AMPATHCHAT no. 89

MAKING SENSE OF THE LABORATORY DIAGNOSIS OF ASTHMA

Dr Sylvia van den Berg and Dr Carla van Heerden

KEY MESSAGES

- The laboratory evaluation of asthma includes routine laboratory tests, as well as the measurement of T2 inflammation biomarkers.
- **Full blood count (FBC) with differential count:** To confirm or exclude the presence of eosinophilia.
- **Sputum eosinophils:** Sputum eosinophilia despite high-dose ICS or oral corticosteroids are associated with more symptomatic disease and poorer patient outcomes.
- **Total serum immunoglobulin E (IgE):** Total serum IgE testing can be used to determine patient suitability for anti-IgE interventions.
- **Specific IgE:** Allergen-specific IgE testing is used to identify triggering allergens.
- **FeNO:** FeNO levels can be used to diagnose and manage asthma.

INTRODUCTION

Asthma is a serious global health problem that affects all age groups. Asthma is one of the most common chronic non-communicable diseases that affects over 260 million people and is responsible for over 450 000 deaths each year worldwide, most of which are preventable. It is also the most common chronic non-communicable childhood disease. Asthma is defined by chronic inflammation of the airways, airway hyper-responsiveness and variable airflow limitation, which can be triggered by viruses, allergens, irritants and exercise. This leads to recurrent episodes of wheezing, breathlessness, chest tightness and/or coughing that can vary over time and in intensity. Symptom episodes are associated with widespread, but variable, airflow obstruction within the lungs that is usually reversible, either spontaneously, or with appropriate asthma treatment such as a fast-acting bronchodilator.

In the past decades, asthma has been increasingly recognised as a heterogeneous disease. Recently acquired knowledge about the different molecular pathways that cause asthma has provided a clearer understanding of asthma's different endotypes, enabling a more accurate diagnosis and personalised therapeutic approach. The one-size-fits-all treatment model has failed to successfully treat all patients with asthma. The ever-expanding repertoire of new biologic agents has made it essential to classify asthma according to its molecular pathways with the appropriate combination of biomarkers.

WHAT IS SEVERE ASTHMA?

About 5–10% of asthmatics suffer from severe asthma. Severe asthma is a leading cause of morbidity and mortality in asthmatic patients. After confirming a diagnosis of asthma and addressing co-morbidities, severe asthma is defined as asthma that requires treatment with high-dose inhaled corticosteroids (ICS) plus a second controller (and/or systemic corticosteroids) to prevent it from becoming 'uncontrolled' or that remains 'uncontrolled' despite this therapy. Other conditions should be excluded, the optimal use of inhalers confirmed, and potential exacerbating factors corrected.

ENDOTYPES AND PHENOTYPES OF ASTHMA

Asthmatic patients were previously grouped into so-called phenotypes, with observable combinations of clinical, biological and physiological characteristics, resulting from hereditary and/or environmental influences. The strategy has now evolved to associate the underlying molecular mechanisms with phenotype (see Table 1). Asthma endotypes describe these distinct pathways at the cellular and molecular levels. Despite a similar clinical picture, patients may respond very differently to treatment.

TABLE 1: ENDOTYPES AND PHENOTYPES OF ASTHMA

Endotype	Phenotype	Clinical characteristics	Molecular mechanism	Biomarkers	Natural history
T2 high	Atopic	Well defined, early onset, steroid sensitive	Allergic sensitisation	Blood/sputum eosinophil count, serum-specific allergen IgE, high FeNO, high total IgE	Identifiable and treatable, preserved lung function
	Late onset	Concomitant CRSwNP, steroid refractory	Staphylococcus aureus enterotoxin	Blood/sputum eosinophil count, high FeNO	Severe from onset, more frequent exacerbation
	AERD	Adult onset	Dysregulated arachidonic acid metabolism	Blood/sputum eosinophil count, urinary LTE4	Severe from onset, more frequent exacerbation
Non-T2	Non-atopic	Adult onset-paucigranulocytic or neutrophilic	NLRP3/IL-1 β, altered micro-RNA expression, Th17	Induced sputum neutrophil count	Variable course and lung function
	Smokers	Older adults	Oxidative stress, mixed T2 high/T2 low	Induced sputum neutrophil count	More frequent exacerbation, lower lung function
	Obesity related	Female sex	Oxidative stress, neutrophils, increased innate immune activation	Serum IL-6	Severe symptoms, preserved lung function
	Elderly	>50 to >65 years at onset	Immunosenescence, Th1/Th17 inflammation	Induced sputum neutrophil count	Steroid resistant

