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Longitudinal changes in sputum and blood inflammatory mediators during FeNO suppression testing

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► Additional supplemental material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/thoraxjnl-2021-217994).

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Received 21 July 2021
Accepted 28 May 2022
Published Online First 8 July 2022

To cite: Couillard S, Shrimanker R, Lemaire-Paquette S, *et al*. *Thorax* 2022;**77**:933–938.

ABSTRACT

To explore whether fractional exhaled nitric oxide (FeNO) non-suppression identifies corticosteroid resistance, we analysed inflammatory mediator changes during a FeNO suppression test with monitored high-intensity corticosteroid therapy. In linear mixed-effects models analysed over time, the 15 clinically distinct 'suppressors' (ie, ≥42% FeNO suppression) normalised Asthma Control Questionnaire scores (mean±SD, start to end of test: 2.8±1.4 to 1.4±0.9, p<0.0001) and sputum eosinophil counts (median (IQR), start to end of test: 29% (6%–41%) to 1% (1%–5%), p=0.0003) while significantly decreasing sputum prostaglandin D_2 (254 (89–894) to 93 (49–209) pg/mL, p=0.004) and numerically decreasing other type-2 cytokine, chemokine and alarmin levels. In comparison, the 19 non-suppressors had persistent sputum eosinophilia (10% (1%–67%) despite high-intensity therapy) with raised end-test inflammatory mediator levels (1.9 (0.9–2.8)-fold greater than suppressors). FeNO non-suppression during monitored treatment implies biological corticosteroid resistance.

INTRODUCTION

Severe asthma represents 1 in 20 asthma cases but comprises half of asthma-related expenditure.[1] The biomarkers fractional exhaled nitric oxide (FeNO) and blood eosinophils are used in the clinic to identify higher risk type 2 inflammatory phenotype which responds favourably to anti-inflammatory therapy.[2 3]

The observation that FeNO predicts inhaled corticosteroid (ICS)-responsiveness has led to the development of the FeNO suppression test to identify non-adherence in difficult-to-treat, FeNO-high asthma.[4 5] One-third of patients have a persistently raised FeNO and disease burden despite objectively measured adherence to high-dose ICS.[4 6–8] This group of 'FeNO non-suppressors' have been presumed 'corticosteroid resistant',[9] but the longitudinal investigation of inflammatory changes over the course of a FeNO suppression test has not been reported. To explore the hypothesis that FeNO non-suppression identifies biological corticosteroid resistance, we analysed induced sputum and blood inflammatory mediator changes during a FeNO suppression test in patients who did and did not suppress FeNO.

METHODS

We performed an observational longitudinal analysis of FeNO suppression tests conducted in our specialist asthma clinic (Oxford, UK).

Patients ≥18 years old with asthma receiving high dosage ICS plus ≥1 other controller were recruited after multidisciplinary evaluation when they had persistently high FeNO (>40 ppb twice)[10] with no confounding pulmonary disease. Participants consented and underwent testing between January 2015 and February 2020; sputum induction and recruitment stopped in March 2020 due to the pandemic.

FeNO suppression tests were conducted according to an adaptation of an early protocol (see Figure E2, online supplement).[4] Briefly, patients with asthma underwent 7–35 days of additional inhaled and/or systemic corticosteroids (+1000 µg inhaled fluticasone propionate per day and, if FeNO did not suppress on day 7 according to the equation below, +80 mg intramuscular (IM) triamcinolone with follow-up 28 days later). Treatment adherence was monitored via a chipped inhaler (INCA) and/or nurse-administered triamcinolone injection. In addition to detailed clinical assessment, Asthma Control Questionnaire (ACQ)-5, spirometry, FeNO measurement (FeNO NIOX VERO), phlebotomy and sputum induction by hypertonic saline nebulisation in clinic on days 0, 7 and/or 35, patients performed daily FeNO measurements at home for days 1–6. Some FeNO suppression tests stopped after 7 days due to patient availability, physician decision or transition to research bronchoscopy protocols.

A positive FeNO suppression test was defined as a $Log_{10}\Delta FeNO \geq 0.24$, where $Log_{10}\Delta FeNO$ is calculated as: (mean (log_{10}FeNO day 0, log_{10}FeNO day 1)) − (log_{10}FeNO day 35 or, if unavailable, mean (log_{10}FeNO day 6, Log_{10}FeNO day 7)). Conversely, patients with a negative FeNO suppression test (ie, <42% fall in FeNO) were categorised as 'non-suppressors'.[4] Medical notes and forms completed on day 0, 7 and 35 were reviewed to assess whether evidence of pre-existing nonadherence issues had been documented. Triggers for categorising patients as 'previously non-adherent' were any of: (1) adequate chipped inhaler data showing <70% acceptable doses taken during the first 7 days of the test, (2) 'non-adherent' noted during clinical review

Table 1 Baseline subject characteristics

Parameter	FeNO suppressed n=15	Not suppressed n=19	P value
Age, years	42±13	57±16	0.006
Male	5 (33)	10 (53)	ns
BMI, kg/m²	26±4	28±5	ns
Comorbidities			
Atopy*	12 (80)	12 (63)	ns
Nasal polyps	7 (47)	7 (37)	ns
Gastro-oesophageal reflux	2 (13)	3 (16)	ns
Cardiovascular disease	2 (13)	1 (5)	ns
Smoking status: never-smoker	12 (80)	11 (58)	ns
Ex-smoker	2 (13)	7 (37)	
Current smoker	1 (7)	1 (5)	
ACQ-5 score at baseline	2.8±1.4	2.5±1.5	ns
Asthma attacks in past year†	1 [0–3]	4 [0–5]	ns
ICS, BDP-CFC eq., µg/day	1561±502	1921±344	0.02
On maintenance OCS	3 (20)	9 (47)	ns
FEV_1, % predicted	89±19	78±17	ns
FEV_1/FVC ratio, % observed	75±17	67±11	ns
FeNO ppb	119 [75-190]	94 [60-136]	ns
Blood eosinophils, cells×10^9/L	0.54 [0.50–0.83]	0.46 [0.36–0.59]	0.03
Total IgE levels, kU/L	545 [35–1551]	229 [77-359]	ns
Sputum eosinophils, %	29 [7-41]	13 [3-39]	ns
Sputum neutrophils, %	46 [19-61]	68 [32-77]	ns
Inadequate adherence identified	8 (53)	2 (11)	0.007
Test duration			
7 days	5 (33)	11 (58)	ns
35 days	10 (67)	8 (42)	
Test optimisation method:			
+FP 1000 µg inhaled-only	12 (80)	13 (68)	ns
+FP then Triamcinolone 80 mg IM	3 (20)	6 (32)	
No of samples (days 0, 7, 35)			
Sputum differential cell count	21 {7, 10, 4}	17 {8, 7, 2}	
Sputum supernatant	25 {9, 9, 7}	31 {13, 12, 6}	
Serum	30 {11, 10, 9}	41 {17, 16, 8}	

Data are presented as no (%), mean±SD, median (IQR), or total no of samples (days 0, 7, 35).
P values reported are unpaired t-tests for parametric variables, Mann-Whitney U tests for nonparametric variables, Fisher's exact test or χ^2 for categorical variables.
*Atopy defined as patient-reported allergic rhinitis, eczema, allergen-worsening of asthma or food allergy.
†Asthma attacks are defined as acute asthma episodes requiring 3 days or more of systemic corticosteroids.
ACQ-5, Asthma Control Questionnaire-5 Item; BDP-CFC eq., beclomethasone dipropionate with CFC propellant equivalent; BMI, body mass index; FeNO, fractional exhaled nitric oxide; FEV1, forced expiratory volume in 1 s (postbronchodilator); FP, fluticasone propionate; FVC, forced vital capacity; ICS, inhaled corticosteroid; IM, intramuscular; ns, not significant; OCS, oral corticosteroids.

by specialist nurse or (3) nursing note stating significant inhaler technique difficulties persisting throughout the test.

Longitudinal (days 0, 7 and 35 whenever available) samples were analysed for 28 clinical, biomarker, sputum and serum inflammatory mediators. Inflammatory proteins were measured in duplicates using multiplex electrochemiluminescent assays (Meso Scale Discovery, USA) or single ELISA (Cayman Chemical, USA).

