Stability of Asthma Phenotypes in the Spanish Cohort of the MEGA Project

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Asthma is considered a general term for various endotypes and phenotypes that share clinical manifestations (wheezing, shortness of breath, cough, and chest tightness) and are accompanied by variable airflow obstruction [1]. Identifying inflammatory endotypes has brought us closer to precision medicine, especially in severe asthma [2]. Stratification according to inflammatory endotype is deemed a central component in the asthma management algorithm [3].

The stability of the proposed phenotypes over time has not been fully clarified. Current evidence points to 4 phenotypes that are relatively stable over 1 year and are differentiated by clinical severity, response to prescribed therapy, and the underlying T2 gene expression profile [4]. However, only some of these phenotypes have been shown to be equally stable over 12 months of follow-up, with many possible factors affecting phenotype stability.

Our study aimed to analyze the stability of inflammatory biomarkers over 2 years in adults with asthma who make up the MEGA project cohort [5-7]. The methodology has been described in previous studies [5,6]. Data on peripheral blood eosinophilia (PBE), induced sputum, fractional exhaled of nitric oxide (FeNO), and pulmonary function were analyzed in 211 patients with complete data available. The inflammatory profile was diagnosed as T2-high when PBE was >150/µL or FeNO was >20 ppb and T2-low when PBE was <150/µL and FeNO was <20 ppb [8]. Eosinophilic sputum was defined as an eosinophil percentage >3%. These data were measured at baseline (V1), 12 months (V2), and 24 months (V3). Quantitative variables were reported as mean and standard deviation and qualitative variables as absolute and relative frequencies. Intergroup comparisons were performed using the χ^2 test or Fisher exact test for qualitative variables and analysis of variance or the Kruskal-Wallis test for quantitative variables. Normality was analyzed using the Kolmogorov–Smirnov test. Correlations were estimated using the Spearman test. A *P* value of <.05 was considered significant. Instat6 (GraphPad) was used for the statistical calculations and graphs.

At V1, 186 (88%) patients had a T2-high phenotype, which remained unchanged in 73.6% and 61.3% of patients at V2 and V3, respectively. In the 25 patients with a T2-low phenotype at V1, 64% and 100%, respectively, remained unchanged. A low correlation was found between the phenotype profiles at V1 and V2 (r=0.24, *P*=0.02 [agreement in 59.5% of the observations]), between V2 and V3 (r=0.49, *P*≤0.01 [agreement in 63.9% of the observations]), and between V1 and V3 (r=0.19, *P*=0.17 [nonsignificant] [agreement in 51.6% of the observations, Figure]).

Of note, sputum data were available for only 45 patients at all follow-up visits. Twenty-four (53.3%) were eosinophilic at V1, although only 16 (35.5%) remained eosinophilic at V2. Moreover, 5 patients (11.1%) with no eosinophilic sputum at V1 had become eosinophilic at V2. At V3, 6 (37.5%) of the 16 eosinophilic sputum samples from V2 remained eosinophilic, in addition to the other 3 patients who were noneosinophilic at V1 and became eosinophilic at V3 (Figure). A moderate correlation was observed between the presence of >3% eosinophilia in sputum between V1 and V2 (r=0.48, *P*=.005); this was lower between V1 and V3 (r= 0.08, *P*=.79 [nonsignificant]). There was no significant difference in inflammatory biomarkers, lung function, or asthma control between patients whose sputum became eosinophilic or noneosinophilic or remained the same as at V1 (all *P*<.05) (Supplementary Table I).

There was no correlation between T2 profile and eosinophilic sputum at any visit (r=0.05 for V1, r=–0.08 for V2, and r=0.04 for V3 [all *P*>.05, nonsignificant]).

