Assessment of corticosteroid response in pediatric patients with severe asthma by using a multidomain approach

Cara J. Bossley, MD(Res),[a,b] Louise Fleming, MD,[a,c] Nicola Ullmann, MD,[a,c] Atul Gupta, MD(Res),[c] Alexandra Adams, MBBS,[a] Prasad Nagakumar, MBBS,[a] Andrew Bush, MD,[a,c] and Sejal Saglani, MD[a,c]

London, United Kingdom

Background: There is no agreed upon definition of systemic corticosteroid response in asthmatic children. Moreover, pediatric severe therapy-resistant asthma (STRA) is heterogeneous, and thus response to steroids is unlikely to be uniform in all patients.

Objective: We sought to evaluate the utility of a multidomain approach incorporating symptoms, lung function, and inflammation to determine steroid responsiveness in pediatric patients with STRA.

Methods: Eighty-two children (median age, 12 years) with STRA received a clinically indicated dose of intramuscular steroid. Changes in 4 separate domains were assessed 4 weeks after intramuscular triamcinolone acetonide: normalization of (1) symptoms (Asthma Control Test score, >19/25 or 50% increase), (2) spirometric results (FEV_1 ≥80% of predicted value or ≥15% increase), (3) fraction of exhaled nitric oxide levels (<24 ppb), and (4) sputum eosinophil counts (<2.5%). Fifty-four of 82 children had complete data in all 4 domains.

Results: Twenty-three (43%) of 54 children had a symptom response, 29 (54%) of 54 had a lung function response, 28 (52%) of 54 had a fraction of exhaled nitric oxide response, and 29 (54%) of 54 had a sputum eosinophil response. Although a similar proportion of children responded to systemic corticosteroids in each domain, there were no reliable predictors of a response pattern. Seven (13%) of 54 were complete responders (response in all domains), 8 (15%) of 54 were nonresponders (no response in any domain), and 39 (72%) of 54 were partial responders (response in ≥1 domain).

Conclusions: A multidomain evaluation of systemic steroid responsiveness using pragmatic clinical assessments confirms childhood STRA is heterogeneous and that a complete response in symptoms and inflammatory and physiologic parameters is rare. Individual response patterns to systemic steroids might be useful in guiding the choice of add-on therapies in each child as a step toward achieving personalized medicine. (J Allergy Clin Immunol 2016;■■■:■■■-■■■.)

Key words: *Pediatric, severe asthma, steroid response, spirometry, inflammation*

Approximately 2% to 5% of all asthmatic children have severe therapy-resistant asthma (STRA), are challenging to treat, and consume significant health care resources.[1] Many children can have asthma that is difficult to treat despite high doses of therapy because of previously unidentified modifiable factors, such as poor adherence or persistent allergen exposure.[2] However, once these basic factors have been addressed, a group with STRA remains.[3]

The mainstay of asthma treatment is inhaled corticosteroids (ICSs). However, children with STRA have disease that remains poorly controlled despite high doses of ICSs; additional controllers, such as long-acting β_2-agonists and leukotriene receptor antagonists; and often maintenance oral steroid therapy. Steroid responsiveness can be measured in many ways, but currently, there is no accepted definition in children. A proposed definition of steroid response[4,5] is a 15% or greater predicted increase in morning FEV_1 in patients with bronchodilator reversibility (BDR) of 12% or greater from baseline and an abnormal FEV_1 (<80% of predicted value) before a systemic steroid trial. However, it is acknowledged that this might not be an appropriate definition for children because many children with a confirmed diagnosis of severe asthma have normal spirometric results, but their symptoms remain poorly controlled.[6-8]

We have previously shown pediatric STRA to be heterogeneous with respect to lung function, inflammation, and remodeling.[3] Although, as a group, children with STRA have lower lung function, increased eosinophilic inflammation, and increased reticular basement membrane thickness and smooth muscle mass compared with patients with mild asthma and nonasthmatic control subjects,[3] there is overlap between groups, as well as considerable variability within patients with STRA. Given this marked variation within the STRA group, it is likely that some children might respond more to steroids with an improvement in lung function, whereas others might only show a response through an improvement in inflammation. Therefore we investigated a multidomain definition of systemic corticosteroid responsiveness, namely change in (1) symptoms, (2) spirometry, and (3) noninvasive markers of inflammation before and 4 weeks

From [a]Respiratory Paediatrics, Royal Brompton Hospital and National Heart & Lung Institute, Imperial College London; [b]Respiratory Paediatrics, Kings College Hospital; and [c]Leukocyte Biology, National Heart & Lung Institute, Imperial College London.

A.B. was supported by the NIHR Respiratory Disease Biomedical Research Unit at the Royal Brompton and Harefield NHS Foundation Trust and Imperial College London. S.S. is an NIHR Career Development Fellow (Grant ID: CDF-2014-07-019). A.B. and S.S. are members of the MRC Asthma UK Centre for Allergic Mechanisms of Disease. A.B. is a Principal Investigator in the Asthma UK Centre for Applied Research.

Disclosure of potential conflict of interest: L. Fleming has received lecture fees from Novartis. A. Gupta has received research support from BMA St James Fellowship and Novartis and has participated in clinical trials sponsored by Boehringer Ingelheim and GlaxoSmithKline. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication July 16, 2015; revised December 12, 2015; accepted for publication December 28, 2015.

Corresponding author: Sejal Saglani, MD, Respiratory Paediatrics, 368 Sir Alexander Fleming Building, Imperial College London, London SW7 2AZ, United Kingdom. E-mail: s.saglani@imperial.ac.uk.

0091-6749/$36.00

© 2016 American Academy of Allergy, Asthma & Immunology

http://dx.doi.org/10.1016/j.jaci.2015.12.1347

Abbreviations used
ACT: Asthma Control Test
BAL: Bronchoalveolar lavage
BDR: Bronchodilator reversibility
FENO: Fraction of exhaled nitric oxide
ICS: Inhaled corticosteroid
STRA: Severe therapy-resistant asthma

after a single clinically indicated dose of intramuscular triamcinolone acetonide. We hypothesized that children with STRA would not have a uniform response pattern. We also related the steroid-response pattern to clinical features and peripheral and airway inflammation before administering the systemic steroid to try to identify predictors of response pattern.

METHODS

Subjects and definition of severe asthma (STRA)

Children aged between 6 and 16 years with problematic severe asthma (ongoing poor control despite prescribed high-dose inhaled steroids [≥800 μg/d], additional controller medications, and assignment to stage 4/5 of the British Thoracic Society/Scottish Intercollegiate Guidelines Network guidelines) referred between 2005 and 2012 were investigated by using our standardized clinical investigation protocol with an outpatient-led nurse assessment and home visit.[2,3] Children with modifiable factors that could be addressed, in particular adherence, were classified as having difficult asthma and excluded from this study (Fig 1).[9] Only the remaining patients, in whom diagnosis had been confirmed (evidence of reversible airflow obstruction and doctor-diagnosed wheeze), adherence had been ensured (general practitioner prescription records, appropriate inhalers being available during the home visit, and proved ability to use the medication delivery device prescribed), and underlying modifiable factors (eg, environmental tobacco smoke and allergen exposure) had been minimized, were given a diagnosis of STRA, which is in line with recent guidelines.[10] All children with STRA underwent our clinical protocol, which includes bronchoscopy, as previously described,[3] followed by a single intramuscular injection of triamcinolone acetonide to determine steroid responsiveness. This was a clinical investigation protocol but not a clinical trial. The intramuscular steroid preparation used (triamcinolone acetonide [Kenalog]; Bristol-Meyers Squibb, New York, NY) was recommended for sustained systemic corticosteroid treatment in patients with asthma[11] and has previously been used in adults[12] and children[13] with difficult asthma. A single dose of intramuscular systemic steroids was administered, and assessments of steroid response were made immediately before and 4 weeks after administration.

