FeNO Non-Suppression Identifies Corticosteroid-Resistant Type-2 Signaling in Severe Asthma

RUNNING TITLE: FeNO non-suppression in severe asthma

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ABREVIATIONS

ACQ-5: asthma control questionnaire (5-item version)

Eos: eosinophil

FeNO: fractional exhaled nitric oxide

FEV1: forced expiratory volume in 1 second

FVC: forced vital capacity

ICS: inhaled corticosteroids

IL: interleukin

INF-γ: interferon-gamma

LTE4: leukotriene E4

PGD2: prostaglandin D2

TARC: thymus activation regulated cytokine

TNF: tumor necrosis factor

TSLP: thymic stromal lymphopoietin

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To the Editor:

Recently, two *post-hoc* analyses of clinical trials in moderate to severe asthma showed that fractional exhaled nitric oxide (FeNO) and the blood eosinophil (Eos) count provide additive prognostic information on the occurrence of severe asthma attacks (1, 2). The effect is large, with a three-fold increased risk in attacks seen in patients with FeNO ≥50 ppb and blood Eos $\geq 0.3 \times 10^9$/L compared to those with a FeNO <25 ppb and blood Eos $<0.15 \times 10^9$/L (2). Importantly, this risk can be reduced with type-2 cytokine and alarmin-directed biologic agents (3–6). The additive, independent and differentially modifiable risk associated with these biomarkers suggests that they identify different yet complementary aspects of type-2 airway inflammation.

Although raised FeNO classically identifies corticosteroid responsiveness, the advent of FeNO-suppression testing for uncontrolled type-2 high asthma has proven that a third of patients have corticosteroid-resistant elevations in FeNO – and disease burden – despite objective evidence of treatment adherence (7, 8). FeNO non-suppression provides a convenient model to control for non-adherence and independently study corticosteroid resistance in severe asthma.

We tested the hypothesis that FeNO and blood Eos relate differently to inflammation observed in the sputum (reflecting airway) and blood (reflecting systemic) compartments. An important feature of our approach was to study patients in whom we had a high degree of confidence in treatment adherence to high-dose inhaled corticosteroids (ICS) and/or systemic corticosteroids.

METHODS

Induced sputum Eos and sputum/serum mediators were analyzed in a pooled cross-sectional analysis of patients with severe asthma and healthy controls.

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We included patients with severe asthma who had sputum analyzed after a FeNO-suppression test (8) or the RASP-UK trial (NCT02717689) (9). Adherence was verified using different approaches. The FeNO suppression cohort underwent remotely monitored ICS via a chipped inhaler and, if FeNO suppressed <42% by day 7, a nurse-administered triamcinolone injection (8). The RASP-UK cohort underwent 8-weekly biomarker or clinically-guided treatment advisories for 1 year (9) followed by a range of objective adherence measurements (prescription refills; cortisol/prednisolone levels if applicable; FeNO-suppression testing if FeNO elevated) prior to being recruited for the associated bronchoscopy study (NCT02883530). Healthy controls were non-smokers, reported no atopy or lung disease, and had normal lung function. All subjects provided written informed consent in ethically approved studies.

Patients and controls underwent same-day detailed clinical assessment, sputum induction and phlebotomy when on maximum intensity treatment; only the FeNO-suppression protocol included serum. Twenty-six sputum, serum and clinical measurements were assessed (see Table). Inflammatory proteins were measured in duplicates using multiplex electrochemiluminescent assays (Meso Scale Discovery, USA) or single enzyme-linked immunosorbent assays (Cayman Chemical, USA). Spearman correlations were computed between FeNO, blood Eos and analytes, controlling for a false discovery rate <0.05. To translate significant correlations, Jonckheere-Terpstra ordinal trend tests were performed across FeNO (<25, 25-<50, ≥50 ppb) and blood Eos (<0.15, 0.15-<0.3, $\geq 0.3\times10^9$/L) categories. Statistical analyses were performed using SPSS v27 (USA) with a two-sided α of 0.05.

