



Phenotypic predictors of response to oral glucocorticosteroids in severe asthma



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KEYWORDS

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Summary

Background: Systemic glucocorticosteroids are side-effect prone but often necessary for the treatment of severe asthma (SA). Our goal was to assess the usefulness of medical history, physiological variables and biomarkers as predictors of response to oral steroids.

Methods: After 4 weeks of treatment optimization, 84 patients with SA and 62 with mild-to-moderate asthma (MA) underwent a 2 week double-blind placebo-controlled oral prednisolone intervention (0.5 mg/kg BW daily) (NCT00555607).

Results: Responders had a lower FEV₁% (73.7 vs 88.0), lower FEV₁/FVC ratio (0.65 vs 0.73), lower quality of life (SGRQ score 39.1 vs 31.4), lower total sputum cell number (1.0 vs 4.5×10^6) and higher number of sputum eosinophils (16.8% vs 6.3%) ($p < 0.05$). For all asthmatics, the degree of improvement in FEV₁ correlated with sputum eosinophils, level of asthma control, FeNO, quality of life, age of asthma onset and blood eosinophils. In SA, sputum eosinophils $\geq 3\%$ (OR 9.91), FEV₁ $\leq 60\%$ (OR 3.7), and SGRQ > 42.2 (OR 3.25) were associated with a good response to oral prednisolone. The highest sensitivity and specificity to predict more than 12% increase in FEV₁ in SA after oral prednisolone was found for sputum eosinophils $\geq 3\%$ and FeNO > 45 ppb.

Conclusions: Sputum eosinophils and FeNO were the best predictors of favorable response to oral prednisolone in severe asthmatics. A guided approach to glucocorticosteroid treatment should be recommended as it favors better control of the disease and presumably a lower rate of adverse events.

The study has been registered at the site: clinicaltrials.gov with number: NCT00555607.

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¹ BIOAIR – Longitudinal Assessment of Clinical Course and BIOMarkers in Severe Chronic AIRway Disease. See Contributors to the BIOAIR study in the [Appendix A](#) section.

Abbreviations

ACQ	Juniper's asthma control questionnaire
ASA	acetylsalicylic acid
BIOAIR	the study of longitudinal assessment of clinical course and BIOmarkers in Severe Chronic AIRway disease
BMI	body mass index
COPD	chronic obstructive pulmonary disease
CRP	C-reactive protein
ED	emergency department
FeNO	fraction of exhaled nitric oxide
FEV ₁	forced expiratory volume in 1 s
FIV	forced inspiratory volume in 1 s
FVC	forced vital capacity

ICS	inhaled corticosteroids
LABA	long-acting β_2 agonist
MA	mild-to-moderate asthma
NSAID	non-steroidal anti-inflammatory drug
OR	odds ratio
OST	oral steroid treatment
ROC	receiver operating characteristic
RR	relative risk
Rs	Spearman's rank correlation coefficient
SA	severe asthma
SABA	short-acting β_2 agonist
SGRQ	St. George's respiratory questionnaire
WBC	white blood cell count

Introduction

Systemic glucocorticosteroids represent a necessary therapeutic option, especially in problematic, severe asthma and in asthma exacerbations [1–3]. However, use of this treatment is often limited by significant side effects, including osteoporosis, arterial hypertension, diabetes, hypothalamic–pituitary–adrenal axis suppression, obesity, cataracts, skin thinning and glaucoma [1]. Optimization of the risk/benefit relationship for systemic glucocorticosteroid treatment is vital in the management of patients with severe asthma.

Asthma is no longer regarded as being a single disease entity, but instead a syndrome comprised of several pathophysiological endotypes and clinical phenotypes [4–6]. This concept is supported by the heterogenous response patterns observed for asthma medications, such as those documented for inhaled corticosteroids [7], β -agonists [8] and leukotriene receptor antagonists [9,10]. Similarly, the efficacy of systemic glucocorticosteroids is variable as reflected by the identification of a subgroup of difficult-to-treat or steroid resistant asthma patients [11]. The identification of a specific phenotype of patients who are most likely to benefit from oral steroid treatment would improve asthma management. It is hypothesized that treatment guided by objective predictors will result in better disease control, a lower rate of adverse events and diminished health care costs.

The objective of this study was therefore to perform an in-depth analysis of how patients' baseline characteristics influenced their response to a two week oral steroid trial. One hundred and forty six patients with mild-to-moderate or severe asthma were included in this investigation that used medical history, physiological variables and biomarkers as possible predictors of favorable response. Differences in the characteristics of responders and non-responders to treatment were analyzed, and correlations between studied variables with the degree of improvement after oral steroid treatment were examined. The sensitivity and specificity of the potential predictors of oral steroid responsiveness were also evaluated.

Methods

Study design

Details regarding the BIOAIR study design and methodology can be found in Ref. [12] and in the [on line depository](#). Patients with severe asthma (SA) and mild-to-moderate asthma (MA) were screened and included into the study. After a four week treatment optimization period an oral steroid (prednisolone 0.5 mg/kg of body weight/day or placebo) was added to regular treatment in a double-blind placebo-controlled manner for two weeks. The study ([clinicaltrials.gov](#) NCT00555607) was approved by the ethics review board (IRB number: 01-329).

