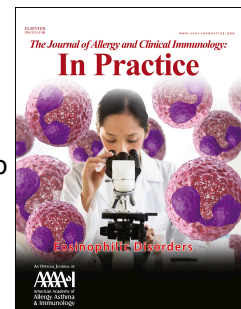


Journal Pre-proof

The relationship between FeNO and effectiveness of mepolizumab and benralizumab in severe eosinophilic asthma

A.P. Hearn, J. Kavanagh, G. d'Ancona, C. Roxas, L. Green, L. Thomson, M. Fernandes, B.D. Kent, J. Dhariwal, A.M. Nanzer, D.J. Jackson



PII: S2213-2198(21)00064-7

DOI: <https://doi.org/10.1016/j.jaip.2021.01.008>

Reference: JAIP 3384

To appear in: *The Journal of Allergy and Clinical Immunology: In Practice*

Received Date: 13 October 2020

Revised Date: 30 December 2020

Accepted Date: 5 January 2021

Please cite this article as: Hearn A, Kavanagh J, d'Ancona G, Roxas C, Green L, Thomson L, Fernandes M, Kent B, Dhariwal J, Nanzer A, Jackson D, The relationship between FeNO and effectiveness of mepolizumab and benralizumab in severe eosinophilic asthma, *The Journal of Allergy and Clinical Immunology: In Practice* (2021), doi: <https://doi.org/10.1016/j.jaip.2021.01.008>.

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2021 Published by Elsevier Inc. on behalf of the American Academy of Allergy, Asthma & Immunology

The relationship between FeNO and effectiveness of mepolizumab and benralizumab in severe eosinophilic asthma

Hearn AP^{1,2}, Kavanagh J^{1,2}, d'Ancona G¹, Roxas C¹, Green L¹, Thomson L¹, Fernandes M¹, Kent BD^{1,3,4}, Dhariwal J¹, Nanzer AM¹, Jackson DJ^{1,2}

¹Guy's Severe Asthma Centre, Guy's Hospital, Guy's & St Thomas' NHS Trust, London, UK

²School of Immunology & Microbial Sciences, King's College London, UK

³Department of Respiratory Medicine, St James' Hospital, Dublin, Ireland

⁴School of Medicine, Trinity College Dublin, Ireland

Authors

Dr Andrew P Hearn (MBBS), School of Immunology and Microbial Sciences, King's College London, UK

Dr Joanne Kavanagh (MBChB), School of Immunology and Microbial Sciences, King's College London, UK

Ms Grainne d'Ancona (MSc), Guy's Severe Asthma Centre, Guy's Hospital, Guy's & St Thomas' NHS Trust, London, UK

Ms Cris Roxas (MSc), Guy's Severe Asthma Centre, Guy's Hospital, Guy's & St Thomas' NHS Trust, London, UK

Ms Linda Green (MSc), Guy's Severe Asthma Centre, Guy's Hospital, Guy's & St Thomas' NHS Trust, London, UK

Ms Louise Thomson (BSc), Guy's Severe Asthma Centre, Guy's Hospital, Guy's & St Thomas' NHS Trust, London, UK

Ms Marianna Fernandes (BSc), Guy's Severe Asthma Centre, Guy's Hospital, Guy's & St Thomas' NHS Trust, London, UK

Dr Brian D Kent (MD), School of Medicine, Trinity College Dublin, Ireland

Dr Jaideep Dhariwal (PhD), Guy's Severe Asthma Centre, Guy's Hospital, Guy's & St Thomas' NHS Trust, London, UK

Dr Alexandra M Nanzer (PhD), Guy's Severe Asthma Centre, Guy's Hospital, Guy's & St Thomas' NHS Trust, London, UK

Dr David J Jackson (PhD), School of Immunology and Microbial Sciences, King's College London, UK

Corresponding author:

Dr David Jackson, Clinical Lead, Guy's Severe Asthma Centre, Guy's & St Thomas' NHS Trust; School of Immunology & Microbial Sciences, King's College London, SE1 9RT, UK.