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RESULTS

We included 74 patients with severe asthma and 10 healthy controls. Patients included from the FeNO-suppression cohort (*n*=34) and RASP-UK cohort (*n*=40) were similar. Asthmatics were 55% male, 74% atopic, 85% never-smokers. The mean (±SD) age was 53±15 years; Asthma Control Questionnaire score (ACQ-5) 1.6±1.2; beclomethasone dipropionate-equivalent dose 2391±1084μg/day; post-bronchodilator FEV1 85±19% predicted; FEV1/FVC ratio 70±11%; and 53% were assessed on systemic corticosteroids. There were 60 sputum supernatants and 30 serum samples available for analysis in asthma.

We observed significant correlations between FeNO and sputum Eos, interleukin (IL)-4,-5,-33, thymic stromal lymphopoietin (TSLP), eotaxin-3, thymus activation regulated cytokine (TARC), and asthma attacks in the past year. Blood eosinophils correlated with serum IL-5 (Table). We observed no correlation between ACQ-5 score and the 26 analytes. Sputum eosinophils inversely correlated with lung function and closely mirrored the correlations observed with FeNO (Figure).

FeNO non-suppression was associated with higher sputum Eos (fold-difference in median values, FeNO <25 to ≥50 ppb: 17-fold, *p* for trend =0.001), IL-4 (7.6-fold, *p*=0.0006), IL-5 (8.9-fold, *p*=0.006), IL-33 (1.8-fold, *p*=0.02), TSLP (5-fold, *p*=0.002), eotaxin-3 (10-fold, *p*=0.00003), TARC (3.5-fold, *p*=0.005), and asthma attacks in the past year (3-fold, *p*=0.03). Greater blood Eos (<0.15 to ≥0.3×10^9/L) was associated with higher serum IL-5 (1.9-fold, *p*=0.04)(Table).

The highest FeNO and blood Eos categories generally had greater sputum Eos, sputum/serum type-2 cytokine, chemokine and alarmin levels than healthy controls (Table).

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The directions of trends were consistent when removing systemic corticosteroid-treated patients or when separating the RASP-UK and FeNO-suppression cohorts. Exploratory multiple regression showed no additive effect for biomarkers to identify inflammation levels.

DISCUSSION

We found that, in severe asthma, FeNO non-suppression identifies increased airway type-2 cytokines (IL-4, IL-5), chemokines (eotaxin-3, TARC), alarmins (IL-33, TSLP) and sputum eosinophilia. In contrast, blood Eos correlate with serum IL-5 and not with any assessed measure of airway inflammation. We base these conclusions on our cross-sectional study of patients with extremely high corticosteroid exposure and proven adherence.

Our results are consistent with the cross-sectional bronchial biopsy-based ADEPT study (10), but extend their findings by showing correlations between FeNO and almost all of the assessed components of the type-2 immune response for a population with confirmed treatment adherence. The most striking finding of our study was the different relationship between FeNO, blood Eos and markers of airway and systemic type-2 inflammation. Our findings imply that FeNO and blood Eos relate to different components and compartments of type-2 inflammation: FeNO reflects airway type-2 activity and the chemotactic pull to the airways, whilst blood Eos reflect the systemic pool of available eosinophils and circulating IL-5.

Our study has several limitations. Its cross-sectional design assessed correlation, not causality. The analysis of serum analytes was underpowered (β=0.43 for r=0.40 with critical p<0.041), and we pooled two cohorts which used different approaches to confirm treatment adherence; though a sensitivity analysis analyzing both independently was supportive of our results. Unexpectedly, sputum IL-13 did not correlate with FeNO. This may reflect the complex dimeric receptor system

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signaling both IL-4/-13, a greater steroid-sensitivity of IL-13, and/or a slightly underpowered analysis.

To conclude, we found that FeNO and blood eosinophils provide different and complementary mechanistic information in severe asthma. How airway signaling (reflected by FeNO) and an increased systemic eosinophil pool (reflected by blood Eos) relate to the pathogenesis of asthma attacks and the response to treatment remains an important question.

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REFERENCES

1. Shrimanker R, Keene O, Hynes G, Wenzel S, Yancey S, Pavord ID. Prognostic and predictive value of blood eosinophil count, fractional exhaled nitric oxide, and their combination in severe asthma: A post hoc analysis. *Am J Respir Crit Care Med* 2019;200:1308–1312.

