Assessment of corticosteroid response in paediatric severe asthma using a multi-domain approach

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Clinical Implications:

There is no agreed definition of corticosteroid response for paediatric severe asthma. We propose a multi-domain approach which may help to guide the choice of optimal add-on therapies.

Capsule summary:

Using an assessment of symptoms, spirometry and inflammation, we show systemic corticosteroid responsiveness is heterogeneous in children with severe asthma and there are no clinical or inflammatory predictors of response pattern.

Key words: paediatric, severe asthma, steroid response, spirometry, inflammation

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ABSTRACT:

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

Objective: To evaluate the utility of a multi-domain approach incorporating symptoms, lung function and inflammation to determine steroid responsiveness in paediatric STRA.

Methods: 82 children (median age 12 years) with STRA received a clinically indicated dose of intra-muscular steroid. Changes in four separate domains; normalisation of i) symptoms (asthma control test >19/25 or 50% increase), ii) spirometry (FEV_1>80% predicted, or 15% increase), iii) exhaled nitric oxide (FeNO) (<24ppb) and (iv) sputum eosinophils (<2.5%) were assessed 4 weeks after intra-muscular triamcinolone acetonide. 54/82 children had complete data in all 4 domains.

Results: 23/54 (43%) had a symptom response, 29/54 (54%) lung function response, 28/54 (52%) FeNO response and 29/54 (54%) 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. 7/54 (13%) were complete responders (response in all domains), 8/54 (15%) non-responders (no response in any domain) and 39/54 (72%) partial responders (response in $\geq$1 domain).

Conclusions: A multi-domain evaluation of systemic steroid responsiveness using pragmatic clinical assessments confirms childhood STRA is heterogeneous and a complete response in symptoms, inflammatory and physiological parameters is rare. Individual response patterns to systemic steroids may be useful in guiding the choice of add-on therapies in each child as a step towards achieving personalised medicine.

INTRODUCTION:

Approximately 2-5% of all asthmatic children have severe therapy resistant asthma (STRA), are challenging to treat and consume significant healthcare resources.[1] Many children may 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 (ICS). However, children with STRA remain poorly controlled despite high doses of ICS, 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 ≥15% predicted increase in morning first second forced expired volume (FEV_1) in patients with bronchodilator reversibility (BDR) ≥12% from baseline and an abnormal FEV_1 (≤80%) prior to a systemic steroid trial. However, it is acknowledged that this may not be an appropriate definition for children since many children with a confirmed diagnosis of severe asthma have normal spirometry, but remain poorly controlled.[6-8]

We have previously shown paediatric STRA is heterogeneous with respect to lung function, inflammation and remodelling.[3] Although as a group, children with STRA have lower lung function, increased eosinophilic inflammation and increased RBM thickness and smooth muscle mass compared to mild asthmatics and non-asthmatic controls,[3] there is overlap between groups as well as considerable variability within

STRA. Given this marked variation within the STRA group, it is likely that some children may respond more to steroids with an improvement in lung function, whereas others may only show a response by an improvement in inflammation. We therefore investigated a multi-domain definition of systemic corticosteroid responsiveness, namely change in i) symptoms ii) spirometry, and iii) non-invasive markers of inflammation before and four weeks after a single clinically indicated dose of intramuscular triamcinolone acetonide. We hypothesised that children with STRA would not have a uniform response pattern. We also related steroid response pattern to clinical features, peripheral and airway inflammation prior to administering the systemic steroid, to try to identify predictors of response pattern.

METHODS:

Subjects and definition of severe asthma (STRA)