No statistically significant differences were found in peripheral blood eosinophil values during the first 12 months of follow-up. However, there were significant differences

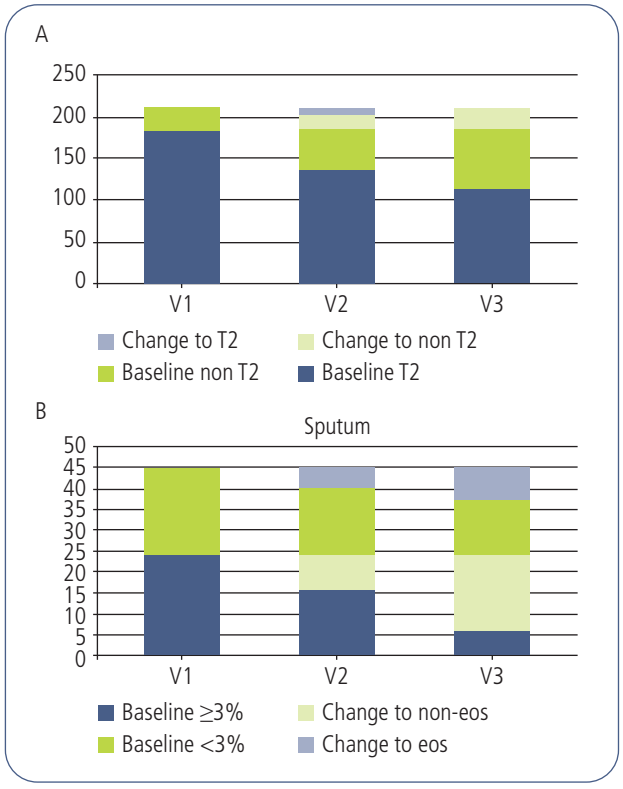

Figure. Changes in phenotype over 2 years. A, T2 profile. B, Sputum profile. eos indicates eosinophilic.

between V1 and V3 (P<.0001). No significant differences in FeNO levels or total IgE levels were found between the follow-up visits. To determine whether the number of patients with fixed obstruction varied between the follow-up visits, we compared the data from V1 with those recorded at V2 and V3 and found no statistically significant differences (Supplementary Table I).

The low correlation between the phenotype profiles could be explained by the loss of data during follow-up. However, this bias is minimized, since the analyses were performed for paired data. Another possible explanation is the instability inherent to asthma, given that, by definition, it is a variable disease. However, this is not a novel finding, as reported elsewhere for pediatric asthma with significant phenotypic variability [9]. A surprising finding from the analysis of this cohort was the absence of a good correlation between sputum eosinophilia and the classification of patients as T2-high [7], even considering that the cut-off point for eosinophilia was set at 150/µL. Other studies demonstrated that the absence of sputum eosinophilia was a consistent finding 4 weeks and 5 months after the first visit [10]. Another interesting finding of our study was that peripheral blood eosinophil samples remained stable during the first 12 months of follow-up, although significant differences were found at 24 months (Supplementary Table 1), suggesting that more than 12 months of follow-up is necessary to objectify the instability of the inflammatory phenotypes. Therefore, studies with only 12 months of follow-up [3,10] may underestimate this finding. Pulmonary function remained stable, with little change observed in patients affected by fixed obstruction over a 2-year follow-up period (Supplementary Table I). These findings can be explained by the observation that bronchial remodeling is poorly reversible with current asthma treatment.

In summary, we present our results after a 2-year follow-up of patients with asthma. The instability of inflammatory phenotypes in induced sputum and peripheral blood eosinophil data was observed at 2 years, although greater stability was observed during the first year of follow-up.