Abbreviations: Type 2 (T2); Fractional exhaled nitric oxide (FeNO); Chronic rhinosinusitis with nasal polyps (CRSwNP); Aspirin-exacerbated respiratory disease (AERD); Urinary leukotriene E4 (LTE4); Nucleotide-binding oligomerisation domain, Leucine rich Repeat and Pyrin domain 3/Interleukin-1β (NLRP3/IL-1 β); T helper cell (Th)

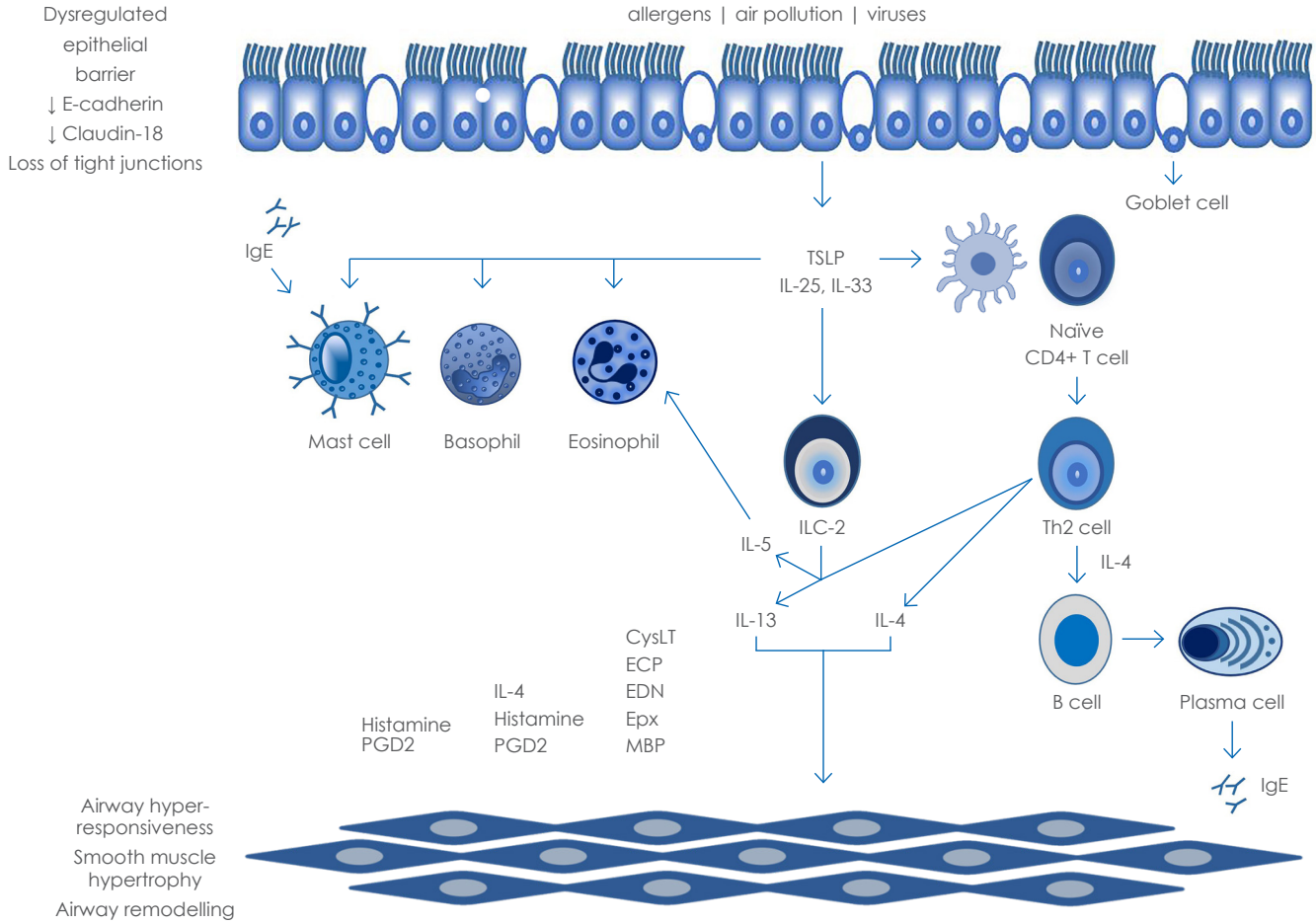

FIGURE 1: T2-HIGH INFLAMMATORY PATHWAYS IN ASTHMA

A dysregulated epithelial barrier facilitates translocation of allergens, air pollution and viruses, leading to the release of alarmins such as thymic stromal lymphopoietin (TSLP), IL-25 and IL-33. TSLP primes dendritic cells to induce the differentiation of naïve T cells into Th2 cells. Th2 cells activate B cells via IL-4 to differentiate into plasma cells that generate IgE required for mast cell responses to allergens. The alarmins IL-25 and IL-33 can activate group 2 innate lymphoid cells (ILC2s), mast cells, eosinophils and basophils. Activated ILC2s, like Th2 cells, produce IL-5 and IL-13. IL-5 promotes eosinophil differentiation and survival. IL-13, IL-4 and inflammatory mediators from mast cells, basophils and eosinophils have effects on airway hyper-responsiveness, smooth muscle hypertrophy, and airway remodeling. CysLT, cysteinyl leukotrienes; ECP, eosinophil cationic protein; EDN, eosinophil-derived neurotoxin; EPx, eosinophil peroxidase; MBP, major basic protein; PGD2, prostaglandin D2.

