Non invasive assessment of severe asthma

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SUMMARY. Many non invasive measurements are available that can help in the diagnosis, assessment and treatment of severe asthma. The fraction of exhaled nitric oxide (FeNO) helps in identification of severe asthma phenotypes, assessment of asthma control and detection of types of asthma that will benefit from treatment with corticosteroids or that will need tailored therapy with new drugs. Induced sputum examination is used mainly for distinguishing between the eosinophilic and other phenotypes, and for the monitoring of treatment. High resolution computed tomography (HRCT) of the chest helps to confirm the diagnosis of severe asthma and to detect underlying diseases, and is useful for monitoring airways remodelling. Questionnaires are used in the assessment of asthma control. Other methods, such as the electronic nose (e-nose) and exhaled breath condensate show promise of being useful. These non-invasive methods are very important in the assessment and management of severe asthma, taking into account that although asthma is generally a benign disease, severe asthma is very difficult to treat and requires constant monitoring. Invasive methods have limited utility for severe asthma monitoring since they are not suitable for repeated sampling. *Pneumon 2011, 24(4):430-444.*

INTRODUCTION

Severe asthma is a heterogeneous disease that continues to be poorly understood and frustrating to treat[1]. Patients with severe asthma consume disproportionately the health care resources incurred by asthma, partly because severe asthma comprises a part of asthma that is still not well understood and is difficult to manage.

The approach to severe asthma should have at least three components: (a) confirmation that the disease is definitely asthma, (b) evaluation of confounding/exacerbating factors, and (c) evaluation of the asthma phenotype, as in severe asthma a variety of totally different phenotypes merge[1]. This review of the non-invasive tools used in the diagnosis and assessment of asthma delineates the settings and the ways in which they can help in the understanding and implementation of these three components in the investigation of severe asthma, regardless of their limits and confounding

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factors. Their contribution to the treatment and monitoring of severe asthma is also covered.

NON-INVASIVE ASSESSMENT OF ASTHMA PHENOTYPES

Asthma is a complex of multiple, separate syndromes that overlap, and thus identification of the asthma phenotypes represents a major challenge in the current management of asthma. This represents an urgent need in the case of patients with persistent and difficult-to-treat disease, where the phenotypic diversity is greater[2]. This heterogeneity is expressed in the variety of different underlying disease mechanisms and treatment responses in severe asthma. Airway inflammation is a key component of severe asthma and its pattern is very heterogeneous, which is why inflammation-guided management is more effective in patients with severe asthma. In this group, the standard recommended treatment appears not always to lead to satisfactory asthma control, and an individualized approach, tailored to the separate phenotypes, is needed. Non-invasive measurement of airway inflammation facilitates the effective investigation of this diversity, enabling appropriate targeting of treatment.

A range of sampling procedures and biomarkers is available for the non-invasive assessment of airway inflammation in severe asthma. Biomarkers of inflammation in severe asthma can be sampled in a variety of biological specimens, including induced sputum, exhaled breath, peripheral blood and urine, using a variety of sampling procedures[3]. Each of these procedures has distinct performance characteristics in terms of ease of collection, reproducibility, safety, and cost of collection and measurement. Non-invasive methods for assessing airway inflammation in severe asthma are actually the only methods that can be used in everyday clinical practice, since the invasive techniques, such as bronchial biopsy or bronchoalveolar lavage (BAL), are not suitable for repeated sampling, and thus not practical for monitoring. Non-invasive methods for the assessment of inflammation in patients with severe asthma have been developed to address this need. These measurements involve the assessment of cells and mediators in body fluids, and quantification of exhaled gases such as nitric oxide (NO), with the exhaled NO fraction (FeNO), and volatile organic compounds (VOCs) in the exhaled breath condensate (EBC), measured using the electronic nose (e-nose). Techniques of non-invasive assessment of severe asthma analysed in this review include the following:

1) Fraction of exhaled nitric oxide (FeNO).
2) Induced sputum analysis
3) Exhaled breath condensate (EBC).
4) Electronic nose (e-Nose).
5) High resolution computed tomography (HRCT)
6) Questionnaires

As illustrated in Table 1 in synopsis, these procedures can help in all aspects of the study of severe asthma.

The use of these non invasive parameters is based on documentation of clinical and research evidence of their relationship with severe asthma.

FRACTION OF EXHALED NITRIC OXIDE (FENO)

FeNO is the most extensively studied of the exhaled gas concentrations that are used for the assessment of airway inflammation[4,5] . Measurement of FeNO has been found to be highly reproducible, well-tolerated, safe, relatively quick and simple to perform, and the necessary equipment is becoming more affordable[3,6].

