Fractional exhaled nitric oxide and the response to prednisolone for asthma attacks in patients treated with anti-IL5/5Rα therapy: a prospective observational study

Received: 18 March 2025
Accepted: 5 Sept 2025

To the Editor:

Anti-interleukin (IL)5/5Rα monoclonal antibody (mAb) therapies deplete blood eosinophils and reduce the annualised asthma attack rate by over 50% [1]. The clinical benefits of oral corticosteroids (OCS) to treat breakthrough attacks are uncertain. Fractional exhaled nitric oxide (F_{ENO}) has the potential to discriminate between ongoing type-2 airway inflammation or infection in breakthrough attacks on mepolizumab [2].

We designed a prospective, observational study to investigate the relationship between F_{ENO} and the clinical responses to prednisolone treatment for outpatient attacks in anti-IL5/5Rα-treated patients.

A peer-reviewed protocol for the BreakthrOugh asthma attacks treated with Oral STeroids (BOOST) study was published with a pre-specified statistical analysis plan [3]. BOOST was a sub-study of the Oxford Airways Study conducted in the outpatient Oxford specialist asthma service (Oxfordshire Research Ethics Committee reference: 18/SC/0361). Participants provided written informed consent to the Oxford Airways Study.

The inclusion criteria were adults aged 18 years or over treated with anti-IL5 or anti-IL5Rα mAb therapy for at least 2 months prescribed according to UK guidelines for severe eosinophilic asthma. An asthma attack was defined as ⩾48 h respiratory symptom deterioration despite increased inhaler usage [4] and no alternative diagnosis after clinical history, physical examination and chest radiograph. The exclusion criteria were treatment with OCS in the 4 weeks prior to the attack visit; pregnancy; systemic immunosuppressive treatment (non-asthma); participant requiring hospitalisation.

Eligible participants were treated with 40 mg of oral prednisolone once daily for 7 days as per usual care [5]. Antibiotic therapy initiated prior to the attack visit was completed. Participants could have treatment for one asthma attack in the study.

Study visits were at stable state (>8 weeks from an attack), attack, and 7 and 28 days after attack. At each visit, participants underwent post-bronchodilator spirometry, F_{ENO} (Circassia), physical examination, vital signs, medication check, full blood count, serum C-reactive protein (CRP), sputum differential cell count [6], and the Asthma Control Questionnaire-5 (ACQ-5). Visual analogue scale (VAS) scores for respiratory symptoms were completed electronically at each visit and daily from attack until day 28. At the attack visit a throat swab for multiplex viral PCR (BioFire Diagnostics), sputum for bacterial culture and a neutrophil elastase test (NEATstik, ProAxsis) were carried out.

Comparison of all outcomes were between F_{ENO}-high (⩾25 ppb) and F_{ENO}-low (<25 ppb) groups defined at attack. The F_{ENO}-low threshold was based on earlier studies showing a low likelihood of corticosteroid-responsive eosinophilic airway inflammation in patients with a F_{ENO} <25 ppb [2, 7].

The primary outcome was the proportion of treatment failure (unscheduled healthcare visit for asthma or repeated acute asthma oral treatment) at day 28 between F_{ENO}-high and F_{ENO}-low groups. Treatment failure was self-reported by patients and corroborated with health records. Secondary outcomes were changes in forced expiratory volume in 1 s (FEV_1), ACQ-5 score, and total VAS symptoms at day 7.

Shareable abstract (@ERSpublications)
F_{ENO} testing at attack can identify the patients on anti-IL5/IL5Rα biologics who have the most lung function and symptom benefits from prednisolone https://bit.ly/3KhMB68

Cite this article as: Howell I, Mahdi M, Mahmood HR, *et al*. Fractional exhaled nitric oxide and the response to prednisolone for asthma attacks in patients treated with anti-IL5/5Rα therapy: a prospective observational study. *Eur Respir J* 2025; 66: 2501229 [DOI: 10.1183/13993003.01229-2025].