Asthma can be characterised into two major endotypes based on the level of type 2 (T2) inflammation: T2 high and T2 low endotypes. Most patients with allergic asthma exhibit T2 inflammation with or without eosinophilia, also referred to as a T2-high asthma phenotype. T2 high is the most common type of inflammation in allergic diseases and involves T helper (Th)-2 lymphocytes, the cytokines IL-5, Il-13 and IL-4, and group 2 innate lymphoid cells (ILC2). T2 inflammation is mainly driven by eosinophilic inflammation (see Figure 1). This endotype can be treated successfully with current available asthma treatment and biologics.

T2-low endotypes have a similar disease phenotype, but the mechanism of T2-low inflammation is less well understood. Th1/Th17 or Th1/Th22 pathway activation, innate immune defects, tissue remodeling and neurogenic inflammation are implicated. T2-low endotypes also have a less clear path to treatment.

BIOMARKERS OF ASTHMA

Biomarkers indicating T2 inflammation include blood and sputum eosinophil counts, fraction of exhaled nitric oxide (FeNO) values, periostin and IgE levels. Biomarkers are considered valuable in the diagnosis, monitoring and prediction of risk and prognosis, in particular asthma.

Asthma can be categorised in T2 (type 2) high (T2 asthma) or T2 low (non-T2 asthma) based on the presence or absence of T2 inflammation (see Figure 2).

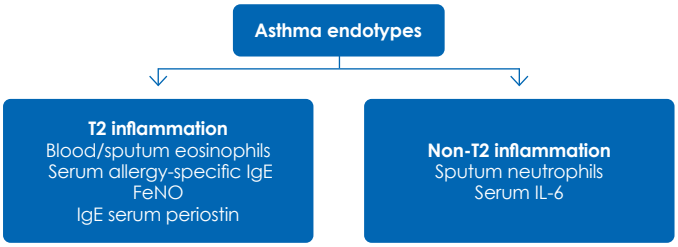

FIGURE 2: ASTHMA ENDOTYPES

It is important to understand the underlying patho-physiology (endotype) in managing a patient with asthma, as it enables clinicians to better diagnose, stratify and monitor their patients. In the case of T2 biomarkers, specific T2-targeted therapies with considerable efficacy have been approved in T2-high asthma (e.g. omalizumab, dupilumab, mepolizumab, reslizumab, benralizumab).

Eosinophils

There is a clear association between T2-inflammation and eosinophils. Airway eosinophilia is associated with asthma exacerbation, an increase in airway hyper-reactivity and poor clinical outcome. The eosinophilic inflammatory process is driven by the key cytokines IL-5, IL-4 and IL-13. Sputum eosinophilia predicts response to treatment with corticosteroids and may be used as a therapeutic target for guiding treatment with inhaled corticosteroids (ICS). Treatment adjustments according to sputum eosinophil levels are associated with reductions in the rate of severe exacerbations and a decreased need for hospital admission. Sputum eosinophils >2% is a reliable marker for airway inflammation. A good-quality sputum is required.

The peripheral blood eosinophil count (BEC) is easily measured and is usually available for most patients. The BEC is a useful predictive biomarker in patients with severe asthma. Raised levels of blood eosinophils >0.3 x 109/L are associated with the early detection of exacerbation risk, loss of asthma control and lung function deterioration. It also predicts the degree of reduction in severe exacerbation rates in patients with severe eosinophilic asthma treated with ICS, and predicts response to treatment with monoclonal antibodies targeting IL-5 and the IL-5 receptor. Higher levels predict a greater response to treatment.

It is important to note that eosinophils are associated with T2 inflammation, but are not essential in the development of all atopic diseases. It is therefore a requirement to use more than one biomarker for T2 inflammation.

