A sputum gene expression signature predicts oral corticosteroid response in asthma

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ABSTRACT Biomarkers that predict responses to oral corticosteroids (OCS) facilitate patient selection for asthma treatment. We hypothesised that asthma patients would respond differently to OCS therapy, with biomarkers and inflammometry predicting response.

Adults with stable asthma underwent a randomised controlled cross-over trial of 50 mg prednisolone daily for 10 days (n=55). A six-gene expression biomarker signature (*CLC*, *CPA3*, *DNASE1L3*, *IL1B*, *ALPL* and *CXCR2*) in induced sputum, and eosinophils in blood and sputum were assessed and predictors of response were investigated (changes in forced expiratory volume in 1 s (ΔFEV_1), six-item Asthma Control Questionnaire score (ΔACQ6) or exhaled nitric oxide fraction (ΔF_{eNO})).

At baseline, responders to OCS (n=25) had upregulated mast cell *CPA3* gene expression, poorer lung function, and higher sputum and blood eosinophils. Following treatment, *CLC* and *CPA3* gene expression was reduced, whereas *DNASE1L3, IL1B, ALPL* and *CXCR2* expression remained unchanged. Receiver operating characteristic (ROC) analysis showed the six-gene expression biomarker signature as a better predictor of clinically significant responses to OCS than blood and sputum eosinophils.

The six-gene expression signature including eosinophil and Th2 related mast cell biomarkers showed greater precision in predicting OCS response in stable asthma. Thus, a novel sputum gene expression signature highlights an additional role of mast cells in asthma, and could be a useful measurement to guide OCS therapy in asthma.

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Introduction

Oral corticosteroids (OCS) are commonly used in the treatment of asthma for moderate to severe exacerbations and to achieve control in severe asthma [1]; however, their clinical efficacy is variable [2], and cannot be accurately predicted by airflow limitation and symptoms. Objective measures of airway and systemic inflammation are not generally used to guide treatment initiation and determine response. We have recently shown that a novel sputum gene expression biomarker signature of six genes predicts both inflammatory phenotype and response to inhaled corticosteroids [3]. This novel methodology that incorporates the exploration of inflammation at the molecular level might provide further insight into which patients might respond to OCS. Inflammometry, the practice of assessing inflammation [4] offers the prospect of targeted therapy in asthma, and is utilised in patient selection for newer asthma treatments [5, 6]. Whereas blood eosinophil counts can predict asthma phenotype [7, 8], their role in predicting OCS response has not been well established.

We hypothesised that the use of OCS to treat stable asthma would lead to clinical improvement in some, but not all subjects, and that this response would be predicted by the expression of a six-gene expression biomarker signature in induced sputum and eosinophils in blood and sputum. Therefore, this study aimed to describe clinical improvements in adults with stable asthma, and determine the molecular and inflammometry predictors of the clinical efficacy of short-term OCS therapy.

Methods

Study design

The randomised double blinded, placebo-controlled crossover trial was registered with the Australian and New Zealand Clinical Trials Registry (ACTRN12611000562976), and conducted at the Hunter Medical Research Institute (HMRI), Newcastle, Australia following the Declaration of Helsinki guidelines. Approval was provided by the Hunter New England Health Human Research Ethics Committee (HREC) and registered with the University of Newcastle HREC (11/06/15/3.03). Written informed consent was obtained. Nutrition data and adverse events from this trial have been previously reported [9].

Subjects

Subjects (n=60) were recruited through the John Hunter Hospital Severe Asthma Clinic, NSW, Australia, HMRI volunteer databases and by advertisement. Non-smoking (ceased ⩾6 months) subjects over 18 years of age with confirmed stable asthma were included. Subjects were excluded owing to pregnancy, breastfeeding, diabetes mellitus, other respiratory disorders and maintenance OCS therapy. See online supplement for further information on asthma stability and severity definitions.

Clinical intervention & assessment

Subjects underwent assessment before and after 10 days of active treatment (prednisolone 25 mg capsule taken twice daily), and identical placebo treatment in random order (Richard Stenlake compounding Chemist, Bondi, Australia), with an intervening 4-week wash out period. Assessment included: exhaled nitric oxide fraction (F_{ENO}) (Ecomedics CLD 88sp Analyzer, Ecomedics, Duernten, Switzerland), spirometry (Medgraphics, PFS/D and BreezeSuite software, MedGraphics, Saint Paul, Minnesota, USA) and sputum induction with nebulised (ULTRA-NEB™ ultrasonic nebuliser, DeVilbiss, Model 2000, Tipton, West Midlands, United Kingdom) hypertonic saline (4.5%) [10]. Predicted values were calculated using NHANES III data [11]. Combined bronchial provocation and sputum induction were performed at the baseline visit only, to establish airway hyperresponsiveness (AHR). Fasting blood samples were collected and full blood count was performed (Hunter Area Pathology Service, Coulter STKS Cell Analyzer, (Miami, FL, USA). The six-item Juniper Asthma Control Questionnaire (ACQ6) was also completed at each visit [12].

