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Reliability of Total Serum IgE Levels to Define Type 2 High and Low Asthma Phenotypes

Giuseppe Guida [1,2,*,†], Francesca Bertolini [2,†], Vitina Carriero [2], Stefano Levra [2], Andrea Elio Sprio [3], Martina Sciolla [2], Giulia Orpheu [2], Elisa Arrigo [2], Stefano Pizzimenti [1], Giorgio Ciprandi [4] and Fabio Luigi Massimo Ricciardolo [1,2,5]

1 Severe Asthma and Rare Lung Disease Unit, San Luigi Gonzaga University Hospital, Orbassano, 10043 Turin, Italy; s.pizzimenti@sanluigi.piemonte.it (S.P.); fabioluigimassimo.ricciardolo@unito.it (F.L.M.R.)
2 Department of Clinical and Biological Sciences, University of Turin, Orbassano, 10043 Turin, Italy; francesca.bertolini@unito.it (F.B.); vitina.carriero@unito.it (V.C.); stefano.levra@unito.it (S.L.); martina.sciolla@unito.it (M.S.); giulia.orpheu@unito.it (G.O.); elisa.arrigo@unito.it (E.A.)
3 Department of Research, ASOMI College of Sciences, 19112 Marsa, Malta; ae.sprio@gmail.com
4 Allergy Clinic, Casa di Cura Villa Montallegro, 16145 Genoa, Italy; gio.cip@libero.it
5 Institute of Translational Pharmacology, National Research Council (IFT-CNR), Section of Palermo, 90146 Palermo, Italy
* Correspondence: giuseppe.guida@unito.it; Tel.: +39-0119026776
† These authors contributed equally to this work.

Abstract: *Background:* High total IgE levels are weak predictors of $T2_{High}$ and have been reported in nonallergic asthma. Therefore, the role of total serum IgE (IgE) in the $T2_{High}$ phenotype is still debated. Objective: This study investigated the reliability of stratifying asthmatics into IgE_{High} and IgE_{Low} within the $T2_{High}$ and $T2_{Low}$ phenotypes. *Methods:* This cross-sectional single-center study investigated the association of clinical, functional, and bio-humoral parameters in a large asthmatic population stratified by IgE $\geq$ 100 kU/L, allergen sensitization, B-EOS $\geq$ 300/µL, and $F_ENO \geq 30$ ppb. *Results:* Combining T2 biomarkers and IgE identifies (1) $T2_{Low}$-IgE_{Low} (15.5%); (2) $T2_{Low}$-IgE_{High} (5.1%); (3) $T2_{High}$-IgE_{Low} (33.6%); and $T2_{High}$-IgE_{High} (45.7%). $T2_{Low}$-IgE_{Low} patients have more frequent cardiovascular and metabolic comorbidities, a higher prevalence of emphysema, and higher LAMA use than the two $T2_{High}$ subgroups. Higher exacerbation rates, rhinitis, and anxiety/depression syndrome characterize the $T2_{Low}$-IgE_{High} phenotype vs. the $T2_{Low}$-IgE_{Low} phenotype. Within the $T2_{High}$, low IgE was associated with female sex, obesity, and anxiety/depression. *Conclusions:* High IgE in $T2_{Low}$ patients is associated with a peculiar clinical phenotype, similar to $T2_{High}$ in terms of disease severity and nasal comorbidities, while retaining the $T2_{Low}$ features. IgE may represent an additional biomarker for clustering asthma in both $T2_{High}$ and $T2_{Low}$ phenotypes rather than a predictor of $T2_{High}$ asthma "*per se*".

Keywords: total serum IgE; asthma; phenotype; Type-2 inflammation; biomarkers; T2-high asthma; T2-low asthma; comorbidities; logistic analysis

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1. Introduction

Asthma is a heterogeneous disease concerning onset, natural course, and response to treatment [1].

Demographic, clinical, pathophysiological, and genetic characteristics of asthmatics guided the identification of recognizable clusters termed «asthma phenotypes» [2]. This approach fosters patient-tailored, efficient strategies to reduce the socio-economic asthma burden. Biomarkers measure biological or pathogenic processes (endotypes) to identify asthma phenotypes [3].

Asthma includes type-2 high ($T2_{High}$) and low ($T2_{Low}$) airway inflammation phenotypes [1,4]. Type-2 cytokine-driven eosinophilic inflammation is common (50–60%) in

adults, albeit variable according to the proportion of inhaled corticosteroid (ICS)-treated patients [5]. Sputum eosinophil (S-EOS) count alone, generally >3%, is insufficient for differentiating between $T2_{High}$ and $T2_{Low}$ phenotypes [6]. Surrogate biomarkers, easily applicable compared with S-EOS, have been widely applied in asthma phenotyping and investigated as pathogenic-related molecules [7,8]. Peripheral eosinophils (B-EOS), total serum IgE, and fractional exhaled nitric oxide (F_ENO) are routinely used for identifying the type-2 asthma phenotype. Generally, the assessment of FENO < 25 ppb has a higher negative predictive value for likely eosinophilic airway inflammation in asthma. However, both F_ENO and B-EOS often lack specificity [9], as they do not completely segregate type-2 asthma phenotypes even when combined.

Immunoglobulin E (IgE) is a protein involved in the immune response. In particular, IgE is known for its role in allergic reactions. Allergen-specific IgE binds the high-(FcεRI) and low-affinity receptors (FcεRII) for IgE located on effector cells and antigen-presenting cells. The FcεRI is constituted by α, β, and γ subunits (FcεRIα, FcεRIβ, and FcεRIγ). FcεRIα is IgE receptor-specific, whereas FcεRIβ and FcεRIγ are also part of other Fc receptors [10].

Allergy is per se a type-2 biomarker [11]. Allergen-specific serum IgE can generally account for up to 20–25% of total serum IgE, determining the overall value of total blood serum IgE [10]. Although total serum IgE is not a marker of asthma severity or airflow limitation, elevated levels of IgE may be associated with severe subgroups [12]. A possible role of the serum-specific IgE/total IgE ratio in predicting the clinical relevance of an allergen exposure or response to allergen immunotherapy is still under investigation.

Total serum IgE levels are usually higher in allergic versus nonallergic asthma, but they may substantially overlap. One study described three clusters of allergic asthma, with cluster 1 having a higher proportion of patients with total serum IgE levels < 100 (kU/L) (31.5%) compared with the others [13]. The Spanish MEGA cohort study did not identify total serum IgE levels as a distinctive characteristic among the four clusters described [14].

High IgE levels have, conversely, been extensively reported in nonallergic asthma. The bronchial mucosa of nonallergic asthma includes not only a similar expression of T2 cytokines and eosinophils as that of allergic asthma but also a similar number of FCεR1β positive cells [15]. Polymorphisms in FCεR1β have been associated with elevated total and specific IgE levels. In addition, elevated expression of local ε germline transcript (Iε) and the ε heavy chain of IgE (Cε) in the bronchial mucosa of both allergic and nonallergic asthmatics suggests local IgE synthesis [16]. How mucosal IgE production influences total serum IgE levels and if it correlates with disease activity is not known.

The usefulness of total serum IgE in differentiating allergic and nonallergic asthma is not so well defined compared with other T2 diseases, such as atopic dermatitis [17].

Therefore, it can be generally assumed that IgE is a common product of the type-2 inflammation pathway, shared by both allergic and nonallergic asthma. Unfortunately, the sensitivity and specificity of total serum IgE as a predictor of airway eosinophilia are quite weak and significantly lower than those of B-EOS and F_ENO [18].

To date, total serum IgE is approved as the biomarker for determining eligibility in severe asthma patients for omalizumab (a humanized recombinant monoclonal antibody with binding specificity at the FcεRI binding site of IgE) treatment [19].

The other currently used biologics in severe asthma treatment are represented by monoclonal antibodies directed towards the main effectors of T2 inflammation: mepolizumab, an anti-IL-5 antibody; benralizumab, an anti-IL-5R antibody; and dupilumab, a fully human monoclonal antibody against the IL-4Rα subunit of the IL-4/13 receptor [20]. The modulatory effects that these biologicals can provoke on IgE concentrations are still to be clarified [21].

A post-hoc analysis of moderate-to-severe asthma showed a high degree of overlap among allergen-specific IgE (positive skin prick and/or serum > 0.35 kU/L), B-EOS ≥ 300 cells/μL, and F_ENO (≥35 ppb) [22]. In this scenario, the International Severe Asthma Registry (ISAR) study reported that the likelihood of total serum IgE > 75 kU/L for the possible concomitant elevation of another biomarker was 59% for B-EOS > 300 and 65%

for F_ENO > 25 ppb [9]. Interestingly, 14% of the whole cohort reported elevated total serum IgE as a single $T2_{High}$ marker, but only 52% and 12% of them had allergic rhinitis or atopic dermatitis, respectively. In a Danish study, the single elevation of total serum IgE of >150 IU/mL (reported in 18% of patients) was consistent with higher S-EOS in only 35%, being the majority associated with a neutrophilic or paucigranulocytic phenotype ($T2_{Low}$) [23].

The $T2_{Low}$ phenotype has been extensively described in asthma, often characterized as non-eosinophilic asthma, accounting for slightly more than 50% in both steroid-naïve and steroid-treated patients [24] and variably associated with poor corticosteroid response [25]. Mean total serum IgE has been reported within a range of 84 to 126 kU/L and from 78 to 107 kU/L in paucigranulocytic and neutrophilic asthma, respectively [24,26]. A more recent study analyzed the longitudinal variability of sputum granulocytes and reported a low eosinophil group with a median total serum IgE level of 114.9 kU/L [27].

These data suggest the limited value of total serum IgE as a biomarker predictive of allergic or type-2 inflammation in asthma. This study aimed to replicate, in a cross-sectional, single-center, large asthmatic cohort, the characteristics of IgE_{High} and IgE_{Low} subpopulations in terms of demographic, clinical, and functional traits and to compare them within the commonly recognized $T2_{High}$ and $T2_{Low}$ phenotypes. We also reasoned that high total serum IgE levels may be detected in non-allergic states, e.g., obesity, viral infections, air pollution, or smoking [28].