Demographics for FeNO suppressors versus non-suppressors were compared by unpaired t-tests for parametric variables, Mann-Whitney tests for nonparametric variables, and Fisher's exact test or χ^2 for categorical variables. To test our hypothesis that FeNO non-suppressors exhibit biological resistance, longitudinal analyses were performed for the 28 outcome repeated measures (days 0, 7 and 35 whenever data were available; plus home-FeNO measurement on days 1–6) in linear mixed-effects models with a random intercept on same patients for (1) FeNO non-suppressors alone and FeNO suppressors alone, respectively, assessing significance of change over timepoints in each subgroup; and (2) FeNO suppressors versus FeNO non-suppressors, assessing significance of the group × time interaction (ie, whether change over time was different according to group status). Significant findings in the longitudinal groupwise analyses were further explored in pooled linear mixed effects models assessing the relationship between selected continuous outcomes (ie, the dependent variable; log-transformed when required) and FeNO (independent variable; log-transformed). Modelling assumptions were all verified visually with appropriate diagnostic plots. The primary set of linear mixed-effect models' p values (84 models) were controlled for a false discovery rate <0.05 using the Benjamini-Hochberg procedure[11]; other statistics used a two-sided $\alpha=0.05$. Linear mixed-effects models were computed in RStudio 2021.09.01 build 372 (RStudio, USA) with R V.4.1.2 (R Foundation), and other statistics were performed in SPSS V.28 (IBM) and GraphPad Prism V.9.3.1 (GraphPad, USA).

RESULTS

Eighty-seven patients were referred for FeNO suppression testing between January 2015 and February 2020; 34 completed tests were included (see online supplemental appendix 1). There were two protocol deviations when FeNO non-suppressors were not administered IM triamcinolone on day 7 due to incorrect application of the FeNO suppression equation stated in the study methods (eg, using only 1 day to determine if suppressed, rather than the mean of several days).

Nineteen patients did not suppress FeNO: these were significantly older, on higher background ICS dosage, had lower baseline blood eosinophil count and had little or no adherence/inhaler technique issues noted (table 1). Specimen availability was low, especially for sputum differential cell counts, but there was no difference in the number of sputum inductions achieved between groups and no trend for better/worst sampling success according to study day.

The clinical, biomarker and sputum/serum inflammatory longitudinal responses during the FeNO suppression tests are shown in table 2, and linear mixed-effect models' outputs are detailed in online supplemental appendix 2. In FeNO suppressors alone, ACQ-5 scores improved significantly during the test (days 0, 7, 35; mean±SD: 2.8±1.4, 1.6±0.9, 1.3±1.0, p<0.0001 over time), as did sputum eosinophils (median (IQR): 29 (6–41), 3 (1–11), 2 (1–5) %, p=0.0003) and sputum PGD_2 (254 (89–894), 174 (37–341), 93 (53–196) pg/mL, p=0.004). In FeNO non-suppressors alone, only the longitudinal change in sputum IL-4 (1.0 (0.3–1.1), 1.0 (0.5–1.2), 0.1 (0.1–0.3) pg/mL, p=0.004) was retained after correcting for multiplicity of testing. When comparing FeNO suppressors and non-suppressors, only the longitudinal change in FeNO was significantly different after correcting for multiplicity of testing (↓3.4 (2.3–4.2) vs ↓1.5 (1.1–1.7)-fold, p<0.0001 for group × time interaction).

The results of the above subgroup longitudinal analyses were further explored for ACQ-5, sputum eosinophils, sputum PGD_2 and sputum IL-4. The continuous relationship

Table 2 Before-and-after clinical and inflammatory changes according to FeNO suppression test result

Analyte (pg/mL or stated) LLOD*		FeNO suppressed			FeNO not suppressed			
		Before	After	P for time (n analysed)	Before	After	*P* for time (n analysed)	P for group ×time
Clinical	**ACQ-5 score**	2.8±1.4	1.4±0.9	**<0.0001** (n=15)	2.5±1.5	1.9±1.3	ns (n=19)	ns
	FEV_1 (L)	2.79±0.86	3.05±0.96	0.009 (n=15)	2.39±0.92	2.56±0.89	0.04 (n=19)	ns
	FEV_1 (% pred)	89±19	98±19	0.02 (n=15)	78±17	83±18	ns (n=19)	ns
	FEV_1/FVC (%)	75±17	78±12	ns (n=15)	67±11	70±10	ns (n=19)	ns
Biomarker	**FeNO (ppb)**	119 [75–190]	35 [20–55]	**<0.0001** (n=15)	94 [60–136]	56 [43–123]	**<0.0001** (n=19)	**<0.0001**
	Blood Eos (×109/L)	0.54 [0.50–0.83]	0.42 [0.10–0.60]	0.02 (n=15)	0.46 [0.26–0.58]	0.24 [0.19–0.36]	ns (n=19)	ns
Induced sputum mediators	**Eosinophils (%)**	29.3 [6.5–41.3]	1.3 [1.0–5.3]	**0.0003** (n=11)	13.0 [2.9–38.8]	10.0 [1.1–67.0]	ns (n=10)	ns
	Neutrophils (%)	46.3 [9.8–61.3]	16.0 [4.7–74.7]	ns (n=11)	67.8 [32.0–77.3]	40.3 [8.5–70.3]	ns (n=10)	ns
	IL-4 0.2	0.4 [0.1–1.0]	0.1 [0.1–0.6]	ns (n=11)	1.0 [0.3–1.1]	0.5 [0.1–1.0]	**0.002** (n=13)	ns
	IL-5 0.5	3.7 [1.2–20.9]	1.4 [0.6–6.0]	0.045 (n=11)	7.8 [1.9–14.5]	3.9 [2.0–7.4]	ns (n=13)	ns
	IL-13 4.2	6.9 [5.7–15.8]	8.8 [6.0–15.5]	ns (n=11)	7.7 [5.4–10.5]	7.7 [6.3–10.8]	ns (n=13)	ns
	IL-33 0.6	1.4 [0.3–1.4]	0.3 [0.3–0.7]	0.02 (n=11)	1.6 [1.4–2.0]	1.4 [0.4–1.7]	0.02 (n=13)	ns
	TSLP 0.9	3.6 [1.3–13.9]	3.0 [1.1–7.9]	0.008 (n=11)	7.0 [5.0–13.4]	6.9 [4.1–10.3]	ns (n=13)	ns
	Eotaxin-3 4.2	63 [24–410]	58 [14–257]	ns (n=9)	361 [20–677]	169 [48–329]	ns (n=11)	ns
	TARC 0.4	10 [7–79]	16 [5–42]	ns (n=9)	36 [8–208]	31 [17–48]	ns (n=11)	ns
	LTE_4 7.8	305 [74–830]	106 [46–218]	0.01 (n=11)	226 [54–905]	80 [47–677]	ns (n=13)	ns
	PGD_2 19.5	254 [89–894]	93 [49–209]	**0.004** (n=11)	279 [151–366]	176 [119–320]	0.04 (n=13)	0.01
	IFN-γ 0.3	0.6 [0.2–1.7]	0.2 [0.2–0.3]	ns (n=9)	0.2 [0.2–0.4]	0.4 [0.2–1.1]	ns (n=11)	ns
	TNF 0.4	1.8 [0.2–9.8]	0.5 [0.2–2.4]	ns (n=9)	1.7 [0.9–4.0]	1.7 [0.2–7.3]	ns (n=11)	ns
Serum mediators	**IL-4** 0.1	0.1 [0.1–0.1]	0.1 [0.1–0.1]	ns (n=14)	0.1 [0.1–0.1]	0.1 [0.1–0.1]	ns (n=19)	ns
	IL-5 0.4	1.4 [0.6–3.4]	0.8 [0.5–1.2]	ns (n=14)	1.5 [0.4–2.4]	0.6 [0.5–1.6]	ns (n=19)	ns
	IL-13 6.7	9.5 [3.3–12.0]	3.3 [3.3–8.5]	ns (n=14)	3.3 [3.3–12.8]	6.1 [3.3–10.5]	ns (n=19)	ns
	IL-33 0.4	0.8 [0.2–0.8]	0.2 [0.2–0.8]	ns (n=14)	0.6 [0.2–0.8]	0.4 [0.2–0.8]	ns (n=19)	ns
	TSLP 0.5	1.8 [0.8–3.1]	1.8 [1.1–2.5]	ns (n=14)	2.7 [1.8–3.6]	2.4 [1.6–3.8]	ns (n=19)	ns
	Eotaxin-3 4.2	14 [6–30]	15 [10–30]	ns (n=14)	19 [10–35]	17 [9–32]	0.03 (n=19)	ns
	TARC 0.2	281 [167–561]	318 [160–560]	0.01 (n=14)	247 [144–395]	248 [92–406]	ns (n=19)	ns
	IFN-γ 0.3	0.6 [0.2–1.1]	0.4 [0.3–0.7]	ns (n=14)	0.3 [0.2–1.0]	0.3 [0.2–0.8]	ns (n=19)	ns
	TNF 0.4	0.8 [0.2–1.3]	1.0 [0.5–1.9]	ns (n=14)	1.1 [0.2–2.1]	0.9 [0.2–2.0]	ns (n=19)	ns

Data are presented as mean±SD or median (IQR); units of measured are in pg/mL unless otherwise stated.
Bold p-values are those retained after controlling for multiplicity of testing (false discovery threshold 0.05 across 84 analyses). P values reported were obtained by linear mixed effects models.
*Cytokine levels that were not quantified were assigned the arbitrary value of 0.5×the LLOD (value below the row label when appropriate) to allow analysis.
ACQ-5, 5-item Asthma Control Questionnaire; Eos, eosinophils; FeNO, fractional exhaled nitric oxide; FEV1, forced expiratory volume in 1 s s (postbronchodilator); FVC, forced vital capacity; IFN, interferon; IL, interleukin; LLOD, lower limit of detection; LTE4, leukotriene E4; ns, not significant; PGD2, prostaglandin D2; TARC, thymus activation regulated cytokine (CCL17); TNF, tumour necrosis factor; TSLP, thymic stromal lymphopoietin.

between FeNO suppression and these analytes are detailed in online supplemental appendix 3. In effect, a 42% decrease in FeNO is associated with a significant change in ACQ-5 (↓0.31 (95% CIs: 0.20 to 0.42) points, p<0.0001), sputum eosinophils (↓ 1.37 (1.10 to 1.72)-fold, p=0.009) and sputum PGD_2 (↓ 1.16 (1.01 to 1.32)-fold, p=0.04). There was no significant relationship between the degree of FeNO suppression and sputum IL-4.