Responder analysis

Clinical response to OCS was defined using current American Thoracic Society (ATS)/European Respiratory Society (ERS) criteria, including a significant change in lung function categorised by an increase in FEV_1 (forced expiratory volume in 1 s) by ⩾12% and ⩾200 mL [13], or a decrease in F_{ENO} of 20% if baseline F_{ENO} ⩾50 ppb, or a decrease of ⩾10 ppb if F_{ENO} <50 ppb at baseline [14], or a significant change in asthma control categorised by a decrease in ACQ6 of ⩾0.5 [15].

Induced sputum inflammatory cell counts

Opaque mucocellular lower respiratory tract sputum portions were selected from saliva, dispersed using dithiothreitol and trypan blue, and total cell count and viability were determined. Cytospins were prepared

and stained (May-Grunwald Giemsa), and a differential cell count was obtained from 400 non-squamous cells [16, 17]. See online supplement for phenotype classification.

Sputum gene expression analysis

100 µL of selected sputum plugs was homogenised and stored in Buffer RLT (Qiagen, Hilden, Germany) at −80°C, until subsequent RNA extraction. The RNA was extracted from sputum using the AllPrep RNA/DNA/Protein Mini Kit (Qiagen, Hilden, Germany), and quantitated using the Quant-iT RiboGreen RNA Assay Kit (Life Technologies, Scoresby, Australia) as per manufacturer's instructions. Sputum RNA (200 ng) was reverse transcribed to cDNA and used to detect gene expression of the Charcot–Leyden crystal protein (*CLC*), carboxypeptidase A3 (*CPA3*), deoxyribonuclease I-like 3 (*DNASE1L3*), interleukin 1B (*IL1B*), alkaline phosphatase, tissue nonspecific isozyme (*ALPL*), and chemokine (C-X-C motif) receptor 2 (*CXCR2*), using standard TaqMan methods [18]. Statistical analysis was performed on the change in cycle threshold (ΔCt) between the target gene and the housekeeping gene (β-actin), or the normalised result, calculated using $2^{-\Delta\Delta Ct}$ relative to β-actin and the mean of the baseline value [3].

Statistical analysis

Data were analysed with the STATA 11 software (StataCorp, College Station, Texas, USA) and reported as mean±SD or median [interquartile range]. Statistical comparisons were analysed using t-tests, the two-sample Wilcoxon rank-sum and Wilcoxon signed-rank tests and Chi-squared test. The mean differences between treatment and placebo and absolute response to OCS in non-responders and responders in intervention outcomes were tested using generalised linear mixed models, as previously published [9]. Multiple logistic regression was used to calculate the predicted value of a subject responding to OCS, based on their level of expression of the six-gene biomarker signature combination, as previously described [3]. Receiver operating characteristic (ROC) curves and the area under the curves (AUC) were calculated and tested for equality. Significance was accepted if $p<0.05$.

Results

Baseline characteristics of participants

Sixty subjects were randomised, of which 55 completed the first treatment phase and 49 completed the second treatment phase of the study (figure S1) [9]. Subjects had a mean age of 53.6 years, and most had an eosinophilic (n=31, 56%) inflammatory phenotype (table 1). Few subjects in this cohort displayed a neutrophilic asthma phenotype (n=2); thus, inflammatory phenotype was classified into eosinophilic (EA) and non-eosinophilic asthma, based on the presence of sputum eosinophils.

Characteristics of responders to OCS

54 subjects completed the course of prednisolone treatment. Responders to prednisolone exhibited a reduction in ACQ6 of 0.5 (n=17, 68%), a reduction in F_{eNO} of 20% if baseline F_{eNO} ⩾50 ppb, or a reduction of ⩾10 ppb if F_{eNO} <50 ppb at baseline (n=13, 52%), or improvement in FEV_1 of ⩾12% and ⩾200 mL from baseline (n=9, 36%) (figure S2). Those who had a clinically significant response to prednisolone treatment (n=25) had poorer lung function, greater airflow obstruction and AHR (table 1). Most responders (n=19, 76%) had EA with greater baseline sputum (p=0.004) and blood eosinophils (p=0.001) (table 1). Responders showed higher levels of *CPA3* expression (p=0.025) and tended to have higher expression of *DNASE1L3* (1.4 [0.9, 4.2] *versus* 1.1 [0.4, 2.7]) and *CLC* (6.1 [0.6, 11.5] *versus* 1 [0.3, 6.4]), compared to non-responders (table 1). Blood eosinophils were significantly reduced in responders as well as non-responders, confirming adherence (figure 1, table 2). Changes in blood eosinophils, sputum eosinophils, macrophages and lymphocytes were significantly greater in responders (table 2).

