Revealing the gap: fractional exhaled nitric oxide and clinical responsiveness to biological therapy in severe asthma – a retrospective study

To the Editor:

Patients with severe asthma often require treatment with a biological drug directed at pivotal immune regulators, including interleukin (IL)-4, IL-5, IL-13, immunoglobulin E (IgE) and, more recently, thymic stromal lymphopoietin [1]. In this regard, biomarkers of type 2-high inflammation, such as exhaled nitric oxide fraction (F_{ENO}), have been progressively and successfully utilised for the endotyping of severe asthma patients [2] in order to improve their therapeutical management. However, there has been relatively little focus on monitoring the dynamics of these biomarkers after treatment initiation and on understanding the correlation between drug-induced changes and the observed clinical response [3]. In the current retrospective study, we evaluated a cohort of patients with severe asthma undergoing treatment with different biologics and investigated the association between the documented clinical response and changes in F_{ENO} levels after 6 months of therapy.

Patients diagnosed with severe asthma were evaluated for inclusion. The inclusion criteria comprised: age $\geqslant$18 years, clinical diagnosis of severe asthma [4], satisfactory spirometry and F_{ENO} results at baseline and at follow-up, treatment with any biological drug for severe asthma. Exclusions were applied to patients with contraindications to biological drug therapy, those unable to perform acceptable and repeatable spirometry tests, those lost to follow-up, those with significant missing data in their records, and current or former smokers (defined as abstinent from smoking for $\geqslant$6 months) with a smoking history $\geqslant$10 pack-years.

After the protocol approval by the Institutional Review Board Campania 2 (number AOC-0010488-2024), we screened patients for inclusion and collected relevant demographical and clinical data from our records, as well as blood eosinophil count (BEC), F_{ENO}, lung function parameters and patient-reported outcomes (Asthma Control Test (ACT) and Asthma Control Questionnaire (ACQ)5). F_{ENO} had been assessed with an electrochemical device (Vivatmo Pro; Bosch, Germany) following the latest available recommendations [5, 6], while lung function parameters had been measured with an automated equipment (Vmax Encore; Vyasis Healthcare, Italy), in line with the most recent guidelines [5, 7]. The study procedures were performed both at baseline, before starting the biological drug treatment and after 6 months of therapy. Following the 2022 consensus paper on minimal clinically important differences for asthma endpoints [8], a F_{ENO} reduction of $\geqslant$20% was considered as clinically significant. Statistical analysis was performed with the SPSS package version 29.0 (IBM, USA).

Of 192 asthmatic patients in total from our database, 97 were eligible and were included in the final analysis. The included subjects had a median annual exacerbation rate of 2.0 (interquartile range (IQR) 1.0–3.0) and mostly presented with an eosinophilic phenotype, demonstrated by a median BEC of 449.5 cells per mm^3 (IQR 305.2–663.8 cells per mm^3). 27 (27.8%) patients reported a smoking history, with a mean±SD exposure score of 5.0±1.2 pack-years. Collectively, F_{ENO} was elevated at baseline (median 31.0 ppb, IQR 23.0–60.0 ppb). No patient was taking oral steroids (OCS) at enrolment and asthma control was poor (median ACT score 16.5 (IQR 11.0–20.0) and ACQ5 of 4.0 (IQR 3.1–4.3)). In order to assess the presence of selection bias, we compared the included subjects to those excluded, and observed no statistically significant difference in demographics, asthma control or lung function (data not shown).
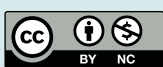
Shareable abstract (@ERSpublications)
A proportion of patients with severe asthma treated with biological drugs undergoes a significant decline in F_{ENO}. However, variations in F_{ENO} are largely independent of the clinical efficacy of the biological drug therapy. https://bit.ly/3xWszYJ