The four analytes found to significantly change in both subgroup and pooled continuous analyses according to

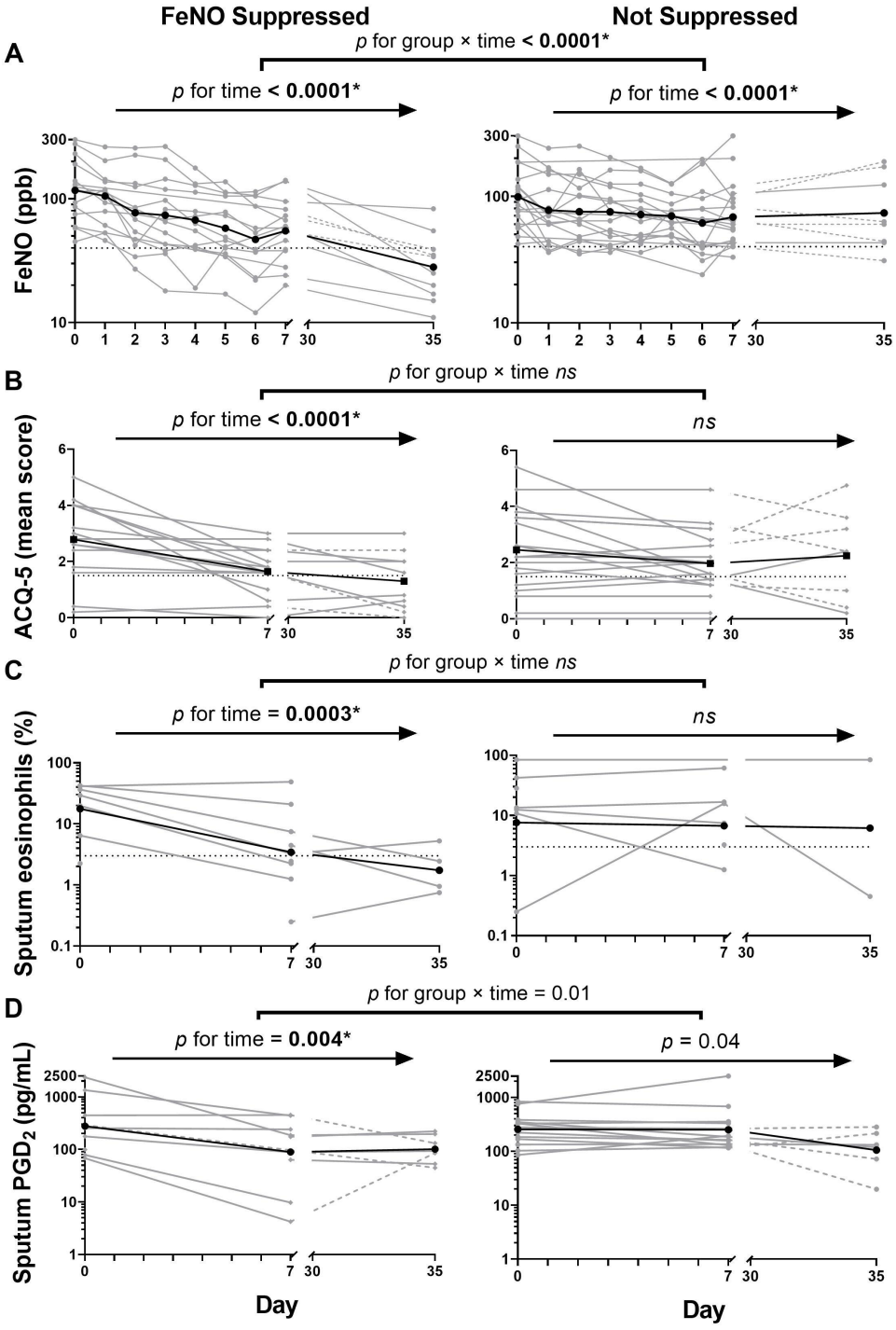

Figure 1 Longitudinal changes in selected analytes during a fractional exhaled nitric oxide (FeNO) suppression test stratified by its results. (A) FeNO (individual and geometric mean values), (B): 5-item Asthma Control Questionnaire (ACQ-5) (individual and mean values); (C): sputum eosinophils (individual and geometric mean values); (D): sputum prostaglandin D_2 (PGD_2) (individual and geometric mean values). Bold *p values are those retained after controlling for a false discovery rate <0.05; dashed segments (- - -) indicate patients administered IM triamcinolone on day 7; dotted horizontal lines (···) delineate the limits of normal/controlled asthma for FeNO (<40 ppb), ACQ-5 (<1.5) and sputum eosinophils (<3%).[10] ns, not significant.

FeNO suppression (FeNO, ACQ-5, sputum eosinophils and PGD_2) are plotted (figure 1).

It is noteworthy that more outcome measures decreased with $p<0.05$ in FeNO suppressors than non-suppressors (11/28 vs 6/28, $p=0.14$ on χ^2 test), and in nearly all cases the end-test median values for sputum and serum inflammatory mediators were numerically greater in FeNO non-suppressors than suppressors (1.9 (0.9–2.8)-fold; 15/22 values greater in FeNO non-suppressors, $p=0.02$ on χ^2 test). Patients who did not suppress FeNO also had significantly greater FeNO values at test termination than suppressors (56 (43–123) vs 35 (20–55) ppb, unpaired t-test on log-FeNO values $p=0.001$). These trends were especially striking for sputum eosinophils (figure 1C), which decreased 5.4 (2.4–10.3)-fold in FeNO suppressors (~normal

median end-test value: 1 (1–5) %, n=7) while increasing 1.3 (0.6–1.6)-fold in non-suppressors (~high median end-test value: 10 (1–67) %, n=6).

Finally, sensitivity analyses were conducted to assess whether the final degree of FeNO suppression or ACQ-5 improvement varied according to study duration (7 days or 35 days) and the optimisation method (ICS-only or ICS+IM triamcinolone) (online supplemental appendix 4). The results suggest that, although methods to ensure optimal FeNO suppression varied, the magnitude of change did not differ significantly between study durations and interventions.

DISCUSSION

We found that patients who failed to suppress FeNO after a suppression test had no improvement in symptoms and FeNO, reflected by raised sputum eosinophil counts, sputum PGD_2, and other inflammatory protein levels at the end of the test. In contrast, FeNO suppressors improve significantly in these domains, often reaching normal values. These results suggest that the assessment of biological corticosteroid resistance can be based on a failure to suppress FeNO during monitored high-intensity corticosteroid therapy.

The criterion for FeNO suppression was derived to identify pre-existing nonadherence—not to assess corticosteroid-resistant type-2 inflammation.[4] Nevertheless, patients who failed to suppress FeNO have consistently been found to be older males with higher baseline asthma morbidity and lesser longitudinal improvements in symptom scores, lung function, and FeNO.[4 6–8] Our data confirm these distinct clinical characteristics and provide translational data supporting the concept that FeNO non-suppression identifies corticosteroid resistance.[9] They also highlight how monitoring adherence allows better interpretation of FeNO fluctuations.[12] An important strength of our study is that we rigorously controlled for multiplicity of testing. Furthermore, we validated the significant findings from longitudinal subgroup analyses (FeNO suppressed, not suppressed) by modelling them according to the degree suppression of FeNO. Hence, FeNO suppression (taken both as a categorical and a continuous variable) translates to a normalisation of the ACQ-5 score, sputum eosinophil count and sputum PGD_2; a mast cell-produced eicosanoid with proinflammatory and bronchoconstrictive effects.[13] Conversely, the clinically distinct FeNO non-suppressors have corticosteroid-refractory symptoms and airway inflammation.

