Mechanisms mediating paediatric severe asthma: translational approaches to uncover phenotypes

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Asthma in children

• 1.1 million children in the UK have asthma (about 3 in every class)

• 2-5% of all children with asthma have severe disease, which cannot be controlled

• They utilise 50% of all healthcare resources for asthma

• New treatments are found from studies in adults
Environmental insult:
Allergen, infection, pollution

Inflammation
Airway remodelling

Healthy airway wall

Asthmatic airway wall

Development of allergic asthma

Reversible airflow obstruction: wheeze
Early loss in lung function in asthma persists to adulthood: worst in severe disease

Children with severe asthma at increased risk of COPD
Paediatric Problematic Severe Asthma

Age: 6-16 years, prescribed maximal treatment, poor asthma control

Detailed MDT assessment
- Correct diagnosis
- Address co-morbidities

Adherence issues – electronic monitoring

Remediable factors which can be addressed

Difficult asthma (DA)

Ongoing poor control

Severe, therapy resistant asthma (STRA)

Age-specific definition of high dose inhaled steroids

<table>
<thead>
<tr>
<th>Inhaled steroid</th>
<th>Age 6-12 years</th>
<th>Age &gt;12 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beclomethasone dipropionate</td>
<td>≥800 mcg/day</td>
<td>≥2000 mcg/day</td>
</tr>
<tr>
<td>Budesonide</td>
<td>≥800 mcg/day</td>
<td>≥1600 mcg/day</td>
</tr>
<tr>
<td>Fluticasone propionate</td>
<td>≥500 mcg/day</td>
<td>≥1000 mcg/day</td>
</tr>
<tr>
<td>Mometasone furoate</td>
<td>≥500 mcg/day</td>
<td>≥800 mcg/day</td>
</tr>
</tbody>
</table>

Chung K-F Eur Respir J 2014;43:343-73
UK National review of asthma deaths 2014: adherence

• 102/128 (80%) of asthma deaths in the UK in people who had picked up less than expected number of prescriptions for maintenance therapy

• 39% had picked up >12 prescriptions year for acute bronchodilators in previous year

• 20% of deaths in people who should have been referred to a specialist for difficult asthma

UK – 3rd highest death rate from asthma in 10-24 year olds

The Nuffield Trust Adolescent Health Report 2019
2\textsuperscript{nd} highest asthma mortality rate for children aged 10-14 years per 100,000 population

The Nuffield Trust Adolescent Health Report 2019

Assessment of adherence: British guidelines

Adherence to long-term asthma treatment should be routinely and regularly addressed by all healthcare professionals within the context of a comprehensive programme of accessible proactive asthma care.

Electronic monitoring

Electronic monitoring is the gold standard for assessing adherence in the research context, although not normally available in routine clinical practice.\textsuperscript{214,215} Dose counting is also used as a comparator, although unlikely to be feasible in a clinical context.\textsuperscript{214,215}

BTS-SIGN 2016 guidelines
Electronic monitoring of adherence

- Smartinhalers®: electronic measurement of adherence
  - clip onto an inhaler

- Contain a microchip - records date and time of medication use

- Usage data is downloaded via App or PC via Bluetooth

- Reminder functions to take preventative medication can be enabled

Example smartinhaler read out

![Graph showing daily usage with average adherence of 33%]

Average Adherence: 33%

Jochmann A Eur Respir J 2017
Categorisation according to adherence

- Median Adherence: 74% (range 21-99%)

<table>
<thead>
<tr>
<th></th>
<th>Good adherence ≥80%</th>
<th>Moderate adherence 60-79%</th>
<th>Poor adherence &lt;60%</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (%)</td>
<td>39 (42)</td>
<td>25 (27)</td>
<td>29 (31)</td>
</tr>
</tbody>
</table>

- 58% with sub-optimal adherence despite being monitored
- No predictors of adherence from clinical characteristics at baseline visit
- No difference in self reported adherence in MARS score (each group median score >20/25: adherent)

