Change in type-2 biomarkers and related cytokines with prednisolone in uncontrolled severe oral corticosteroid dependent asthmatics: an interventional open-label study

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ABSTRACT

Type-2 biomarkers and related cytokines (IL-5, IL-13), lung function and asthma symptoms were measured in 44 poorly-controlled severe oral corticosteroid (OCS)-dependent asthmatics for up to 88 days after a 7-day prednisolone boost (0.5 mg/kg). High-dose OCS reduced median blood eosinophils (−60 cells/µl; 95% CI −140 to 10), peristin (−8.4 ng/mL; −11.6 to −2.8), FeNO (−19.0 ppb; −28.5 to −4.0), IL-5 (−0.17 pg/mL; −0.28 to −0.08) and IL-13 (−0.15 pg/mL; −0.27 to −0.03). There were small improvements in mean FEV1 (0.16 L; 0.05 to 0.27) and (Asthma Control Questionnaire) ACQ-7 score (0.3; 0.0 to 0.7). Study measures returned to baseline 1-month postintervention. Following rescue OCS, 1 month is sufficient before using type-2 biomarkers to guide long-term treatment.

Trial registration number NCT01948401.

INTRODUCTION

The effect of high-dose oral corticosteroids (OCS) in OCS-dependent asthmatics is currently unknown. This group is important as they represent a large proportion of the severe asthma population, are patients with multiple corticosteroid-associated morbidities and drive much of the healthcare cost of asthma. Type-2 biomarkers are used to guide treatment for novel biological therapies, making it important to understand the longitudinal stability of their profile following high-dose OCS. The aims of this study were to examine the trajectory of the type-2 biomarker profile in severe OCS-dependent asthmatics after additional high-dose OCS.

METHODS

Participants aged between 12 and 75 with well characterised severe uncontrolled asthma were recruited from UK Severe Asthma Registry centres between July 2013 and February 2014. All patients were adherent with a maintenance dose of at least 10 mg prednisolone for at least 6 months prior to study initiation based on prednisolone/cortisol levels. Full details of study inclusion criteria are given in the online supplementary file 1. The study received Ethical Approval from National Research Ethics Service East of England (Ref: 13/EE/0099) and was registered on ClinicalTrials.Gov (https://clinicaltrials.gov/ct2/show/NCT01948401). Subjects were offered a 7-day OCS escalation (0.5 mg/kg) and invited to attend four follow-up visits 1 (visit 2), 32 (visit 3), 60 (visit 4) and 88 (visit 5) days postintervention. Patient outcomes including type-2 biomarkers (blood eosinophils, FeNO, serum peristin), cytokines (serum IL-5, IL-13), spirometry (FEV1, FVC) and patient-reported symptoms (ACQ-7) were collected at baseline and during follow-up visits (see online supplementary tables e1, e2). Patients receiving additional rescue OCS for an asthma exacerbation during follow-up were excluded from future study visits.

Descriptive data are presented using the mean (SD) or median (IQR). For normally distributed variables, mean change from baseline was calculated for each follow-up visit, and paired t-tests used to formally test for differences. For non-normally distributed variables, median difference from baseline was calculated (with bootstrapped 95% CIs), and the Wilcoxon signed-rank test used to test for differences. We conducted separate short-term (visit, visit 2 and visit 3) and long-term (all study visits) analysis. For each outcome, patients with missing data at baseline, visit 2 or visit 3 where excluded from the short-term analysis. Similarly, patients with missing data at any study visit were excluded from the long-term analysis.

