INFLAMMATION BIOMARKERS IN THE ASSESSMENT AND MANAGEMENT OF SEVERE ASTHMA – TOOLS AND INTERPRETATION
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# OVERVIEW

Asthma is a heterogeneous disease, meaning that there are many different subtypes. Groups of patients with similar disease characteristics can be clustered into asthma phenotypes. Well-recognised inflammatory phenotypes in severe asthma include allergic asthma, eosinophilic asthma and non-eosinophilic asthma (Figure 1).

In the severe asthma population, treatment shifts from a step-wise to a targeted approach. Disease pathology is caused by differing mechanisms in the different inflammatory phenotypes. Targeted therapy with monoclonal antibodies (mAbs) works by specifically blocking these disease pathways. Recognition and assessment of individual phenotypes is necessary to support a targeted therapy approach. Detailed assessment is required to inform the selection of targeted therapies which are likely to benefit individual patients.

## BIOMARKERS

Biomarkers are quantifiable factors (e.g. protein, molecule or cell type) that provide information about a biological process. Biomarker measurement provides insight into the mechanisms that are causing symptoms in an individual patient. Biomarkers are essential components of new management approaches using endotypes and “treatable traits”. In severe asthma assessment and management, biomarkers have been proposed to assess optimum inhaled maintenance therapy, determine treatment adherence, guide selection of targeted therapies and predict and assess response to treatment (1). Fractional exhaled nitric oxide (FeNO), sputum and blood eosinophil numbers have been proposed as useful biomarkers in the asthma population.

In this document, we provide an overview of each of these biomarkers and a summary of assessment approaches and interpretation of findings.

### FRACTIONAL EXHALED NITRIC OXIDE (FENO)

The fraction of exhaled nitric oxide (FeNO) can be non-invasively quantified in breath. Nitric oxide is produced by epithelial cells lining the airways. The production of nitric oxide is increased in the presence of Type 2 inflammation, where it is largely driven by IL-13. FeNO levels correlate with levels of Type-2 inflammation and are partially predictive of increased sputum eosinophil numbers (2). Elevated FeNO levels are associated with increased rates of asthma attacks and wheeze (3). FeNO levels are also influenced by a number of external variables including ambient air quality, smoking, sinus disease, allergic rhinitis, diet and virus infection. These factors need to be controlled and considered when interpreting FeNO results. FeNO levels are not used to select mAb treatments nor are they included in the Australian Pharmaceutical Benefits Scheme (PBS) eligibility criteria for mAb therapy. FeNO cut-off levels (≥ 20ppb) have been suggested in identifying patients more likely to have Type 2 inflammation and as a potential predictor of patients more likely to respond to omalizumab (anti-IgE) therapy (4).

### ASSESSMENT

Portable FeNO analysers are available from a number of manufacturers. Testing is performed by breathing into the analyser device. Most patients are able to perform the test well following training.

FeNO analysers vary in costs and ongoing maintenance and consumables requirements. Determining which analyser is most cost-effective for a practice will depend on the expected number of tests per year.

Differences in FeNO values may result from measurements on different analyser types. This should be considered when interpreting changes in response to treatment or when using FeNO levels to inform treatment decisions, if a different type of analyser has been used.

FeNO is listed as a reimbursable test on the Australian Medical Benefits Schedule (MBS), when performed in a pulmonary function laboratory (item #11507).
INTERPRETATION

Detailed guidelines for the interpretation of FeNO in clinical applications are available from the American Thoracic Society (https://www.atsjournals.org/doi/abs/10.1164/rccm.9120-11ST) (5). The ATS guidelines recommend a FeNO cut-off value of <25ppb (<20 ppb in children) to suggest that eosinophilic inflammation and ICS responsiveness are unlikely. A cut-off value of >50ppb (>35ppb in children) is recommended to predict the presence of eosinophilic inflammation and a likely response to ICS therapy. The BTS / SIGN British Guideline on the Management of Asthma recommend the use of a cut-off value of ≥40ppb to indicate likely eosinophilic inflammation or atopy in adults (≥35ppb in children 5-16 years of age) (https://www.brit-thoracic.org.uk/standards-of-care/guidelines/btssign-british-guideline-on-the-management-of-asthma/) (6).

FENO AND ICS ADHERENCE

An elevated FeNO measurement may also indicate non-adherence to prescribed corticosteroid treatment. If non-adherence is suspected, a “FeNO suppression test” may be considered (7). FeNO suppression testing consists of serial FeNO measurements in conjunction with objectively observed ICS administration for 5-7 days (7), using an electronic monitoring device, for example, an Inhaler Compliance Assessment (INCA) device.