Bronchoscopy, bronchoalveolar lavage, and endobronchial biopsy

Bronchoscopy and bronchoalveolar lavage (BAL) were performed after achievement of general anesthesia, and samples were processed for analysis of eosinophilic inflammation, as previously described (see the Methods section in this article's Online Repository at www.jacionline.org for details).[3] Blood for total IgE and eosinophil measurements was taken at the time of bronchoscopy.

Administration of systemic steroid and assessments of steroid response

An assessment with the (1) the Asthma Control Test (ACT) for symptom control (see Fig E1 in this article's Online Repository at www.jacionline.org), (2) spirometry,[14] (3) sputum eosinophil measurement, and (4) fraction of exhaled nitric oxide (FENO; at a flow rate of 50 mL/s) measurement was made on the morning of bronchoscopy. An intramuscular injection of triamcinolone acetonide (80 mg at age ≥12 years and 40 mg at age <12 years)[13] was administered at the end of the bronchoscopy, and tests 1 to 4 were repeated 4 weeks later to determine steroid response (Table I).

Multidomain definition of steroid response

Some children had normal values for some domains before receiving the systemic corticosteroid. Thus they did not have steroid-unresponsive abnormalities in those domains. Because all children were already prescribed high-dose maintenance inhaled steroids and we had ensured adherence as far as possible, we assumed those with normal values before the systemic corticosteroid trial that remained normal in that domain were steroid responsive. We believed in these children it was unethical to confirm this by reducing their treatment until abnormalities appeared in all domains. Therefore data analysis was initially undertaken for all patients. Subsequently, the analysis was restricted to include only those children whose symptoms did not improve after triamcinolone (ie, abnormal at baseline) to assess additional responsiveness after systemic corticosteroids. Response to steroids was examined in each domain and in a combination of domains.

Symptom (ACT) response. Symptom control was assessed by using the ACT[15] because most patients were 12 years or older, and for consistency, this was used in preference to the childhood ACT, which was not available at the start of data collection. A positive response was defined as an ACT score of greater than 19 of 25[16] or an increase of 50% or greater.

FEV_1 response. Spirometry was performed according to American Thoracic Society/European Respiratory Society guidelines.[14] Response was defined as attaining a prebronchodilator FEV_1 of 80% of predicted value or greater or an increase of 15% or greater.

FENO response. FENO measurements at 50 mL/s were made with a chemiluminescence analyzer (NIOX; Aerocrine, Solna, Sweden) in accordance with American Thoracic Society/European Respiratory Society guidelines.[17] Response was defined as a normal value (<24 ppb).[18]

Sputum eosinophil response. Sputum induction was performed with 3.5% saline, as previously described.[3] Sputum response was defined as normalization of sputum eosinophil counts (<2.5%).[19]

Complete response, partial response, and nonresponse to triamcinolone acetonide

A complete corticosteroid response was defined as symptom, FEV_1, sputum eosinophil, and FENO responses. A response in at least 1 domain was a partial response, and an absence of response in all domains was nonresponse. If sputum was unavailable, responses were assessed in 3 domains only.

Statistical analysis

Baseline values for all domains in the discovery and validation cohorts were not normally distributed and were therefore analyzed by using nonparametric tests. The Mann-Whitney *U* test was used for continuous variables, and categorical data were analyzed by using the Fisher exact or χ^2 tests. Data were analyzed by using the Wilcoxon test for nonparametric data and the paired *t* test for parametric data to compare changes before and after triamcinolone. Data were analyzed with SPSS, version 17 (SPSS, Chicago, Ill). Logistic regression analysis was performed, and the results were presented for univariate analysis with the statistical software Stata, version 12.1 (StataCorp, College Station, Tex).

RESULTS

Subjects and demographics

Eighty-two (51 male) children (median age, 12 years; range, 6.5-17.3 years) underwent investigations and received systemic corticosteroids (Fig 1). All children had data for at least 1 steroid-response domain (symptoms, FEV1, FENO, and/or sputum eosinophils), and a subgroup of 54 (37 male) children had data for all 4 domains before and after systemic steroid injection (Table II).

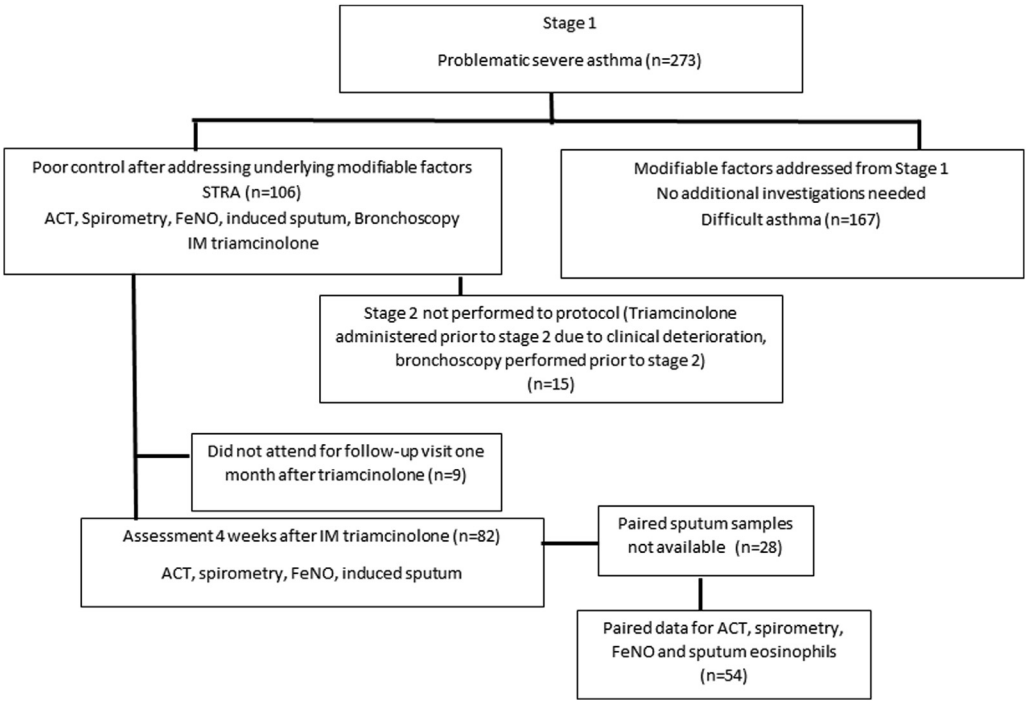

FIG 1. Flow diagram showing the number of children initially assessed with problematic asthma, those excluded having received a diagnosis of difficult asthma with underlying modifiable factors, and those remaining who were included with STRA. *IM*, Intramuscular.

