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Real-World Finetuning of Biomarkers in Severe Asthma: Longitudinal Latent Class Analysis From the Italian Registry on Severe Asthma (IRSA)

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To the Editor,

Blood eosinophils, fractional exhaled nitric oxide (FeNO), and total IgE (tIgE) are the biomarkers used in clinical practice to guide the prescription of biologics in severe asthma (SA). However, the choice of the appropriate biologic is hampered by their overlapping therapeutic targets, and more efficient methods are needed to phenotype patients. Cluster analyses are useful tools [1, 2], but the standard clustering methods tend to add complexity, instead of simplifying phenotyping, due to the high number of generated clusters with less robust prediction of clinical features or treatment responses [3]. The aim of this study is to identify real-world clusters of patients with SA, based on baseline levels of biomarkers, and to understand their predictive role in response to biologics.

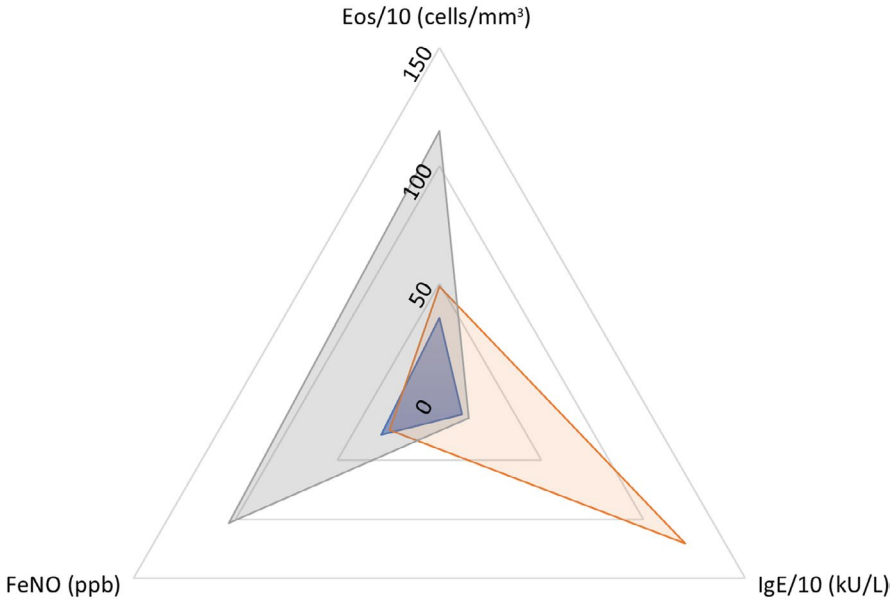
All the biologic-naïve patients with a 12-month follow-up were selected, from the Italian Registry on Severe Asthma (IRSA) [4], for the latent class analysis (LCA), an alternative patient-centered, unsupervised, machine learning method that was conducted using baseline eosinophils, FeNO, and tIgE as predictors. IRSA was approved by the Ethics Committee of the Coordinating Center (Milan), and all patients provided written informed consent.

Three classes were identified by LCA in the 320 patients included in this study (Figure 1). They showed an important imbalance in the number of patients and in some baseline characteristics (Table 1). Class 1 included the majority of SA patients and presented the lowest biomarker values, including non/low-T2 SA (approximately 13% of the IRSA population [5]). Class 2, the smallest group, showed a distinctive hyper-IgE phenotype, associated with the highest frequency of active smokers and males, no clinically significant IgE-related conditions, and the best baseline status (i.e., lung function, asthma control, exacerbations), with the lowest use of oral corticosteroids. Class 3 was the hypereosinophilic subgroup, with the highest frequency of rhinosinusitis with nasal polyps, and the worst baseline status. It also was the only Class with significantly abnormal FeNO.

After baseline, the start of biologics was less frequent in Class 1. Anti-IL5(R)s were the most common biologics in all classes. They were initiated more frequently in Class 3, and omalizumab and dupilumab were more frequently started in Class 2. Overall, at 1-year follow-up (Table 1), all patients improved, mostly the ones on biologics. However, Class 3 achieved the best improvements concerning asthma control and exacerbations, while a slight better improvement was observed in lung

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	Class 1 (*N* = 226, 70.6%)	Class 2 (*N* = 27, 8.4%)	Class 3 (*N* = 67, 20.9%)
Eos/mm3	353.5 ± *229.5*	488.0 ± *377.0*	1,147.4 ± *612.9*
IgE kU/L	111.6 ± *2.8*	1,204.6 ± *1.8*	143.7 ± *3.0*
FeNO ppb	28.5 ± *16.9*	24.4 ± *16.8*	103.3 ± *72.9*