Jochmann A Eur Respir J 2017

3 sub-groups identified after monitoring period

- Electronic monitoring
  - Good adherence
    - Good control N=22 (24%)
  - Poor adherence
    - Good control N=24 (26%)
    - Poor control N=30 (32%)
  - Good adherence
    - Poor control N=17 (18%)
    - Step up treatment

Jochmann A Eur Respir J 2017
Investigation of paediatric problematic severe asthma

Stage 1
Confirm diagnosis
Investigation of additional diagnoses
Spirometry and BDR
Measurement of inflammation
Allergy testing (SPTs)
Assessment of adherence
Psychosocial questionnaire
Home visit

Stages 2 and 3
Bronchoscopy
Immunology screen
Allergy testing (RASTs)
Measurement of inflammation
Assessment of steroid responsiveness

Identify modifiable factors
Interventions recommended
Ongoing poor control
Control improved

Targeted treatment plan

Bossley C JACI 2012;129:974-82

Demographics after introduction of electronic monitoring of adherence

<table>
<thead>
<tr>
<th></th>
<th>STRA and electronic monitoring (n=37)</th>
<th>STRA: prescription uptake only (n=53)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>13.21 (8.99-14.42)</td>
<td>11.97 (9.97-14.4)</td>
<td></td>
</tr>
<tr>
<td>Male gender</td>
<td>24/37 (65%)</td>
<td>33/53 (62%)</td>
<td></td>
</tr>
<tr>
<td>Percent predicted FEV₁</td>
<td>89.40 (71.50-102.0)</td>
<td>68.5 (54.8-86.5)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Total IgE (IU/mL)</td>
<td>416.50 (144-1514)</td>
<td>386 (115-1286)</td>
<td></td>
</tr>
<tr>
<td>Medication</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Beclomethasone (µg)</td>
<td>1200 (1000-1600)</td>
<td>1600 (800-1600)</td>
<td></td>
</tr>
<tr>
<td>- Oral</td>
<td>3/15 (20%)</td>
<td>24/53 (45%)</td>
<td></td>
</tr>
<tr>
<td>FeNO (normal &lt;24ppb)</td>
<td>18.85 (9.00-50.15)</td>
<td>50.3 (29.3-69.7)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>ACT score (/25)</td>
<td>14 (13-20)</td>
<td>13 (9-17)</td>
<td></td>
</tr>
</tbody>
</table>

Nagakumar and Adams - Unpublished
Difficult Asthma patients reduce inhaled steroid dose over time

DA

STRA

Difficult Asthma improve lung function over time

DA

STRA

Sharples J ERJ 2012;40:264-7
DA had fewer asthma attacks up to 6 years later despite reduced steroid dose

Demographics of children with STRA, DA and Disease controls

<table>
<thead>
<tr>
<th></th>
<th>STRA (n=25)</th>
<th>DA (n=8)</th>
<th>Disease Control (n=8)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>12.9 (8.9-16.1)</td>
<td>14 (8.1-16.5)</td>
<td>9.8 (6.1-16.5)</td>
<td></td>
</tr>
<tr>
<td>Percent predicted FEV₁</td>
<td>87.5 (66,134)</td>
<td>82.5 (63,110)</td>
<td>86 (63-115)</td>
<td>ns</td>
</tr>
<tr>
<td>Total IgE (IU/mL)</td>
<td>321.5 (21,1938)</td>
<td>161 (81,801)</td>
<td>38.5 (19-1252)</td>
<td>0.04</td>
</tr>
<tr>
<td>ICS (mg/day)</td>
<td>1 (0.8-2)</td>
<td>1 (0.8-2)</td>
<td>0.4 (0-1.6)</td>
<td>0.02</td>
</tr>
<tr>
<td>FeNO (ppb)</td>
<td>12.5 (8-43)</td>
<td>12 (10-31)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Omalizumab</td>
<td>N=5</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Sharples J ERJ 2012;40:264-7

Prasad Nagakumar In revision
Inflammation in sputum samples

Blood eosinophils similar in DA and STRA, but sputum eosinophils elevated in STRA