2. Materials and Methods

2.1. Patients and Study Design

In the current observational cross-sectional real-life study, we consecutively enrolled adults (age $\geq$ 18 years) as outpatients with guideline-validated asthma diagnoses [1,29], attending the "Severe Asthma Centre" of the San Luigi Gonzaga University Hospital (Orbassano, Turin, Italy) between January 2018 and December 2022. The patients signed informed consent to participate in this study. The San Luigi Gonzaga University Hospital Ethical Review Board approved the study (protocol number: 4478/2017) in accordance with the Declaration of Helsinki.

2.2. Collection of Clinical, Functional, and Bio-Humoral Parameters

Demographic data, clinical history, functional and humoral parameters, and medications were retrieved from chart data for each patient after at least a complete assessment, adjustment, and review management cycle, according to GINA guidelines [1]. Data were not collected in cases of current or recent asthma exacerbations (AEs), self-reported poor adherence (<50%) to treatment, or inadequate inhalation technique. A subset of severe asthmatics was divided into those who had already started a biologic (biologics ongoing) and those who were candidates to start a biologic treatment (pre-biologics). In order to avoid the influence of biologic treatment, B-EOS, total serum IgE, pulmonary function tests, and F_ENO were collected before treatment initiation for severe asthma patients on biologics. Parasite infestation was not ruled out due to the low prevalence in Italy. Patients enrolled in the study did not have active cancer disease and had to be cancer disease-free (remission) for at least 5 years.

Pulmonary function and lung volumes were assessed by spirometry, performed before and 15 min after albuterol administration (400 µg), and plethysmography using a Vmax Encore 62 (Carefusion, Höechberg, Germany). F_ENO was analyzed at a flow rate of 50 mL/s with FeNO+ (Medisoft, Sorinnes, Belgium). Asthma Control Test (ACT) questionnaire was used to evaluate asthma control perception [30].

Asthma treatment strategy and asthma severity were graded according to the GINA guidelines [2]. We consider chronic the use of oral corticosteroids (OCS) for at least 3 consecutive months in the last year. AEs were also retrieved and reported, and $\geq$2 AEs in the previous year identified frequent exacerbator phenotype [31,32]. Life-span history of serious AEs requiring emergency room access and hospitalization was reported.

Allergen sensitization to perennial (house dust mites, cat and dog dander, and molds) and seasonal inhalants was detected by positive skin prick tests and/or serum allergen-specific IgE. Patients sensitized to ≥2 allergens were considered polysensitized.

$T2_{High}$ inflammation occurred whether at least one condition among B-EOS > 300 cells/μL, $F_ENO \geq 30$ ppb or allergic sensitization existed [33]. We selected F_ENO cut-off value ≥ 30 ppb, as previously found in our population [33]. Total serum IgE levels ≥ 100 kU/L were not considered per se as T2 inflammatory biomarkers, but this cut-off was used to categorize patients into two subgroups. This cut-off is derived from the median value of total serum IgE in our population, which is 102 kU/L, and from other cluster analyses that used 100kU/L as a discriminator for high and low IgE [13].

Patients were stratified according to both T2 inflammatory status and total serum IgE concentrations (IgE_{High}, ≥100 kU/L).

2.3. Statistical Analysis

In descriptive analyses, the ROUT method detected outliers to be excluded [34]. The deviation from normality was evaluated by the D'Agostino-Pearson test. Accordingly, one-way ANOVA (with Tukey post-hoc test) or Kruskal–Wallis H test (with Dunn post-hoc test) compared differences among groups. Chi-squared (χ^2) tests (with Fisher's exact post-hoc approach) [35] compared frequencies among groups.

In binomial and multinomial logistic regression models (LRMs), odds ratios (ORs) were calculated as exponentiation of the B-coefficient for each factor, which represents the odds of a unit change occurring in the independent variable in the analysis when all the others are kept constant. The 95% confidence interval (CI) was also reported. A *p*-value less than 0.05 was set as the significant cut-off. Variables for which a statistically significant difference resulted in comparing the groups were selected for further binomial logistic regression analysis. Variables giving significant odds or odds ≥ 2 were put into the multinomial LRM. The collinearity test for continuous variables was applied through inspection of correlation coefficients and accepted for variance inflation factors (VIF) values between 1 and 10.

Descriptive statistics and regression models were analyzed with IBM SPSS Statistics version 28 (IBM Corp., Armonk, NY, USA).

3. Results

3.1. Population General Characteristics

Descriptive statistics for the whole cohort of 547 asthmatic patients are reported in Table 1A–C. Most patients were female (62.7%), had late disease onset (76.4%), and had a long asthma history (24 ± 16 years); 28.4% were current or ex-smokers. Allergic sensitization was detected in 54.5% of patients, with the majority polysensitized (82.9%) (Table 1A). About 1/2 of patients (52.6%) could be classified into GINA Step 4 or 5 grades of severity, and 3/4 (75.5%) were partially or not controlled, while 15.9% had severe asthma treated with biological drugs (Table 1B). Upper airway inflammatory diseases resulted in the most common comorbidities, accounting for 64.0% and 35.4% of rhinitis and chronic rhinosinusitis (CRS), respectively (Table 1C).

Table 1. (**A**): Demographic, clinical, and bio-humoral parameters of asthmatic patients stratified according to T2 inflammatory phenotype. (**B**): Pulmonary function parameters, disease severity, and pharmacological treatments. (**C**): Comorbidities.

(A)				
Characteristic	**Overall (N = 547)**	**T2-Low (N = 113)**	**T2-High (N = 434)**	***p***
Age (years)	59 ± 15 [60]	63 ± 15 [68]	57 ± 15 [59]	***
Sex (Male)	204 (37.3%)	33 (29.2%)	171 (39.4%)	*
BMI (N = 544)	27.2 ± 5.6 [26.2]	28.8 ± 5.3 [29]	26.7 ± 5.6 [25.9]	***

Table 1. *Cont.*

(A)				
Characteristic	**Overall (N = 547)**	**T2-low (N = 113)**	**T2-high (N = 434)**	***p***
Age at asthma onset	35 ± 20 [35.0]	42 ± 20 [42]	33 ± 19 [34]	***
Early onset	129 (23.6%)	15 (13.3%)	114 (26.3%)	**
Asthma duration (years)	24 ± 16 [20]	22 ± 17 [17]	24 ± 16 [21]	
Vitamin D (ng/mL) (N = 427)	26.9 ± 13.5 [25]	27.5 ± 19.4 [23.2]	26.7 ± 11.7 [25.0]	
AE/years (N = 497)	1.12 ± 2.00 [0]	0.92 ± 1.07 [0]	1.17 ± 2.01 [0]	
Frequent Exacerbator phenotype (N = 497)	118 (23.7%)	19 (19.8%)	99 (24.7%)	
Serious AE (N = 513)	175 (34.1%)	36 (20.6%)	139 (33.7%)	
Fibrinogen (mg/dL) (N = 326)	341.5 ± 82.4 [331]	342.6 ± 74.4 [332]	341.2 ± 84.5 [331]	
Current smokers (≥10PY) (N = 544)	30 (5.5%)	6 (5.3%)	24 (5.5%)	
Past smokers (≥10PY) (N = 543)	125 (22.9%)	32 (28.3%)	93 (21.4%)	
Leukocytes (WBC cells/μL) (N = 542)	7293 ± 2147 [7000]	7220 ± 2122 [6775]	7312 ± 2156 [7070]	
Lymphocytes (cells/μL) (N = 396)	2319 ± 794 [2190]	2153 ± 575 [2060]	2363 ± 838 [2230]	*
Monocytes (cells/μL) (N = 366)	576 ± 214 [550]	553 ± 164 [530]	582 ± 225 [560]	
Neutrophils (cells/μL) (N = 482)	4039 ± 1533 [3775]	4209 ± 1738 [3890]	3995 ± 1475 [3760]	
Basophils (cells/μL) (N = 351)	47 ± 38 [40]	43 ± 32 [40]	47 ± 40 [40]	
Eosinophils (cells/μL) (N = 541)	344 ± 451 [240]	156 ± 65 [150]	392 ± 493 [300]	***
F_ENO (ppb) (N = 498)	38.1 ± 35.2 [28]	14.5 ± 7.3 [13.7]	44.0 ± 36.9 [36.0]	***
Total serum IgE (kU/L)	270.3 ± 536.8 [102]	131.2 ± 337.8 [33.0]	306.6 ± 572.3 [126.5]	**
Allergic sensitization	298 (54.5%)	-	298 (68.7%)	
Poly-sensitization	247/298 (82.9%)	-	247/298 (82.9%)	
Seasonal allergen sensitization	246/298 (82.6%)	-	246/298 (82.6%)	
Perennial allergen sensitization	225/298 (75.5%)	-	225/298 (75.5%)	
Moulds sensitization	55/298 (18.5%)	-	55/298 (18.5%)	
(B)				
Characteristic	**Overall (N = 547)**	**T2-low (N = 113)**	**T2-high (N = 434)**	***p***
FVC (%pred.) (N = 536)	100.2 ± 19.2 [100.0]	99.6 ± 20.1 [98.5]	100.3 ± 18.7 [100.0]	
FVC (L) (N = 536)	3.11 ± 1.05 [2.98]	2.77 ± 0.97 [2.65]	3.19 ± 1.06 [3.12]	***
FEV_1 (%pred.) (N = 540)	84.1 ± 21.4 [84.0]	84.5 ± 22.2 [83.0]	84.0 ± 22.2 [85.0]	
FEV_1 (L) (N = 540)	2.14 ± 0.84 [2.07]	1.94 ± 0.82 [1.84]	2.19 ± 0.84 [2.12]	**
FEV_1/FVC (%) (N = 536)	68.4 ± 12.4 [68.9]	68.8 ± 10.8 [69.5]	68.2 ± 12.8 [68.8]	
Δ-post-BD FVC (mL) (N = 437)	230.8 ± 453.4 [160.0]	234.7 ± 484.2 [170]	229.7 ± 445.6 [160]	
Δ-post-BD FEV_1 (mL) (N = 469)	228.4 ± 243.8 [190.0]	203.9 ± 247.7 [160.0]	234.7 ± 242.7 [190.0]	
RV (%pred.) (N = 464)	127.3 ± 37.2 [122.0]	129.0 ± 33.4 [126.0]	126.7 ± 38.2 [122.0]	
RV/TLC (%) (N = 411)	45.3 ± 12.2 [44.9]	48.4 ± 11.6 [48.2]	44.5 ± 12.2 [43.9]	**
TLC (%pred.) (N = 446)	107.0 ± 15.0 [106.0]	106.7 ± 15.8 [108.0]	107.0 ± 14.8 [106.0]	
FRC (%pred.) (N = 270)	112.3 ± 25.1 [109.0]	117.4 ± 22.7 [118.0]	110.8 ± 25.7 [109.0]	
FVC (%pred.)-post (N = 438)	105.8 ± 19.3 [107.0]	105.6 ± 21.8 [106.5]	105.9 ± 18.6 [107.0]	
FEV_1 (%pred.)-post (N = 465)	91.1 ± 21.6 [92.0]	90.3 ± 21.6 [90.0]	91.3 ± 21.6 [93.0]	
FEV_1/FVC (%)-post (N = 436)	69.3 ± 11.1 [70.0]	69.0 ± 10.6 [69.0]	69.4 ± 11.2 [70.0]	
SpO2 (%) (N = 529)	96 ± 1 [97]	96 ± 2 [96]	97 ± 2 [97]	
Heart Rate (bpm) (N = 530)	78 ± 12 [77]	77 ± 12 [77]	78 ± 12 [78]	
ACT	20.1 ± 4.3 [21.0]	18.8 ± 4.7 [20.0]	20.5 ± 4.1 [22.0]	***
24–25 (Controlled)	134 (24.5%)	19 (16.8%)	115 (26.5%)	*
20–23 (Partially controlled)	213 (38.9%)	41 (36.3%)	172 (39.6%)	
≤19 (Not controlled)	200 (36.6%)	53 (46.9%)	147 (33.9%)	*
Activity limitation	4.0 ± 1.1 [4.0]	3.7 ± 1.2 [4.0]	4.1 ± 11 [5.0]	**
Asthma severity grade				
GINA Step 1	52 (9.5%)	12 (10.6%)	40 (9.2%)	
GINA Step 2	53 (9.7%)	7 (6.2%)	46 (10.6%)	
GINA Step 3	154 (28.2%)	35 (31.0%)	119 (27.4%)	
GINA Step 4	116 (21.2%)	34 (30.1%)	82 (18.9%)	**
GINA Step 5	172 (31.4%)	25 (22.1%)	147 (33.9%)	*
BCM HFA dose (μg)	364.1 ± 259.3 [300.0]	365.5 ± 263.1 [400.0]	363.7 ± 258.6 [300.0]	
Chronic OCS use	37 (6.8%)	12 (10.6%)	25 (5.8%)	