FIGURE 1 | Latent class analysis: classes in severe asthma according to baseline biomarkers (*N* = 320). Data are arithmetic (Eos and FeNO) or geometric (IgE) means ± standard deviation. Eos and IgE are divided by 10 in the figure for graphical scaling purposes. Eos, peripheral eosinophils; FeNO, fractional exhaled nitric oxide; IgE, total immunoglobulin E.

function for Class 2. After adjustment for confounders, the magnitude of the improvement was lower, especially for Class 1, but the differences among classes were confirmed and magnified (Table S1). Interestingly, the multiple regression analysis showed that polyps seem to be an important effect modifier. Therefore, it is likely that the reduction in exacerbations is significantly mediated by the improvement in polyps, as suggested by the polyps subanalysis (Table S2) when confounding factors are ruled out. Regarding class-specific efficacy of biologics, Class 2 showed the highest benefit in patients on biologics, versus patients without biologics, for lung function, asthma control, and frequency of exacerbators (Table S3). A comparison with results obtained by other cluster analyses, from previous studies, is reported in Table S4.

The strength of this study is the LCA technique, applied for the first time on a registry-based population with SA for biomarker-based phenotyping. The patient-centered nature of LCA, compared to other standard clustering methods, is considered a more statistically robust method to uncover latent subgroups [6]. The limits of the study were the lack of adjustment for possible factors that can affect biomarkers over time, and the follow-up limited to 1 year, partially mitigated by the multiple regression analysis, the broad geographical distribution of the centers, and the long-term consecutive enrolment (sensitivity analyses in Tables S5–S7). In addition, the single-nation design and the limited number of patients in some of the resulting classes hampered a robust comparison of the classes. Therefore, further studies in different cohorts are needed.

Our findings unveiled three biomarker-based classes, with distinct phenotypes, that are easily detectable in clinical practice. The results suggest that phenotyping through a population-driven usage of biomarkers might be more helpful in clinical practice, than using standard clinical trial-driven T2-high/low classifications. The LCA showed that additional factors can fine-tune the biological selection, in real life, beyond the traditional T2 cut-off alone. In particular, the hyper-IgE trait (Class 2) within the T2 inflammation was associated with a better baseline status, as well as greater efficacy of the biologics used in this Class; additionally, polyps can be a key treatable trait for achieving maximum reduction of exacerbations (Class 3).

TABLE 1 | Characteristics of the latent class analysis classes at baseline and after 1 year of follow-up ($N = 320$).

	Class 1 ($N = 226$)	Class 2 ($N = 27$)	Class 3 ($N = 67$)	p value*
Baseline characteristics				
Females	143 (63.3%)	8 (29.6%)	41 (61.2%)	**0.003**
Age	56.7 (13.8)	51.3 (15.9)	55.3 (12.8)	0.140
Smoking status				**0.008**
Never	167 (73.9%)	15 (55.6%)	54 (80.6%)	
Former	46 (20.4%)	7 (25.9%)	13 (19.4%)	
Active	13 (5.8%)	5 (18.5%)	0 (0.0%)	
Pack-year	13.9 (12.1)	13.4 (14.5)	9.0 (9.4)	0.412
Age at symptom's onset	30.1 (18.1)	28.9 (16.7)	32.1 (16.0)	0.658
Age at asthma diagnosis	30 [17,44]	30 [21,42]	35 [21,47]	0.344
Allergic asthma	136 (60.2%)	19 (70.4%)	33 (49.3%)	0.196
Biological and functional characteristics				
Peripheral eosinophils/mm3				**< 0.001**
< 150	54 (23.9%)	5 (18.5%)	3 (4.5%)	
150–300	55 (24.3%)	5 (18.5%)	0 (0.0%)	
> 300	114 (50.4%)	17 (63.0%)	64 (95.5%)	
FEV1 (L)	1.9 (0.8)	2.5 (1.1)	1.9 (0.6)	**0.007**
FEV1 (%)	69.1 (20.8)	74.0 (24.1)	64.9 (18.3)	0.207
FVC (L)	2.8 (1.1)	3.6 (1.4)	2.9 (0.9)	**0.036**
FVC (%)	84.4 (20.5)	87.2 (26.1)	82.0 (17.8)	0.603
FEV1/FVC (%)	68.7 (15.0)	70.6 (22.8)	67.6 (14.2)	0.762
ACT score	15.4 (4.5)	16.7 (4.6)	14.4 (4.4)	0.067
ACT < 20	178 (78.8%)	19 (70.4%)	55 (82.1%)	0.261
Exacerbations (last 12 months)				
Incidence rate	0.91 (0.28)	0.85 (0.36)	1.00 (0.00)	**0.020**
Patients with ≥ 1	203 (89.8%)	23 (85.2%)	64 (95.5%)	**0.021**
Access to Emergency Dep	65 (28.8%)	6 (22.2%)	21 (31.3%)	0.597
Hospitalization	45 (19.9%)	2 (7.4%)	11 (16.4%)	0.281
Comorbidities				
Sinusitis	111 (49.1%)	9 (33.3%)	37 (55.2%)	0.099
Nasal polyps	90 (39.8%)	10 (37.0%)	41 (61.2%)	**0.003**
Osteoporosis	48 (21.2%)	3 (11.1%)	5 (7.5%)	**0.015**
Gastroesophageal reflux	33 (14.6%)	2 (7.4%)	7 (10.4%)	0.576
Body mass index ≥ 30	48 (21.2%)	3 (11.1%)	4 (6.0%)	**0.014**
ASA hypersensitivity	40 (17.7%)	4 (14.8%)	7 (10.4%)	0.363
Bronchiectasis	15 (6.6%)	1 (3.7%)	6 (9.4%)	0.359
Cataract	20 (8.8%)	2 (7.4%)	5 (7.5%)	0.911
Diabetes	18 (8.0%)	1 (3.7%)	1 (1.5%)	0.144