Descriptive statistics categorised by F_{ENO} groups are presented. The proportion of treatment failure at day 28 between F_{ENO} groups was calculated by Fisher's exact test and time to treatment failure by Kaplan–Meier analysis and stratified log-rank test. Linear mixed effects models compared continuous data from the secondary outcomes. The random effect was the participants, and fixed effects were the study visit, F_{ENO} group, and their interaction. The model managed missing data using the missing at random assumption.

BOOST was a pilot study to support designing definitive placebo-controlled trials. The sample size of 60 asthma attacks was based on an anticipated treatment failure rate of 20% at 28 days [8, 9], with 30% absolute difference between F_{ENO} groups, two-sided α=0.05, β=0.8, and a 10% dropout.

Between September 2022 until April 2024, 111 patients established on anti-IL5/5Rα treatment with acute respiratory symptoms were assessed for study inclusion. 51 patients were excluded: 23 clinically did not have an asthma attack, and 28 had an asthma attack but met exclusion criteria. 60 were enrolled and treated with open label prednisolone. 21 participants were F_{ENO}-low (<25 ppb), and 39 participants were F_{ENO}-high ($\geqslant$25 ppb) at attack.

In all participants, the mean age was 56 years (range 22–78 years), 63% were female, 85% were of white ethnicity, mean body mass index was 29.6 $kg \cdot m^{-2}$, 23% had chronic rhinosinusitis/nasal polyps, 72% had never smoked and there were no current smokers. These characteristics were similar between F_{ENO}-low and F_{ENO}-high groups. The F_{ENO}-low group had more years with asthma (28 *versus* 16 years; p=0.04). Routine medications were similar between groups. All participants were treated with an inhaled corticosteroid-containing inhaler at a mean 1680 µg beclomethasone dipropionate equivalent dose. 45% were on an antimuscarinic inhaler and 3% on prophylactic azithromycin. Median duration on anti-IL5/5Rα biologic was 23 months (interquartile range 7–43 months). More F_{ENO}-low participants were on an anti-IL5Rα biologic (62% *versus* 33%; p=0.03). F_{ENO}-low and -high groups had similar stable state FEV_1 (2.34 *versus* 2.23 L), ACQ-5 (1.61 *versus* 2.09) and total VAS (13.8 *versus* 21.9 mm). F_{ENO}-low attacks had quicker symptom deterioration before attack (5 *versus* 10 days; p=0.02), associated fever (43 *versus* 18%; p=0.04), and viral positivity (57 *versus* 29%; p=0.03) compared to F_{ENO}-high attacks. Sputum culture positivity at attack (20% *versus* 8%) and antibiotic prescription in the 2 weeks before attack (9% *versus* 8%) were similar in F_{ENO}-low and F_{ENO}-high groups. Median blood (0.00 *versus* 0.04×10^9 L^{-1}) and sputum (0.0 *versus* 0.6%) eosinophil counts at attack were low in F_{ENO}-low and F_{ENO}-high groups at attack. No participant had blood eosinophils $\geqslant$0.3×10^9 L^{-1} at any visit. Median attack F_{ENO} was lower in the F_{ENO}-low group (12 *versus* 69 ppb; p<0.001). Sputum neutrophils (42% *versus* 68%), NEATstik positivity (29% *versus* 44%), median CRP (7 *versus* 5 $mg \cdot L^{-1}$), and median IgE (97 *versus* 167 $KU \cdot L^{-1}$) were similar in F_{ENO}-low and F_{ENO}-high groups at attack.

All 60 patients were followed up. There was no significant difference in proportion of treatment failure at day 28 between the F_{ENO}-low (n=9, 43%) *versus* the F_{ENO}-high (n=11, 28%) group (OR 1.89, 95% CI 0.54 to 6.64; p=0.27). The time to first treatment failure within 28 days was not significant between the F_{ENO}-low *versus* the F_{ENO}-high group (HR 1.87, 95% CI 0.77 to 4.52; log-rank p=0.2).