Prasad Nagakumar In revision
Blood eosinophils rarely reflect airway eosinophilia in children with STRA

![Blood and airways](image)

Ullmann N Allergy 2013;68:402-6

No correlation between blood and sputum eosinophils in STRA (*) or DA (▲)

![Graph](image)

Prasad Nagakumar In revision
**Persistent airway eosinophilia and remodelling in STRA**

![Graph showing Persistent airway eosinophilia and remodelling in STRA](image)

- **Eosinophils**
  - p=0.001
  - Log10 BAL eosinophil %

- **Reticular basement membrane thickness**
  - p<0.001
  - RBM thickness (µm)

- **Airway smooth muscle**
  - p=0.003
  - Vv (ASM/subepithelium)

- **Bossley CJ**
- **JACI 2012**

**No increase in Th2 cytokines in stable paediatric STRA**

![Graph showing No increase in Th2 cytokines in stable paediatric STRA](image)

- **Submucosal IL-5 expression**
  - Submucosal IL-5+ cells/mm²

- **Submucosal IL-13 expression**
  - Submucosal IL-13+ cells

- **Bossley CJ et al JACI 2012;129:974-82**
Neonatal mouse model of experimental allergic asthma

Inflammation

Remodelling

Airways resistance

Airway hyperresponsiveness

Saglani AJRCMB 2009

No effect on AHR, eosinophilia or remodelling with therapeutic steroids in neonatal house dust mite induced allergic airways disease

Intra-nasal budesonide
0.6mg/kg daily

Saglani JACI 2013
Significant reduction in IL-13 levels, but no change in IL-33 with therapeutic budesonide

GWAS of a specific childhood asthma phenotype of severe exacerbations

Saglani JACI 2013

Bonnelykke K Nat Genetics 2014
Increased submucosal IL-33+ cells in bronchial biopsies from children with STRA

IL-33 expression in paediatric endobronchial biopsies

IL-33 is associated with increased reticular basement membrane thickness in STRA

Positive correlation between IL-33 expression and RBM thickness

Saglani JACI 2013;132:676-85
rIL-33 and pulmonary remodelling

Day 3

Week 1

Week 2

PBS

i.m. rIL33 or PBS

collagen

fibronectin

Week 1

Week 2

rIL-33

NS

collagen (μg)

fibronection (fold change)

rIL-33

NS

IL-5, IL-13

Th2 differentiation

Type 2 Innate lymphoid cell

Antigen presenting cell

Eosinophilia

Adaptive immune response

Saglani Thorax 2011
Innate lymphoid cells (ILC)

- Morphologically similar to lymphocytes (T helper cells) but lack specific antigen receptor (T cell receptor) and cell surface markers like CD3, CD4, CD19
- Produce cytokines similar to T helper cell subsets
- Play a role in immunity and inflammation at mucosal surfaces (lung, gut, skin)

Th2 cells vs ILC2 cells

<table>
<thead>
<tr>
<th>Th2</th>
<th>ILC2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lineage surface markers</td>
<td>Lineage negative</td>
</tr>
<tr>
<td>T cell receptor, CD4+, CD3+</td>
<td><strong>No T cell receptor</strong></td>
</tr>
<tr>
<td>Lymphoid morphology</td>
<td>Lymphoid morphology</td>
</tr>
<tr>
<td>Intra-nuclear transcription factor: GATA3</td>
<td>Intra-nuclear transcription factor: GATA3</td>
</tr>
<tr>
<td>Induced via adaptive immune responses:</td>
<td>Induced via innate epithelial cytokines:</td>
</tr>
<tr>
<td>IgE, DCs, lymph nodes</td>
<td><strong>IL-33, IL-25, TSLP</strong></td>
</tr>
<tr>
<td>Type 2 cytokine production:</td>
<td>Type 2 cytokine production</td>
</tr>
<tr>
<td>IL-4, IL-5, IL-13, IL-9</td>
<td>IL-4, IL-5, IL13, IL-9</td>
</tr>
</tbody>
</table>
Increased innate lymphoid cells (ILC2s) in BAL from children with STRA compared to controls