Email: David.jackson@gstt.nhs.uk.

Phone: +44 2071887188

COI:

Conflicts of interest: DJ, BK, GdA, reports advisory board and speaker fees and congress travel support from GSK, Astrazeneca, Chiesi, Napp, and Teva pharmaceuticals. JK reports congress travel support from Teva. JD reports congress support from Sanofi. AN reports speaker fees from Astrazeneca and congress travel support from Napp. .

Funding: none declared

Word count: 1064

Clinical Implications

The clinical effectiveness of mepolizumab and benralizumab is independent of baseline FeNO level. In SEA patients with a FeNO-high phenotype, benralizumab is associated with a fall in FeNO suggesting that IL-5R-expressing cells including eosinophils and basophils are an important source of IL-13.

Monoclonal antibodies (mAbs) directed against type 2 (T2) inflammatory pathway have revolutionised the treatment of severe eosinophilic asthma (SEA) by improving symptom control and exacerbation frequency, and reducing exposure to oral corticosteroids (OCS).(1, 2) Mepolizumab(1) and benralizumab(2) target interleukin-5 (IL-5) and IL-5 receptor-alpha (IL-5R) respectively, inhibiting the eosinophilic inflammatory cascades which underpin the pathogenesis of poor symptom control and exacerbation development in SEA.

Blood eosinophil count has been shown to be a reliable predictor of response to anti-IL-5/5R biologics. Fraction of exhaled nitric oxide (FeNO) has emerged as an additional biomarker of T2 inflammation, primarily reflecting IL-13 activity. The phase 3 studies of the anti-IL-4R mAb dupilumab (which targets both IL-4 and IL-13 signalling) demonstrated that elevated baseline FeNO is associated with improved clinical benefit.(3) Whilst the phase 2b DREAM study(4) of mepolizumab did measure FeNO, it was not systematically measured in the phase 3 programs of either mepolizumab or benralizumab; it is therefore unknown if baseline FeNO levels predict response to anti-IL-5/5R therapies. In addition, it is unknown whether anti-IL-5/5R mAbs themselves lead to a significant change in FeNO level. IL-13 is produced by several cells, including eosinophils and basophils, so it is mechanistically plausible that FeNO could fall with therapies that deplete these cells.(5)

We conducted a retrospective review of all patients with SEA who commenced mepolizumab or benralizumab between April 2017 and April 2019 at our regional, tertiary asthma centre in the United Kingdom. We compared treatment responses to mepolizumab and benralizumab across patients grouped by baseline FeNO level. Clinical outcome measures including exacerbation rate, ACQ-6, mAQLQ, FEV1 and maintenance OCS dose reduction after 12 months of biologic therapy were compared across baseline FeNO groups. We further assessed changes in FeNO levels following anti-IL-5/IL-5R therapy. Data were analysed with SPSS software (version 24; IBM SPSS, Chicago, IL). Parametric variables were compared with *t*-tests (paired or independent) and non-parametric variables with the Mann-Whitney U test (unrelated) or Wilcoxon Signed Rank (related). Differences between multiple groups were identified using ANOVA test with Tukey's post-hoc test (normally distributed) or Kruskal-Wallis one-way analysis of variance (non-normally distributed). Jonckheere-Terpstra test with Kendall's tau test were used to identify trends (non-normally distributed).

We identified 229 individual cases of treatment with either benralizumab (n=130) or mepolizumab (n=99). Baseline characteristics are reported in table 1. Clinical outcomes of these patients have previously been described (1, 2). Of the 229 individual cases, 37 patients initially appear in the mepolizumab cohort and went on to be treated with benralizumab due to a suboptimal response to the former. For these patients, each treatment episode was analysed individually for both therapies given the differences in mechanism of action and the potential for each drug to affect FeNO to a different extent. The cohort was grouped according to baseline FeNO (0-24ppb, 25-49ppb, 50-74ppb, ≥75ppb). There were no differences in any of the baseline characteristics across the four FeNO subgroups apart for mean BMI (FeNO 0-24ppb subgroup: 32.48+/-8.79; FeNO ≥75ppb subgroup: 28.20+/-6.29, p=0.007) and FEV1% predicted (FeNO 0-24ppb subgroup: 51.4%+/-29.1%; FeNO ≥75ppb subgroup: 56.3+/-36.1], p=0.025).