Children aged between 6-16 years with problematic severe asthma (on-going poor control despite prescribed high dose inhaled steroids (≥800mcg/day), additional controller medications and at stage 4/5 of the BTS/Sign guidelines) referred between 2005-2012 were investigated using our standardised clinical investigation protocol with an outpatient led nurse assessment and home visit.[2;3] Children with modifiable factors which could be addressed, in particular adherence, were classified as difficult asthmatics and excluded from this study (Figure 1).[9] Only the remaining patients, in whom diagnosis had been confirmed (evidence of reversible airflow obstruction and doctor diagnosed wheeze), adherence had been assured (GP prescription records, appropriate inhalers being available during the home visit, and proven ability to use the medication delivery device prescribed) and underlying modifiable factors (such as

environmental tobacco smoke and allergen exposure) had been minimised were diagnosed as STRA 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, not a clinical trial. The intra-muscular steroid preparation used (Kenalog®) was recommended for sustained systemic corticosteroid treatment in patients with asthma[11] and has previously been used in adults[12] and children with difficult asthma.[13] A single dose of intra-muscular systemic steroids was administered and assessments of steroid response were made immediately before and 4 weeks after administration.

Bronchoscopy, broncho-alveolar lavage (BAL) and endobronchial biopsy

Bronchoscopy and BAL were performed under general anaesthesia, and samples were processed for analysis of eosinophilic inflammation as previously described.[3] (see online supplement (OLS) for details). Blood for total IgE and eosinophils was taken at the time of bronchoscopy.

Administration of systemic steroid and assessments of steroid response

An assessment of: (1) the asthma control test (ACT) for symptom control (Figure E1, OLS), (2) spirometry[14], (3) sputum eosinophils and (4) exhaled nitric oxide (FeNO) (at flow rate 50ml/sec) were made on the morning of bronchoscopy. An intramuscular injection of triamcinolone acetonide (80mg age ≥12 years; 40mg <12 years)[13] was administered at the end of the bronchoscopy and tests 1-4 were repeated 4 weeks later to determine steroid response (Table 1).

Multi-domain 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. As 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, and remained normal in that domain, were steroid responsive. We felt it was unethical in these children to confirm this by reducing their treatment until abnormalities appeared in all domains. Data analysis was therefore initially undertaken for all patients. Subsequently, to assess additional responsiveness following systemic corticosteroids, the analysis was restricted to include only those children who could improve after triamcinolone (i.e. abnormal at baseline). Response to steroids was examined in each domain and a combination of domains.

Symptom (ACT) response

Symptom control was assessed using the ACT,[15] as most patients were ≥12 years, and for consistency, this was used in preference to the childhood ACT, which was not available at the start of data collection. Positive response was defined as ACT score attaining >19/25[16] or an increase of ≥50%.

FEV_1 response

Spirometry was measured according to American Thoracic Society (ATS)/European Respiratory Society (ERS) guidelines.[14] Response was defined as attaining a pre-bronchodilator FEV_1≥80% predicted, or an increase of ≥15%.

FeNO response

FeNO measurements at 50ml/sec were made with a chemiluminescence analyser (NIOX Aerocrine, Sweden) in accordance with ATS/ERS guidelines.[17] Response was defined as a normal value (<24ppb[18]).

Sputum eosinophil response

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

Complete, partial and non-response to triamcinolone acetonide

Complete corticosteroid response was defined as symptom, FEV_1, sputum eosinophil and FeNO response. A response in at least one domain was a partial response, and an absence of response in all domains was non-response. If sputum was unavailable, response was assessed in three domains only.

Statistical Analysis

Baseline values for all domains in the discovery and validation cohorts were not normally distributed and were therefore analysed using non-parametric tests. The Mann-Whitney U test was used for continuous variables and categorical data was analysed using the Fisher's exact or Chi squared tests. To compare changes before and after triamcinolone, data were analysed using the Wilcoxon test for non-parametric and paired t-test for parametric data. The data was analysed using the Statistical Package for Social Sciences (SPSS) version 17. Logistic regression

187 analysis was performed and the results presented for univariate analysis using the
188 statistical software Stata version 12.1.

189

190 **RESULTS:**

191 **Subjects and demographics**

192 Eighty two (51 male) children (median age 12 [range 6.5-17.3]) years, underwent
193 investigations and received systemic corticosteroids (Figure 1). All children had data
194 for at least one steroid response domain (symptoms, FEV1, FeNO and /or sputum
195 eosinophils) and a sub-group of fifty-four (37 male) children had data for all four
196 domains before and after the systemic steroid injection (Table 2).