Subjects

Asthma was diagnosed by pulmonary specialists according to published criteria [1,13]. Patients included in the severe asthma (SA) group had been under specialist treatment for at least one year and had experienced at least one exacerbation requiring oral steroid treatment in the past year, despite continuous treatment with high doses of inhaled corticosteroids (ICS; at least 1600 μ g/day budesonide or beclomethasone, 800 μ g/day fluticasone or equivalent), and long-acting β -agonists, and oral theophylline for at least one year. Patients in the mild-to-moderate asthma (MA) group had stable disease and received daily treatment with a maximum of 800 μ g/day budesonide or beclomethasone, 500 μ g/day fluticasone or equivalent. The MA patients used short-acting β -agonists as needed but did not require treatment with long-acting β -agonists and had not experienced exacerbations or hospitalizations in the past year.

Measurements

Lung function was assessed as forced expiratory volume in 1 s (FEV₁), and forced vital capacity (FVC), according to published guidelines [14,15]. A reversibility test as well as

skin prick tests to common aeroallergens were performed at the screening visit. Induced sputum was obtained using inclusion and exclusion criteria according to published recommendations [16]. Fraction of exhaled nitric oxide (FeNO) was measured using a NIOX analyser (Aerocrine AB, Solna, Sweden) according to guidelines [17]. All patients completed the St. George's respiratory questionnaire (SGRQ) [18], and the Juniper's Asthma Control Questionnaire (ACQ) [19].

Data collection and statistical methods

Data was entered into a central database through a web-based Case Record Form (eCRF) system developed specifically for the BIOAIR study. Patients' baseline characteristics are expressed as mean $\pm$ standard error of the mean (SEM). Continuous variables were analyzed by Mann–Whitney *U* test and categorical variables by χ^2 test. The outcome of treatment was defined as change in FEV₁% predicted (Δ FEV₁ = FEV₁% predicted post – FEV₁% predicted pre steroid treatment). Cut-offs Δ FEV₁ > 0% and Δ FEV₁ $\geq$ 12% were evaluated as definitions of a favorable outcome of oral steroid treatment. To identify baseline characteristics that may predict the outcome of OST, subjects with a positive outcome were compared with those who did not respond at all to OST. Correlations of studied variables with degree of improvement in FEV₁ after OST were evaluated with the application of Spearman's rank correlation coefficient. The following baseline characteristics were analyzed: sex, age, reversibility, body mass index (BMI), sputum eosinophils, sputum neutrophils, blood

eosinophils, smoking history, FeNO, acetylsalicylic acid (ASA) hypersensitivity, FEV₁% predicted, FEV₁/FVC, childhood onset (<16 years), SGRQ and Juniper ACQ. Odds ratios (OR) and relative risk (RR) in univariate analysis were calculated. For numerical variables median, 25 percentile and 75 percentile as possible cut-off values were evaluated. Chi-square test (or Fisher's exact test wherever appropriate) was applied. Multivariate logistic regression model including all potential predictors with a univariate *p* value of less than 0.1 was built and adjusted for atopy, sex, age and smoking status. Receiver operating characteristic (ROC) curves were drawn to study sensitivity and specificity of evaluated variables to predict a good response to OST. SPSS version 17.0 software (SPSS Inc, Chicago, Ill.) was used for the statistical analysis. *p* < 0.05 was considered a statistically significant difference for all tests.

Results

Patients' baseline characteristics

Demographic data and baseline characteristics of the 151 recruited patients are displayed in Table 1. SA patients were older than those with MA (50.2 vs 43.4 years), had a higher BMI (28.3 vs 25.2), and lower lung function (FEV₁% of predicted: 73.2 vs 89.9%) as compared with MA. The SA group was also characterized by a lower quality of life (44.8 vs 23.2 SGRQ units) and worse asthma control (2.0 vs 1.0 ACQ units). In comparison to MA, sputum eosinophils were significantly higher in SA patients (5.9% vs 17.9%).

Table 1 Demographic data and baseline characteristics of the study cohort [mean values ($\pm$ SEM), unless stated differently]. ICS – inhaled corticosteroids, CRP – serum C-reactive protein, atopy defined as at least one positive skin prick test (ND – not determined).

