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The Influence of Peripheral Blood Eosinophil Counts in Asthma Comorbidities in Adults: A Real Life Study

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Abstract: Asthma and eosinophilia are two closely related pathologies whose interaction is key in the era of precision medicine. However, this relationship is rarely taken into account without the influence of therapeutic prescriptions. In this study involving 1296 subjects, the relationship between eosinophilia and asthma was analyzed without taking into account other biases. We observed that rhinitis only appears in non-asthmatic patients with elevated blood eosinophil levels, while atopy was elevated in parallel to eosinophilia regardless of whether the patients were asthmatic or not. In terms of lung function, a decrease was observed for higher blood eosinophil levels, which is especially relevant in the FEV1/FVC ratio. FENO is elevated in relation to higher eosinophilia, but total IgE is only elevated in patients with high peripheral blood eosinophil levels and asthma. Finally, the only feature of asthma that is altered by increased peripheral eosinophilia is persistent asthma, with all other features remaining unchanged.

Keywords: asthma; biomarkers; eosinophils; eosinophilic asthma; food allergy

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1. Introduction

The range of eosinophilia in the general population and certain diseases is wide and variable. Eosinophilia is associated with severity and control in different diseases such as asthma, food allergy, atopic dermatitis and eosinophilic oesophagitis. However, it is also observable in mild, well-controlled forms of these diseases [1].

Under physiological conditions, only a small group of eosinophils is released from the bone marrow. In contrast, eosinophilopoiesis is greatly increased in Th2 responses associated with helminth infections or allergic diseases. This increased eosinophil production is driven by the cytokines IL-3, IL-5 and granulocyte growth factor (GM-CSF) [2].

IL-5 is the most lineage-specific cytokine for eosinophils. It is responsible for the expansion of eosinophils from the bone marrow, their release into the blood and their survival after migration into tissues [2]. Considering pharmacoeconomic factors and by taking into account the high budgetary impact of their introduction in therapeutics, it seems necessary to use IL-5/IL-5R inhibitors in patients with severe refractory eosinophilic asthma who present with eosinophilia ($\geq$500 cells/µL in the blood). [3]. The consensus is however less clear for all anti-IL-5/IL-5R drugs with other eosinophil cut-off points such as 150, 300 or 400 [3].

Scientific evidence shows that eosinophils are present in many patients with airways and pulmonary tissues characterised by asthma. In the case of asthma, eosinophilia is associated with asthma exacerbations and plays a role in bronchial remodeling. They increase in uncontrolled or severe asthma and decrease with corticosteroid therapy and strategies targeting airway eosinophilia. They are significantly more effective in improving asthma control and decreasing exacerbations than non-targeted therapies [4]. Nevertheless, the use of other biological tools such as anti-IL-4Rα does not significantly decrease the

number of blood eosinophils but achieves similar asthma outcomes [5]. Since the eosinophil and its key activating cytokines have been the focus of new lines of therapy in many eosinophilic diseases, we consider it important to understand the extent to which the eosinophil is responsible for asthmatic pathology.

2. Materials and Methods

2.1. Study

We conducted a retrospective, cross-sectional study that included patients visiting the allergy department at Hospital General de Villalba in Madrid (Spain). All patients who underwent spirometry for suspected asthma between November 2014 and November 2017 were recruited. Standard data collection methods were used in all participants. The study's criteria for performing spirometry followed the existing hospital protocols and the clinical practice and were not influenced by the study. This research project was approved by the Clinical Research Ethics Committee of the hospital, in accordance with Personal Data Protection Act 15/1999, Biomedical. Research Act 14/2007, and Biomedical Research Royal Decree 1716/2011.

2.2. Study Subjects

We recruited patients over 18 years of age who attended the Allergy Clinic of the General Hospital of Villalba (Madrid, Spain) and underwent a good quality spirometric maneuver due to suspected asthma. We included 1296 subjects. Seven hundred and thirty-three patients had been diagnosed with asthma based on the Global Initiative for Asthma (GINA) criteria [6]. In 563 patients, asthma was excluded according to GINA guidelines [6]. A standardised clinical history was completed for each patient. All the clinical charts of the patients were analysed. Only those with clear information for the diagnosis of asthma were included. Patients unable to perform an adequate spirometric maneuver were excluded. Other inclusion/exclusion criteria were not considered. The final database contains 1296 study subjects.