Table 1. *Cont.*

(B)				
Characteristic	**Overall (N = 547)**	**T2-low (N = 113)**	**T2-high (N = 434)**	***p***
LABA use	447 (81.7%)	95 (84.1%)	352 (81.1%)	
LAMA use	94 (17.2%)	28 (24.8%)	66 (15.2%)	*
Biologics ongoing	39 (7.1%)	-	39 (7.1%)	
Pre Biologics	48 (8.8%)	-	48 (8.8%)	
All Biologics	87 (15.9%)	-	87 (15.9%)	
Omalizumab	37 (6.8%)	-	37 (6.8%)	
Mepolizumab	23 (4.2%)	-	23 (4.2%)	
Benralizumab	18 (3.3%)	-	18 (3.3%)	
Dupilumab	9 (1.6%)	-	9 (1.6%)	
Nasal CS use	325 (59.4%)	42 (37.2%)	283 (65.2%)	***
Antileukotriene	74 (13.5%)	7 (6.2%)	67 (15.4%)	*
(C)				
	Overall (N = 547)	**T2-low (N = 113)**	**T2-high (N = 434)**	***p***
Aspirin intolerance	75 (13.7%)	13 (17.3%)	62 (14.3%)	
Rhinitis	350 (64.0%)	49 (43.4%)	301 (69.4%)	***
CRSsNP	96 (17.5%)	14 (12.4%)	82 (18.9%)	
CRSwNP	98 (17.9%)	10 (8.8%)	88 (20.3%)	**
Bronchiectasis	61 (11.2%)	13 (11.5%)	48 (11.1%)	
Emphysema	67 (12.2%)	24 (21.2%)	43 (9.9%)	**
Pneumonia history	71 (13.0%)	18 (15.9%)	52 (12.2%)	
OSAS	29 (5.3%)	8 (7.1%)	21 (4.8%)	
GERD	119 (21.8%)	32 (28.3%)	87 (20.0%)	
Obesity	124 (22.7%)	36 (31.9%)	88 (20.3%)	*
Diabetes mellitus	38 (6.9%)	13 (11.5%)	25 (5.8%)	*
Arterial hypertension	167 (30.5%)	50 (44.2%)	117 (27.0%)	***
Acute myocardial infarction	24 (4.4%)	9 (8.0%)	15 (3.5%)	*
Heart failure	8 (1.5%)	3 (2.7%)	5 (1.2%)	
Arrhythmia	38 (6.9%)	9 (8.0%)	29 (6.7%)	
Anxiety-depression	76 (13.9%)	15 (13.3%)	61 (14.1%)	
Osteoporosis	44 (8.0%)	10 (8.8%)	34 (7.8%)	
Chronic Pain	33 (6.0%)	9 (8.0%)	24 (5.5%)	
Arthropathy	48 (8.8%)	12 (10.6%)	36 (8.3%)	

The results are expressed as mean with standard deviation or as a number of subjects with percentage. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$; median is reported in brackets []; AE: asthma exacerbations; frequent exacerbator ($\geq$2 exacerbation/year). The results are expressed as mean with standard deviation or as a number of subjects with percentage. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$; median is reported in brackets []; we consider chronic the use of OCS for at least 3 consecutive months in the last year. FVC: forced vital capacity; FEV_1: forced expiratory capacity in the first second; BD: bronchodilator; RV: residual volume; TLC: total lung capacity; FRC: functional residual capacity; GINA: Global Initiative for Asthma; BCM HFA: beclomethasone extrafine formulation equivalent dose; LABA: Long-Acting Beta-Agonists; LAMA: long-acting muscarinic antagonist. The results are expressed as the number of subjects by percentage. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$; CRSsNP: chronic rhinosinusitis without polyps; CRSwNP: chronic rhinosinusitis with polyps; OSAS: obstructive sleep apnea syndrome; GERD: gastroesophageal reflux disease. Obesity = BMI $\geq$ 30.

3.2. Patients' Stratification into T2/IgE Phenotypes

Patients classifiable as $T2_{High}$ due to the presence of one or more markers of T2 inflammation were 434 (79.3%). In detail, 298 (68.7%) had allergic sensitization, 232 (42.4%) had $F_ENO \geq 30$ ppb, and 227 (41.5%) reported B-EOS $\geq$ 300 cells/μL. The remaining 113 subjects (20.7%) were defined as $T2_{Low}$ (Table 1A–C). Patients defined as IgE_{High} (IgE $\geq$ 100 kU/L) were 278 (50.8%), while 269 (49.2%) were considered IgE_{Low}. The descriptive statistics of clinical and functional parameters among groups of patients stratified by a single biomarker were reported in Supplementary Table S1. The combination of T2 markers and total IgE status allowed us to classify four groups of patients: (1) $T2_{Low}$ and IgE_{Low}; (2) $T2_{Low}$ and

IgE_{High}; (3) $T2_{High}$ and IgE_{Low}; and (4) $T2_{High}$ and IgE_{High}. The distribution of one to three positive T2 markers among IgE_{Low} (Figure 1A) and IgE_{High} (Figure 1B) patients is schematized by the Euler diagram.

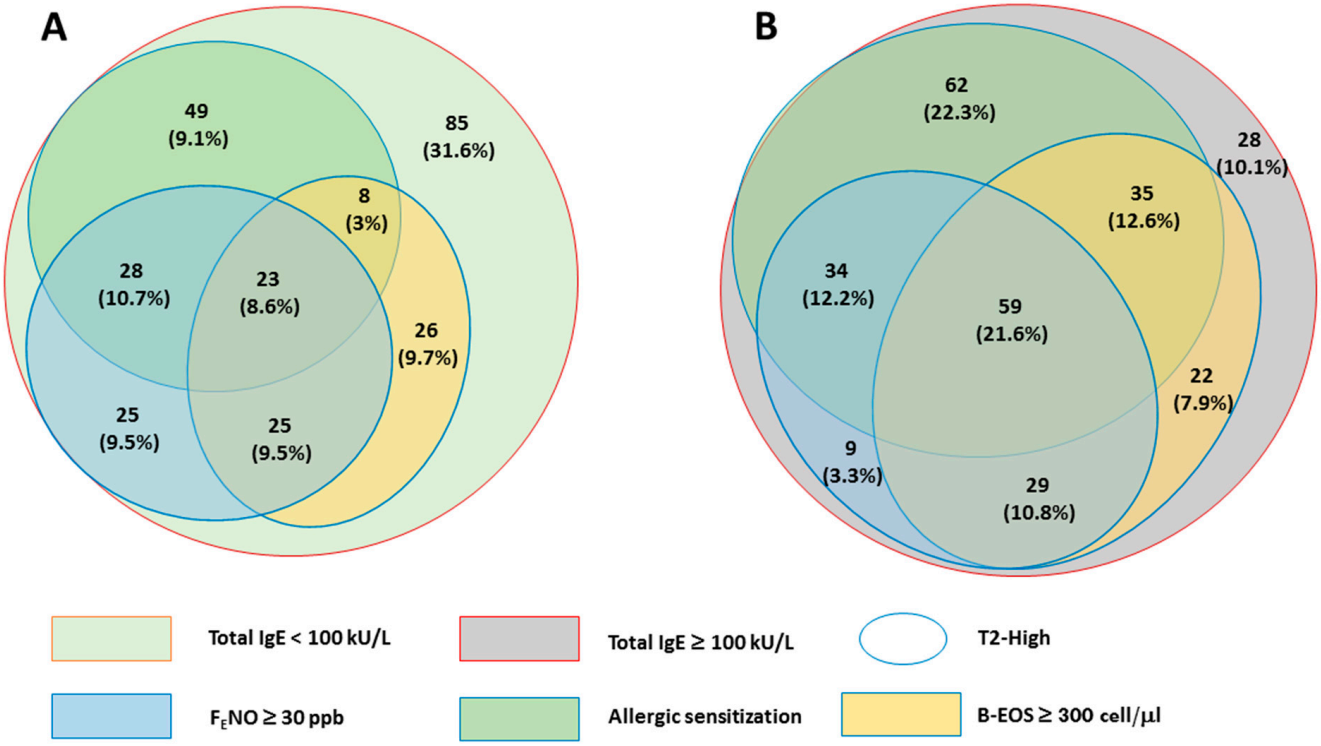

Figure 1. Area-proportional Euler diagrams of the type 2 biomarker positivity. (**A**) Population with Total Serum IgE < 100 kU/L; (**B**) Population with Total Serum IgE ≥ 100 kU/L.