Between the attack and day 7, the F_{ENO}-high group had significantly greater improvements in their FEV_1 (373 mL *versus* 3 mL, mean difference 370 mL, 95% CI 113 to 628 mL; p=0.006), ACQ-5 (−1.68 *versus* −0.27, mean difference −1.41, 95% CI −0.70 to −2.11; p<0.001), and total VAS symptoms (−25.9 *versus* −12.9, mean difference −13.0, 95% CI −4.2 to −21.9; p<0.005) compared to the F_{ENO}-low group (figure 1).

In multivariable regression, after adjusting for biologic class, sex, asthma duration (continuous), stable FEV_1 (continuous), stable ACQ-5 (continuous), viral status, attack sputum eosinophil percentage (continuous), and attack blood eosinophil count (continuous), only attack F_{ENO} (continuous) was associated with a significant improvement in FEV_1 (L) (0.004 per ppb, 95% CI 0.001 to 0.007; p=0.02) and ACQ-5 (−0.01 per ppb, 95% CI −0.003 to −0.018; p=0.005) between attack and day 7.

The BOOST study showed no statistical difference in rate of treatment failure between F_{ENO} groups at day 28. This result likely reflects a small study sample size and more late treatment failures in the F_{ENO}-high group than anticipated. Nonetheless, we demonstrated that F_{ENO}-high attacks were associated with more rapid and larger symptom and lung function improvement after 7 days prednisolone treatment compared to F_{ENO}-low attacks in a population where blood eosinophils were suppressed by anti-IL-5/5Rα treatment.

Raised F_{ENO} at attack has been associated with sputum eosinophilia in an anti-IL5-treated population [2]. In BOOST this was not seen, potentially reflecting greater suppression of blood eosinophilia in patients treated with benralizumab [10]. The relationship between F_{ENO} with symptom and lung function recovery

