TITLE: Biomarker-guided management reduces exacerbations in non-eosinophilic asthma in pregnancy: a secondary analysis of a randomised controlled trial

Running head: Non-eosinophilic asthma in pregnancy

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SUMMARY AT A GLANCE:

This study shows that a FENO-guided algorithm for asthma treatment adjustment in pregnancy better targets treatment to phenotype compared to a symptom control algorithm, with reduced exacerbations in non-eosinophilic asthma.

ABSTRACT:

Background and objective: The aim of this secondary analysis of a randomised controlled trial (RCT) of asthma management in pregnancy was to determine the treatment decision differences between a symptom control algorithm and a FENO-guided algorithm, and whether the approach was effective in non-eosinophilic asthma (NEA).

Methods: In this double-blind parallel group RCT, women with asthma were randomised prior to 22 weeks gestation to treatment adjustment according to a symptom control algorithm (control group), or a FENO-guided algorithm (inhaled corticosteroid [ICS] dose adjusted according to FENO with long-acting beta-agonist [LABA] added for uncontrolled symptoms). NEA was classified as baseline blood eosinophils $<0.26 \times 10^9$/L and FENO ≤29ppb. Exacerbations requiring medical intervention were recorded.

Results: Among 220 non-smokers (n=109 control, n=111 FENO), 1006 treatment decisions were made, with significant group differences after the first and second algorithm applications. 53% of women had NEA. Treatment was better targeted to phenotype in the FENO group: ICS use increased in eosinophilic asthma (EA, 48%-86%), while ICS/LABA increased in NEA (11%-30%). Fewer women in the FENO group had exacerbations during pregnancy in NEA only (18.9% FENO, vs 44% control, P=0.006).

Conclusions: The FENO algorithm was more effective in treating NEA, resulting in reduced exacerbations, compared to a symptom control algorithm. This was not the result of ICS overtreatment, since the benefits occurred at a lower median daily ICS dose. Two applications of the FENO-guided algorithm, one month apart, were sufficient to achieve beneficial effects in terms of asthma exacerbations, among pregnant women with asthma.

Keywords: asthma, eosinophils, nitric oxide, phenotype, pregnancy

Short title: Biomarker-guided management in non-eosinophilic asthma in pregnancy

INTRODUCTION

Asthma is a chronic inflammatory condition of the airways, with recognised heterogeneity of the inflammatory response. Airway eosinophilia and elevated fractional exhaled nitric oxide (FENO) result from T2 inflammation and predict corticosteroid responsiveness[1]. Management algorithms based on titrating corticosteroids according to the level of T2 inflammation are effective in reducing the number of asthma exacerbations in adults[2, 3] and pregnant women[4]. However, a substantial proportion of asthma patients have non-eosinophilic inflammation. In a prospective follow-up study, 47% of asthma patients not treated with inhaled corticosteroids (ICS) were persistently non-eosinophilic, and among ICS-treated asthma patients, this number was 72%[5].

Non-eosinophilic asthma (NEA) appears to respond less to corticosteroid treatment compared to eosinophilic asthma (EA)[6]. Increasing the bronchodilator component of treatment could theoretically be more effective in patients with poor symptom control, but low levels of eosinophilic airway inflammation. The optimal treatment strategy in NEA has not been evaluated formally, and guidelines on asthma management do not provide recommendations for treating NEA. Hence there is a need for better understanding of potential treatment strategies for NEA.

Asthma is the most common chronic medical condition to affect pregnant women[7], and in particular, exacerbations occur frequently, in up to 45% of women[8], with adverse consequences for mother and baby[9, 10]. The Managing Asthma in Pregnancy (MAP) study tested the efficacy of a FENO-guided treatment algorithm compared to a symptom control algorithm (control group). The FENO-guided algorithm reduced the exacerbation rate by 50%[4], altered the treatment profile (increased ICS and LABA use), reduced mean ICS dosage[4] and improved offspring outcomes in infancy and early childhood[11, 12], compared to a symptom control algorithm. However, there is debate in the literature about the efficacy

of the FENO-guided approach in non-pregnant adults and children, with recent systematic reviews concluding that there is statistically significant benefit in terms of severe exacerbations or ICS use, but more research is warranted to determine which patient groups are likely to benefit the most[13]. Study design and algorithm limitations also confound the interpretation of FENO-guided management trials[14], and were not accounted for in systematic reviews.

The aims of these secondary analyses of the MAP study were to describe the differences in treatment decisions according to algorithm, and to determine whether the effect of the FENO algorithm on exacerbations in pregnancy differed between NEA and EA.