Notwithstanding the results of our subgroup longitudinal analyses which confirmed our study hypothesis, we were unable to show a comparative difference between the two groups across time, possibly because the assessment of the group×time statistical interaction was underpowered to detect the likely difference. Sputum availability in our cohort was also problematic and the study was thus generally underpowered despite robust linear mixed-effect modelling efforts to use all the data at hand. Reports on sputum induction success rates reach 92%[14]; our rate was 44% for differential cell counts and 65% for sputum supernatants. Serum samples were more available (83%) but less useful to assess FeNO-related mechanisms. Another limitation of this study is its observational design with consequent heterogenous testing durations and interventions, although sensitivity analyses did not show any significant impact of these factors on FeNO and symptom improvements. Despite these limitations, the number of inflammatory mediator changes in contrasting directions between suppressors and non-suppressors were unlikely to be just stochastic.

To conclude, our longitudinal subgroup support the notion that patients with uncontrolled asthma who fail to suppress FeNO despite monitored high-intensity corticosteroid therapy have distinct clinical, biomarker and inflammatory mediator responses which imply biological corticosteroid resistance. Further comparative biological analyses between FENO suppressors and non-suppressors require larger validation cohorts and sample sets.

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Acknowledgements The authors thank the colleagues and patients in the Oxford University Hospitals Foundation Trust's severe asthma clinic for help, advice and for proof reading.

Contributors SC collated the data, analysed specimens, drafted and approved the final manuscript. RS participated in data collection, specimen analysis and approved the final manuscript. SL-P performed advanced statistics. GMH, CB, CC, SJT, AM, SP, SM, TP, IP and TH participated in patient recruitment, data collection and approved the final manuscript. TH participated in manuscript preparation, approved the final publication and is the guarantor of this publication.

Funding This work was supported by a non-restricted research grant from Sanofi Genzyme for investigator-initiated type two innovation research; by the NIHR Oxford BRC; and in part by the Wellcome Trust (211050/Z/18/Z) and Beit Fellowship (211050/Z/18/A /A).

Disclaimer The views expressed are those of the authors and not necessarily those of the NHS, the NIHR or the Department of Health.

Competing interests SC and TP: received a non-restricted research grant from Sanofi-Genzyme for investigator-initiated type 2 innovation research, within the submitted work; SC: received speaker honoraria from GlaxoSmithKline, Sanofi-Regeneron and AstraZeneca, outside the submitted work. RS has no conflicts to declare. SL-P has no conflicts of interest to declare. GMH reports funding from an Oxford-UCB Prize Fellowship and the NIHR BRC, outside the submitted work. CB, CC, SM, AM, SP and SJT have no conflicts to declare. TP is an employee of Springer Nature, outside the submitted work. IDP: In the last 5 years, IP has received speaker's honoraria for speaking at sponsored meetings from AstraZeneca, Boehringer Ingelheim, Aerocrine AB, Almirall, Novartis, Teva, Chiesi, Sanofi/Regeneron, Menarini, and GSK, and payments for organising educational events from AstraZeneca, GSK, Sanofi/Regeneron, and Teva. He has received honoraria for attending advisory panels with Genentech, Sanofi/Regeneron, AstraZeneca, Boehringer Ingelheim, GSK, Novartis, Teva, Merck, Circassia, Chiesi, and Knopp, and payments to support FDA approval meetings from GSK. He has received sponsorship to attend international scientific meetings from Boehringer Ingelheim, GSK, AstraZeneca, Teva, and Chiesi. He has received a grant from Chiesi to support a phase 2 clinical trial in Oxford. He is co-patent holder of the rights to the Leicester Cough Questionnaire and has received payments for its use in clinical trials from Merck, Bayer, and Insmed. In 2014-2015 he was an expert witness for a patent dispute involving AstraZeneca and Teva. TH: has received grants from the Wellcome Trust, grants from The Guardians of the Beit Fellowship, grants from Pfizer Inc., Kymab Ltd, grants from University of Oxford, and grants from the NIHR Oxford BRC outside the submitted work. He has received personal fees from Astra Zeneca, personal fees from TEVA, personal fees from Omniprex and personal fees from Peer Voice outside the submitted work.

Patient consent for publication Not applicable.

Ethics approval Study protocols were approved by local UK ethics committees (references 08/H0406/189 and 18/SC/0361). Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

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Longitudinal changes in sputum and blood inflammatory mediators during FeNO suppression testing

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Supplementary Material

Couillard S, *et al. Thorax* 2022;0:1–6. doi: 10.1136/thoraxjnl-2021-217994

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APPENDIX 1 – Flowchart of patient enrolment process

Eighty-seven FeNO suppression tests were planned between 2015 and 2020; 34 completed tests were retained after applying inclusion/exclusion criteria (Figure E1).

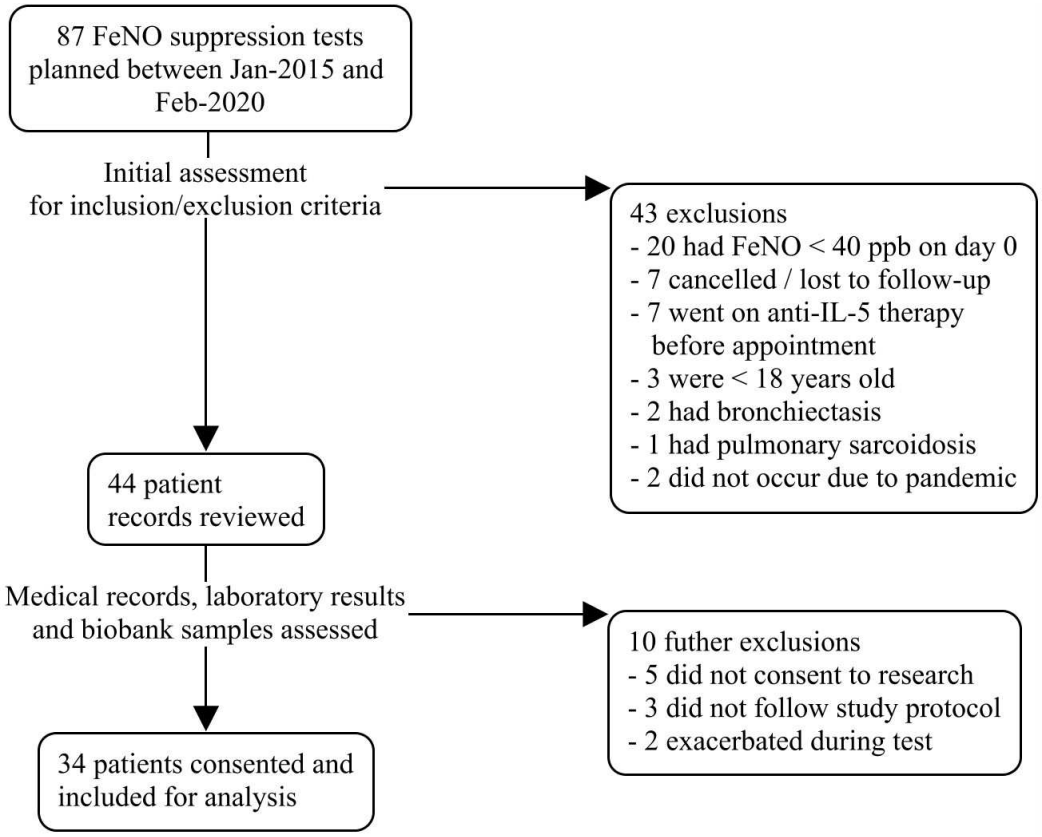

FIGURE E1. Flowchart for fractional exhaled nitric oxide (FeNO) suppression study inclusion. IL-5, interleukin 5.

