








# Utility of the Basophil Reactivity Test in the Clinical Management of People with Severe Uncontrolled Asthma

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**Introduction:** The prognosis of asthma has improved significantly since the availability of monoclonal antibodies (mAbs). However, there are no robust predictive markers of response to help clinicians select one of the multiple biologicals recommended in clinical practice guidelines. The aim of this study was to evaluate the utility of basophil reactivity, measured through the basophil activation test (BAT), as a marker of response to mAbs.

**Methods:** We measured basophil reactivity, using anti-immunoglobulin E (anti-IgE) antibodies as a stimulus, in 72 consecutive patients with severe uncontrolled asthma before initiation of treatment with mAbs. Forty-nine patients received omalizumab, 28 received mepolizumab, and 23 received benralizumab at some point. The Spanish Asthma Management Guidelines (GEMA) informed clinical management throughout the study. We studied clinical characteristics, laboratory values, and measures of respiratory function and asthma control.

**Results:** Basophil reactivity (at the highest anti-IgE dilution at which basophil activation was positive) was inversely associated with asthma control and response to any mAb. The patients with higher basophil reactivity ( $\geq 29\%$  versus  $< 29\%$ ) had lower mAb complete response, more frequent mAb switches, and worse baseline lung function and Asthma Control Test (ACT) scores. The BAT was associated with poor response above the cut-off values of 10.5% for mepolizumab, 15.5% for omalizumab, and 28% for benralizumab.

**Conclusion:** Patients with basophil reactivity greater than or equal to 29% were less likely to achieve full control of asthma when treated with omalizumab, mepolizumab, or benralizumab, independently of classic clinical or biological markers of type 2 asthma.

**Keywords:** basophil activation test, asthma, monoclonal antibodies, omalizumab, mepolizumab, benralizumab

## Introduction

The clinical management of severe asthma presents an important challenge, especially in people who cannot achieve full control with optimised inhaled therapy and all the measures recommended by clinical practice guidelines.<sup>1,2</sup> In these cases, biological drugs, or monoclonal antibodies (mAbs), have substantially improved the disease prognosis.<sup>3</sup>

Until the arrival of tezepelumab, all mAbs (omalizumab, reslizumab, mepolizumab, benralizumab, and dupilumab) were indicated for type 2 (T2)-high or eosinophilic asthma. Although induced sputum is considered the gold standard technique for characterising the underlying inflammatory substrate, its diagnostic utility is far from perfect, and it has some drawbacks that prevent its widespread use.<sup>4</sup> The biological markers used for pheno-endotyping in clinical practice are peripheral eosinophils, immunoglobulin E (IgE), and fractional exhaled nitric oxide (FeNO). With these limitations, clinicians may have difficulty classifying patients into the two main disease categories (T2 high and T2 low), or may be

unsure which mAb to select for asthma with a clearly eosinophilic phenotype.<sup>5</sup> It is highly unlikely that clinicians who follow the relevant guidelines will fail to prescribe biological treatment to a person who could benefit from it.<sup>6,7</sup> However, the selection of appropriate mAbs is a common problem, as there are no predictive biomarkers of response to biological drugs or of better response to one drug versus another from the same phenotypic group, even in the most favourable clinical scenario.<sup>8–13</sup> Moreover, complete blockade of the eosinophil pathway cannot achieve complete control of asthma in 20% to 30% of cases, even in people with a clear T2 profile.<sup>14</sup> It is logical to presume that other pathogenic mechanisms play a predominant role in these patients.<sup>15</sup>

Mast cells and basophils constitute one of the most important groups of effector cells in asthma.<sup>16</sup> They are involved in the release of various interleukins (primarily IL-4 and IL-13) and are present in significantly greater numbers in bronchial biopsies, induced sputum, and bronchoalveolar lavage in people with severe asthma.<sup>17,18</sup> Postmortem studies of fatal asthma have reported significantly elevated basophil counts in the airways.<sup>19</sup> Basophils express on their surface a variety of receptors that regulate their effector function.<sup>20</sup> The reactivity of basophils can be assessed with “Basophil stimulation assay with Anti Ig-E” (BSA-IgE) part of the basophil activation test (BAT) that is commonly used in the study of medication and food allergies, in immunotherapy effectiveness monitoring, in desensitisation procedures, and in the study of allergic processes where conventional methods cannot detect specific IgE.<sup>21,22</sup>

