	< 0.001

r: Pearson coefficient. *: statistically significant at P ≤ 0.05.

to the control group. Interestingly, CCR7- cells (TEM) were also higher in patients than control. Moreover, our results showed that the percentage of CCR7+ memory CD4+ T cells was markedly increased in allergic (mean ± SD = 23.7 ± 5.4) than in non-allergic BA patients (mean ± SD = 13.8 ± 2.7; P < 0.001) (**figures 3**, **4 a**). Due to the heterogeneity of asthma phenotypes and clinical variation, we next investigated whether the increase of CCR7+ memory CD4+ T cells is a common feature of different asthma subtypes. Allergic asthma patients were divided into 3 subgroups, based on their asthma control test. We found that poorly controlled and not well controlled patients had nearly similar percentages of circulating CCR7+ memory CD4+ T cells (mean ± SD = 24.0 ± 7.8; 21.2 ± 6.2, respectively; P = 0.199), but both subgroups of patients had a significant higher percentage of CCR7+ memory CD4+ T cells than well controlled patients (mean ± SD = 17.8 ± 5.6) (**figure 4 b**). Regarding the relation between dyspnea scale categories and CCR7+ memory CD4+ T cells, our results revealed that the percentage of CCR7+ memory CD4+ T cells was upregulated with increasing score.

We further investigated whether percentage of the CCR7+ memory CD4+ T cells could impact the % predicted FEV1, where a negative correlation was observed between CCR7+ memory CD4+ T cells and FEV1% pred as well as other pulmonary functions (P <0.001). On the other hand, metacholine challenge test had a positive correlation with the percentage of CCR7+ memory cells CD4+ T cells (P = 0.015), **table V**.

Figure 2 - *The percentages of CCR4⁺ CD4⁺ T cells in BA patients:* ***(a)*** *representative dot plots were shown from allergic asthmatic patients, non-allergic asthmatic patients and healthy individuals,* ***(b)*** *representative dot plots were shown from BA patients who were classified into well controlled, not well controlled and poorly controlled patients based on asthma control test,* ***(c)*** *representative dot plots were shown from BA patients who were classified according to their IgE serum levels into categories (< 20, 20-100, > 100 IU/ml).*



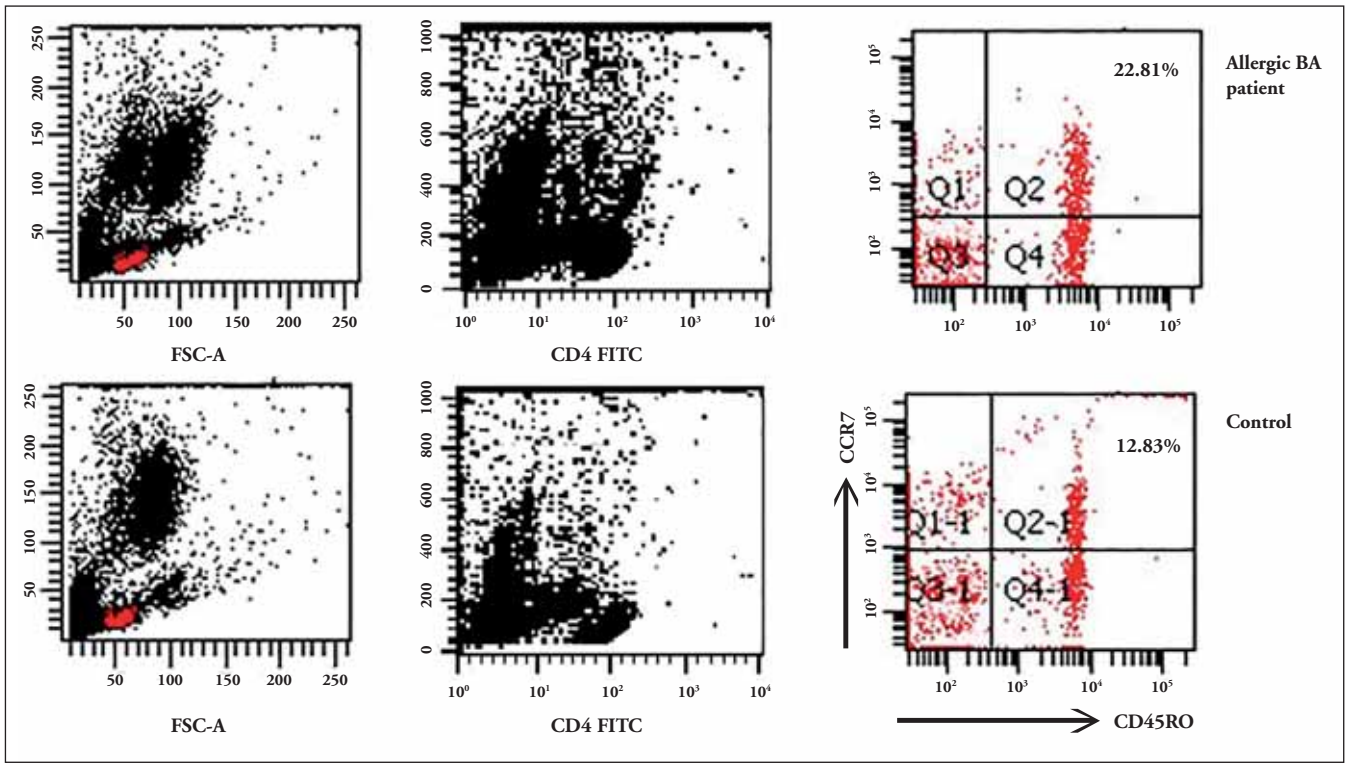
Moreover, we found that the percentage of CCR7⁺ memory CD4⁺ T cells was significantly increased in patients with IgE level > 100 IU/ml compared to those with IgE level < 100 IU/ml (P < 0.001) (**figure 4 c**). Of interest the percentage of CCR7⁻ cells showed no difference between the studied subgroups (P = 0.828) (**figure 5**). Above all, we found that the percentage of CCR7⁺ CD45RO⁺ CD4⁺ T memory cells was positively correlated with CCR4⁺ CD4⁺ T cell (r = 0.555, P < 0.001) (**table IV**) and negatively correlated with % of CCR7⁻ CD45RO⁺ CD4⁺ T cell (r = - 0.470, P < 0.001) (**table V**). However, no correlation was observed with phagocytic activity (r = 0.073, P = 0.429) (**table V**).

Discussion

Despite the improved understanding of the role of airway inflammation in asthma pathogenesis, the sequence of events that lead to persisting airway inflammatory cells and airway hyperresponsiveness in asthma remains to be clarified. A decline in apoptosis in peripheral blood lymphocytes might explain the extensive exacerbations but not the persistent inflammatory reactions seen exclusively in severe BA (34).

It has been shown that sustained allergic inflammation in the lower airway may require an abundant presence of readily primed memory T cells in peripheral blood that can respond to

Figure 3 *- The percentages of CCR7⁺ CD45RO⁺ CD4⁺ T cells in allergic BA patients and healthy control where CD3⁺ CD4⁺ T cells were stained with anti-human CD45RO and anti-human CCR7 antibodies.*