Based on a F_{ENO} reduction of $\geqslant 20\%$, we then identified 50 F_{ENO} decliners and 47 nondecliners. The main results are summarised in table 1. At baseline, a significant difference was found in the values of F_{ENO}, which were, of course, higher among decliners compared to nondecliners (34.5 ppb, (IQR 27.8–69.5 ppb) *versus* 25.0 ppb (IQR 18.0–46.0 ppb), p=0.004). Conversely, decliners had lower baseline ACQ5 scores (p=0.005). After treatment, variations (Δ) of comparable magnitudes were observed in the two groups for all the main outcomes (always nonsignificant). The only exception that met statistical significance was forced vital capacity (FVC), both expressed as absolute values (median ΔFVC 0.06 L (IQR −0.11–0.24 L) among nondecliners *versus* 0.20 L (IQR 0.07–0.44 L) among decliners, p=0.017) and as percentage of the predicted value (median ΔFVC 2.0% (IQR −3.0–6.0%) predicted among nondecliners *versus* 5.0% (1.0–14.0%) predicted among decliners, p=0.008). Among F_{ENO} decliners, ΔF_{ENO} was associated by linear correlations both with baseline ACT (r= −0.346, p=0.019) and baseline ACQ5 (r=0.530, p=0.005), as well as with baseline F_{ENO} values (r= −0.921, p<0.001); such data were further confirmed by using Spearman's nonparametric coefficients. After adjusting for age, sex, smoking history and presence of nasal polyps, ACQ5 was found to be the most important predictor of ΔF_{ENO} (r^2=0.407, β=0.679; p=0.004), with higher baseline values predicting lower decreases in F_{ENO}.

In our study of severe asthma patients, we have demonstrated that variations in F_{ENO} following biological therapy are mostly independent from clinical outcomes and the specific drug utilised. No difference was observed between F_{ENO} decliners and nondecliners in terms of age, sex, annual exacerbation rate, smoking history, lung function, blood eosinophil count and ACT score at baseline, and no significant difference was found at follow-up in lung function and asthma control. However, we observed a striking difference between the two groups in F_{ENO} at baseline, which were, of course, higher among decliners, thus suggesting a higher degree of bronchial inflammation among such patients; we also observed a strong relationship between baseline F_{ENO} and the magnitude of ΔF_{ENO}, as the higher the inflammation at baseline, the wider the change. While F_{ENO} changes do not mean clinical improvement *per se*, it is interesting to notice that F_{ENO} decliners presented with a numerically higher forced expiratory volume in 1 s (FEV_1) improvement (220 *versus* 140 mL) and a significantly higher FVC improvement (200 *versus* 70 mL), which suggests a more effective improvement of lung function in those patients with a more marked reduction of bronchial inflammation.

Our results are partially in line with those reported by MENIGOZ *et al.* [9] in a retrospective real-world study investigating the efficacy of anti-IL-5/anti-IL-5 receptor (IL-5R) treatment in patients with severe eosinophilic asthma. F_{ENO} changes were not associated with therapeutic response, as measured by ACT and FEV_1. Another real-life study on 99 patients treated with mepolizumab concluded that baseline F_{ENO} was not different in patients defined as clinical "non-responders", "responders" or "super-responders" [10].

Finally, in the present study we report that a 6-month course of biologic treatment with anti-IgE, anti-IL-5/IL-5R or anti-IL-4/IL-13 caused a significant decrease in F_{ENO} in a variable number of patients with uncontrolled severe eosinophilic asthma as compared to baseline, regardless of the type of biologic considered. This observation is in line with previous studies on the effects of biologics on F_{ENO} [11, 12], although other studies failed to show significant variations of F_{ENO} during omalizumab treatment [13]. Interestingly enough, we observed a lack of concordance between the trajectories of F_{ENO} decline and changes in BEC, which tended towards reduction in both groups, thus suggesting either that different inflammatory pathways or treatment dynamics might be involved.

In our study, the stronger predictor of F_{ENO} decline was the baseline ACQ5 score, with higher values being associated with smaller changes in F_{ENO}, thus suggesting a lower reduction of bronchial inflammation among more severe patients after treatment with biologics.

To date, researchers and clinicians have focused mainly on the role of biomarkers in predicting the response to biological treatment. However, much less attention has been paid to the dynamics of biomarkers during biologic treatment and to the relationship with the clinical response induced by such treatment. This is a novel finding presented by our study.

However, some important limitations should be addressed, such as: the study's retrospective design; the presence of unbalanced subgroups; a median exacerbation rate of 2.0 (IQR 1.0–3.0), which is slightly less than in most trials involving severe asthma patients; and an overall baseline population that did not use OCS on a regular basis. Finally, we could not infer any effect on acute exacerbations because although no exacerbation was reported during the study, exacerbation rates can only be calculated after a whole year of observation.