In both diagrams, the blue line delimits the $T2_{High}$ subgroup: the presence of allergic sensitization is represented in green; blood eosinophils ≥ 300 cells/µL are reported in yellow; $F_ENO \geq 30$ ppb is colored in azure; the presence of multiple T2 biomarkers is reported with the overlap of the respective biomarker ovals. The $T2_{Low}$ subgroup, with (red) or without (teal) high total serum IgE levels, is represented outside the blue line.

3.3. Biomarker Expression

Both B-EOS and F_ENO were significantly higher in the $T2_{High}$ compared with the $T2_{Low}$ groups, irrespective of their total serum IgE values (Tables 1A and 2A). In detail, a mean $T2_{High}$ B-EOS of 392 ± 493 cells/µL, 325 ± 299 cells/µL in IgE_{Low}, and 443 ± 593 cells/µL in IgE_{High} resulted, respectively. Mean B-EOS were 156 ± 65 cells/µL ($p < 0.001$) in $T2_{Low}$, 153 ± 65 cells/µL in IgE_{Low}, and 161 ± 69 cells/µL in IgE_{High}, respectively; mean $T2_{High}$ F_ENO was 44.0 ± 36.9 ppb, 43.0 ± 32.4 ppb in IgE_{Low}, and 44.7 ± 40.1 ppb in IgE_{High}; significantly increased compared with $T2_{Low}$ F_ENO of 14.7 ± 7.3 ppb (14.7 ± 7.3 ppb in IgE_{Low} and 13.6 ± 7.3 ppb in IgE_{High}, respectively). Mean total IgE levels were significantly higher in the whole $T2_{High}$ group (306.6 ± 572.3 kU/L) compared with the $T2_{Low}$ group (131.2± 337.8 kU/L) but were comparable within both the IgE_{High} (499.6 ± 693.3 kU/L in $T2_{High}$ and 437.6 ± 584.8 kU/L in $T2_{Low}$, respectively) and the IgE_{Low} subgroups (44.3 ± 27.6 kU/L in $T2_{High}$ and 30.2 ± 23.5 kU/L in $T2_{Low}$). The $T2_{High}$-IgE_{High} subjects reported higher allergen sensitization and prevalence of perennial allergens compared with the $T2_{High}$-IgE_{Low} subjects ($p < 0.001$ and $p < 0.05$, respectively).

Table 2. (**A**): Demographic, clinical, and bio-humoral parameters of asthmatic patients stratified according to serum Total Serum IgE and T2 inflammatory phenotype. (**B**): Pulmonary function parameters, disease severity, and pharmacological treatments. (**C**): Comorbidities.

(A)

Characteristics	Overall (N = 547)	T2-Low		T2-High	
		IgE < 100 kU/L (N = 85)	IgE ≥ 100 kU/L (N = 28)	IgE < 100 kU/L (N = 184)	IgE ≥ 100 kU/L (N = 250)
Age (years)	59 ± 15 [60]	65 ± 15 £££/# [68]	61 ± 17 [63.5]	59 ± 14 * [59.5]	56 ± 16 *** [58]
Sex (Male)	204 (37.3%)	19 (22.4%) £££/§§/#	14 (50.0%) **	61 (33.2%) £/*	110 (44.0%) ***/#
BMI (N = 544)	27.2 ± 5.6 [26.2]	28.8 ± 5.0 £££ [28.6]	28.6 ± 6.1 [30]	27.6 ± 5.7 £ [26.9]	26.1 ± 5.5 ***/# [25.6]
Age at asthma onset	35 ± 20 [35]	43 ± 20 ##/£££ [45]	38 ± 18 [40]	34 ± 19 ** [35]	33 ± 19 *** [33]
Early onset	129 (23.6%)	10 (11.8%) #/££	5 (17.9%)	46 (25.0%) *	68 (27.2%) **
Asthma duration (years)	24 ± 16 [20]	22 ± 17 [17]	22 ± 15 [18]	25 ± 18 [20]	23 ± 15 [21]
Vitamin D (ng/mL) (N = 427)	26.9 ± 13.5 [25]	26.6 ± 14.0 [24.3]	30.3 ± 31.7 [22.3]	26.5 ± 10.9 [25.0]	26.9 ± 12.2 [25.2]
AE/years (N = 497)	1.12 ± 2.00 [0]	0.65 ± 1.06 £ [0]	1.90 ± 2.91 [1]	0.91 ± 1.66 [0]	1.36 ± 2.31 * [1]
Frequent Exacerbator phenotype (N = 497)	118 (23.7%)	12 (16.0%) £	7 (33.3%)	37 (21.4%)	62 (27.2%) *
Serious AE (N = 513)	175 (34.1%)	27 (34.6%)	9 (40.9%)	55 (30.9%)	84 (35.7%)
Fibrinogen (mg/dL) (N = 326)	341.5 ± 82.4 [331.0]	347.5 ± 77.1 [333.0]	328.4 ± 65.9 [323.0]	341.1 ± 86.1 [334.0]	341.3 ± 83.6 [330.0]
Current smokers (≥10PY) (N = 544)	30 (5.5%)	6 (7.1%)	0 (0.0%)	12 (6.5%)	12 (4.8%)
Past smokers (≥10PY) (N = 543)	125 (22.9%)	20 (23.5%) §	12 (42.9%) */#/£	39 (21.2%) §	54 (21.6%) §
Leukocytes (cells/μL) (N = 542)	7293 ± 2147 [7000]	7153 ± 2016 [6730]	7417 ± 2441 [6855]	7079 ± 1872 [6670]	7483 ± 2332 [7220]
Lymphocytes (cells/μL) (N = 396)	2319 ± 794 [2190]	2156 ± 533 [2110]	2144 ± 715 [1930]	2313 ± 797 [2155]	2398 ± 867 [2300]
Monocytes (cells/μL) (N = 366)	576 ± 214 [550]	560 ± 146 [540]	529 ± 218 [490]	550 ± 211 [520]	605 ± 232 [580]
Neutrophils (cells/μL) (N = 482)	4039 ± 1533 [3775]	4153 ± 1729 [3820]	4382 ± 1790 [3935]	3843 ± 1342 [3650]	4106 ± 1559 [3790]
Basophils (cells/μL) (N = 351)	47 ± 38 [40]	40 ± 33 [40]	33 ± 28 [30]	50 ± 33 [50]	45 ± 44 [40]
Eosinophils (cells/μL) (N = 541)	344 ± 451 [240]	153 ± 65 £££/# [150]	161 ± 69 £ [160]	325 ± 299 */£ [245]	443 ± 593 ***/§/# [330]
F_ENO (ppb) (N = 498)	38.1 ± 35.2 [28]	14.7 ± 7.3 £££/### [14.0]	13.6 ± 7.3 £££/### [13.0]	43.0 ± 32.4 ***/§§§ [36.5]	44.7 ± 40.1 ***/§§§ [35.0]
Total serum IgE (kU/L)	270.3 ± 536.8 [102]	30.2 ± 23.5 £££/§§§ [26.2]	437.6 ± 584.8 ***/### [200.5]	44.3 ± 27.6 £££/§§§ [40.9]	499.6 ± 693.3 ***/### [271.5]
Allergic sensitization	298 (54.5%)	0 (0.0%)	0 (0.0%)	108/184 (58.7%) £££	190/250 (76.0%) ###
Poly-sensitization	247/298 (82.9%)	-	-	86/108 (79.6%)	161/190 (84.7%)
Seasonal allergen sensitization	246/298 (82.6%)	-	-	87/108 (80.6%)	159/190 (83.7%)
Perennial allergen sensitization	225/298 (75.5%)	-	-	73/108 (67.6%) £	152/190 (80.0%) #
Moulds sensitization	55/298 (18.5%)			17/108 (15.7%)	38/190 (20.0%)

(B)

Characteristics	Overall (N = 547)	T2-low		T2-high	
		IgE < 100 kU/L (N = 85)	IgE ≥ 100 kU/L (N = 28)	IgE < 100 kU/L (N = 184)	IgE ≥ 100 kU/L (N = 250)
FVC (%pred.) (N = 536)	100.2 ± 19.2 [100.0]	102.3 ± 19.2 § [100.5]	91.4 ± 23.9 * [96.0]	100.5 ± 19.9 [100.5]	100.1 ± 17.8 [100.0]
FVC (L) (N = 536)	3.11 ± 1.05 [2.98]	2.72 ± 0.89 £££/# [2.65]	2.93 ± 1.17 [2.70]	3.10 ± 1.02 * [2.98]	3.26 ± 01.08 *** [3.20]
FEV_1 (%pred.) (N = 540)	84.1 ± 21.4 [84.0]	86.3 ± 20.5 [87.0]	79.1 ± 26.5 [80.5]	85.1 ± 20.9 [86.0]	83.2 ± 21.4 [83.0]
FEV_1 (L) (N = 540)	2.14 ± 0.84 [2.07]	1.88 ± 0.71 ££ [1.84]	2.08 ± 1.06 [1.74]	2.15 ± 0.78 [2.11]	2.23 ± 0.88 ** [2.16]
FEV_1/FVC (%) (N = 536)	68.4 ± 12.4 [68.9]	68.6 ± 10.3 [69.8]	69.2 ± 12.2 [67.5]	68.9 ± 10.0 [69.6]	67.7 ± 14.6 [67.9]
D-post-BD FVC (mL) (N = 437)	230.8 ± 453.4 [160.0]	247.0 ± 542.8 [1.70]	192.8 ± 182.4 [1.70]	221.8 ± 393.3 [1.60]	235.2 ± 479.2 [1.60]