The current BAT technique is based on quantifying changes in activation marker expression after basophil degranulation caused by stimulation with the specific allergen.<sup>23</sup> There are two common measures of basophil activation: reactivity, or the percentage of basophils that express CD63+ or CD203c when stimulated by the allergen; and sensitivity, or the threshold of allergen concentration that provokes a positive response. Both methods are recommended by the European Academy of Asthma, Allergy and Clinical Immunology (EAACI).<sup>24</sup>

The aims of this study were: to assess basophil reactivity measured by basophil stimulation assay with Anti-IgE-mediated basophil activation in patients with severe asthma before initiation of biological treatment, and to correlate the results with patients’ clinical characteristics, asthma severity, and clinical response.

## Methods

We conducted a prospective study in the Asthma Unit of Dr Balmis General University Hospital, Alicante (Spain). We included consecutive patients aged 18 years and older with a diagnosis of uncontrolled severe asthma according to the Spanish Asthma Management Guidelines (GEMA). We recorded the comorbidities most frequently associated with asthma and optimised treatment in all patients. The choice of biological drug was based on the GINA (Global Initiative for Asthma) and GEMA recommendations in force during the study and the availability of each drug in the Spanish public health system at the time of prescription. In routine clinical practice, there is no difference between the two guidelines when used to select a biological drug. We performed the BSA-IgE before initiation of mAbs. The results did not influence the choice of mAb in any case. Patients had to use the same mAb for six months before switching to another mAb.

The Ethics Committee of Alicante Hospital, Alicante Health and Biomedical Research Institute (ISABIAL) approved the protocol (reference 2024–116). We conducted this study in accordance with the ethical principles outlined in the Declaration of Helsinki for research involving human subjects. All participants provided their informed consent to participate.

We studied clinical characteristics (age, sex, atopy, history of smoking), comorbidities (psychological disorders, sleep apnoea, obesity, chronic rhinosinusitis with nasal polyposis), laboratory values (fractional exhaled nitric oxide (FeNO), eosinophilia, IgE), and measures of respiratory function and asthma control (Asthma Control Test (ACT) score, forced expiratory volume in 1 second (FEV1), exacerbations).

We used the GEMA definitions of controlled, partially controlled, and uncontrolled severe asthma.<sup>2</sup>

The mAbs listed in our protocol were omalizumab, mepolizumab, and benralizumab. Omalizumab is used for its anti-IgE effect, mepolizumab for its action against IL-5, and benralizumab for its high affinity and specificity to the  $\alpha$  subunit of the IL-5 receptors (IL-5R $\alpha$ ), which is specifically expressed on the surface of eosinophils and basophils. Dupilumab and tezepelumab were unavailable for clinical use when we designed the study, so no patients treated with these mAbs were included.

For the BSA-IgE, we used blood samples anticoagulated with sodium heparin. All samples were analysed within four hours after collection. To estimate basophil reactivity, we measured response to activation through the high-affinity IgE receptor (FcεRI) using a rabbit anti-human IgE (RAHIgE) polyclonal antibody (Sigma-Aldrich, Darmstadt, Germany). The binding of this antibody to IgE molecules bound to FcεRI induces receptor cross-linking and cell degranulation, even at very low concentrations. To semi-quantitatively estimate the response, we used increasing dilutions of anti-IgE (1/1000, 1/2500, 1/5000, 1/10,000, 1/20,000, 1/40,000, 1/80,000, 1/160,000, and 1/320,000). We used the anti-IgE reagent of the Basotest™ kit for the assay (Celonic, Heidelberg, Germany). Aliquots of 100 µL of heparinised whole blood were preactivated at 37°C for 10 minutes using a stimulation buffer with IL-3. Next, 100 µL of the anti-IgE dilution, the positive control included in the kit (N-formyl-methionyl-leucyl-phenylalanine; fMLP), and the negative control were added to the tubes, which were incubated at 37°C for 30 minutes. After incubation, the basophils were stained with a mixture of CD123-PE, HLA-DR-PerCP-Cy5, and CD63-FITC antibodies (BD Biosciences, San Jose, CA) and incubated on ice. After lysis of the erythrocytes and three washes with phosphate-buffered saline (PBS) pH 7.4, the cells were resuspended in a volume of 400 µL and analysed in a FACSCanto II cytometer (Becton Dickinson). We employed flow cytometry to determine the extent of basophil activation. The activated basophil count was assessed by measuring expression of CD63 (a molecule that is not expressed on the surface of resting basophils) after stimulus as a marker of degranulation. The number of positive cells was calculated by comparison with the basal expression in the absence of stimulus. We recorded: the percentage of basophils that expressed CD63 in response to each RAHIgE dilution; and the percentage of basophil activation at the highest dilution (lowest concentration) that produced a positive BAT result.