RESULTS

Forty-four subjects were recruited and 17 completed long-term follow-up (6 did not consent to long-term follow-up, 2 did not attend study visits, 19 required rescue steroids) (online figure e1). Cohort details are given in table 1. In the short-term analysis, there were median decreases of 60 cells/µl (95% CI: 10, 140; p=0.004), 8.4 ng/mL (95% CI: 2.8, 11.6; p<0.001) and 19.0 ppb (95% CI: 4.0, 28.5; p<0.001) between baseline and visit 2 for blood eosinophils, serum peristin and FeNO, respectively (table 2, online figure e2). Serum IL-5 and IL-13 concentrations were reduced between baseline and visit 2 by 0.17 pg/mL (95% CI 0.08 to 0.28; p=0.002) and 0.15 pg/mL (95% CI 0.03 to 0.27; p=0.002), respectively. Blood eosinophils, serum peristin, FeNO and serum IL-5 concentration returned to baseline levels by visit 3 (visit 4 for cytokines), although serum IL-13 was slightly higher (0.07 pg/mL; 95% CI −0.01 to...
0.33; p=0.028) than baseline (table 2, online figure e2) at this point. FEV₁ (160 mL; 95% CI 50 to 270; p=0.005) and FVC (160 mL; 95% CI 40 to 280; p=0.009) showed small improvements after high-dose OCS (table 2, online figure e2), but were close to baseline by visit 3.

These short-term changes were replicated in patients with complete data to study end, although standard errors were noticeably larger due to a smaller sample size. Each of the study measures was close to baseline by visit 4 and remained relatively stable until visit 5 (figure 1, online supplementary table e3).

**DISCUSSION**

Seven days of high-dose OCS treatment significantly reduced type-2 biomarkers and related cytokine levels, but was associated with only small improvements in lung function and patient-reported symptoms. In general, all study measures had returned to baseline levels 1 month postintervention and remained stable until 3 months postintervention.

To our knowledge, this is the first study to investigate the effect of high-dose OCS in severe OCS-dependent asthmatics. Our data concur with studies of broader severe asthma populations which reported significant reductions in blood eosinophils and FeNO after high-dose OCS. Changes in serum type-2 cytokine levels have been variably reported.

Increased FEV₁ and improved ACQ scores have been reported after high-dose OCS in patients not on maintenance steroids.

Our results suggest that, after rescue steroids, clinicians should wait 1 month before using type-2 biomarkers to guide treatment in OCS-dependent asthmatics. This is particularly relevant for patients where type-2 biomarkers are used to guide treatment for biological therapies, where failure to account for recent high-dose OCS could lead to ineffective therapies being unnecessarily withheld. The high exacerbation rate observed within our study suggests that there is a rationale for how to interpret type-2 biomarker results in light of recent rescue steroids is commonly faced in routine clinical practice. Our data confirm that high-dose OCS inhibit both the IL-5 and IL-13 pathways, but despite significant reductions in type-2 inflammation, patients had significant residual impaired lung function and high symptoms scores. This suggests that broad therapeutic targeting of the type-2 cytokine axis may still leave significant unmet clinical need.

The study has some potential weaknesses as it is observational and hence open to confounding. The first postintervention follow-up was 1 month after the intervention period, therefore a more precise timing of biomarker levels returning to baseline prior to this time-point could not be determined. However, type-2 biomarkers were decreased 2 weeks after prednisolone treatment for acute exacerbation, suggesting that shorter time periods are unlikely to be sufficient. We necessarily excluded patients who had additional rescue OCS to treat exacerbations during follow-up which reduced the sample size in our long-term analysis and may have hindered generalisability.

In summary, type-2 biomarker and serum cytokine levels are significantly suppressed after high-dose OCS treatment in OCS-dependent asthmatics, but return to baseline levels after 1 month. Following rescue steroids, 1 month is a sufficient time period before using type-2 biomarkers to guide long-term treatment decisions.

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**Table 1** Baseline characteristics of the study population

