Fraction of exhaled nitric oxide is associated with disease burden in the German Asthma Net severe asthma cohort

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To the Editor:

The fraction of exhaled nitric oxide (F_{ENO}) is a biomarker for type 2 asthma, reflecting the degree of local pulmonary inflammation linked to immune pathways, including interleukin (IL)-13 [1]. In clinical practice, F_{ENO} is a reliable marker for inhaled corticosteroid (ICS) responsiveness [2] and the efficacy of biological therapies, such as those targeting IL-4/IL-13 pathways [3, 4], as well as the detection of steroid nonadherence or resistance in severe asthma [2]. The prospective Severe Asthma Registry of the German Asthma Net (GAN) enrols patients with severe asthma for in-depth assessment of phenotypes, underlying mechanisms and therapeutic strategies; GAN has been approved by respective ethics committees, with all included patients having signed informed consent [5]. Prior studies of F_{ENO} either included patients with asthma of any severity [6] or did not involve a comprehensive analysis in a large cohort [7]. We therefore used cross-sectional data from GAN to determine the correlation of F_{ENO} with epidemiological, laboratory, clinical, lung function, or quality of life parameters and the need for oral corticosteroid (OCS) maintenance therapy in a carefully selected severe asthma cohort to better characterise the severe asthma subtype with high F_{ENO} values.

At the time of data acquisition (October 2019), GAN included 1689 patients with severe asthma, as defined by the European Respiratory Society/American Thoracic Society [1], from multiple tertiary referral centres, mainly in Germany, but also in Slovenia, Austria and Croatia [5]. F_{ENO} was measured using any available device, according to the manufacturer's instructions [8]. Patients were included in the analysis if a F_{ENO} measurement was available and excluded only if essential data were missing. Consistent with German and international guidelines [1, 9], F_{ENO} values ⩾25 ppb were considered elevated; exacerbations were defined as events requiring OCS for ⩾3 days, doubling of established OCS dose, or hospitalisation; and thresholds for lung function parameters and exacerbation frequency were established. Controlled asthma was defined by Asthma Control Questionnaire-5 (ACQ-5) score <1.5, or Asthma Control Test (ACT) score ⩾20, with better asthma quality of life defined by mini Asthma Quality of Life Questionnaire (mAQLQ) score ⩾5.4 [1, 9]. Hypoxaemia was defined as partial pressure of oxygen in the blood (P_{O_2}) <72 mmHg, and obesity as body mass index (BMI) ⩾30 $kg \cdot m^{-2}$. Total IgE cut-off was aligned with the German criteria for anti-IgE therapy of 75 $U \cdot mL^{-1}$ [9]. Information bias was addressed by requiring an online form to be completed on assessment of the patient. The study was approved by the ethics committee of the Medical University of Vienna (EK 1849/2019), as well as by further local committees as per local requirements. Since the registry was initiated as a longitudinal project, data acquisition was not selective or biased towards any hypotheses. The significance level for hypothesis testing was set to 0.05. Due to the exploratory character of the study no adjustment for multiple testing was performed and p-values should be interpreted in a descriptive manner. Analyses were performed in R 4.0.3 program (R Core Team 2021), SPSS version 26 (IBM, Armonk, New York, USA), GraphPad Prism 8.3 (GraphPad, San Diego, USA), and Excel 2013 (Microsoft, Redmond, USA), using two-sample unequal variance t-tests, for F_{ENO}, as well as for patient characteristics as dichotomous variables. A sensitivity analysis was performed, and the predictive value of F_{ENO} on exacerbation rate was determined by calculating the positive predictive value. The influence of patient parameters on F_{ENO} was analysed with regression analysis. The target variable F_{ENO} was transformed through 10's logarithm to adapt to the deviation of the residuals' distribution. For continuous patient parameters, univariate linear regressions and for dichotomous variables, t-tests were performed. A multiple covariance analysis was performed for all patient parameters

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In a severe asthma cohort of 1007 patients, high F_{ENO} was associated with chronic rhinosinusitis/polyps, later asthma onset, poor lung function and asthma control, low quality of life, frequent exacerbations and the need for maintenance OCS. #GANregistry https://bit.ly/3sNrtIQ

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TABLE 1 Correlation between fraction of exhaled nitric oxide (F_{ENO}) values and patient parameters and demographics