The threshold we considered negative was 2.5% following the current recommendation.<sup>24</sup>

## Statistical Analysis

We compared all variables between the three clinical categories (severe controlled asthma, severe partially controlled asthma, and severe uncontrolled asthma). We used SPSS v.16 for the statistical analysis. For normally distributed continuous data, we presented means and standard deviations; for non-normally distributed continuous data, we presented medians and interquartile ranges. We presented categorical data as percentages. To compare variables, we used the Student *t* test or the Mann–Whitney *U*-test. For associations between two categorical variables, we used the Chi-square test. We evaluated the diagnostic yield of BAT by estimating the sensitivity, specificity, and Youden index for different cut-offs. For the receiver operating characteristics (ROC) analysis, we considered two categories: controlled versus uncontrolled plus partially controlled. For all comparisons, a two-sided *P* value below 0.05 was considered statistically significant.

## Results

Between August 2019 and December 2022, we took BSA-IgE measurements in 77 people, of whom we excluded five: one was lost to follow-up, one died of a concomitant neoplasm, one was pregnant, and two had profound basopenia precluding BAT assessment.

[Table 1](#) shows the general baseline characteristics (before initiation of any biological drug) of the 72 patients, according to the degree of control achieved with treatment. [Supplementary Table 1](#) is an extended version of this one. In seven patients (9.7%; three with controlled severe asthma and four with partially controlled severe asthma), the BSA-IgE was negative. [Supplementary Table 2](#) presents the characteristics of these patients.

The factors normally used to characterise the T2 phenotype, presence of comorbidities, frequency of exacerbations, and cumulative dose of prednisone in the last year, had similar mean values or frequencies at all levels of response. The only variables that differed between responders and the remaining patients was smoking history, maximum historic eosinophil count, baseline lung function, and baseline ACT score. Complete response was significantly associated with lower basophil reactivity.

[Figure 1](#) shows the diagnostic yield of basophil reactivity for all treatments, and [Figure 2](#) for omalizumab, mepolizumab, and benralizumab separately. We found the best specificity of good response with omalizumab (86.7%).