Overall, no statistically significant differences in clinical effectiveness of anti-IL-5/5R mAbs were observed in relation to baseline FeNO: Mean change in % exacerbation rate (FeNO 0-24: 65.8% [±71.0%], FeNO 25-49: 56.3% [±95.8%], FeNO 50-74: 51.1% [±61.7%], FeNO ≥75: 67.2% [±46.3%] $p=0.71$); median reduction in maintenance OCS dose (FeNO 0-24: 100% [IQR 33-100%], FeNO 25-49: 100% [IQR 25-100%], FeNO 50-74: 90% [IQR 25-100%], FeNO ≥75: 100% [75-100%], $p=0.125$); mean reduction in ACQ-6 (FeNO 0-24: 0.35 [±0.96], FeNO 25-49: 0.55 [±1.17], FeNO 50-74: 0.60 [±1.51], FeNO ≥75: 0.89 [±1.20] $p=0.14$); mean increase in mAQLQ (FeNO 0-24: 0.48 [±0.96], FeNO 25-49: 0.70 [±1.20], FeNO 50-74: 0.77 [±1.60], FeNO ≥75: 0.90 [±1.25] $p=0.35$); and mean change in FEV1 (FeNO 0-24: 0.02 [±0.35], FeNO 25-49: 0.11 [±0.40], FeNO 50-74: 0.03 [±1.60], FeNO ≥75: 0.08 [±0.43], $p=0.60$) (figure 1 A-E).

Across the combined mepolizumab and benralizumab cohort there was no significant change in median FeNO at 1 year (baseline: 43ppb [IQR 26-75], 1 year: 38ppb [24-66], $p<0.21$). However, reduction in FeNO levels were observed in those with the highest baseline levels: FeNO 50-74ppb subgroup, median baseline FeNO 61ppb (IQR 57-65) to 48ppb (29-68), $p=0.058$; FeNO ≥75 subgroup: baseline FeNO 96ppb (85-134) to 64ppb (37-104), $p<0.001$. Conversely, no significant differences or trends were seen in patients with baseline FeNO ≤50ppb (figure 1F). When this relationship was analysed by relative change in FeNO, a significantly greater FeNO reduction in the FeNO high groups was confirmed ($r=-0.357$, $p<0.001$) (supplementary figure E1). This pattern persisted in the benralizumab-only cohort, in which individuals with baseline FeNO ≥75ppb had a reduction in baseline FeNO from 100ppb (IQR 88-145) to 58ppb (33-102) at 1 year, $p<0.001$. In the mepolizumab cohort a non-significant trend towards reduction was observed in the FeNO ≥75ppb group: 88ppb [82-117] to 68ppb [37-118], $p=0.086$, (supplementary figure E2).

Our real world finding that the clinical effectiveness of anti-IL-5/5R mAbs is independent of baseline FeNO is in concordance with the DREAM study which showed that patients with FeNO ≥50ppb had equivalent outcomes on mepolizumab to those with levels <50ppb.(4) Our data suggest that this relationship is preserved even in those with the highest FeNO levels (≥75ppb). FeNO therefore cannot be used to predict response to anti IL-5/5R, unlike dupilumab.(3)