Parameters associated with F_{ENO} ⩾25 ppb

Parameter	N	F_{ENO} ⩾25 ppb	F_{ENO} <25 ppb	F_{ENO} ⩾25 ppb *versus* <25 ppb[#] p-value	F_{ENO} ⩾25 ppb *versus* <25 ppb[#] 95% CI
Age, years	1005	53±15	45±17	<0.001	−10.02, −5.86
P_{O_2}, mmHg	443	74±9	77±12	0.002	1.22, 5.28
FEV_1, % predicted	981	66±21	70±23	0.033	0.26, 6.15
FEV_1, L	983	2.0±0.7	2.1±0.9	0.006	0.04, 0.26
FEV_1/FVC, %	950	64±14	68±16	<0.001	1.57, 5.59
Exacerbations per year	1007	3.5±4.5	2.9±3.4	0.019	−1.09, −0.10

F_{ENO} levels in categories of patient demographics and characteristics

Parameter	N	Category	F_{ENO}, ppb	Comparison of F_{ENO} values between categories[#] p-value	Comparison of F_{ENO} values between categories[#] 95% CI
BMI	1002	<30 kg·m^{-2}	52±49	0.001	4.12, 15.67
		⩾30 kg·m^{-2}	42±37		
CRSwNP	1007	CRSwNP	54±49	<0.001	−16.75, −5.52
		No CRSwNP	43±42		
Age at asthma onset	804	⩾12 years	54±49	<0.001	−24.16, −12.15
		<12 years	36±33		
P_{O_2}	443	⩾72 mmHg	40±36	0.001	−25.27, −6.28
		<72 mmHg	56±60		
FEV_1/FVC	950	<70%	53±49	0.001	−16.52, −4.92
		⩾70%	44±42		
FVC/IVC ratio	51	<0.93	55±53	0.041	−51.46, −1.16
		⩾0.93	29±27		
ACQ-5 score	781	⩾1.5	51±51	<0.001	−19.60, −7.07
		<1.5	38±35		
ACT score	927	<20	51±49	0.01	−12.89, −1.75
		⩾20	43±35		
mAQLQ score	746	<5.4	50±51	0.006	−16.89, −2.89
		⩾5.4	40±36		
Exacerbations per year	1007	⩾2	52±49	0.008	−13.28, −1.99
		<2	45±42		
Total IgE	427	⩾75 U·mL^{-1}	53±50	0.048	−17.78, −0.08
		<75 U·mL^{-1}	44±40		
Maintenance OCS	1007	Yes	56±54	0.001	−16.84, −4.51
		No	45±40		

Linear regression analysis, t-test[¶]

Parameter	Estimate	t-value	p-value
BMI, kg·m^{-2}	−0.01	−2.94	0.003
Age, years	0.01	6.95	<0.001
P_{O_2}, mmHg	−0.02	−3.81	<0.001
FEV_1, % predicted	0.00	−2.05	0.040
FEV_1, L	−0.10	−2.73	0.006
FEV_1/FVC, %	−0.01	−4.03	<0.001
ACQ-5 score	0.06	3.07	0.002
Exacerbations per year	0.02	3.54	<0.001
Blood eosinophils per µL	0.00	5.91	<0.001
BMI ⩾30 kg·m^{-2}[¶]		2.7	0.008
CRSwNP[¶]		−4.5	<0.001
Age at asthma onset ⩾12 years[¶]		−5.7	<0.001
Asthma control, defined by ACQ-5[¶]		−2.8	0.005
Maintenance OCS[¶]		−3.4	<0.001

Continued

TABLE 1 Continued

Multiple linear regression analysis			
Parameter	**Estimate**	**t-value**	**p-value**
Age, years	0.004	5.323	<0.001
BMI, $kg \cdot m^{-2}$	−0.007	−3.292	0.001
CRSwNP	0.087	3.577	<0.001
FEV_1, % predicted	0.001	1.302	0.193
FEV_1/FVC, %	−0.003	−2.247	0.025
Exacerbations per year	0.009	2.968	0.003
Maintenance OCS	0.049	1.930	0.054

#: p-values and confidence intervals are for the mean difference between groups or categories from the t-test; other data are mean±SD. ¶: dichotomous independent parameters. P_{O_2}: partial pressure of oxygen in blood; FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; BMI: body mass index; CRSwNP: chronic rhinosinusitis with nasal polyposis; IVC: inspiratory vital capacity; ACQ-5: Asthma Control Questionnaire-5; ACT: Asthma Control Test; mAQLQ: mini Asthma Quality of Life Questionnaire; OCS: oral corticosteroids. In the univariate regression analyses and t-tests with the target variable log(10) F_{ENO}, for continuous independent patient variables, regression estimate, t-statistic and p-value are reported, for dichotomous independent variables, t-test t-statistic and p-value are provided. In the multiple linear regression analysis with the target variable log(10) F_{ENO}, 64 patients were excluded due to missing data.

with a p-value <0.05 and at least 90% non-missing values; forced expiratory volume in 1 s (FEV_1) in L was excluded because of multicollinearity.