Effects of OCS treatment

For the group as a whole, the change in FEV_1 % predicted was significant (Δ=3.12, (0.59, 5.65) 95% CI). However, no significant differences were noted between groups in the changes in forced vital capacity (FVC), FEV_1/FVC, F_{eNO} and the ACQ6 score with OCS treatment, compared to those with the placebo (table S1). Expression of *CLC, CPA3* and *DNASE1L3* genes was reduced following OCS treatment, whereas expression of *IL1B, ALPL* and *CXCR2* showed no change (figure 2). Changes in *CLC*, *CPA3* and *DNASE1L3* gene expression were correlated with improvement in FEV_1 ($r_s=-0.62$, $p<0.001$; $r_s=-0.41$, $p=0.001$; $r_s=-0.69$, $p<0.001$, respectively) (figure S3).

Within groups, F_{eNO} (−9.85±23.2 (mean±SD)), ACQ6 (−0.23±0.56) and FEV_1 (L) (0.14±0.28) improved significantly following OCS treatment (figure 3). Sputum eosinophils were significantly reduced to below 3% in 83% of the subjects (figure 3a), and blood eosinophils were significantly reduced by −0.29 ±0.36×10^9·L^{-1} ($p<0.001$) (table S1), and below 0.26×10^9·L^{-1} in the majority (91%) of subjects (figure 1).

TABLE 1 Clinical and inflammatory characteristics at baseline, by response to OCS (ΔACQ6/ΔF_{ENO}/ΔFEV1)

Outcome	All asthma	Non-Responders	Responders[#]	p-value[f]
Subjects n	54	29	25	
Age years (range)	53.6 (21–78)	54.1 (21–78)	52.4 (24–72)	0.693
BMI kg·m^{-2}	30.7±6.4	30.1±6.8	31.5±6.2	0.451
Male n (%)	23 (41.8)	13 (45)	10 (40)	0.721
Pre B_2 FEV1 % predicted	82 [69, 89]	87 [74, 94]	78 [65, 85]	**0.038**
Pre B_2 FVC % predicted	90 [78, 99]	88 [79, 100]	92 [78, 99]	0.901
Pre B_2 FEV1/FVC %	72 [64, 77]	74 [68, 82]	70 [60, 74]	**0.015**
ACQ6 med [IQR]	0.9 [0.3, 1.6]	0.7 [0.2, 1]	1.3 [0.5, 2]	**0.006**
Airway hyperresponsiveness n (%)	30 (55)	10 (35)	20 (80)	**0.001**
PD_{15} med [IQR]	4.9 [2.9, 10.5]	5.5 [1.0, 6.4]	4.3 [2.9, 10.5]	0.984
Dose response slope %fall/mL med [IQR]	1.4 [0.5, 3.7]	0.7 [0.4, 2.1]	2.6 [1.4, 4.2]	**0.001**
Severe asthma n (%)	22 (40)	9 (31)	13 (52)	0.118
Ex-smokers n (%)	21 (38)	10 (35)	10 (40)	0.675
Pack-years med [IQR]	3 [1, 20]	3 [0, 20]	2 [1, 20]	0.849
ICS use n (%)	43 (78)	24 (83)	16 (64)	0.117
ICS+LABA n (%)	38 (70)	22 (76)	16 (64)	0.341
OCS cumulative dose[§] in 2 yrs mg med [IQR]	400 [238, 675]	350 [290, 562]	495 [150, 750]	0.877
Non-eosinophilic asthma[¶] n (%)	21 (38)	15 (52)	6 (24)	**0.037**
Eosinophilic asthma n (%)	31 (56)	12 (41)	19 (76)	**0.010**
***F*ENO ppb**	22.5 [13.1, 37.4]	15.2 [8.7, 27.3]	30 [18.5, 71.8]	**0.002**
Induced sputum median [IQR]				
CLC mRNA[+]	2.1 [0.4, 9.8]	1 [0.3, 6.4]	6.1 [0.6, 11.5]	0.157
CPA3 mRNA	1.6 [0.4, 5.9]	1.1 [0.2, 2.1]	2.8 [0.8, 8.2]	**0.025**
DNASEIL3 mRNA	1.2 [0.4, 3.9]	1.1 [0.4, 2.7]	1.4 [0.9, 4.2]	0.263
IL-1β mRNA	0.7 [0.5, 1.2]	0.7 [0.4, 0.9]	0.7 [0.5, 1.2]	0.251
ALPL mRNA	0.9 [0.4, 2.1]	0.7 [0.4, 1.7]	1.4 [0.6, 2.1]	0.263
CXCR2 mRNA	0.8 [0.5, 1.5]	0.9 [0.5, 1.5]	0.7 [0.4, 1.7]	0.777
Eosinophils %	4.5 [1.3,12.8]	2 [0.8, 6]	10.8 [2.5, 28.3]	**0.004**
Blood median [IQR]				
Eosinophils ×10^9·L^{-1}	0.3 [0.2,0.4]	0.2 [0.1, 0.3]	0.4 [0.2, 0.4]	**0.001**