| Number of patients | 44 |
|---------------------|----|
| **Demographics**    |    |
| Age (years, mean (SD)) | 52.9 (10.5) |
| 0–20                | 0 (0%) |
| 21–40               | 4 (9.1%) |
| 41–60               | 29 (65.9%) |
| 60+                 | 10 (22.7%) |
| Missing             | 1 (2.3%) |
| Female (n, %)       | 24 (54.5%) |
| Body mass index (mean (SD)) | 30.4 (4.4) |
| ACQ-7 score (mean (SD)) | 3.2 (1.2) |
| AQLQ (mean (SD))    | 3.8 (1.3) |
| **Asthma history/treatment** |    |
| Exacerbations in prior year (median (IQR)) | 5.5 (2.5, 8.0) |
| 0                   | 2 (4.5%) |
| 1                   | 5 (11.4%) |
| 2–3                 | 8 (18.2%) |
| 4–6                 | 17 (38.6%) |
| 7–10                | 5 (11.4%) |
| 11+                 | 7 (15.9%) |
| Hospitalisations in prior year (median (IQR)) | 0.0 (0.0, 1.0) |
| 0                   | 23 (52.3%) |
| 1                   | 11 (25.0%) |
| 2–3                 | 8 (18.2%) |
| 4+                  | 2 (4.5%) |
| Immunotherapy ever (n, %) | 3 (6.8%) |
| Intubated ever (n, %) | 4 (9.1%) |
| Maintenance OCS dose (median, IQR) | 10 (10, 20) |
| BDP equivalent ICS dose (median, IQR) | 2000 (2000, 2000) |
| **Lung function**   |    |
| FEV₁ (L, mean (SD)) | 1.91 (0.54) |
| % FEV₁ predicted (mean (SD)) | 62.4 (16.8) |
| FVC (L, mean (SD))   | 3.01 (0.71) |
| % FVC predicted (mean (SD)) | 76.9 (15.2) |
| **Type-2 biomarkers** |    |
| Blood eosinophils (cells/µl, median (IQR)) | 200 (120,360) |
| Serum periostin (ng/mL,median (IQR)) | 49.4 (39.3, 56.3) |
| FeNO (ppb, median (IQR)) | 47.5 (18.0, 66.0) |
| IGE (IU/mL, median (IQR)) | 101 (22,255) |
| **Type-2 cytokines in serum** |    |
| IL-5 concentration (pg/mL, median (IQR)) | 0.32 (0.13, 0.69) |
| IL-13 concentration (pg/mL, median (IQR)) | 0.46 (0.21, 0.75) |

Data are shown as median (IQR), mean (SD) or n (%) as appropriate. AQLQ, asthma quality of life questionnaire; BDP, beclometasone dipropionate; ICS, inhaled corticosteroid; IGE, immunoglobulin E; OCS, oral corticosteroid.
Table 2  Short-term change in study measures between baseline visit (visit 1) and 1 day (visit 2) and 32 days (visit 3) after completing 7 days of high-dose oral corticosteroids (0.5 mg/kg)