APPENDIX 2 – Detailed outputs from linear mixed effects modelling

TABLE E1
Linear mixed effect model outputs within groups to assess suppression over time

	Analyte	Variable	FeNO suppressed (N=15) Coeff.	Lower 95%CI	Upper 95%CI	P-value (n)	FeNO not suppressed (N=19) Coeff.	Lower 95%CI	Upper 95%CI	P-value (n)
Clincal	**ACQ-5 score**	Time 2 (Day 7)	-1.15	-1.71	-0.58	2.8E-06	-0.48	-0.97	0.00	0.08
		Time 3 (Day 35)	-1.50	-2.15	-0.85	(n=15)	-0.61	-1.29	0.07	(n=19)
	FEV_1, L	Time 2 (Day 7)	0.16	0.01	0.31	0.009	0.05	-0.12	0.22	0.04
		Time 3 (Day 35)	0.26	0.09	0.44	(n=15)	0.31	0.07	0.55	(n=19)
	FEV_1, % pred	Time 2 (Day 7)	6.15	0.20	12.11	0.02	1.81	-3.98	7.60	0.06
		Time 3 (Day 35)	9.62	2.66	16.57	(n=15)	9.75	1.59	17.91	(n=19)
	FEV_1/FVC, %	Time 2 (Day 7)	2.01	-1.79	5.81	0.4	0.06	-3.48	3.61	0.5
		Time 3 (Day 35)	2.82	-1.61	7.26	(n=15)	2.73	-2.25	7.72	(n=19)
Biomark	**Log_e(FeNO, ppb)**	Time 2 (Day 7)	0.47	0.37	0.60	**3.9E-07**	0.69	0.61	0.80	**2.2E-16**
		Time 3 (Day 35)	0.23	0.17	0.31	(n=15)	0.72	0.59	0.88	(n=19)
	Log_e(Blood Eos, $\times 10^9$/L)	Time 2 (Day 7)	0.62	0.41	0.94	0.02	0.73	0.49	1.09	0.09
		Time 3 (Day 35)	0.57	0.37	0.88	(n=15)	0.61	0.39	0.97	(n=19)
Induced sputum mediators	**Log_e(Eosinophils, %)**	Time 2 (Day 7)	0.24	0.11	0.53	**0.0003**	1.18	0.23	6.11	0.9
		Time 3 (Day 35)	0.15	0.05	0.43	(n=11)	0.71	0.05	9.24	(n=10)
	Log_e(Neutrophils, %)	Time 2 (Day 7))	1.08	0.56	2.06	0.9	0.23	0.06	0.97	0.1
		Time 3 (Day 35)	1.21	0.50	2.94	(n=11)	0.71	0.08	6.46	(n=10)
	Log_e(IL-4, pg/mL)	Time 2 (Day 7)	0.63	0.36	1.10	0.1	1.18	0.73	1.89	**0.002**
		Time 3 (Day 35)	0.54	0.29	1.01	(n=11)	0.39	0.21	0.71	(n=13)
	Log_e(IL-5, pg/mL)	Time 2 (Day 7)	0.29	0.11	0.78	0.045	0.69	0.29	1.66	0.6
		Time 3 (Day 35)	0.42	0.14	1.25	(n=11)	0.60	0.20	1.82	(n=13)
	Log_e(IL-13, pg/mL)	Time 2 (Day 7)	0.86	0.63	1.19	0.5	1.00	0.71	1.42	1.0
		Time 3 (Day 35)	1.03	0.72	1.46	(n=11)	1.01	0.65	1.56	(n=13)
	Log_e(IL-33, pg/mL)	Time 2 (Day 7)	0.60	0.33	1.09	0.02	0.93	0.55	1.58	0.02
		Time 3 (Day 35)	0.40	0.21	0.76	(n=11)	0.41	0.21	0.77	(n=13)
	Log_e(TSLP, pg/mL)	Time 2 (Day 7)	0.52	0.34	0.80	0.008	0.80	0.42	1.53	0.4
		Time 3 (Day 35)	0.82	0.51	1.31	(n=11)	0.60	0.27	1.31	(n=13)
	Log_e(Eotaxin-3, pg/mL)	Time 2 (Day 7)	0.27	0.05	1.51	0.3	1.69	0.25	11.28	0.6
		Time 3 (Day 35)	0.36	0.07	1.85	(n=9)	0.62	0.10	3.77	(n=11)

(Table E2 continued)

	Analyte	Variable	FeNO suppressed (N=15) Coeff.	Lower 95%CI	Upper 95%CI	P-value (n)	FeNO not suppressed (N=19) Coeff.	Lower 95%CI	Upper 95%CI	P-value (n)
Sputum mediators	**Log_e(TARC, pg/mL)**	Time 2 (Day 7)	0.48	0.19	1.21	0.2	0.74	0.27	2.02	0.5
		Time 3 (Day 35)	0.48	0.20	1.14	(n=9)	0.59	0.22	1.56	(n=11)
	Log_e(LTE_4, pg/mL)	Time 2 (Day 7)	0.28	0.12	0.65	0.01	0.72	0.36	1.46	0.5
		Time 3 (Day 35)	0.40	0.16	1.01	(n=11)	0.61	0.24	1.55	(n=13)
	Log_e(PGD_2, pg/mL)	Time 2 (Day 7)	0.28	0.13	0.62	**0.004**	0.99	0.63	1.55	0.04
		Time 3 (Day 35)	0.34	0.14	0.81	(n=11)	0.49	0.28	0.89	(n=13)
	Log_e(IFN-γ, pg/mL)	Time 2 (Day 7)	0.40	0.12	1.33	0.3	1.75	0.80	3.85	0.3
		Time 3 (Day 35)	0.53	0.17	1.66	(n=9)	1.50	0.69	3.25	(n=11)
	Log_e(TNF, pg/mL)	Time 2 (Day 7)	0.76	0.25	2.30	0.4	0.57	0.18	1.76	0.5
		Time 3 (Day 35)	0.49	0.17	1.38	(n=9)	0.55	0.18	1.66	(n=11)
Serum	**Log_e(IL-4, pg/mL)**	Time 2 (Day 7)	0.68	0.43	1.07	0.2	(incalculable as all values equal)			1.0
		Time 3 (Day 35)	0.74	0.46	1.18	(n=14)				(n=19)
	Log_e(IL-5, pg/mL)	Time 2 (Day 7)	0.63	0.35	1.14	0.08	0.75	0.49	1.13	0.2
		Time 3 (Day 35)	0.51	0.28	0.94	(n=14)	0.64	0.37	1.09	(n=19)
	Log_e(IL-13, pg/mL)	Time 2 (Day 7)	0.84	0.65	1.09	0.1	1.02	0.91	1.15	0.6
		Time 3 (Day 35)	0.73	0.54	0.98	(n=14)	0.94	0.81	1.10	(n=19)
	Log_e(IL-33, pg/mL)	Time 2 (Day 7)	0.86	0.75	0.99	0.1	0.90	0.78	1.04	0.3
		Time 3 (Day 35)	0.91	0.78	1.07	(n=14)	0.90	0.75	1.10	(n=19)
	Log_e(TSLP, pg/mL)	Time 2 (Day 7)	0.88	0.57	1.35	0.5	0.89	0.59	1.34	0.06
		Time 3 (Day 35)	0.74	0.46	1.21	(n=14)	0.53	0.31	0.89	(n=19)
	Log_e(Eotaxin-3, pg/mL)	Time 2 (Day 7)	1.20	0.87	1.66	0.07	0.76	0.52	1.11	0.03
		Time 3 (Day 35)	0.79	0.55	1.14	(n=14)	0.52	0.32	0.86	(n=19)
	Log_e(TARC, pg/mL)	Time 2 (Day 7)	0.84	0.68	1.03	0.007	0.87	0.18	4.22	0.9
		Time 3 (Day 35)	0.68	0.54	0.86	(n=14)	1.21	0.17	8.54	(n=19)
	Log_e(IFN-γ, pg/mL)	Time 2 (Day 7)	0.62	0.37	1.03	0.09	1.06	0.60	1.88	0.9
		Time 3 (Day 35)	1.04	0.59	1.82	(n=14)	1.15	0.56	2.39	(n=19)
	Log_e(TNF, pg/mL)	Time 2 (Day 7)	0.94	0.65	1.37	0.6	0.84	0.54	1.31	0.6
		Time 3 (Day 35)	0.81	0.53	1.24	(n=14)	1.10	0.61	1.97	(n=19)

Values and 95% confidence intervals (CI) were obtained by linear mixed-effects models with a random intercept on same patients. **Bold** *P*-values are those retained after controlling for multiplicity of testing: a false discovery threshold 0.05 was applied across 84 analyses including Group × Time p-values indicated in table E2. ACQ-5, 5-item asthma control questionnaire; Eos, eosinophils; FeNO, fractional exhaled nitric oxide; FEV_1, forced expiratory volume in 1 second (post-bronchodilator); FVC, forced vital capacity; IFN, interferon; IL, interleukin; LTE_4, leukotriene E_4; PGD_2, prostaglandin D_2; TNF, tumour necrosis factor; TARC, thymus activation regulated cytokine (CCL17); TSLP, thymic stromal lymphopoietin.