Data are presented as mean±SD, unless otherwise stated. p-values in bold are statistically significant. ACQ6: six-item Asthma Control Questionnaire; F_{ENO}: exhaled nitric oxide fraction; FEV1: forced expiratory volume in 1 s; FVC: forced vital capacity; Med: median; PD_{15}: provocation dose; ICS: inhaled corticosteroid; OCS: oral corticosteroid; ppb: parts per billion. [#]: Responders=ΔACQ6≥0.5 or ΔF_{ENO} (≥20%↓ if V1 F_{ENO}≥50 ppb or ≥10 ppb↓ if V1<50 ppb) or ΔFEV1≥12% and 200 mL); [¶]: non-eosinophilic asthma defined by sputum eosinophils <3%, eosinophilic asthma defined by sputum eosinophils ≥3%, [+]: mRNA data are expressed as normalised result, calculated using $2^{-\Delta\Delta Ct}$ relative to β-actin and the mean of the baseline visit.[§]: Prednisone equivalents. [f]: p-values represent differences between non-responders and responders.

Changes in sputum and blood eosinophils were correlated (r_s=0.45, p=0.003), and correlated with changes in FEV1 (r_s=−0.75, p<0.001 and r_s=−0.51, p<0.001, respectively).

Biomarkers predicting clinically significant response to OCS

Receiver operating characteristic (ROC) curves were created to analyse the diagnostic value of baseline sputum gene expression and inflammometry to predict response to OCS (figure 4). Expression of the

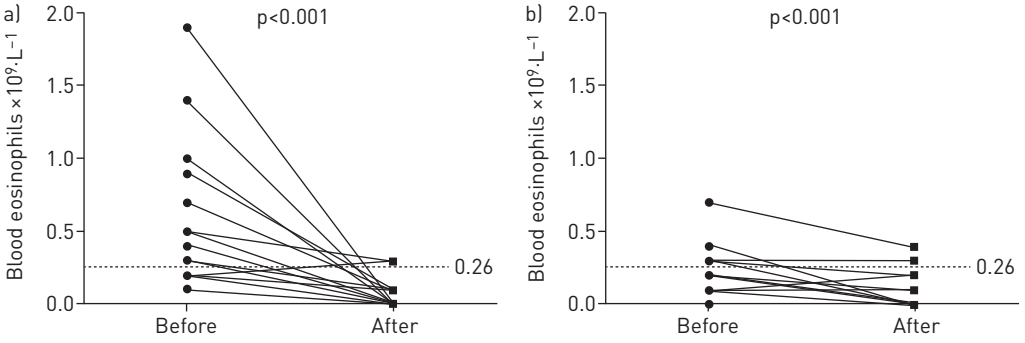

FIGURE 1 Change in blood eosinophils after oral corticosteroid (OCS) treatment, in (a) responders and (b) non-responders.

TABLE 2 Changes in clinical and inflammatory outcomes after oral corticosteroid treatment in non-responders and responders

Outcome	Change in non-responders	Change in responders	β-coef[#]	CI[¶]	p-value[+]
Subjects n	29	25			
Pre B_2 FEV_1 L	0.03±0.12	0.27±0.35	0.25	0.11, 0.39	**<0.001**
Pre B_2 FEV_1 % pred	0.94±3.91	8.57±11.46	7.63	3.10, 12.16	**0.001**
Pre B_2 FVC % pred	0.98±6.01	4.58±9.08	3.60	−0.56, 7.76	0.090
Pre B_2 FEV_1/FVC %	−0.79±4.09	4±5.49	4.79	2.16, 7.41	**<0.001**
ACQ6	0.08±0.33	−0.58±0.58	−0.67	−0.91, −0.42	**<0.001**
F_{ENO} ppb	0.36±6.01	−20.06±29.1	−20.43	−32.57, −8.28	**0.001**
Sputum cell counts					
Eosinophils %	−4.42±8.78	−13.43±16.71	−9.02	−16.72, −1.31	**0.022**
Neutrophils %	4.36±22.66	−0.75±27.13	−5.11	−19.82, 9.60	0.496
Macrophages %	−5.90±21.02	10.40±28.15	16.30	1.76, 30.84	**0.028**
Lymphocytes %	−1.30±2.01	−0.09±0.81	1.21	0.28, 2.15	**0.011**
Blood cell counts					
Eosinophils $\times 10^9 \cdot L^{-1}$	−0.15±0.13	−0.44±0.45	−0.29	−0.47, −0.10	**0.002**
Neutrophils $\times 10^9 \cdot L^{-1}$	4.98±2.66	5±3.45	0.03	−1.72, 1.72	0.978
Lymphocytes $\times 10^9 \cdot L^{-1}$	0.18±0.88	0.29±0.79	0.12	−0.35, 0.59	0.627

Data are presented as mean±SD. p-values in bold are statistically significant. FEV_1: forced expiratory volume in 1 s; % pred: % predicted; FVC: forced vital capacity; ACQ6: six-item Asthma Control Questionnaire score (Juniper); F_{ENO}: exhaled nitric oxide fraction. [#]: Coefficient showing difference in outcome change, [¶]: Confidence interval for coefficient, [+]: difference in change between non-responders and responders.