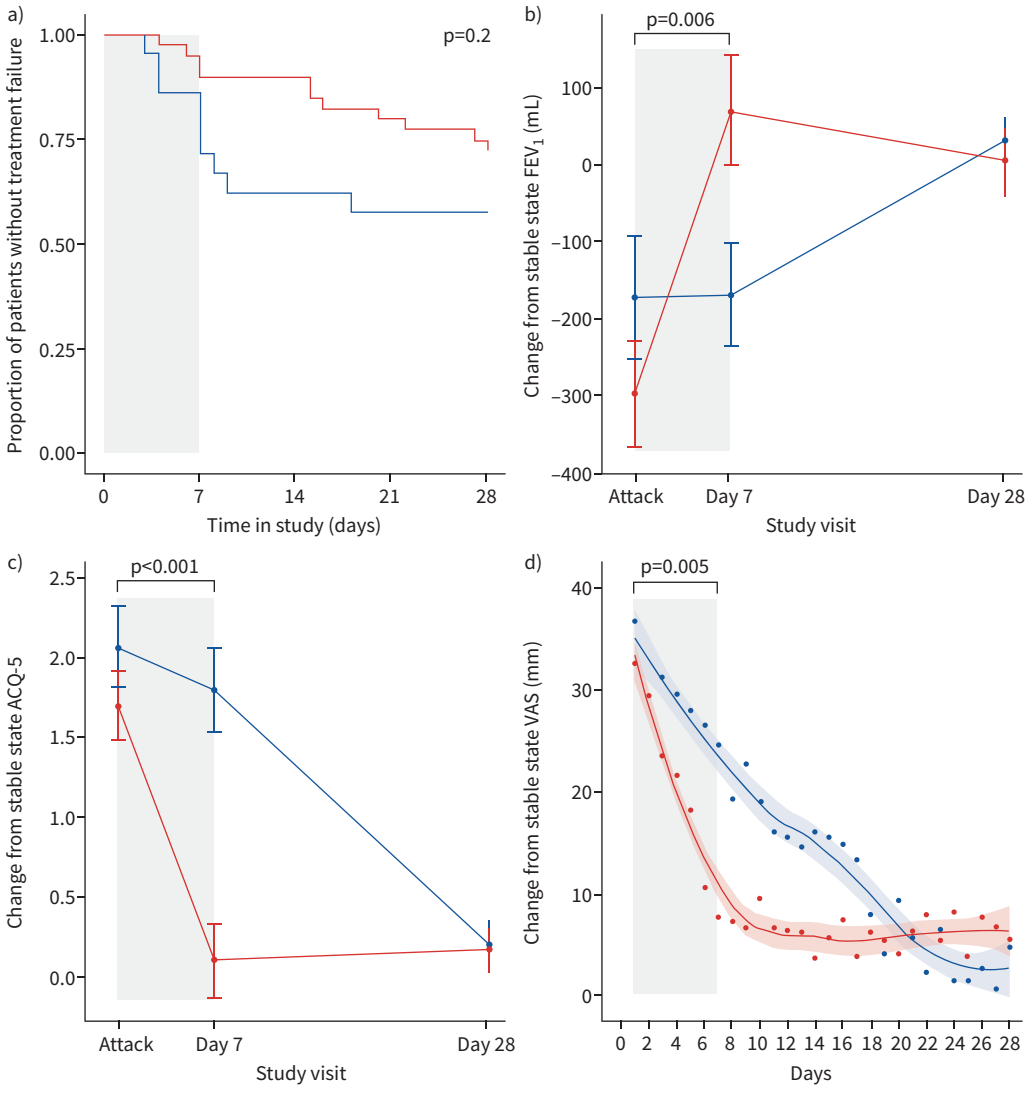

FIGURE 1 Comparison of clinical outcomes between fractional exhaled nitric oxide (F_{ENO})-high and F_{ENO}-low groups from attack to day 28. a) Kaplan–Meier plot of time to first treatment failure; b) forced expiratory volume in 1 s (FEV_1); c) Asthma Control Questionnaire-5 (ACQ-5); d) total visual analogue scale (VAS) symptoms. The red line indicates the F_{ENO}-high group, and the blue line indicates the F_{ENO}-low group. The vertical grey shaded area indicates when prednisolone treatment was given between the attack and day 7 visits. In plots b–d, the 0 value on the *y*-axis corresponds to stable state. All mean values, and the mean difference in change from attack to day 7 between F_{ENO}-high and F_{ENO}-low groups, were calculated by linear mixed effects models. The curves in plot d were fit by LOESS regression.

after OCS treatment was independent of sputum eosinophils. This questions whether sputum eosinophils alone predict OCS responsiveness and suggests involvement of broader, active steroid-responsive inflammation [11]. Broader suppression of type-2 inflammation may be beneficial in patients with ongoing attacks, despite anti-IL5/5Rα treatment.

F_{ENO}-low attacks were associated with viral infection and displayed small, non-clinically meaningful improvements to symptoms and lung function after OCS treatment. This is consistent with a prior study conducted in a mAb-naive population [12]. Together, these studies question the value of OCS treatment for blood eosinophil and F_{ENO} suppressed outpatient asthma attacks. The decision to prescribe medical treatment is contingent on its risk–benefit balance, and OCS have a significant risk of side-effects [13]. Biomarker-directed, placebo-controlled trials of OCS treatment for outpatient asthma attacks are required to re-evaluate whether using OCS for type-2 low attacks is clinically effective. Since symptoms and lung

function declined similarly at attack in both F_{ENO} groups, alternative treatable mechanisms need to be explored in the type-2 low population [14, 15].

The strengths of the BOOST study are the prospective design with a predefined F_{ENO} threshold for analysis based on a low risk of steroid responsiveness below 25 ppb [7], and the detailed serial assessment of clinical and airway inflammatory variables around a time of great clinical interest. The main limitations are that this was a small, single-centre, unblinded and non-randomised study. There was a risk of confounding due to differing baseline characteristics between F_{ENO} groups. The conclusions of this work are preliminary and need to be confirmed in definitive, larger trials.