	Severe asthma	Mild-to-moderate asthma	<i>p</i>
Number of patients (<i>n</i>)	85	66	ND
Age (y) (min–max)	50.2 $\pm$ 1.4 (18–72)	43.4 $\pm$ 1.6 (21–70)	0.001 ^a
Females (%)	57.1%	59.7%	0.858 ^b
FEV ₁ (% pred)	73.2 $\pm$ 2.3	89.9 $\pm$ 2.5	< 0.0001 ^a
FEV ₁ (L)	2.08 $\pm$ 0.08	2.76 $\pm$ 0.09	< 0.0001 ^a
FEV ₁ /FVC	0.67 $\pm$ 0.01	0.70 $\pm$ 0.01	0.093 ^a
Reversibility	8.7 $\pm$ 0.7	10.5 $\pm$ 0.7	0.091 ^a
ICS (median [mean $\pm$ SD]) beclomethasone eq.	1600 μ g [2044 $\pm$ 912]	775 μ g [606 $\pm$ 223]	< 0.0001 ^a
OCS (median [mean $\pm$ SD; min–max]) prednisolone eq.	10 mg (14.15 $\pm$ 11.8; 2–50)	–	ND
BMI (kg/m ²)	28.3 $\pm$ 0.6	25.2 $\pm$ 0.5	< 0.0001 ^a
QoL (SGRQ)	44.8 $\pm$ 2.1	23.2 $\pm$ 2.2	< 0.0001 ^a
ACQ (Juniper)	2.03 $\pm$ 0.1	1.03 $\pm$ 0.7	< 0.0001 ^a
CRP (mg/L)	5.6 $\pm$ 0.8	4.6 $\pm$ 1.2	0.081 ^a
Atopy (%)	37.6%	46.9%	1.0 ^b
FeNO (ppb)	46.9 $\pm$ 6.6	39.3 $\pm$ 4.4	0.957 ^a
Sputum cells ($\times 10^6$)	3.36 $\pm$ 1.1	1.82 $\pm$ 0.3	0.425 ^a
Sputum eosinophils (%)	17.9 $\pm$ 3.7	5.9 $\pm$ 1.8	0.014 ^a
Sputum neutrophils (%)	40.4 $\pm$ 3.8	45.8 $\pm$ 4.4	0.433 ^a
Blood eosinophils ($\times 10^8$ /L)	3.75 $\pm$ 0.4	3.20 $\pm$ 0.4	0.531 ^a

p < 0.05 in bold.

^a Mann–Whitney *U* test.

^b Chi-square test.

Oral steroid trial

The oral steroid trial was completed by 146 patients, including 84 SA and 62 MA subjects. Five patients did not complete the OST and were excluded from further analyses. There were no major adverse events reported during the oral steroid intervention. The OST resulted in a mean 6.6% increase in FEV₁% predicted in SA ($p = 0.002$, steroid treatment vs placebo). No significant effects of the steroid intervention were observed in MA (-0.47% , $p = 0.38$). The oral steroid treatment did not induce significant changes in the FEV₁/FVC ratio in either of the studied groups. As many as 58.3% ($n = 49$) of the patients with SA were improved (improvement defined as any degree of increase in FEV₁, $\Delta\text{FEV}_1 > 0\%$) by OST and in 17.8% ($n = 15$) of SA patients, lung function increased by more than 12% ($\Delta\text{FEV}_1 \geq 12\%$).

Responders' and non-responders' characteristics

There were 79 patients in the whole asthma group who responded with any degree of improvement to OST, 23 who improved by at least 12% in FEV₁, and 67 patients who did not respond to the treatment ($\Delta\text{FEV}_1 < 0\%$) (Table 2, Fig. E3).

Responders defined by $\Delta\text{FEV}_1 > 0\%$ were characterized by a lower FEV₁% predicted (73.7 vs 88.0), lower FEV₁/FVC ratio (0.65 vs 0.73), lower quality of life (SGRQ score 39.1 vs 31.4), lower number of total sputum cells (1.0 vs 4.5) and higher percentage of eosinophils in induced sputum (16.8 vs 6.3) as compared with non-responders (Table 2).

Responders defined by $\Delta\text{FEV}_1 \geq 12\%$ were also characterized by a lower FEV₁% predicted (66.0 vs 88.0), lower FEV₁/FVC ratio (0.6 vs 0.73), lower quality of life (SGRQ score 44.5 vs 31.4), lower number of total sputum cells (0.8 vs 4.5) and higher percentage of eosinophils in induced

Table 2 Characteristics table for the whole study population, non-responders $\Delta\text{FEV}_1 < 0\%$, responders $\Delta\text{FEV}_1 > 0\%$ and responders $\Delta\text{FEV}_1 \geq 12\%$ (mean $\pm$ SEM). QoL – quality of life (St George's respiratory questionnaire, CRP – serum c-reactive protein, ACQ – asthma control questionnaire). Mann–Whitney test for difference in means unless stated otherwise. p Values < 0.05 were considered as statistically significant differences between groups.