Fraction of exhaled nitric oxide (FeNO)

Measurement of FeNO has been used as a biomarker for T2 inflammation. The T2 inflammatory cytokines, IL-5 and IL-13 mediate production of inducible nitric oxide synthase and cause a subsequent increase in nitric oxide. In asthma, the bronchial airway is usually rich in nitric oxide. This can be measured non-invasively as a fraction in exhaled breath by a handheld device, making it a useful tool in clinical practice. Fractional excretion of nitric oxide is a reproducible and non-invasive indirect biomarker of IL-13-mediated T2-airway inflammation. Higher values of FeNO are found in T2-high compared with T2-low asthma and help confirm diagnosis of allergic asthma in adults and children. FeNO values <25 ppb (particles per billion) or <20 ppb in children <12 years old generally rule out airway eosinophilic inflammation, whereas values >50 ppb in adults or >35 ppb in children less than 12 years old are strongly suggestive of eosinophilic airway inflammation and responsiveness to inhaled corticosteroids. Normal FeNO values alone do not exclude bronchial asthma and may vary with different asthma endotypes.

FeNO can be measured with an easy and non-invasive point-of-care test to monitor airway inflammation. FeNO measurement is performed with a filter-containing a single-patient use mouthpiece, which directs air to the device sensor. FeNO levels can be used to diagnose and manage asthma (see Table 2). The utility of FeNO measurement is further expanded on in Figure 3. A low FeNO in untreated asthma indicates either T2 low asthma or an alternate diagnosis.

TABLE 2: THE USE OF FENO IN THE DIAGNOSIS AND MANAGEMENT OF ASTHMA

	Undiagnosed asthma	Diagnosed asthma
Symptomatic and FeNO ≥50 ppb (adults) or ≥35 ppb (children <12 years old)	• Suggests type 2 (eosinophilic) airway inflammation • Diagnosis of asthma probable and patient likely to benefit from ICS • Predicts reversibility of airway obstruction	• Ongoing airway inflammation • Inadequate ICS dosage or technique • Poor ICS adherence • Corticosteroid resistance • Continuous allergen exposure • Risk of exacerbation

FeNO breath analysers are now available in South Africa at selected Ampath facilities and doctors' rooms. Factors that may influence FeNO include age, gender, body weight, atopy, smoking, food, exercise, use of bronchodilators, upper respiratory viral infections, upper respiratory allergic diseases and nasal polyps, alcohol and certain drugs like asthma medications and prostaglandins. Foods that may affect FeNO measurement include nitrate-containing food like beetroot.