The evidence supporting the rationale behind the concept of using FeNO as a guide in the diagnosis and management of asthma include: (a) Increased FeNO in patients with asthma is highly correlated with eosinophilic airway inflammation, (b) The use of inhaled corti-

ΠΙΝΑΚΑΣ 1. Non invasive evaluation of severe asthma

	Confirmation of diagnosis	Evaluation of confounding/ exacerbating factors	Evaluation of asthma phenotype	Assessment of treatment	Asthma Control
Chest HRCT scan	+	+			
Questionnaires			+	+	+
Induced sputum analysis	+	+	+	+	+
FeNO	+		+	+	+

HRCT = high resolution computed tomography, FeNO = fraction of exhaled nitric oxide

costeroids (ICS) in asthma results in a fall in FeNO, and a dose response relationship is observed between ICS and FeNO, (c) Raised FeNO predicts steroid responsiveness in patients with non-specific respiratory symptoms[6]. Thus, monitoring of FeNO may also guide asthma treatment in clinical practice.

FeNO in the identification of asthma phenotypes

As emphasised above the identification of phenotypes represents a major challenge in persistent and difficult-to-treat asthma[2]. There is no standardized method, however, for defining asthma phenotypes[7]. The most recent main studies relating FeNO to the identification of asthma phenotypes are reviewed below.

Schleich and co-workers showed in a retrospective study that FeNO values of ≥41 ppb were able to identify the presence of sputum eosinophilia (≥3%), with reasonable accuracy (sensitivity 65% and specificity 79%)[8]. The threshold for the identification of the eosinophilic phenotype varies according the dose of ICS, atopy and current smoking[8]. In a recent cross-sectional study, Tseliou and colleauges demonstrated that FeNO levels of >19 ppb were associated with a sensitivity of 0.78 and a specificity of 0.73 for sputum eosinophilia, while FeNO levels of <19 ppb were associated with a sensitivity of 0.63 and specificity of 0.90 for sputum neutrophilia, irrespective of the presence of eosinophils[9]. Thus, in patients with severe refractory asthma, FeNO threshold values can identify those with predominant eosinophilia or neutrophilia. These two studies have provided strong evidence for the use of FeNO for the prediction of sputum cell counts in patients with asthma.

In a population of patients with severe refractory asthma, Silkoff and co-workers used FeNO measurements to identify the persistent eosinophilic phenotype [10]. FeNO values of >72.9 ppb were associated with a sensitivity of 0.56 and a specificity of 1.0 for the identification of tissue eosinophilia, regardless of steroid therapy [10]; thus, a subgroup of patients with severe refractory asthma with persistent eosinophilia was identified by FeNO measurements, despite steroid therapy. Further studies will be needed on the use of FeNO in monitoring response to treatment over time in subjects with severe refractory asthma. Van Veen and colleagues investigated in 98 patients with severe asthma the predictive value of inflammatory markers, namely exhaled NO, blood and sputum eosinophils and bronchial hyperresponsiveness (BHR) for the decline in forced expiratory volume in 1 second (FEV_1), over 5 years[11]. The results of this study showed that patients with high FeNO levels (>20ppb) and normal lung function at baseline (FEV_1, ≥80% predicted) had a 90% risk of having an accelerated decline in lung function (225 ml/year), compared to 30% in those with FeNO levels of <20 ppb at baseline. The study demonstrates that FeNO measurements can help to identify patients with severe asthma who are at risk of developing persistent airflow limitation and who might benefit from novel asthma treatment or individualized treatment strategies[11].

In study reported in 2010 by the Severe Asthma Research Program (SARP) of the National Heart, Lung and Blood Institute (NHLBI), 5 different phenotypes of severe asthma, as defined by the American Thoracic Society (ATS), were distinguished, using cluster analysis. The FeNO did not differ among the 5 identified clusters, in contrast to sputum eosinophils and neutrophils [12]. Focusing on FeNO in the same SARP cohort, Dweik and co-workers showed that neither FeNO levels nor the proportion of patients with increased FeNO values (i.e. >35 ppb) differed between patients with non-severe and severe asthma, despite the higher doses of corticosteroids being taken by the latter[13]. High FeNO values in patients with severe asthma identified those patients with more severe airflow obstruction, BHR and hyperinflation, and were associated with the most frequent use of emergency care, in a retrospective analysis, possibly identifying patients with more severe disease in clinical practice [13]. The researchers concluded that grouping of asthma by FeNO provides an independent classification of asthma severity, and that among patients with severe asthma this grouping identifies the most reactive and troublesome asthma phenotype.

The value of measuring FeNO in patients with severe asthma and the way these values should be incorporated in the clinical work-up and guidance of these patients is not yet completely clear. High FeNO levels persist in subgroups of patients with severe asthma who are on high doses of oral corticosteroids or ICS, which might be due to relative steroid resistance, persistent systemic eosinophilic inflammation or continued inflammation in regions of the airways which are not effectively reached by ICS, such as the nasosinal region or the peripheral airways.

FeNO and asthma control, prediction of steroid responsiveness, steroid reduction and exacerbations

The management of severe asthma requires careful and ongoing evaluation in order to assess the state of the disease, its response to treatment and possible

complications. As is well known, the Global Initiative for Asthma (GINA) has proposed a new approach to asthma management, with asthma control being the focus of treatment decisions[14]. It is also known that patients with severe disease are those in whom asthma is less well controlled, and thus an individualizedapproach, tailored to separate phenotypes is needed. The role of FeNO in the management of severe asthma in patients with an established diagnosis of asthma has been evaluated in numerous studies.