197

198 **Assessments of steroid response**

199 The response pattern in each domain for all children (n=82) and the sub-group of
200 children that had data available in all four domains (n=54), including those with a
201 normal value at baseline, are summarised in Figure 2 and Table 3. There was no
202 significant difference in the proportion of children that responded in each domain
203 when all patients were compared to those that had data available for all domains.

204

205 **Response pattern in each domain in children that had data for all four**
206 **parameters (n=54)**

207 In order to prevent any chance of selection bias influencing the results, only those
208 children that had a response recorded in all four domains (a complete dataset

recorded) were assessed in detail both for patterns of response and predictors of response.

Symptom response

23/54 (43%) of children had an improvement in symptoms after systemic corticosteroids (Figure 2A, Table 3). 8/54 children (15%) had a normal ACT before the steroid trial, 16/46 (35%) of those with an abnormal ACT at baseline improved or normalised (Figure 2B).

FEV_1 response

29/54 (54%) of all children were steroid responsive in the FEV_1 domain (Figure 2C, Table 3). 20/54 (37%) had a normal FEV_1 (≥80% predicted) before systemic corticosteroids (this is explained by their maintenance high-dose inhaled steroid therapy). 14/34 (41%) of those with an abnormal FEV_1 (<80%) at baseline improved after systemic corticosteroids (Figure 2D).

FeNO response

28/54 (52%) children had normal FeNO levels after systemic corticosteroids (Figure 2E, Table 3). 15/52 (28%) children had a normal FeNO value before systemic steroids, 17/37 (46%) of those with an elevated FeNO at baseline had a reduction to normal levels after systemic steroids (Figure 2F).

Sputum eosinophil response

Fifty-four children had paired sputum samples before and after triamcinolone acetonide. Of these, 29/54 (54%) had sputum eosinophils <2.5% post triamcinolone acetonide (Figure 2G, Table 3). 15/54 (28%) had normal sputum eosinophils before

triamcinolone. 17/39 (44%) patients who started with an abnormal sputum eosinophil count, had normalisation of sputum eosinophils after triamcinolone acetonide (Figure 2H.

Discordance in FeNO and sputum eosinophil domains

There was concordance in 39/54 (72%) for FeNO or sputum response. 21/54 were responders in both domains, and 18/54 were non-responders in both domains. There was discordance in 15/54 (28%); 7/54 had FeNO but not sputum response and 8/54 had sputum but not FeNO response. Relationships between invasive and non-invasive assessments of airway eosinophils (FeNO and sputum and BAL eosinophils) are shown in Figures E2-E4 of the OLS.

Complete, partial and non-responders

7/54 (13%) responded in all domains (complete responders), 39/54 (72%) responded in at least 1 domain (partial responders) and 8/54 (15%) did not respond in any domain (non-responders).

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 (2.3-14.5)).

Comparison to current definitions of systemic steroid responsiveness

A definition of ≥15% predicted increase in FEV_1 in patients with bronchodilator reversibility (BDR) ≥12% from baseline and an abnormal FEV_1 (≤80%) was only applicable to 25/54 (46%) children as the remainder had an FEV_1>80% and/or BDR<12% before administration of triamcinolone acetonide. Of these patients, 12/25 (48%) had a positive steroid response.

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

There were no consistent clinical or inflammatory features at baseline associated with a response to systemic corticosteroids in any of the four domains (Table 4). A lower Asthma Control Test score (indicating more symptoms) before systemic steroids was associated with a poor response in the symptom domain (p=0.0002). 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 (CI 1.4-35.8, p=0.019) (Table E1 OLS). However, atopy, serum IgE, blood or BAL eosinophils were not associated with any pattern of steroid response (Table 4).

DISCUSSION:

We have proposed here that systemic corticosteroid response should be assessed in multiple domains, including normalisation or improvement of spirometry, symptom score and inflammation (sputum eosinophils and/or FeNO) in children with severe asthma since this approach reflects disease heterogeneity. None of these domains was abnormal in every child with STRA, underscoring the need for a multi-domain assessment. A similar number of children (approximately 40-50%) responded in each

domain with no clinical or pathological features of a response pattern being apparent. Very few STRA children (13%) were complete responders to triamcinolone and a similar number (15%) were non-responders. The majority showed a response (72%) in at least one domain.