| Measure                                      | Count | Baseline absolute value | Visit 2 (1 day postintervention) | Visit 3* (32 days postintervention) | Absolute value | Difference (95% CI) | P value | Absolute value | Difference (95% CI) | P value |
|----------------------------------------------|-------|-------------------------|----------------------------------|-------------------------------------|----------------|----------------------|---------|----------------|---------------------|---------|
| **Type-2 biomarkers**                        |       |                         |                                  |                                     |                |                      |         |                |                      |         |
| Blood eosinophils (N/ul)†                    | 27    | 190 (100, 290)          | 130 (100, 170)                  | −60 (−140 to 10)                    | 0.004          | 180 (110, 400)       | −10 (−85 to 135) | 0.895          |
| Serum periostin (ng/mL)†                     | 29    | 48.2 (39.3, 55.1)       | 39.8 (34.8, 50.5)               | −8.4 (−11.6 to −2.8)               | <0.001         | 47.5 (41.8, 57.7)    | −0.7 (−4.2 to 5.3) | 0.469          |
| FeNO (ppb)†                                  | 27    | 49.0 (18.0, 77.0)       | 30.0 (17.0, 46.0)               | −19.0 (−28.5 to −4.0)              | <0.001         | 37.0 (22.0, 56.0)    | −12.0 (−21.0 to 5.0) | 0.139          |
| **Type-2 cytokines in serum**                |       |                         |                                  |                                     |                |                      |         |                |                      |         |
| IL-5 concentration (pg/mL)†                 | 18    | 0.22 (0.11, 0.54)       | 0.05 (0.00, 0.14)               | −0.17 (−0.28 to −0.08)             | 0.002          | 0.27 (0.16, 0.49)    | 0.05 (−0.07 to 0.28) | 0.913          |
| IL-13 concentration (pg/mL)†                | 17    | 0.31 (0.19, 0.50)       | 0.16 (0.11, 0.22)               | −0.15 (−0.27 to −0.03)             | 0.002          | 0.38 (0.32, 0.82)    | 0.07 (−0.01 to 0.33) | 0.028          |
| **Lung function**                           |       |                         |                                  |                                     |                |                      |         |                |                      |         |
| FEV, (L)†                                   | 28    | 1.98 (0.55)             | 2.14 (0.61)                     | 0.16 (0.05 to 0.27)               | 0.005          | 1.95 (0.55)          | −0.03 (−0.15 to 0.09) | 0.607          |
| FEV, (% predicted)†                          | 28    | 64.0 (17.5)             | 69.2 (18.4)                     | 5.2 (1.8 to 8.5)                   | 0.004          | 63.6 (18.8)          | −0.4 (−4.1 to 3.4)  | 0.848          |
| FVC (L)†                                    | 28    | 3.11 (0.70)             | 3.28 (0.75)                     | 0.16 (0.04 to 0.28)               | 0.009          | 3.06 (0.73)          | −0.06 (−0.21 to 0.10) | 0.460          |
| FVC (% predicted)†                           | 28    | 78.3 (14.4)             | 82.1 (14.2)                     | 3.9 (1.1 to 6.7)                   | 0.009          | 77.0 (15.7)          | −1.2 (−5.0 to 2.6)  | 0.516          |
| **Patient-reported outcomes**               |       |                         |                                  |                                     |                |                      |         |                |                      |         |
| ACQ score‡                                   | 30    | 3.0 (1.1)               | 2.6 (1.2)                       | −0.3 (−0.7 to 0.0)                 | 0.045          | 3.2 (1.1)            | 0.2 (−0.0 to 0.4)   | 0.090          |
| AQLQ score‡                                  | 30    | 3.9 (1.3)               | 4.1 (1.4)                       | 0.2 (0.0 to 0.4)                   | 0.095          | 4.0 (1.3)            | 0.1 (−0.1 to 0.2)   | 0.514          |

Subjects with missing data at any of the short-term visits or who required additional rescue corticosteroids for exacerbation in addition to the study intervention were excluded. Data are shown as median (IQR) or mean (SD) as appropriate.

*Except IL-5 and IL-13 where visit 4 (60 days postintervention) is presented.
†Median (IQR).
‡Mean (SD).

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Competing interests CH, AC, JGM, TS are (or were at time of study) employees of Genentech Inc., a Member of the Roche Group and own Roche stock. RC has attended Advisory Board Meetings for AstraZeneca, GSK, Novartis and Teva and been a speaker at meetings for AstraZeneca. She has attended conferences supported by Boehringer, Teva, AstraZeneca and received educational grants from Novartis and Aerocrine. AHM received personal and department funds for talks and advisory board meetings and was sponsored to attend national and international conferences from pharmaceutical companies that include GlaxoSmithKline, AstraZeneca, Novartis, Napp, Boehringer Ingelheim, Roche, Chiesi. AM-G has attended advisory boards with GlaxoSmithKline, Novartis, AstraZeneca, Boehringer Ingelheim and Teva. He has received speaker fees from Novartis, AstraZeneca, Vectura, Boehringer Ingelheim, Sanofi and Teva. He has participated in research with Hoffman La Roche, GlaxoSmithKline, Boehringer Ingelheim and AstraZeneca. He has attended international conferences with Teva and Boehringer Ingelheim and has consultancy agreements with AstraZeneca, Sanofi and Vectura. RN has received an unrestricted grant of £10,000 from Novartis in 2010 towards development of clinical services at the University Hospital of South Manchester. He has run preceptorship programmes in 2015 and 2016. These programmes have resulted in payment to the University Hospital of South Manchester for amounts not exceeding £10,000. He has also performed lecturing at Pharmacologically sponsored meetings for the following pharmaceutical companies in the last 3 years: Astra Zeneca (£1,000), Boehringer Ingelheim (£2,000) Boston scientific (£3,000), Chiesi (£1,000), Novartis (£10,000), Napp (£2,000), Teva (£2,000). He has sat on advisory boards for the following companies in the last 3 years: Astra Zeneca, Boehringer Ingelheim, Boston scientific, Chiesi, GSK, Novartis, Vectura, Teva, and received reimbursement not exceeding £5,000 per company. He has received sponsorship support to attend international academic meetings from Astra Zeneca, Boehringer Ingelheim, Novartis, Chiesi and Teva. RN (or any members of his family) has no shares or any pecuniary interest in any pharmaceutical industry and has no shareholdings or dividends and is not a paid consultant for any company. LGH is Academic Lead for the Medical Research Council Stratified Medicine UK Consortium in Severe Asthma which involves industrial partnerships with a number of pharmaceutical companies.