2.3. Studied Variables

Anthropometric characteristics and respiratory functional variables obtained from spirometries performed according to the ERS/ATS criteria [7] were recorded. The GLI equations were used as reference values [8] and the percentage with respect to the predicted value and the z-score were calculated. A bronchodilator test was considered positive when the FEV_1 increase was higher than 12% and >200 mL. An FEV_1 increase of <12% and/or <200 mL with an FVC increase of >10% or an $FEF_{25–75}$ increase of >35% was considered as a partial bronchodilator response. As inflammatory variables, the peripheral blood eosinophil count and FeNO were recorded. FeNO was obtained using a Fenompro ® testing device and its determination was performed following the ERS/ATS recommendations [9]. The following asthma characteristics were also recorded: time since diagnosis, age of asthma onset, allergic asthma, persistent asthma, asthma symptomatic period (patient is exposed to asthma trigger) and current asthma symptoms (patient is in symptomatic period and symptoms when spirometry is performed). Presence of rhinitis, atopy (defined as sensitization to any food or aeroallergen, independently of its clinical relevance), peripheral blood IgE, food allergy, drug allergy, allergic contact dermatitis, other concomitant comorbidities and treatment used 48 h prior to the spirometry were collected. Treatments were classified according to current asthma guidelines [6,10].

2.4. Statistical Analysis

Patients were divided into categories of asthmatics or non-asthmatics. Variables were classified into gaussian and non-gaussian distributions [11] and were described according to its features [11]. For non-gaussian distributions, the U-Mann Whitney test was selected for head-to-head comparisons. Discrete variables were analyzed using Chi-square test and continuous variables were analyzed using ANCOVA test. The medication steps according

to GINA were considered as a covariate in this analysis. When data were distributed into a two-by-two table, the Odds Ratio (OR) and Relative Risk (RR) were also calculated for risk evaluation. Patients were normalized by corticosteroid intake. Corticosteroid intake was divided into different levels according to GINA guidelines [6]. Data analysis was performed using IBM® SPSS® statistics 25 (Sacramento, CA, USA) and graphs were conducted using GraphPad Prism 9 (GraphPad Software Inc., San Diego, CA, USA).

3. Results

Firstly, the different peripheral blood eosinophil counts cut-off points were examined for patients with asthma. Statistically significant differences were found in every selected cut-off point (150, 300, 500 and 800) for some items (Table 1). Nevertheless, there were important differences between the sensibility of these thresholds when detecting them. Furthermore, 300 Eos/μL was the cut-off point that detected more statistically significant differences and indicated a higher significance, followed by 500, which also objectified these differences with a high sensibility (Tables 1–3). It was found that 150 Eos/μL is less effective in detecting these changes and 800 Eos/μL does not detect most differences and its statistical significance is markedly lower. The results were analyzed by comparing sexes and age strata (19–39, 40–64 and >65 years), with no notable differences.

When looking into patients without asthma, we observed that a 150 Eos/μL cut-off point was able to detect differences in drug (probability value (p) 0.033, relative risk (RR) 0.408, odds ratio (OR) 0.389) and contact allergies (p 0.017, RR 4.438, OR 4.567). Moreover, rhinitis (p 0.017, RR 0.894, OR 0.606) and atopy (p 0.023, RR 0.408, OR 0.389) are more common in patients with more than 150 eosinophils, showing a higher risk of developing these conditions.

It is also remarkable that women are always more prevalent in all the eosinophil strata. However, men increase their prevalence when eosinophils are higher, showing statistically significant differences between genders when using 150 (p 0.005, RR 0.673, OR 1.750) and 300 (p 0.009, RR 0.530, OR 1.767). FeNO is also consistently higher in higher eosinophil groups (150 p 0.015, 300 p 0.001, 500 p 0.001 and 800 p 0.005), whilst Total IgE comorbidities and functional pulmonary tests remained stable despite the eosinophil counts.

Looking at all studied populations, including patients with and without asthma, women are also more frequent in every studied cut-off point. However, men become more prevalent when peripheral blood eosinophil counts increase (Table 2). Asthma diagnosis also increases in higher eosinophil groups for every cut-off point aside from 800, showing a decrease in the risk of developing this condition when eosinophil counts are lower (150 p 0.001, OR 0.540, RR 0.749; 300 p 0.001, OR 0.513, RR 0.768; 500 p 0.001, OR 0.391, RR 0.721). The tendency can be observed in the 800 Eos/μL, but risk and statistical differences were not significant. Rhinitis (150 p 0.001, RR 0.914, OR 0.508; 300 p 0.003, RR 0.935, OR 0.513; 500 RR 0.949) and atopy (150 p 0.001, RR 0.863, OR 0.499; 300 p 0.001, RR 0.886, OR 0.472; 500 p 0.002, RR 0.885, OR 0.426) are also more frequent when eosinophils are elevated, having a lower risk of existing when eosinophils decrease.