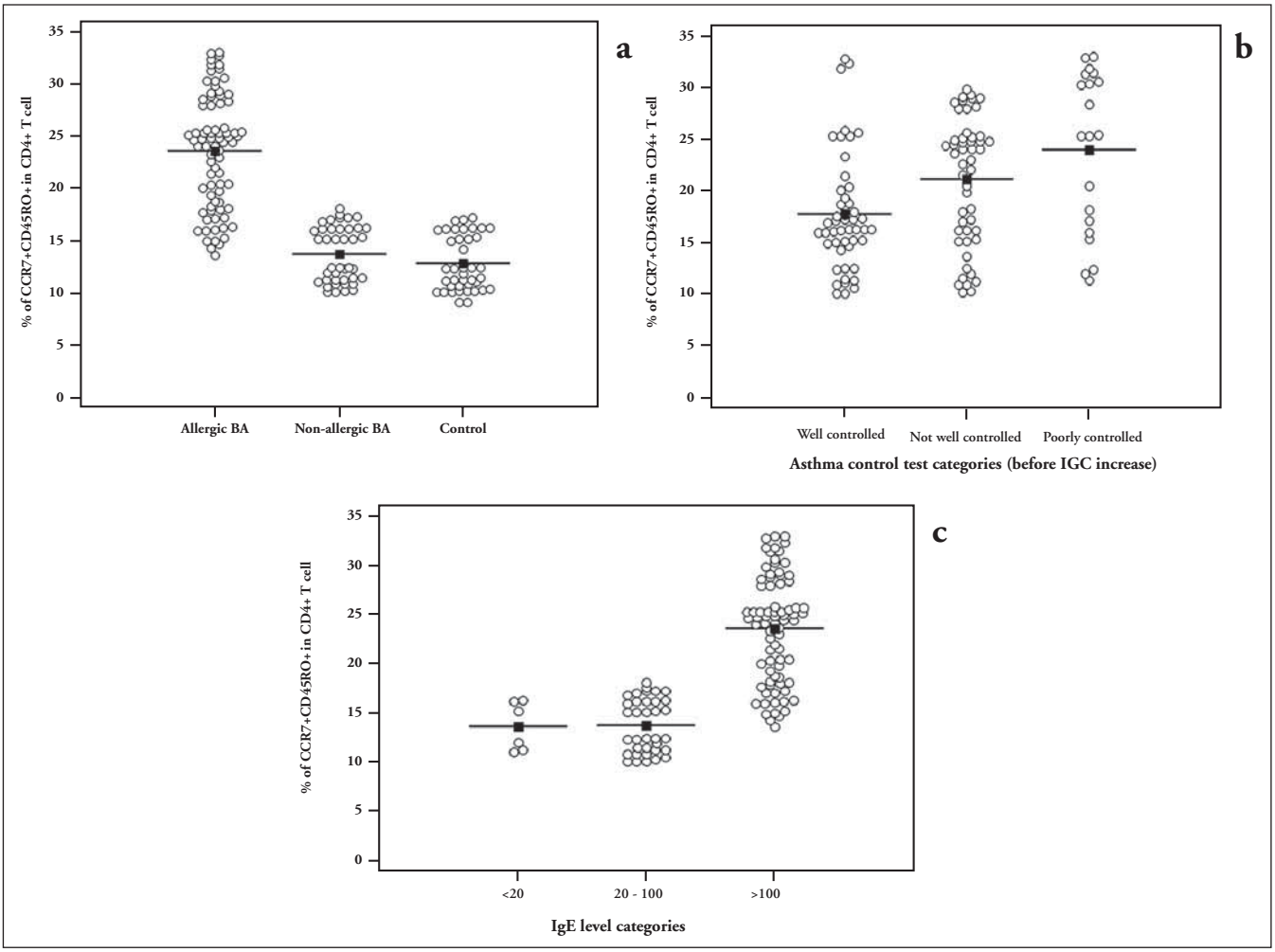
allergens (35). CD4⁺ memory T cells were shown to be involved in recurrent episodes of inflammation in both murine models of BA and BA patients (36, 37). We hypothesized that memory T cells in BA patients display distinctive phenotypes that can sustain chronic inflammation in the lung; and that the expression of certain chemokine receptors on T cells is associated with disease severity and worsening of symptoms.

Over the last few decades, chemokine family and their receptors attracted so much attention for their numerous roles in regulating leukocyte functions throughout inflammation and immune reactivity. A number of studies have speculated that the CCR7 plays essential roles in immune-cell trafficking in various tissue compartments during inflammation and in immunosurveillance (38). Therefore, we analyzed memory (CD45RO⁺) CD4⁺ T cells based on their chemokine receptor (CCR7) expression and the results showed that the percentage of CCR7⁺ CD45RO⁺ CD4⁺ T memory cells was elevated significantly in allergic BA patients compared to both non-allergic BA patients and controls, with an obvious non-significant difference between the latter two groups (P = 0.956). This may be explained by the fact that the immunoregulation through CCR7 expression in T cells plays a role in allergen-specific sensitization in the airway where natural allergen exposure in patients with allergic respiratory syndrome affects T cell activation and their memory status (39). More importantly, we found that the percentage of CCR7⁺ T memory cells was inversely correlated to improved pulmonary function tests, and positively correlated to disease severity scores, suggesting a central role of CCR7⁺ memory T cells in persistence of chronic inflammatory reactions in allergic BA patients' lungs with or without the existence of a specific allergen, and that the memory compartment of severe asthmatic patients expressing CCR7 is significantly expanded.

CCR7⁺ memory T cells (TCM) were also directly correlated to total IgE level being a critical factor for the development of bronchial hyperresponsiveness in asthmatics (40). In concordance, it has been suggested that CCR7 may promote immune inflammation and that the role of cytokines and IgE in allergic asthma may be associated with the expression level of CCR7 where its downregulation was associated with reduced inflammatory cell infiltration and IL-4 levels (41). They were also directly correlated to disease duration. TCMs are thought to have long-lived behavior and show superior engraftment capacities compared with other memory T cell subsets (42).

On the other hand, our results revealed an elevated percentage of CCR7⁻ effector memory T cells (TEM) in BA patients as well, with

Figure 4 - *The percentages of CCR7⁺ CD45RO⁺ CD4⁺ T cells in BA patients:* ***(a)*** *representative dot plots were shown from allergic asthmatic patients, non-allergic asthmatic patients and healthy individuals,* ***(b)*** *representative dot plots were shown from BA patients who were classified into well controlled, not well controlled and poorly controlled patients based on asthma control test,* ***(c)*** *representative dot plots were shown from BA patients who were classified according to their IgE serum levels into categories (< 20, 20-100, > 100 IU/ml).*

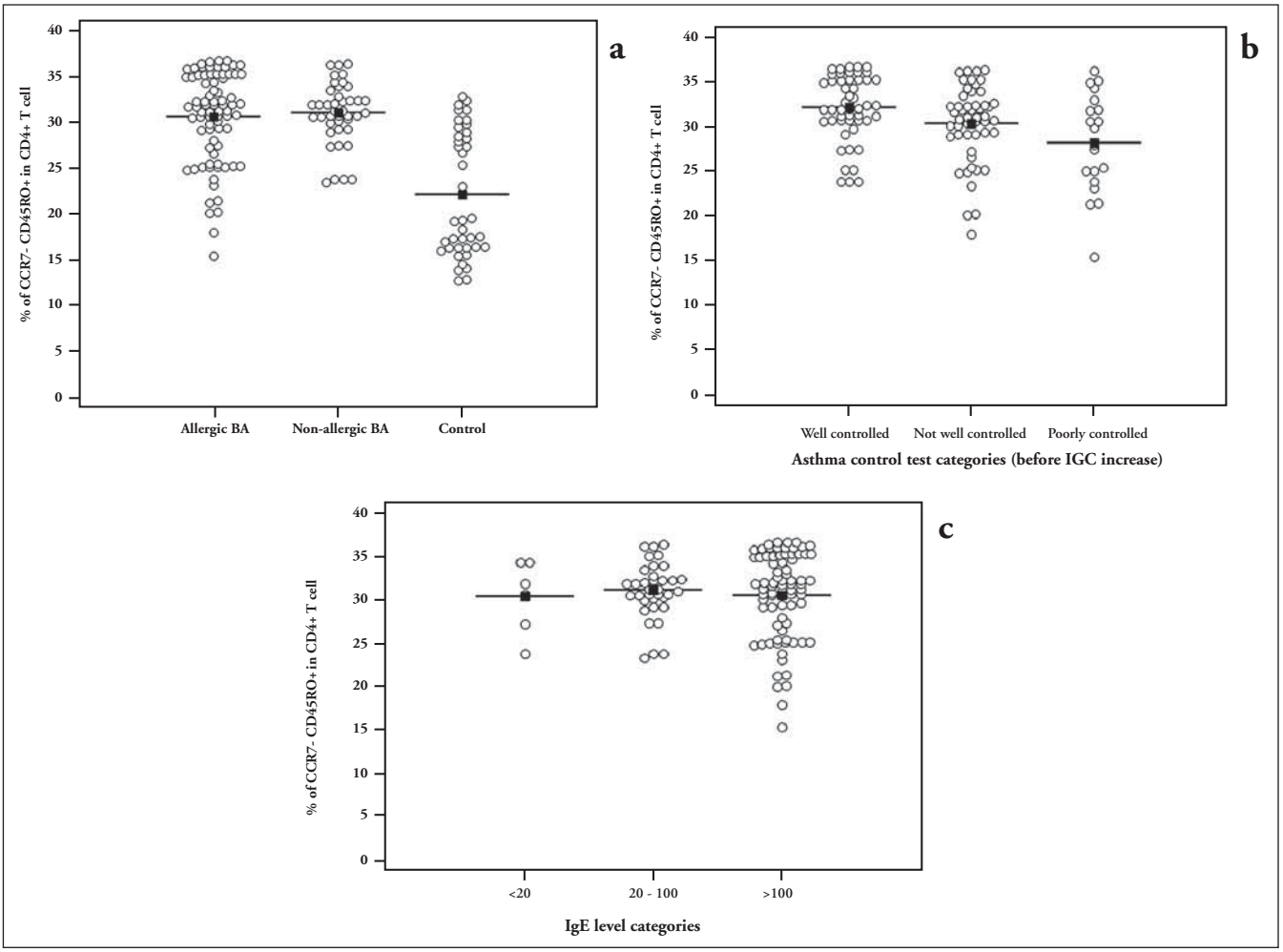


no difference between allergic and non-allergic BA patients, endorsing that the increase of CCR7⁺ CD45RO⁺ CD4⁺ T cells (TCM) in BA patients was not due to a decrease of CCR7⁻ CD45RO⁺ CD4⁺ T cells (TEM) in their blood. TEM cells did not show a significant correlation with any clinical variables, including ACT and % predicted FEV1 scores. In fact an imbalance in memory CD45RO⁺ T cells in peripheral blood of patients with allergic disease have been reported; however, results are inconsistent (43-45).