An early study on a small population provided evidence that eosinophilic airway inflammation, as evaluated by induced sputum examination and FeNO, may precede the onset of symptoms, and thus the loss of asthma control, during steroid reduction[15]. More than 5 years ago, based on a placebo-controlled study, Smith and co-workerssuggested that FeNO values of >47 ppb are a robust predictor of responsiveness to corticosteroids in patients with undiagnosed respiratory symptoms, independent of the diagnostic label[16]. In a more recent study that implemented steroid withdrawal for the identification of eosinophilic and non-eosinophilic asthma, Cowan and colleagues showed that FeNO is a predictor of steroid response in both types of asthma, despite the absence of eosinophilia in the non-eosinophilic form, providing a complementary role for FeNO in that setting[17].

Prospective studies in paediatric populations have suggested that high FeNO values may also predict loss of asthma control during steroid withdrawal[18,19]; with similar or even better performance than induced sputum eosinophil counts[19]. This was not the case in adults, in whom FeNO was not predictive of loss of asthma control, in contrast to increased sputum eosinophil counts[20,21]. It appears, therefore, that in the adult setting, FeNO may not be as useful as sputum eosinophils for monitoring "safe" steroid reduction. Several studies have evaluated FeNO in relation to prediction of loss of asthma control and subsequent exacerbations. In a cross-sectional study of 174 adults with asthma, Lopes and colleagues implemented principal components factor analysis to show that FeNO, the Asthma Control Questionnaire (ACQ) and FEV_1 may have complementary roles in the evaluation of asthma control [22]. In a cross-sectional study of 134 patients with asthma from a tertiary hospital asthma clinic, FeNO was not found associated with uncontrolled asthma, as evaluated by ACQ scores ≥1.5, in contrast to sputum eosinophils and methacholine airway hyper- responsiveness (AHR) [23]. In contrast, in a prospective study with post-hoc analysis, Michils and co-workers showed that a single measurement of FeNO of >45 ppb is related to poor asthma control, with a negative predictive value (NPV) of 88%[24]; this relationship was more marked in steroid-nave patients, and blunted in patients receiving high doses of ICS[24]. In the same study, a reduction in FeNO by 40% was associated with optimization of asthma control (NPV 79%), whereas an increase of 30% was associated with loss of control (NPV 82%), but this was blunted in patients receiving high doses of ICS[24]. The same authors subsequently showed that in people with asthma who were smokers a decrease in FeNO of >20% in two consecutive measurements precluded of asthma control improvement (NPV 72%), while an increase in FeNO <30% was unlikely to be associated with deterioration in asthma control (NPV 84%) [25]. The ability of FeNO to predict changes in asthma control was lost in patients receiving high doses of ICS. In a more recent study, Papaioannou and co-workers, an increase in FeNO >30% was highly predictive of loss of control, with a positive predictive value (PPV) of 89%, while an increase of <20% was unlikely to be associated with loss of control (NPV 81%)[26]. In addition, in patients with coexisting allergic rhinitis, which is another significant confounder in the evaluation of FeNO, an increase in FeNO levels >40% from baseline was highly indicative of loss of asthma control (PPV 92%)[26]. These data suggest that serial measurements of FeNO may have an important role in the evaluation of asthma control, in contrast to single measurements, that are of limited usefulness in the general population.

An earlier study had shown that increased FeNO levels predict the development of asthma exacerbations in the two weeks following the initial evaluation[27]. Subsequently, Gelb and co-workers showed, in a population of 44 patients with asthma followed for 3 years that those with FeNO values ≥28 ppb had a relative risk of 3.4 for a subsequent first exacerbation (PPV 77%, NPV 87%)[28]. These results provided evidence of the complementary roles of FeNO and FEV_1 in the stratification of patients at risk for subsequent asthma exacerbations[28]. In a recent study of Pérez-de-Llano and co-workers study, 102 patients with suboptimal asthma control underwent a stepwise increase in their asthma treatment consisting of a combination of high-dose ICS and a long-acting β_2-agonist (LABA) for one month. The patients were asked to return one month after the increase in medication[29], at which time those whose asthma remained uncontrolled received additional oral corticosteroids for another month, followed by a final examination one month later. Of the patients in this study, 48% did not achieve control, despite receiving the best

available treatment and optimal management efforts. The addition of oral prednisolone led to a modest 7% increase in the percentage of well-controlled asthma. In the study, FeNO was an excellent marker for predicting therapeutic response; with a cut-off value of 30 ppb for FeNO, the test showed sensitivity of 87.5% and specificity of 90.6% for the identification of the patients who would benefit from the stepwise treatment strategy to achieve control. The authors conclude that this biomarker can not only identify patients with difficult-to-treat asthma, but also predict those who will respond to steroids (maximal step-up therapy with inhaled, and subsequently oral), and thus achieve optimal asthma control, proving its clinical utility. Based on these findings, FeNO appears to have a role in the identification of patients who may benefit from intense treatment with corticosteroids, possibly providing an opportunity for minimization of steroid-related adverse effects and unnecessary drug costs in non-responders[7,30].