TABLE I. Multidomain definition of systemic corticosteroid response

Domain	Response pattern 4 wk after intramuscular triamcinolone
Lung function (FEV_1)	Increase in FEV_1 (% predicted) to normal (≥80%) or ≥15% improvement
Symptoms (ACT score)	Increase in ACT score to normal (≥20/25) or ≥50% or 5 points (whichever is greater)
FENO	Improvement to normal (<24 ppb)
Sputum eosinophils	Improvement to normal (<2.5%)

Assessments of steroid response

The response pattern in each domain for all children (n = 82) and the subgroup of children with data available in all 4 domains (n = 54), including those with a normal value at baseline, are summarized in Fig 2 and Table III. There was no significant difference in the proportion of children who responded in each domain when all patients were compared with those who had data available for all domains.

Response pattern in each domain in children with data for all 4 parameters (n = 54)

Only those children with a response recorded in all 4 domains (a complete data set recorded) were assessed in detail both for patterns of response and predictors of response to prevent any chance of selection bias influencing the results.

Symptom response

Twenty-three (43%) of 54 children had an improvement in symptoms after systemic corticosteroids (Fig 2, *A*, and Table III). Eight (15%) of 54 children had a normal ACT score before the steroid trial, and 16 (35%) of 46 of those with an abnormal ACT score at baseline improved or normalized (Fig 2, *B*).

FEV_1 response

Twenty-nine (54%) of 54 children were steroid responsive in the FEV_1 domain (Fig 2, *C*, and Table III). Twenty (37%) of 54 had normal FEV_1 (≥80% predicted) before systemic corticosteroids (as explained by their maintenance high-dose inhaled steroid therapy). Fourteen of (41%) 34 of those with an abnormal FEV_1 (<80%) at baseline showed improvement after systemic corticosteroids (Fig 2, *D*).

FENO response

Twenty-eight (52%) of 54 children had normal FENO levels after systemic corticosteroids (Fig 2, *E*, and Table III). Fifteen (28%) of 52 had a normal FENO level before systemic steroids, and 17 (46%) of 37 of those with an increased FENO level at baseline had a reduction to normal levels after systemic steroids (Fig 2, *F*).

Sputum eosinophil response

Fifty-four children had paired sputum samples before and after triamcinolone acetonide. Of these, 29 (54%) of 54 had sputum eosinophil counts of less than 2.5% after triamcinolone acetonide (Fig 2, *G*, and Table III). Fifteen (28%) of 54 had normal sputum eosinophil counts before triamcinolone. Seventeen of (44%) 39 patients who started with an abnormal sputum eosinophil count had normalization of sputum eosinophil counts after triamcinolone acetonide (Fig 2, *H*).

TABLE II. Demographics of all children before receiving systemic corticosteroids

	All (n = 82)	Patients with data for all domains (n = 54)
Atopy	54/82 (66%)	39/54 (72%)
Male/female sex	51:31	37:17
Age (y)	12.3 (7-16.2)	12.7 (7-16.2)
Duration of symptoms (y)	8.96 (2.3-15.1)	8.48 (2.3-14.5)
Weight (kg)	43.8 (18.7-115)	45.5 (22.4-115)
Weight *z* score	0.48 (−4.5 to 3.7)	0.61 (−4.5 to 3.7)
Height (cm)	145 (76-188)	146 (76-188)
Height *z* score	0.04 (−3.9 to 2.88)	0.09 (−3.3 to 2.9)
Intubation for asthma	22/82 (27%)	13/54 (24%)
Medications: daily dose of ICS (μg/d), budesonide equivalent	1600 (800-3200)	1600 (800-3200)
Combination ICS/LABA	82/82 (100%)	54/54 (100%)
Leukotriene receptor antagonist	62/82 (76%)	40/54 (74%)
No. on daily systemic steroids	26/82 (32%)	19/52 (37%)
Theophylline	20/82 (24%)	15/54 (28%)
Antihistamine	30/82 (37%)	20/54 (37%)
Baseline ACT score	13 (6-23)	13 (6-23)
Normal ACT score (>19/25)	9/82 (11%)	8/54 (15%)
Baseline FEV_1 (% predicted)	72 (24-134)	72.5 (36-134)
Baseline FEV_1 (L)	1.72 (0.45-4.02)	1.85 (0.7-4.02)
No. with FEV_1 "normal" (≥80% of predicted value)	30/82 (37%)	20/54 (37%)
Baseline FVC (% predicted)	90 (36-134)	90 (52-134)
Baseline FVC (L)	2.36 (0.64-5.36)	2.56 (1.38-5.39)
Baseline BDR (%)	12.8 (−6 to 134)	14 (0-29)
Baseline $FENO_{50}$ (ppb)	47 (5.6-225)	47 (9-225)
No. with normal FENO (<24 ppb)	20/83 (24%)	15/54 (28%)
Induced sputum eosinophil counts (%)	5.1 (0-92)	4.85 (0-92)
No. with normal sputum eosinophil counts (<2.5%)	16/59 (27%)	15/54 (28%)

Values are presented as medians (ranges), unless otherwise stated.
$FENO_{50}$, Fraction of exhaled nitric oxide at a flow rate of 50 mL/s; *FVC*, forced vital capacity; *LABA*, long-acting β-agonist.

Discordance in FENO and sputum eosinophil domains

There was concordance in 39 (72%) of 54 children for FENO or sputum response. Twenty-one of 54 were responders in both domains, and 18 of 54 were nonresponders in both domains. There was discordance in 15 (28%) of 54 children; 7 of 54 had a FENO but not a sputum response, and 8 of 54 had a sputum but not a FENO response. Relationships between invasive and noninvasive assessments of airway eosinophils (FENO values and sputum and BAL eosinophil counts) are shown in Figs E2-E4 in this article's Online Repository at www.jacionline.org.

Complete responders, partial responders, and nonresponders

Seven (13%) of 54 children responded in all domains (complete responders), 39 (72%) of 54 responded in at least 1 domain (partial responders), and 8 (15%) of 54 did not respond in any domain (nonresponders).

Medications

There were no significant differences in response to triamcinolone in any domain between those children prescribed and not prescribed maintenance oral steroids nor was there any ICS dose effect. We do not have data concerning duration of prescribed controller therapy, but children had all been symptomatic for many years (median, 8.48 years; range, 2.3-14.5 years).

Comparison with current definitions of systemic steroid responsiveness

A definition of 15% of predicted value or greater increase in FEV_1 in patients with a BDR of 12% or greater from baseline and an abnormal FEV_1 (<80% predicted) was only applicable to 25 (46%) of 54 children because the remainder had an FEV_1 of greater than 80%, a BDR of less than 12%, or both before administration of triamcinolone acetonide. Of these patients, 12 (48%) of 25 had a positive steroid response.