This is the first time a multi-domain approach has been proposed for an assessment of steroid response in severe asthma. As many children with STRA have normal spirometry despite significant symptoms,[20] and/or exacerbations, 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 >80% predicted could not be applied to 50% of our children with STRA because baseline FEV_1 measurements were $\geq$80% predicted. Many children had normal results in any one 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 multi-domain approach is that it allows for the recognised 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.

306 To our knowledge, this is the first report of systemic corticosteroid responsiveness in
307 children with true STRA. We have ensured as far as possible that underlying
308 modifiable factors were identified and addressed prior to inclusion of subjects.[2;3] A
309 weakness of previous reports of systemic corticosteroid responsiveness in children
310 was the clinical mix of patients, including those with “difficult asthma” with underlying
311 modifiable factors and genuine STRA.[9] Another significant improvement on previous
312 reports[5;6] is the use of an intra-muscular steroid to ensure adherence.
313
314 We speculate that this multi-domain approach may usefully be applied clinically when
315 considering outcomes expected from add-on therapies used as steroid sparing
316 agents in the individual child. For example, if a child has a systemic corticosteroid
317 response in an inflammatory domain, specifically a sputum eosinophil response, then
318 they may benefit from a trial of a monoclonal antibody to IL-5, provided it can be
319 shown that this is the pathway driving airway eosinophilia. If, however, they have a
320 lung function response, then an agent such as a monoclonal antibody to IL-13 may
321 be a better choice, since clinical trials have shown benefit in FEV_1[23] and
322 mechanistically IL-13 induces airway hyperresponsiveness.[24] This approach of
323 choosing an add-on therapy is of particular relevance to children because blood
324 eosinophils cannot always be used as a reliable marker of airway eosinophils in
325 paediatric STRA,[25] and serum periostin cannot be used as a biomarker to predict
326 response to anti-IL13 antibody as it is produced from bone during growth and
327 development.[26] Omalizumab is currently the only licenced add-on therapeutic for
328 children with STRA, but as newer biologicals become available, it may be possible to
329 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 multi-domain assessment was incomplete data in each domain for all 82 children. This is particularly relevant to sputum cytology, since obtaining samples from children can be difficult. However, our success rate for sputum induction was approximately 80%, similar to previous paediatric reports.[27;28] In order to overcome this difficulty, we included FeNO as an alternative non-invasive inflammatory marker. We accept the two cannot be used interchangeably and therefore both domains were analysed separately.[29] We acknowledge that sputum eosinophil counts vary unpredictably over time, but we submit that if sputum eosinophil count remains elevated despite parenteral corticosteroids, then the process driving sputum eosinophilia is steroid responsive, and this is the case irrespective of whether subsequently sputum eosinophils fall. We also acknowledge the limitations of longitudinal FeNO measurements.[30;31] However, since this is currently the only non-invasive test of inflammation that can be performed reliably and repeatedly in children, and as we were looking for a change in values over time[32;33] rather than absolute values, we considered this a reasonable alternative. In order 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 that responded in each domain.

What remained uncertain with our initial approach was whether complete normalisation in each domain after a single triamcinolone dose should be expected,

or whether a fixed improvement of >50% may be more appropriate. The relatively large number (45%) of children who had persistent eosinophilic airway inflammation after a single injection of triamcinolone, and the large number of non-responders that were atopic, suggested one dose may not be enough to determine a complete response, even though we had given the recommended high dose.[34] We have given a sub-group of patients up to three 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 since we accepted a 50% improvement in symptoms as a response, but for lung function and the inflammatory domains only normalisation 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 further to understand steroid resistance in children, but an essential pre-requisite to such studies is a definition in children, which is the purpose of this manuscript.

In summary, we propose a novel multi-domain approach to identifying systemic corticosteroid response pattern in children with STRA since 50% do not meet the criteria for definitions of steroid response encompassing lung function alone. Using this approach, we have shown that approximately 40-50% 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.