Parallel to their cytokine expression, subsets of effector T cell express distinct chemokine receptor patterns with an evidence of constant recirculation through the lungs and an immunosurveillance role. The subsequent variation in the local cytokine milieu might induce a change in chemokine receptor expression to allow correct migration within the surrounding airways. Th2 cells have been delineated by expression of CCR4 and CCR8 (35). CCR4 has been long thought to take part in the recruitment of Th2 cells following allergen exposure, owing to its high expression on Th2 cells (46). However, the role of CCR4⁺ T cells in the BA pathogenesis is still controversial (8, 47). In addition, resident pulmonary APCs can present allergen long after cessation of allergen exposure and have been shown to promote Th2 cell differentiation *in situ* (48).

An increase in the percentage of CCR4 expressing CD4⁺ T cells in BA patients has been previously described (8) which was also reported in our study; though, a correlation between proportion of CCR4⁺

Figure 5 *- The percentages of CCR7- CD45RO+ CD4+ T cells in BA patients:* ***(a)*** *representative dot plots were shown from allergic asthmatic patients, non-allergic asthmatic patients and healthy individuals,* ***(b)*** *representative dot plots were shown from BA patients who were classified into well controlled, not well controlled and poorly controlled patients based on asthma control test,* ***(c)*** *representative dot plots were shown from BA patients who were classified according to their IgE serum levels into categories (< 20, 20-100, > 100 IU/ml).*



CD4+ T cells in peripheral blood or in the lungs and the severity of asthma has been declined (49). We described an inverse correlation of CCR4+ CD4+ cells and pulmonary functions. This could be explained by upregulated CCR4 specific ligands on airway epithelial cells upon allergen challenge suggesting an involvement of this receptor/ligand axis in the regulation of CD4+ T lymphocyte recruitment into the BA patients' bronchi. These findings were in line with the above results raising the possibility that the increased expression of CCR4 can be attributed to the expansion of Th2 cells, which could contribute to both chronic disease and allergen induced exacerbations. A direct correlation to IgE level was shown in our study as T cell help is a crucial factor for plasma cell differentiation and immunoglobulins production. Moreover, CCR4+ cells showed a direct correlation to TCM cells and an inverse correlation to TEMs. Furthermore, our results revealed no significant association between neutrophil phagocytic activity and either of studied T cell subsets; however, a correlation to asthma control test was observed. Instead, it has been reported that neutrophils' phagocytic activity was most pronounced in BA patients irrespective of disease severity (50).

Conclusions

In this study, we report an increase of circulating long-lived TCM cells along with CCR4+ CD4+ effector cells in adult patients with allergic BA. This study also describes evidence of a

clinical relevance of the existence of these cells as well as an association with increased disease severity, decreased lung functions, and increased production of immunoglobulin-E. Hence, these results might open a new horizon for proper understanding of the pathogenesis and progression of allergic BA in human, and further direct our efforts toward the rational design of new modalities of proper treatment candidates.

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Conflict of interests

The authors declare that they have no conflict of interests.

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Economic burden of severe asthma in Turkey: a cost of illness study from payer perspective

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Key words

Severe asthma; asthma attack; practice patterns; direct costs; cost analysis; Turkey.

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Summary

Objective. *To estimate economic burden of severe asthma in Turkey from payer perspective based on expert panel opinion on practice patterns in clinical practice.* ***Methods.*** *This cost of illness study was based on identification of per patient annual direct medical costs for the management of severe asthma in Turkey from payer perspective. Average per patient direct medical cost was calculated based on cost items related to outpatient visits, laboratory and radiological tests, hospitalizations and interventions, drug treatment and equipment, and co-morbidities/complications.* ***Results.*** *Based on total annual per patient costs calculated for outpatient admission ($ 177.91), laboratory and radiological tests ($ 82.32), hospitalizations/interventions ($ 1,154.55), drug treatment/equipment ($ 2,289.63) and co-morbidities ($ 665.39) cost items, total per patient annual direct medical cost related to management of severe asthma was calculated to be $ 4,369.76 from payer perspective. Drug treatment/equipment (52.4%) was the main cost driver in the management of severe asthma in Turkey, as followed by hospitalizations/interventions (26.4%) and co-morbidities (15.2%).* ***Conclusions.*** *In conclusion, our findings indicate that managing patients with severe asthma pose a considerable burden to health economics in Turkey, with medications as the main cost driver.*

Introduction

Asthma is a chronic disease with a high prevalence (4.3%) in adults and is a global health, social and economic problem affecting 300 million individuals worldwide (1-3). The prevalence of asthma in Turkey was documented to be 7.4% in Global Initiative for Asthma (GINA) - Global Burden of Asthma Report (4), while approximately 3-4 million people in Turkey have been considered to suffer from asthma (5). Asthma has been associated with significant economic burden in terms of both direct and indirect costs leading to considerable increase in medical expenditures and productivity loss in conjunction with the high prevalence of the disease (6, 7).

Severe asthma was defined by World Health Organization in 2009 as "uncontrolled asthma which can result in risk of frequent severe exacerbations (or death) and/or adverse reactions to medications and/or chronic morbidity" and categorized into 3 groups including untreated severe asthma, difficult-to-treat severe asthma, and treatment-resistant severe asthma (8). Accordingly, while patients with severe asthma comprise only 5% to 10% of overall asthmatic population (9-11), severe asthma was associated with use of almost 80% of health resources allocated to the disease and considered to be responsible for 50% of all direct and indirect healthcare costs (12-18).

Both asthma severity and presence of asthma exacerbations are considered amongst the important risk factors for increased asth-

ma cost (6, 12, 13, 16, 19, 20), particularly in terms of increased health care utilization and costs due to increased hospitalizations (20-22). Considerable percentage of patients with severe asthma have a poor therapeutic response to available pharmacotherapy and continue to experience exacerbations despite the use of intensive therapy (15, 18, 23-25). This seems notable given the almost 2-fold cost increment in asthma-related costs among patients with severe asthma who experience exacerbations compared to patients with controlled severe disease (25, 26).

In past studies on economic burden of managing asthma, higher average annual direct costs were reported in patients with persistent asthma as compared with non-asthmatic control subjects (27), in severe persistent asthma as compared with mild and moderate persistent asthma (6, 14, 28, 29), in poorly controlled asthma as compared with controlled asthma (21, 26, 30), and in patients with than without exacerbation (6, 21, 25, 31), while limited data are available on the impact of acute asthma exacerbations in severe asthma patients (20, 25, 31).

Asthma exacerbation has a high prevalence in Turkey which results in increased hospital costs, long-term treatment and complications and thus further cost increment. While the treatment is reimbursed totally by the Turkish healthcare system, in a monopsony market based on government reimbursement and overall coverage of health insurance for the entire population, treatment costs for diseases necessitating long-term and expensive treatments often extends the amount of reimbursement, particularly in tertiary care hospitals. In fact, expert panel-based cost-of-illness studies have recently become popular in literature despite they are an established consensus-finding method in clinical and health services research considered very important in terms of public health investigations and health strategy development. Given the cost increment associated with uncontrolled disease status and ongoing restrictions in the healthcare budgets, this type of data should be more extensively addressed by researchers and be discussed on appropriate platforms to enable awareness raising and more efficient use of healthcare resources.

This cost of illness study was therefore designed to determine economic burden of severe asthma in Turkey from payer perspective and in relation to acute attack and attack-free periods.

Methods

Design

This cost of illness study was based on identification of per patient annual direct medical costs for the management of severe asthma in Turkey based on expert panel opinion on practice patterns in clinical practice. Direct medical cost was calculated based on cost items related to outpatient visits, laboratory and radiological tests, hospitalizations/interventions, drug treatment/equipment and co-morbidities.