TABLE E2
Linear mixed effect model outputs in the whole sample for Group × Time interaction

	Analyte (n included)	Variable	Coefficient	Lower 95%CI	Upper 95%CI	P-value
Clinical	**ACQ-5 score (n=34)**	Group	0.33	-0.52	1.19	0.4
		Time 2 (Day 7)	-0.48	-0.98	0.01	0.09
		Time 3 (Day 35)	-0.58	-1.27	0.11	
		Group × Time 2	-0.66	-1.41	0.08	0.09
		Group × Time 3	-0.92	-1.86	0.03	
	FEV_1, L (n=34)	Group	0.40	-0.23	1.04	0.2
		Time 2 (Day 7)	0.05	-0.10	0.20	0.02
		Time 3 (Day 35)	0.31	0.10	0.53	
		Group × Time 2	0.11	-0.12	0.34	0.5
		Group × Time 3	-0.05	-0.35	0.24	
	FEV_1, % pred (n=34)	Group	10.78	-2.37	23.94	0.1
		Time 2 (Day 7)	1.81	-3.75	7.36	0.046
		Time 3 (Day 35)	9.88	2.04	17.72	
		Group × Time 2	4.35	-4.02	12.71	0.5
		Group × Time 3	-0.35	-11.06	10.36	
	FEV_1/FVC, % (n=34)	Group	7.31	-1.07	15.69	0.09
		Time 2 (Day 7)	0.06	-3.39	3.52	0.5
		Time 3 (Day 35)	2.89	-2.00	7.77	
		Group × Time 2	1.95	-3.26	7.15	0.7
		Group × Time 3	-0.13	-6.80	6.54	
Biomarker	**Log_e(FeNO, ppb) (n=34)**	Group	1.18	0.80	1.76	0.4
		Time 2 (Day 7)	0.69	0.58	0.83	0.0002
		Time 3 (Day 35)	0.72	0.56	0.93	
		Group × Time 2	0.68	0.52	0.89	**3.0E-07**
		Group × Time 3	0.32	0.22	0.45	
	Log_e(Blood Eos, $\times 10^9$/L) (n=34)	Group	1.44	0.89	2.35	0.1
		Time 2 (Day 7)	0.73	0.49	1.08	0.08
		Time 3 (Day 35)	0.61	0.39	0.96	
		Group × Time 2	0.85	0.48	1.52	0.9
		Group × Time 3	0.93	0.50	1.75	
Sputum mediators	**Log_e(Eosinophils, %) (n=21)**	Group	2.10	0.41	10.80	0.4
		Time 2 (Day 7)	1.25	0.36	4.30	0.8
		Time 3 (Day 35)	0.67	0.10	4.59	
		Group × Time 2	0.19	0.03	1.03	0.1
		Group × Time 3	0.21	0.02	2.55	
	Log_e(Neutrophils, %) (n=21)	Group	0.41	0.09	1.92	0.3
		Time 2 (Day 7)	0.22	0.07	0.72	0.04
		Time 3 (Day 35)	0.84	0.13	5.38	
		Group × Time 2	3.52	0.69	17.86	0.3
		Group × Time 3	1.04	0.10	11.18	

(Table E2 continued)

	Analyte (n analysed)	Variable	Coefficient	Lower 95%CI	Upper 95%CI	P-value
Sputum mediators	**Log$_e$(IL-4, pg/mL) (n=24)**	Group	0.55	0.25	1.21	0.1
		Time 2 (Day 7)	1.17	0.73	1.88	0.003
		Time 3 (Day 35)	0.40	0.22	0.73	
		Group × Time 2	0.54	0.26	1.13	0.09
		Group × Time 3	1.33	0.56	3.16	
	Log$_e$(IL-5, pg/mL) (n=24)	Group	0.58	0.17	1.97	0.4
		Time 2 (Day 7)	0.69	0.29	1.62	0.6
		Time 3 (Day 35)	0.61	0.21	1.79	
		Group × Time 2	0.42	0.11	1.59	0.4
		Group × Time 3	0.69	0.15	3.24	
	Log$_e$(IL-13, pg/mL) (n=24)	Group	1.18	0.71	1.95	0.5
		Time 2 (Day 7)	0.99	0.73	1.35	0.99
		Time 3 (Day 35)	1.02	0.68	1.51	
		Group × Time 2	0.87	0.54	1.42	0.8
		Group × Time 3	1.03	0.58	1.82	
	Log$_e$(IL-33, pg/mL) (n=24)	Group	0.66	0.37	1.21	0.2
		Time 2 (Day 7)	0.92	0.55	1.54	0.03
		Time 3 (Day 35)	0.43	0.22	0.81	
		Group × Time 2	0.66	0.30	1.47	0.6
		Group × Time 3	0.91	0.37	2.28	
	Log$_e$(TSLP, pg/mL) (n=24)	Group	0.49	0.21	1.17	0.1
		Time 2 (Day 7)	0.82	0.43	1.54	0.4
		Time 3 (Day 35)	0.56	0.25	1.25	
		Group × Time 2	0.71	0.27	1.90	0.6
		Group × Time 3	1.33	0.42	4.18	
	Log$_e$(Eotaxin-3, pg/mL) (n=20)	Group	0.84	0.12	5.78	0.9
		Time 2 (Day 7)	1.42	0.23	8.57	0.7
		Time 3 (Day 35)	0.56	0.10	3.18	
		Group × Time 2	0.20	0.02	2.44	0.4
		Group × Time 3	0.64	0.06	6.99	
	Log$_e$(TARC, pg/mL) (n=20)	Group	0.47	0.10	2.20	0.3
		Time 2 (Day 7)	0.73	0.27	1.95	0.5
		Time 3 (Day 35)	0.59	0.22	1.56	
		Group × Time 2	0.67	0.17	2.58	0.8
		Group × Time 3	0.81	0.22	2.97	
	Log$_e$(LTE$_4$, pg/mL) (n=24)	Group	1.04	0.28	3.82	0.96
		Time 2 (Day 7)	0.72	0.36	1.44	0.5
		Time 3 (Day 35)	0.61	0.25	1.53	
		Group × Time 2	0.39	0.13	1.18	0.2
		Group × Time 3	0.65	0.18	2.42	

(Table E2 continued)

	Analyte (n analysed)	Variable	Coefficient	Lower 95%CI	Upper 95%CI	P-value
Sputum mediators	**Log$_e$(PGD$_2$, pg/mL) (n=24)**	Group	1.11	0.46	2.67	0.8
		Time 2 (Day 7)	0.99	0.58	1.70	0.1
		Time 3 (Day 35)	0.50	0.25	1.01	
		Group × Time 2	0.29	0.12	0.67	0.01
		Group × Time 3	0.68	0.25	1.88	
	Log$_e$(IFN-γ, pg/mL) (n=20)	Group	2.34	0.70	7.75	0.2
		Time 2 (Day 7)	1.93	0.60	6.14	0.4
		Time 3 (Day 35)	1.90	0.63	5.78	
		Group × Time 2	0.20	0.04	1.02	0.1
		Group × Time 3	0.28	0.06	1.30	
	Log$_e$(TNF, pg/mL) (n=20)	Group	0.63	0.14	2.90	0.6
		Time 2 (Day 7)	0.56	0.18	1.77	0.5
		Time 3 (Day 35)	0.55	0.18	1.69	
		Group × Time 2	1.35	0.28	6.65	0.9
		Group × Time 3	0.87	0.19	4.01	
Serum mediators	**Log$_e$(IL-4, pg/mL) (n=23)**	Group	1.49	1.16	1.93	0.002
		Time 2 (Day 7)	1.00	0.80	1.26	1.000
		Time 3 (Day 35)	1.00	0.75	1.32	
		Group × Time 2	0.68	0.47	0.98	0.09
		Group × Time 3	0.74	0.49	1.11	
	Log$_e$(IL-5, pg/mL) (n=23)	Group	1.37	0.73	2.57	0.3
		Time 2 (Day 7)	0.77	0.49	1.21	0.4
		Time 3 (Day 35)	0.69	0.39	1.23	
		Group × Time 2	0.78	0.38	1.60	0.7
		Group × Time 3	0.70	0.30	1.61	
	Log$_e$(IL-13, pg/mL) (n=23)	Group	1.07	0.65	1.75	0.8
		Time 2 (Day 7)	1.02	0.87	1.20	0.8
		Time 3 (Day 35)	0.95	0.77	1.18	
		Group × Time 2	0.83	0.64	1.08	0.2
		Group × Time 3	0.78	0.57	1.07	
	Log$_e$(IL-33, pg/mL) (n=23)	Group	0.89	0.55	1.43	0.6
		Time 2 (Day 7)	0.90	0.79	1.03	0.3
		Time 3 (Day 35)	0.90	0.76	1.08	
		Group × Time 2	0.95	0.77	1.18	0.9
		Group × Time 3	1.00	0.77	1.30	
	Log$_e$(TSLP, pg/mL) (n=23)	Group	0.54	0.30	0.99	0.046
		Time 2 (Day 7)	0.87	0.58	1.31	0.052
		Time 3 (Day 35)	0.52	0.31	0.88	
		Group × Time 2	1.13	0.59	2.16	0.4
		Group × Time 3	1.67	0.78	3.58	

(Table E2 continued)