Table 2. *Cont.*

Characteristics	Overall (N = 547)	T2-low		T2-high	
		IgE < 100 kU/L (N = 85)	IgE ≥ 100 kU/L (N = 28)	IgE < 100 kU/L (N = 184)	IgE ≥ 100 kU/L (N = 250)
		(B)			
D-post-BD FEV_1 (mL) (N = 469)	228.4 ± 243.8 [190.0]	201.1 ± 272.2 [1.45]	213.0 ± 147.6 [2.00]	224.4 ± 254.7 [1.90]	241.9 ± 234.4 [1.90]
RV (%pred.) (N = 464)	127.3 ± 37.2 [122.0]	127.3 ± 33.2 [122.0]	134.9 ± 33.1 [137.5]	122.9 ± 31.2 [120.0]	129.9 ± 42.6 [123.5]
RV/TLC (%) (N = 411)	45.3 ± 12.2 [44.9]	48.0 ± 11.1 [48.3]	49.6 ± 13.1 [46.8]	45.1 ± 12.1 [43.6]	44.1 ± 12.3 [44.8]
TLC (%pred.) (N = 446)	107.0 ± 15.0 [106.0]	107.4 ± 16.2 [108.0]	105.2 ± 14.3 [107.0]	105.9 ± 14.5 [106.0]	107.8 ± 14.9 [106.0]
FRC (%pred.) (N = 270)	112.3 ± 25.1 [109.0]	117.4 ± 25.0 $^{\#}$ [117.0]	117.2 ± 22.6 [121.0]	105.5 ± 22.8 $^{£/*}$ [105.0]	114.6 ± 26.9 $^{\#}$ [111.5]
FVC (%pred.)-post (N = 438)	105.8 ± 19.3 [107.0]	107.7 ± 17.1 [107.0]	98.5 ± 30.8 [105.0]	106.5 ± 19.5 [105.0]	105.5 ± 17.9 [107.0]
FEV_1 (%pred.)-post (N = 465)	91.1 ± 21.6 [92.0	92.3 ± 19.9 [91.0]	81.7 ± 24.8 [81.0]	92.3 ± 21.6 [95.0]	90.6 ± 21.6 [92.0]
FEV_1/FVC (%)-post (N = 436)	69.3 ± 11.1 [70.0]	69.2 ± 10.5 [70.0]	68.2 ± 11.1 [66.0]	70.4 ± 10.0 [71.0]	68.6 ± 11.9 [69.5]
SpO2 (%) (N = 529)	96 ± 1 [97.0]	96 ± 1 [96]	97 ± 2 [96]	97 ± 1 [97]	97 ± 2 [97]
Heart Rate (bpm) (N = 530)	78 ± 12 [77.0]	76 ± 12 [76]	79 ± 12 [78]	77 ± 12 [76]	79 ± 12 [79]
ACT	20.1 ± 4.3 [21.0]	19.2 ± 4.3 [21.0]	17.7 ± 5.9 [19.5]	20.6 ± 4.1 [22.0]	20.4 ± 4.1 [21.5]
24–25 (Controlled)	134 (24.5%)	14 (16.5%)	5 (17.9%)	50 (27.2%)	65 (26.0%)
20–23 (Partially controlled)	213 (38.9%)	32 (37.6%)	9 (32.1%)	74 (40.2%)	98 (39.2%)
≤19 (Not controlled)	200 (36.6%)	39 (45.9%) $^{\#}$	14 (50.0%)	60 (32.6%) *	87 (34.8%)
Activity limitation	4.0 ± 1.1 [4.0]	3.8 ± 1.1 [4.0]	3.4 ± 1.4 $^{\#/£}$ [4.0]	4.1 ± 1.1 § [5.0]	4.1 ± 1.0 § [4.0]
Asthma severity grade					
GINA Step 1	52 (9.5%)	9 (10.6%)	3 (10.7%)	19 (10.3%)	21 (8.4%)
GINA Step 2	53 (9.7%)	3 (3.5%) $^{§/\#/£}$	4 (14.3%) *	19 (10.3%) *	27 (10.8%) *
GINA Step 3	154 (28.2%)	31 (36.5%) $^{§/£}$	4 (14.3%) *	57 (31.0%)	62 (24.8%) *
GINA Step 4	116 (21.2%)	27 (31.8%) $^{\#/£}$	7 (25.0%)	34 (18.5%) *	48 (19.2%) *
GINA Step 5	172 (31.4%)	15 (17.6%) $^{\#/§/£££}$	10 (35.7%) *	55 (29.9%) *	92 (36.8%) ***
BCM HFA dose (µg)	364.1 ± 259.3 [300.0]	338.8 ± 242.6 [300]	446.4 ± 308.5 [400]	346.4 ± 245.6 [200]	376.7 ± 267.6 [300]
Chronic OCS use	37 (6.8%)	7 (8.2%)	5 (17.9%) $^{\#\#/£}$	6 (3.3%) §§	19 (7.6%) §
LABA use	447 (81.7%)	73 (85.9%)	22 (78.6%)	145 (78.8%)	207 (82.8%)
LAMA use	94 (17.2%)	21 (24.7%) $^{£}$	7 (25.0%)	29 (15.8%)	37 (14.8%) *
Biologics ongoing	39 (7.1%)	0 (0.0%)	0 (0.0%)	9 (4.9%)	30 (12%)
Pre Biologics	48 (8.8%)	0 (0.0%)	0 (0.0%)	16 (8.7%)	32 (12.8)
All Biologics	87 (15.9%)	0 (0.0%)	0 (0.0%)	25 (13.6%) $^{££}$	62 (24.8%) $^{\#\#}$
Omalizumab	37 (6.8%)	0 (0.0%)	0 (0.0%)	10 (5.4%) $^{£}$	27 (10.8%) $^{\#}$
Mepolizumab	23 (4.2%)	0 (0.0%)	0 (0.0%)	5 (2.7%) $^{£}$	18 (7.2%) $^{\#}$
Benralizumab	18 (3.3%)	0 (0.0%)	0 (0.0%)	7 (3.8%)	11 (4.4%)
Dupilumab	9 (1.6%)	0 (0.0%)	0 (0.0%)	3 (1.6%)	6 (2.4%)
Nasal CS use	325 (59.4%)	28 (32.9%) $^{\#\#\#/£££}$	14 (50.0%)	117 (63.6%) ***	166 (66.4%) ***
Antileukotriene	74 (13.5%)	7 (8.2%) $^{£}$	0 (0.0%) $^{££/\#}$	25 (13.6%) §	42 (16.8%) $^{§§/*}$

Table 2. *Cont.*

	(C)				
	Overall	**T2-low**		**T2-high**	
	(N = 547)	**IgE < 100 kU/L (N = 85)**	**IgE ≥ 100 kU/L (N = 28)**	**IgE < 100 kU/L (N = 184)**	**IgE ≥ 100 kU/L (N = 250)**
Aspirin intolerance	75 (13.7%)	8 (9.4%)	5 (17.9%)	24 (13.0%)	38 (15.2%)
Rhinitis	350 (64.0%)	34 (40.0%) ###/£££	15 (53.6%) £	120 (65.2%) ***	181 (72.4%) ***/§
CRSsNP	96 (17.5%)	11 (12.9%)	3 (10.7%)	34 (18.5%)	48 (19.2%)
CRSwNP	98 (17.9%)	6 (7.1%) #/££	4 (14.3%)	33 (17.9%) *	55 (22.0%) **
Bronchiectasis	61 (11.2%)	9 (10.6%)	4 (14.3%)	19 (10.3%)	29 (11.6%)
Emphysema	67 (12.2%)	19 (22.4%) ##/££	5 (17.9%)	16 (8.7%) **	27 (10.8%) **
Pneumonia history	71 (13.0%)	14 (16.5%)	4 (14.3%)	28 (15.2%)	25 (10%)
OSAS	29 (5.3%)	4 (4.7%)	4 (14.3%) £	10 (5.4%) §	11 (4.4%) §
GERD	119 (21.8%)	23 (27.1%) £	9 (31.1%)	44 (23.9%)	43 (17.2%) *
Obesity	124 (22.7%)	25 (29.4%) £	11 (39.3%) ££	46 (25%) £	42 (16.8%) */#/§§
Diabetes mellitus	38 (6.9%)	11 (12.9%) £	2 (7.1%)	13 (7.1%)	12 (4.8%) *
Arterial Hypertension	167 (30.5%)	40 (47.1%) #/£££	10 (35.7%)	61 (33.2%) */£	56 (22.4%) ***/#
Acute myocardial infarction	24 (4.4%)	8 (9.4%) £	1 (3.6%)	7 (3.8%)	8 (3.2%) *
Heart failure	8 (1.5%)	3 (3.5%) £	0 (0.0%)	5 (2.7%) £	0 (0.0%) */#
Arrhythmia	38 (6.9%)	7 (8.2%)	2 (7.1%)	15 (8.2%)	14 (5.6%)
Anxiety-depression	76 (13.9%)	13 (15.6%)	2 (7.1%)	35 (19.0%) £	26 (10.4%) #
Osteoporosis	44 (8.0%)	8 (9.4%)	2 (7.1%)	13 (7.1%)	21 (8.4%)
Chronic Pain	33 (6.0%)	8 (9.4%)	1 (3.6%)	13 (7.1%)	11 (4.4%)
Arthropathy	48 (8.8%)	11 (12.9%)	1 (3.6%)	19 (10.3%)	17 (6.8%)

The results are expressed as mean with standard deviation or as a number of subjects with percentage. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$ vs. IgE_{Low}-$T2_{Low}$; # = $p < 0.05$, ## = $p < 0.01$, ### = $p < 0.001$ vs. IgE_{Low}-$T2_{High}$; § = $p < 0.05$, §§ = $p < 0.01$, §§§ = $p < 0.001$ vs. IgE_{High}-$T2_{Low}$; £ = $p < 0.05$, ££ = $p < 0.01$, £££ = $p < 0.001$ vs. IgE_{High}-$T2_{High}$; median is reported in brackets []; AE: asthma exacerbations; Frequent exacerbator (≥2 exacerbation/year); The results are expressed as mean with standard deviation or as a number of subjects with percentage. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$ vs. IgE_{Low}-$T2_{Low}$; # = $p < 0.05$, ## = $p < 0.01$, ### = $p < 0.001$ vs. IgE_{Low}-$T2_{High}$; § = $p < 0.05$, §§ = $p < 0.01$, §§§ = $p < 0.001$ vs. IgE_{High}-$T2_{Low}$; £ = $p < 0.05$, ££ = $p < 0.01$, £££ = $p < 0.001$ vs. IgE_{High}-$T2_{High}$; median is reported in brackets []; we consider as chronic the use of OCS for at least 3 consecutive months in the last year. FVC: forced vital capacity; FEV_1: forced expiratory capacity in the first second; BD: bronchodilator; RV: residual volume; TLC: total lung capacity; FRC: functional residual capacity; GINA: Global Initiative for Asthma; BCM HFA: beclomethasone extrafine formulation equivalent dose; LABA: Long-Acting Beta-Agonists; LAMA: long-acting muscarinic antagonist. The results are expressed as the number of subjects by percentage.* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$ vs. IgE_{Low}-$T2_{Low}$; # = $p < 0.05$, ## = $p < 0.01$, ### = $p < 0.001$ vs. IgE_{Low}-$T2_{High}$; § = $p < 0.05$, §§ = $p < 0.01$, §§§ = $p < 0.001$ vs. IgE_{High}-$T2_{Low}$; £ = $p < 0.05$, ££ = $p < 0.01$, £££ = $p < 0.001$ vs. IgE_{High}-$T2_{High}$; CRSsNP: chronic rhinosinusitis without polyps; CRSwNP: chronic rhinosinusitis with polyps; OSAS: obstructive sleep apnoea syndrome; GERD: gastroesophageal reflux disease. Obesity = BMI ≥ 30.