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Conflicts of Interest

MJR declares lecture fees from AstraZeneca, GSK, Chiesi, Leti, Cipla, and Allergy Therapeutics. DB declares a Rio Hortega research grant and lecture fees from AstraZeneca, GSK, Chiesi, Allergopharma, and Leti. JMO declares lecture fees from Gebro Pharma. IB declares lecture fees from AstraZeneca, GSK, Chiesi, Sanofi, and Novartis. VdelP declares grants from ISCIII, CIBERES, and AstraZeneca and lecture fees from AstraZeneca and GSK. JDO declares lecture fees from Sanofi, GSK, AstraZeneca, Chiesi, Leti Pharma, and ALK. FJGB declares lecture fees from ALK, AstraZeneca, Bial, Chiesi, Gebro Pharma, GlaxoSmithKline, Menarini, Novartis, Rovi, Roxall, Sanofi, Stallergenes-Greer, and Teva. CMR declares lecture fees from AstraZeneca, GSK, Sanofi, Chiesi, and Gebro. JM declares grants from AstraZeneca, GSK, Viatris (Mylan-Meda Pharmaceuticals), Regeneron Pharmaceuticals, Inc, Sanofi-Genzyme, and NOUCOR/Uriach Group and lecture fees from AstraZeneca and GSK, Menarini, Mitsubishi-Tanabe Pharma, MSD, Viatris (Mylan-Meda Pharmaceuticals), Novartis, Proctor & Gamble, Regeneron Pharmaceuticals, Inc Sanofi-Genzyme, UCB Pharma, NOUCOR/Uriach Group. XM declares grants from AstraZeneca, GSK, Sanofi, and Novartis. CP declares grants from CIBERES and AstraZeneca and lecture fees from AstraZeneca. VP declares lecture fees from AstraZeneca, Chiesi, GSK, and Medwell. SQ declares lecture fees from AstraZeneca, GSK, Chiesi, Allergy Therapeutics, Sanofi, Novartis, and Gebro. LS declares lecture fees from Sanofi, GSK, AstraZeneca, Stallergenes-Greer, Hal Allergy, and Immunotek. The remaining authors declare that they have no conflicts of interest.

References

1. https://ginasthma.org/gina-reports/ [Accessed March 20, 2022].
2. Agache I, Beltran J, Akdis C, Akdis M, Canelo-Aybar C, Canonica GW, 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(5):1023-42.

3. Kuruvilla ME, Lee FE, Lee GB. Understanding Asthma Phenotypes, Endotypes, and Mechanisms of Disease. Clin Rev Allergy Immunol. 2019;56(2):219-33.
4. Loza MJ, Djukanovic R, Chung KF, Horowitz D, Ma K, Branigan P, et al. Validated and longitudinally stable asthma phenotypes based on cluster analysis of the ADEPT study. Respir Res. 2016;17(1):165.
5. Rial MJ, Álvarez-Puebla MJ, Arismendi E, Caballero ML, Cañas JA, Cruz MJ, et al. Clinical and inflammatory characteristics of patients with asthma in the Spanish MEGA project cohort. Clin Transl Allergy. 2021;11(1):e12001
6. Muñoz X, Álvarez-Puebla MJ, Arismendi E, Arochena L, Ausín MDP, Barranco P, et al. The MEGA Project: A Study of the Mechanisms Involved in the Genesis and Disease Course of Asthma. Asthma Cohort Creation and Long-Term Follow-Up. Arch Bronconeumol (Engl Ed). 2018:S0300-2896(18)30009-7.
7. Betancor D, Olaguibel JM, Rodrigo-Muñoz JM, Arismendi E, Barranco P, Barroso B, et al. How reliably can algorithms identify eosinophilic asthma phenotypes using non-invasive biomarkers? Clin Transl Allergy. 2022;12(8):e12182.
8. McDowell PJ, Busby J, Hanratty CE, Djukanovic R, Woodcock A, Walker S, et al. Exacerbation Profile and Risk Factors in a Type-2-Low Enriched Severe Asthma Cohort: A Clinical Trial to Assess Asthma Exacerbation Phenotypes. Am J Respir Crit Care Med. 2022;206(5):545-53.
9. Fleming L, Tsartsali L, Wilson N, Regamey N, Bush A. Sputum phenotypes are not stable in Children with asthma. Thorax. 2012;67:675-81.
10. Simpson JL, McElduff P, Gibson PG. Assessment and reproducibility of non-eosinophilic asthma using induced sputum. Respiration. 2010;79:147-51.

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