2. Busse W, Wenzel S, Bateman E, Casale T, FitzGerald J, Rice M, Deniz Y, Patel N, Harel S, Rowe P, Graham N, O'Riordan T, Pavord I. Baseline FeNO as a Prognostic Biomarker for Subsequent Severe Asthma Exacerbations in Patients With Uncontrolled, Moderate-to-Severe Asthma Receiving Placebo in the LIBERTY ASTHMA QUEST Study: A Post Hoc Analysis. *Lancet Respir Med* 2021: In Press;

3. Castro M, Corren J, Pavord ID, Maspero J, Wenzel S, Rabe KF, Busse WW, Ford L, Sher L, FitzGerald JM, Katelaris C, Tohda Y, Zhang B, Staudinger H, Pirozzi G, Amin N, Ruddy M, Akinlade B, Khan A, Chao J, Martincova R, Graham NMH, Hamilton JD, Swanson BN, Stahl N, Yancopoulos GD, Teper A. Dupilumab Efficacy and Safety in Moderate-to-Severe Uncontrolled Asthma. *N Engl J Med* 2018;378:2486–2496.

4. Pavord ID, Korn S, Howarth P, Bleecker ER, Buhl R, Keene ON, Ortega H, Chanez P. Mepolizumab for severe eosinophilic asthma (DREAM): A multicentre, double-blind, placebo-controlled trial. *Lancet* 2012;380:651–659.

5. Corren J, Parnes JR, Wang L, Mo M, Roseti SL, Griffiths JM, van der Merwe R. Tezepelumab in Adults with Uncontrolled Asthma. *N Engl J Med* 2017;377:936–946.

6. Regeneron/Sanofi. Regeneron and Sanofi Announce Positive Topline Phase 2 Results for

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IL-33 Antibody in Asthma | Regeneron Pharmaceuticals Inc. 2019;at <https://investor.regeneron.com/news-releases/news-release-details/regeneron-and-sanofi-announce-positive-topline-phase-2-results> (visited 12 May 2021) .

7. Heaney LG, Busby J, Bradding P, Chaudhuri R, Mansur AH, Niven R, Pavord ID, Lindsay JT, Costello RW. Remotely monitored therapy and nitric oxide suppression identifies nonadherence in severe asthma. *Am J Respir Crit Care Med* 2019;199:454–464.

8. McNicholl DM, Stevenson M, McGarvey LP, Heaney LG. The utility of fractional exhaled nitric oxide suppression in the identification of nonadherence in difficult asthma. *Am J Respir Crit Care Med* 2012;186:1102–1108.

9. Heaney LG, Busby J, Hanratty CE, Djukanovic R, Woodcock A, Walker SM, Hardman TC, Arron JR, Choy DF, Bradding P, Brightling CE, Chaudhuri R, Cowan DC, Mansur AH, Fowler SJ, Niven RM, Howarth PH, Lordan JL, Menzies-Gow A, Harrison TW, Robinson DS, Holweg CTJ, Matthews JG, Pavord ID, Adcock IM, Azim A, Bellamy M, Borg C, Bourne M, *et al.* Composite type-2 biomarker strategy versus a symptom–risk-based algorithm to adjust corticosteroid dose in patients with severe asthma: a multicentre, single-blind, parallel group, randomised controlled trial. *Lancet Respir Med* 2021;9:57–68.

10. Silkoff PE, Laviolette M, Singh D, FitzGerald JM, Kelsen S, Backer V, Porsbjerg CM, Girodet PO, Berger P, Kline JN, Chupp G, Susulic VS, Barnathan ES, Baribaud F, Loza MJ, Strambu I, Lam S, Eich A, Ludwig-Sengpiel A, Leigh R, Dransfield M, Calhoun W, Hussaini A, Chanez P. Identification of airway mucosal type 2 inflammation by using clinical biomarkers in asthmatic patients. *J Allergy Clin Immunol* 2017;140:710–719.