Data on real life clinical practice

Data on real-life practice patterns in the management of severe asthma in Turkey including outpatient clinic admission rates, laboratory and radiological investigations, selected medications, hospitalizations and interventions were based on expert panel consensus. Expert panel members were from selected from 14 tertiary healthcare centers for Pulmonology and Allergy and Immunology diseases providing severe asthma patient care across Turkey, by the Project Advisory Board of the study according to the geographical distribution of specialists in Turkey. The participating 17 experts (professors, also international speakers and national influencers), who had at least 15 years of experience in Pulmonology and Allergy and Immunology were invited to participate in the meeting and informed about the study via e-mail and asked to fill a standardized form reflecting data from their clinic before attending the meeting. Hence each expert participated in the consecutive meetings to achieve the proposed consensus based on data provided for different clinics reflecting actual patient data used to fill out the forms, based on sampling of overall 20,879 patient admissions per year. The panel critically analyzed the previously published literature data on real-life practice patterns in the management of severe asthma in Turkey and agreed on a series of statements supported by scientific evidence and expert clinical opinion. The local ethics committee of Ankara University, School of Medicine, approved the study (Approval number: 14-685-16)

Cost analysis

Average per patient direct medical costs were calculated based on cost items outpatient visits, laboratory and radiological tests, hospitalizations/interventions, drug treatment/equipment and co-morbidities from payer perspective (only direct medical costs using prices of the public payer "Social Security Institution (SSI)" in Turkey), using cost of illness method developed by WHO (32). For drugs, retail prices from the updated price list and updated institution discount list of SSI for May 2016 were taken into account in calculation of the unit costs (33). Average usage rate for active ingredients was calculated based on data provided by each clinic regarding percent use of active ingredients, while the average of all brands included in the current reimbursement system was calculated using the unit costs. Costs related to diagnostic tests were calculated considering the Health Implementation Notification by SSI (34). Physician visits costs were calculated using unit prices also based on the same SSI notification (34). Salaries and labor force of healthcare staff giving service to pediatric asthma patients was provided from the Healthcare Organization Questionnaire composed of Staff Inventory Form and Information Form on the Labor Force Spent during an intervention filled for each study center. Hospitalization costs were calculated using unit prices based on Healthcare Organization Price List in Health Practice Declaration and Treatment Assist Practice Declaration. Monetary results were converted by using

2.97 USD/TL May 2016 exchange rate. Direct non-medical costs of different origin (*e.g.*, transfers of patient and caregivers for examinations and/or hospitalization, home care, *etc*.) and indirect costs were not included in the cost analysis. For each cost item, calculation was based on the formula:
Percentage of patients utilizing the item x number of item utilization x unit cost.
Total cost was reached via sum of all cost items.

Statistical analysis

Descriptive statistics were used to summarize results on practice patterns for the pediatric asthma management. Expenses related to management of pediatric asthma were the main cost-analysis related parameter of the study. Cost model was based on the following equation: *Cost* = $\sum$ *(Frequency; %) x (Unit price; TL) x (patient ratio; %).*

Results

Overall patient profile

The present cost of illness study was based on expert panel opinion regarding practice patterns in the management of severe asthma in Turkey and included overall 25,579 patient admissions (severe asthma in 21.25%) per year from 14 clinics across Turkey. Accordingly cost calculations were based on the prevalence of severe asthma (21.25%), rates of controlled (32.56%), partially controlled (40.06%) and uncontrolled (27.39%) disease in severe asthma patients, prevalence of severe asthma in patients with severe asthma attack (60.83%), rates for controlled (30.0%) and uncontrolled (55.33%) disease in severe asthma patients with severe asthma attack and percentage (72.50%) of acute asthma attacks in patients with severe asthma being treated with hospitalization.

Outpatient admission cost item

Outpatient admission was estimated to occur in 100.00% of patients and for 11 times per patient per year at Pulmonology and Allergy and Immunology Diseases outpatient clinics, in 34.2% of patients at Cardiology, in 29.5% of patients at Ear Nose and Throat and in 27.4% of patients at Endocrinology outpatient clinics, each for once a year per patient. Acute asthma attack was considered to be associated with admission to Pulmonology and Allergy and Immunology Diseases outpatient clinics in 50.3% of patients and for 4 times per patient per year (**table I**).
Based on unit costs, total per patient annual cost related to outpatient admissions was calculated to be $ 177.91 (**table I**).

Laboratory and radiological tests cost item

Most common laboratory tests were considered to be spirometry (99.7%), PA/lateral chest X-ray (94.8%) and reversibility test (95.9%) during attack-free period, while respiratory function test (91.4%), reversibility test (82.5%) and skin prick test (84.5%) during an acute asthma attack. High resolution lung CT (56.0%) and lung CT (35.7%) were the most commonly required radiological tests in attack-free period and during an acute asthma attack, respectively (**table II**).
Based on unit costs, total per patient annual cost related to laboratory and radiological tests was calculated to be $ 82.32 (**table II**).

Table I *- Outpatient admission cost item: clinical practice, unit costs and total cost.*

Outpatient admissions	Annual admission rate (%)	Annual visit # per patient	Unit cost per admission ($)	Total cost
Pulmonology and allergy and immunology diseases	100.0	11	13.39	147325.10
Ear nose and throat	29.5	1	2.02	595.56
Endocrinology	27.4	1	2.02	554.14
Gastroenterology	2.5	1	2.02	50.51
Rheumatology	2.9	1	2.02	58.99
Cardiology	34.2	1	2.02	691.72
Psychiatry	17.1	1	2.02	345.05
Ophthalmology	14.9	1	2.02	301.41
Physical therapy and rehabilitation	21.4	1	2.02	432.12
Chest diseases (acute attack)	50.3	4	13.39	26925.67
Emergency (acute attack)	3.1	4	5.22	643.01
Total				177923.28
Per patient outpatient admission costs ($)				**177.91**

Hospitalizations/interventions cost item

Hospitalizations were considered to occur twice in a year at ward in 39.8% of patients (each for 8 days) and at ICU in 1.5% of patients (each for 4 days) with severe asthma during attack-free period. Overall, a patient with severe asthma was considered to have 4 attacks per year and 3 attacks per year (72.5% of total attacks) were considered to result in hospitalization. Hospitalization for an acute attack was considered to occur tree times in a year at ward in 47.6% (8 days for each) and at ICU in 2.1% (1 day for each) of patients with severe asthma (**table III**).

Based on unit costs, total per patient annual cost related to hospitalizations and interventions was calculated to be $ 1,154.55 ($ 525.1 for attack-free period and $ 629.5 for acute asthma attack related hospitalizations) (**table III**).

Drug treatment and equipment cost item

Based on prescription rates in Turkey, maintenance doses and annual dose and unit cost per box for each drug regimen and unit costs of equipment, total per patient annual cost related to drug treatment and equipment was calculated to be $ 2,289.63 ($ 2,199.7 for attack-free period and $ 89.8 during acute asthma attack) (**table IV**).

Co-morbidities cost item

Most common co-morbidities in severe asthma patients in Turkey were considered to be rhinitis (47.4%), reflux (43.8%), sinusitis (42.2%) and allergic rhinitis (33.3%). Based on prevalence of comorbid disorders in patients with severe asthma in Turkey and related unit costs, total per patient annual cost related to co-morbidities and complications was calculated to be $ 665.39 (**table V**).

Per patient total annual direct medical cost

Based on total annual per patient costs calculated for outpatient admission ($ 177.91), laboratory and radiological tests ($ 82.32), hospitalizations/interventions ($ 1,154.55), drug treatment/equipment ($ 2,289.63) and co-morbidities ($ 665.39) cost items, total per patient annual direct medical cost related to management of severe asthma was calculated to be $ 4,369.76 from payer perspective (**table VI**).

Drug treatment/equipment (52.4%) was the main cost driver in the management of severe asthma in Turkey, as followed by hospitalizations/interventions (26.4%) and co-morbidities (15.2%) (**table VI**).

Discussion

Our findings revealed that per patient annual direct medical cost of severe asthma in Turkey was $ 4,369.76 from payer perspective and drug treatment ($ 2,289.63; 52.4%) was the major cost driver as followed by hospitalizations ($ 1,154.55; 26.4%) and co-morbidities ($ 665.39; 15.2%). Drug treatment cost was higher for attack free period as compared with acute attack ($ 2,199.7 *vs* $ 89.8), while anti-IgE treatment was responsible for 83% of the medication cost during attack-free period.