Relationship between steroid-response pattern and clinical features and airway inflammation before systemic steroid injection

There were no consistent clinical or inflammatory features at baseline associated with a response to systemic corticosteroids in any of the 4 domains (Table IV). A lower ACT score (indicating more symptoms) before systemic steroids was associated with a poor response in the symptom domain (P = .0002). A history of intubation was associated with a better response in the FEV_1 domain, and this held true when logistic regression analysis was performed. A past history of intubation was associated with a response in the FEV_1 domain, with an odds ratio of 7.02 (95% CI, 1.4-35.8; P = .019; see Table E1 in this article's Online Repository at www.jacionline.org). However, atopy, serum IgE levels, and blood or BAL eosinophil counts were not associated with any pattern of steroid response (Table IV).

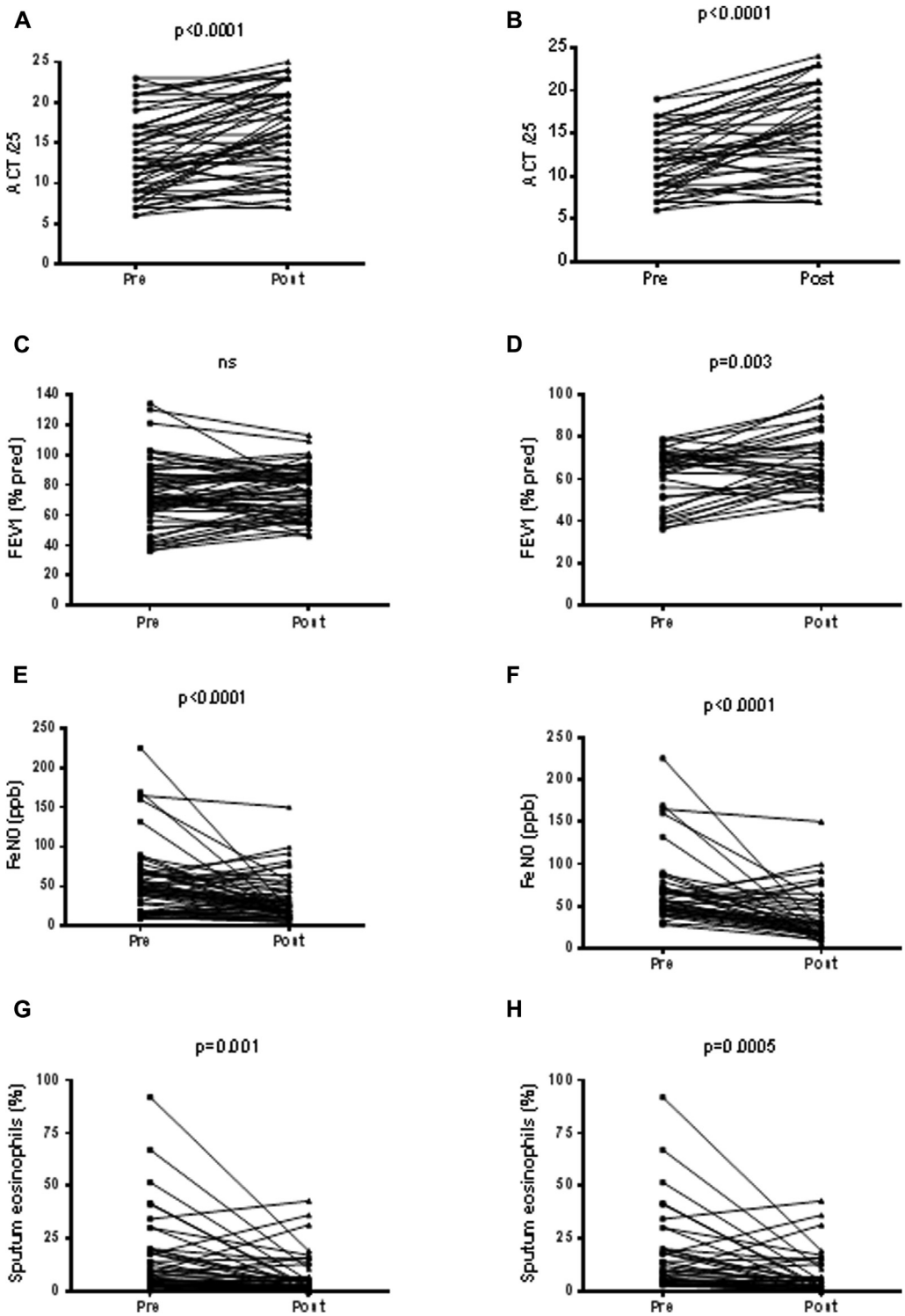

FIG 2. Steroid-response pattern in each domain for every child. **A, C, E,** and **G,** Subjects who had all data available (n = 54). **B, D, F,** and **H,** Subjects who had all data available and an abnormal value before triamcinolone.

DISCUSSION

We have proposed here that systemic corticosteroid response should be assessed in multiple domains, including normalization or improvement of spirometric results, symptom scores, and inflammation (sputum eosinophil counts, FENO levels, or both) in children with severe asthma because this approach reflects disease heterogeneity. None of these domains was abnormal in every child with STRA, underscoring the need for a multidomain assessment. A similar number of children (approximately 40% to 50%) responded in each domain, with no clinical features of

TABLE III. Steroid response in each domain for all children (n = 82) and the subgroup (n = 54) with data available for all domains

	Patients with ≥3 domains (ACT, FEV_1, FENO ± sputum [n = 82])			Patients with all 4 domains (n = 54)		
Domain	**Before triamcinolone**	**After triamcinolone**	***P* value**	**Before triamcinolone**	**After triamcinolone**	***P* value**
Symptom (ACT score)	13 (6-23)	16.5 (5-25)	<.001	13 (6-23)	16.5 (7-25)	<.001
ACT response	34/82 (41%)			23/54 (43%)		
FEV_1 (% predicted)	72 (24-134)	76.5 (44-113)	.09	72.5 (36-134)	76.5 (46-113)	.29
FEV_1 response	47/82 (57%)			29/54 (54%)		
FENO (ppb)	47 (3.6-225)	24.6 (1-150)	<.0001	47 (9-225)	22 (5-150)	<.0001
FENO response	40/82 (49%)			28/54 (52%)		
Sputum eosinophils (%)	5.1 (0-92)	1.6 (0-42.8)	.001	4.85 (0-92)	2.0 (0-42.8)	.001
Sputum response	37/66 (56%)			29/54 (54%)		

Data are presented as median (range) differences before and after triamcinolone, as assessed by using the Wilcoxon test.
FENO, FENO at a flow rate of 50 mL/s.

a response pattern being apparent. Very few children with STRA (13%) were complete responders to triamcinolone, and a similar number (15%) were nonresponders. The majority showed a response (72%) in at least 1 domain.