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TABLE
Analytes according to FeNO and blood Eos-based stratification strategies

	Analyte (pg/mL or stated) LLOD†	FeNO (ppb) <25 (n=17)	25-<50 (n=30)	≥50 (n=27)	*r* (*p*)	Blood Eos (×10⁹/L) <0.15 (n=21)	0.15-<0.30 (n=22)	≥0.30 (n=31)	*r* (*p*)	Healthy controls (n=10)
Biomarker	**FeNO (ppb)**	16 [13-20]	39 [32-42] *	83 [60-123] *		38 [23-55]	38 [26-74] *	45 [25-89] *	0.24 (0.04)	19 [11-28]
Biomarker	**Blood Eos (×10⁹/L)**	0.17 [0.1-0.54]	0.24 [0.1-0.35]	0.26 [0.19-0.55] *	0.24 (0.04)	0.09 [0.05-0.12]	0.23 [0.19-0.25]	0.54 [0.36-0.66] *		0.14 [0.09-0.18]
Sputum	**Eos (%)**	0.8 [0.4-5.3]	2.7 [1.1-17.8] *	12.8 [3.3-35.5] *	**0.51 (0.0002)**	2.7 [0.7-6.1]	5.1 [0.5-30.5] *	4.3 [1-21] *	ns	0.3 [0.3-0.4]
Sputum	**IL-4** 0.2	0.1 [0.1-0.3]	0.4 [0.1-1.1] *	0.8 [0.2-1.2] *	**0.48 (<0.0001)**	0.3 [0.1-1] *	0.4 [0.1-0.9]	0.3 [0.1-1] *	ns	0.1 [0.1-0.1]
Sputum	**IL-5** 0.5	1.2 [0.4-4.6]	4.6 [1.9-7.8]	10.9 [2.9-29.8] *	**0.47 (0.0002)**	2.3 [1.1-9.7]	5.3 [1.5-15.1] *	4.7 [1.8-10.8] *	ns	0.3 [0.2-2.7]
Sputum	**IL-13** 4.2	6.4 [2.1-8.8]	7 [5.8-14.2] *	8.4 [6.4-13.9] *	0.26 (0.04)	7 [5.1-11.5] *	8.3 [4-12.5] *	7.6 [6-12.2] *	ns	2.1 [2.1-2.1]
Sputum	**IL-33** 0.6	0.9 [0.3-1.3]	0.9 [0.3-2.1] *	1.7 [0.7-2.9] *	**0.35 (0.006)**	0.9 [0.3-1.9]	1.4 [0.5-2.6] *	1 [0.3-2.3] *	ns	0.3 [0.3-0.3]
Sputum	**TSLP** 0.9	2.4 [1-9.3]	6.4 [2.3-10.7] *	11.9 [5-20.7] *	**0.41 (0.001)**	4.9 [1.5-16.9]	9.1 [1.9-2.6]	7.1 [2.5-15] *	ns	0.9 [0.5-1.8]
Sputum	**Eotaxin-3** 4.2	34 [2-71]	133 [23-369] *	353 [245-804] *	**0.55 (<0.0001)**	76 [23-264]	215 [9-418] *	191 [29-390] *	ns	2 [2-26]
Sputum	**TARC** 0.4	17 [9-89]	27 [18-77]	58 [38-301] *	**0.32 (0.02)**	35 [19-107] *	41 [9-101]	36 [17-88]	ns	6 [2-21]
Sputum	**LTE4** 7.8	59 [23-114]	138 [42-465] *	133 [42-730] *	ns	64 [23-139]	94 [48-343]	163 [49-676] *	ns	7 [4-70]
Sputum	**PGD2** 19.5	241 [173-384]	217 [119-354]	209 [135-439]	ns	213 [133-505]	219 [183-389]	222 [117-439]	ns	89 [43-200]
Sputum	**IFN-γ** 0.3	0.3 [0.2-0.5]	0.4 [0.2-1.8]	0.6 [0.2-1.5]	ns	0.5 [0.2-1.7]	0.4 [0.2-2.6]	0.3 [0.2-0.8]	ns	0.2 [0.2-2.1]
Sputum	**TNF** 0.4	1.5 [0.4-10.2]	2 [0.8-7.5]	3.3 [1.5-6.7]	ns	2.5 [1-6.7]	3.2 [0.5-8.5]	2 [0.7-8.6]	ns	2.9 [0.4-16.7]