METHODS

Pregnant women with diagnosed asthma and symptoms and/or medication use in the past year, were recruited from the antenatal clinics of John Hunter Hospital, Newcastle, Australia, to a randomised controlled trial, known as the MAP study (Australian and New Zealand Clinical Trials Registry number 12607000561482)[4]. Asthma diagnosis was confirmed by diagnostic interview with the study physician (PGG). Exclusion criteria were current smoking, >3 courses of oral corticosteroid (OCS) or hospital admission for exacerbation in the prior 3 months, maintenance OCS or oral theophylline, chronic lung disease other than asthma, concomitant chronic medical illness and drug/alcohol dependence[4]. Women were randomised (<22 weeks' gestation) 1:1 to have their asthma treatment adjusted monthly according to symptom control (control group), or according to FENO and symptom control (FENO group, Supplementary Table S1, see previous publication for original trial details[4]). Written informed consent was obtained prior to participation and ethics approval granted by the Hunter New England Health Human Research Ethics Committee (approval number 07/02/21/3.06).

This is a secondary analysis of monthly treatment decisions made according to the symptom control algorithm or the FENO-guided algorithm. We also examined exacerbations and treatment changes among a sub-group who had a blood eosinophil measure in early pregnancy (before randomisation, visit 2 [median 20.4 weeks gestation IQR 19.0, 21.6]) and a FENO measurement at randomisation, allowing for inflammatory phenotyping.

FENO was measured according to American Thoracic Society (ATS) criteria[15] at a controlled flow rate of 50 ml/s using the Ecomedics chemiluminescence analyser (Ecomedics, Duernten, Switzerland). Peripheral blood eosinophil counts were measured using an automated analyser (Beckman Coulter LH780, Miami FL, USA) by the Hunter

Area Pathology Service (Newcastle, NSW, Australia). Women were classified as NEA if blood eosinophils were <0.26 x 10^9/L and FENO was ≤29ppb at baseline. The remainder were classified as EA. The blood eosinophil cut-point was previously established to have optimal receiver operating characteristics for predicting sputum eosinophilia (>3%) in our population[16]. The FENO cut-point of 29ppb was previously derived from the lower 95% confidence interval (CI) of FENO among pregnant asthmatic women with unstable eosinophilic asthma, and was the cut-point for ICS dose up-titration[4]. Lung function was measured using an EasyOne Spirometer (NicheMedical, North Sydney, Australia), exhaled carbon monoxide (ECO) using a piCO Smokerlyzer Breath CO Monitor (Bedfont, UK), the asthma control questionnaire (ACQ7)[17] was used to assess asthma control (uncontrolled asthma defined as ACQ7≥1.5)[18], and the common cold questionnaire (CCQ)[19] screened for viral infection. Nasal and throat swabs were collected from women with a probable cold via CCQ, and tested for respiratory viruses with real-time quantitative polymerase chain reaction (PCR)[20].

Maintenance treatment algorithms (Supplementary Table S1) were applied monthly from randomisation (12-22 weeks' gestation) until delivery, except when women were taking exacerbation treatment[4]. The research assistant, participant and prescribing physician were all blinded to the intervention, and FENO and ACQ were measured in both groups[4]. Possible treatment decisions were: no change, increase ICS, decrease ICS, increase ICS/LABA, decrease ICS/LABA, increase LABA, decrease LABA and increase ICS/decrease LABA. Exacerbations requiring medical intervention (hospital admission, emergency department [ED] presentation, OCS course, or unscheduled doctor visit for asthma) were assessed prospectively.

Statistical analysis was conducted using GraphPad Prism version 6.01 (GraphPad Software, Inc, La Jolla, CA) and Stata version 15 (Stata Corp, StataCorp LLC, College Station TX,

USA). Results are presented as mean ± standard deviation (SD) or median (interquartile range, IQR) with Student's t-test or Mann Whitney test, as appropriate. The Chi square test or Fisher's exact test was used to compare proportions. Bonferroni adjusted P values were considered significant for multiple comparisons.

RESULTS

220 non-smoking mothers were randomised to the MAP Study (n=109 control group, n=111 FENO group)[4] and 1006 treatment decisions were made during the trial (217 at visit 2 [randomisation], 209 at visit 3, 202 at visit 4, 192 at visit 5, 144 at visit 6, 40 at visit 7, and 1 each at visits 8 and 9). Baseline characteristics (prior to the first treatment change) were similar between women randomised to the control group and the FENO group, with no significant differences in use of ICS (41.5% control, 41.4% FENO) or ICS/LABA (20.8% control, 19.8% FENO), ICS dose (table 1), FENO and ACQ score (data not shown). There were significant group differences in treatment decisions made after the first and second applications of the algorithm. At the first application (visit 2), the FENO group was significantly less likely to have a "no change" decision (52%) compared to the control group (74%, P=0.001). However, the other treatment decisions were not significantly different after Bonferroni correction (P<0.0083) due to multiple comparisons. At the second application of the algorithm (visit 3), groups differed significantly for "no change" (53% FENO vs 71% control, P=0.007) and increase ICS/LABA (14% FENO vs 1% control, P=0.0006). There were no other significant differences in treatment decisions between groups (Figure 1).