This is the first time a multidomain approach has been proposed for an assessment of steroid response in patients with severe asthma. Because many children with STRA have normal spirometric results despite significant symptoms,[20] exacerbations, or both, an alternative definition to that currently used in adults is needed. In support of this, the current proposed definition of systemic corticosteroid response that incorporates a baseline FEV_1 of greater than 80% of predicted value could not be applied to 50% of our children with STRA because baseline FEV_1 measurements were 80% or greater of predicted value. Many children had normal results in any 1 domain before the steroid trial. However, given the use of high-dose inhaled steroids by all children and our assessments to ensure adherence, we assumed that normal values before the systemic corticosteroid trial suggested steroid response was present in that domain or at least that the child was not steroid unresponsive in that domain.

The advantage of the proposed multidomain approach is that it allows for the recognized heterogeneity within STRA in children,[3,21,22] which was confirmed by the fact that a similar number of children responded in each domain and no single domain had a majority response. We related both clinical and inflammatory features to response to steroids. However, consistent associations were difficult to identify and also reflect the heterogeneity of the group.

To our knowledge, this is the first report of systemic corticosteroid responsiveness in children with true STRA. We have ensured, as far as possible, that underlying modifiable factors were identified and addressed before inclusion of subjects.[2,3] A weakness of previous reports of systemic corticosteroid responsiveness in children was the clinical mix of patients, including those with "difficult asthma" with underlying modifiable factors and genuine STRA.[9] Another significant improvement on previous reports[5,6] is the use of an intramuscular steroid to ensure adherence.

We speculate that this multidomain approach can usefully be applied clinically when considering outcomes expected from add-on therapies used as steroid-sparing agents in the individual child. For example, if a child has a systemic corticosteroid response in an inflammatory domain, specifically a sputum eosinophil response, then they might benefit from a trial of an mAb to IL-5, provided it can be shown that this is the pathway driving airway eosinophilia. However, if they have a lung function response, then an agent, such as an mAb to IL-13, might be a better choice because clinical trials have shown benefit in FEV_1,[23] and mechanistically, IL-13 induces airway hyperresponsiveness.[24] This approach of choosing an add-on therapy is of particular relevance to children because blood eosinophil counts cannot always be used as a reliable marker of airway eosinophils in pediatric patients with STRA,[25] and serum periostin cannot be used as a biomarker to predict response to anti–IL-13 antibody because it is produced from bone during growth and development.[26] Omalizumab is currently the only licensed add-on therapeutic for children with STRA, but as newer biologicals become available, it might be possible to predict which one should be used in which patient by assessing the domain-specific steroid-response phenotype. The efficacy of this approach needs to be confirmed prospectively.

A limitation of our multidomain assessment was incomplete data in each domain for all 82 children. This is particularly relevant to sputum cytology because obtaining samples from children can be difficult. However, our success rate for sputum induction was approximately 80%, which is similar to that in previous pediatric reports.[27,28] To overcome this difficulty, we included FENO as an alternative noninvasive inflammatory marker. We accept that the 2 cannot be used interchangeably, and therefore both domains were analyzed separately.[29] We acknowledge that sputum eosinophil counts vary unpredictably over time, but we submit that if the sputum eosinophil count remains increased despite parenteral corticosteroids, then the process driving sputum eosinophilia is steroid responsive, and this is the case irrespective of whether sputum eosinophil counts decrease subsequently. We also acknowledge the limitations of longitudinal FENO measurements.[30,31] However, because this is currently the only noninvasive test of inflammation that can be performed reliably and repeatedly in children and because we were looking for a change in values over time[32,33] rather than absolute values, we considered this a reasonable alternative. To avoid bias in the patterns of steroid response reported, we have presented data for the 54 patients in whom all parameters were available before and after the triamcinolone acetonide injection separately. However, there were no significant differences in the proportion of children who responded in each domain.

What remained uncertain with our initial approach was whether complete normalization in each domain after a single triamcinolone dose should be expected or whether a fixed improvement of greater than 50% might be more appropriate. The relatively large number (45%) of children with persistent eosinophilic airway inflammation after a single injection of

TABLE IV. Relationships between clinical features, inflammation, and corticosteroid response in each domain for children with data available for all domains (n = 54)

	Response	Nonresponse	*P* value
ACT response			
No.	23	31	
Duration of symptoms (decimal years)	10.45 (4-14.45)	7.90 (2.25-14.26)	**.04**
Atopy	16/23 (70%)	23/31 (74%)	.77
Intubation	4/23 (17%)	9/31 (45%)	.36
FEV_1 (% predicted)	81 (37-134)	71 (36-130)	.27
BDR (%)	10.7 (0-33)	14 (0-30)	.84
FENO	44 (9-169)	50 (29-225)	.13
IgE (IU/mL)	298 (9-19832)	584 (6-6737)	.24
Sputum eosinophils (%)	5 (0-51.6)	3.2 (0-92)	.37
Serum eosinophils (%)	5.15 (1.2-11.5)	5.55 (0-19.7)	.79
BAL eosinophils (%)	4 (0-34.7)	2.7 (0-51)	.11
FEV_1 response			
No.	29	25	
Duration of symptoms (decimal years)	8.25 (2.25-14.48)	8.5 (3.5-14.3)	.88
Atopy	20/29 (69%)	19/25 (76%)	.76
Intubation	11/29 (38%)	2/25 (8%)	**.01**
FEV_1 (% predicted)	81 (36-130)	70 (37-134)	.27
BDR (%)	17 (0.2-30)	12 (0-33)	.41
FENO	44.8 (9-225)	47.8 (11-169)	.38
IgE (IU/mL)	318 (6-19832)	486 (15-3792)	.77
Sputum eosinophils (%)	4 (0-92)	5 (0-67)	.59
Serum eosinophils (%)	4.1 (0-19.7)	6.1 (0-14.7)	.96
BAL eosinophils (%)	2.7 (0-51)	3 (0-34.7)	.36
FENO response			
No.	28	26	
Duration of symptoms (decimal years)	6.75 (3.5-14.3)	10.15 (2.25-14.48)	**.02**
Atopy	20/28 (71%)	19/26 (73%)	1.0
Intubation	7/28 (25%)	6/26 (23%)	1.0
FEV_1 (% predicted)	74 (36-134)	71.5 (37-103)	.67
BDR (%)	10.7 (0-30)	14 (0-33)	.37
FENO	40 (9-225)	51 (11-165)	**.08**
IgE (IU/mL)	363 (6-6737)	450 (9-19832)	.47
Sputum eosinophils (%)	3.3 (0-92)	8.1 (0-67)	.08
Serum eosinophils (%)	2.7 (0-19.7)	7 (0-17.1)	.18
BAL eosinophils (%)	4 (0-51)	2.7 (0-13)	.65
Sputum eosinophil response			
No.	29	25	
Duration of symptoms (decimal years)	7.65 (3.5-14.48)	6.88 (2.25-14.25)	.30
Atopy	18/29 (62%)	21/25 (84%)	.12
Intubation	8/29 (28%)	5/25 (20%)	.55
FEV_1 (% predicted)	72 (36-130)	73 (37-134)	.95
BDR (%)	9.7 (0-31)	17.7 (0-33)	**.03**
FENO	40.5 (9-225)	52 (14-165)	.10
IgE (IU/mL)	299 (6-6737)	563 (15-19832)	.25
Sputum eosinophils (%)	3 (0-41.6)	8.89 (0.33-92)	**.008**
Serum eosinophils (%)	4.65 (0-19.7)	6.1 (0-17.1)	.95
BAL eosinophils (%)	3.65 (0-34.7)	2.7 (0-51)	.58