Annual direct per patient medical cost for severe asthma was reported to be $ 658 in Thailand (35), $ 135 to $ 733 in Brazil (17), $ 1277 in Spain (14), $ 1563 in previously in Turkey (36), $ 1635 in South Korea (20), $ 2214 in Korea (16), CHF 3075 in Switzerland (31), VND 13,196,280 in Vietnam (37), € 2635 in Spain (38), $ 6354 in USA (28), € 8221.5 in France (39), while estimated at $ 4369 in the present analysis.

In a systematic review of 29 cost-of-illness studies of asthma, the annual incremental socio-economic cost of asthma was reported to range from € 416 to € 5,317 in adults and to further increase with level of severity from € 964 for intermittent asthma to € 11,703 for severe persistent asthma (40). Authors concluded that a large variation exists in the severe asthma costs per affected person by country limiting their comparability (40). This seems consistent with the similarly non-uniform data regarding the total annual asthma costs between countries that ranges from $ 346 in the USA and US $ 1,395 in Sweden (14, 28). Notably, the assessment of asthma burden is considered a challenge with remarkable variations in asthma prevalence and disease severity within and among countries even with use of similar research protocols (41).

Our findings revealed medications (52.4%) to be the main cost driver in severe asthma, particularly in the attack-free period, while hospitalization (26.4%) was the second-most contributor to overall direct cost, particularly during acute exacerbation. This supports that prescription medications rather than hospitalizations comprise the largest percentage of total costs attributable to asthma in the adult population (42-44).

In a retrospective analysis of a national administrative claims database in USA, authors reported that an increased mean annual asthma-related costs in severe versus persistent asthma ($ 6,496 *vs* $ 2,739) which was shown to be driven mainly by 3-fold greater mean annual asthma medication costs in severe asthma, while twice as many asthma-related hospitalizations in severe asthma represented the second largest category of asthma-related costs (18).

Likewise, total per-person direct annual costs of asthma were reported to be $ 3180 in USA with medications ($ 1605; 50%) rather than hospital admissions ($ 463; 15%) accounted for the largest share of direct costs (39). However, subgroup analysis of patients with severe asthma revealed direct per patient medical cost to be $ 6354 with consideration of both medications ($ 2404) and hospitalizations ($ 2122) as the major cost drivers (39). Similarly, in a systemic review of cost of severe asthma in Brazil, average annual direct costs per patient for severe asthma was reported to range from $135 to $ 733 from public health system perspective and to range from $ 764 to $ 929 from the family perspective with hospitalizations and medications indicated to be the key cost drivers of severe asthma (17).

Drug cost and hospitalization were also documented to be main cost items responsible for the burden of severe asthma on health

Table II *- Laboratory and radiological tests cost item: clinical practice, unit and total cost.*

Laboratory/radiological tests	Annual rate (%)	Annual test # per patient	Unit cost ($)	Total cost ($)
At diagnosis				
Spirometry	99.7	1	8.42	8395.08
Complete blood count	92.9	1	0.00	0.00
PA/lateral chest X-ray	94.8	1	0.00	0.00
Reversibility test	95.9	1	0.00	0.00
Sputum smear	10.8	1	0.00	0.00
High resolution lung CT	56.0	1	18.52	10372.36
DLCO	41.1	1	14.98	6153.41
Specific IgE measurement	29.4	1	7.14	2097.85
Serum total IgE measurement	81.5	1	0.00	0
Skin Prick Test	89.9	1	20.21	18163.11
Sinus CT	37.0	1	18.52	6848.24
Eosinophil count	95.1	1	0.00	0
BMD	26.5	1	7.00	1852.34
During follow up				
Respiratory function test	91.4	1	8.42	7693.87
Reversibility	82.5	1	0.00	0
Bronchial provocation test	3.6	1	12.90	460.61
Exhaled CO measurement	10.7	1	8.42	901.55
Lung CT	35.7	1	18.52	6614.91
Skin Prick test	84.5	1	0.00	0
Sweat test	0.5	1	11.41	61.64
Specific IgE measurement	25.3	1	0.00	0
Lung MRI	0.6	1	21.89	129.12
Bronchoscopy	8.5	1	50.34	4258.52
Tuberculin test	1.2	1	0.00	0
Bronchial biopsy	2.6	1	77.03	2010.46
Lung volume diffusing capacity	35.7	1	14.98	5350.33
Arterial blood gas analysis	32.3	1	0.00	0
Exercise test	4.25	1	10.00	424.96
Alpha-1 antitrypsin test	3.0	1	2.79	84.98
Theophylline level	3.9	1	4.71	185.26
Endoscopy	22.1	1	33.70	74.34
BMD	13.1	1	0.0	44.21
High resolution lung CT	41.7	1	18.52	140.27
Total				82317.42
Per patient laboratory and radiological tests cost ($)				**82.32**

BMD: Bone mineral density; CO: carbon monoxide; CT: computerized tomography; DLCO: Carbon monoxide diffusing capacity; Ig: immunoglobulin; MRI: magnetic resonance imaging; PA: posteroanterior.

Table III - *Hospitalization/interventions cost item: clinical practice, unit costs and total cost.*

	Annual # of hospitalization	Rate (%)	LOS per admission (days)	Unit daily cost ($)	Total cost ($)
Attack-free period					
Pulmonology ward and allergy and ICU	2	39.8	8	73.19	465715.15
immunology	2	1.5	4	503.69	59292.26
Diseases chest total					525007.07
For an acute attack					
Pulmonology ward and allergy and ICU	3	47.6	8	73.19	835953.54
Immunology	3	2.1	1	503.69	32380.47
Diseases chest total					629542.14
Total					1154549.36
Per patient hospitalization cost ($)					**1,154.55**

ICU: Intensive care unit, LOS: Length of hospital stay.

economics in a past from Turkey which evaluated the direct costs of asthma in the same patient group within eight years interval (36). Authors reported no significant difference from 2000 to 2008 in mean annual asthma cost ($ 659.8 *vs* $ 830.2) and in the cost increment due to severe asthma ($ 1563 *vs* $ 152.8 for mild and $ 857.4 for moderate asthma), while drugs (45%) and hospitalization (40%) were reported to be the main components of the direct costs (36).

Contribution of attack-free disease to the majority (96%) of total drug costs in the present study supports the reported increase in the percentage of total cost attributable to hospitalization (from 4% to 48%) and decrease in the percentage of total cost attributable to drugs (from 46% to 26%) with decrease in asthma control level in Turkey (30). A decline in the percentage (from 47% to 19%) of total costs attributed to medications as the disease severity increases from mild to severe was also reported in asthma patients from USA (28).

This seems also to be in agreement with findings from a past study on the economic burden of asthma in Asia-Pacific region which revealed maintenance costs (medication, physician visits) in controlled asthma, whereas higher urgent care costs (emergency care and hospitalizations) in case of poor asthma to be the main driver of asthma-related costs (45).

Notably, analysis of costs by disease severity and exacerbation status in asthma patients from Switzerland revealed attribution of medication and hospitalization costs to 70.4% and 9.6% of total cost, respectively in patients without exacerbations, while to 28.1% and 63.4% of total costs, respectively in patients with exacerbation (31). Accordingly, our findings emphasize medications as the main cost driver predominantly in the attack-free period with the cost of drugs approximately doubling the cost of hospitalization for severe asthma. Although these findings support the consideration of asthma drugs to account for most of the incremental ($ 1056) per patient annual direct costs attributed to severe uncontrolled asthma (46), it should be noted that addressing the biologic use is important in cost-analysis of managing patients with severe asthma who are the primary target for biologic therapies (47). Although biologics have been reported to be associated with decrease in the frequency of asthma exacerbations, unplanned health care use, including emergency admissions and hospitalizations in clinical trials with severe asthma patients (48), the major concern about use of biologics is the cost (49). Data from pharmaceutical manufacturers indicated the wholesale acquisition cost of an individual unit of these biologics to range from $ 879 to > $ 47502, while ICER report recommended that biologic costs would need to be reduced 62% to 80% from their 2018 wholesale acquisition cost, depending on the biologic, to meet the cost-effectiveness threshold (50). Hence, the cost of omalizumab (the only biological drug available in the current study), calculated based on local references (51-53), contributed to 83% of overall medication cost in the attack-free period in our study. This seems notable given that cost-effectiveness data for omalizumab revealed wholesale acquisition cost to be $ 39,048 and discount from WAC required to achieve cost-effectiveness threshold prices to be 66-77 % (49). Nonetheless, the sustainability of biological drugs is considered doubtful and difficult to be demonstrated, if the payer does not take into account the indirect and intangible costs.