The proportion of women using ICS differed significantly after Bonferroni correction (P<0.0071) between the groups from the second treatment change (visit 3, 71.6% FENO vs 43.9% control, P<0.0001, Supplementary Figure S1) to the fifth treatment change (visit 6, 75% FENO vs 40.8% control, P<0.0001), while the proportion of women using ICS/LABA differed between groups from the second treatment change (39% FENO vs 16% control, P=0.0002). Table 1 shows the median ICS dose in the control group and FENO group at each visit during pregnancy. The dose was significantly lower in the FENO group at visit 3, 5 and 6 (after Bonferroni correction, P<0.0083).

In order to address the second aim of the study, classification of inflammatory phenotype was made for 195 women (88.6%) with both baseline FENO and blood eosinophil count available. Participants were grouped as eosinophilic (EA) (n=92, 47%) or non-eosinophilic asthma (NEA) (n=103, 53%, Figure 2). Further details about the reproducibility of the phenotype are contained in the Supplement. Baseline characteristics are shown in Table 2. Atopy was significantly more common, and the age at diagnosis significantly younger in EA. EA subjects had more prior asthma exacerbations and lower lung function than NEA. Asthma medication use was similar between groups.

Application of the FENO-guided algorithm led to significantly more use of ICS in women with EA (48% to 86%, P=0.0002). Use of the FENO-guided algorithm was associated with higher ICS/LABA in women with NEA (11% to 30%), but this was not statistically significant (P<0.0031) after Bonferroni correction (P=0.017, Table 3). After the first treatment change, the control and FENO groups significantly differed in the proportion of women with eosinophilic asthma treated with ICS (57% with the control algorithm, 86% with the FENO algorithm, P=0.003, Table 3). In order to determine performance of the algorithm, we defined the treatment target ratio as the ratio of the % of correctly targeted treatment with the FENO algorithm to the % correctly targeted treatment with the symptom control algorithm, where ICS is the correctly targeted treatment for EA, while LABA is the correctly targeted treatment for NEA. The FENO algorithm correctly targeted ICS to 86% of women with EA, and LABA to 30% of women with NEA (mean 58%, Table 3). The symptom control algorithm correctly targeted ICS to 57% of women with EA, and LABA to 14% of women with NEA (mean 36%), giving a treatment target ratio of 1.6.

Among women with EA who were randomised to the control algorithm, 19 women had exacerbations requiring medical intervention post randomisation (38.0%, 95% CI 25.9 –

51.9%). This was not different to the proportion of EA randomised to the FENO-guided algorithm with exacerbations (n=16, 38.1%, 95% CI 25.0 – 53.2%, P=0.993). However, among women with NEA, there were significantly fewer women with exacerbations in the FENO-managed group compared to the control group (18.9%, 95% CI [10.6 – 31.4%] vs 44.0%, 95% CI [31.2 – 57.7%], P=0.006, Figure 3).

Exacerbations among women with NEA in the FENO-guided group occurred at a mean of >5 weeks later in pregnancy, than the control group (P=0.203, Supplementary Table S2). FENO during exacerbations was not different between the control and FENO-guided algorithms in women with NEA (P=0.235), and the majority of exacerbations were non-eosinophilic (FENO<29ppb) and associated with a viral trigger (Supplementary Table S2). Among women with EA, exacerbations also occurred later in pregnancy in those treated with the FENO-guided algorithm (P=0.539) and 24% of exacerbation events in the control group and 21% in the FENO group were associated with high FENO, with a viral trigger identified for the majority of exacerbations. ACQ7 scores following the first treatment change are shown in Supplementary Table S3.

DISCUSSION

This is the first study to show that the FENO-guided algorithm is a more effective way to treat NEA and results in a reduction in asthma exacerbations, compared to a symptoms-based treatment algorithm. The FENO algorithm better targets treatment to phenotype, such that more women with EA get ICS, and more women with NEA get LABA. Two to three applications of the algorithm, one month apart, are sufficient to achieve these changes. After 2-3 applications of the algorithm, the percentage of women on ICS does not change any further, and there is no longer any difference between the 2 algorithms in terms of the "no change" treatment decision. The reduction in exacerbations occurred at a lower median daily ICS dose, that is, they are not the result of ICS overtreatment. Targeted ICS use (with more ICS titration) may have contributed to reduced exacerbations.

The MAP study was the first to show that exacerbations were reduced with a FENO-guided algorithm, applied monthly during pregnancy[4]. In this sub-analysis, we demonstrate that FENO-guided management reduced exacerbations among the sub-group of women with NEA. This group made up 53% of the total population, and was characterised by having both low peripheral blood eosinophils and low FENO. There was a significant increase in the requirement for LABA in the NEA group after the first treatment change, suggesting that this early control of symptoms may have explained the reduction in exacerbations.