Lin⁻ CD45⁺CD127⁺CRTH2⁺ cells

Nagakumar JACI 2016

Higher sputum ILC2 and Th2 cells in STRA compared to DA

Sputum

ILC2

Th2

Blood

Prasad Nagakumar In revision
Assessment of Problematic Asthma

Stage 1
Problematic severe asthma

Modifiable factors addressed from Stage 1
No additional investigations needed

Difficult asthma

Poor control after excluding DA – Stage 2
STRA
ACT, Spirometry, FeNO, induced sputum, Bronchoscopy, IM triamcinolone

Assessment 4 weeks after IM triamcinolone
ACT, spirometry, FeNO, induced sputum

How do airway cells respond to steroids *in vivo* in children with STRA?

Child with STRA
Visit 1: i.m. triamcinolone
Visit 2: 4 weeks later
Spirometry, exhaled nitric oxide, symptom control score, sputum induction

ILC2

Th2

Prasad Nagakumar unpublished
No change in FEV₁, but persistent airway eosinophilia after triamcinolone

Exacerbations (n=11)

Sputum Eosinophils (n=5)

Reduction in sputum IL-5 after triamcinolone despite elevated eosinophils

Prasad Nagakumar In revision
Clinically significant exacerbations reduced with anti-IL5 Ab (mepolizumab) in adult severe asthma

Blood eosinophils reduced, no impact on sputum eosinophils, lung function or symptoms

Pavord I Lancet 2012;380:651-9
Anti-IL5 treatments for paediatric STRA?

• Total children >12 years included in mepo studies - N=34

• Safety and dosing study of mepo in 6-11 year olds

• NO efficacy data from children under 12 years

• Mepolizumab approved by European Medicines Agency as adjunctive treatment for severe refractory eosinophilic asthma in children aged 6 to 17 years

Anti-IL5 Ab and other biologics for paediatric STRA?

Concerns:
1. No appropriate studies of biologics in children

2. Therapeutic targets chosen from mechanistic studies in adults

3. What is the optimal biologic for the individual?

Saglani S Lancet Resp Med 2019 March
Paediatric STRA characterized by severe atopy and polysensitisation

Aeroallergens - sum of SPT wheals

Food allergens - sum of SPT wheals

Omalizumab (anti-IgE Ab): British Guidelines

- Severe, persistent allergic (IgE mediated) asthma in patients >6 years
  - 4 or more courses OCS in the previous year

- Positive skin test or in vitro reactivity to a perennial aeroallergen

• Serum IgE 30-1500 IU/ml
  • 30% of STRA have IgE >1500IU/ml
Predictors of response to anti-IgE Ab (Omalizumab): exacerbating phenotype

<table>
<thead>
<tr>
<th>Variable</th>
<th>Continued (n=20)</th>
<th>Stopped (n=10)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Predicted FEV₁</td>
<td>75 (35.3-106)</td>
<td>79.7 (50.7-112.9)</td>
<td>NS</td>
</tr>
<tr>
<td>Asthma control test</td>
<td>11 (5-22)</td>
<td>8 (5-11)</td>
<td>0.005</td>
</tr>
<tr>
<td>FeNO</td>
<td>31.38 (6.53-176.5)</td>
<td>49.95 (10.97-99.96)</td>
<td>NS</td>
</tr>
<tr>
<td>Subjects with hospital admission in last 16 weeks</td>
<td>n=11</td>
<td>n=0</td>
<td>0.041</td>
</tr>
<tr>
<td>Subjects needing oral steroid burst last 16 weeks</td>
<td>n=19</td>
<td>n=3</td>
<td>0.028</td>
</tr>
<tr>
<td>Serum IgE (IU)</td>
<td>379 (105-1155)</td>
<td>717.5 (216-6284)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Response to omalizumab in 60% of subjects who are eligible

NIHR funded trial:
Treating severe paediatric asthma; an open label randomised controlled trial comparing mepolizumab and omalizumab (TREAT)