In conclusion, FeNO levels are similar in severe and non-severe asthma[13,31-34] indicating that FeNO cannot be used to detect severe asthma, although it may have a role in defining subtypes of severe asthma, as described above[3].

2. INDUCED SPUTUM

Sputum induction is a semi-invasive method, less invasive than bronchoscopy with BAL and biopsy. It has been accepted in the non-invasive category of methods and isused to determine the characteristics and intensity of the lower airway inflammatory response in asthma. It has notable advantages over exhaled NO. The main limitation of induced sputum is that results are not available immediately and that the specimens need to be examined in a specialized laboratory. Sputum processing provides a differential cell count which enumerates the eosinophils and neutrophils.

It should be remember that the induced sputum procedure carries risks, especially in severe asthma with low FEV_1. Inhalation challenges may cause excessive bronchoconstriction with subsequent suffocation, particularly in patients with severe asthma. As sputum induction entails repeated inhalations with hypertonic saline, strict precautions have to be taken when performing these procedures. Factors affecting safety during induced sputum testing are listed in Table 2.

A low FEV_1 is a relative contraindication to performing sputum induction in patients with asthma. There is no guarantee of a safe procedure in any patient, as sudden and severe bronchospasm may occasionally develop during sputum induction even in those with normal lung function and after pre-treatment with a β_2-agonist[36]. The precise cause of this excessive bronchoconstriction is not known, but widespread mast cell degranulation and extensive stimulation of afferent nerve endings have been postulated. Poor asthma control, in particular if associated with overuse of β_2-agonists during the weeks before sputum induction has been shown to be a risk factor[37,38]. Overuse of short-acting β_2-agonists has already been suggested as potential predictor of excessive airway narrowing in patients with exacerbations of asthma[39].

TABLE 2. Factors affecting safety during sputum induction

1. Airflow limitation before induction
2. Degree of asthma control
3. Previous (over)use of short-acting bronchodilators
4. Pre-treatment with short acting β-2 agonists,
5. Concentration of the saline solution
6. Nebuliser output
7. Duration of saline inhalation
8. Frequency of lung function measurements during the procedure

The key points of a 2002 European Respiratory Society (ERS) taskforce, which reviewed and summarized to the ways of significantly attenuating the risk of excessive bronchoconstriction[40] are: (a) apply standard operating procedures, including details of safety and hygiene precautions; (b) be aware of the degree of asthma severity and current clinical stability of all volunteers; (c) premedicate with 200 mg salbutamol; (d) record the pre- and post-bronchodilator FEV_1; (e) monitor airflow regularly during induction, and (f) always stop if FEV_1 falls >20% from post bronchodilator baseline value; (g) use a modified protocol for subjects with severe asthma[37]. The ERS taskforce recommends starting with 0.9% NaCl sterile saline solution, performing induction for 30 sec, 1 min, and 5 min (FEV_1 after each period). If this is unsuccessful, an increase to 3%, and induction for 30 sec, 1 min, and 2 min is recommended, and if there is still no success, further increase to 4.5% and induction for 30 sec, 1 min, 2 min, 4 min and 8 min. Delvaux and colleagues found that the addition of 400μg inhaled salbutamol through an ultrasonic nebuliser markedly improves bronchoprotection against saline induced bronchoconstriction in patients with moderate to severe asthma undergoing sputum induction, without affecting cell counts and inflammatory markers[41]. It is recommended, however, to keep the nebulisation and

pre-treatment protocol consistent from subject to subject and over repeated challenges in the same individual.

Although it is not recommended though to use a spontaneous sputum sample in research studies because of lower cell viability and poorer quality of samples (and preparations) compared to induced sputum[42] it could be used for high-risk patients with severe asthma, who often produce spontaneous sputum. As the inflammatory cell profile and mediators in spontaneous and induced sputum are similar, if the patient is not able to undergo sputum induction, the findings in spontaneous samples can be used in the analysis[42].

In conclusion, sputum induction can be safely conducted even in severe asthma if done very carefully. Sample analysis and interpretation, however, may be difficult.

Phenotyping severe asthma by sputum cell counts[125]

The eosinophilic phenotype

Sputum eosinophilia is a feature of asthma, and some studies have reported a significantly greater increase in sputum eosinophil numbers in severe asthma than in moderate asthma[43-45]. One-half to two thirds of patients with severe asthma have persistent eosinophils in the large airway tissues, despite continued high-dose systemic and inhaled steroids[1]. The presence of eosinophils may represent a subtype of severe asthma characterized by a higher level of active symptoms, lower FEV_1, and a greater likelihood of exacerbations and near-fatal events, than subtypes without eosinophils[1]. Recently the phenotypes of severe asthma have been explored using cluster analysis[6,46]. This analysis identified two clusters unique to refractory asthma, according to the criteria of the American Thoracic Society (ATS)[74], which were characterized by marked discrepancy between the day-to-day clinical expression of the disease and eosinophilic airway inflammation.