Recent systematic reviews have summarised the studies testing the use of FENO-guided algorithms for the management of asthma[21] in adults[22] and children[23]. None of these studies reported sub-group analyses for participants classified as having NEA. This is a significant limitation because the goal of the FENO algorithm is to target treatment to phenotype, and failure to adequately do this may explain negative study results. Two prior studies have reported select findings in relation to inflammation. Calhoun et al found that

baseline FENO or sputum eosinophil count did not predict treatment failure in the trial (which included exacerbations) using a multivariate model, suggesting that inflammatory phenotype did not influence outcome by management strategy[24]. Shaw et al performed a sub-group analysis for participants with EA (sputum eosinophils and FENO > 26ppb) and found a lower exacerbation frequency when asthma was managed by FENO (0.38 exacerbations per patient per year vs 0.67 in the control group, P value was not reported)[25]. In addition, they examined a sub-group with low sputum eosinophils (<3%) but high FENO (>26 ppb) and found no difference in exacerbation frequency (0.09 exacerbations per patient per year in the FENO managed group vs 0 in the control group). However, data for a non-eosinophilic group with low sputum eosinophils and low FENO was not reported[25].

In the landmark study by Green et al[2], where adults with asthma were randomised to receive management guided either by sputum eosinophils or according to guidelines, sub-group analysis suggested that among adults with a normal sputum eosinophil count (<1.9%, that is, NEA), there was a significant change in ICS dose when asthma was managed by sputum eosinophils (ICS decreased by 961 μg/day)[2]. Conversely, adults with NEA who were managed by guidelines had an increase in ICS dose over the course of the trial by 464μg/day, giving a significant mean difference between the groups of 1425μg/day (P=0.001). Despite this, there was no statistical difference in exacerbations between management groups in adults with NEA (P=0.47, no further details given), although the study was likely underpowered for this outcome (n=13 in the sputum management group vs n=11 in clinical guidelines group)[2]. Our study had a larger sample size, and also included data on LABA use, which was not reported by Green et al.

Although ICS were better targeted with the FENO algorithm, exacerbations were similar in EA between the control and FENO groups. This may be because there is a greater risk of

exacerbation with EA compared to NEA[26], and/or because even low doses of ICS reduce exacerbation risk in EA. Despite the high prevalence of exacerbations, women in both the control and FENO groups had a significant improvement in asthma control after the first application of the algorithm. The mechanisms for our observation of incomplete suppression of exacerbations in EA remain unclear.

There are several limitations to this study. This was a post-hoc analysis of MAP study data and we did not collect sputum from pregnant women, and hence cannot determine whether our cut-points of blood eosinophils and FENO represent EA and NEA according to sputum cell count. However, we did classify almost 90% of our original cohort according to phenotype using both blood eosinophils and FENO. Not all women used ICS prior to the study, however, this was unlikely to interfere with the results, since ICS use was commenced at baseline when symptoms were uncontrolled.

In conclusion, women with NEA during pregnancy benefit from treatment adjustment according to a FENO-guided algorithm, with a reduction in asthma exacerbations. The FENO-algorithm was superior in targeting treatment to phenotype than the symptom control algorithm. In pregnancy, 2 to 3 applications of the algorithm, one month apart, was effective for achieving reduced exacerbations.

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The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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TABLES:

Table 1: Changes in ICS dose over time by algorithm group

	Control group	FENO group	P value
Visit 2 [randomisation]	800 (400, 1000) μg/day	800 (400, 800) μg/day	0.985
Visit 3	800 (400, 800) μg/day	400 (400, 800) μg/day	**0.003**
Visit 4	800 (400, 800) μg/day	400 (200, 800) μg/day	0.011
Visit 5	800 (400, 800) μg/day	400 (200, 800) μg/day	**0.004**
Visit 6	800 (400, 1600) μg/day	200 (200, 800) μg/day	**<0.001**
Visit 7	400 (400, 800) μg/day	200 (200, 800) μg/day	0.02

Values are median (interquartile range).

P values in bold are statistically significant after Bonferroni correction (p=0.0083)

Table 2: Baseline subject characteristics (Eosinophilic vs non-eosinophilic asthma)

	Eosinophilic asthma (n=92)	**Non-eosinophilic asthma (n=103)**	**P Value**
Maternal age, years	28.2 (5.1)	28.8 (5.7)	0.491†
Maternal BMI, kg/m^2	27.7 [23.8, 31.4]	29.1 [24.8, 32.8]	0.259‡
Gestational age, weeks	16.6 [14.7, 18.6]	16.4 [14.3, 18.3]	0.411‡
Gravidity	2 [1, 3]	2 [1, 3]	0.623‡
Parity	1 [0, 2]	1 [0, 1]	0.413‡
Ex-Smoker, n (%)	33 (39)	39 (40)	0.900§
Pack years	2.0 [1.0, 5.0]	3.6 [1.6, 7.0]	0.117‡
Atopy, n(%)	**79 (93)** *n=85*	**59 (61)** *n=97*	**<0.001**§
Asthma History			
Age at diagnosis, years	**6 [3, 10]** *n=87*	**8 [5, 15]** *n=97*	**0.033**‡
Any hospital admissions for asthma past 2 years, n(%)	3 (3)	1(1)	0.345¶
Any emergency department presentations for asthma past 2 years,	12 (13)	10 (10)	0.462§