	Asthma group	Non-responders $\Delta\text{FEV}_1 < 0\%$	Responders $\Delta\text{FEV}_1 > 0\%$	p Value (responders $\Delta\text{FEV}_1 > 0\%$ vs non- responders)	Responders $\Delta\text{FEV}_1 \geq 12\%$	p Value (responders $\Delta\text{FEV}_1 \geq 12\%$ vs non- responders)	p Value (responders $\Delta\text{FEV}_1 > 0\%$ vs responders $\Delta\text{FEV}_1 \geq 12\%$)
Number of patients (n)	146	67	79	—	23	—	—
Severe asthmatics (n)	84	35	49	—	15	—	—
Age (y) (min–max)	47.5 $\pm$ 1.1 (18–72)	46 $\pm$ 1.82 (18–72)	49 $\pm$ 1.31 (26–69)	0.28	52 $\pm$ 2.27 (31–67)	0.97	0.27
Females (%)	57.5	56.7	58.2	0.715 ^a	60.9	0.64 ^a	0.82 ^a
FEV ₁ (% pred)	80.22 $\pm$ 1.84	88.0 $\pm$ 2.44	73.74 $\pm$ 2.5	0.0002	66.04 $\pm$ 4.3	0.0001	0.148
FEV ₁ /FVC	0.69 $\pm$ 0.010	0.73 $\pm$ 0.013	0.65 $\pm$ 0.015	0.0002	0.6 $\pm$ 0.03	0.0002	0.086
Reversibility	9.48 $\pm$ 0.509	10.01 $\pm$ 0.72	9.04 $\pm$ 0.71	0.215	8.29 $\pm$ 0.96	0.190	0.694
Atopy (%)	40.4	40.3	41.7	1.000 ^a	34.8	0.5 ^a	0.5 ^a
Age of onset	26.78	27.57	26.13	0.74	36.71	0.043	0.012
BMI (kg/m ²)	27.04 $\pm$ 0.407	27.07 $\pm$ 0.55	27.01 $\pm$ 0.6	0.631	27.13 $\pm$ 1.06	0.781	0.939
QoL (SGRQ)	35.50 $\pm$ 1.84	31.41 $\pm$ 2.43	39.11 $\pm$ 2.7	0.044	44.5 $\pm$ 4.16	0.007	0.293
ACQ (Juniper)	1.59 $\pm$ 0.094	1.44 $\pm$ 0.12	1.72 $\pm$ 0.141	0.24	2.13 $\pm$ 0.23	0.010	0.101
CRP (mg/L)	5.01 $\pm$ 0.64	5.05 $\pm$ 1.10	4.98 $\pm$ 0.742	0.59	4.06 $\pm$ 1.25	0.7	0.451
FeNO (ppb)	44.0 $\pm$ 4.16	48.0 $\pm$ 8.04	41.71 $\pm$ 4.7	0.9	70.86 $\pm$ 13.06	0.073	0.014
Sputum cells ($\times 10^6$)	2.6 $\pm$ 0.61	4.49 $\pm$ 1.26	1.02 $\pm$ 0.20	0.0003	0.77 $\pm$ 0.2	0.004	0.947
Sputum eosinophils (%)	12.06 $\pm$ 2.27	6.28 $\pm$ 2.08	16.8 $\pm$ 3.6	0.015	29.70 $\pm$ 8.31	0.002	0.088
Sputum neutrophils (%)	42.61 $\pm$ 2.96	42.84 $\pm$ 4.51	42.4 $\pm$ 4.0	0.95	41.4 $\pm$ 7.66	0.86	0.905
Blood eosinophils ($\times 10^9$)	0.35 $\pm$ 0.03	0.311 $\pm$ 0.043	0.38 $\pm$ 0.04	0.072	0.50 $\pm$ 0.092	0.025	0.205

$p < 0.05$ in bold.

^a Chi-square test.

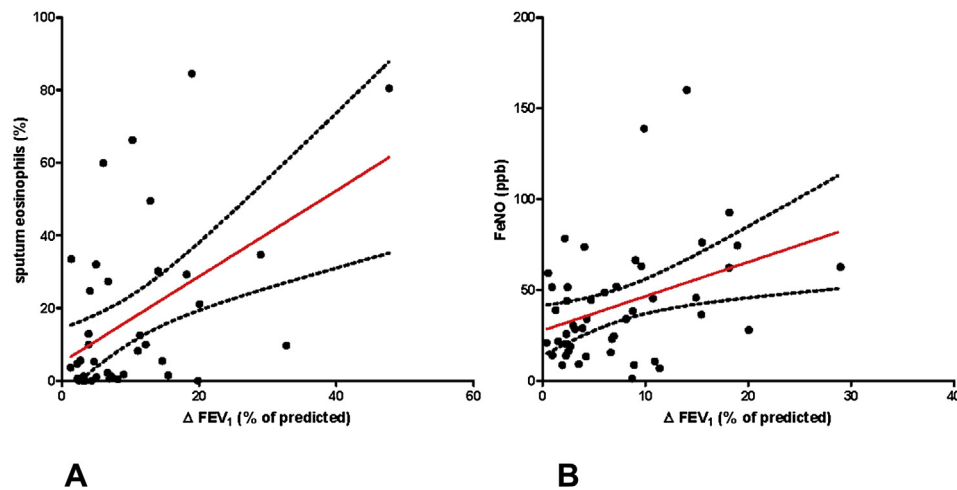


Figure 1 Correlation of studied variables with degree of improvement of FEV₁ after oral steroid treatment. Panel A: sputum eosinophils (% cells) $R_s = 0.41$, $p = 0.008$; panel B: FeNO (ppb) $R_s = 0.32$, $p = 0.03$.

sputum (29.7 vs 6.3) as compared with non-responders (Table 2). In addition, these more pronounced responders were also characterized by a later age of onset of the disease (36.7 vs 27.6) and higher number of eosinophils in peripheral blood (0.5 vs 0.3×10^9). Responders defined by $\Delta\text{FEV}_1 \geq 12\%$ also had significantly higher FeNO (70.9 vs 41.7) and a later age of asthma onset (36.7 vs 26.1) as compared to responders defined by a $\Delta\text{FEV}_1 > 0\%$ cut-off.