The finding that treatment with anti-IL-5/5R was associated with a reduction in FeNO in those with the highest baseline FeNO levels is intriguing and may have been missed in previous controlled trials where the baseline levels of FeNO were on average lower. Although major sources of IL-13 in T2 inflammation include Th2 and ILC2 cells(6), it is known that eosinophils themselves produce functional IL-13.(5) Consequently, eosinophil-depleting mAbs may lower IL-13 (and therefore FeNO) levels by removing this cellular source of IL-13. Similarly, IL-13 is produced by IL-5R expressing basophils, which are also significantly depleted by benralizumab therapy.(6, 7) In our study this observation appeared to be driven by the benralizumab cohort, echoing data from clinical trials showing benralizumab(8) but not mepolizumab(9) reduces FeNO. Taken together this suggests that depletion of cells expressing IL-5R may be driving the FeNO reduction. Mechanistic studies investigating the functional source of IL-13 in severe asthma are required to further explore these possibilities. It should be noted that FeNO reductions observed here are more modest than the near normalisation of FeNO typically associated with treatment with dupilumab.(3)

In summary we have shown that in a real world setting of over 200 SEA patients treated with anti-IL-5/5R mAbs, baseline FeNO does not predict response to these therapies, with equivalent treatment effectiveness of anti-IL-5/5R mAbs in patients with very high and more modest FeNO levels. FeNO level should not therefore be used in isolation to direct treatment away from an anti-IL5/5R approach. Well-designed head to head studies are needed to decisively establish whether an anti-IL-5/5R or anti IL-4R approach would most benefit a FeNO-high patient with SEA.

Journal Pre-proof

139

	Whole Cohort n= 229	Mepolizumab n = 99	Benralizumab n = 130	P value Mepolizumab vs Benralizumab
Age (years)	53.1 (13.4)	53.5 (13.2)	52.8 (14.0)	0.716
Female subjects, n (%)	132 (57.6)	52 (52.5)	80 (61.5)	0.150
BMI (kg/m ²)	30.57 (6.90)	29.88 (5.76)	31.1 (7.1)	0.124
Atopy, n (%)	163 (71.2)	67 (68.4)	96 (73.8)	0.306
Adult-onset disease (≥ 18 years), n (%)	134 (58.5)	66 (66.7)	68 (52.3)	0.057
Nasal polyposis, n (%)	85 (37.1)	46 (46.5)	39 (30.2)	0.013
Smoking history, n (%)	n=218	n=89	n=128	0.011
Never smoker	141 (64.7)	53 (59.6)	88 (68.8)	
Ex-smoker	70 (32.1)	32 (36.0)	38 (29.7)	
Current smoker	6 (2.8)	4 (4.5)	2 (1.6)	

140

141

142

143

144

145

146

Peak blood eosinophil count in the year preceding anti-IL5/alpha (cells x 10 ⁹)	0.6 (0.4 – 0.8)	0.6 (0.4 – 0.8)	0.6 (0.4-0.9)	0.223
Baseline blood eosinophil count (cells x 10 ⁹)	0.2 (0.1-0.4)	0.2 (0.1-0.4)	0.2 (0.1-0.4)	0.550
FeNO (ppb)	52 (31-77.5)	51.5 (37-78)	53 (30.5-77)	0.827
Exacerbation rate in year prior to anti-IL5/IL5R	4.54 (3.30)	4.04 (2.57)	4.92 (3.35)	0.546
Previous Omalizumab treatment, n (%)	36 (15.7)	20 (20.2)	16 (12.3)	0.204
High-dose ICS/LABA treatment, n (%)	229 (100)	99 (100)	130 (100.0)	
Number on mOCS (%)	142 (62.0)	68 (68.7)	74 (56.9)	0.026
mOCS dose (prednisolone, mg/day)	5 (0-10)	10 (10-15)	10 (5-20)	0.057
FEV1 (L)	1.85 (0.70)	1.98 (0.68)	1.76 (0.69)	0.732
FEV1 (% predicted)	64.8 (20.9)	66.0 (19.4)	63.8 (20.6)	0.670
ACQ-6	2.83 (1.34)	2.73 (1.27)	2.90 (1.39)	0.227
Mini-AQLQ	3.72 (1.41)	4.05 (1.34)	3.46 (1.49)	0.397

Table 1: Baseline Characteristics

For normally distributed data, number quoted is mean (standard deviation). For non-parametric variables, number quoted is median (inter-quartile range).