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386 **Table 1. Multi-domain definition of systemic corticosteroid response**

Domain	**Response pattern 4 weeks after intra-muscular triamcinolone**
Lung function (FEV1)	Increase in FEV_1 (% predicted) to: normal (≥ 80%) or ≥15% improvement
Symptoms (ACT)	Increase in Asthma Control Test (ACT) score to: normal (≥ 20/25) or ≥ 50% or 5 points (whichever is greater)
Exhaled nitric oxide (FeNO)	Improvement to normal (< 24ppb)
Sputum eosinophils	Improvement to normal (<2.5%)

Table 2. 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	51:31	37:17
Age, years	12.3 (7-16.2)	12.7 (7-16.2)
Duration of symptoms, years	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 Inhaled corticosteroid (mcg/day) – 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%)
Systemic corticosteroids Daily dose (mg/day)	26/82 (32%)	19/52 (37%)
Theophylline	20/82 (24%)	15/54 (28%)
Anti-histamine	30/82 (37%)	20/54 (37%)
Baseline ACT	13 (6-23)	13 (6-23)
ACT normal (>19/25)	9/82 (11%)	8/54 (15%)
Baseline % predicted FEV_1	72 (24-134)	72.5 (36-134)
Baseline FEV_1 (litres)	1.72 (0.45-4.02)	1.85 (0.7-4.02)
Number FEV_1 'normal' (>80% predicted)	30/82 (37%)	20/54 (37%)
Baseline % predicted FVC	90 (36-134)	90 (52-134)
Baseline FVC (litres)	2.36 (0.64-5.36)	2.56 (1.38-5.39)
Baseline bronchodilator reversibility (%)	12.8 (-6 to 134)	14 (0-29)
Baseline $FeNO_{50}$ (ppb)	47 (5.6-225)	47 (9-225)
Number normal FeNO (<24ppb)	20/83 (24%)	15/54 (28%)
Induced sputum eosinophils (%)	5.1 (0-92)	4.85 (0-92)
Number with normal sputum eosinophils (<2.5%)	16/59 (27%)	15/54 (28%)

Median (range) unless otherwise stated. ACT, asthma control test; $FeNO_{50}$; Fractional exhaled nitric oxide at flow rate 50ml/second; FEV_1, first second forced expiratory volume; FVC, forced vital capacity; ICS, inhaled corticosteroid; LABA, long acting β agonist; ppb, parts per billion

Table 3. Steroid response in each domain for all children (n=82) and the sub-group (n=54) that had data available for all domains

	Patients with at least 3 domains (ACT, FEV_1, FeNO +/- sputum) (n=82)			**Patients with all 4 domains (n=54)**		
Domain	**Pre triamcinolone**	**Post triamcinolone**	**p**	**Pre triamcinolone**	**Post triamcinolone**	**p**
Symptom (ACT)	13 (6-23)	16.5 (5-25)	<0.001	13 (6-23)	16.5 (7-25)	<0.001
ACT response	34/82 (41%)			23/54 (43%)		
FEV_1 (% predicted)	72 (24-134)	76.5 (44-113)	0.09	72.5 (36-134)	76.5 (46-113)	0.29
FEV_1 response	47/82 (57%)			29/54 (54%)		
FeNO (ppb)	47 (3.6-225)	24.6 (1-150)	<0.0001	47 (9-225)	22 (5-150)	<0.0001
FeNO response	40/82 (49%)			28/54 (52%)		
Sputum eosinophils (%)	5.1 (0-92)	1.6 (0-42.8)	0.001	4.85 (0-92)	2.0 (0-42.8)	0.001
Sputum response	37/66 (56%)			29/54 (54%)		

ACT: asthma control test; FeNO: fractional exhaled nitric oxide at flow rate 50ml/sec; FEV_1: first second forced expiratory volume; ppb: parts per billion. Data presented as median (range), differences pre and post triamcinolone assessed using the Wilcoxon test.