	Analyte (n anlaysed) / Variable	Coefficient	Lower 95%CI	Upper 95%CI	P-value
Serum mediators	**Log_e(Eotaxin-3, pg/mL) (n=23)** Group	0.67	0.32	1.40	0.3
	Time 2 (Day 7)	0.76	0.54	1.06	0.01
	Time 3 (Day 35)	0.52	0.34	0.81	
	Group × Time 2	1.58	0.92	2.72	0.2
	Group × Time 3	1.51	0.79	2.90	
	Log_e(TARC, pg/mL) (n=23) Group	2.85	0.70	11.53	0.1
	Time 2 (Day 7)	0.87	0.25	3.05	0.9
	Time 3 (Day 35)	1.21	0.26	5.72	
	Group × Time 2	1.07	0.14	8.10	0.99
	Group × Time 3	0.90	0.09	8.46	
	Log_e(IFN-γ, pg/mL) (n=23) Group	1.09	0.53	2.24	0.8
	Time 2 (Day 7)	1.07	0.64	1.78	0.9
	Time 3 (Day 35)	1.16	0.60	2.24	
	Group × Time 2	0.59	0.26	1.35	0.4
	Group × Time 3	0.91	0.35	2.36	
	Log_e(TNF, pg/mL) (n=23) Group	0.94	0.36	2.47	0.9
	Time 2 (Day 7)	0.84	0.56	1.24	0.5
	Time 3 (Day 35)	1.10	0.66	1.83	
	Group × Time 2	1.14	0.61	2.14	0.6
	Group × Time 3	0.76	0.35	1.62	

Values and 95% confidence intervals (CI) were obtained by linear mixed-effects models with a random intercept on same patients. The P-values surrounded by a solid square were included in the control for multiplicity of testing: a false discovery threshold 0.05 was applied across 84 analyses including those of table E1. **Bold** *P*-values are those retained after controlling for multiplicity of testing. ACQ-5, 5-item asthma control questionnaire; Eos, eosinophils; FeNO, fractional exhaled nitric oxide; FEV_1, forced expiratory volume in 1 second (post-bronchodilator); FVC, forced vital capacity; IFN, interferon; IL, interleukin; LTE_4, leukotriene E_4; PGD_2, prostaglandin D_2; TNF, tumour necrosis factor; TARC, thymus activation regulated cytokine (CCL17); TSLP, thymic stromal lymphopoietin.

APPENDIX 3 – Results of pooled linear mixed effect models exploring the continuous relationships between FeNO and key analytes

Methods

Analytes found to suppress significantly in the primary longitudinal subgroup analyses on FeNO suppressors and non-suppressors (ACQ-5, sputum eosinophils, sputum PGD2, sputum IL-4) were submitted to further analyses exploring their continuous relationship between log-FeNO suppression and analyte-suppression. The 34 patients were analysed in linear mixed effect models with a random intercept for patient to be able to consider each available timepoint, with the independent variable the natural logarithm of FeNO. An unstructured covariance structure was assumed based on AIC and modeling assumptions were all verified visually. Coefficients and their 95% confidence interval are presented to assess the association between significant markers and FeNO suppression.

Results

TABLE E3. Association between selected mediators and ln(FeNO) according to linear mixed models on the entire sample.

Outcome variable	Coefficients	95% C.I.		*p*
ACQ-5	0.89	0.57	1.20	**<0.0001**
Sputum IL-4 (Log-transformed)	0.27	-0.06	0.60	0.1
Sputum eosinophils (Log-transformed)	0.91	0.27	1.55	**0.009**
Sputum PGD2 (Log-transformed)	0.42	0.04	0.80	**0.04**

Bold *p*-values indicate statistical significance ($p<0.05$). ACQ-5, 5-item asthma control questionnaire; C.I., confidence intervals; IL, interleukin; PGD2, prostaglandin D2.

APPENDIX 4 – Sensitivity analyses for different optimisation methods and test durations

Methods

Summary of different optimisation methods and test durations

An overview of the FeNO suppression test Oxford protocol is provided in Figure E2. Briefly, patients with asthma underwent 7 to 35 days of additional inhaled and/or systemic corticosteroids (+1000µg inhaled fluticasone propionate per day and, if FeNO did not suppress on day 7 according to the equation provided in study methods, +80mg intramuscular (IM) triamcinolone with follow-up 28 days later). Some FeNO suppression tests stopped after 7 days due to patient availability, physician decision, or transition to research bronchoscopy protocols.

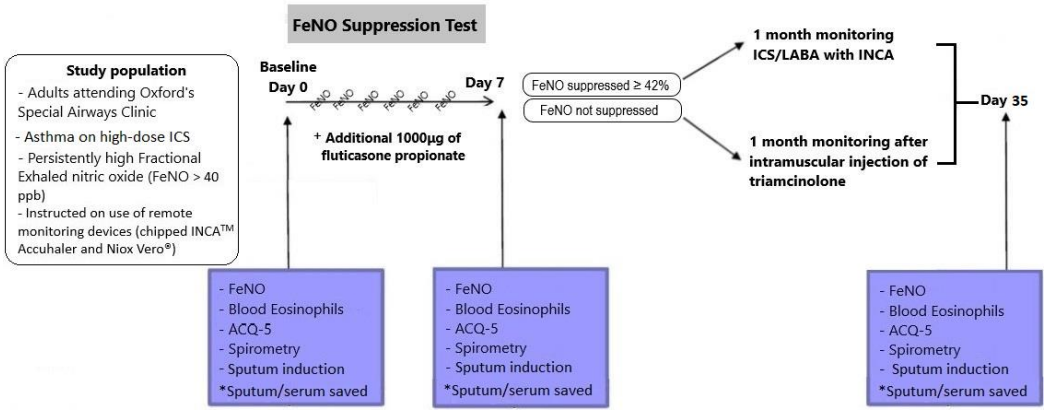

FIGURE E2. The FeNO suppression test, as performed by the Oxford Special Airways Clinic. ACQ-5, 5-item asthma control questionnaire; FeNO, fractional exhaled nitric oxide; GINA, global initiative for asthma; ICS, inhaled corticosteroid; LABA, long-acting beta2-agonist. Figure adapted from Heaney et al. (2019).

Assessing impact of the optimisation method

Sensitivity analyses of positive findings were conducted to assess whether the final optimisation method for FeNO suppression (*i.e.*: +1000µg inhaled fluticasone propionate or +80mg triamcinolone intramuscularly) resulted in significantly different before/after changes. All study patients were grouped based on the optimisation method. Before *vs* after changes in $\log_{10}$-transformed FeNO and differences in ACQ-5 scores were compared within each group using paired *t*-tests. Fold-changes in FeNO and differences in ACQ (after – before) were compared between the two groups with a Mann-Whitney test and an unpaired t-test, respectively. Furthermore, areas under the curve (AUC) were computed for the log-transformed FeNO values (dependent variable) *vs* time course of FeNO suppression testing (independent variable; segmented in days 0 to 7 and days 7 to 35), with their 95% CI analysed for differences.

Assessing the impact of the test duration

To assess if study duration method (*i.e.* 7- or 35-day test) impacted degree of FeNO suppression and ACQ-5 improvements, all included patients were grouped based on study duration. Before *vs* after changes in $\log_{10}$-transformed FeNO and differences in ACQ-5 scores were compared within each group using paired *t*-tests. Fold-changes in FeNO and differences in ACQ (after – before) were compared between the two groups with a Mann-Whitney test and an unpaired t-test, respectively.

Statistics were analysed with a two-sided α of 0.05.

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Results

Sensitivity analyses on the optimisation method

Patients who did and did not suppress FeNO received triamcinolone 80mg intramuscularly on day 7 in similar proportions (3/15 *vs* 6/19; see main manuscript text, Table 1). The results of the sensitivity analyses are shown in Figure E3 and suggest that, although both optimisation methods were used in different circumstances to ensure optimal FeNO suppression, the magnitude of change did not differ significantly between methods for FeNO (median [IQR], ICS-only vs ICS+IM triamcinolone: ↓2.1[1.4-3.8] *vs* 1.6 [1.3-2.2] -fold, p=0.35) and ACQ (mean±SD: -1.0 ±1.3 *vs* –0.7±0.8, p=0.48). These considerations were further explored in a time course plot (Figure E4), with AUC analyses showing similar degrees of $\log_{10}$-FeNO suppression for both optimisation methods (Table E4).