When examined for asthma patients, and contrary to non-asthmatics, total IgE and FeNO were found to be elevated in the groups with higher eosinophil count (p = 0.001) in all cut-off points for FeNO and p between 0.001 and 0.003 for IgE, except at the 800 Eos/μL threshold where total IgE was higher in the >800 Eos/μL but not significantly.

Moreover, in this sample we can observe changes in spirometric values. For the 150 Eos/μL threshold, we observed differences in FEV1% (p 0.001), FVC% (p 0.033), FEV1/FVC (p 0.010), FEV1/FVC Zscore (p 0.002) and FEF 25–75% (p 0.007). Additionally, the bronchodilation test results led to more positive results in the group >150 Eos/μL (p 0.030). This tendency can also be observed in other cut off points such as 300 Eos/μL FEV1% (p 0.001), FVC% (p 0.006), FEV1/FVC (p 0.001), FEV1/FVC Zscore (p 0.001), FEF 25–75% (0.001) and bronchodilation test (0.019) and with 300 Eos/μL FEV1% (p 0.004), FVC (0.047), FEV1/FVC (p 0.002), FEV1/FVC Zscore (p 0.001), FEF 25–75% (p 0.001) and the bronchodilation test

result (0.047). The 800 Eos/μL cut-off point only detected differences in FEV1/FVC (*p* 0.032) and FEV1/FVC (*p* 0.007).

Finally, when analyzing patients with asthma, asthma traits were also studied. Having lower eosinophil counts decreases the risk and prevalence of persistent asthma (150 *p* 0.028, RR 0.749, OR 0.651; 300 *p* 0.001, RR0.649, OR 0.499; 500 *p* 0.001, RR 0.657, OR 0.491) and the amount of patients whose spirometry was performed during their asthma symptomatic period (150 *p* 0.015, RR 0.844, OR 0.644; 300 *p* 0.003, RR 0.843, OR 0.601; 500 *p* 0.009, RR 0.829, OR 0.545; 800 *p* 0.039, RR 0.742, OR 0.292). No other asthma traits were modified by eosinophil counts.

Table 1. Clinical characteristics of asthma patients over 18 years old.

	<300 Eosinophils	>300 Eosinophils	*p*	RR	OR
Simple size	501	232	-	-	-
Age	39.50 ± 12.96	37.91 ± 13.00	-	-	-
Men	155 (31%)	85 (37%)	-	-	-
Women	346 (69%)	147 (63%)	-	-	-
BMI	26.74 ± 5.05	27.12 ± 5.66	-	-	-
FEV 1 Volume	3.15 ± 0.83	3.10 ± 0.80	-	-	-
FEV 1%	100.25 ± 15.03	95.74 ± 15.06	0.001	-	-
FEV 1 Zscore	−0.72 ± 1.48	−0.92 ± 1.37	-	-	-
FVC Volume	3.96 ± 1.02	4.00 ± 0.99	-	-	-
FVC%	106.80 ± 14.02	104.57 ± 12.51	0.039	-	-
FVC Zscore	−0.54 ± 1.47	−0.53 ± 1.40	-	-	-
FEV 1/FVC	79.75 ± 7.44	77.54 ± 8.09	0.001	-	-
FEV 1/FVC Zscore	−0.36 ± 1.06	−0.72 ± 1.15	0.001	-	-
FEF 25–75 Volume	3.11 ± 1.16	2.86 ± 1.12	0.007	-	-
FEF 25–75%	80.37 ± 25.64	72.15 ± 25.10	0.001	-	-
+ Bronchodilation	41 (11%)	31 (17%)	-	-	-
* Bronchodilation	39 (11%)	19 (11%)	-	-	-
− Bronchodilation	285 (78%)	130 (72%)	-	-	-
Asthma diagnosis	-	-	-	-	-
Asthma onset prior to 12 years old	65 (13%)	29 (13%)	-	-	-
Asthma onset prior to 40 years old	384 (77%)	177 (76%)	-	-	-
Allergic asthma	394 (79%)	176 (76%)	-	-	-
Asthma in symptomatic period	304 (61%)	167 (72%)	0.003	0.843	0.601
Persistent asthma	150 (30%)	107 (46%)	0.001	0.649	0.499
Current asthma symptoms	159 (32%)	86 (37%)	-	-	-
Rhinitis	475 (95%)	224 (97%)	-	-	-
Atopy	446 (89%)	222 (96%)	0.003	0.930	0.365
Food allergy	76 (15%)	31 (13%)	-	-	-
Drug allergy	35 (7%)	19 (8%)	-	-	-
Contact dermatitis	18 (4%)	6 (3%)	-	-	-
Other allergies	11 (2%)	2 (1%)	-	-	-
FeNO (ppb)	28.70 (33.20)	51.45 (66.73)	0.001	-	-
Eosinophil counts (Eos/μL)	200 (200)	500 (300)	0.001	-	-
Total IgE (kU/L)	135.00 (281.55)	190.00 (403.5)	0.001	-	-
Comorbidities	66 (13%)	34 (15%)	-	-	-
Atopic dermatitis	24 (4%)	10 (4%)	-	-	-