In conclusion, the BOOST study demonstrates that F_{ENO} testing at attack can identify the patients on anti-IL5/IL5Rα treatment who have the most lung function and symptom benefit from prednisolone.

Imran Howell [1], Mahdi Mahdi[1], Hafiz R. Mahmood[1], Laura Bermejo-Sanchez[1], Catherine Borg[1], Sanjay Ramakrishnan [2], James Melhorn [1], Gabriel Lavoie[3], Nayia Petousi [1], Timothy S.C. Hinks [1], Mona Bafadhel [4] and Ian D. Pavord[1]

[1]Respiratory Medicine Unit and Oxford Respiratory NIHR BRC, Nuffield Department of Medicine, University of Oxford, Oxford, UK. [2]Institute for Respiratory Health and UWA Medical School, University of Western Australia, Perth, Australia. [3]Département de médecine, Université de Montréal, Québec, QC, Canada. [4]King's Centre for Lung Health, School of Immunology and Microbial Sciences, King's College London, London, UK.

Corresponding author: Imran Howell (imran.howell@ndm.ox.ac.uk)

Data availability: Deidentified participant data and code scripts are available on request after publication.

Ethics statement: BOOST was a sub-study of the Oxford Airways Study (Integrated Research Application System Project number: 234581; Oxfordshire Research Ethics Committee B Reference: 18/SC/0361). Participants provided written informed consent to the Oxford Airways Study at their first study visit.

Author contributions: I. Howell, M. Bafadhel and I.D. Pavord were responsible for study conception. I. Howell, S. Ramakrishnan, J. Melhorn, N. Petousi, T.S.C. Hinks, M. Bafadhel and I.D. Pavord were responsible for study design. I. Howell, M. Mahdi, H.R. Mahmood, L. Bermejo-Sanchez, C. Borg, S. Ramakrishnan, J. Melhorn and G. Lavoie were responsible for data collection. I. Howell was responsible for the statistical analysis and the first draft of the manuscript. All authors were responsible for the interpretation of the data and for reviewing and approving the final submitted manuscript. The corresponding author attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted. I. Howell and I.D. Pavord are the guarantors of the content of the manuscript, including the data and analysis.

Conflict of interest: I. Howell reports support for the present study from the National Institute for Health and Care Research Oxford Biomedical Research Centre, grants from the BMA Foundation, support for attending meetings from GSK, and a leadership role on the British Thoracic Society science and research committee. S. Ramakrishnan reports support for the present study from the Charlies Foundation for Research and the National Institute for Health and Care Research Clinical Research Network, payment or honoraria for lectures, presentations, manuscript writing or educational events from AstraZeneca, Chiesi, Sanofi, Boehringer Ingelheim and GlaxoSmithKline, and support for attending meetings from AstraZeneca and Boehringer Ingelheim. T.S.C. Hinks reports support for the present study from a Wellcome Trust Fellowship (211050/Z/18/z) and grants from Pfizer. M. Bafadhel reports support for the present study from the National Institute for Health and Care Research (NIHR) under Research Professor award NIHR304263, grants from AstraZeneca, Asthma+Lung UK, Roche, GlaxoSmithKline and the National Institute for Health and Care Research (NIHR), payment or honoraria for lectures, presentations, manuscript writing or educational events from AstraZeneca, Roche and Sanofi, support for attending meetings from AstraZeneca and Chiesi, participation on a data safety monitoring board or advisory board with AlbusHealth and Areteia, and leadership role on the British Thoracic Society science and research committee. I.D. Pavord reports grants from Chiesi, payment or honoraria for lectures, presentations, manuscript writing or educational events from AstraZeneca, Boehringer Ingelheim, Aerocrine, Almirall, Novartis, Teva, Chiesi, Sanofi/Regeneron, Menarini and GlaxoSmithKline, payment for organising educational events from AstraZeneca, GlaxoSmithKline, Sanofi/Regeneron and Teva, payment for expert testimony in a patent dispute involving AstraZeneca and Teva, support for attending meetings from Boehringer Ingelheim, GlaxoSmithKline, AstraZeneca, Teva and Chiesi, patents