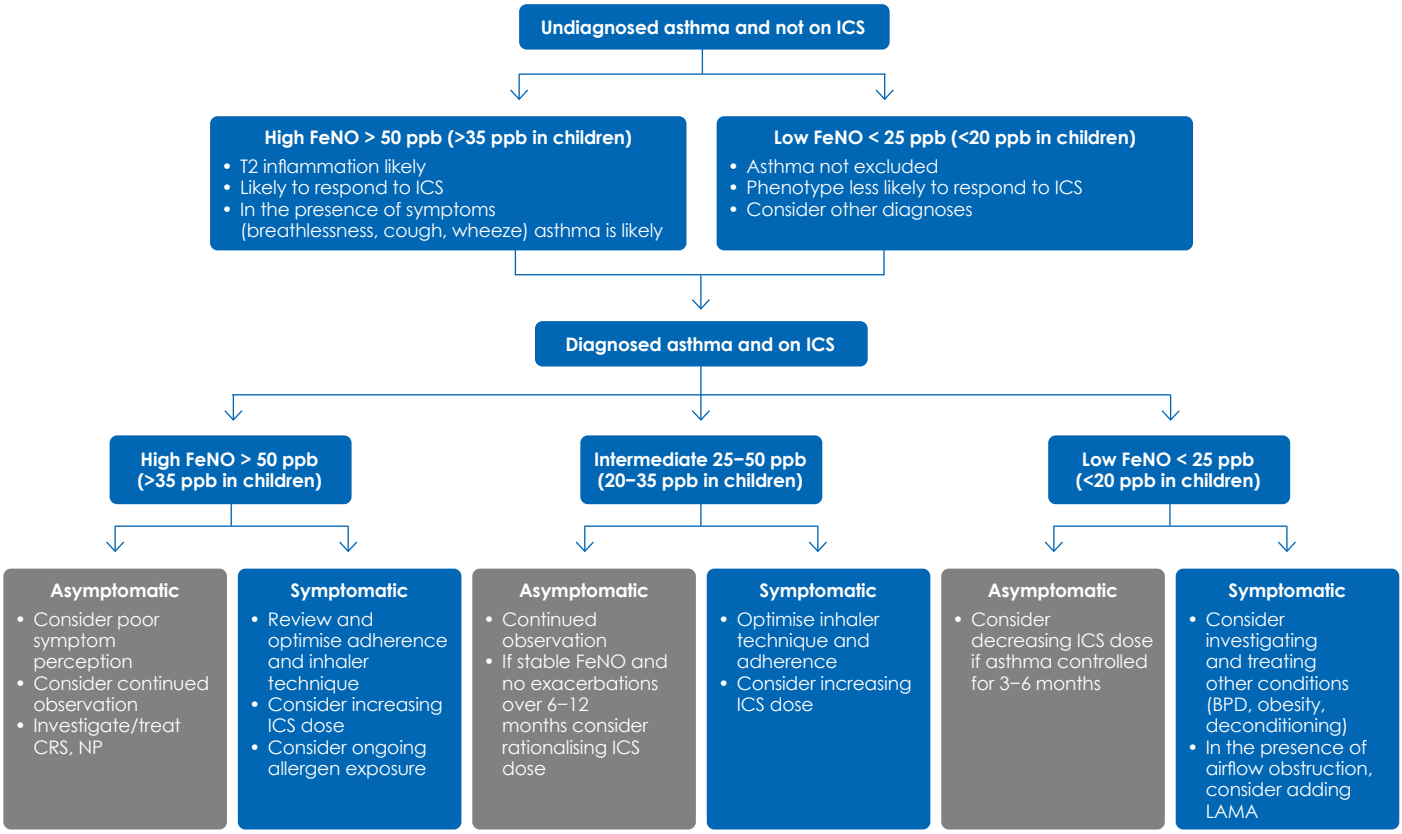

FIGURE 3: AN ALGORITHM HIGHLIGHTING THE USEFULNESS OF FENO IN MAKING THE DIAGNOSIS OF ASTHMA AND AFTER THE DIAGNOSIS OF ASTHMA HAS BEEN MADE. CUT-OFFS APPLY FOR CHILDREN <12 YEARS OLD.

BPD: Breathing pattern disorder; CRS: Chronic rhinosinusitis; FeNO: Fractional exhaled nitric oxide; ICS: Inhaled corticosteroid; LAMA: Long-acting muscarinic antagonist; NAEPPCC: National Asthma Education and Prevention Programme Coordinating Committee; NICE: National Institute for Health and Care Excellence; NP: Nasal polyposis; ppb: Parts per billion; T2: Type 2. *Adapted from: Rupani and Kent, 2022.*

The European Respiratory Society, American Thoracic Society and Global Initiative for Asthma (GINA) Guidelines suggest that the treatment of severe asthma should be guided by clinical criteria and biomarkers such as blood and/or sputum eosinophils and FeNO. Blood eosinophils and FeNO provide additive mechanistic information. FeNO reflects T2 inflammation and the chemotactic pull to the airways, whereas blood eosinophils reflect the amount of circulating IL-5 and systemic pool of available effector cells. The Oxford Asthma Attack Risk Scale (ORACLE) combines clinical risk factors, blood eosinophils and FeNO measurement to predict the risk of future asthma exacerbations (Figure 4). The prototype ORACLE shows potential to quantify the excess risk of asthma attacks in type-2 high asthma, which is removed by anti-inflammatory therapy.