**Table 1** General Baseline Characteristics Before Starting Any Biological Drug

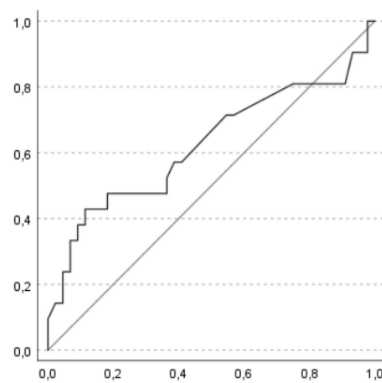
Patient Characteristics	Total n = 72	Responders n = 47 (65.3%)	Partial/non-Responders n = 25 (34.7%)	P value
Age (years), mean $\pm$ SD	57.6 $\pm$ 13.5	57.9 $\pm$ 14.6	56.9 $\pm$ 11.5	0.755
Sex (woman), n (%)	47 (65.3%)	29 (61.7%)	18 (72%)	0.382
History of smoking, n (%)	32 (44%)	16 (34%)	16 (64%)	0.015
Pack years, mean $\pm$ SD	26.6 $\pm$ 16.2	27.1 $\pm$ 20.4	26.2 $\pm$ 11.6	0.878
CRSwNP, n (%)	21 (29.2%)	14 (29.8%)	7 (28%)	0.874
Atopy, n (%)	48 (66.7%)	34 (72.0%)	14 (56%)	0.161
OSA, n (%)	34 (47.2%)	22 (46.8%)	12 (48%)	0.814
Psychological disorders, n (%)	20 (27.8%)	11 (23.4%)	9 (36%)	0.475
BMI (kg/m <sup>2</sup> ), mean $\pm$ SD	29.5 $\pm$ 5.3	28.7 $\pm$ 4.6	31.0 $\pm$ 6.2	0.086
Baseline ACT score, mean $\pm$ SD	15.8 $\pm$ 5.53	17.6 $\pm$ 5.4	12.6 $\pm$ 4.3	0.033 <sup>a</sup>
Baseline FEV1 (mL), mean $\pm$ SD	2006.9 $\pm$ 849.7	2170.0 $\pm$ 912.2	1700.4 $\pm$ 625.3	0.043 <sup>a</sup>
Baseline FEV1%, mean $\pm$ SD	70.9 $\pm$ 21.4	74.6 $\pm$ 21.9	63.9 $\pm$ 18.8	0.024 <sup>a</sup>
Baseline FVC (mL), mean $\pm$ SD	3043.37 $\pm$ 1037.68	3096.65 $\pm$ 1101.86	2943.20 $\pm$ 917.85	0.554
Baseline FVC%, mean $\pm$ SD	89.22 $\pm$ 16.81	88.95 $\pm$ 17.34	89.72 $\pm$ 16.09	0.856
Baseline FVC/FVC %, mean $\pm$ SD	66.81 $\pm$ 11.41	67.42 $\pm$ 11.65	65.64 $\pm$ 11.06	0.531
FeNO (ppb), Mdn (IQR)	18.5 (10.75–37.0)	22.0 (10.0–42.0)	15 (11–22.5)	0.139
Baseline EOS count ( $\mu$ L), Mdn (IQR)	225.0 (110.3–495.0)	240.0 (120.0–550.0)	190 (90–260)	0.115
Max. historical Eos count (Eos/ $\mu$ L), Mdn (IQR)	610.0 (445.0–1292.5)	930.0 (510.0–1470.0)	510 (315–735)	0.003 <sup>a</sup>
Eos cationic protein $\mu$ g/L, Mdn (IQR)	28.1 (13.6–42.2)	32.4 (16.5–61.4)	21.8 (10.7–36.2)	0.139
IgE (IU/mL), Mdn (IQR)	296.5 (1.35–598.75)	286.0 (132–658)	327.0 (131.0–579.0)	0.022 <sup>a</sup>
CS dependence, n (%)	10 (13.9%)	6 (12.8%)	4 (16%)	0.732
Dose of prednisone (mg/day), mean $\pm$ SD	10.0 $\pm$ 4.1	10.83 $\pm$ 4.9	8.75 $\pm$ 2.5	0.552
Cumulative CS dose (mg) in last year Mdn (IQR)	720 (320–1130)	540 (360–1130)	720 (360–1220)	0.398
Exacerbations in previous year, mean $\pm$ SD	2.5 $\pm$ 1.84	2.5 $\pm$ 1.90	2.5 $\pm$ 1.76	0.865
BAT % Mdn (IQR)	12.0 (9.0–25.5)	11.5 (8.25–20.6)	14 (9.5–38.5)	0.010 <sup>a</sup>

Note: <sup>a</sup>Significant P value < 0.05.

**Abbreviations:** ACT, Asthma Control Test; BMI, body mass index; Baso, basophil; BAT, basophil activation test; CRSwNP, chronic rhinosinusitis with nasal polyps; CS, corticosteroid; Eos, eosinophils; FeNo, fractional exhaled nitric oxide; FEV1, forced expiratory volume in one second; IgE, immunoglobulin E; IQR, interquartile range; IU, international units; Mdn, Mdn; ppb, parts per billion; OSA, obstructive sleep apnoea; SD, standard deviation.