Correlation of studied variables with degree of improvement in FEV₁

Significant positive correlations were found between the degree of improvement in FEV₁ after the OST with sputum eosinophils ($R_s = 0.41$, $p = 0.008$), level of asthma control (ACQ score, $R_s = 0.33$, $p = 0.005$), FeNO ($R_s = 0.32$, $p = 0.029$), quality of life (SGRQ score, $R_s = 0.26$, $p = 0.035$), age of onset of the disease ($R_s = 0.28$, $p = 0.02$) and blood eosinophils ($R_s = 0.24$, $p = 0.04$) (Fig. 1 and Fig. E1, on-line repository). Significant negative correlations were found between the degree of improvement in FEV₁ after OST with FEV₁% predicted ($R_s = -0.35$, $p = 0.0015$) and FEV₁/FVC ratio ($R_s = -0.23$, $p = 0.045$) (Fig. E2, on-line repository). No significant correlations were found for any other evaluated variable (atopy, BMI, CRP, total sputum cell number and neutrophils in induced sputum). It should be noted that there was no correlation between the degree of improvement in FEV₁ after OST and reversibility test (improvement after β_2 agonist) at baseline ($R_s = -0.02$, $p = 0.186$). A significant correlation between blood and sputum eosinophils was found ($R_s = 0.5$, $p < 0.0001$) (Fig. 2).

Univariate analysis

Predictors of response to oral steroid treatment, using the outcome of $\Delta\text{FEV}_1 > 0\%$ for the SA group, include sputum eosinophils $\geq 3\%$ (OR 4.57), FEV₁ $\leq 74\%$ (OR 3.30), FEV₁ $\leq 60\%$ (OR 3.76) and SGRQ > 42.2 (OR 2.62). Using the outcome of $\Delta\text{FEV}_1 \geq 12\%$ to indicate response to treatment,

blood eosinophils $> 0.44 \times 10^9/\text{L}$ (OR 3.72), FeNO > 25 (OR 13.38) and FeNO > 50 (OR 6.94) were statistically significant (Table 3). For the MA group, using the outcome of $\Delta\text{FEV}_1 > 0\%$, statistically significant predictors of response to steroid treatment were blood eosinophils $> 0.24 \times 10^9/\text{L}$ (OR 3.11) and FEV₁/FVC < 0.7 (OR 3.29). Using the outcome of $\Delta\text{FEV}_1 \geq 12\%$, statistically significant predictors of response were FEV₁ $\leq 60\%$ (OR 31.20), FEV₁/FVC < 0.7 (OR 5.83) and childhood onset (< 16 y) (OR 0.07) (Table 3).

Multivariate analysis

When adjusted for possible confounding factors (sex, age, smoking history and atopy) and sorted for the outcome $\Delta\text{FEV}_1 > 0\%$, multivariate logistic regression models in the SA group showed that sputum eosinophils $\geq 3\%$ (OR 9.91), sputum eosinophils $\geq 2\%$ (OR 8.7), FEV₁ $\leq 60\%$ (OR 3.7), FEV₁ $\leq 74\%$ (OR 3.7) and SGRQ > 42.2 (OR 3.25) were associated with a good response to OST. When the outcome $\Delta\text{FEV}_1 \geq 12\%$ was used, blood eosinophils $> 0.44 \times 10^9/\text{L}$ (OR 4.7) were statistically significant (Table 3). In the MA

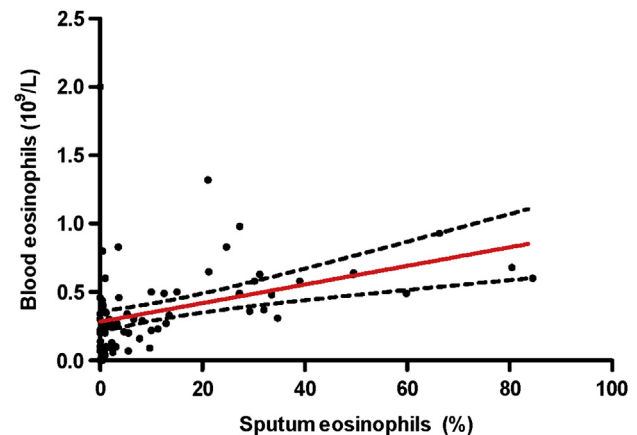


Figure 2 Correlation between blood and sputum eosinophils in asthma patients ($R_s = 0.5$, $p < 0.0001$).