• 10 centres in UK (Brompton/Imperial – lead site): start May 2019

• Head to head, non-inferiority comparison of mepolizumab and omalizumab in children with STRA

• Age 6-16 years

• Primary outcome – number of exacerbations over 52 weeks
NIHR EME Grant: Treating severe paediatric asthma; an open label randomised controlled trial comparing mepolizumab and omalizumab (TREAT)

PROBLEMATIC SEVERE ASTHMA (PSA) \[n=500 over 3 years\]
Adherence monitoring (run-in period), 3 month electronic monitoring to identify STRA

~30%

Enhanced run-in to identify STRA and exclude DA

Good adherence, Persistent poor control
SEVERE THERAPY RESISTANT ASTHMA (STRA) \[n=150\]

Invasive and non-invasive phenotyping and mechanistic studies:
BAL, brushings, biopsy, sputum, blood, urine

Omalizumab
N=75

Mepolizumab
N=75

Primary outcome: Number of asthma attacks by 52 weeks

No change in IL-17 levels after triamcinolone

Prasad Nagakumar
unpublished
Elevated BAL IL-17 associated with severe asthma in adults

Irvin et al JACI 2014

No increased IL-17A in paediatric severe asthma

Andersson C JACI 2017
Similar numbers of sputum Th17 and Lin⁻IL17⁺ cells in STRA, DA and Controls

![Bar chart showing the percentage of CD4⁺ cells and Lin⁻IL17⁺ cells in STRA, DA, and CI across weeks 1, 2, and 3.](chart1.png)

Prasad Nagakumar unpublished

No change in AHR or inflammation after blocking IL-17 in neonatal HDM induced AAD

![Graph showing AHR and BAL inflammation levels in different groups.](chart2.png)

3 weeks prevention regimen

<table>
<thead>
<tr>
<th>Anti-IL-17 Ab / isotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDM/PBS</td>
</tr>
</tbody>
</table>

Saglani Unpublished
No benefit from anti-IL17RA antibody in adult severe asthma

Busse W Am J Respir Crit Care Med 2013

Increased IL-17A receptor expression in epithelium

Andersson C JACI 2017
Increased IL-8 secretion from primary HBECs after stimulation with IL-17A +/- budesonide

Increased intra-epithelial neutrophils in STRA
Epithelial neutrophils associated with better lung function, symptom control and less steroids

Summary

- Difficult asthma and STRA are distinct clinical and molecular phenotypes
  - DA – “steroid sensitive” without airway eosinophilia

- STRA characterised by steroid resistant airway eosinophilia and remodelling
  - Need huge care before using blood eosinophils as a biomarker in children
  - Role of anti-IL5 Ab therapies in STRA remains uncertain

- Airway IL-17 levels not increased in paediatric STRA compared to controls, maintained despite steroids, no benefit of blocking IL-17

- Increased intra-epithelial neutrophils in sub-group of STRA
- NOT associated with worse disease in children, may be protective
Children are not small adults...

- Huge care before direct extrapolation of data from adult studies to children
- Need mechanistic studies in early life models and using samples from children
- Need to look at mechanisms comparing both blood and airway samples

Translational Research Programme:

Clinical phenotyping
- Diagnostic confirmation
- Basic management
- Adherence monitoring
- Symptom control, QOL
- PEFR Fluctuation
- Nocturnal monitoring
- Breathing pattern disorders

Invasive and non-invasive airway sampling
- Molecular phenotype
- Steroid response
- High dose ICS and HPA axis
- In vitro culture systems

Neonatal model of AAD
- Mechanisms mediating disease
- Novel therapeutic targets
Acknowledgements

**Inflammation, Repair & Development**
- Clare Lloyd
- Lisa Gregory
- Simone Walker
- Laura Denney
- Avneet Manghera
- Cecilia Andersson

**Royal Brompton Hospital**
- Andy Bush
- Louise Fleming
- Prasad Nagakumar
- Cara Bossley
- Alexandra Adams
- James Cook