Values are presented as n/number tested or median (range), as indicated. Differences were analyzed with the Mann-Whitney *U* or χ^2 tests.

triamcinolone and the large number of atopic nonresponders suggested that 1 dose might not be enough to determine a complete response, even though we had given the recommended high dose.[34] We have given a subgroup of patients up to 3 consecutive monthly injections, but additional injections did not alter the response pattern seen after the first injection (data not shown). We acknowledge that our definition of a response in each domain was arbitrary because we accepted a 50% improvement in symptoms as a response, but for lung function and the inflammatory domains, only normalization was accepted. It is difficult to know whether systemic corticosteroid "resistance" is a true entity in children,[35] and it is likely that it is a spectrum, other than in those rare children with mutations in the corticosteroid receptor gene.[36] Clearly, mechanistic data are required in the future to further understand steroid resistance in children, but an essential prerequisite to such studies is a definition in children, which is the purpose of this article.

In summary, we propose a novel multidomain approach to identifying the systemic corticosteroid-response pattern in children with STRA because 50% do not meet the criteria for definitions of steroid response encompassing lung function alone. Using this approach, we have shown that approximately 40% to 50% of children respond in each domain, with little evidence of single clinical predictors of a response in a specific domain. This approach allows us to capture systemic corticosteroid responsiveness in a more phenotype-specific way, which will be the basis of future mechanistic studies and, we speculate, will help identify optimal add-on therapies.

We thank all the patients and parents for agreeing to take part in our study. Also, we thank Winston Banya, statistician, for his advice.

Clinical implications: There is no agreed upon definition of corticosteroid response for pediatric patients with severe asthma. We propose a multidomain approach that might help guide the choice of optimal add-on therapies.

REFERENCES

1. Braman SS. The global burden of asthma. Chest 2006;130(suppl):4S-12S.
2. Bracken M, Fleming L, Hall P, Van Stiphout N, Bossley C, Biggart E, et al. The importance of nurse-led home visits in the assessment of children with problematic asthma. Arch Dis Child 2009;94:780-4.
3. Bossley CJ, Fleming L, Gupta A, Regamey N, Frith J, Oates T, et al. Pediatric severe asthma is characterized by eosinophilia and remodeling without T(H)2 cytokines. J Allergy Clin Immunol 2012;129:974-82.
4. Adcock IM, Ito K. Steroid resistance in asthma: a major problem requiring novel solutions or a non-issue? Curr Opin Pharmacol 2004;4:257-62.
5. Little SA, Chalmers GW, Macleod KJ, McSharry C, Thomson NC. Non-invasive markers of airway inflammation as predictors of oral steroid responsiveness in asthma. Thorax 2000;55:232-4.
6. Bossley CJ, Saglani S, Kavanagh C, Payne DN, Wilson N, Tsartsali L, et al. Corticosteroid responsiveness and clinical characteristics in childhood difficult asthma. Eur Respir J 2009;34:1052-9.
7. Fitzpatrick AM, Teague WG. Severe asthma in children: insights from the National Heart, Lung, and Blood Institute's Severe Asthma Research Program. Pediatr Allergy Immunol Pulmonol 2010;23:131-8.
8. Yim RP, Koumbourlis AC. Steroid-resistant asthma. Paediatr Respir Rev 2012;13:172-6.
9. Bush A, Hedlin G, Carlsen KH, de Benedictis F, Lodrup-Carlsen K, Wilson N. Severe childhood asthma: a common international approach? Lancet 2008;372:1019-21.
10. Chung KF, Wenzel SE, Brozek JL, Bush A, Castro M, Sterk PJ, et al. International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. Eur Respir J 2014;43:343-73.
11. Kenalog Intra-articular/intramuscular injection. Available at: http://www.medicines.org.uk/emc/medicine/11366. Accessed 2015.
12. ten Brinke A, Zwinderman AH, Sterk PJ, Rabe KF, Bel EH. "Refractory" eosinophilic airway inflammation in severe asthma: effect of parenteral corticosteroids. Am J Respir Crit Care Med 2004;170:601-5.
13. Panickar JR, Kenia P, Silverman M, Grigg J. Intramuscular triamcinolone for difficult asthma. Pediatr Pulmonol 2005;39:421-5.

14. American Thoracic Society. Standardization of spirometry: 1994 update. Am J Respir Crit Care Med 1995;152:1107-36.
15. Nathan RA, Sorkness CA, Kosinski M, Schatz M, Li JT, Marcus P, et al. Development of the asthma control test: a survey for assessing asthma control. J Allergy Clin Immunol 2004;113:59-65.
16. Schatz M, Sorkness CA, Li JT, Marcus P, Murray JJ, Nathan RA, et al. Asthma Control Test: reliability, validity, and responsiveness in patients not previously followed by asthma specialists. J Allergy Clin Immunol 2006;117:549-56.
17. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. Am J Respir Crit Care Med 2005;171:912-30.
18. Warke TJ, Fitch PS, Brown V, Taylor R, Lyons JD, Ennis M, et al. Exhaled nitric oxide correlates with airway eosinophils in childhood asthma. Thorax 2002;57:383-7.
19. Cai Y, Carty K, Henry RL, Gibson PG. Persistence of sputum eosinophilia in children with controlled asthma when compared with healthy children. Eur Respir J 1998;11:848-53.
20. Bacharier LB, Strunk RC, Mauger D, White D, Lemanske RF Jr, Sorkness CA. Classifying asthma severity in children: mismatch between symptoms, medication use, and lung function. Am J Respir Crit Care Med 2004;170:426-32.
21. Fitzpatrick AM, Teague WG, Meyers DA, Peters SP, Li X, Li H, et al. Heterogeneity of severe asthma in childhood: confirmation by cluster analysis of children in the National Institutes of Health/National Heart, Lung, and Blood Institute Severe Asthma Research Program. J Allergy Clin Immunol 2011;127:382-9.
22. Haldar P, Pavord ID, Shaw DE, Berry MA, Thomas M, Brightling CE, et al. Cluster analysis and clinical asthma phenotypes. Am J Respir Crit Care Med 2008;178:218-24.
23. Corren J, Lemanske RF, Hanania NA, Korenblat PE, Parsey MV, Arron JR, et al. Lebrikizumab treatment in adults with asthma. N Engl J Med 2011;365:1088-98.
24. Wills-Karp M, Luyimbazi J, Xu X, Schofield B, Neben TY, Karp CL, et al. Interleukin-13: central mediator of allergic asthma. Science 1998;282:2258-61.
25. Ullmann N, Bossley CJ, Fleming L, Silvestri M, Bush A, Saglani S. Blood eosinophil counts rarely reflect airway eosinophilia in children with severe asthma. Allergy 2013;68:402-6.
26. Kashima TG, Nishiyama T, Shimazu K, Shimazaki M, Kii I, Grigoriadis AE, et al. Periostin, a novel marker of intramembranous ossification, is expressed in fibrous dysplasia and in c-Fos-overexpressing bone lesions. Hum Pathol 2009;40:226-37.
27. Covar RA, Spahn JD, Martin RJ, Silkoff PE, Sundstrom DA, Murphy J, et al. Safety and application of induced sputum analysis in childhood asthma. J Allergy Clin Immunol 2004;114:575-82.
28. Lex C, Payne DN, Zacharasiewicz A, Li AM, Wilson NM, Hansel TT, et al. Sputum induction in children with difficult asthma: safety, feasibility, and inflammatory cell pattern. Pediatr Pulmonol 2005;39:318-24.
29. Fleming L, Tsartsalis L, Wilson N, Regamey N, Bush A. Longitudinal relationship between sputum eosinophils and exhaled nitric oxide in children with asthma. Am J Respir Crit Care Med 2013;188:400-2.
30. Jartti T, Wendelin-Saarenhovi M, Heinonen I, Hartiala J, Vanto T. Childhood asthma management guided by repeated FeNO measurements: a meta-analysis. Paediatr Respir Rev 2012;13:178-83.
31. Pike K, Selby A, Price S, Warner J, Connett G, Legg J, et al. Exhaled nitric oxide monitoring does not reduce exacerbation frequency or inhaled corticosteroid dose in paediatric asthma: a randomised controlled trial. Clin Respir J 2013;7:204-13.
32. Hewitt RS, Modrich CM, Cowan JO, Herbison GP, Taylor DR. Outcomes using exhaled nitric oxide measurements as an adjunct to primary care asthma management. Prim Care Respir J 2009;18:320-7.
33. van der Valk RJ, Baraldi E, Stern G, Frey U, de Jongste JC. Daily exhaled nitric oxide measurements and asthma exacerbations in children. Allergy 2012;67:265-71.
34. Panickar JR, Bhatnagar N, Grigg J. Exhaled nitric oxide after a single dose of intramuscular triamcinolone in children with difficult to control asthma. Pediatr Pulmonol 2007;42:573-8.
35. Adcock IM, Barnes PJ. Molecular mechanisms of corticosteroid resistance. Chest 2008;134:394-401.
36. Bray PJ, Cotton RG. Variations of the human glucocorticoid receptor gene (NR3C1): pathological and in vitro mutations and polymorphisms. Hum Mutat 2003;21:557-68.