six-gene expression biomarker signature predicted response to OCS with greater AUC values than blood or sputum eosinophils, for changes in FEV_1, ACQ and F_{ENO} combined (figure 4a) and FEV_1 (figure 4b), and was significantly better at predicting improvement in ACQ6 (figure 4c) and F_{ENO} (figure 4d) than sputum eosinophils. Two cut points for predictor variables were evaluated; one chosen to minimise false negatives (highest sensitivity) and the second, to minimise false positive results (highest specificity) (table 3). Six-gene signature values ⩾0.63 had 95% specificity for OCS response (FEV_1, ACQ6 or F_{ENO}) and a positive predictive value (PPV) of 90%. Whereas six gene signature values <0.36 had high sensitivity to predict non-response to OCS (87% sensitivity) and a negative predictive value (NPV) of 84%. Baseline blood eosinophils ⩾$0.4\times10^9 \cdot L^{-1}$ had 50% sensitivity (specificity 92%) and a PPV of 86%. Baseline blood eosinophils <$0.3\times10^9 \cdot L^{-1}$ had a NPV of 70%. Sputum eosinophils ⩾4.8% had a sensitivity of 67% (specificity 77%) and PPV of 71%, whereas sputum eosinophils <2.5% had a sensitivity of 79% and NPV of 78%.

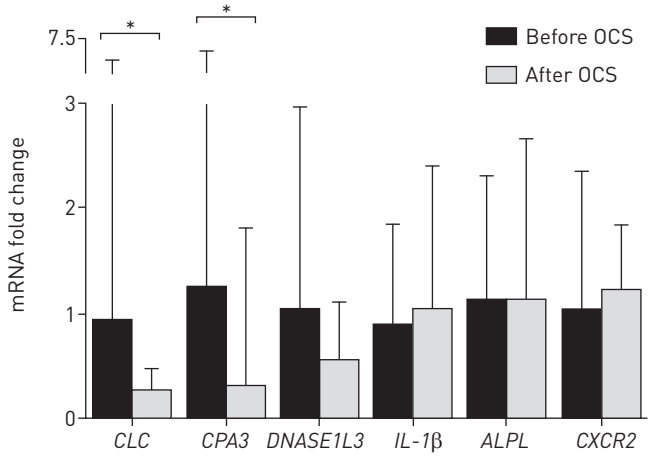

FIGURE 2 Changes in sputum gene expression of the six-gene biomarker signature before and after oral corticosteroid (OCS) treatment. Eosinophil markers are *CLC*, *CPA3* and *DNASE1L3*. Neutrophil markers are *IL1B*, *ALPL* and *CXCR2*.

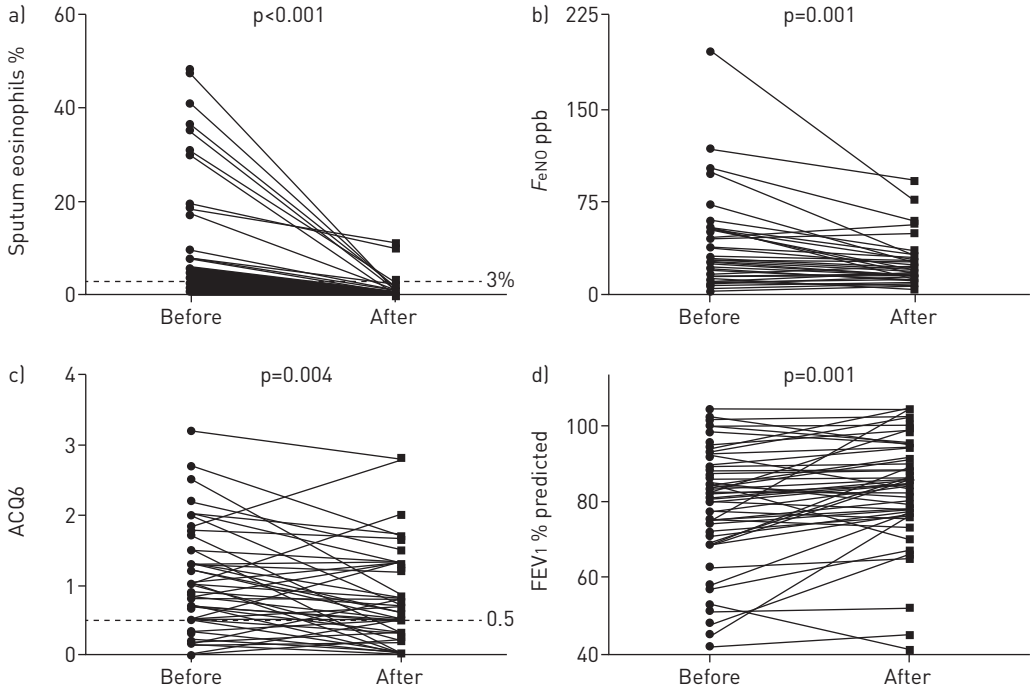

FIGURE 3 Changes in clinical outcomes after oral corticosteroid (OCS) treatment in, (a) sputum eosinophils (b) exhaled nitric oxide, (c) ACQ6 (six-item Asthma Control Questionnaire score) and (d) FEV1 (forced expiratory volume in 1 s).