Table 1 Data are presented as *n* (%) and mean ± standard deviation, FEV1: Forced expiratory volume, FVC: Forced vital capacity, FEF 25–75: Forced expiratory flow between 25% and 75% of the spirometry, + Bronchodilation: Variation in FEV1 > 12% and >200 mL in bronchodilation test, * Bronchodilation: Variation in FVC > 10% and/or variation in FEF 25–75 > 30% with a variation in FEV1 < 12% and/or <200 mL in bronchodilation test, − Bronchodilation: Variation in FEV1 < 12% and/or <200 mL variation in FVC < 10% and variation in FEF 25–75 < 30%, %: Percentage.

Table 2. Clinical characteristics of asthmatics and non-asthmatic patients.

	<300 Eosinophils	**>300 Eosinophils**	***p***	**RR**	**OR**
Simple size	956	340	-	-	-
Age	41.47 ± 14.01	38.26 ± 13.37	0.001	-	-
Men	286 (30%)	130 (38%)	0.005	0.782	1.450
Women	670 (70%)	210 (62%)	0.005	1.135	1.450
BMI	26.85 ± 5.32	27.00 ± 5.52	-	-	-
FEV 1 Volume	3.14 ± 0.84	3.15 ± 0.81	-	-	-
FEV 1%	102.49 ± 14.65	98.09 ± 14.90	0.001	-	-
FEV 1 Zscore	−0.61 ± 1.45	−0.71 ± 1.48	-	-	-
FVC Volume	3.90 ± 1.02	4.01 ± 0.99	-	-	-
FVC %	107.82 ± 13.84	105.48 ± 12.73	0.006	-	-
FVC Zscore	−0.52 ± 1.47	−0.42 ± 1.45	-	-	-
FEV 1/FVC	80.54 ± 6.93	78.77 ± 7.78	0.001	-	-
FEV 1/FVC Zscore	−0.20 ± 0.99	−0.55 ± 1.11	0.001	-	-
FEF 25–75 Volume	3.16 ± 1.14	3.02 ± 1.14	-	-	-
FEF 25–75%	83.66 ± 24.86	76.47 ± 25.11	0.001	-	-
+ Bronchodilation	44 (6%)	31 (12%)	0.019	-	-
* Bronchodilation	61 (9%)	23 (9%)	0.019	-	-
− Bronchodilation	582 (85%)	206 (79%)	0.019	-	-
Asthma diagnosis	501 (52%)	232 (68%)	0.001	0.768	0.513
Asthma onset prior to 12 years old	-	-	-	-	-
Asthma onset prior to 40 years old	-	-	-	-	-
Allergic asthma	-	-	-	-	-
Asthma in symptomatic period	-	-	-	-	-
Persistent asthma	-	-	-	-	-
Current asthma symptoms	-	-	-	-	-
Rhinitis	828 (87%)	315 (93%)	0.003	0.935	0.513
Atopy	750 (79%)	301 (86%)	0.001	0.886	0.472
Food allergy	122 (13%)	40 (12%)	-	-	-
Drug allergy	59 (6%)	29 (9%)	-	-	-
Contact dermatitis	27 (3%)	7 (2%)	-	-	-
Other allergies	20 (2%)	4 (1%)	-	-	-
FeNO (ppb)	24.70 (23.70)	43.75 (59.45)	0.001	-	-
Eosinophil counts (Eos/μL)	200 (200)	500 (300)	0.001	-	-
Total IgE (kU/L)	118.00 (229.8)	161.00 (372.9)	0.001	-	-
Comorbidities	160 (17%)	61 (18%)	-	-	-
Atopic dermatitis	31 (3%)	10 (3%)	-	-	-