The steps involved in a FeNO suppression test are as follows:

1. **Day 0 – Clinic visit:**
   a. Baseline FeNO measurement to assess levels prior to observed ICS treatment
   b. Baseline assessment of asthma control (e.g. asthma control questionnaire (ACQ)), lung function via spirometry and airway inflammation (e.g. sputum induction), where available
   c. Directly observed ICS treatment

4. **Days 1-7 – Home**
   a. Home monitoring of FeNO levels may be considered, if resources are available
   b. Ongoing ICS treatment using an electronic inhaler monitoring device (e.g. INCA device)
   c. Identified non-adherence should inform patient-centred discussions to improve outcomes. If FeNO levels remain elevated, this is suggestive of likely ICS treatment adherence accompanied by corticosteroid-refractory inflammation.

<table>
<thead>
<tr>
<th>Assessment</th>
<th>Medication Change</th>
<th>Dose Levels</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FeNO Level (ppb)</strong></td>
<td>ICS</td>
<td>ICS Doses</td>
</tr>
<tr>
<td>Smoker</td>
<td>Non-Smoker</td>
<td></td>
</tr>
<tr>
<td>&gt;22</td>
<td>&gt;29</td>
<td>↑ one level</td>
</tr>
<tr>
<td>14-22</td>
<td>19-29</td>
<td>No change</td>
</tr>
<tr>
<td>&lt;14</td>
<td>&lt;19</td>
<td>↓ one level</td>
</tr>
<tr>
<td><strong>Asthma Control Questionnaire (ACQ)</strong></td>
<td>LABA</td>
<td>LABA Doses</td>
</tr>
<tr>
<td>&gt;1.5</td>
<td></td>
<td>2 x 12µg bd</td>
</tr>
<tr>
<td>&lt;1.5</td>
<td></td>
<td>1 x 12µg bd</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 x 6µg bd</td>
</tr>
</tbody>
</table>

In clinical trials of mAb treatment, elevated FeNO levels were predictive of response to benralizumab (anti-IL-5Ra; ≥50ppb, in combination with elevated peripheral blood eosinophils) (10), omalizumab (anti-IgE; ≥19.5 ppb) (11) and lebrikizumab (anti-IL-13; ≥30 ppb) (12, 13) (reviewed in (1)). FeNO levels were reduced following treatment with lebrikizumab (12-14) or dupilumab (15, 16) (reviewed in (1), however mAbs targeting the IL-5 pathway (e.g. mepolizumab, benralizumab) do not suppress FeNO levels (17).
SPUTUM EOSINOPHILS

Sputum assessment provides insights into the pattern of airway inflammation, by counting immune cells isolated from the airway lumen. Quantification of immune cell types in sputum samples identifies patient subgroups with elevated eosinophils (eosinophilic; ≥3%), neutrophils (neutrophilic; ≥61%), both (mixed granulocytic) or neither (paucigranulocytic)(18). Elevated sputum eosinophil counts are present in approximately a third to half of people with asthma. Sputum eosinophil numbers correlate with asthma exacerbation risk in the severe asthma population (19), and correlate partially with FeNO and blood eosinophil numbers (20).

ASSESSMENT

Sputum analysis consists of the following steps:

1. **Sputum induction** – typically performed with nebulised sterile saline solution (isotonic or hypertonic). Spirometry (FEV1) is performed prior to sputum induction, and at intervals throughout the procedure. Unlike saline challenge patients are pretreated with inhaled short-acting beta agonist (SABA), as induction can induce bronchoconstriction. In some instances, collection of spontaneously produced sputum may be possible.

2. **Sample processing** – consists of steps to homogenise the sputum sample (e.g. dithiothreitol (DTT) treatment), total cell number quantification, preparation onto slides and differential staining (e.g. Giemsa staining).

3. **Microscopy analysis** – immune cell types are counted based on morphological criteria and typically expressed as a percentage of total cells.

Note that sputum induction, processing and analysis requires specialised staffing and is time-consuming. Sputum assessment is not available in all clinical settings.

A step-by-step guide to sputum induction, processing and analysis is available from the European Respiratory Society (http://breathe.ersjournals.com/content/9/4/300 (21).

INTERPRETATION

The presence of elevated sputum eosinophils (e.g. ≥3%) identifies a patient population that may respond to corticosteroid therapy or specific treatments targeting eosinophilic inflammation.

Sputum eosinophil numbers have also been used to guide step-up / step-down treatment with inhaled corticosteroids. A 2018 systematic review synthesised the results from four randomised clinical trials on the effects of sputum eosinophil-guided management approaches for asthma. When management was guided by sputum eosinophil numbers the proportion of patients experiencing an asthma exacerbation was reduced (OR 0.36, 95% CI 0.21-0.62)(8). No consistent differential effects of sputum-guided corticosteroid adjustments were observed on other outcomes assessed (e.g. daily ICS dose, asthma control or lung function).