Table 4. Relationships between clinical features, inflammation and corticosteroid response in each domain for children with data available for all domains (n=54)

ACT Response	Response	Non-Response	p
N	23	31	
Duration of symptoms (Decimal years)	10.45 (4-14.45)	7.90 (2.25-14.26)	**0.04**
Atopy	16/23 (70%)	23/31 (74%)	0.77
Intubation	4/23 (17%)	9/31 (45%)	0.36
FEV_1 (% predicted)	81 (37-134)	71 (36-130)	0.27
Bronchodilator Reversibility (%)	10.7 (0-33)	14 (0-30)	0.84
FeNO	44 (9-169)	50 (29-225)	0.13
IgE (IU/ml)	298 (9-19832)	584 (6-6737)	0.24
Sputum eosinophils (%)	5 (0-51.6)	3.2 (0-92)	0.37
Serum eosinophils (%)	5.15 (1.2-11.5)	5.55 (0-19.7)	0.79
BAL eosinophils (%)	4 (0-34.7)	2.7 (0-51)	**0.11**
FEV_1 Response			
N	29	25	
Duration of symptoms (Decimal years)	8.25 (2.25-14.48)	8.5 (3.5-14.3)	0.88
Atopy	20/29 (69%)	19/25 (76%)	0.76
Intubation	**11/29 (38%)**	**2/25 (8%)**	**0.01**
FEV_1 (% predicted)	81 (36-130)	70 (37-134)	0.27
Bronchodilator Reversibility (%)	17 (0.2-30)	12 (0-33)	0.41
FeNO	44.8 (9-225)	47.8 (11-169)	0.38
IgE (IU/ml)	318 (6-19832)	486 (15-3792)	0.77
Sputum eosinophils (%)	4 (0-92)	5 (0-67)	0.59
Serum eosinophils (%)	4.1 (0-19.7)	6.1 (0-14.7)	0.96
BAL eosinophils (%)	2.7 (0-51)	3 (0-34.7)	0.36
FeNO Response			
N	28	26	
Duration of symptoms (Decimal years)	**6.75 (3.5-14.3)**	**10.15 (2.25-14.48)**	**0.02**
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)	0.67
Bronchodilator Reversibility (%)	10.7 (0-30)	14 (0-33)	0.37
FeNO	40 (9-225)	51 (11-165)	**0.08**
IgE (IU/ml)	363 (6-6737)	450 (9-19832)	0.47
Sputum eosinophils (%)	3.3 (0-92)	8.1 (0-67)	**0.08**
Serum eosinophils (%)	2.7 (0-19.7)	7 (0-17.1)	0.18
BAL eosinophils (%)	4 (0-51)	2.7 (0-13)	0.65
Sputum Eosinophil Response			
N	29	25	
Duration of symptoms (Decimal years)	7.65 (3.5-14.48)	6.88 (2.25-14.25)	0.30
Atopy	18/29 (62%)	21/25 (84%)	0.12
Intubation	8/29 (28%)	5/25 (20%)	0.55
FEV_1 (% predicted)	72 (36-130)	73 (37-134)	0.95
Bronchodilator Reversibility (%)	9.7 (0-31)	17.7 (0-33)	**0.03**
FeNO	40.5 (9-225)	52 (14-165)	0.10
IgE (IU/ml)	299 (6-6737)	563 (15-19832)	0.25
Sputum eosinophils (%)	3 (0-41.6)	8.89 (0.33-92)	**0.008**
Serum eosinophils (%)	4.65 (0-19.7)	6.1 (0-17.1)	0.95
BAL eosinophils (%)	3.65 (0-34.7)	2.7 (0-51)	0.58

n/number tested or median (range). BAL, bronchoalveolar lavage; FEV_1, first second forced expiratory volume; FeNO, fractional exhaled nitric oxide, Ig, immunoglobulin. Differences analysed using the Mann Whitney U test or Chi squared tests.

Legends for Figures

Figure 1. Flow diagram showing number of children initially assessed with problematic asthma, those excluded having been diagnosed as having difficult asthma with underlying modifiable factors, and those remaining that were included with severe therapy resistant asthma (STRA).

Figure 2. Steroid response pattern in each domain for every child. (A,C,E,G) all subjects who had all data available (n=54), (B,D,F,H) only those subjects who had an abnormal value before the triamcinolone injection.

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