Table 2 Data are presented as *n* (%) and mean ± standard deviation, FEV1: Forced expiratory volume, FVC: Forced vital capacity, FEF 25–75: Forced expiratory flow between 25% and 75% of the spirometry, + Bronchodilation: Variation in FEV1 > 12% and >200 mL in bronchodilation test, * Bronchodilation: Variation in FVC > 10% and/or variation in FEF 25–75 > 30% with a variation in FEV1 < 12% and/or <200 mL in bronchodilation test, − Bronchodilation: Variation in FEV1 < 12% and/or < 200 mL variation in FVC < 10% and variation in FEF 25–75 < 30%, %: Percentage.

Table 3. Clinical characteristics of non-asthmatics patients over 18 years old.

	<300 Eosinophils	>300 Eosinophils	*p*	RR	OR
Simple size	455	108	-	-	-
Age	43.63 ± 14.79	38.99 ± 14.17	0.003	-	-
Men	131 (29%)	45 (42%)	0.009	0.530	1.767
Women	324 (71%)	63 (58%)	0.009	1.221	1.767
BMI	26.97 ± 5.60	26.74 ± 5.22	-	-	-
FEV 1 Volume	3.12 ± 0.85	3.26 ± 0.84	-	-	-
FEV 1%	104.97 ± 13.83	103.15 ± 13.28	-	-	-
FEV 1 Zscore	−0.49 ± 1.40	−0.26 ± 1.62	-	-	-
FVC Volume	3.83 ± 1.02	4.02 ± 0.98	-	-	-
FVC%	108.95 ± 13.57	107.43 ± 13.04	-	-	-
FVC Zscore	−0.52 ± 1.47	−0.17 ± 1.55	0.034	-	-
FEV 1/FVC	81.42 ± 6.22	81.42 ± 6.37	-	-	-
FEV 1/FVC Zscore	−0.03 ± 0.88	−0.18 ± 0.94	-	-	-
FEF 25–75 Volume	3.21 ± 1.12	3.36 ± 1.11	-	-	-
FEF 25–75%	87.29 ± 23.47	85.75 ± 22.58	-	-	-
+ Bronchodilation	3 (1%)	0 (0%)	-	-	-
* Bronchodilation	22 (7%)	4 (5%)	-	-	-
− Bronchodilation	297 (92%)	76 (95%)	-	-	-
Asthma diagnosis	-	-	-	-	-
Asthma onset prior to 12 years old	-	-	-	-	-
Asthma onset prior to 40 years old	-	-	-	-	-
Allergic asthma	-	-	-	-	-
Asthma in symptomatic period	-	-	-	-	-
Persistent asthma	-	-	-	-	-
Current asthma symptoms	-	-	-	-	-
Rhinitis	353 (78%)	91 (84%)	-	-	-
Atopy	304 (67%)	79 (73%)	-	-	-
Food allergy	46 (10%)	9 (8%)	-	-	-
Drug allergy	24 (5%)	10 (9%)	-	-	-
Contact dermatitis	9 (2%)	1 (1%)	-	-	-
Other allergies	9 (2%)	2 (2%)	-	-	-
FeNO (ppb)	22.60 (16.93)	29.50 (38.18)	0.001	-	-
Eosinophil counts (Eos/μL)	200 (100)	500 (200)	0.001	-	-
Total IgE (kU/L)	92.90 (130.60)	100. 99 (308.20)	-	-	-
Comorbidities	104 (23%)	27 (25%)	-	-	-
Atopic dermatitis	7 (2%)	0 (0%)	-	-	-

Table 3 Data are presented as *n* (%) and mean ± standard deviation, FEV1: Forced expiratory volume, FVC: Forced vital capacity, FEF 25–75: Forced expiratory flow between 25% and 75% of the spirometry, + Bronchodilation: Variation in FEV1 > 12% and >200 mL in bronchodilation test, * Bronchodilation: Variation in FVC > 10% and/or variation in FEF 25–75 > 30% with a variation in FEV1 < 12% and/or <200 mL in bronchodilation test, − Bronchodilation: Variation in FEV1 < 12% and/or <200 mL variation in FVC < 10% and variation in FEF 25–75 < 30%, %: Percentage.