planned, issued or pending for the Leicester Cough Questionnaire with Merck, Bayer and Insmed, and participation on a data safety monitoring board or advisory board with Genentech, Sanofi/Regeneron, AstraZeneca, Boehringer Ingelheim, GlaxoSmithKline, Novartis, Teva, Merck, Circassia, Chiesi and Knopp. The remaining authors have no potential conflicts of interest to disclose.

Support statement: This study was funded by an NIHR Oxford Biomedical Research Centre grant and a BMA Foundation James Trust grant. Funding information for this article has been deposited with the Open Funder Registry.

References

1 Howell I, Howell A, Pavord ID. Type 2 inflammation and biological therapies in asthma: targeted medicine taking flight. *J Exp Med* 2023; 220: e20221212.
2 McDowell PJ, Diver S, Yang F, *et al.* The inflammatory profile of exacerbations in patients with severe refractory eosinophilic asthma receiving mepolizumab (the MEX study): a prospective observational study. *Lancet Respir Med* 2021; 9: 1174–1184.
3 Howell I, Mahdi M, Bafadhel M, *et al.* Recovery of breakthrough asthma attacks treated with oral steroids while on monoclonal antibody therapy: protocol for a prospective observational study (BOOST). *JMIR Res Protoc* 2023; 12: e46741.
4 GINA. Global Strategy for Asthma Management and Prevention 2023. www.ginasthma.org
5 BTS/SIGN. British Guideline on the Management of Asthma 2019. www.brit-thoracic.org.uk/quality-improvement/guidelines/asthma/
6 Djukanović R, Sterk PJ, Fahy JV, *et al.* Standardised methodology of sputum induction and processing. *Eur Respir J* 2002; 20: Suppl. 37, 1s–2s.
7 Dweik RA, Boggs PB, Erzurum SC, *et al.* An official ATS clinical practice guideline: interpretation of exhaled nitric oxide levels (F_ENO) for clinical applications. *Am J Respir Crit Care Med* 2011; 184: 602–615.
8 Chapman KR, Verbeek PR, White JG, *et al.* Effect of a short course of prednisone in the prevention of early relapse after the emergency room treatment of acute asthma. *N Engl J Med* 1991; 324: 788–794.
9 Hill J, Arrotta N, Villa-Roel C, *et al.* Factors associated with relapse in adult patients discharged from the emergency department following acute asthma: a systematic review. *BMJ Open Respir Res* 2017; 4: e000169.
10 Logan J, Wetherall K, Gillespie L, *et al.* S104 Study of asthma exacerbations in patients on the IL-5 receptor blocker, benralizumab – the BenRex study. *Thorax* 2024; 79: Suppl. 2, A74.
11 Howell I, Yang F, Brown V, *et al.* Airway proteomics reveals broad residual anti-inflammatory effects of prednisolone in mepolizumab-treated asthma. *J Allergy Clin Immunol* 2024; 154: 1146–1158.
12 Celis-Preciado C, Leclerc S, Duval M, *et al.* Phenotyping the responses to systemic corticosteroids in the management of asthma attacks (PRISMA). *Eur Respir J* 2025; 65: 2402391.
13 Skov IR, Madsen H, Henriksen DP, *et al.* Low-dose oral corticosteroids in asthma associates with increased morbidity and mortality. *Eur Respir J* 2022; 60: 2103054.
14 Fahy JV, Jackson ND, Sajuthi SP, *et al.* Type 1 immune responses related to viral infection influence corticosteroid response in asthma. *Am J Respir Crit Care Med* 2025; 211: 194–204.
15 McDowell PJ, Busby J, Hanratty CE, *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: 545–553.