n(%)			
Any oral corticosteroid use for asthma past 2 years, n(%)	**25 (27)**	**14 (14)**	**0.018**§
Pregnant in past 2 years, n(%)	31 (34)	42 (41)	0.308§
Asthma control (ACQ7)	**0.86 [0.43, 1.43]**	**0.71 [0.29, 1.14]** *n=102*	**0.027**‡
Uncontrolled asthma (ACQ7 >1.5), n(%)	22 (24)	15 (15)	0.103§
FeNO, ppb	**27.2 [14.8, 44.5]**	**8.3 [5.6, 13.2]**	**<0.001**‡
Blood Eosinophils, 10^9/L	**0.3 [0.3, 0.44]**	**0.1 [0.1, 0.2]**	**<0.001**‡
Lung Function	*N=65*	*N=88*	
Pre-bronchodilator FEV$_1$, % predicted	**92.4 (13.1)**	**99.0 (11.8)**	**0.001**†
Pre-bronchodilator FVC, % predicted	**101.1 (12.7)**	**106.4 (13.6)**	**0.017**†
Pre-bronchodilator FEV$_1$/FVC ratio	0.80 (0.07)	0.82 (0.07)	0.058†
Treatment			
Maintenance ICS use, n(%)	43 (47)	39 (28)	0.210§
Maintenance ICS dose among ICS users, BDP	800 [400, 1600]	800 [400, 800]	0.094‡

equivalent, μg/day			
Proportion of ICS users on ICS/LABA combination therapy, n(%)	25 (58)	17 (44)	0.188§
Intervention (FENO-based) algorithm, n(%)	50 (54)	50 (49)	0.418§
Data presented as mean (sd), median [IQR] or n(%).†Students t-test; ‡Mann-Whitney test; §Chi-Squared test; ¶ Fisher exact test			

Table 3: Use of asthma medications before and after the first application of the treatment algorithm

	Eosinophilic asthma			**Non-eosinophilic asthma**		
Any ICS Medication	**Control algorithm n=49**	**FENO-based algorithm n=42**	***P Value***	**Control algorithm n=49**	**FENO-based algorithm n=53**	***P Value***
Before Treatment Change	22/49 (44.9%)	20/42 (47.6%)	*0.795*	17/49 (34.7%)	21/53 (39.6%)	*0.607*
After Treatment Change	28/49 (57.1%)	36/42 (85.7%)	***0.003***	18/49 (36.7%)	25/53 (47.2%)	*0.286*
P Value	*0.225*	***0.0002***		*0.833*	*0.433*	
ICS/LABA Medication						
Before Treatment Change	10/49 (20.4%)	14/42 (33.3%)	*0.163*	10/49 (20.4%)	6/53 (11.3%)	*0.207*
After Treatment Change	12/49 (24.5%)	14/42 (33.3%)	*0.352*	7/49 (14.3%)	16/53 (30.2%)	*0.055*
P Value	*0.628*	*1.0*		*0.424*	*0.017*	

P values in bold are statistically significant after Bonferroni correction (p=0.0031)

Note: Women using short acting beta agonists only were included in the study as pregnancy is known to increase asthma symptoms in some women

FIGURE LEGEND:

Figure 1: The proportion of women with each treatment decision in the Control group and FENO group over the course of monthly visits during pregnancy

Figure 2: Participant Flow Chart for inflammatory phenotyping

MAP: Managing Asthma in Pregnancy Study, Eos: eosinophils, FENO: fractional exhaled nitric oxide, EA: Eosinophilic Asthma, NEA: Non-Eosinophilic Asthma,

Figure 3: Proportion of women with exacerbations requiring medical intervention among women with eosinophilic and non-eosinophilic asthma according to treatment algorithm

SUPPLEMENTARY INFORMATION

TITLE: Biomarker-guided management reduces exacerbations in non-eosinophilic asthma in pregnancy

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Results

We examined the reproducibility of the asthma phenotyping in 106 participants who had multiple visits with both FENO and blood eosinophil measures available. 73% of women (n=77) had the same phenotype on every occasion where it could be assessed (median number of visits 4, 25^{th}-75^{th} percentile 2-6). The median similarity of phenotypes across visits was 100% (100-100, 25^{th}-75^{th} percentile). Among the 29 women where phenotype changed during pregnancy, 9 (31%) had the same phenotype at least 80% of the time, 13 (44.8%) had the same phenotype between 60-80% of the time, 4 (13.8%) had the same phenotype 50% of the time, 2 (6.9%) had the same phenotype 40% of the time, and 1 woman (with only 2 measurements) had a different phenotype each time. Interestingly, where at least 4 visits were available to phenotype, and the phenotype changed, it changed from eosinophilic to non-eosinophilic 57% of the time, suggesting success of the treatment algorithms in reducing eosinophilia. Simpson et al found short-term reproducibility of sputum-based asthma phenotyping of 94% over 2 visits one month apart, and 86% over 2 visits 5 years apart[1].