3.4. Characterization of T2High and T2Low Phenotypes

3.4.1. Demographic Data, Lung Function, and Disease Presentation

$T2_{High}$ and $T2_{Low}$ patients significantly differed in terms of age, sex, and BMI, with $T2_{High}$ patients being significantly younger, more frequently male, and thinner. $T2_{High}$ had a shorter age of onset but did not differ in asthma duration (Table 1A). Significantly higher absolute FVC and FEV_1 measures were detected in $T2_{High}$ patients, as well as a lower RV/TLC ratio. Disease activity significantly differed between the two groups, with $T2_{Low}$ subjects being worst controlled ($p < 0.05$) and more frequently in the GINA Step 4 level of treatment ($p < 0.01$) compared with $T2_{High}$ patients, often needing LAMA add-on treatment, while $T2_{High}$ patients were more frequently in GINA Step 5 ($p < 0.05$) (Table 1B).

3.4.2. Comorbidities

Rhinitis and nasal polyps were significantly more prevalent in $T2_{High}$ compared with $T2_{Low}$ patients (69.4% vs. 43.4%, $p < 0.001$, and 20.3% vs. 8.8%, $p < 0.01$, respectively). On the other hand, $T2_{Low}$ patients reported higher rates of emphysema (21.2% vs. 9.9%, $p < 0.01$), obesity (31.9% vs. 20.3%, $p < 0.05$), diabetes mellitus (11.5% vs. 5.8%, $p < 0.05$), arterial hypertension (44.2% vs. 27.0%, $p < 0.001$), and acute myocardial infarction (8.0% vs. 3.5%, $p < 0.05$) (Table 1C).

3.5. Characterization of T2 Phenotype According to Total Serum IgE Levels

3.5.1. Demographic Data, Lung Function, and Disease Presentation

The two $T2_{High}$ groups showed significant differences compared with the $T2_{Low}$-IgE_{Low} group but less compared with the $T2_{Low}$-IgE_{High} one (Table 2A,B).

Both $T2_{High}$-IgE_{High} and $T2_{High}$-IgE_{low} were characterized by a lower age ($p < 0.001$ and $p < 0.05$), earlier onset ($p < 0.01$ and $p < 0.05$), a higher proportion of male subjects ($p < 0.001$ and $p < 0.05$), and better lung function in terms of absolute FVC ($p < 0.001$ and $p < 0.05$) compared with the $T2_{Low}$-IgE_{Low} group.

$T2_{High}$-IgE_{High} had a lower BMI ($p < 0.001$), a higher AE rate ($p < 0.05$), and a significantly higher FEV_1 ($p < 0.001$) compared with $T2_{Low}$-IgE_{Low}, while $T2_{High}$-IgE_{Low} had a FRC% significantly lower than $T2_{Low}$-IgE_{Low} ($p < 0.05$).

Both $T2_{High}$-IgE_{High} and $T2_{High}$-IgE_{Low} were more often classified in GINA Step 5 and less in GINA Step 4 and used more frequently nasal corticosteroids (CS) compared with the $T2_{Low}$-IgE_{Low} group ($p < 0.001$).

Some differences are also evident between the two $T2_{High}$ groups. $T2_{High}$-IgE_{Low} patients were more often female with a significantly higher BMI than the $T2_{High}$-IgE_{High} patients ($p < 0.05$).

Within the two $T2_{Low}$ groups, $T2_{Low}$-IgE_{High} subjects showed a higher prevalence of male sex ($p < 0.01$), past smokers ($p < 0.05$), GINA Step 5 and Step 2 ($p < 0.05$), and lower FVC% ($p < 0.05$) compared with the $T2_{Low}$-IgE_{Low} group (Table 1A,B). The $T2_{Low}$-IgE_{High} group was also characterized by a higher rate of past smokers compared with all other groups ($p < 0.05$) and higher OCS and lower anti-leukotriene use compared with the $T2_{High}$ subgroups.

3.5.2. Comorbidities

The main comorbidities that differentiated $T2_{High}$-IgE_{High} from $T2_{Low}$-IgE_{Low} included a higher prevalence of rhinitis ($p < 0.001$) and chronic rhinosinusitis with nasal polyps (CRSwNP) ($p < 0.01$) and a lower occurrence of emphysema ($p < 0.01$), cardiovascular comorbidities ($p < 0.05$), GERD ($p < 0.05$), diabetes mellitus ($p < 0.05$), and obesity ($p < 0.05$). More frequently, rhinitis ($p < 0.001$) and CRSwNP ($p < 0.01$) were observed in $T2_{High}$-IgE_{Low} patients compared with $T2_{Low}$-IgE_{Low} patients (Table 2C). $T2_{Low}$-IgE_{High} had a lower rate of rhinitis ($p < 0.05$) but a higher rate of OSAS ($p < 0.01$) and obesity ($p < 0.05$) compared with $T2_{High}$-IgE_{High}. Finally, within the $T2_{High}$ subgroups, the presence of low IgE was associated with higher obesity ($p < 0.05$), arterial hypertension ($p < 0.05$), and anxiety/depression syndrome ($p < 0.05$).

3.6. Predictive Statistics (LRM)

When applying a binomial LRM to variables to predict T2 High versus T2 Low status, significantly higher odds were obtained for rhinitis (OR: 2.0), use of nasal CS (OR: 3.3), and GINA Step 5 (OR: 4.8), while lower risks were associated with emphysema (OR: 0.3) and poor ACT (OR: 0.3) (Table 3). The risks for obesity, diabetes, arterial hypertension, and acute myocardial infarction were not significant.

Table 3. Binomial logistic regression analysis of variables for asthmatic patients stratified according to T2 inflammatory phenotype.

Characteristic	$T2_{High}$ vs. $T2_{Low}$		
	OR	**LB**	**UB**
Age	0.997	0.957	1.038
BMI	0.947	0.869	1.033
Age at asthma onset	0.983	0.958	1.009
FVC (L)	2.731	0.825	9.041
FEV_1 (L)	0.265	0.045	1.572
RV/TLC	0.190	0.000	179.421
Lymphocytes	1.000	0.000	1.001
Sex (male)	1.107	0.409	2.996
Early onset	0.791	0.215	2.906
Rhinitis	**2.017 ***	0.990	4.112
CRSwNP	0.629	0.213	1.854
Emphysema	**0.268 ***	0.093	0.769
Obesity	1.115	0.392	3.174
Diabetes mellitus	1.381	0.371	5.138
Arterial hypertension	1.118	0.507	2.468
Acute myocardial infarction	0.663	0.152	2.890
ACT	*		
ACT $\leq$ 19 (Not controlled)	**0.312 ***	0.087	1.124
ACT $\geq$ 24 (well controlled)	0.309	0.055	1.720
LAMA use	0.550	0.198	1.526
Nasal CS use	**3.345 *****	1.637	6.835
Antileukotriens	5.546	0.913	33.673
Asthma severity grade	*		
GINA (Step 1)	2.179	0.395	12.004
GINA (Step 2)	1.113	0.278	4.449
GINA (Step 3)	0.899	0.198	4.081
GINA (Step 4)	4.375	0.874	21.904
GINA (Step 5)	**4.759 ****	1.645	13.768

* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$. Bold font highlights statistically significant results. LB: lower bound; UB: upper bound; FVC: forced vital capacity; FEV_1: forced expiratory capacity in the first second; RV: residual volume; TLC: total lung capacity; CRSwNP: Chronic Rhinosinusitis with Nasal Polyps; LAMA: long-acting muscarinic antagonist; GINA: Global Initiative for Asthma.

When applying a binomial LRM for variables to predict the odds for each single subgroup compared with the rest of the population (Supplementary Table S2), major significant effects were found for factors identifying the $T2_{Low}$-IgE_{Low} group, either positively (BMI OR: 1.2; FEV_1 OR: 19.5; emphysema OR: 5.2; LAMA use OR: 11.3) or negatively (nasal CS use OR: 0.2). A forest graphic representation of binomial LMR is reported in Figure 2A–D.