Table 3 Statistically significant univariate and multivariate (adjusted for sex, age, smoking status and atopy) odds ratios and relative risks of baseline characteristics as predictors of response to oral steroid treatment.

	Univariate analysis outcome: $\Delta\text{FEV}_1 \geq 12\%$					Multivariate analysis outcome: $\Delta\text{FEV}_1 \geq 12\%$				
	Factor	OR	95% CI	RR	95% CI	p	Factor	OR	95% CI	p
Severe asthma group	eNO > 25	13.38	0.70–252.9	–	–	0.03	Blood eosinophils > $0.44 \times 10^9/\text{L}$	4.67	1.16–18.8	0.03
	eNO > 50	6.94	1.13–42.38	4.82	1.06–21.78	0.03		4.67	1.16–18.8	0.03
	Blood eosinophils > $0.44 \times 10^9/\text{L}$	3.72	1.09–12.68	2.891	1.08–7.68	0.029		4.67	1.16–18.8	0.03
Mild-to-moderate asthma group	$\text{FEV}_1 \leq 60\%$	31.20	2.7–36	8.5	3.11–23.49	0.006	$\text{FEV}_1 \leq 60\%$	24.82	1.6–385.8	0.022
	$\text{FEV}_1/\text{FVC} < 0.7$	5.83	1.1–31.89	4.62	1.02–21.06	0.048		24.82	1.6–385.8	0.022
	Childhood onset (<16 y)	0.07	0.004–1.4	0.00	–	0.034		24.82	1.6–385.8	0.022

group, multivariate analysis using the outcome of $\Delta\text{FEV}_1 > 0\%$ revealed that blood eosinophils $> 0.24 \times 10^9/\text{L}$ (OR 3.3) and $\text{FEV}_1/\text{FVC} < 0.7$ (OR 3.3) were associated with a good response to OST. Using the outcome of $\Delta\text{FEV}_1 \geq 12\%$ in the MA group showed that $\text{FEV}_1 \leq 60\%$ (OR 24.8) was statistically significant (Table 3).

Sensitivity and specificity of evaluated variables to predict a good response to OST

To evaluate the possibility of using the studied baseline characteristics as predictors of response to OST, receiver operating characteristic (ROC) curves were constructed and analyzed (selected curves are presented in Fig. 3). Of those asthma patients with sputum eosinophils $\geq 3\%$ at baseline, 68.2% improved after the OST (positive predictive value 68.2%, sensitivity 60.0%, specificity 68.2%) and 28.6% showed an improvement of at least 12% in FEV_1 (positive predictive value 28.6%, sensitivity 83.3%, specificity 60.7%) (Table E2, on-line repository). The negative predictive value of this test suggests that in the vast majority of asthma patients (94.7%), with baseline sputum eosinophil numbers below 3%, we cannot expect an improvement in FEV_1 greater than 12% after oral steroid treatment. Having a baseline $\text{FEV}_1 < 80\%$ of predicted and an SGRQ score > 30 also had high predictive value for a good response to oral steroid treatment (69.6% and 60.9%, respectively) (Table E2, on-line repository). These variables also show high negative predictive value (93.3% and 92.9%, respectively).

Of those severe asthma patients with sputum eosinophils $\geq 3\%$ at baseline, 76.2% improved after the OST (positive predictive value 69.6%, sensitivity 76.2%, specificity 64.7%) and 30.4% showed an improvement in FEV_1 of at least 12% (positive predictive value 30.4%, sensitivity 87.5%, specificity 50.0%) (Table E2, on-line repository). The negative predictive value of this test suggests that in the vast majority of severe asthma patients (93.3%), with baseline sputum eosinophil numbers below 3%, we cannot expect an improvement in FEV_1 greater than 12% after oral steroids. A high negative predictive value was found also for $\text{FeNO} > 45$ ppb. In the majority of SA patients (92.0%) with a baseline FeNO below 45 ppb, a significant improvement in $\text{FEV}_1 (>12\%)$ after oral steroids should not be expected (Table E2, on-line repository).

Discussion

Identifying asthma patients who are most likely to benefit from oral steroids may have significant practical implications, as this could optimize the risk to benefit ratio of a very side-effect prone treatment. This is the first comprehensive evaluation of phenotypic predictors of response to oral glucocorticosteroids in severe asthma. We assessed several possible predictors of a favorable response including medical history, physiological variables and biomarkers. We found that there are significant phenotypic differences between responders and non-responders to the OST (oral steroid treatment). Responders are characterized by a lower lung function (FEV_1 and FEV_1/FVC ratio), lower quality of life, lower total sputum cell number and higher number of sputum eosinophils as compared to