The differences in FeNO (150 *p* 0.001; 300 *p* 0.001, 500 *p* 0.001; 800 *p* 0.037) and total IgE (150 *p* 0.016: 300 *p* 0.001; 500 *p* 0.011; 800 *p* 0.018) are more significant than the differences detected when analyzing the overall sample.

Atopy still shows a higher prevalence and increased risk with higher eosinophil counts (150 p 0.007, RR 0.926, OR 0.480; 300 p 0.003, RR 0.930, OR 0.365) in low eosinophil count cut-off points, but these differences are minimised for higher thresholds such as 500 or 800 Eos/µL. Contrarily, no differences exist when analysing rhinitis.

Spirometric value changes were observed in the 300 (FEV1% p 0.001, FVC% p 0.039, FEV1/FVC p 0.001, FEV1/FVC Zscore p 0.001, FEF 25–75 p 0.007, FEF 25–75% p 0.001), 500 (FEV1/FVC p 0.008, FEV1/FVC Zscore p 0.004, FEF 25–75% p 0.004) and 800 (FEV1/FVC p 0.017, FEV1/FVC Zscore p 0.012) cut-off points, but no statistically significant differences were spotted in the 150 Eos/µL limit. When looking into patients without asthma, we can observe that the 150 Eos/µL cut-off point led to differences in drug (probability value (p) 0.033, relative risk (RR) 0.408, odds ratio (OR) 0.389) and contact (p 0.017, RR 4.438, OR 4.567) allergies. Moreover, rhinitis (p 0.017, RR 0.894, OR 0.606) and atopy (p 0.023, RR 0.408, OR 0.389) is more common among patients with more than 150 eosinophils, showing a higher risk of developing these conditions.

It is also remarkable that women are always more prevalent in all the eosinophil strata, but men increase their prevalence when eosinophils are higher, showing statistically significant differences between genders using 150 (p 0.005, RR 0.673, OR 1.750) and 300 (p 0.009, RR 0.530, OR 1.767). FeNO is also always higher in higher eosinophil groups (150 p 0.015, 300 p 0.001, 500 p 0.001 and 800 p 0.005), whilst Total IgE comorbidities and functional pulmonary test remained stable despite the eosinophil counts.

4. Discussion

The results obtained in this study suggest that eosinophil counts are related to asthma its comorbidities and traits. Due to this study design, the dynamic changes on these counts have not been analysed, but the different cut-off points suggested in the literature in terms of phenotype or endotype asthma patients were studied.

Nowadays, peripheral blood eosinophil counts are a cornerstone to phenotype patients [12,13]. Notwithstanding, this phenotyping process is in continuous change. However, its modifications are mainly influenced by the studies performed to describe the effects and functioning of biologic drugs to contribute to the treatment prescription algorithm [14,15].

This study aimed to describe the influence of eosinophil counts on asthma and its interactions with different comorbidities. Despite the importance of creating a treatment algorithm to personalise the treatment of patients with asthma, we believe that it is important to deepen the analysis of the relationship between asthma and peripheral blood eosinophil counts without the existent therapies bias.

Consequently, cut-off points described in the bibliography, 150, 300, 500 and 800 Eos/µL, have been studied to examine which cut-off point is the best selection. Interestingly, the best cut-off point for discriminating asthma in this cohort was 300 Eos/µL, given that in a different cohort, 300 Eos/µL was described as the best cut-off point for correlating peripheral blood and sputum eosinophilia [12]. These results are reasonably consistent with those published in other cohorts of asthmatics [16–20].

The presence of atopy is directly related to elevated eosinophil counts in this study, independent of having or not having asthma, whereas rhinitis only appears in patients with elevated eosinophilia, but without asthma, and this fact does not seem to be replicated in other studies [21]. In the analysis of the data from this cohort, the risk of rhinitis only increases in non-asthmatic patients and was not significant in patients with elevated eosinophilia and asthma. In the same way, we can see how this pattern repeated in IgE and FENO, where the elevation of FENO will occur in all groups with elevated eosinophilia, regardless of being asthmatic or not. In contrast, IgE is only significantly elevated in patients with asthma. Consequently, we can deduce that the elevation of FENO is secondary to eosinophilia, while the elevation of IgE is dependent on the existence of asthma and eosinophilia.