Figure S1- *The proportion of women using ICS (a) or ICS/LABA (b) in the control and FENO groups. * indicates a statistically significant difference between groups*

The algorithm used for treatment adjustment in the MAP study is given in Table S1[2]. Women in the control group had their treatment adjusted based on symptoms. When the result of the asthma control questionnaire (ACQ) indicated uncontrolled asthma (>1.5), treatment was increased one level. When ACQ indicated well controlled asthma (<0.75), treatment was decreased by one level. Between these cut-points, no change to treatment was made. The dose levels were salbutamol as required, budesonide 200 μg/day x 2 (twice per day), budesonide 400 μg/day x 2, budesonide 400 μg/day + formoterol 12 μg/day x 2, budesonide 800 μg/day + formoterol 24 μg/day x 2.

Women in the FENO group had their treatment adjusted based on both symptoms and FENO. When FENO was high (>29ppb), ICS dose was increased. When FENO was low (<16ppb), ICS dose was decreased, while in the mid-range (FENO 16-29ppb), no change to ICS dose was made. In the lower and mid FENO ranges, LABA was added when symptoms remained uncontrolled (ACQ >1.5). The ICS dose steps were 0, budesonide 100 μg/day x 2, budesonide 200 μg/day x 2, budesonide 400 μg/day x 2, budesonide 800 μg/day x 2. The beta2 agonist steps were salbutamol as required, formoterol 6 μg/day x 2, formoterol 12 μg/day x 2 and formoterol 24 μg/day x 2.

Table S1-*: Treatment Algorithms*[2]

Symptom control algorithm		
	Symptoms (ACQ score)	Treatment adjustment
	>1.5	↑ 1 level
	0.75-1.5	-

	<0.75	↓ 1 level	
FENO-based treatment algorithm			
FENO (ppb) Non-smokers	Symptoms (ACQ score)	ICS dose change	LABA dose change
>29	N/A	↑ ICS x 1 level	-
16-29	<= 1.5	-	-
16-29	>1.5	-	↑ LABA x 1 level
<16	<=1.5	↓ ICS x 1 level	-
<16	>1.5	↓ ICS x 1 level	↑ LABA x 1 level

Table S2- *Features of exacerbation events according to baseline inflammatory phenotype*

	Eosinophilic asthma		Non-eosinophilic asthma	
	Control algorithm	FENO-based algorithm	Control algorithm	FENO-based algorithm
Number of exacerbation events	27 (in 19 women)	18 (in 16 women)	31 (in 22 women)	11 (in 9 women)
Gestational age at exacerbation (weeks)	27.6 (25.0, 32.7)	29.1 (24.0, 34.9)	24.3 (23, 31.5)	29.9 (25.7, 31.9)
OCS required	10 (37.0%)	6 (33.3%)	3 (9.7%)	3 (27.3%)
FENO (ppb)	13.7 (7.7, 28.7) *n=21*	20.9 (12.4, 28.5) *n=14*	7.7 (5.3, 10.0) *n=29*	12.6 (5.6, 23.3) *n=10*
Proportion with high FENO (>29 ppb)	5/21 (23.8%)	3/14 (21.4%)	1/31 (3.2%)	0/11 (0%)
Viral trigger *	19 (70.4%)	10 (55.6%)	21 (67.7%)	8 (72.7%)
Laboratory confirmed viral infection	5/6 tested MPV, FluB, EV, RV x 2	0/6 tested	5/7 tested MPV x 3, CoV, RV+EV	0/3 tested
ACQ at	1.9 (1.5, 2.5)	1.7 (1.3, 2.7)	1.8 (1.3, 2.3)	1.6 (1.1, 2,1)

exacerbation				

Abbreviations used: OCS oral corticosteroids, FENO fractional exhaled nitric oxide, MPV metapneumovirus, FluB Influenza B, EV enterovirus, RV rhinovirus, CoV Coronavirus, ACQ asthma control questionnaire

ACQ7 scores significantly improved in both groups with EA following the first treatment change (Supplementary Table S3). In the FENO group, ACQ7 was in the partially controlled range (median 0.86, interquartile range [IQR] 0.29 - 1.14) before the treatment change, and in the controlled range (median 0.43, IQR 0.14 – 0.57) after treatment change.

Table S3- *Asthma control questionnaire score before and after the first application of the treatment algorithm*

	Eosinophilic asthma			**Non-eosinophilic asthma**		
ACQ7	**Control algorithm n=50**	**FENO-based algorithm n=42**	***P Value****	**Control algorithm n=50**	**FENO-based algorithm n=53**	***P Value****
Before Treatment Change	1.0 (0.57, 1.71)	0.86 (0.29, 1.14)	0.047	0.71 (0.14, 0.96)	0.93 (0.43, 1.29)	0.034
After Treatment Change	0.71 (0.29, 1.14)	0.43 (0.14, 0.57)	0.0501	0.57 (0.18, 1.14)	0.71 (0.29, 1.14)	0.329
P Value*	0.013	0.022		0.982	0.353	
*P Values Bonferroni adjusted for 8 comparisons, significance accepted at p=0.00625						