ABBREVIATIONS: ACQ6 = Asthma Control Questionnaire 6; anti-IL5/R = anti Interleukin 5 / Interleukin 5 Receptor; BMI = Body Mass Index; FeNO = Fractional exhaled Nitric Oxide; FEV1 = Forced Expiratory Volume in 1 second; ICS = Inhaled Corticosteroid; LABA = long-acting β_2 agonist; mOCS = maintenance Oral Corticosteroid; Mini-AQLQ = Mini Asthma Quality of Life Questionnaire; ppb = parts per billion; SEA = Severe Eosinophilic Asthma

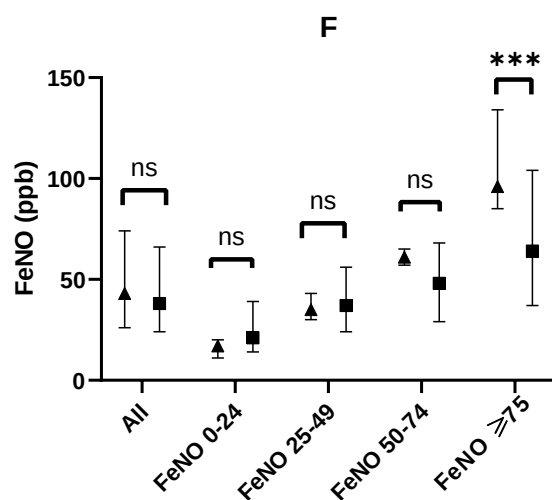
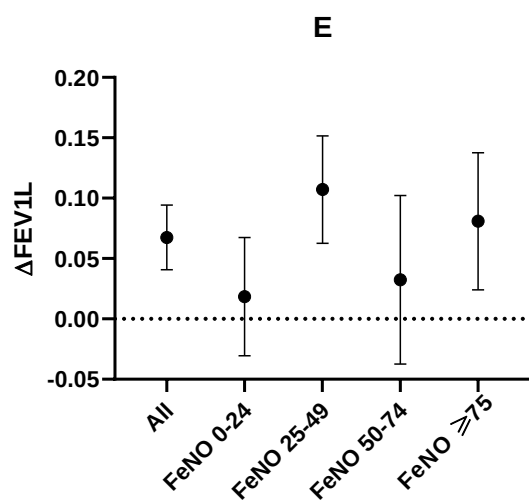
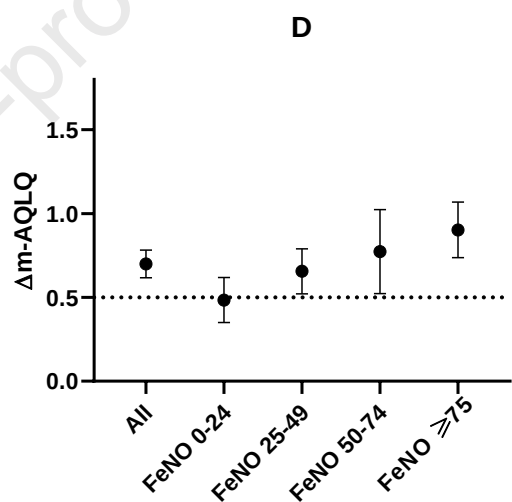
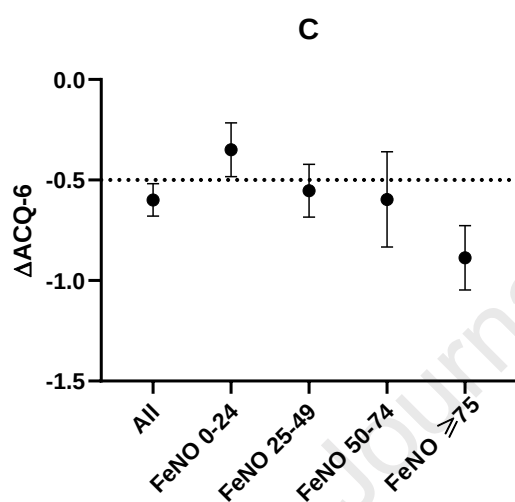
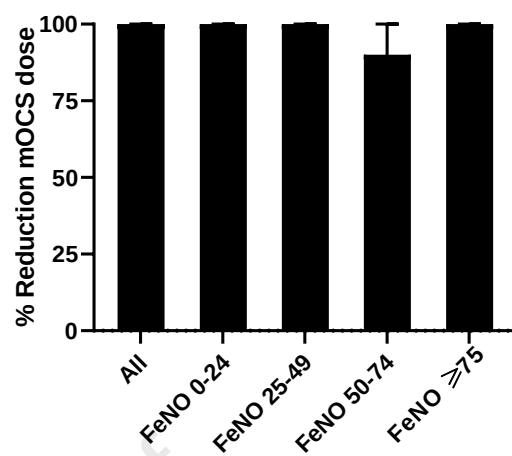
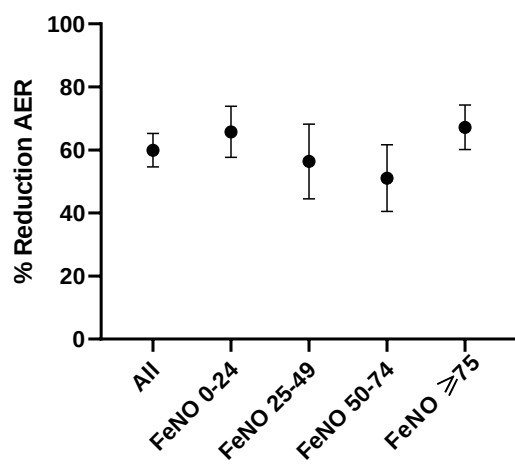
Figure 1. Relationship between baseline FeNO and clinical effectiveness of anti-IL-5/5R therapy