Notably, the only asthma characteristic analysed in this study that is modified by higher eosinophil levels is the higher prevalence and increased risk for persistent asthma. Due to this increase, the number of patients who were included in this study in the symptomatic period also increased, since patients with persistent asthma are always in the symptomatic period. This is a remarkable observation in this study since most of the included patients suffer from seasonal pollen asthma, which is intermittent asthma.

Regarding lung function, elevated eosinophils in peripheral blood generate a decrease in spirometric volumes where FEV1/FVC is especially relevant both in % and in terms of the Z-score, which is consistent with other cohorts [12,16,17]. However, a decrease in the percentages with respect to the predicted value of FEV1 and FVC was observed, suggesting that these corrective factors are influenced by eosinophilia more than calculated in these equations.

Despite stratified analysis by sex and age, no significant differences were found, so the analysis of eosinophilia should be performed equally regardless of sex and age stratum.

This was an observational study and therefore there are two important weaknesses to keep in mind. First, there was no control over the classification of the study groups, nor were the study groups randomly divided. Secondly, it is possible to create biases or to find cause and effect relationships where none exist. Therefore, further studies are needed to confirm the findings found in this cohort.

In conclusion, eosinophilia alters the characteristics of asthma which is not only useful to obtain a therapeutic algorithm and should therefore be taken into account for the correct phenotyping of the disease.

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References

1. Mesnil, C.; Raulier, S.; Paulissen, G.; Xiao, X.; Birrell, M.A.; Pirottin, D.; Janss, T.; Starkl, P.; Ramery, E.; Henket, M.; et al. Lung-resident eosinophils represent a distinct regulatory eosinophil subset. *J. Clin. Investig.* **2016**, *126*, 3279–3295. [CrossRef] [PubMed]
2. Pavord, I.D. Eosinophilic Phenotypes of Airway Disease. *Ann. Am. Thorac. Soc.* **2013**, *10*, S143–S149. [CrossRef] [PubMed]
3. Buhl, R.; Humbert, M.; Bjermer, L.; Chanez, P.; Heaney, L.G.; Pavord, I.; Quirce, S.; Virchow, J.C.; Holgate, S. Severe eosinophilic asthma: A roadmap to consensus. *Eur. Respir. J.* **2017**, *49*, 1700634. [CrossRef] [PubMed]
4. Nelson, R.K.; Bush, A.; Stokes, J.; Nair, P.; Akuthota, P. Eosinophilic Asthma. *J. Allergy Clin. Immunol. Pract.* **2020**, *8*, 465–473. [CrossRef] [PubMed]
5. Agache, I.; Beltran, J.; Akdis, C.; Akdis, M.; Canelo-Aybar, C.; Canonica, G.W.; Casale, T.; Chivato, T.; Corren, J.; Del Giacco, S.; et al. Efficacy and safety of treatment with biologicals (benralizumab, dupilumab, mepolizumab, omalizumab and reslizumab) for severe eosinophilic asthma. A systematic review for the EAACI Guidelines—Recommendations on the use of biologicals in severe asthma. *Allergy* **2020**, *75*, 1023–1042. [CrossRef] [PubMed]
6. 2021 GINA Report, Global Strategy for Asthma Management and Prevention. Available online: https://ginasthma.org/gina-reports/ (accessed on 28 February 2022).