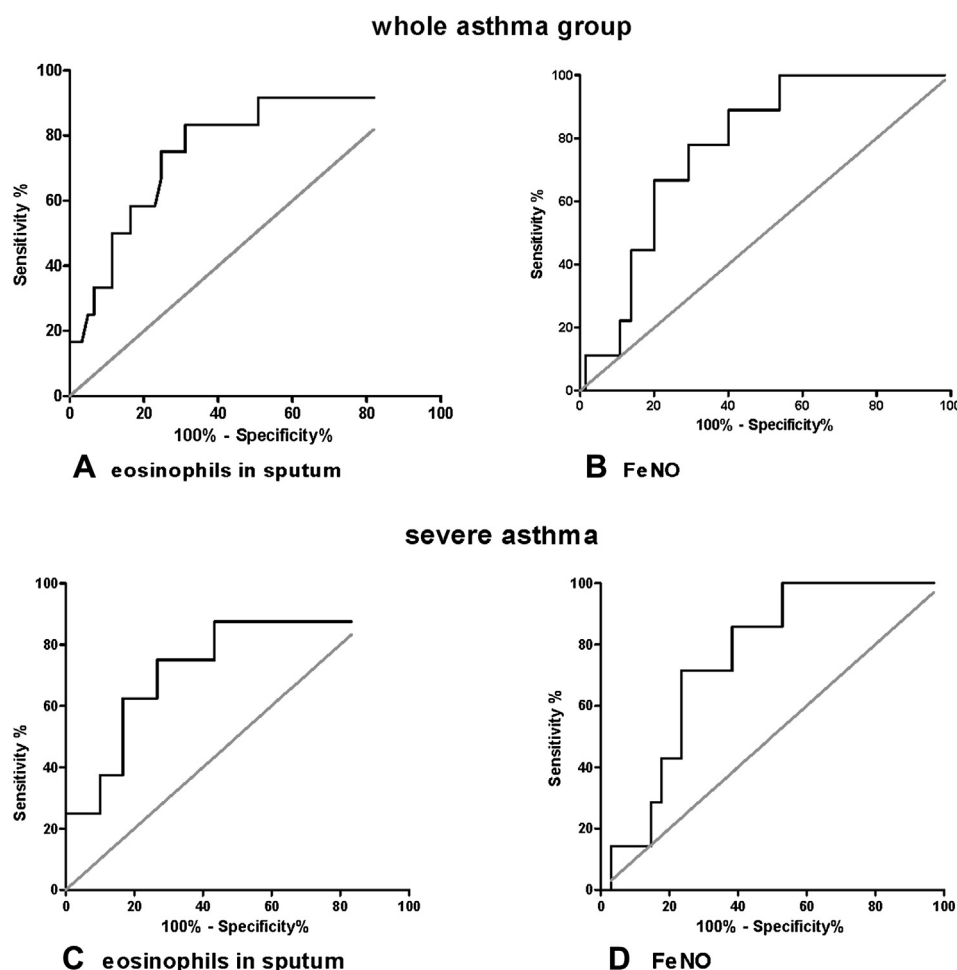


Figure 3 Receiver operating characteristic curves (ROC) for eosinophils in induced sputum (A and C) and FeNO (B and D) as parameters to predict good response to oral steroid treatment in the whole study group (upper panels) and severe asthmatics (lower panels).

non-responders. Moreover, patients who responded to the oral steroid treatment with more than a 12% improvement in FEV₁ had significantly higher FeNO levels and later onset of the disease as compared to those who did not respond so well to oral steroids. The strongest correlation between the degree of improvement in lung function after OST was found for sputum eosinophils. The predictive value of sputum eosinophils was thereafter confirmed by multivariate modeling, where after adjustment for possible confounding factors such as age, sex, atopy and smoking, sputum eosinophils together with lung function and quality of life remained strongly associated with a good response to OST in severe asthmatics.

The usefulness of sputum eosinophils in guiding steroid therapy in asthma has previously been proposed by several authors [20–22], however those reports have evaluated the use of inhaled [21,23] treatment in mild-to-moderate, rather than severe asthmatics. The few previous studies of oral steroid use include relatively small numbers of patients [24,25]. In clinical practice we are able to achieve good asthma control in the majority of mild-to-moderate asthmatics with currently available controller medications, mainly inhaled steroids. This is, however not the case

in severe, treatment resistant asthmatics where systemic steroids or other new biological treatments are necessary. Thus, our goal was to evaluate predictors of responsiveness to OST in severe asthma and to our knowledge this is the first report dealing with this issue. The strength of our study lies in the prospective, double-blinded study design, and the involvement of several leading clinical centers which enabled us to include over 80 carefully phenotyped patients with well-defined severe asthma. Moreover, the impact of oral steroids was evaluated on top of the intensive anti-inflammatory therapy introduced to patients during the four week treatment optimization phase. In our study we aimed to evaluate not only biomarkers but also several phenotypic predictors and physiological variables similar to those previously investigated in preschool children, where subjects with specific characteristics showed a more favorable response to inhaled steroids [26].