The diagnostic value of the six-gene expression biomarker signature, sputum eosinophils and blood eosinophils to predict response to individual OCS response items (FEV1, ACQ6 or F_{eNO}) is presented in table S2.

Discussion

This study investigated the clinical, molecular and inflammatory predictors of response to short-term OCS therapy in adults with stable asthma. The mRNA for *CLC* (an eosinophilic granule protein) and *CPA3* (a mast cell granule marker) were elevated in OCS responders and significantly reduced with OCS treatment. A composite six-gene expression biomarker signature in induced sputum that included *CLC* and *CPA3* was found to be highly predictive of a response to OCS, and superior to blood or sputum eosinophils. This suggests that combining an eosinophilic with a mast cell marker gives excellent predictability of corticosteroid response. The OCS responders were also characterised by poorer lung function, AHR and higher levels of blood and sputum eosinophils. These results demonstrate the value of a precision medicine approach in the treatment of asthma, and highlight a potential role for targeting mast cells in asthma therapy.

Improvement in clinical outcomes was best predicted by the expression of a novel six-gene expression biomarker signature in induced sputum; however, baseline blood eosinophils also predicted response with less precision, which is consistent with the findings of other investigations [19].

The six-gene expression biomarker signature was previously developed and validated using transcriptomic analysis [3, 18]. It included expression of *CLC, CPA3* and *DNASE1L3*, which are increased in subjects with EA and *IL1B*, *ALPL* and *CXCR2* which are increased in neutrophilic asthma [3]. In the present study, expression of the *CPA3* gene was increased at baseline in OCS responders and that of *CLC* and *CPA3* was reduced following OCS treatment, whereas *DNASE1L3, IL1B, ALPL* and *CXCR2* showed no change. These findings show that the signature is responsive to change. The CLC protein, also known as galectin-10, comprises up to 10% of the total proteins in eosinophils [20] and is expressed by both basophils [21] and regulatory T-cells [22]. A dominant protein in mast cell granules, CPA3, is present in a subtype of mast cells that also contain tryptase. This mast cell subtype is dominant in severe asthma and Th2-high asthma [23, 24]. Expression of *CPA3* has been found to predict the response to inhaled corticosteroids (ICS) in asthma [24, 25]. In the present study, we combined CPA3, a Th2 related mast cell marker, with an eosinophil marker, and found that the biomarker signature had an excellent ability to predict response to OCS. Whereas both sputum and blood eosinophils are predictors of OCS response, we found that the