References

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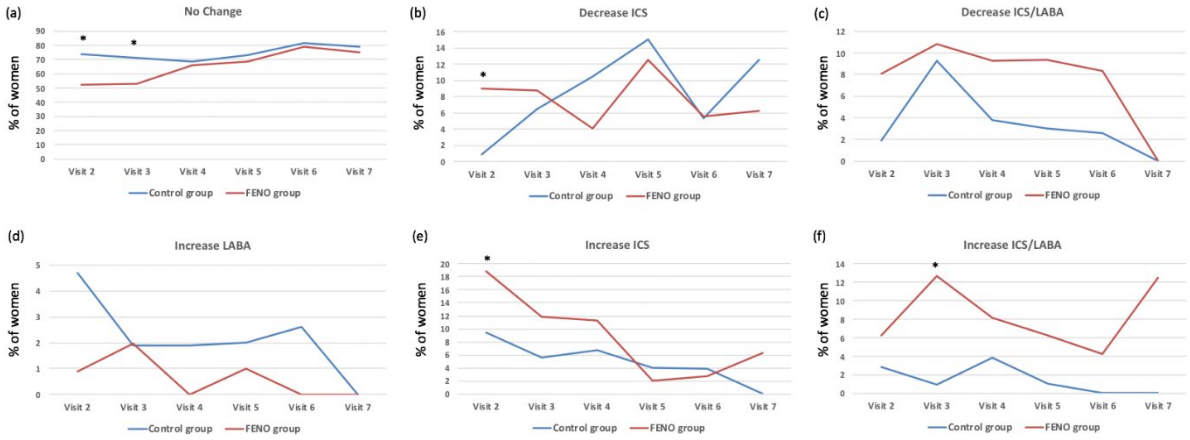
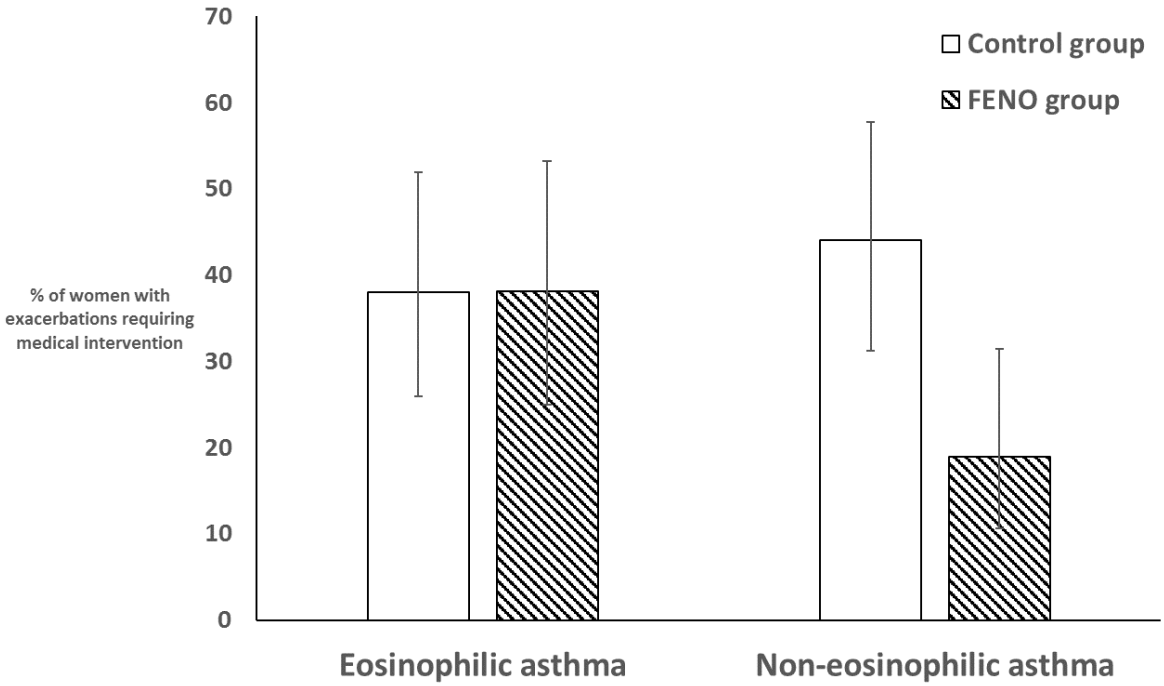
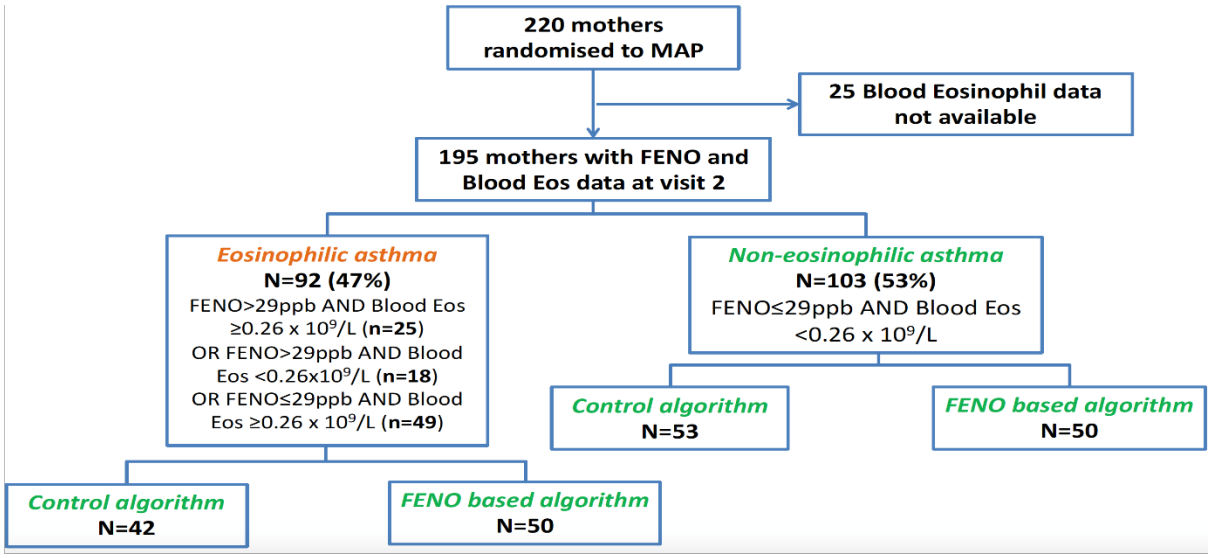