It should be noted that the 72.50% of acute attacks in severe asthma patients were considered to be treated by hospitalization in the present cost analysis. Given that the smaller proportion in hospital costs and the higher proportion in medication costs is considered suggestive of better control of asthma (6), significant contribution of experiencing exacerbations to total hospitalization costs but not

Table IV *- Drug treatment and equipment cost item: clinical practice, unit costs and total cost.*

Attack-free period					
Equipment	**Patients (%)**	**Duration**	**# of equipment**	**Unit cost ($)**	**Total cost ($)**
Peak flow meter	23.1	1/year	1	0.00	0
Nebulizer	53.2	1/year	1	0.45	241.64
Drugs	**Prescription (%)**	**Daily dose**	**Duration**	**Unit cost (box/year; $)**	
SABA	85.2	According to posology		31.89	27154.77
LABA + ICS	99.9			150.98	150869.78
Systemic steroids	27.0			22.71	6137.06
Leukotriene antagonists	69.9			104.32	72882.33
SAMA	17.5			314.31	55003.47
Triotropium/LAMA	30.1			170.80	51446.79
Theophylline SR	17.1			30.76	5265.43
Theophylline FA	7.8			57.58	4478.11
Anti-IgE	26.1			499.51	1826147.74
Clarithromycin	1.9			8.81	166.76
Total (attack free)					2199793.89
For acute attack					
Nebulized ICS	64.6	According to posology		11.00	7108.18
Systemic steroids	97.1			3.91	3797.95
LABA-FA	37.1			7.69	2857.47
ICS/LABA	45.4			13.39	6073.43
SABA	100.0			3.10	3103.37
SAMA	72.9			4.31	3136.91
Theophylline FA	43.6			1.55	673.68
Magnesium sulphate	24.8			17.06	4221.82
Total (acute attack)					89821.10
Total					2289614.99
Per patient drug/equipment cost ($)					**2,289.63**

ICS: Inhaled corticosteroids; FA: fast-acting; Ig: Immunoglobulin; LABA: Long-acting beta-2 agonists; LAMA: Long-acting muscarinic antagonist; SABA: Short-acting beta-2 agonists; SAMA: Short-acting muscarinic antagonist; SR: slow release.

to medication costs in severe asthma patients in our analysis seems to emphasize the likelihood of inappropriate treatment and failure to reach targeted treatment intensity recommended by guidelines in a considerable portion of patients with severe asthma (54, 55). In a cost of illness study estimating the direct cost per asthma exacerbation in Turkey, high hospitalization rates reported in patients with asthma attack was considered to be highly suggestive of unnecessary and inappropriate hospitalization since asthma attack was mild to moderate in more than 75% of patients (21). Given that asthma attacks leading to hospitalization account for 90% of the total costs of attacks (56), our findings emphasize the likelihood of a cost-saving with appropriate hospitalization and better management of asthma attacks in severe asthma patients, since effective implementation of best practice results in significant cost savings in asthma management (57). Presence of co-morbidities were reported to be associated with significant cost increment in asthma patients along with consideration of even low-cost high prevalence diseases such as lower respiratory tract infections as significant cost drivers (6, 31, 58, 59). Accordingly, in our analysis co-morbidities (26.4%) were the third largest category of asthma-related costs following the medication and hospitalization costs in patients with severe asthma.

Table V *- Co-morbidities cost item: clinical practice, unit costs and total cost.*

Comorbidities	Patients (%)	Unit cost ($)	Total cost ($)
Rhinitis	47.4	26.34	12486.92
Allergic rhinitis	33.3	157.43	52358.69
Sinusitis	42.2	19.68	8306.75
Polyp	20.4	331.39	67438.85
Reflux	43.8	228.51	100104.60
Hypertension	33.4	58.06	19393.85
Obesity	10.6	0.00	0.00
Sleep apnea	5.9	926.82	54285.41
Psychiatric disease	17.7	91.52	16158.78
CAD	2.9	345.19	9862.70
Rheumatic disease	2.1	5.22	107.45
Cataract	2.4	138.05	3253.97
PID	0.8	38815.49	321614.04
Total			665372.03
Per patient comorbidities cost ($)			**665.39**

CAD: Coranary artery disease; PID: Primary Immune Deficiency.

This seems notable given that in a systemic review of 68 cost-of-illness studies of asthma, disease severity and presence of co-morbidities were found to be indicated amongst the factors that can contribute to higher total hospital costs by studies which revealed hospitalization as the major cost driver of direct asthma costs (6). Certain limitations to this study should be considered. First, being focused only on direct costs, lack of data on indirect costs (loss of productivity due to the illness) or intangible costs of illness (costs of suffering for the patient and his/her family) seems to be the major limitation of the present study which likely to result in a downward bias in our estimates of the economic cost of severe asthma. Second, use of expert consensus based data rather than national database on practice patterns to identify direct medical costs might raise a concern with the validity and reliability of the data. Third, while a cost-of-illness study gives a perspective on the economic burden of asthma in a population, it does not reflect what is happening with the individual patient or family unit. Fourth, cost analysis was based on severe asthma care in tertiary care centers and therefore practice patterns and related contribution to overall of direct cost of severe asthma may differ from non-tertiary care centers. Nevertheless, providing cost estimates for management of severe asthma patients with respect attack-free and attack periods in Turkey, our findings represent a valuable contribution to the literature.

Conclusions

In conclusion, our findings indicate that managing patients with severe asthma pose a considerable burden to health economics in Turkey, with medications as the main cost driver, particularly in the attack-free period alongside the likelihood of hospitalization to account for a larger share of costs in patients with acute exacerbation. Hence, our findings emphasize the likelihood of cost-savings with implementation of appropriate hospitalization practices, more effective strategies to prevent asthma attacks and to improve asthma control status as well as with better management of asthma attacks in severe asthma patients. Future studies addressing both direct and indirect costs of severe asthma with the potential impact of factors such as patient adherence, inhaler techniques or smoking on cost estimates may help to extend the knowledge about the impact of severe asthma on functioning and quality of life and morbidity at individual and family level and to develop cost-effective strategies in the disease management.

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Table VI *- Per patient annual direct medical cost related to management of severe asthma.*

Cost items	Per patient annual cost ($)	Contribution to total cost (%)
Outpatient admission	177.91	4.1
Laboratory test	82.32	1.9
Hospitalization/intervention	1,154.55	26.4
Drug/equipment	2,289.63	52.4
Comorbidities/complications	665.39	15.2
Total direct per patient cost ($)	**4,369.76**	

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Conflict of interests

The authors declare that they have no conflict of interests.

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What do asthmatic patients think about telemedicine visits?

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Asthma; COVID-19; survey; telemedicine; virtual visits.

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Summary
Introduction. *Due to the Coronavirus disease 2019 (COVID-19) outbreak and the national emergency state, virtual visits were implemented as an alternative to in-person visits. With this study we aimed to establish asthma patients' general satisfaction with the quality of health care provided by virtual visits (phone or video calls).* ***Materials and methods.*** *A questionnaire (9 questions) was published on the Facebook page of the Portuguese Association of Asthmatics. It was available online for general self-reported asthmatic patients to answer during one month, starting on 11st May 2020. The survey only allowed one answer per registered user.* ***Results.*** *Fifty-five responses were obtained. Patients were satisfied with communication with providers (> 88%); nevertheless, one-half evaluated the virtual visit as inferior when compared to in-person visits. About one third attributed a classification of 6 or less (0-10 scale, 0 being the worst and 10 the best consultation possible), but still most of the patients would either recommend it or use this kind of medical visits in the future, even outside the actual pandemic context. Patients also referred some important limitations, as lack of physical examination and the fact that the medical visit was more impersonal. Only 27% had technical issues accessing virtual visits. Positive aspects were also named, such as virtual visits being practical and avoiding the need to move to the hospital.* ***Discussion and conclusions.*** *Our survey revealed that small changes could further increase patients' satisfaction, adherence and confidence in telemedicine. Although presenting some limitations, virtual visits seem to be generally well accepted by asthmatic patients and it might be a good alternative for in-person visits, at least in such difficult times when social distancing is recommended.*

Introduction

Worldwide coronavirus disease 2019 (COVID-19) brought a lot of challenges to healthcare organizations, including safety measures, with the need to restrict the number of face-to-face visits (1). Telemedicine is capable to overcome the distance and safety barriers in this context and might be as effective as in-person visits for outpatient management of asthma (2), enabling mild to moderate-severe patients to get the supportive care they need. Several authors documented that virtual visits (VV), that could be either video or phone calls, for asthma patients allow positive outcomes, such as more symptom-free days and fewer emergency department visits or hospitalizations, improving asthma control (3, 4). Moreover, it was demonstrated that VV are comparable to in-person visits, enabling its occasional replacement with same outcomes in asthma control (5).
Every patient might be at risk of SARS-CoV-2 exposure (6) and to reduce such risk, as it successfully occurred in many other medical specialties around the globe (7-11), allergy centers implemented VV as an alternative to in-person visits (12). As telemedicine programs were nationally applied, we became curious about the acceptability and satisfaction of asthmatic patients with this type of virtual visits. With this study we aimed to establish self-reported asthma patients' general satisfaction with the quality of health care provided in VV during the recent National Emergency State in Portugal.