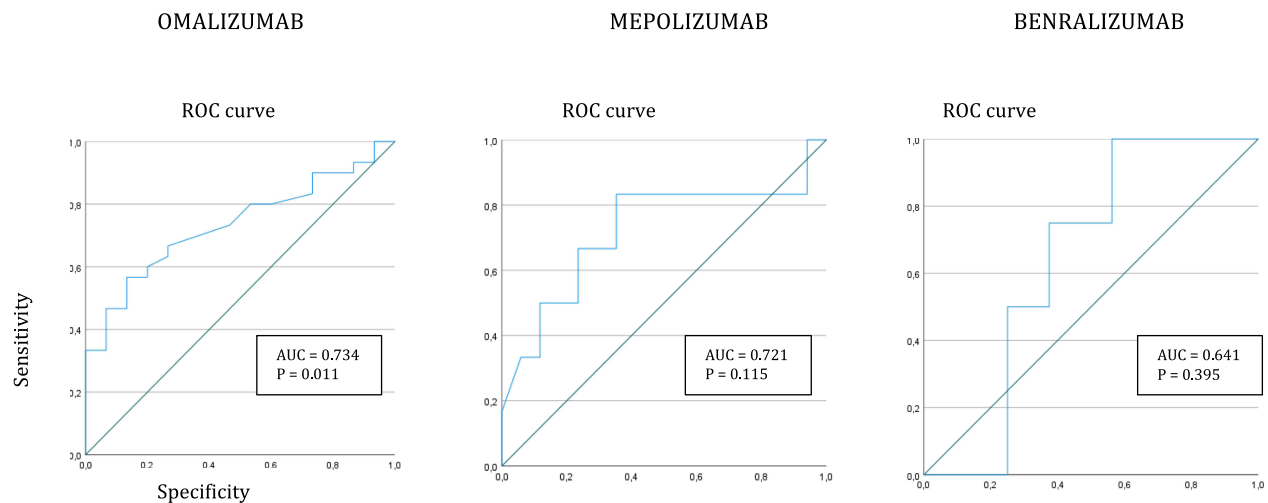
Mepolizumab and benralizumab showed similar specificity results (64.7% and 62.5%, respectively), but the activation cut-off point was much higher for benralizumab (28%, versus 10.5% for mepolizumab).

In patients with a BSA-IgE result greater than or equal to 29% (cut-off point; see Figure 1), the frequency of incomplete responses and the number of mAb switches were significantly higher, whereas the baseline ACT score and lung function were significantly worse. There were no differences in the rest of the clinical characteristics (Table 2).



AUC	0,623
p	0.135
Youden index	0.315
Cut-off point	> 29%

**Figure 1** Receiver operating characteristics (ROC) curve for all treatments, with cut-off points according to the Youden index for optimal sensitivity and specificity.  
**Abbreviation:** AUC, area under the ROC curve.



	Omalizumab	Mepolizumab	Benralizumab
Sensitivity (95% CI)	0.567 (0.3896 to 0.7443)	0.833 (0.5345 to 0.999)	0.75 (0.3256 to 0.999)
Specificity (95% CI)	0.867 (0.689 to 0.999)	0.647 (0.4051 to 0.8888)	0.625 (0.38 to 0.87)
Positive predictive value (95% CI)	0.894 (0.7555 to 0.999)	0.4167 (0.1377 to 0.6956)	0.333 (0.025 to 0.6409)
Negative predictive value (95% CI)	0.48 (0.2841 to 0.6758)	0.888 (0.6835 to 0.999)	0.9 (0.714 to 0.999)
Youden index	0.433	0.48	0.375
Cut-off point	> 15.5%	> 10.5%	> 28%

**Figure 2** Receiver operating characteristics (ROC) curves for each biological, with cut-off points according to the Youden Index for optimal sensitivity and specificity.  
**Abbreviations:** AUC, area under the ROC curve; CI, confidence interval.

## Discussion

Basophils are involved in airway inflammation and remodeling.<sup>25</sup> They correlate directly with eosinophils and inversely with sputum neutrophils in asthma patients. Compared with blood basophils, sputum basophils show higher expression of activation markers.<sup>26</sup> In one study on papain-induced asthma, basophil-derived IL-4 induced IL-5 and CCL11 expression in ILC2 cells, causing eosinophil infiltration.<sup>27</sup> A model of IgE-dependent dermatitis showed that the production of IL-4

**Table 2** Comparison of the General Baseline Characteristics of Patients Before the Start of Biological Treatment According to Basophil Activation Test (BAT) Results