** Indicates significant difference between Control and FENO groups*





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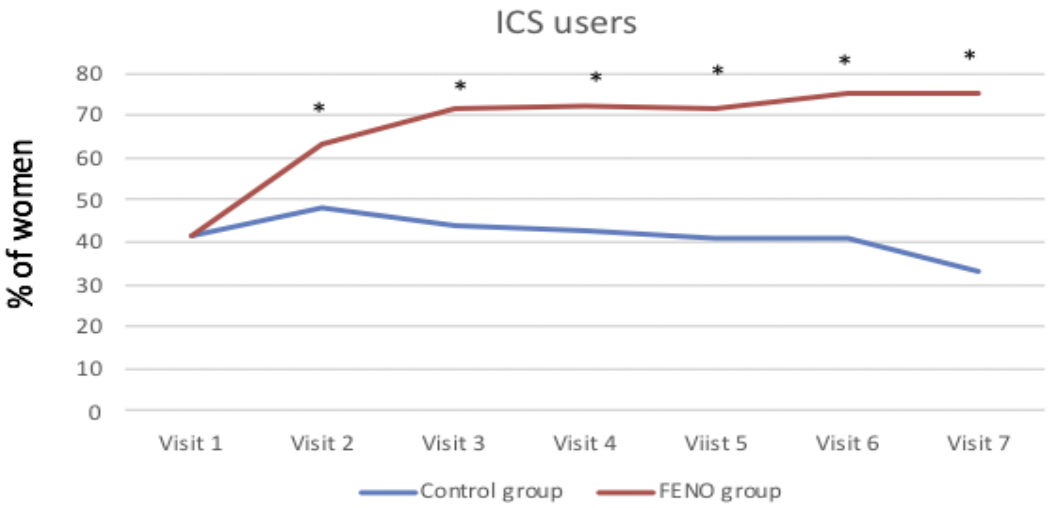

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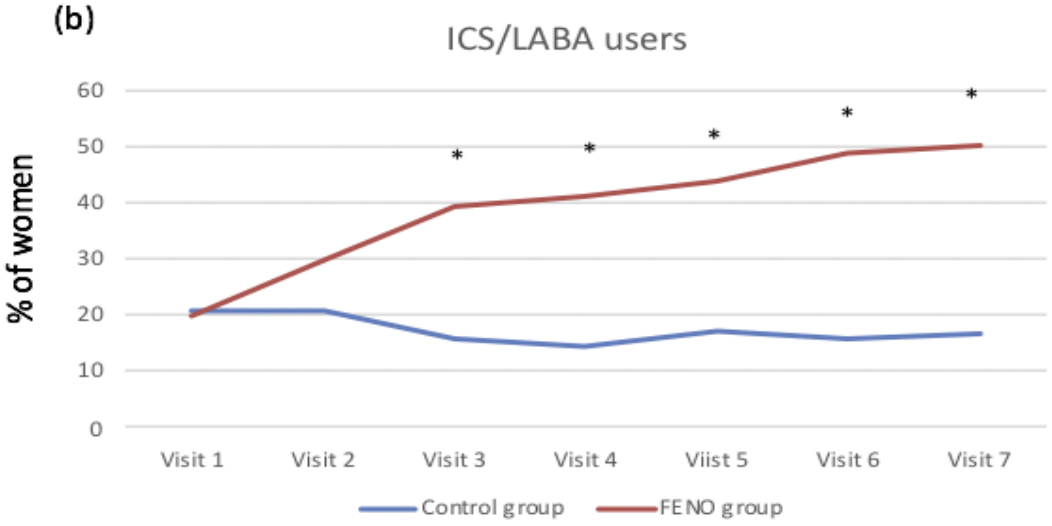

**** Indicates significant difference between Control and FENO groups***