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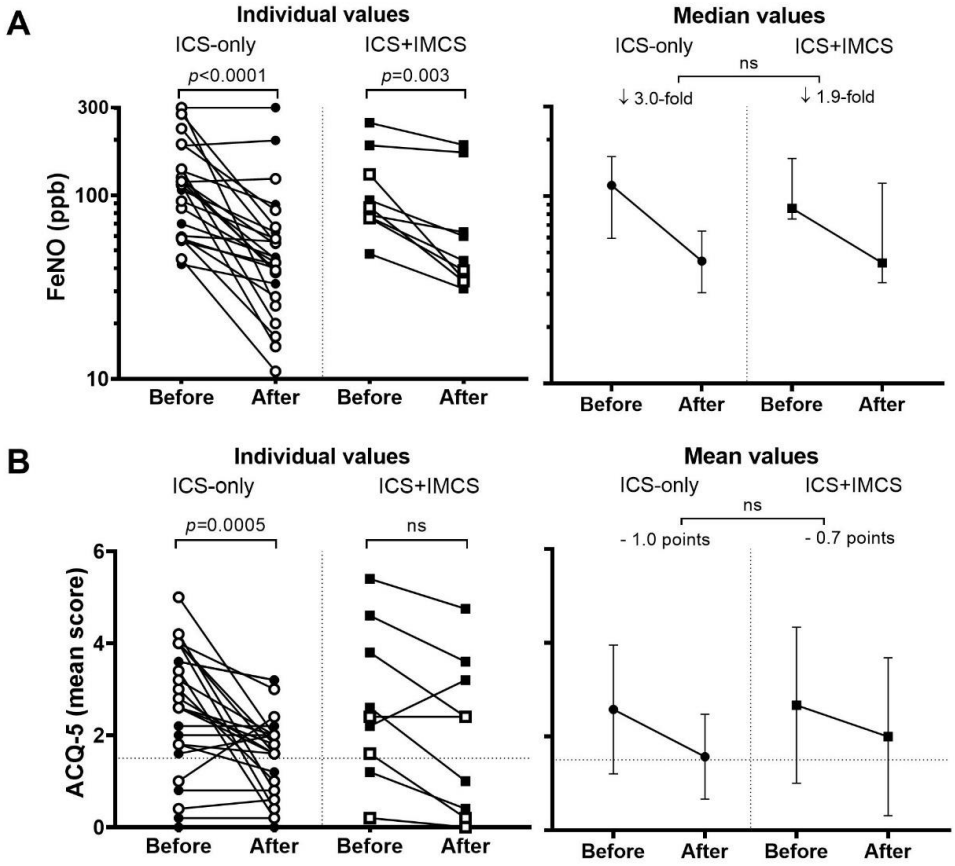

FIGURE E3. Before and after changes in selected analytes following a FeNO suppression test according to the optimisation method. Panel A**:** FeNO, Panel B: 5-item asthma control questionnaire (ACQ-5), with the 1.5-point threshold for good symptom control delimited by the dotted line. ICS, inhaled corticosteroid (*i.e.*: additional fluticasone propionate 1000µg inhaled daily throughout); IMCS, intramuscular corticosteroid (*i.e.*: triamcinolone 80mg intramuscularly on day 7); ○□, FeNO suppressed; ●■, FeNO not suppressed.

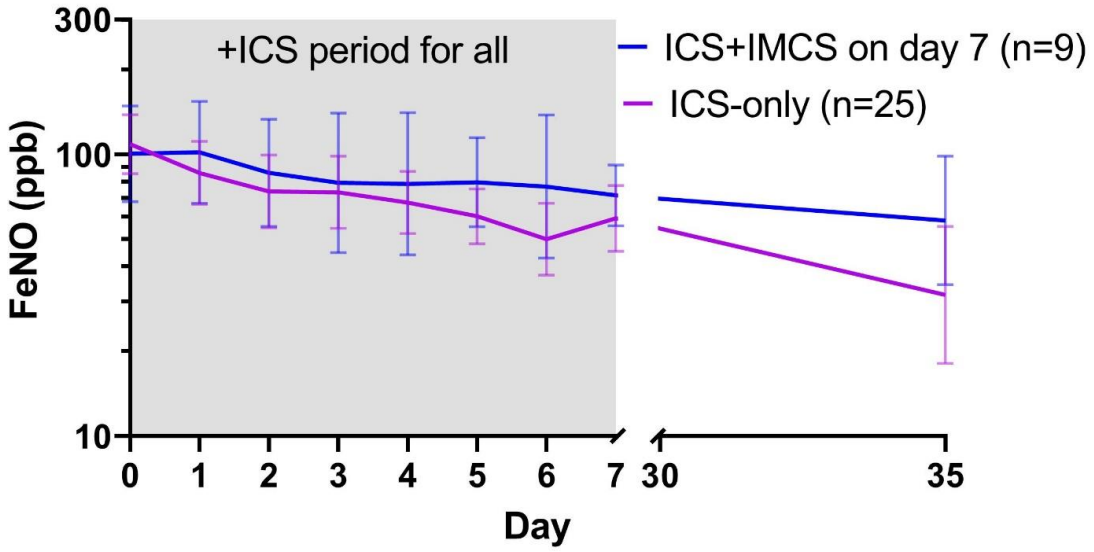

FIGURE E4. FeNO suppression time course according to optimisation method. Full lines connect the geometric mean values at each day of measurement (**bold** day numbers), with error bars corresponding to the 95% CI.

TABLE E4
Area under the curve per segment of the FeNO suppression test according to optimisation method.

Optimisation method (n)	**Total area under the curve per segment**					
	Days 0 to 7		**Days 7 to 35**		**Days 0 to 35**	
ICS+IMCS on day 7 (n=9)	13.5	(12.7-14.2)	50.7	(41.7-59.7)	63.0	(54.4-73.9)
ICS-only (n=25)	12.9	(11.9-13.9)	45.8	(33.7-58.0)	56.9	(45.6-71.8)
p	ns		ns		ns	

Areas under the curve (95% confidence intervals) shown are computed for mean $\log_{10}$-transformed FeNO values according to time in each subgroup. ICS, inhaled corticosteroid; IMCS, intramuscular corticosteroid; ns, $p \geq 0.05$.

Sensitivity analyses on the duration of the FeNO suppression test

Patients who did and did not suppress FeNO underwent 7-day tests in similar proportions (5/15 *vs* 11/19; see main manuscript text, Table 1). The results of the sensitivity analyses are shown in Figure E5 and suggest that the magnitude of change did not differ significantly between testing durations for FeNO (median [IQR], 7-day vs 35-day tests: ↓ 1.6[1.3-2.4] *vs* 2.1 [1.5-4.1] -fold, p=0.11) and ACQ (mean±SD: -0.6 ±0.9 *vs* -1.2 ± .14, p=0.20).

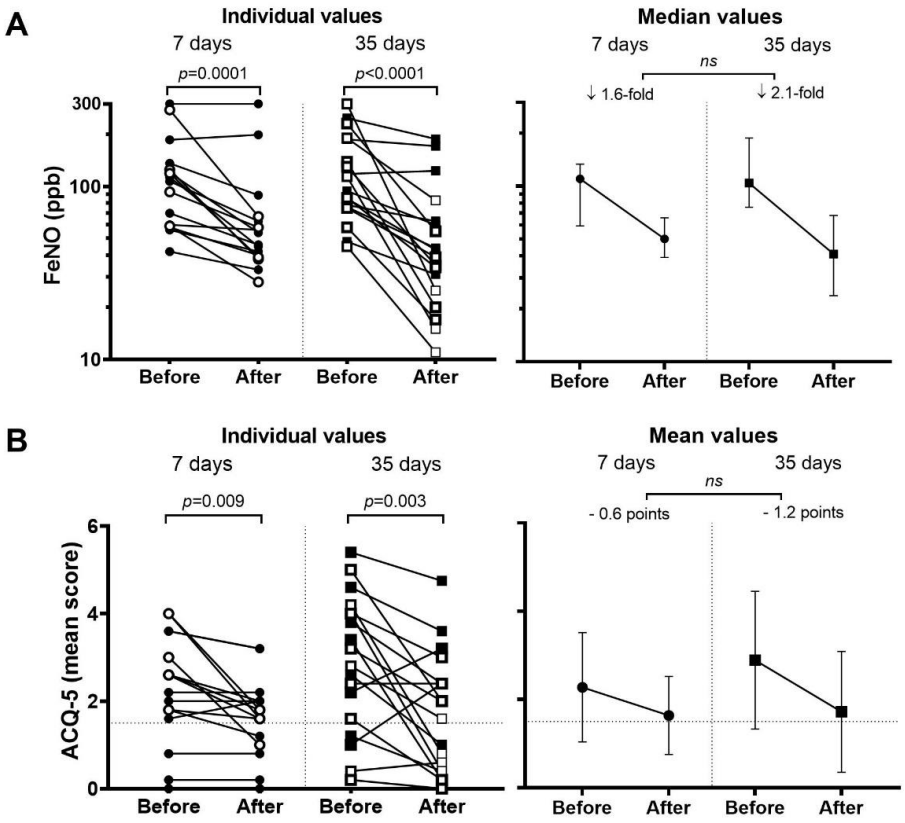

FIGURE E5. Before and after changes in selected analytes following a FeNO suppression test according to duration of test. Panel A**:** FeNO, Panel B: 5-item asthma control questionnaire (ACQ-5), with the 1.5-point threshold for good symptom control delimited by the dotted line. ○□, FeNO suppressed; ●■, FeNO not suppressed.