METHODS

Subjects

Children older than 5 years referred to the Royal Brompton Hospital with problematic severe asthma between April 2005 and June 2012 were initially investigated by using the difficult asthma protocol, as shown in the CONSORT diagram (Fig 1). The diagnosis of problematic severe asthma was defined as follows:

1. persistent (most days for >3 months) chronic symptoms (use of short-acting β_2-agonists >3/wk) of airway obstruction despite high ICS doses (≥800 μg/d budesonide equivalent) and/or regular oral corticosteroids, long-acting β_2-agonists, and current (or previous failed trial of) montelukast OR
2. recurrent severe exacerbations requiring more than 1 admission to the intensive care unit, more than 2 hospital admissions requiring intravenous medications, or 2 or more courses of oral corticosteroids in the past year despite therapy for persistent symptoms as described in (1) above OR
3. at least 1 very sudden (≤6 hours) severe attack (requiring hospitalization) without warning despite therapy for persistent symptoms, as described in (1) above.

After assessment,[E1] those reclassified as having difficult asthma were excluded. The remaining patients with STRA were further investigated.

Ethics

The study was approved by the local research ethics committee, with all procedures performed after obtaining written informed parental consent and age-appropriate child assent.

Atopic status

Atopy was defined as 1 or more positive serum specific IgE measurements or skin prick test responses to aeroallergens. Quantification of atopy was made by summing the results of specific IgE levels to cat, dog, house dust mite, tree, grass, and *Aspergillus* species allergens.

Asthma control

Asthma control was assessed by using the ACT (Fig E1). A score of 20 or greater was taken to represent adequate control.[E2]

Spirometry and BDR

Spirometry was performed with a Compact Vitalograph 2120 (Vitalograph, Maid, Moreton, United Kingdom) before and 15 minutes after administration of 1 mg of salbutamol through a large-volume spacer. BDR was defined as a greater than 12% change from baseline FEV_1.

Sputum induction and analysis

Sputum was induced by means of inhalation of an aerosol of 3.5% nebulized hypertonic saline if FEV_1 was 65% of predicted value or greater after pretreatment with 1 mg of salbutamol through a large-volume spacer device.[E3] If FEV_1 was less than this or the subject had previously been unable to tolerate hypertonic saline, then normal saline was used. Nebulizations of 5-minute intervals were performed up to 20 minutes or until an adequate sample was produced. Spirometry was performed after each expectoration to check that the FEV_1 had not decreased by 20% or greater from baseline, in which case the child would be given a further dose of salbutamol, and the procedure was stopped.

Sputum processing and analysis

Sputum samples were processed within 2 hours of expectoration. Mucus plugs were selected from the expectorated sputum.[E4] Dithiothreitol 0.1% of a volume 4 times the volume of the selected sputum sample was added to the sputum and gently mixed by means of gentle aspiration with a wide-bore pipette and further on a mixing roller (Denley Spiramix 5; Denley Instruments, Colchester, United Kingdom) for 15 minutes. The liquefied sputum was filtered through a 48-μm-pore nylon gauze into a clean centrifuge tube. Four milliliters of PBS for every 1 g of selected sputum was used to wash out the remaining contents of the original tube through the gauze filter. After centrifugation at 1500 rpm (400*g*) at 4°C for 10 minutes (Sorvall RT6000D; Kendro, Bishops Stortford, United Kingdom), supernatants were separated and stored at −80°C. Cell viability and total cells were established with the Trypan blue exclusion method. A suspension of approximately 200,000 cells per milliliter was made up by means of dilution, where necessary, with PBS. Cytospin preparations were made with 100 to 300 μL of this suspension per slide and centrifuged at 450 rpm for 3 minutes. After air drying, slides were stained with the REASTAIN Quik-Diff staining kit (Reagena, Toivala, Finland), fixed with methanol, and mounted with DPX mounting medium.

Samples were presumed adequate if 80% or less squamous cells were present and 400 or more inflammatory cells were present and distinguishable. Slides were coded so as to blind the investigator. A minimum of 400 cells was counted, and a percentage count was calculated.

Intraobserver variabilities of sputum eosinophil counts were 8.6%, 8.3%, and 1% for 3 subjects counted on 3 separate occasions.

BAL processing and analysis

BAL cytology was processed by the hospital cytology department. Eosinophilia was defined as greater than 1.19%[E5] and neutrophilia as greater than 3.5%[E6] of the total cell count.