Patient consent for publication Not required.

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REFERENCES
1 O’Neill S, Sweeney J, Patterson CC, et al. The cost of treating severe refractory asthma in the UK: an economic analysis from the British Thoracic Society difficult asthma registry. Thorax 2015;70:376–8.
2 Sousa AR, Marshall RP, Warnock LC, et al. Responsiveness to oral prednisolone in severe asthma is related to the degree of eosinophilic airway inflammation. Clin Exp Allergy 2017;47:890–9.
3 Oishi K, Hirano T, Suetake R, et al. A trial of oral corticosteroids for persistent systemic and airway inflammation in severe asthma. Allergol Int 2013;62:359–65.
4 Matsunaga K, Hirano T, Akamatsu K, et al. Predictors for identifying the efficacy of systemic steroids on sustained exhaled nitric oxide elevation in severe asthma. Immun Inflamm Dis 2017;5:261–4.
5 Bentley AM, Hamid O, Robinson DS, et al. Prednisolone treatment in asthma. Reduction in the numbers of eosinophils, T cells, tryptase-only positive mast cells, and modulation of IL-4, IL-5, and interferon-gamma cytokine gene expression within the bronchial mucosa. Am J Respir Crit Care Med 1996;153:551–6.
6 Djukanović R, Homeyard S, Gratziou C, et al. The effect of treatment with oral corticosteroids on asthma symptoms and airway inflammation. Am J Respir Crit Care Med 1997;155:826–32.
Brief communication

**Figure 1** Trajectories of type-2 biomarkers and cytokines, patient-reported outcomes (ACQ and AQLQ) and lung function (FEV1 and FVC) over serial study visits—subjects with missing data at any of the study visits or who required additional rescue corticosteroids for exacerbation in addition to the high-dose OCS between baseline visit and study 2 were excluded. Data are shown as median or mean difference (with 95% CIs) as appropriate.

1. **Type 2 Biomarkers**
   - Blood Eosinophils (N/μl, n=14)
   - Serum Periostin (ng/ml, n=16)

2. **Type 2 Cytokines**
   - Serum IL-5 Concentration (pg/ml, n=18)
   - Serum IL-13 Concentration (pg/ml, n=18)

3. **Lung Function**
   - FEV1 (litres, n=16)
   - FEV1 (% predicted, n=16)
   - FVC (litres, n=16)
   - FVC (% predicted, n=16)

4. **Patient reported outcomes**
   - ACQ Score (n=17)
   - AQLQ Score (n=17)

7. Chakir J, Hamid Q, Bossé M, et al. Bronchial inflammation in corticosteroid-sensitive and corticosteroid-resistant asthma at baseline and on oral corticosteroid treatment. *Clin Exp Allergy* 2002;32:578–82.
8. Fukakusa M, Bergeron C, Tulic MK, et al. Oral corticosteroids decrease eosinophil and CC chemokine expression but increase neutrophil, IL-8, and IFN-gamma-inducible protein 10 expression in asthmatic airway mucosa. *J Allergy Clin Immunol* 2005;115:280–6.
9. Kupczyk M, Haque S, Middelveld RJM, et al. Phenotypic predictors of response to oral glucocorticosteroids in severe asthma. *Respir Med* 2013;107:1521–30.
10. Semprini R, Shortt N, Ebmeier S, et al. Change in biomarkers of type-2 inflammation following severe exacerbations of asthma. *Thorax* 2019;74.