A favorable outcome for systemic steroids is sometimes defined as at least a 15% improvement in FEV₁ after 14 days of treatment with an oral steroid (40 mg/d prednisolone) in patients who demonstrated more than a 15% improvement in FEV₁ following the use of an inhaled β_2 agonist [27]. It is doubtful if such a definition is appropriate for the subgroup

of severe asthmatics, since many of those patients are characterized by a component of irreversible airway obstruction, probably due to chronic inflammation and airway remodeling [27–29]. Moreover, the majority of severe asthma patients do not show a 15% improvement in FEV₁ following the use of an inhaled β_2 agonist. We therefore decided to evaluate two different levels of favorable outcome for the OST, namely, any degree of improvement ($>0\%$), and at least a 12% increase in FEV₁. We believe this approach is more applicable to this particular patient population and may provide valuable information regarding treatment options in the severe asthma cohort.

Possible limitations of our study include the duration of treatment (2 weeks) and the definition of the primary outcome (change in lung function, FEV₁). We have however justified the short course of oral steroids as this is the most common approach used in clinical practice for a period of treatment with systemic glucocorticosteroids [1]. It remains a goal for future studies to assess whether our results can be generalized to longer treatment periods and the management of exacerbations. However, our choice of the shorter treatment duration for this first comprehensive study of predictors of steroid response did impose restrictions regarding selection of the primary study outcome. Although endpoints such as the number of exacerbations, quality of life or asthma control index could be considered clinically relevant, additional determinants, of a favorable outcome, a significantly longer duration of treatment would be necessary to have sufficient power to detect changes in those variables.

Of interest was the fact that in our severe asthma cohort the percentage of eosinophils and level of exhaled nitric oxide were relatively high. This raises the question whether these asthmatics are compliant with current therapy or should receive a higher dose of ICS or additional anti-inflammatory treatment (LTRA) before starting oral steroids. Compliance with treatment regimens is one of the major challenges in everyday clinical practice, especially with respect to systemic or inhaled glucocorticosteroids in asthma patients [30]. We do believe however, that this is unlikely to pose a problem in the current study. First, high adherence to the treatment protocol was achieved by the involvement of highly devoted clinical centers and the frequent and careful follow-up of included patients. This was confirmed by a surrogate marker of steroid usage, namely changes in white blood cell numbers. There was thus a significant increase in the total number of white cells and neutrophils and a decrease in numbers of peripheral and sputum eosinophils in all patients randomized to receive OST as compared with baseline values.

We found that none of the evaluated patients' baseline characteristics, or currently available biomarkers, were better predictors of steroid responsiveness than determination of eosinophil numbers in induced sputum. Sputum eosinophilia allows us to predict a response in lung function following oral steroid treatment in clinical practice. The study results show that of those severe asthma patients with baseline sputum eosinophils $\geq 3\%$, almost 70% will respond favorably to OST, a prediction with acceptable sensitivity and specificity (76.2% and 64.7%, respectively). This is in line with the study of Little et al. [24] who, in a group of 37 patients with stable, chronic asthma found that

sputum eosinophils $\geq 4\%$ had a positive predictive value of 68%, with a sensitivity of 54% and specificity of 90% for an increase in FEV₁ $\geq 15\%$ after a short course of oral steroids. In another study, ten Brinke et al. [22] demonstrated that the vast majority of patients with difficult-to-treat asthma and persistent eosinophilia in induced sputum responded well to systemic steroid treatment.

For those subjects in whom sputum induction is not applicable, other practical approaches may be proposed. We found a good correlation between blood and sputum eosinophils (Fig. 2). This has further been confirmed in our multivariate regression model, as blood eosinophils were also predictors of good response to oral steroids. Of particular practical interest is the high negative predictive value of a low sputum eosinophil number and a moderate elevation of FeNO. In our study, in the vast majority of the whole asthma population (more than 95%) and in the majority of severe asthma patients (more than 90%) who had baseline sputum eosinophils below 3% or FeNO levels below 45 ppb, there was no significant improvement in lung function after the OST. Generalizing from these observations, we would suggest that other treatments (including new biological agents) should be considered for patients with low sputum eosinophils and unremarkable FeNO levels, as apart from inducing adverse events, oral glucocorticosteroids would seem to have less potential to improve lung function.

In conclusion, we found major differences in characteristics between responders and non-responders to oral glucocorticosteroids among asthma patients. Sputum eosinophils and FeNO represented the most promising options for predicting a favorable outcome to OST. This is the first study to document this effect in severe asthmatics. Guided approaches to oral glucocorticosteroid treatment should be recommended as this would result in better control of the disease, help to lower the rate of adverse events and therefore ultimately diminish health care costs.

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Authors' contribution: Primary data aggregation and statistical analyses were done by MK, SH, RM, BD and SED, and then discussed and interpreted with the other co-authors at

face-to-face meetings. MK wrote the first draft of the manuscript. Revisions were made by MK together with SED and in an interactive process including all co-authors. All authors participated in the study design, conduction of the trial, collection and interpretation of data, and critical review of the draft versions of the report. The authors thank all participating patients, all investigators listed and several additional team members that made the BIOAIR study possible as well as the indicated funding.

Appendix A. Contributors to the BIOAIR study

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Appendix B. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.rmed.2013.07.014>.

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