Total Patients n = 65 <sup>a</sup>	%BAT ≥ 29 n = 15	%BAT < 29 n = 50	P value
Age (years), mean ± SD	59.0 ± 8.2	57.5 ± 14.9	0.648
Sex (woman), n (%)	11 (73.3%)	34 (68%)	0.461
History of smoking, n (%)	6 (40%)	32 (64%)	0.170
Pack years, mean ± SD	22.5 ± 12.5	29.33 ± 18.1	0.345
BMI, mean ± SD	29,80±6,42	28,97±5,05	0,608
CRSwNP, n (%)	3 (20%)	14 (28%)	0.380
Atopy	8 (53%)	37 (74%)	0.218
Baseline ACT score, mean ± SD	13.13 ± 5.9	16.55 ± 5.3	0.034 <sup>c</sup>
Baseline FEV1 (mL), mean ± SD	1530.0 ± 631.1	1925.0 ± 03.6	0.014 <sup>c</sup>
Baseline FEV1 (%), mean ± SD	50.0 ± 22.6	72 ± 20.8	0.014 <sup>c</sup>
Baseline FVC (mL), mean ± SD	2710.20 ± 747.20	3050.80 ± 1059.27	0.251
Baseline FVC (%), mean ± SD	85.40 ± 12.86	90.66 ± 17.43	0.284
Baseline FEV1/FVC (%) mean ± SD	64.93 ± 10.40	66.70 ± 11.37	0.584
Eos cationic protein µg/L, median (IQR)	16.9 (11.15–45.2)	32.2 (14.3–44.9)	0.413
Baseline FeNO (ppb), Mdn (IQR)	14.0 (8.75–18.5)	22.0 (11.0–40.0)	0.103
IgE (IU/mL), Mdn (IQR)	350 (54–1277)	306 (192–540)	0.603
Complete response to any biological, n (%)	6 (40%)	38 (76%)	0.021 <sup>c</sup>
Need for switch, n (%)	8 (53.3%)	10 (20%)	0.029 <sup>c</sup>
Total number of switches <sup>b</sup>	11 (73%)	11 (22%)	0.008 <sup>c</sup>

**Notes:** <sup>a</sup>We excluded the seven non-responders. <sup>b</sup>Excluding omalizumab prescriptions not indicated in current Spanish Asthma Management Guidelines (GEMA) 5.3. <sup>c</sup>Significant P value < 0.05.

**Abbreviations:** ACT, Asthma Control Test; CRSwNP, chronic rhinosinusitis with nasal polyps; CS, corticosteroid; BMI, body mass index; Eos, eosinophils; FEV1, forced expiratory volume in one second; FeNo, fractional exhaled nitric oxide; IgE, immunoglobulin E; IQR, interquartile range; IU, international units; Mdn, median; ppb, parts per billion, SD, standard deviation.

from basophils directly stimulated the endothelium to increase the expression of vascular cell adhesion molecule 1 (VCAM-1), which facilitated the in-vivo entry of eosinophils into lesion sites.<sup>28</sup> In addition, the role of basophils in the pathophysiology of chronic spontaneous urticaria (CSU) is well defined; research has even identified immunological phenotypes of varying severity based on basophil reactivity in this pathology.<sup>29</sup> The higher or lower sensitivity to FcεRI activation observed in basophils may also occur in eosinophils (which also express FcεRI). The mechanism by which IgE-mediated activation influences disease expression is unknown. It may be related to the existence of IgE against self-antigens, as occurs in other diseases such as bullous pemphigoid (IgE against BP 180).<sup>30</sup> This mechanistic observation may help elucidate eosinophil/basophil-IL-4 associations commonly seen in different diseases. These findings indicate that basophils may be involved in eosinophilic asthma, and assessment of basophils may be a useful additional indicator of T2-high asthma.

In clinical practice, mAbs have had a positive impact on asthma control and patient quality of life, and they are cost-effective when prescribed by specialists in asthma units.<sup>31</sup> However, one recent study on the prescription of mAbs for asthma in Spain found that omalizumab is the drug with the highest number of switches, especially to mepolizumab

(60%), followed by mepolizumab, which is changed to benralizumab in 14% of cases.<sup>32</sup> Although all the patients in our study were classified as having T2-high asthma according to GEMA 5.3<sup>2</sup> criteria, and although the overall complete response rate was 65.3% (somewhat higher than previously reported<sup>33</sup>), 26.3% of our patients had an incomplete response, and 8.3% achieved no response despite switching treatments. As mentioned, no patients had T2-low asthma, although it was not an exclusion criterion. Therefore, we have no information on the response in this subtype.