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Serum	**IL-4** 0.1	0.1 [0.1-0.1]		0.1 [0.1-0.1]		0.1 [0.1-0.1]	ns	0.1 [0.1-0.1]	0.1 [0.1-0.1]	0.1 [0.1-0.1]	ns	0.1 [0.1-0.1]
	IL-5 0.4	1.1 [1-1.2]	*	0.6 [0.5-0.9]	*	0.6 [0.4-1.6]	* ns	0.4 [0.4-0.6]	0.6 [0.6-1.6]	* 0.8 [0.6-1.5]	* **0.41 (0.03)**	0.2 [0.2-0.4]
	IL-13 6.7	3.3 [3.3-3.3]		3.3 [3.3-9.9]		3.3 [3.3-14.1]	ns	3.3 [3.3-3.3]	8.8 [3.3-13.3]	3.3 [3.3-10]	ns	9.2 [7.8-9.8]
	IL-33 0.4	0.2 [0.2-0.3]		0.8 [0.2-0.8]		0.2 [0.2-0.8]	ns	0.2 [0.2-0.8]	0.4 [0.2-0.8]	[0.2-0.8]	ns	0.4 [0.4-0.4]
	TSLP 0.5	1.3 [1-2.1]		1.8 [0.6-2.5]		2.7 [1.8-4.5]	* ns	1.7 [1.2-3.6]	2.3 [1.9-4.4]	* 1.8 [0.8-2.7]	ns	1.4 [0.9-1.5]
	Eotaxin-3 4.2	9 [6-30]		18 [7-32]		16 [13-29]	ns	18 [14-31]	29 [12-34]	* 13 [7-18]	ns	8 [4-13]
	TARC 0.2	427 [108-571]		252 [147-463]		226 [89-430	ns	314 [195-664]	190 [92-252]	344 [146-480]	ns	206 [124-285]
	IFN-γ 0.3	0.4 [0.4-0.5]		0.5 [0.2-0.7]		0.3 [0.2-1.2]	ns	0.3 [0.2-0.6]	0.7 [0.2-2.3]	0.2 [0.2-0.6]	ns	0.4 [0.2-0.7]
	TNF 0.4	1.8 [0.9-3.8]	*	0.6 [0.2-2]		1.2 [0.2-4.2]	* ns	1.7 [0.2-2.3]	* 0.6 [0.2-2]	0.9 [0.3-1.9]	* ns	0.2 [0.2-0.3]
Clinical	**ACQ-5 score**	1.2 [0.5-1.8]		1.6 [0.2-2.2]		2 [0.8-3]	ns	1.6 [0.5-2.1]	1.7 [0.7-2.9]	1.2 [0.6-2.2]	ns	
	FEV1 (% predicted)	88 [78-103]		85 [75-98]		81 [72-96]	ns	81 [77-94]	83 [74-97]	85 [76-99]	ns	
	FEV_1/FVC (%)	72 [64-82]		68 [61-79]		72 [60-77]	ns	71 [62-82]	68 [61-77]	72 [61-80]	ns	
	Asthma attacks (past year)	1 [0-3]		1 [0-4]		3 [0-5]	**0.25 (0.03)**	1 [0-5]	1.5 [0-4]	1 [0-4]	ns	

Data are presented as median [interquartile range] in pg/mL unless stated otherwise. Spearman correlation coefficients (*r*) and associated *p*-values are **in bold** if retained after controlling for a false discovery rate <0.05 across the 52 computed correlations. *adjusted *p*-value <0.05 compared to healthy controls on Kruskal-Wallis test adjusted for 6 comparisons. †Cytokine levels that were not quantified were assigned the arbitrary value of 0.5×the lower limit of detection (LLOD) to allow analysis. ACQ-5, 5-item asthma control questionnaire; Eos, eosinophils; FeNO, fractional exhaled nitric oxide; FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; IFN, interferon; IL, interleukin; LTE4, leukotriene E4; ns, $p \geq 0.05$; PGD2, prostaglandin D2; TNF, tumor necrosis factor; TARC, thymus activation regulated cytokine (CCL17); TSLP, thymic stromal lymphopoietin.