Other studies however, do not show that sputum eosinophilia is a distinguishing feature between severe and non severe asthma[3,31,46,47]. This lack of consistency in research findings may be explained by the following:

a) Severe asthma consists of many heterogenic phenotypes.

Different definitions of severe asthma are used in different studies [i.e., ATS Workshop on Refractory Asthma, Global Initiative for Asthma (GINA)].

The sputum eosinophil count is influenced by smoking and by adherence to corticosteroid treatment. This is why studies that include ex-smokers, or that directly assess adherence and exclude non-adherent patients, do not show that an elevated eosinophil percentage is specific for severe asthma[31,48].

Recent evidence suggests that the macrophage colour on stained sputum slides reflects the eosinophil load, which is increased in severe asthma, even in subjects without current sputum eosinophilia[49].

The neutrophilic phenotype

In some cases, when there is an absence of eosinophils, there may be an increase in neutrophils. Neutrophilia is not always exclusive for the absence of eosinophils, and the two cell types may be present concomitantly [1]. An increase in sputum neutrophils is frequently observed as a distinguishing feature of severe asthma[56-60], and has been seen in patients with severe/difficult asthma on high doses of inhaled/oral steroids[60,61]. The mechanisms of this neutrophilic inflammation are not clear, nor are the clinical implications, but the possible explanations could be summarized in the following:

a) Corticosteroid therapy increases neutrophils. Patients with severe asthma require treatment with high doses of ICS or oral corticosteroid, either as daily maintenance therapy or in frequent bursts for exacerbations. Neutrophils may thus be the only "residual" indication of inflammation, with the steroids having effectively reduced the eosinophils[63]. It is also known that steroids suppress neutrophil apoptosis[63], so the treatment of severe asthma itself may increase the numbers of neutrophils[63].

b) The mechanism of sputum neutrophilia in severe asthma involves the release of the potent neutrophil chemokine, interleukin-8 (IL-8)[56-58], as along with increased recruitment of activated neutrophils from the blood[64].

c) Neutrophilic inflammation may be an expression of a different disease, such as bronchiolitis obliterans (BO) [62].

Airway neutrophils in severe asthma are activated and release increased amounts of myeloperoxidase (MPO) and leukotriene (LT) B4[58].

Studies have been conducted to find out which inflammatory markers are specific to severe asthma. In addition to the changes in mediators found as part of the cellular inflammation in severe asthma, such as in IL-8 and LTB4, a range of mediator changes indicate activation of other biological systems in severe asthma. In addition, the levels of osteopontin (OPN) in the sputum supernatant of patients have been found to be higher in those with severe asthma than those with less severe forms of the disease[76]. OPN is associated with mediators involved in both the inflammatory and remodelling processes, such

as TGF-β1, IL-13 and cysteinyl LT, only in severe refractory asthma. None of the soluble markers have been systematically evaluated for their prediction of treatment response or future risk in severe asthma.

Tailored treatment for severe asthma based on phenotyping

Patients with severe asthma need to be treated with corticosteroids. Dose titration traditionally is effected by assessing the clinical response to treatment and attempting to define the lowest dose of ICS that maintains control. Although this approach is effective for many patients, there is evidence that the use of the induced sputum eosinophil count to titrate treatment results in a lower exacerbation rate with no overall increase in medication[6,65]. The benefits of sputum eosinophil directed management are more clearly seen in patients with severe asthma[6]. Traditional symptom guided management may lead to over- or undertreatment of the severe asthma phenotypes, leading to a poor outcome. Retrospective analysis of an earlier study[6,65] showed that inflammation guided asthma management enabled identification of these phenotypes and facilitated appropriate targeting of treatment. In the inflammation predominant cluster, the main benefit of this approach was a reduction in severe asthma exacerbations while in the symptom-predominant cluster excess corticosteroid treatment was avoided[6]. At least 50% of patients with severe asthma, however, have very little identifiable inflammation, and for them this approach is not feasible.

The clinical subtypes of severe asthma include: frequent severe exacerbations, poor control, incompletely reversible airway obstruction (IRAO), and asthma with near-fatal attacks (brittle asthma). Non-invasive markers have been related to certain of these subtypes[3].

Sputum eosinophilia in severe asthma is associated with a clinically favourable short-term response to ICS[50-52,65]. The step-up in the corticosteroid treatment regime can be from high-dose inhaled to oral[50], or from oral to parenteral, corticosteroid[3], but airway eosinophilia in severe asthma can be refractory to corticosteroids. The mechanism of this lack of response to corticosteroids appears to be related to persistent IL-5 secretion. IL-5 is a potent cytokine that promotes growth, differentiation and activation of eosinophils and also inhibits eosinophil apoptosis. Absence of sputum eosinophilia thus indicates that the response to corticosteroids will be absent or of low magnitude[50], and the eosinophil load as determined by macrophage colour is associated with successful corticosteroid withdrawal [49]. There is also evidence of suppressed eosinophil apoptosis in more severe forms of asthma[53].