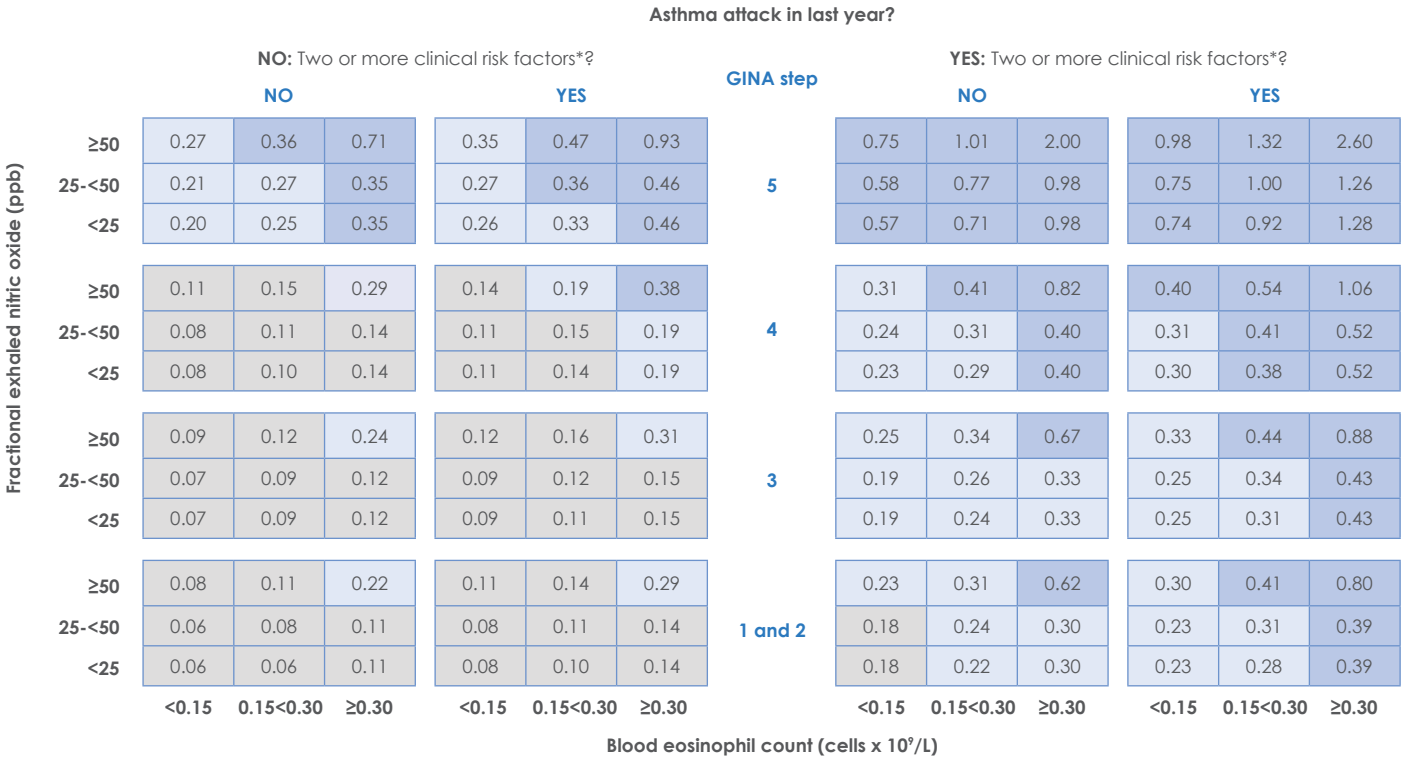

FIGURE 4: OXFORRD ASTHMA ATTACK RISK SCALE (ORACLE)

Numbers in each cell are predicted annual asthma attack rates for patients over the age of 12 if treatment is not changed. An asthma attack is an episode of acute asthma that requires treatment with systemic steroids >3 days and/or hospitalisation. The blood eosinophil count is contemporaneous or the highest result in the last 12 months. Fractional exhaled nitric oxide level is contemporaneous. Risk factors are defined by the Global Initiative for Asthma (GINA) Guidelines as: poor symptom control (asthma control questionnaire score >1.5), low lung function (forced expiratory volume in 1 second <80% predicted), adherence issues, reliever overuse (>200 dose of salbutamol cannister/month), intubation or intensive care unit admission for asthma previously, co-morbidities (one of chronic rhinosinusitis, obesity and psychiatric disease) and environmental exposures (one of smoking, allergen and pollution). GINA treatment steps: in Step 1, the patient takes a low-dose ICS whenever they take their short-acting beta2-agonist (SABA) for symptom relief; in Step 2, they take a daily low-dose ICS; in Step 3, the maintenance treatment is changed to a daily low-dose ICS-long-acting beta2-agonist (LABA); and in Steps 4 and 5, the dose of ICS-LABA is increased. *Adapted from: Couillard et al., 2022.*

Periostin

Periostin is a biomarker of T2 inflammation in adults. It is a secreted extracellular matrix protein produced by bronchial epithelial cells and fibroblasts under the stimulus of IL-4 and IL-13. Periostin is associated with airway remodelling and is therefore a marker for chronic rather than acute T2 inflammation. The levels vary in children due to bone growth.

IgE

High total IgE should not be used as a screening test for atopy or for possible type I immediate hypersensitivity reactions. Total IgE levels have some correlation with asthma severity, increased risk for loss of asthma control and severe asthma exacerbations in children and adults.

Specific IgE (sIgE) to aeroallergens has a relatively high sensitivity in asthma diagnosis and serves as a good predictor for atopy. Specific IgE is associated with more severe asthma. The number of sIgE sensitisations correlates with asthma severity and exacerbation risk in children.

The clinical suspicion of allergic sensitisation is confirmed by demonstrating the presence of allergen-specific IgE antibodies *in vivo* (skin tests) or *in vitro*.

In-vivo testing

Several types of skin tests are used in allergy diagnostics:

- Skin prick test (SPT)
- Intradermal test (IDT)

In-vitro testing

The in-vitro diagnosis of IgE-mediated, mixed or non-IgE medicated allergic diseases involves different laboratory procedures:

- Total IgE assay. This is considered non-specific and only provides generalised information.
- Serum-specific IgE assay against allergen sources/ molecules can be performed by a singleplex (ImmunoCAP®) or multiplex strategy (ALEX²®). The Phadiatop® assay (Phadia) is the most reliable laboratory test available for the screening of patients for allergy to inhaled allergens and is considered an excellent rule-out test. The test detects specific IgE to common aero-allergens in a single quantitative assay. If identification of the specific allergen/s responsible for symptoms is required, a positive Phadiatop® result should be followed by a panel of common inhalant ImmunoCAP® (specific IgE) or skin tests, or a multiplex IgE panel for multiple allergens. The ALEX²® test is a multiplex array containing IgE extracts and natural or recombinant allergen

components. Patients that will benefit the most from multiplex testing are summarised in Table 3.