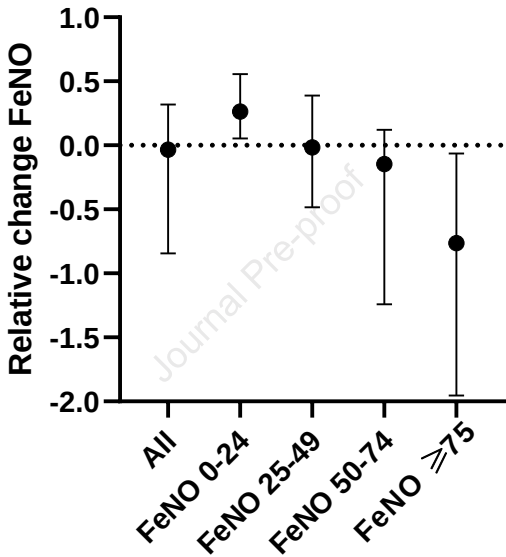
Treatment outcomes in relation to FeNO groups (A-E) and change in FeNO at 1 year (F). A: percentage reduction in annualised exacerbation rate (AER), B: percentage reduction in mOCS dose. C: absolute change in asthma control questionnaire-6, D: absolute change in mini-asthma quality of life questionnaire, E: absolute change in forced expiratory volume in 1 second. F: baseline (triangle) and 1 year (square) FeNO. Each cohort is broken down according to baseline FeNO (0-24ppb [n=51], 25-49ppb [n=80], 50-74ppb [n=41], ≥ 75 ppb [n=57]) P values given as: * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$. Outcomes in panels A-E show no significant differences across groups.