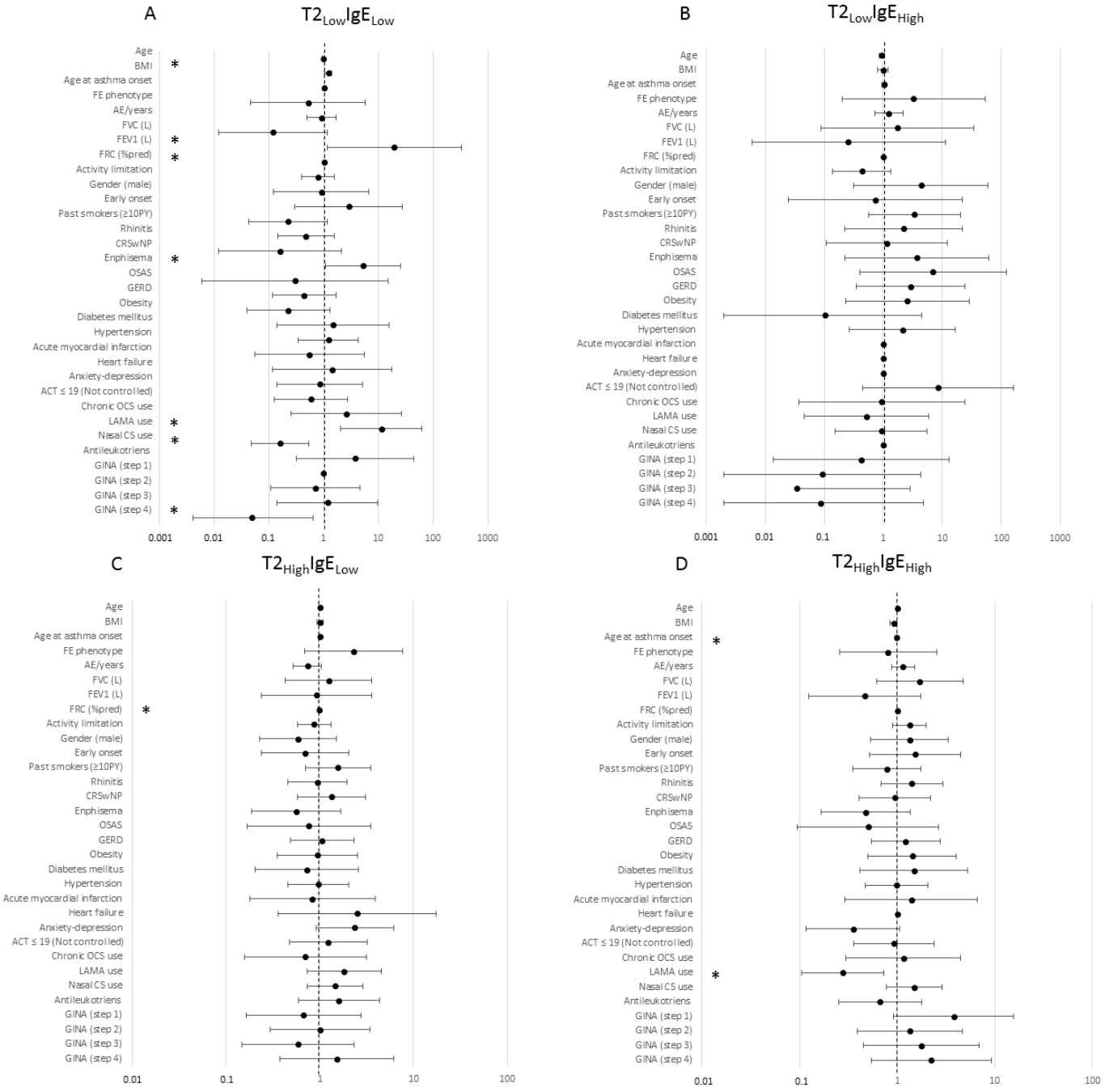

Figure 2. Forest diagrams representing odds from binomial LRM for patients stratified according to Total IgE and T2 inflammatory phenotypes. (**A**) $T2_{Low}$-IgE_{Low} group; (**B**) $T2_{Low}$-IgE_{High} group; (**C**) $T2_{High}$-IgE_{Low} group; (**D**) $T2_{High}$-IgE_{High} group. Odds for each single group are compared with the rest of the population (the union of the other three groups). X axis is expressed on a logarithmic scale. *: statistically significant.

After adjusting for colinearity, multinomial LRM was applied to predict the odds for variables associated with each subgroup compared with each of the others (Supplementary Table S3). The main factors associated with $T2_{Low}$-IgE_{Low} compared with $T2_{High}$-IgE_{High} were high BMI (OR: 1.1), emphysema (OR: 4.0), heart failure (OR: 8.0×10^8), LAMA use (OR: 9.9), and poor control (OR: 3.6), while negative odds were found for rhinitis and nasal CS use compared with both the $T2_{High}$ subgroups ($p < 0.05$).

Within the $T2_{Low}$ groups, factors associated with high total serum IgE were the frequent exacerbator phenotype (OR: 7.9), rhinitis (OR: 8.5), and anxiety/depression (8.5×10^9, $p < 0.001$).

Finally, the presence of anxiety/depression, use of LAMA, and lower FRC% could predict entry into the IgE_{Low} group within the $T2_{High}$. A forest graphic representation of binomial LMR is reported in Figure 3A–F.

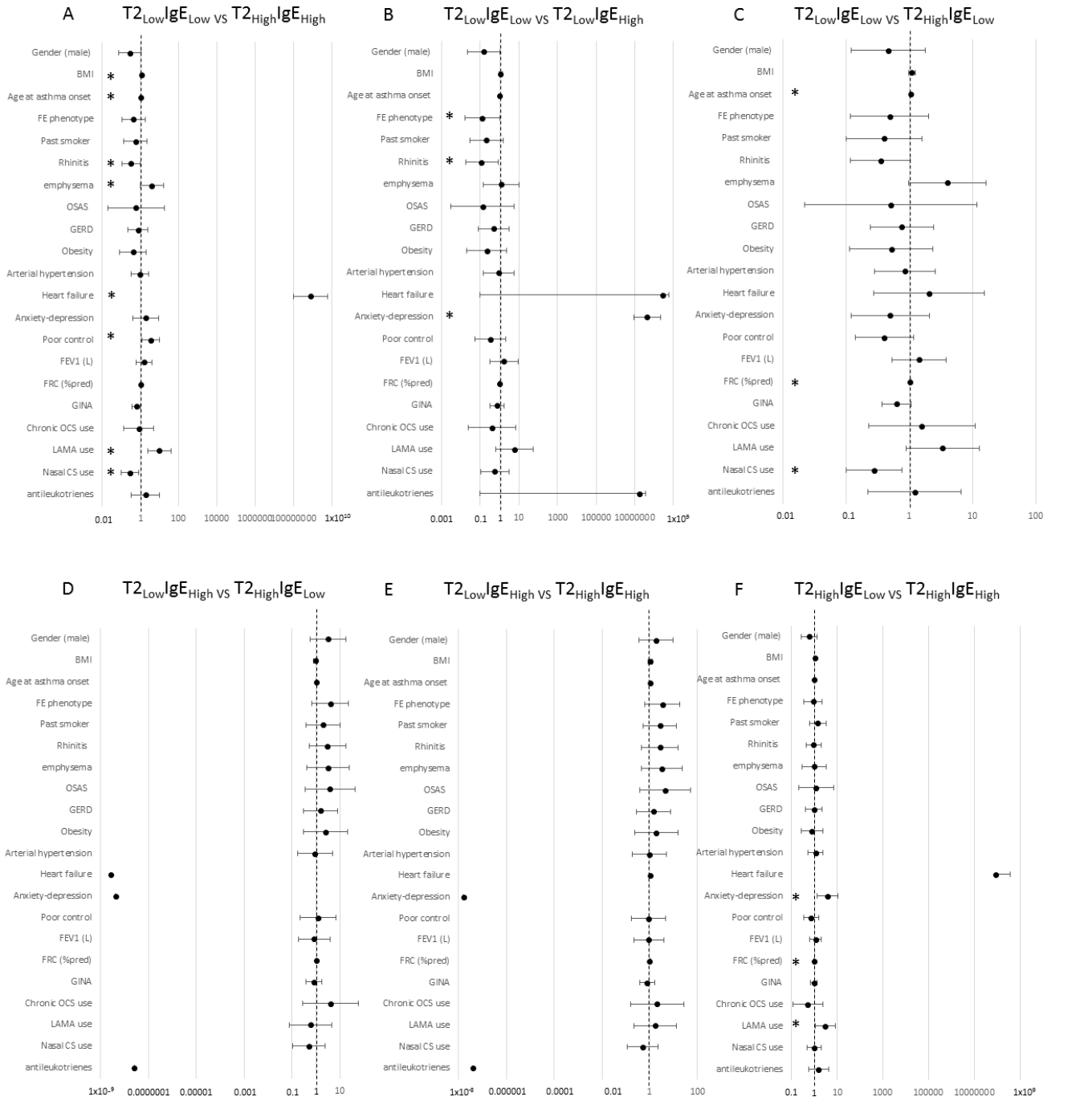

Figure 3. Forest Diagrams representing odds from Multinomial LRM for patients stratified according to Total IgE and T2 inflammatory phenotype. (**A**) $T2_{Low}$-IgE_{Low} vs. $T2_{High}$-IgE_{High}; (**B**) $T2_{Low}$-IgE_{Low} vs. $T2_{Low}$-IgE_{High}; (**C**) $T2_{Low}$-IgE_{Low} vs. $T2_{High}$-IgE_{Low}; (**D**) $T2_{Low}$-IgE_{High} vs. $T2_{High}$-IgE_{Low}; (**E**) $T2_{Low}$-IgE_{High} vs. $T2_{High}$-IgE_{High}; (**F**) $T2_{High}$-IgE_{Low} vs. $T2_{High}$-IgE_{High}. Odds for each single group are compared with each of the other three groups. X axis is expressed in a logarithmic scale. *: statistically significant.

4. Discussion

Total serum IgE levels above a predefined cut-off have been widely used as a biomarker of type-2 inflammation in asthma. This paradigm derives from the expected association between high total serum IgE and the presence of allergen-specific IgE. High total serum IgE has also been found in "intrinsic" asthma, likewise characterized by eosinophilic airway inflammation [15]. However, many studies demonstrated IgE's low predictivity of airway eosinophilia compared with B-EOS and F_ENO [18], independently from the cut-off [23]. A systematic review and meta-analysis (including 942 patients) yielded for total IgE a sensitivity and specificity in detecting sputum eosinophils (>3%) of 0.64 and 0.71 [36]. The number of studies (n = 2) and the diagnostic accuracy (AUC range 0.62–0.64) of IgE dropped when S-EOS, bronchoalveolar lavage, or endobronchial biopsies were used as the reference standard. Moreover, a prospective trial reported that total serum IgE was less accurate in detecting S-EOS in allergic and obese asthmatics than in nonallergic and normal-weight asthmatics [18]. Therefore, the question of whether total serum IgE, either high or low, impacts the type-2_{High} and type-2_{Low} asthma phenotypes is still open.