Identification of the different inflammatory phenotypes of asthma may be particularly important in relation to the development of new highly selective immunomodulatory agents for the treatment of severe asthma where traditional treatment is insufficient. Mepolizumab, a monoclonal antibody to IL-5, is an effective inhibitor of eosinophilic airway inflammation. Its preclinical development was delayed after disappointing findings on its effects on asthma symptoms and lung function. This lack of evidence was subsequently found to be due to the selection criteria of study patients, which were based on physiological findings rather than the presence of eosinophilic airway inflammation, and to the choice of outcome measures not closely linked to eosinophilic airway inflammation. More recent trials in patients selected by eosinophilic inflammatory phenotype showed much more encouraging results from the use of mepolizumab against asthma exacerbations. It is apparent that eosinophilia in severe asthma can be suppressed by specific monoclonal anti-IL-5 therapy, indicating that IL-5 is related to severe asthma with refractory eosinophilia, and such therapy is also associated with significant reduction in severe asthma exacerbations[66,67] in the eosinophilic severe asthma phenotype identified by Haldar and colleagues [67]. There is also evidence for a role of the eosinophil-active chemokine, eotaxin, in severe asthma with eosinophilia[54]. Studies have not yet systematically assessed IL-5 and eotaxin levels in induced sputum samples in severe asthma to determine whether or not they might be useful markers of eosinophilic disease of refractory eosinophilic asthma. There are certain technical difficulties that need to be overcome in the assay of these cytokines using induced sputum supernatant[55].

The degree of sputum eosinophilia has been linked to exacerbation frequency in severe asthma (Table 3)[68,46]. Elevated FeNO does not appear to show the same relationship to exacerbation risk[69]. Irreversible airflow obstruction can develop in severe asthma, and may be detected in up to 50% of cases[48]. This IRAO subtype of severe asthma is variably related to FeNO[69,48] and sputum eosinophil numbers, being not seen in some studies[69], but reported in others[73,11]. Anti-IL-5 therapy for refractory eosinophilic asthma led to a significant improvement in FEV_1, indicating that eosinophilia is linked to airflow obstruction in the eosinophil subtype of severe asthma[66]. The mixed granulocytic pattern appears to be more common in severe asthma with IRAO[47,48]. Search has been made for mediator profiles that might distinguish severe asthma

TABLE 3. Relationship between severe asthma clinical subtypes and inflammatory biomarkers

Clinical subtype	Inflammatory biomarker
Poor asthma control	Eosinophilia (IS)
Exacerbations	Eosinophilia (IS)
Incompletely reversible airway obstruction	Eosinophils, neutrophils, mixed granulocytic pattern (IS), oncostatin M (IS)
Near-fatal (brittle) asthma	None identified

IS: Induced sputum

Adapted from Gibson et al, Noninvasive assessment of inflammation in severe asthma European Respiratory Society Monograph 2011; 51 Chapter 16, page 212

with IRAO[47,48,72].

Most granulocyte activation markers and inflammatory cytokines did not identify the group with IRAO[48]. Simpson and colleagues[72] reported that levels of the cytokine oncostatin M (OSM) were significantly elevated in asthma with IRAO. This is an important observation, since bronchial biopsy studies have indicated the importance of airway wall remodelling[31,48] and neutrophilic inflammation in severe asthma. OSM is a pleiotropic cytokine with both pro-inflammatory and profibrotic actions that might link the inflammation and remodelling pathways in severe asthma. The levels of induced sputum markers were not found to be different in the near-fatal subtype of severe asthma[75].

Treatments directed at airway smooth muscle hypertrophy, such as bronchial thermoplasty (BT), may be particularly helpful in the non-eosinophilic cluster.

Simpson and colleagues have shown that long-term treatment with clarithromycin in patients with refractory asthma was particularly beneficial in patients with the non-eosinophilic phenotype in whom macrolide therapy was effective in reducing sputum neutrophil numbers and improving quality of life (QoL) in severe asthma (Table 4)[70,71].

In conclusion, in severe asthma when the recommended treatment does not achieve satisfactory control, an individualized approach, tailored according to the separate phenotypes, is needed.

3. EXHALED BREATH CONDENSATE (EBC)[3]

In severe asthma, acidification of exhaled breath can be detected by a low EBC pH[9]. In addition, the levels of a range of other markers are reported to be elevated in the EBC of patients with severe asthma and poor control, including RANTES[77] and endothelin-1[78]. There is need for studies of EBC biomarker reproducibility, both within individual studies and in replication of results across centres.

4. ELECTRONIC NOSE (e-NOSE)[3]

The pattern of exhaled VOCs can be assessed using the e-nose[68,69]. The technique involves recognition of a pattern of exhaled VOCs, detected using a biological sensor and subjected to integrative analysis to yield a pattern, often termed a smell-print. Although its use has not yet been evaluated in severe asthma, this promising technology is undergoing further investigation. The technique is non-invasive and safe, but its reproducibility and utility in severe asthma are not yet established.