Journal Pre-proof

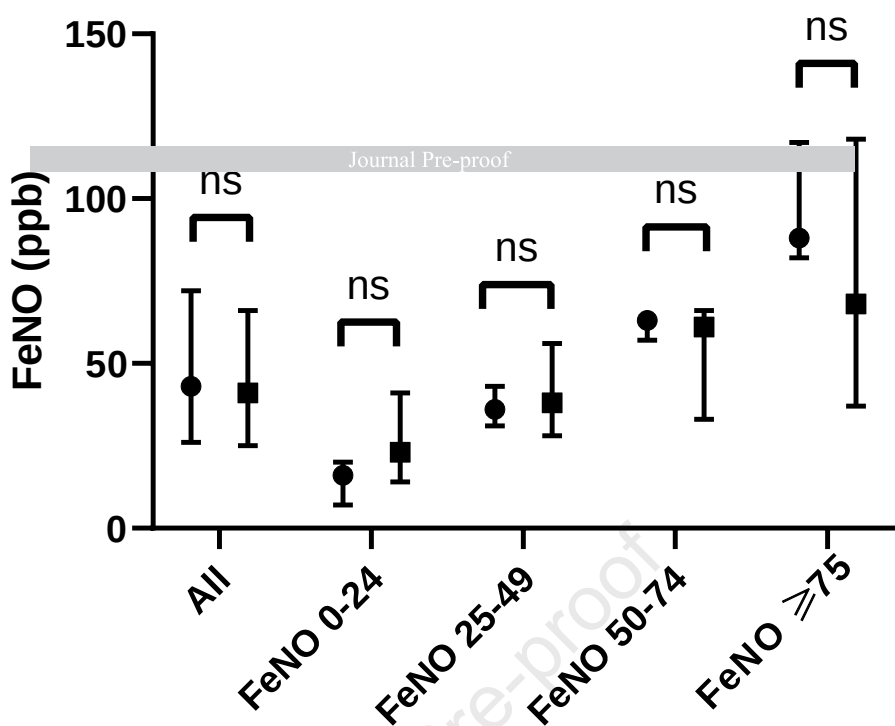
References

1. Kavanagh JE, d'Ancona G, Elstad M, Green L, Fernandes M, Thomson L, et al. Real-World Effectiveness and the Characteristics of a "Super-Responder" to Mepolizumab in Severe Eosinophilic Asthma. *Chest*. 2020;158(2):491-500.
2. Kavanagh JE, Hearn AP, Dhariwal J, Gráinne dA, Douiri A, Roxas C, et al. Real World Effectiveness of Benralizumab in Severe Eosinophilic Asthma. *Chest*. 2020. In press
3. Castro M, Corren J, Pavord ID, Maspero J, Wenzel S, Rabe KF, et al. Dupilumab Efficacy and Safety in Moderate-to-Severe Uncontrolled Asthma. *N Engl J Med*. 2018;378(26):2486-96.
4. Pavord ID, Korn S, Howarth P, Bleecker ER, Buhl R, Keene ON, et al. Mepolizumab for severe eosinophilic asthma (DREAM): a multicentre, double-blind, placebo-controlled trial. *Lancet*. 2012;380(9842):651-9.
5. Schmid-Grendelmeier P, Altnauer F, Fischer B, Bizer C, Straumann A, Menz G, et al. Eosinophils express functional IL-13 in eosinophilic inflammatory diseases. *J Immunol*. 2002;169(2):1021-7.
6. Lei A, He Y, Yang Q, Li X, Li R. Role of myeloid cells in the regulation of group 2 innate lymphoid cell-mediated allergic inflammation. *Immunology*. 2020;161(1):18-24.
7. Lambrecht BN, Hammad H, Fahy JV. The Cytokines of Asthma. *Immunity*. 2019;50(4):975-91.
8. Castro M, Wenzel SE, Bleecker ER, Pizzichini E, Kuna P, Busse WW, et al. Benralizumab, an anti-interleukin 5 receptor alpha monoclonal antibody, versus placebo for uncontrolled eosinophilic asthma: a phase 2b randomised dose-ranging study. *Lancet Respir Med*. 2014;2(11):879-90.
9. Haldar P, Brightling CE, Hargadon B, Gupta S, Monteiro W, Sousa A, et al. Mepolizumab and exacerbations of refractory eosinophilic asthma. *N Engl J Med*. 2009;360(10):973-84.





Mepolizumab Cohort



Benralizumab Cohort