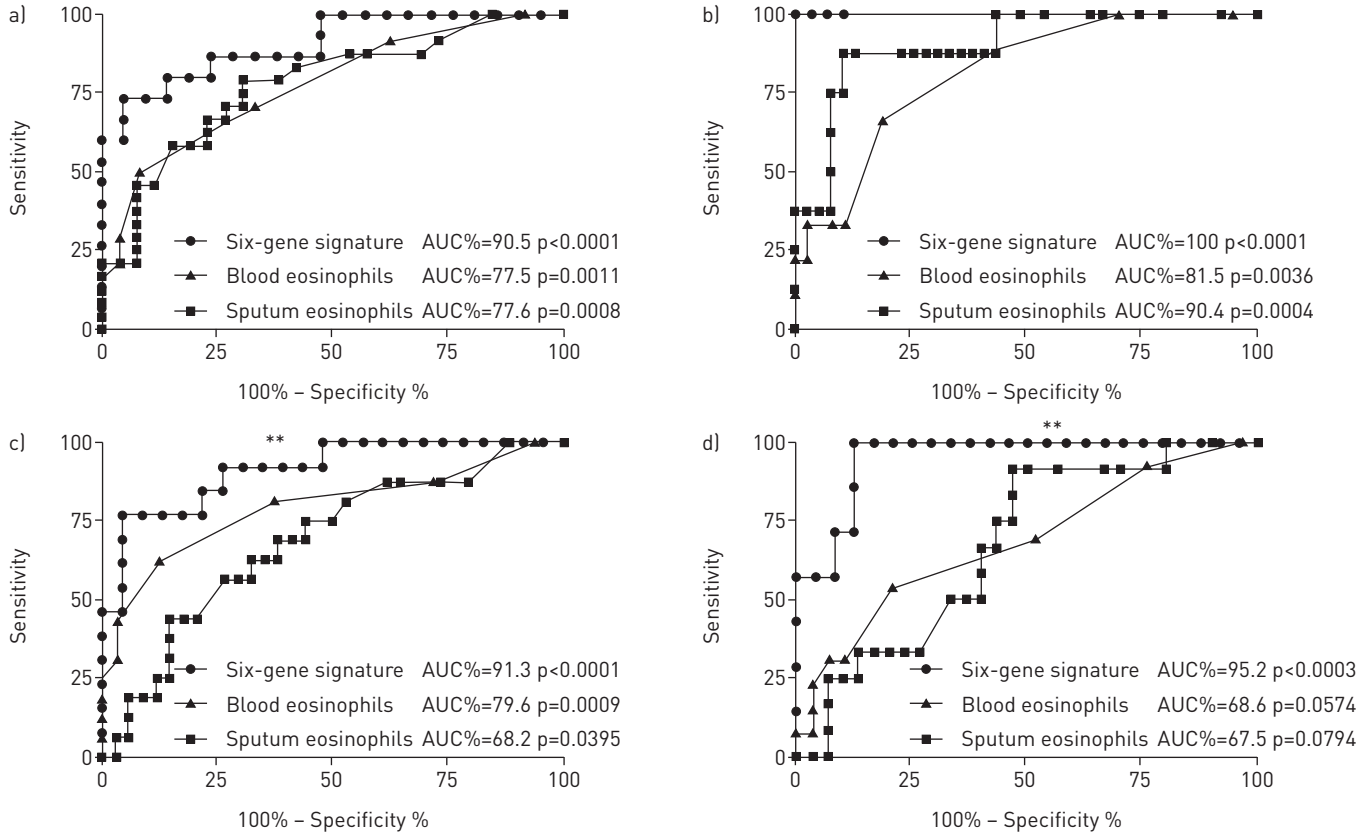

FIGURE 4 Receiver operating characteristic curve comparison of the six-gene biomarker signature (circles ●) compared with blood eosinophils (triangles ▲) and induced sputum eosinophils (squares ■) to predict a clinically significant response to oral corticosteroids. The six-gene biomarker signature showed superior performance at predicting OCS (oral corticosteroid) responsiveness, in terms of changes in (a) FEV_1 (forced expiratory volume in 1 s), ACQ6 (six-item Asthma Control Questionnaire score) and F_{ENO} (exhaled nitric oxide), and (b) FEV_1 and was significantly improved at predicting improvement in (c) ACQ6 and (d) F_{ENO}, in comparison to sputum eosinophils. **: p<0.05.

addition of a Th2 related mast cell marker was superior to these other biomarkers. The signature also contained other biomarkers, such as DNASE1L-3, an endonuclease, which is active during cellular apoptosis by degrading DNA. It was first linked to EA *via* a transcriptomic discovery study [18], and other than its role as a marker of EA, there is yet little evidence to clarify any further role in asthma. Expression of IL1B is increased in neutrophilic asthma, and induced by the NRLP-3 inflammasome [26]. *ALPL* produces an enzyme that is related to the TNFα/NFkβ family, and is supposedly linked to neutrophilic inflammation in asthma [27]. Chemokine (C-X-C motif) receptor 2 assists in neutrophil migration to the site of inflammation, including the airways following acute lung injury [28]. The neutrophil markers *IL1B, ALPL* and *CXCR2* were all unaltered by OCS treatment in the present study. The six-gene expression

TABLE 3 Analysis of diagnostic value of six-gene expression signature, blood eosinophils and sputum eosinophils for OCS responsiveness

Responders *versus* Non-Responders	ROC	Minimal false negative results[#]				Minimal false positive results[¶]			
	AUC (%)	Cut point	Sensitivity	Specificity	PPV/NPV	Cut point	Sensitivity	Specificity	PPV/NPV
ΔFEV1, ACQ6 or F_{ENO}[+]									
Six-gene signature	90.5	⩾0.36	86.7	76.2	70.6/84.2	⩾0.63	73.3	95.2	90.1/80.0
Sputum Eosinophils	77.6	⩾2.5	79.2	69.2	70.4/78.3	⩾4.8	66.7	76.9	71.4/69.0
Blood Eosinophils	77.5	⩾0.3	70.8	66.7	68.0/69.6	⩾0.4	50.0	91.7	85.7/64.7

ROC:receiver operating characteristic; AUC: area under the curve; PPV: positive predictive value; NPV: negative predictive value; FEV_1: forced expiratory volume in 1 s; ACQ6: six-item Asthma Control Questionnaire score; F_{ENO}: exhaled nitric oxide. [#]: Minimal false negatives correspond to the point of the ROC curve with the highest sensitivity (true positive rate, useful for ruling disease out); [¶]: minimal false positives correspond to the point with the highest specificity (false positive rate, useful for ruling disease in); [+]: response=ΔACQ6⩾0.5 or ΔF_{ENO} (⩾20%↓ if V1 F_{ENO}⩾50 ppb or ⩾10 ppb↓ if V1<50 ppb) or ΔFEV1⩾12% and 200 mL).

biomarker signature was found to have excellent diagnostic value in determining subjects who might respond to OCS, as it provided a more accurate, sensitive assessment of active airway inflammatory mechanisms than other biomarkers. This provides further evidence to support a precision medicine approach to asthma.