Materials and methods

The authors conducted an online survey consisting of eight multiple-choice questions and one optional open-ended question for asthma patients that had recently participated in VV (either phone or video calls), partially adapted from the questionnaire used by Donelan K. *et al.* (13). The survey addressed three main domains of virtual visits: communication with the provider, quality of the visit and technical difficulties in online access. Quality of the visit was accessed asking the patient to rate it in a scale from 0 to 10, 0 being the worst and 10 the best consultation possible and asking them to compare to an in-person visit. The online survey only enabled the same user to answer once, except if using another computer/e-mail. The survey was published on the Portuguese Association of Asthmatics Facebook page, being available online for one month starting on 11th May 2020. Patients were self-reported asthmatic patients having access to this Facebook page. The questionnaire was specifically addressed to self-reported asthma patients and no information regarding personal characteristics was asked, enabling a shorter survey, and overcoming potential privacy issues.

Results

We obtained 55 replies to our survey. The survey (freely translated to English language) and respective answers are shown in **table I**. Patients were satisfied with communication with the provider (87,5% said the clinician listened carefully to their questions or complaints, and 90,9% said the clinician exposed things clearly). The length of the appointment was adequate for 76,3% as they were satisfied with the amount of time the doctor spent with them. One-half of the patients evaluated the teleconsultation as inferior when compared to in-person visits and about one third attributed a classification of 6 or less to it. Only 27,3% had some technical issues accessing the virtual visit and the majority of patients would either recommend it or use this model of visits in the future, even outside actual pandemic context.

In addition to the answers to the pre-established questions, patients were given the possibility to point out some critics or compliments in the last question (optional and open-ended). Twenty-seven patients answered to this optional question (**table II**). In this open-ended question patients signaled as negative aspects the lack of physical examination and the fact that the medical visit was more impersonal. Compliments were given to the fact that it was a very practical and fast way to access a medical appointment and avoided to move by transportations to the hospital.

Discussion

As it was also previously found by other authors outside this pandemic context (13), patients reported an overall satisfaction with VV during the COVID-19 outbreak. Communication between patients and providers was not compromised in this model of appointments (> 85% were satisfied with both explanation and active listening by the doctor). Other reports documented similar results, as it was found in a systematic review of 32 studies suggesting that VV were acceptable to patients in several circumstances (14).

Nevertheless, to obtain an increase of VV in daily practice much can be learned from this survey, and some aspects have to be improved in the future. Furthermore, the pandemic context might interfere with patient's expectations and lead to a perception of an overall satisfaction that otherwise would not be noted. About one-half of patients ranked their last VV as inferior to in-person visits and one-third of them attributed a classification of 6 or less to these appointments. Complementing this information with limitations pointed out in the open answer question, the major concern for the patients was the lack of physical examination, so it can be hypothesized that this is the main factor preventing further acceptance to this telemedicine tool. Some smartphone apps have been tested for the detection and analysis of both cardiac and pulmonary auscultation sounds, and might constitute a future solution to overcome this limitation of virtual visits (15, 16).

Although the identified limitations, most patients would recommend VV to their friends and family members and would use it in the future. This reveals that small changes could further increase patients' satisfaction, adherence and confidence in telemedicine for healthcare assistance.

In addition, patients acknowledge that this kind of appointments is a valuable tool for disease follow-ups and prescription renewal. These results are inconsistent with those found by Duplaga M. *et al.* (17) that stated, patients suffering from chronic respiratory diseases have a high acceptance of e-health applications (appointment booking, prescription renewal, and access to laboratory test results and educational resources) but do not recognize telemedicine as a valuable solution directly related to medical care (communication with healthcare providers and disease monitoring) (17).

Surprisingly, technical issues were a minor difficulty, with only one quarter of the patients reporting technical problems accessing to the VV. Other potential patients' concerns, such as legal, safety or privacy issues (18) were not contemplated in our questionnaire, but remained unreported in the open-ended question.

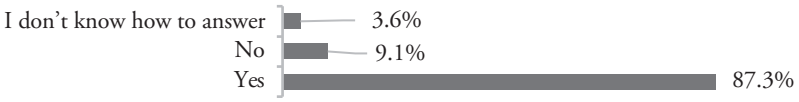
The authors believe that the aspects pointed out by asthma patients are excellent opportunities to improve adherence to VV by asthma patients in the near future. For instance, doctors might clarify patients that in follow-up visits a good clinical history and attention to some physical signals visible by video might partially replace physical examination, despite not being able to perform an important observation step that is pulmonary and cardiac auscultation. This could surpass patients' fears and insecurities that their illness might not be well managed without physical examination, promoting more recognition of the potential of VV.

Table I *- Answers of patients with asthma that participated in virtual medical appointments during the Emergency State.*

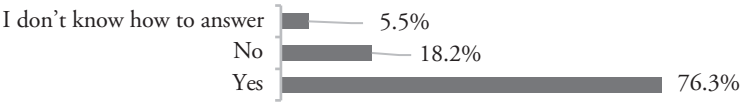
PATIENTS VIRTUAL VISITS SURVEY (n = 55)

The clinician exposed things clearly or in an easy way for me to understand?

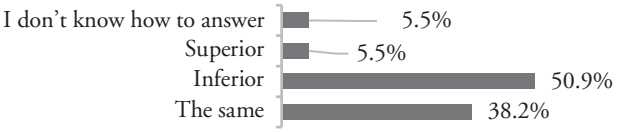
The clinician listened carefully to my complains and questions?



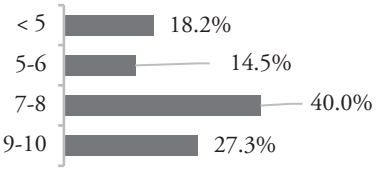
Am I satisfied with the amount of time the doctor spent with me on this visit?



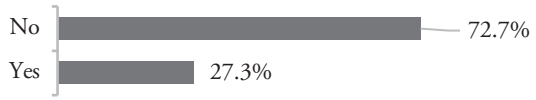
In comparison to the quality of a face-to-face visit, this virtual consultation was:



Rate your virtual visit in a scale from 0 to 10, 0 meaning it was the worst medical visit and 10 the best possible medical visit:



Did you feel any difficulties accessing to this virtual medical visit?



Would you recommend this model of medical visit to your friends and family?

In the future, even if actual contingencies change, will you adhere to this model of appointments if it continues to be available?

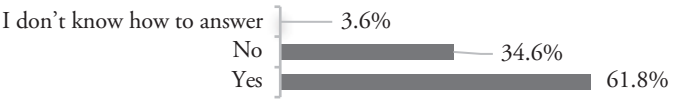


Table II *- Main critics and compliments pointed out in the open question and the number of patients referring each aspect (one patient may have contributed with more than one critic and/or compliment).*

Some critics and compliments to VIRTUAL VISITS mentioned in open QUESTION	
COMPLIMENTS	**CRITICS**
Practical and fast (n = 6)	Only applies when the reason for the appointment does not include observation (n = 8).
Good for prescription renewal (n = 1)	Very impersonal appointment, doctors seem to follow a pre-defined protocol: they are limited to asking questions and the user is limited to just answering (n = 2).
Avoids commuting to the hospital (n = 7)	Sometimes the internet connection fails (video failures, sound or problems with the delay) (n = 1).
Good for follow-up appointments (n = 3)	
It allows us not to be helpless in times when face-to-face appointment would be impractical (n = 1)	

On the other hand, the creation of a simplified and prioritized way to the doctor arrange a face-to-face medical visit for an indispensable physical examination/treatment discussion after a VV could be a win-win alternative to address this matter.

Limitations

One limitation is that this survey was applied to any patient with self-reported asthma that were observed in a VV during or before the Emergency weeks in Portugal. There was no discrimination between VV (real-time audio or video). Real-time video-appointments might enable a more empathic relation between patients and their doctors and provide visual signs that could complement physical examination. It would be interesting to investigate whether there are differences in patient satisfaction between these two different telemedicine tools. Although being in line with the literature (14), the size of our sample is another limitation. Despite being available for four weeks, only 55 patient's answers were retrieved, limiting the generalization of results. Another particularity is that the survey didn't include questions addressing patient's general characteristics, such as genre or age, and patients with access to Facebook usually are younger and technology-friendly, which can explain the low rate of technical difficulties accessing VV. This can influence the perception of acceptance and thus might not be extrapolated to the general population or even to other chronic respiratory diseases. As COVID-19 might interfere with patients' expectations, we cannot extrapolate that similar results could be achieved in a post-pandemic period.