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FIGURE LEGEND

Correlation matrix for fractional exhaled nitric oxide (FeNO), blood eosinophils (Eos), and selected analytes in severe asthma. **Bold** Spearman coefficient of correlations (*r*) and *p*-values indicate statistically significant values in the primary analysis which controlled for a false discovery rate <0.05; the rest of the matrix is exploratory. Asthma attacks are defined as acute events requiring ≥3 days of systemic corticosteroids in the past year. ACQ-5, 5-item asthma control questionnaire; FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; IL, interleukin; ns, $p \geq 0.05$; TARC, thymus activation regulated cytokine (CCL17); TSLP, thymic stromal lymphopoietin.

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r \ *p*	FeNO	Blood Eos	Sputum Eos	Sputum IL-4	Sputum IL-5	Sputum IL-13	Sputum IL-33	Sputum TSLP	Sputum Eotaxin-3	Sputum TARC	Serum IL-5	ACQ-5 score	FEV1	FEV1/FVC ratio	Asthma attacks (past yr)
FeNO		0.04	**0.0002**	**<0.0001**	**0.0002**	0.04	**0.006**	**0.001**	**<0.0001**	**0.02**	ns	ns	ns	ns	**0.03**
Blood Eos	0.24		ns	ns	ns	ns	ns	ns	ns	ns	**0.03**	ns	ns	ns	ns
Sputum Eos	**0.51**	0.25		0.0005	<0.0001	0.02	ns	0.002	0.001	0.005	ns	ns	0.04	0.02	ns
Sputum IL-4	**0.48**	0.06	0.49		<0.0001	0.0004	<0.0001	<0.0001	<0.0001	<0.0001	ns	ns	ns	ns	ns
Sputum IL-5	**0.47**	0.14	0.55	0.71		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.006	ns	ns	0.01	ns
Sputum IL-13	0.26	0.05	0.33	0.44	0.60		<0.0001	<0.0001	<0.0001	<0.0001	ns	ns	ns	ns	ns
Sputum IL-33	**0.35**	0.03	0.25	0.81	0.65	0.48		<0.0001	<0.0001	<0.0001	ns	ns	ns	ns	ns
Sputum TSLP	**0.41**	0.14	0.44	0.65	0.84	0.57	0.61		<0.0001	<0.0001	ns	ns	ns	ns	0.049
Sputum Eotaxin-3	**0.55**	0.15	0.51	0.79	0.89	0.63	0.70	0.83		<0.0001	ns	ns	ns	ns	ns
Sputum TARC	**0.32**	0.02	0.42	0.63	0.83	0.51	0.56	0.85	0.70		0.04	ns	ns	0.04	ns
Serum IL-5	0.03	**0.41**	0.27	0.14	0.62	0.36	0.17	0.40	0.83	0.85		ns	ns	0.01	ns
ACQ-5 score	0.19	0.00	0.22	0.04	0.08	0.09	-0.11	0.07	0.06	0.13	0.00		ns	ns	ns
FEV1	-0.17	0.04	-0.29	-0.16	-0.21	0.05	-0.06	-0.09	-0.14	-0.14	-0.06	-0.05		<0.0001	ns
FEV1/FVC ratio	-0.14	0.01	-0.34	-0.18	-0.31	-0.07	-0.08	-0.23	-0.22	-0.27	-0.48	-0.03	0.69		ns
Asthma attacks	**0.25**	0.00	0.17	0.21	0.18	0.11	0.16	0.26	0.25	0.09	0.11	-0.01	-0.15	0.02	

r: 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, 0.1, 0, -0.1, -0.2, -0.3, -0.4

Correlation matrix for fractional exhaled nitric oxide (FeNO), blood eosinophils (Eos), and selected analytes in severe asthma. **Bold** Spearman coefficient of correlations (*r*) and p-values indicate statistically significant values in the primary analysis which controlled for a false discovery rate <0.05; the rest of the matrix is exploratory. Asthma attacks are defined as acute events requiring ≥3 days of systemic corticosteroids in the past year. ACQ-5, 5-item asthma control questionnaire; FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; IL, interleukin; ns, $p \geq 0.05$; TARC, thymus activation regulated cytokine (CCL17); TSLP, thymic stromal lymphopoietin.

251x105mm (300 x 300 DPI)

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