Although technically more complex than blood cell counts, sputum gene expression signatures have great potential in detecting underlying mechanisms and guiding personalised treatment and management strategies [18]. Other gene expression profiles have been identified in induced sputum, such as *IL-4, IL-5* and *IL-13*, which are useful in phenotyping asthma as Th2-low and Th2-high [29]. High baseline expression of the genes encoding chloride channel, calcium-activated, family member 1 (*CLCA1*), periostin, and serine peptidase inhibitor, clade B (ovalbumin), member 2 (*serpinB2*) in epithelial cell brushings are related to clinical response to ICS [30]. Measurement of the expression of the six-gene expression biomarker signature is therefore a promising clinical tool, as it has the technical advantages of being quick, automated and more accurate, as compared to using sputum cell counts, which require more technical expertise and are time intensive.

The peripheral blood eosinophil count is less invasive, more cost effective and requires less technical expertise than the measurement of sputum eosinophils. It is an acceptable surrogate marker for sputum eosinophils, and can predict EA in uncontrolled asthma [7], and mild, moderate and severe asthma [8]. Blood eosinophils can also be utilised for targeted biologic therapy, by using baseline levels to predict response and levels during treatment to monitor effectiveness [6]. One limitation of blood eosinophils is that they are reduced by OCS in both responders and non-responders. This means that whereas baseline blood eosinophils might be useful in identifying subjects who require OCS, they have a limited role in monitoring the clinical efficacy of OCS in asthma. Since both CLC and CPA3 were reduced by OCS, the six-gene signature might be able to monitor treatment. However, further study is necessary. Blood eosinophils also have a role as an adherence marker to OCS therapy [31], as was evident in the present study; blood eosinophil levels were significantly reduced following prednisolone treatment. It might be important to assess adherence to OCS therapy, owing to the reluctance of some patients with asthma to take OCS as prescribed [32].

Interestingly, clinical improvements were observed following OCS intervention, despite the stability of subjects at baseline. This is in contrast to another study, which reported that 60 mg prednisone for 7 days in stable asthma did not lead to any improvements in FEV_1, FVC or asthma symptoms [33]. Half of the study population did not respond clinically to OCS, and some subjects exhibited persistent sputum eosinophilia, despite OCS treatment. It is likely that some of these subjects either had already achieved optimum control, and had no room for further clinical improvement, or had reduced steroid sensitivity. Both explanations seem likely. A subset of asthma patients is known to display resistance to OCS due to a range of factors [34]. Steroid resistance is generally reported in severe asthma, whereas in this study, only 30% of the non-responders were classified as severe. This observation might be explained by the paucity of investigations of OCS efficacy in stable or mild to moderate asthma.

An important element of the design of the present study is the inclusion of subjects ranging from mild to severe with varying inflammatory phenotypes, considering that all patients with asthma are at risk of exacerbation and subsequent OCS treatment. Similar studies have been limited because of narrow inclusion criteria, and the inclusion of subjects with high levels of (>2%) sputum eosinophils [35], severe asthma only [36], or high F_{ENO} ($\geqslant$40 ppb) [37]. In addition, some studies have not reported the effects of OCS on a range of biomarkers or gene expression [38–40]. Furthermore, the definitions used to categorise a positive response to corticosteroids are heterogeneous and not based on established international guidelines. This study used a composite definition for a positive response to OCS, which observes guideline-recommended clinically significant improvements in FEV_1, ACQ6 and F_{ENO}. This unique categorisation identifies all subjects with a clinically significant response, and recognises the heterogeneity of asthma.

The intervention dosage and duration of the present study are highly relevant to the examination of responses that might be expected in acute exacerbations. It is a limitation that subjects were studied while stable, although this design was chosen to reduce confounding factors associated with acute episodes of asthma. Thus, further investigation to confirm these results should be performed during exacerbations of asthma. This study provides new knowledge on the OCS response as it includes clinical assessment and biomarkers of both blood and sputum, which are not routinely reported. Assessment of inflammometry markers was performed blinded to clinical characteristics, to prevent unblinding of treatment allocation. The present study did have some limitations. Known side effects and previous OCS therapy might have affected the subject's decision to participate in the study. The analysis had limited power to detect differences in severe asthma response, and because of the relatively small number of subjects with neutrophilic airway inflammation, we were unable to examine the effects of OCS on neutrophilic asthma.

In conclusion, a clinical response to a 10-day course of OCS in adults with stable asthma was evident in subjects with poorer asthma control, AHR and lung function, and eosinophilic airway inflammation. Clinically significant improvements in FEV_1, ACQ6 and F_{ENO} were predicted based on the expression of a novel six-gene expression biomarker signature in induced sputum that included a combination of mast cell and eosinophil markers. Steroid resistance might not be limited to subjects with severe asthma. The present study supports further investigation into precision medicine approaches to asthma, and the use of molecular markers to enhance the prediction of treatment responsiveness in asthma.

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