The ISAR used a predefined total serum IgE cut-off of 75 kU/L or greater as a biomarker of type-2 severe asthma [9], while a Danish cohort study used ≥150 IU/mL [23,37]. In the current study, a total serum IgE cut-off ≥100 kU/L categorized patients, when combined with the expression of type-2 biomarkers, into four subgroups: $T2_{Low}$ and IgE_{Low}; $T2_{Low}$ and IgE_{High}; $T2_{High}$ and IgE_{Low}; and $T2_{High}$ and IgE_{High}. Their distribution revealed $T2_{High}$-IgE_{High} as the most prevalent (45.7%), followed by $T2_{High}$-IgE_{Low} (33.6%). Overall, $T2_{High}$ patients accounted for 79.3% of the total population. These data were consistent with studies derived from moderate to severe unselected populations with $T2_{High}$ prevalence ranging from 79.1% to 45.3% depending on whether allergy, B-EOS, or F_ENO were chosen as selective criteria [22]. In our cohort, factors predictive of the $T2_{High}$ phenotype were younger age, male sex, lower BMI, better lung function (FVC and FEV_1) control, and a more frequent GINA Step 5 level of treatment. Moreover, rhinitis and nasal polyps were significantly more prevalent in $T2_{High}$ patients compared with $T2_{Low}$ patients. On the other hand, $T2_{Low}$ subjects were worst controlled, more frequently in GINA Step 4 level of treatment, often needed LAMA as add-on treatment, and reported higher rates of comorbidities related to past smoking habits (emphysema), metabolic syndrome (obesity, diabetes mellitus), and cardiovascular disease (arterial hypertension, acute myocardial infarction). We applied a binomial logistic regression analysis to identify variables as predictors of $T2_{High}$ versus $T2_{Low}$ status, finding the highest odds for rhinitis, use of nasal CS, and GINA Step 5, and the lowest risks for emphysema and poor ACT. These findings are in line with previous reports [33]. It should be underlined that in our cohort, the high rate of GINA Step 4-5 patients, ex-smokers, and median long duration of disease (24 years) can justify the presence of airway obstruction in many patients independently from the T2 phenotype [33]. Both the $T2_{High}$ and $T2_{Low}$ groups may develop fixed airflow obstruction due to both eosinophilic and neutrophilic-derived airway remodeling [38].

The further sub-analysis detected two distinct populations within the $T2_{High}$ and $T2_{Low}$ phenotypes according to the serum IgE cut-off of 100 kU/L. This cut-off overlaps with the median value within our population (102 kU/L) and has been previously used both for cluster analysis in asthma populations [13] and type 2 biomarkers [33]. The $T2_{Low}$-IgE_{Low} patients have no marker of type-2 inflammation. As expected, these subjects showed B-EOS and F_ENO significantly lower and sharply below the thresholds of the $T2_{High}$ groups. These patients had different clinical presentations and comorbidities than others. First, compared with the $T2_{High}$ groups, they had more frequent cardiovascular comorbidities, known to be associated with an accelerated Th1/Th17 inflammatory milieu [39,40].

When applying a logistic regression analysis, the $T2_{Low}$-IgE_{Low} group was positively identified by high BMI, low FEV_1, emphysema, and LAMA use. Obesity was an important feature of this subset, composed mainly of older females with late-onset asthma, gastroesophageal reflux, and diabetes mellitus. A late-onset, obesity, high neutrophils, low eosinophils, and low IgE cluster in females was reported in a Taiwanese Adult Asthma

Cohorts study [41]. Obesity, diabetes mellitus, and metabolic syndrome are diseases mediated by proinflammatory cytokines such as TNF-α, IL-1β, and IL-6 [42]. The SARP study showed, in a relevant proportion of ICS-naive mild-to-moderate asthmatics, a $T2_{Low}$ asthma phenotype associated with frequent bronchial hyperresponsiveness (BHR) and a lower AE risk than subjects with $T2_{High}$ disease [43]. In this context, different pathogenic processes may be involved, driven by IL-17, IL-6, and IL-23 or, alternatively, due to airway smooth muscle or neural dysfunction [5]. Moreover, different cluster analyses applied to moderate-to-severe asthma cohorts recurrently identified difficult-to-treat subsets of obese asthmatics, adult-onset, primarily affecting women, lacking T2 biomarkers, driven by oxidative stress or hormonal mechanisms. These patients have lower activity levels and frequent corticosteroid use due to symptom burden [44]. Exercise-induced airflow obstruction, ventilatory limitation, and lung mechanics alteration, unloading airway smooth muscle, and favoring fiber shortening during bronchoconstriction are involved in this phenotype [5]. We can suggest that our $T2_{Low}$-IgE_{Low} patients collected both the mild and severe $T2_{Low}$ asthmatics described in the above-mentioned cohorts.

We here describe a small group of patients characterized by low T2 biomarkers and high total serum IgE levels ($T2_{Low}$-IgE_{High}). They differed from the $T2_{Low}$-IgE_{Low} group in having a higher male and ex-smoker prevalence. Applying a logistic regression analysis model, the $T2_{Low}$-IgE_{High} subjects showed significant odds of being AE-prone and suffering from rhinitis and anxiety/depression. In addition, sex (male), past smoking habits, OSAS, and obesity were also factors associated with high but not statistically significant odds for the $T2_{Low}$-IgE_{High} group compared with the $T2_{Low}$-IgE_{Low} group.

A similar phenotype has been described by a cluster analysis of inflammatory biomarker expression in the ISAR, in which cluster 4 was characterized by very high levels of total serum IgE (1932 kU/L), the longest duration of asthma, and a high BMI [9]. The Danish study above reported that patients with severe asthma (11% of the whole population) carried high total serum IgE levels as the only elevated T2 biomarker and were characterized by early onset disease, overweight, higher median pack years, and prednisone use compared with the other severe asthmatics [23]. What is interesting is that the median F_ENO (14 ppb) and B-EOS (140 cell/μL) corresponded to those of our $T2_{Low}$-IgE_{High} group. However, both the clusters above mentioned did not exclude patients with allergic sensitization, as we did. Therefore, we can assume that just a proportion of those populations corresponded to ours. Accordingly, cluster analysis from the U-BIOPRED network, based on sputum omics, reported that about half of the cluster T2 (obese, late-onset severe asthma, smoking history, and chronic airflow obstruction) were not allergic, but a sub-analysis of this group was not available [45]. Smoking and/or exposure to environmental pollutants have been associated with increased total serum IgE levels independently of allergen exposure. Cigarette smoking enhances the production of IgE antibodies, BHR, and leukotriene B4 (a potent stimulator of neutrophil chemotaxis) by leucocytes in elderly asthmatics, and in vitro, it appears to be associated with increased levels of IL-13 [46,47]. Finally, a further mechanism potentially involved in the IgE modulation is the expression of SNPs in FCεRII associated with increased IgE levels, steroid-refractory responses, and asthma exacerbations [48].

In our study, within the $T2_{High}$ subgroups, the presence of low IgE was associated with higher obesity ($p < 0.05$), arterial hypertension ($p < 0.05$), and anxiety/depression syndrome ($p < 0.05$). This last feature, as well as the use of LAMA and a lower FRC%, could predict the entry of the IgE_{Low} group within the $T2_{High}$. A cluster analysis study applied to allergic asthmatics described a similar cluster 1, gathering a high proportion of IgE-low patients (<100 kU/L), prevalently female, with mean F_ENO levels of 44.3 ppb and mean B-EOS of 304.0/mL [13]. Within a severe asthma population, biomarker cluster analysis revealed cluster 2 of older exacerbating females with relatively low IgE [9]. Among the potential mechanisms influencing sex-related IgE levels, candidates are polymorphisms involving the IL21R promoter region, the IL4/IL13 pathway, and the CTLA-4 genes [10,49].

We can speculate that serum total IgE is a biomarker able to identify different and specific (sub)phenotypes in both T2 high and T2 low clusters.

In a recent study, we described among a population of 503 mild to severe asthmatics that about 19.5% of patients could be classified as $T2_{Low}$, triple "negative" for the expression of T2 biomarkers [33]. These patients had positive tests for high BMI, age onset, and smoking pack years. Moreover, these patients were more susceptible to cardiovascular and obesity-related comorbidities. We reported a median total serum IgE value of 44 UI/mL among these nonallergic $T2_{Low}$ patients. Here, we extend the previous observation to a wider population, describing the two subgroups of $T2_{Low}$-IgE_{Low} and $T2_{Low}$-IgE_{High}, which are different from a clinical point of view, as detailed above.

The strength of this study was the large cohort of included patients and the vast number of evaluated parameters. However, the study had some limitations. One limitation was the cross-sectional nature, such as the variability of biological measures along the natural history of a patient. Some data reported during a 5-year retrospective observation showed that 66.7% of participants had more than 50% alternations of IgE in two different measures [50]. However, fluctuations of IgE above or below the threshold of 100 kU/L we have chosen seemed to be limited and associated with a further small subgroup of patients. A longitudinal observation could reveal additional information, such as the influence of IgE levels on concomitant immune-mediated disease development. The elevated risk of cancer of any type in patients with ultra-low IgE levels is one of the most intriguing fields of research [51]. In addition, emotional and socioeconomic issues were not considered in this study. The high prevalence of severe asthma we reported is consistent with the real-life setting of a third-level asthma clinic, in which additional factors influence daily clinical practice [52].

5. Conclusions

This single-center cross-sectional study explored the role of total IgE levels among asthmatic patients classified into $T2_{High}$ and $T2_{Low}$ phenotypes according to the most routinely accepted measurable biomarkers. The role of a high level of IgE in $T2_{Low}$ patients seems to be associated with a peculiar clinical phenotype, distinct not only from $T2_{High}$ patients but also from patients with low total serum IgE and low T2 markers. Moreover, lower levels of total IgE in the context of a type-2 phenotype seem to confer a somehow different profile on the $T2_{High}$ subgroup. Therefore, measuring total IgE could be useful in asthma workup and management as it may suggest clinically relevant information orienting towards forms of asthma with overlapping pathogenetic mechanisms involving IgE and other inflammatory pathways. According to a recent review [53], this study confirmed that different phenotype overlaps could appear in the natural history of asthma: for instance, an allergic T2 high phenotype could be associated with neutrophilic inflammation (Th17, Th1, Th3) and vice versa. A $T2_{low}$ phenotype, such as in smokers or obese asthmatics, could move towards a high IgE response, generating novel phenotypes with pathognomonic clinical signs.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/jcm12175447/s1. Table S1: Descriptive statistics of clinical, bio-humoral and functional parameters among patients stratified by single biomarker cut-offs; Table S2: Binomial LRM of variables for asthmatic patients stratified according to serum Total IgE and T2 inflammatory phenotype; Table S3: Multinomial LRM of variables comparing each group of patients stratified according to serum Total IgE and T2 inflammatory phenotype.

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Conflicts of Interest: The authors declare no conflict of interest.

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