5. HIGH RESOLUTION COMPUTED

TABLE 4. Inflammatory biomarkers in severe asthma

Biomarker	Sample	Specificity for Severe Asthma	Studies (N)
Eosinophils	Induced Sputum	Possibly	Multiple
Neutrophils	Induced Sputum	Possibly	Multiple
FeNO	Exhaled Breath	No	Multiple
8-Isoprostane	Induced Sputum	Yes	1
	Exhaled breath	Yes	1
pH	Exhaled Breath	Possibly	Multiple
IL-8	Induced Sputum	Yes	Multiple

FeNO: exhaled nitric oxide fraction.

Adapted from Gibson et al. Noninvasive assessment of inflammation in severe asthma European Respiratory Society Monograph 2011; 51 Chapter 16, page 212

TOMOGRAPHY (HRCT).

Typical structural changes in the airways of asthma patients, known as "airway remodelling", may lead to relatively irreversible airway narrowing, and are related to the severity of the disease. Airway remodelling is characterized by increase in airway smooth muscle mass, due to hypertrophy and hyperplasia, mucous gland hyperplasia and mucus hypersecretion, and increased vasculature and thickening of the reticular basement membrane (RBM), leading to airway wall thickening and narrowing of the lumen[14,80]. Study of airway remodelling requires biopsies, preferably from open surgical procedures. This would be extremely difficult to apply routinely in patients with asthma, and much of the histological evidence is derived from autopsies. Chest HRCT has been utilized in the study of structural lung alterations in patients with asthma over the last 20 years[81].

The correlation of asthma severity and the extent of permanent abnormalities detected on HRCT of the lungs of patients with both atopic and non-atopic asthma were among the first observations[82]. Investigators were able to confirm these initial findings objectively by measuring the ratio of airway wall thickness to outer diameter (T/D) and the percentage wall area (WA %), defined as wall area/total airway area x 100. According to their findings, all groups of patients with asthma (mild, moderate and end stage) have greater airway wall thickening than normal (control) subjects, but patients with more severe asthma have a greater degree of airway wall thickening than those with mild asthma[83]. Later investigators confirmed that permanent alterations in lung architecture correlate with asthma severity and duration. Some patients with asthma present permanent changes with milder forms of the disease and at earlier stages, and patients with reversible lung function impairment may have irreversible changes in the lung architecture[87]. Little and co-workers studied the association of airway wall thickness in asthma, via measurements of T/D and WA% in airways with a diameter >1.5mm, with lung function and asthma severity. They confirmed the positive association of asthma severity and airway wall thickness, and showed an inverse association of airway wall thickness with carbon monoxide (CO) gas transfer, but found no correlation with FEV_1 or airway inflammation as assessed by induced sputum examination and exhaled NO measurement[93]. Park and co-workers confirmed that the bronchial wall thickness in asthma is not correlated with the clinical features, lung function or AHR.

HRCT evidence of other conditions, such as bronchiectasis, a mosaic pattern and emphysema, were more common in patients with a prolonged history of asthma and FEV_1 <80%[86]. Patients with asthma usually have thickened airway walls, plugged large and small airways, subsegmental atelectasis, and air trapping, but emphysema is rarely seen, even in the more severe cases[84]. Lee and colleagues point out the significance of the HRCT finding of prominence of centrilobular structures in patients with near fatal asthma (NFA), which is more marked than in moderate to severe asthma with similar FEV_1 and degrees of bronchial thickness[90].

A recent interesting finding is the correlation of inflammatory markers with the magnitude of the permanent architectural distortion in the lungs of patients with asthma. Sputum elastase, metalloproteinase (MMP)-9, tissue-inhibitor metalloproteinase (TIMP)-1 and persistent neutrophilic airway inflammation are associated with the magnitude of abnormalities of the airways demonstrated on HRCT scan[88,89]. Vignola and colleagues assessed induced sputum in 30 patients with asthma, 16 of whom were suffering from severe asthma. They found that HRCT scan abnormalities, including bronchiectasis, thickness of peripheral bronchi, emphysema and peripheral linear hyperdensities, were correlated with the degree of airway obstruction and with sputum elastase in severe asthma and with the sputum MMP-9 /TIMP-1 ratio in both mild and severe asthma[88]. Gupta and co-workers measured the dimensions of the right upper lobe apical segmental bronchus (RB1) and the clinical and sputum inflammatory characteristics in 99 patients with severe asthma and 16 healthy control subjects, and found that RB1 WA% was best associated with airflow obstruction and neutrophilic inflammation of the airways in severe asthma[89]. The conflict between these findings and those of other investigators [94] may be due to various methological differences and to the variability and diversity of asthma and lung remodelling. It is clear that further prospective and interventional studies are needed in this field.