7. Miller, M.R.; Hankinson, J.A.T.S.; Brusasco, V.; Burgos, F.; Casaburi, R.; Coates, A.; Crapo, R.; Enright, P.; Van Der Grinten, C.P.M.; Gustafsson, P.; et al. Standardization of spirometry. *Eur. Respir. J.* **2005**, *26*, 319–338. [CrossRef] [PubMed]
8. Quanjer, P.H.; Stanojevic, S.; Cole, T.J.; Baur, X.; Hall, G.L.; Culver, B.H.; Enright, P.L.; Hankinson, J.L.; Ip, M.S.; Zheng, J.; et al. Multi-ethnic reference values for spirometry for the age range 3 to 95 years: The global lung function equations. *Eur. Respir. J.* **2012**, *40*, 1324–1343. [CrossRef] [PubMed]
9. American Thoracic Society; European Respiratory Society. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide. *Am. J. Respir. Crit. Care Med.* **2005**, *171*, 912–930. [CrossRef] [PubMed]
10. GEMA Guideline. Available online: https://www.gemasma.com (accessed on 14 February 2022).
11. Esteban-Gorgojo, I.; Gorgojo, M.P.; Sastre, J.; Quirce, S. Food allergy as an asthma comorbidity in children and adolescents: A practical approach through a real-world study. *Allergol. Immunopathol.* **2021**, *49*, 68–78. [CrossRef] [PubMed]
12. Rial, M.J.; Álvarez-Puebla, M.J.; Arismendi, E.; Caballero, M.L.; Cañas, J.A.; Cruz, M.J.; González-Barcala, F.J.; Luna, J.A.; Martínez-Rivera, C.; Mullol, J.; et al. Clinical and inflammatory characteristics of patients with asthma in the Spanish MEGA project cohort. *Clin. Transl. Allergy* **2021**, *11*, e12001. [CrossRef] [PubMed]
13. Esteban-Gorgojo, I.; Antolín-Amérigo, D.; Domínguez-Ortega, J.; Quirce, S. Non-eosinophilic asthma: Current perspectives. *J. Asthma Allergy* **2018**, *11*, 267–281. [CrossRef] [PubMed]
14. Rial, M.; Cañas, J.; Rodrigo-Muñoz, J.; Valverde-Monge, M.; Sastre, B.; Sastre, J.; del Pozo, V. Changes in Serum MicroRNAs after Anti-IL-5 Biological Treatment of Severe Asthma. *Int. J. Mol. Sci.* **2021**, *22*, 3558. [CrossRef] [PubMed]
15. Delgado, J.; Dávila, I.; Domínguez-Ortega, J. Clinical Recommendations for the Management of Biological Treatments in Severe Asthma Patients: A Consensus Statement. *J. Investig. Allergy Clin. Immunol.* **2021**, *31*, 36–43. [CrossRef] [PubMed]
16. Loza, M.J.; Djukanovic, R.; Chung, K.F.; Horowitz, D.; Ma, K.; Branigan, P.; Barnathan, E.S.; Susulic, V.S.; Silkoff, P.E.; Sterk, P.J.; et al. Validated and longitudinally stable asthma phenotypes based on cluster analysis of the ADEPT study. *Respir. Res.* **2016**, *17*, 1–21. [CrossRef] [PubMed]
17. Abraham, B.; Anto, J.; Barreiro, E.; Bel, E.; Bonsignore, G.; Bousquet, J.; Castellsague, J.; Chanez, P.; Cibella, F.; Cuttitta, G.; et al. The ENFUMOSA cross-sectional European multicentre study of the clinical phenotype of chronic severe asthma. *Eur. Respir. J.* **2003**, *22*, 470–477. [CrossRef]
18. Moore, W.C.; Bleecker, E.R.; Curran-Everett, D.; Erzurum, S.C.; Ameredes, B.T.; Bacharier, L.; Calhoun, W.J.; Castro, M.; Chung, K.F.; Clark, M.P.; et al. Characterization of the severe asthma phenotype by the National Heart, Lung, and Blood Institute's severe asthma research program. *J. Allergy Clin. Immunol.* **2007**, *119*, 405–413. [CrossRef] [PubMed]
19. Pretolani, M.; Soussan, D.; Poirier, I.; Thabut, G.; Aubier, M. Clinical and biological characteristics of the French COBRA cohort of adult subjects with asthma. *Eur. Respir. J.* **2017**, *50*, 1700019. [CrossRef]
20. Schleich, F.N.; Manise, M.; Sele, J.; Henket, M.; Seidel, L.; Louis, R. Distribution of sputum cellular phenotype in a large asthma cohort: Predicting factors for eosinophilic vs. neutrophilic inflammation. *BMC Pulm. Med.* **2013**, *13*, 11. [CrossRef] [PubMed]
21. Burte, E.; Bousquet, J.; Varraso, R.; Gormand, F.; Just, J.; Matran, R.; Pin, I.; Siroux, V.; Jacquemin, B.; Nadif, R. Characterization of Rhinitis According to the Asthma Status in Adults Using an Unsupervised Approach in the EGEA Study. *PLoS ONE* **2015**, *10*, e0136191. [CrossRef] [PubMed]