- Basophil activation test (BAT)/cellular allergen stimulation tests (CAST). These tests are considered specific, but with lesser sensitivity.

TABLE 3: INDICATIONS FOR MULTIPLEX SPECIFIC IGE TESTING ALEX²®

Patients that will benefit the most from multiplex IgE testing include:

- Patients with suspected multiple allergies (polysensitised patients).
- Patients with combined food and pollen allergies.
- Patients who wish to obtain a comprehensive understanding and interpretation of their allergies.
- Multiplex assays performed early in life are useful to predict the risk of developing allergic symptoms later in life.
- When the detection of more than 12 or 13 sIgE is needed, it has been suggested that the multiplex assay may be more cost-effective than the singleplex diagnostic approach.

BIOMARKERS IN NON-T2 ASTHMA

The promoters causing severe asthma in non-T2 asthma involve neutrophils, smooth muscle and metabolic-related processes. Airway neutrophilia is facilitated by local, IL-17 mediated and systemic inflammatory pathways. Unlike the development of T2 high biomarker profiles, biomarkers for non-T2 asthma have not been adequately investigated.

Induced sputum neutrophils

The neutrophilic phenotype constitutes a proportion of non-T2 asthma. The normal range of neutrophils in induced sputum ranges between 30 and 50%. Airway neutrophilia is defined as a neutrophil percentage between 51 and 70%, although this varies with age. Causes of the neutrophilic phenotype include smoking, obesity, acute airway infections, various forms of air pollution and underlying anti-inflammatory therapies, including high-dose corticosteroid treatment.

Serum IL-6

IL-6 is a cytokine associated with neutrophilic airway inflammation and may be an indicator of asthma severity and metabolic dysfunction. making it a potential biomarker in obese asthmatic patients.

USING BIOMARKERS TO CLASSIFY SEVERE ASTHMA: AN ALGORITHMIC APPROACH

An algorithm was proposed by Zervas et al. (2018) to allocate a specific endotype. The first step is to differentiate T2 high from T2 low asthma based on certain clinical features and biomarkers. T2 high asthma is defined as the presence of atopy (SPT/specific IgE +) and/or IgE >100 IU/mL, and/or FeNO 25 ppb and/or blood eosinophil >0.3 x 10^9/L and/or sputum eosinophils >2% (see Figure 5). If a patient was classified as T2 high asthma, it is necessary to assess which endotype, i.e. allergic or eosinophilic, is predominant (see Table 4).