In clinical trials of monoclonal antibody treatment, elevated sputum eosinophil numbers predicted response to mepolizumab (anti-IL-5; ≥3%) (22, 23), reslizumab (anti-IL-5; ≥3%) (24) and benralizumab (anti-IL-5Rα; ≥2%) (25) (reviewed in (1)). Further, sputum eosinophil numbers were reduced following mepolizumab (17, 22), reslizumab (24) and omalizumab (26) treatment (reviewed in (1)).
**BLOOD EOSINOPHILS**

Blood eosinophil quantification has been proposed as a surrogate marker of airway eosinophilia, as quantification is much simpler, inexpensive and requires fewer resources. Blood eosinophil counts partially correlate with sputum eosinophil numbers (20). Elevated blood eosinophil numbers are associated with asthma severity and an increased risk of asthma attacks (27).

Documentation of elevated peripheral blood eosinophil number (≥300/μL; ≥0.3 x 10^9/ml) is currently required for initial application for mepolizumab (anti-IL-5) and benralizumab (anti-IL-5Ra) therapy with Australian Pharmaceutical Benefits Scheme (PBS).

**ASSESSMENT**

Blood eosinophil quantification can be performed as part of a full blood count through standard pathology services.

Alternatively, blood eosinophil proportion can be determined by manual count of a stained blood smear.

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Cut-off Value</th>
<th>Advantages / Disadvantages</th>
<th>Utility</th>
</tr>
</thead>
</table>
| Fractional exhaled nitric oxide (FeNO) | <25 ppb (<20 in children) to rule out eosinophilic inflammation 
 ≥40-50 ppb (≥35 in children) to predict eosinophilic inflammation | Simple and point of care assessment 
 Specialised equipment required | Guidance for ICS dosage 
 Assess treatment adherence (FeNO suppression test) 
 Predicts response to mAb therapy (benralizumab, omalizumab, lebrikizumab) 
 Responsive to mAb therapy that targets IL-13 (e.g. lebrikizumab) but not mepolizumab or benralizumab |
| Sputum eosinophils | ≥3% | Direct measure of airway eosinophils 
 Specialised staffing / training required 
 Time-consuming | Predicts response to corticosteroid therapy 
 Guidance for ICS dosage 
 Predict response to mAb therapy (mepolizumab, benralizumab) 
 Responsive to mAb therapy (mepolizumab, reslizumab, omalizumab) |
| Blood eosinophils | ≥300-400/μL (≥0.3-0.4 x 10^9/mL) | Simple blood test assessment 
 Partially correlates with airway inflammation | Predict response to mAb therapy (mepolizumab, reslizumab, benralizumab, omalizumab) 
 Responsive to mAb therapy (mepolizumab, reslizumab, benralizumab) 
 Forms part of PBS eligibility for mAb therapy |

**INTERPRETATION**

A blood eosinophil count threshold of ≥300-400/μL (≥0.3-0.4 x 10^9/mL) is typically used as a cut-off to identify elevated eosinophil numbers. Caution should be taken when interpreting results in individual patients, as the time since eating, exercise, medication use and time of testing (diurnal variation) affect results (reviewed in (28)). Further, blood eosinophil counts can vary over time, requiring testing at multiple timepoints, with up to 1/3 of individuals with low levels at baseline shown to have elevated numbers at later follow-up in clinical trials (29). Corticosteroid treatment (particularly OCS) reduces blood eosinophil counts. Eosinophil levels vary over the course of the day, with peak levels observed at midnight and lowest levels at midday, in healthy individuals (reviewed in (28)). Thus, blood samples collected later in the day are more likely to indicate a non-eosinophilic phenotype. The degree of variation and pattern differs between individuals with asthma (30), and requires more study.

A small pilot case series has provided proof of concept that in patients with severe asthma taking maintenance oral corticosteroids (OCS), OCS dose adjustment guided by peripheral blood eosinophil counts led to a reduced maintenance OCS dose and fewer asthma attacks while maintaining improved symptom control (31). Larger, randomised studies assessing this approach are required.

In clinical trials of monoclonal antibody treatment, blood eosinophil counts predicted response to mepolizumab (anti-IL-5; ≥150/μL) (17, 32-34), reslizumab (anti-IL-5; ≥400/μL) (35-37), benralizumab (anti-IL-5Ra; ≥300/μL) (10, 25, 38, 39) and omalizumab (anti-IgE; ≥260 or ≥300/μL) (11, 40) (reviewed in (1)). Blood eosinophil counts were also reduced following mepolizumab (17, 22, 32, 33), reslizumab (24, 35) and benralizumab (10, 25, 41) treatment (reviewed in (1)).
RESOURCES USED

  • Severe asthma phenotypes:
    https://toolkit.severeasthma.org.au/severe-asthma/phenotypes/
  • Assessment – phenotyping:
  • Monoclonal antibodies:

American Thoracic Society Guidelines for Interpretation of FeNO for Clinical Applications
  • https://www.atsjournals.org/doi/abs/10.1164/rccm.9120-11ST

Induced Sputum Analysis: Step by Step (European Respiratory Society)
  • breathe.ersjournals.com/content/9/4/300
REFERENCES


31. Wark PA, McDonald VM, Gibson PG. Adjusting prednisone using blood eosinophils reduces exacerbations and improves asthma control in difficult patients with asthma. Respirology. 2015;20(6):1282-4.