(Continues)

	Class 1 ($N=226$)	Class 2 ($N=27$)	Class 3 ($N=67$)	p value*
Atopic dermatitis	8 (3.5%)	2 (7.4%)	2 (3.0%)	0.882
Treatments				
High dose inhaled corticosteroid	149 (65.9%)	18 (66.7%)	52 (77.6%)	0.191
Long-acting muscarinic antagonist	96 (42.5%)	13 (48.1%)	31 (46.3%)	0.746
Oral corticosteroids (OCS)	104 (46.0%)	5 (18.5%)	28 (41.8%)	**0.023**
OCS duration (months)	6.6 (9.9)	5.6 (10.3)	11.1 (23.1)	0.285
OCS daily dose	56.9 (77.9)	45.0 (32.0)	70.1 (134.8)	0.743
Start of biologics	100 (44.2%)	19 (70.4%)	42 (62.7%)	**0.003**
Omalizumab	20 (20.0%)	5 (26.3%)	3 (7.1%)	0.091
Anti-IL5 (R)	69 (69.0%)	9 (47.4%)	37 (88.1%)	**0.001**
Dupilumab	11 (11.0%)	5 (26.3%)	2 (4.8%)	0.133
Improvements after 1 year of follow-up				
ACT score	+4.7 (5.6)	+5.7 (5.3)	+7.7 (5.1)	
ACT ≥ 20	+103 (51.8%)	+14 (60.9%)	+40 (74.1%)	**0.012**
FEV1 (L)	+0.2 (0.6)	+0.5 (1.0)	+0.3 (0.7)	**0.044**
FEV1%pred	+6.0 (20.7)	+11.7 (21.2)	+12.0 (21.9)	0.146
FEV1/FVC	+2.2 (14.2)	+8.3 (21.5)	+2.9 (16.5)	0.247
Exacerbations (last 12 months)				
Incidence rate	−3.5 (4.4)	−3.1 (2.8)	−4.3 (4.3)	0.294
Patients with ≥ 1	−134 (60.4%)	−18 (66.7%)	−50 (78.1%)	**0.032**
Access to Emergency Dep	−53 (23.9%)	−4 (14.8%)	−20 (31.2%)	0.225

Note: Data are mean (standard deviation), median [interquartile ranges], or frequency (%).
Abbreviations: ACT, asthma control test; ASA, acetylsalicylic acid; FEV, forced expiratory volume; FVC, forced vital capacity.
*ANOVA test or chi-squared test used for continuous or categorical variables, respectively, after log-transformation of nonnormal distributions. Statistically significant results are in bold.

Author Contributions

M.B.B., M.M., and L.A. designed the study. M.M. performed the statistical analysis. M.B.B., M.M., L.A., and A.M. wrote the manuscript. All the authors interpreted data, collected the clinical, functional, and laboratory data, and revised the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.