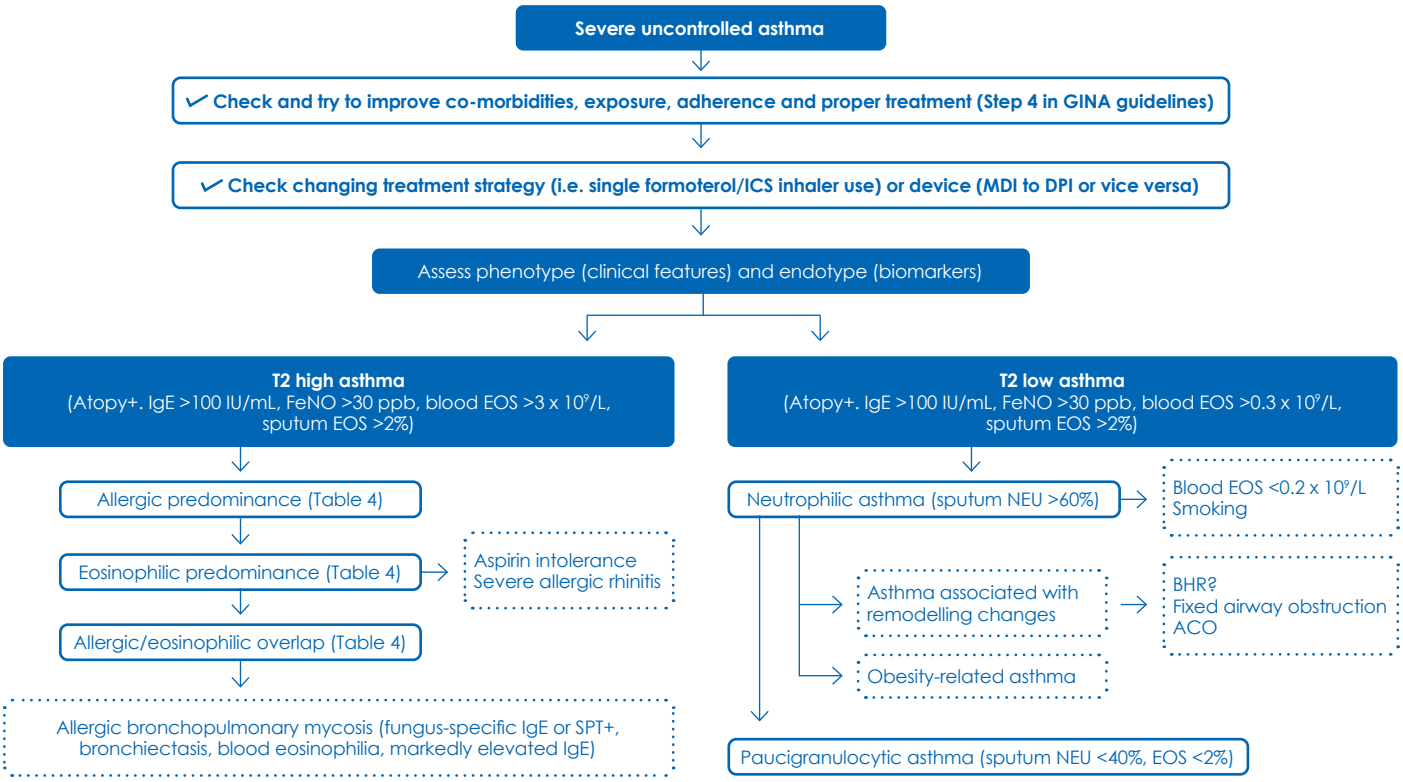

FIGURE 5: A STEPWISE THERAPEUTIC APPROACH IN SEVERE UNCONTROLLED ASTHMATIC SUBJECTS

An algorithmic approach for the treatment of severe uncontrolled asthma. ERJ Open Research. 1 January 2018; 4(1). GINA: Global Initiative for Asthma; ICS: Inhaled corticosteroid; MDI: Metered dose inhaler; DPI: Dry powder inhaler; FeNO: Exhaled nitric oxide fraction; EOS: Eosinophils: SPT: Skin prick test; NEU: Neutrophils; BHR: Bronchial hyperresponsiveness; ACO: Asthma-chronic obstructive pulmonary disease overlap. *Adapted from: Zervas et al., 2018.*

TABLE 4: SKIN PRICK TEST AND EXHALED NITRIC OXIDE FRACTION

Clinical features and biomarkers that can be used to differentiate between allergic and eosinophilic T2 high severe asthma		
	A: Allergic predominant asthma	**B: Eosinophilic-predominant asthma**
1.	Early onset	Late onset
2.	SPT/specific IgE + with clinically significant allergies	SPT/specific IgE ± with no clinically significant allergies
3.	IgE >100 IU/mL	IgE <100 IU/mL
4.	Allergic rhinitis	Nasal polyps
5.	High FeNO (30–50 ppb)	Very high FeNO (>50 ppb)
6.	Blood eosinophils <0.3 x 10^9/L	Blood eosinophils >0.3 x 10^9/L

Check the number of relevant patient characteristics per column. If a patient has more features from column A or B, it is more likely that they have allergic- or eosinophilic-predominant asthma, respectively. If the patient shares features from both columns, it is more likely that they suffer from eosinophilic/allergic overlap asthma. °: Obligatory characteristics for allergic and/or eosinophilic asthma. *Adapted from: Zervas et al., 2018.*

CONCLUSION

Asthma is a very common respiratory disorder and contributes significantly to global morbidity and mortality. A diagnosis of asthma should be suspected in patients with recurrent cough, wheeze, chest tightness and dyspnea, and should be confirmed using objective measures of lung function (spirometry preferred). Asthma represents diverse inflammatory conditions that are characterised by significant complexity and multiple genetic and environmental factors that lead to different disease phenotypes. Atopic diseases are heterogenous: different pathogenic pathways (endotypes) lead to different phenotypes. Asthma biomarkers are measurable indicators of the disease with clear cut-off values and can indicate which endotype of disease is responsible for a specific phenotype. Based on this knowledge, highly selective treatment options, including various biologics, which target specific pathways, are now available. Knowing the phenotype and endotype of a patient with asthma will enable a personalised therapeutic approach and replace the old therapeutic "one-size fits all" model with precision medicine.

REFERENCES AVAILABLE ON REQUEST

PUBLISHED MAY 2024

Contact your local Ampath representative to enquire about care centres offering FeNO testing.