Of the 1007 patients in GAN with available F_{ENO} data, 64% had high F_{ENO} measurements (*i.e.* ⩾25 ppb), 58% were female, and 72% had uncontrolled asthma. The mean age was 50.3 years, BMI 27 $kg \cdot m^{-2}$, FEV_1 2.04 L (67% predicted), and median F_{ENO} (interquartile range) 34 (18–66) ppb.

Compared to patients with low F_{ENO}, those with F_{ENO} ⩾25 ppb had a significantly higher rate of asthma exacerbations, had significantly lower P_{O_2}, FEV_1 (both absolute and % predicted) and FEV_1 to forced vital capacity (FVC) ratio, and were significantly older (table 1). F_{ENO} ⩾25 ppb had a sensitivity of 65% to predict the occurrence of ⩾2 exacerbations per year, with a positive predictive value of 61%, and an area under the curve of 0.53 (95% CI 0.50–0.56). Furthermore, when patients were divided into categories, significantly higher F_{ENO} levels were associated with: BMI <30 $kg \cdot m^{-2}$, the presence of chronic rhinosinusitis with nasal polyposis (CRSwNP), age at asthma onset ⩾12 years, P_{O_2} <72 mmHg, lower lung function values (FEV_1/FVC <70% or FVC/inspiratory vital capacity (IVC) <0.93 (the lower limit of normal [10])), poor asthma control (ACQ-5 ⩾1.5 or ACT <20), worse asthma quality of life (mAQLQ <5.4), frequent exacerbations (⩾2 per year), IgE ⩾75 $U \cdot mL^{-1}$, and maintenance OCS use (table 1). These results were corroborated by linear regression analysis (table 1), and included in a multiple regression analysis. Here, age, CRSwNP, BMI, as well as FEV_1/FVC, and exacerbations per year were independently significantly associated with F_{ENO} levels (table 1). Maintenance OCS therapy showed a borderline significance.

This real-life registry of a representative, carefully characterised, large, severe asthma cohort demonstrated the correlation of F_{ENO} with several epidemiological factors, lung function, asthma control and asthma quality of life. This broadens our insight into severe asthma and strengthens the role of F_{ENO} in identifying patients who are at risk of frequent exacerbations.

Our data support the findings that patients with severe asthma with high F_{ENO} values and CRSwNP may be the ideal candidates for anti-IL-4/IL-13R therapy (dupilumab) therapy, which has been approved in Germany for treatment of severe asthma with type 2 inflammation, as well as CRSwNP that is inadequately controlled by nasal corticosteroids and surgery [3, 9]. Importantly, obesity, considered a hallmark of a non-type 2 phenotype in other cohorts [11], was associated with lower F_{ENO} values. In addition to altered airway mechanics [12], obesity is known to interfere with nitric oxide generation by inducible nitric oxide synthase through a lower ratio of L-arginine to asymmetric dimethylarginine, which could lead to reduced F_{ENO} but increased oxidative stress [13].

Regarding lung function parameters, our association of high F_{ENO} with hypoxaemia has not been described previously. We also observed high F_{ENO} to be associated with reduced FVC/IVC, marking compressive air trapping through reduced lung elastic recoil and increased peripheral airflow resistance [10].

Chronic local inflammation, as indicated by high F_{ENO}, could lead to airway remodelling over time, linking these two phenomena. These results warrant further evaluation.

Some results corroborate those of existing studies [14, 15], including in smaller [7], or less selected asthma cohorts [6], such as the association with age, asthma control, quality of life, exacerbations, and maintenance OCS use. Whilst this cohort was skewed towards type 2 inflammation, cohorts such as the NOVELTY study included a larger portion of non-type 2 asthma patients, and showed similar age, sex and BMI values, but lower eosinophil count and F_{ENO} values [16]. The main strengths of our study in this regard were the careful selection of patients with severe asthma, and the large cohort size. Indeed, discrepant results *versus* previous analyses were mainly due to smaller sample sizes in those studies (suggesting that the findings of our study are more likely to be correct), such as our observations of significant associations of F_{ENO} with FEV_1 % predicted and maintenance OCS use, in contrast to Mansur *et al.* [7], with our findings corroborated by others [6, 14], and the associations that we observed between F_{ENO} and age of asthma onset, compared to Dweik *et al.* [6], who recruited a younger population.

In conclusion, this study involved a comprehensive evaluation of the biomarker, F_{ENO}, in a large, well-characterised cohort of patients with severe asthma. In severe asthma, F_{ENO} seems to be a sensitive marker for patients at increased exacerbation risk, with a good positive predictive value. Translating these results into clinical practice, we suggest that F_{ENO} can act as a marker of disease burden, and could be a useful parameter in the identification and management of patients with increased risk of complications associated with severe asthma, and those who may require intensified therapy.

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