REFERENCES

E1. Bracken M, Fleming L, Hall P, Van Stiphout N, Bossley C, Biggart E, et al. The importance of nurse-led home visits in the assessment of children with problematic asthma. Arch Dis Child 2009;94:780-4.
E2. Schatz M, Sorkness CA, Li JT, Marcus P, Murray JJ, Nathan RA, et al. Asthma Control Test: reliability, validity, and responsiveness in patients not previously followed by asthma specialists. J Allergy Clin Immunol 2006;117:549-56.
E3. Lex C, Payne DN, Zacharasiewicz A, Li AM, Wilson NM, Hansel TT, et al. Sputum induction in children with difficult asthma: safety, feasibility, and inflammatory cell pattern. Pediatr Pulmonol 2005;39:318-24.
E4. Pizzichini E, Pizzichini MM, Efthimiadis A, Evans S, Morris MM, Squillace S, et al. Indices of airway inflammation in induced sputum: reproducibility and validity of cell and fluid-phase measurements. Am J Respir Crit Care Med 1996;154:308-17.
E5. Fitch PS, Brown V, Schock BC, Taylor R, Ennis M, Shields MD. Chronic cough in children: bronchoalveolar lavage findings. Eur Respir J 2000;16:1109-14.
E6. ERS Task Force on bronchoalveolar lavage in children. Bronchoalveolar lavage in children. Eur Respir J 2000;15:217-31.

In the past 4 weeks how much of the time did your asthma keep you from getting as much done at school or home?				
All of the time **1**	Most of the time **2**	Some of the time **3**	A little of the time **4**	None of the time **5**
In the past 4 weeks how often have you had shortness of breath?				
More than once a day **1**	Once a day **2**	3-6 times a week **3**	Once or twice a week **4**	Not at all **5**
During the past 4 weeks how often did your asthma symptoms (wheezing, coughing, shortness of breath, chest tightness or pain) wake you up at night or earlier than usual in the morning?				
4 or more nights a week **1**	2 to 3 nights a week **2**	Once a week **3**	Once or twice **4**	Not at all **5**
In the past 4 weeks how often have you used your rescue inhaler or nebulizer solution?				
3 or more times per day **1**	1 to 2 times per day **2**	2 to 3 times per week **3**	Once a week or less **4**	Not at all **5**
How would you rate your asthma control over the past 4 weeks?				
Not controlled at all **1**	Poorly controlled **2**	Somewhat controlled **3**	Well controlled **4**	Completely controlled **5**

TOTAL SCORE /25

FIG E1. The ACT Questionnaire.

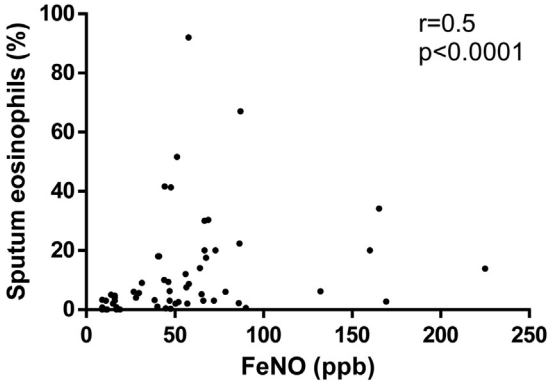

FIG E2. Positive relationship between sputum eosinophil counts (as a percentage) and FENO (Spearman coefficient).

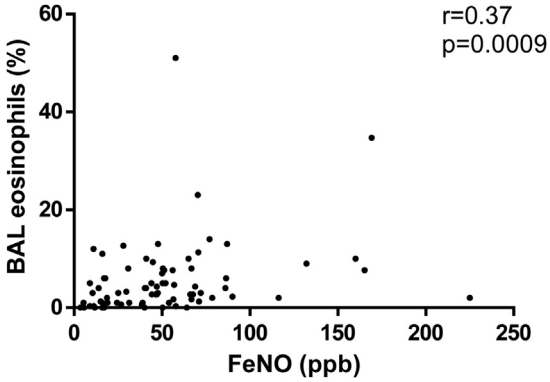

FIG E3. Relationship between FENO levels and BAL eosinophil counts (as a percentage). A positive correlation was found between FENO levels and BAL eosinophil counts by using the Spearman coefficient.

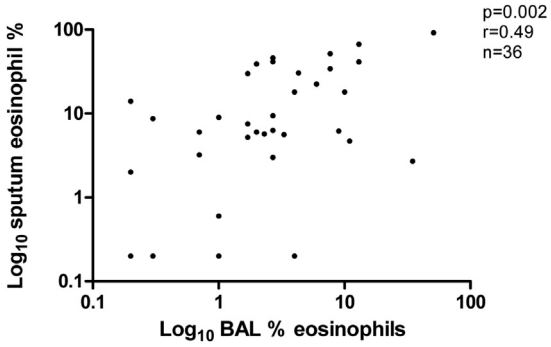

FIG E4. Positive relationship between sputum and BAL eosinophil counts (as a percentage [Spearman coefficient]).

TABLE E1. Logistic regression analysis to determine which clinical and inflammatory features determine steroid response in each domain (54 children with data available for all domains included)

	Odds ratio	CI	*P* value
ACT response			
Duration of symptoms (y)	**1.2**	**1.01-1.44**	**.036**
Intubation	0.51	0.14-1.94	.33
FEV_1 (% predicted)	1.01	0.99-1.04	.34
BDR (%)	1.0	0.94-1.06	.9
FENO normal (<24 ppb)	0.54	0.16-1.82	.32
IgE <100 (IU/mL)	1.14	0.28-4.62	.85
Serum eosinophils <2%	1.18	0.29-4.83	.81
Sputum eosinophils <2.5%	2.61	0.71-9.64	.15
BAL eosinophils <1.19%	2.13	0.56-8.12	.26
FEV_1 response			
Duration	0.99	0.84-1.16	.90
Intubation	**7.02**	**1.38-35.79**	**.019**
BDR (%)	1.02	0.96-1.08	.44
FENO normal (<24 ppb)	1.02	0.31-3.36	.97
IgE <100 (IU/mL)	1.2	0.30-4.74	.78
Serum eosinophils <2%	0.58	0.15-2.21	.43
Sputum eosinophils <2.5%	0.48	0.13-1.65	.24
BAL eosinophils <1.19%	0.90	0.26-3.18	.87
FENO response			
Duration of symptoms (y)	**0.83**	**0.70-0.99**	**.037**
Intubation	1.11	0.32-3.88	.87
IgE <100 (IU/mL)	0.67	0.16-2.69	.57
FEV_1 (%)	1.01	0.99-1.04	.41
BDR (%)	0.97	0.92-1.03	.40
FENO normal (<24 ppb)	0.28	0.08-1.03	.057
Serum eosinophils <2%	0.36	0.09-1.44	.15
Sputum eosinophils <2.5%	2.61	0.71-9.64	.18
BAL eosinophils <1.19%	1.22	0.35-4.32	.75
Sputum response			
Duration	0.95	0.81-1.12	.57
Intubation	1.52	0.43-5.45	.52
IgE <100 (IU/mL)	0.23	0.04-1.20	.08
FEV_1 (%)	1.0	0.98-1.02	.98
BDR (%)	**0.93**	**0.88-0.99**	**.03**
FENO normal (<24 ppb)	0.31	0.08-1.15	.08
Serum eosinophils <2%	0.77	0.20-2.93	.70
Sputum eosinophils <2.5%	**0.19**	**0.05-0.79**	**.02**
BAL eosinophils <1.19%	1.51	0.43-5.33	.52

P values in boldface indicate statistical significance.