Despite such limitations, however, we can assert that biologic drugs effectively improve lung function and quality of life even when they do not directly affect F_{ENO}. Prospective trials are therefore necessary in

TABLE 1 Major clinical and functional parameters at baseline and after 6 months of therapy with biologic drugs in patients with severe asthma stratified and compared by exhaled nitric oxide (F_{ENO}) response

Variable	F_{ENO} nondecliners			F_{ENO} decliners			Nondecliners versus decliners, p-value	
	t_0	t_6	p-value	t_0	t_6	p-value	t_0	t_6
Patients	47			50				
Demographics								
Females	31 (66.0)			26 (52.0)			0.163	
Age, years	55.1±14.2			55.9±11.5			0.769	
Clinical history								
Smoking history	12 (25.5)			15 (30.0)			0.588	
Exacerbations	2.0 (1.0–3.0)			2.0 (1.0–3.0)			0.634	
Markers of T2-high inflammation								
Eosinophil count, cells per mm^3	401.3 (300.0–630.8)	40.2 (0–118.6)	**<0.001**	477.0 (300.4–674.0)	57.4 (10.0–210.0)	**<0.001**	0.642	0.186
Eosinophil count, %	5.8 (3.7–8.9)	0.7 (0–1.4)	**<0.001**	5.6 (4.0–8.9)	0.8 (0–3.1)	**<0.001**	0.921	0.244
Δ Eosinophils, cells per mm^3		−388.6 (−571.1– −243.4)			−329.0 (−565.7– −2.5)			0.221
F_{ENO}, ppb	25.0 (18.0–46.0)	24.0 (20.0–75.0)	**0.045**	34.5 (27.7–69.5)	22.0 (16.7–36.0)	**<0.001**	**0.004**	**0.004**
ΔF_{ENO}, ppb		2.0 (−4.0–14.0)			−14.0 (−30.5− −8.0)			**<0.001**
High F_{ENO}	23 (48.9)			41 (82.0)			**<0.001**	
Patient-reported outcomes								
ACT score	16.4±5.5	21.9±3.8	**<0.001**	15.4±5.7	20.1±4.3	**<0.001**	0.389	**0.035**
ΔACT		5.4±4.4			4.7±5.1			0.494
ACQ5 score	4.3±0.5	2.9±0.7	**<0.001**	3.3±1.3	2.34±1.2	**<0.001**	**0.005**	0.198
ΔACQ5		−1.4±0.6			−1.0±1.2			0.372
Lung function								
FEV$_1$, L	2.13±0.99	2.27±1.13	0.056	2.04±0.75	2.35±0.75	**<0.001**	0.633	0.662
ΔFEV$_1$, L		0.14 (−0.06–0.35)			0.22 (0.12–0.51)			0.054
FEV$_1$, % predicted	74.3±21.4	80.0±22.0	**0.004**	72.72±20.92	83.4±19.1	**<0.001**	0.715	0.423
ΔFEV$_1$, % predicted		5.7±13.0			10.7±12.2			0.057
FVC, L	3.10±1.23	3.17±1.34	0.492	3.11±1.05	3.38±1.02	**<0.001**	0.972	0.398
ΔFVC, L		0.06 (−0.11–0.24)			0.20 (0.07–0.44)			**0.017**
FVC, % predicted	89.6±21.1	91.5±19.2	0.301	88.5±18.3	95.9±16.9	**<0.001**	0.794	0.232
ΔFVC, % predicted		2.0 (−3.0–6.0)			5.0 (1.0–14.0)			**0.008**
FEV$_1$/FVC	66.2±12.0	69.0±11.7	**0.030**	66.1±12.1	69.3±12.4	**0.006**	0.945	0.881
Use of biologic drugs								
Benralizumab	19 (40.5)			21 (42.0)			0.961	
Dupilumab	5 (10.6)			10 (20.0)			0.321	
Mepolizumab	18 (38.3)			9 (18.0)			**0.045**	
Omalizumab	5 (10.6)			10 (20.0)			0.321	
Comorbidities								
CRSwNP	10 (21.3)			14 (28.0)			0.595	
CRSsNP	6 (12.8)			9 (18.0)			0.666	

Data are presented as n, n (%), mean±SD or median (interquartile range), unless otherwise stated. Patients experiencing a decrease in F_{ENO} of ≥20% are classified as F_{ENO} decliners. t_0: baseline; t_6: 6-month follow-up; T2: type 2; Δ: change at 6-month follow-up; ACT: Asthma Control Test; ACQ: Asthma Control Questionnaire; FEV$_1$: forced expiratory volume in 1 s; FVC: forced vital capacity; CRSwNP: chronic rhinosinusitis with nasal polyps; CRSsNP: chronic rhinosinusitis without nasal polyps. Bold indicates p<0.05.

order to identify biomarkers that accurately predict therapeutic response and early markers of response to biotherapy (monitoring biomarkers).

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