Conclusions

As far as we know, this is the first survey applied to patients in our country regarding telemedicine performed during the COVID-19 pandemic. The results suggest that telemedicine is quickly becoming a key add-on to healthcare and might be a good alternative for in-person visits for asthmatic patients, patients, at least in such difficult times when social distancing is recommended, as patients express an overall satisfaction with this type of medical consultations.

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Conflict of interests

The authors declare that they have no conflict of interests.

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The treatment of allergic rhinitis in asthmatic children and adolescents: practical outcomes from the real-world "ControL'Asma" study

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To the Editor,

Allergic rhinitis (AR) affects up to 40% of children and adolescents (1). AR is characterized by a type 2 inflammation, including allergen-specific IgE production, eosinophilic infiltrate, and T helper 2(Th2)-derived cytokines (2). T regulatory cells' specific and functional defect promotes the typical Th2 polarization in allergic patients (2). Asthma is the most common chronic disease of childhood and adolescence (3). Asthma management is, therefore, a daily challenge in pediatric practice (4). Asthma is a heterogeneous disease, usually characterized by chronic airway inflammation (3). To define clinical, functional, and immunopathological patterns allows identifying asthma phenotypes and endotypes (5). In this regard, the allergic asthma phenotype is the most common in childhood and is defined when asthma symptoms and airway eosinophilic inflammation are associated with inhalation of the sensitizing allergen (3). There is also a close link between eosinophilic airway inflammation and airflow limitation (6). Therefore, AR and asthma share common pathogenic mechanisms and are frequently associated (7). In clinical practice, the concomitant treatment of asthma and AR can commonly produce practical problems. The relief of symptoms and control of airway inflammation represents the cornerstone of their management, even though some exceptions (3, 8). Symptoms relief need bronchodilator use in asthma and essentially antihistamines (anti-H1) in AR, but inflammation resolution depends on inhaled corticosteroids (ICS) in asthma and intranasal corticosteroids in AR. However, the overtreatment of both diseases may generate adverse events, mainly concerning corticosteroids that may induce relevant issues (9). Allergen immunotherapy (AIT) could represent the shared treatment committed to restoring allergen tolerance, revert Th2 polarization, and ultimately dampen type 2 inflammation (10).
The Italian Society of Pediatric Allergy and Immunology recently established a prospective study ("ControL'Asma") to investigate the asthma control in children and adolescents managed in clini-

cal practice. This research has been paid attention to the concomitant treatment of AR in children and adolescents with asthma. This cross-sectional study included a series of asthmatic children and adolescents consecutively visited across 10 Italian Pediatric Allergy centers. The centers are in Genoa, Bergamo, Milan, Pavia, Parma, Pisa, Rome (3 centers), and Catanzaro. All patients were currently treated according to the GINA guidelines based on the asthma control level and AR guidelines (11). The visit included careful history, mainly concerning asthma duration, current use of asthma and AR medications, including inhaled corticosteroids dosage (ICS) expressed as beclomethasone equivalence, oral corticosteroids use, rhinitis and allergy comorbidity, clinical examination, lung function testing (including bronchodilation testing), asthma control level according to the GINA guidelines (3). The Ethics Committee initially approved the procedure of the Istituto Giannina Gaslini of Genoa (code number: 22253/2017; in the Italian Project "ControL'Asma" promoted by the Italian Society of Pediatric Allergy and Immunology). All the other Review Ethics Committees further approved the study procedure, and written informed consent was obtained from all parents. Clinical data were recorded by an electronic case report form designed expressly for this study. Descriptive statistics of the study patients were firstly calculated; qualitative data were reported in terms of absolute frequencies and percentages; quantitative data were reported in terms of medians, first and third quartiles (1st-3rd Q).

The normality of distributions was evaluated using the Shapiro-Wilk test.

The statistical software "Statistica" (version 9, StatSoft Corporation, Tulsa, OK, USA) was used for all the analysis, and the software "Stata" (version 11, Stata Corporation, College Station, TX, USA) was used to calculate the Shapiro-Wilk.

Globally, 480 subjects were enrolled; 423 (88.1%) had AR comorbidity. **Table I** reports the clinical characteristics of the patients with both diseases. Signally, there was a male predominance (70%); well-controlled asthma was 55%, moderate-severe AR in 14%. ICS were used in 75% and OCS in 23%, and antileukotrienes in 24%. Antihistamines were used in 57.6%, intranasal corticosteroids in 53%, and both combined in 35. Notably, about ¾ of patients used pharmacotherapy for AR, only 19.5%, in contrast, used AIT. As regards sensitization, house dust mites were the most common sensitizing allergen (78%), followed by grasses (60%), olive tree (36%), cat (31%), hazelnut tree (25%),

Table I *- Description of the study patients with allergic asthma and rhinitis.*

Clinical characteristics		**Sensitization to**	
Age (years)	11.4 (9.4-13.8)*	House dust mites	78.0 %
Males	69.3 %	Grasses	59.7 %
Females	30.7 %	Olive tree	35.8 %
Well-controlled asthma	54.9 %	Cat	30.6 %
Partly controlled asthma	32.5 %	Hazelnut tree	24.7 %
Uncontrolled asthma	12.6 %	Birch	23.4 %
Mild intermittent AR	37.9 %	Dog	20.7 %
Moderate/severe intermittent AR	9.2 %	*Alternaria*	19.6 %
Mild persistent AR	47.6 %	Cypress	16.4 %
Moderate/severe persistent AR	5.2 %	*Parietaria*	16.3 %
ICS low dose	41.0 %	*Compositae*	14.9 %
ICS medium dose	32.1 %		
ICS high dose	3.1 %		
OCS: at least 1 course/year	22.7 %		
LABA	35.5 %		
Anti-LTC	24.3 %		
Intranasal corticosteroids	53.0 %		
Anti-H_1	57.6 %		
Intranasal corticosteroids + Anti-H_1	35.0 %		
Allergen-specific Immunotherapy	19.5 %		

*Median values and 1st and 3rd quartiles.

birch (23.4%), dog (21%), *Alternaria* (20%), cypress (16%), *Parietaria* (16%), and *Compositae* (15%). There were geographical differences concerning the distribution of specific sensitizations consistently with previous studies (12-14). Nevertheless, there was no significant difference in terms of treatments and disease severity among the centers. It mainly depended on the uniform sharing to International guidelines for asthma and rhinitis and the fact that all centers were third-level pediatric allergy clinics.

The current study demonstrated that AR is prevalent comorbidity in children and adolescents with asthma as affected by almost 90% of the whole sample. This outcome underlined the clinical relevance of the concept of united airways disease (15). However, corticosteroids were the most common medication as ICS was used in nearly all subjects and intranasal corticosteroids in more than half. However, antihistamines (mostly oral) were the first-choice treatment for AR. These findings arouse some concern concerning potential adverse events related to medication use. On the other hand, AIT was used only in 20% of patients. It is well known that medications do not cure the allergy, as symptoms and inflammation quickly recur after their suspension (16, 17). AIT should represent the choice treatment of AR and allergic asthma as restores immunological and clinical tolerance toward the causal allergen, may prevent allergy worsening, and its effects are longlasting over time. Also, there is a predominance of sensitization to perennial allergens, such as it means that allergic inflammation persists throughout the year. Anti-inflammatory medications should be used for a long time, with the problem of side effects. As a result, a more rational approach should be pursued in asthmatic children and adolescents.

The current study had some limitations, mainly concerning the cross-sectional design and the lack of biomarkers assessment. However, a follow-up study is ongoing. Moreover, the strength of this study was the nationwide size that provides generalizability of the outcomes. This real-world study may also provide information more adherent to the daily practice that studies involving selected patient populations that rarely mirror the real situation (18).

In conclusion, the present study demonstrated that AR was common asthma comorbidity in children and adolescents. Well-controlled asthma affected only half of the patients despite the use of corticosteroids was widespread and perennial allergy was also predominant. AIT was scarcely prescribed. These outcomes have to convince that more efforts should be made to improve asthma management in children and adolescents.

Contributors

MAT designed the study, collected patients, and discussed the paper, GLM discussed and revised the paper, GC wrote the paper.

Conflict of interests

The authors declare that they have no conflict of interests.

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