In an attempt to find mechanistic explanations for treatment failures, we confirmed that neither previous eosinophil levels nor any other classic T2 biomarker could have predicted a complete response to treatment with any mAb. It is particularly striking that not even chronic rhinosinusitis with nasal polyps, the strongest T2 clinical marker, was predictive of good response. On the other hand, basophil reactivity was significantly lower in patients who achieved complete control compared with the remaining patients. Basophil reactivity greater than or equal to 29% was associated with worse lung function independently of cumulative tobacco exposure. A possible explanation for this finding is airway remodelling, an early feature of severe asthma that typically limits expiratory airflow.<sup>34–36</sup> High basophil reactivity, low eosinophil counts, low baseline lung function, and low baseline ACT score were the only differential characteristics of the group of patients who did not achieve complete asthma control. There are previous studies on this topic with very limited series. One study of 26 children with allergic asthma showed that basophil sensitivity was much higher in children with uncontrolled asthma, in line with our findings.<sup>37</sup> Recently, basophil allergen sensitivity has been proposed as a method for monitoring anti-IgE treatment in different clinical situations.<sup>38</sup>

Although the ROC curves of basophil reactivity among patients using mepolizumab and benralizumab are non-significant, they are similar to those of other biomarkers used in clinical practice, including induced sputum<sup>37,39</sup> and bronchial biopsy.<sup>40</sup> While this is a pilot study, with a relatively small sample size and a design that limits definitive data interpretation, our results suggest that high basophil reactivity may be associated with poor response to mAb treatment (at least with omalizumab, mepolizumab, and benralizumab) and unsuccessful switches. The fact that benralizumab may have some effect on basophils,<sup>41,42</sup> a finding not observed with mepolizumab,<sup>43</sup> could explain the higher cut-off point for benralizumab in the BSA-IgE. The cut-off points calculated with the Youden index (used to obtain the best balance between sensitivity and specificity) have not been optimised or adjusted to improve clinical convenience. We propose that the addition of BSA-IgE could improve the current specificity of GEMA criteria by reducing the therapeutic options in many cases without reducing the probability of response (which depends on the effectiveness and therapeutic ceiling of each biological drug).

Having patients with non-reactive basophils could constitute a limitation in clinical practice, since around 10% of the healthy population has basophils that do not respond *in vitro* to specific stimuli (allergens) or non-specific stimuli (IgE antibodies). In our series, 9.7% of patients were non-reactive.

This study has some design limitations that prevent us from establishing causal associations. However, our findings do seem to shed some light on pathogenic mechanisms other than eosinophils that can be measured in usual practice.

## Conclusions

Patients with severe asthma who had high basophil reactivity at baseline before starting biological treatment had worse lung function, worse control of their disease, and worse response to treatment with omalizumab, mepolizumab, and benralizumab, independently of the classic clinical or biological markers of type 2 asthma. Future studies could explore the response of these patients with high basophil reactivity to blockade of other pathophysiological pathways, such as with dupilumab or tezepelumab. We believe that incorporating a basophil activation assay to the pheno-endotyping of people with severe uncontrolled asthma could help to inform clinical management.

## Abbreviations

ACT, Asthma Control Test; Anti-IgE, anti-immunoglobulin E; BSA-IgE, basophil stimulation assay with Anti Ig-E; BAT, basophil activation test; CSU, chronic spontaneous urticaria; EAACI, European Academy of Asthma, Allergy and Clinical Immunology; FeNO, fractional exhaled nitric oxide; FcεRI, high-affinity IgE receptor; FEV1, forced expiratory volume in 1 second; fMLP, N-formyl-methionyl-leucyl-phenylalanine; GEMA, Spanish Asthma Management Guidelines; GINA, Global Initiative for Asthma; IgE, immunoglobulin E; IL-4, interleukin 4; IL-5, interleukin 5; IL-

5R $\alpha$ , IL-5 receptor; IL-13, interleukin 13; mAbs, monoclonal antibodies; PBS, phosphate-buffered saline; RAHIgE, rabbit anti-human immunoglobulin E; ROC, receiver operating characteristics; T2, type 2; VCAM-1, vascular cell adhesion molecule 1.

## Artificial Intelligence Involvement

No part of this manuscript was produced with the help of any artificial intelligence software or tool.

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## Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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## Disclosure

The authors declare not to have any conflicts of interest that may be considered to influence directly or indirectly the content of the manuscript.

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