Lee and colleagues showed partial reversion of the structural abnormalites on HRCT scan after treatment with ICS[90]. Bumbacea and co-workers showed that patients with severe asthma and fixed airflow obstruction (post dilator FEV_1 <50%) had more HRCT airway abnormalities than patients with reversible airflow obstruction (post dilator FEV_1 >80%), despite being on similar treatment and experiencing equivalent impairment in QoL[91].

Such findings lead to the conclusion that HRCT is constructive in establishing correlations of severe asthma in the research setting. It can be used to in order to correlate

severe asthma and air wall thickening (remodelling)[83,89,93] and to demonstrate the efficacy of medication in structural restoration[90], although the clinical implications of such findings have not yet been validated[93].

All patients with severe asthma need high dosage of ICS or systemic steroids[14,92], but before they are placed on such treatment their diagnosis should be confirmed. The use of HRCT for the investigation of asthma is of great value, and it is even more essential in severe cases[84,85.] Some conditions that can masquerade asthma can easily be differentiated by HRCT scan[84], which can reveal many disorders of lung architecture, such as panacinar emphysema in α_1 antitrypsin deficiency, cysts in lymphangioleiomyomatosis, and central bronchiectasis in ABPA that could result in an obstructive pulmonary picture in young patients. It can also detect such features as air trapping or small nodules that could lead to the diagnosis of small airways disorders, such as BO[84]. Some of the findings of BO (e.g., mosaic pattern) are also observed rarely in asthma (8%)[92], which makes differential diagnosis difficult, especially, in younger patients and children[92-94]. Some patients with severe asthma have changes on HRCT scan that cannot be convincingly discriminated from those of BO. Because the clinical definition of asthma is primarily "physiological," several diseases, including BO, may meet the criteria for asthma, although the pathology of these diseases may vary substantially from that which is classically representative of asthma.

Of the patients attending a "difficult" asthma clinic, 80% had abnormal HRCT findings, including airway wall thickening (62%), bronchiectasis (40%), and emphysema (8%). Neither clinical suspicion nor measurements of airflow limitation could reliably predict the significant bronchial wall changes, and for that reason Gupta and co-workers recommend that all patients with severe asthma should undergo HRCT chest scan[85], while other investigators conclude that HRCT is most useful in the investigation of small airways disease[84]. Severe asthma with permanent obstruction may be difficult to differentiate from chronic obstructive pulmonary disease (COPD), but characteristic imaging features (e.g., centrilobular emphysema) may aid in the diagnosis[86]. Recent reviews confirm the usefulness of chest HRCT in the evaluation and management of severe asthma[1,96], although at present there are no definitive criteria[97]. The evaluation and treatment of confounding or exacerbating factors (e.g., sinusitis, smoking) is also of great importance in the management of severe asthma[14,95]. HRCT can also contribute to this aspect of asthma management, as shown in an asthma clinic with a high percentage of complex-to-treat cases, where an extensive re-characterization of the patients was conducted. Of a total of 463 patients with a diagnosis of resilient asthma, 185 underwent HRCT, which led to a diagnosis of allergic bronchopulmonary aspergillosis (ABPA) in 5%[85,98].

6. QUESTIONNAIRES

The Global Initiative for Asthma (GINA) in 2006 proposed a new approach to asthma management, with asthma control, rather than asthma severity, as the focus of treatment decisions[99]. Subjectivity on the part of either the physician or the patient has always been a problem in the assessment of asthma control[122]. For this reason GINA suggested the use of validated instruments for assessing the clinical control of asthma in a reproducible manner. This is intended to improve the evaluation of asthma control and thus advance communication and partnership between patients and health care professionals, which is the first of the 5 components of asthma therapy[14]. Examples of validated asthma control instruments (ACI) include:

1. Asthma Control Test (ACT) (http://www.asthmacontrol.com)[100-1],
2. Asthma Control Questionnaire (ACQ) (http://www.qoltech.co.uk/acq.html)[102],
3. Asthma Therapy Assessment Questionnaire (ATAQ) (http://www.ataqinstrument.com)[103],
4. Asthma Control Scoring System[104], and
5. Childhood Asthma Control Test (C-ACT)[105].

Comparison of the validated ACIs has failed to show clear predominance of one instrument over the others[106], and each presented both advantages and limitations[107]. Wallenstein and colleagues demonstrated the equivalence of the ACT and ACQ regarding reliability, validity, screening accuracy, and responsiveness[106]. Halbert and colleagues, in a review of the relevant literature identified 5 validated ACIs, among which were ACT, ACQ and ATAQ. They concluded that all the ACIs are efficient and reliable and that no one of them could be recommended over the others[107]. Most are short, easily administered and easy to interpret, and clinicians can thus decide which ACI to use according to practicality, availability and adaptability to their specific needs[106-107].

A large number of studies has validated the adjustment of the ACIs to various different ethnic and social groups and confirmed their efficacy and validity in a wide variety

of settings[108-113]. Investigators have used validated ACIs in local languages (e.g., Greek, Portuguese, Spanish, etc.) and also the English version in English speaking ethnic groups (e.g., multiethnic Asian populations[113]) and shown that these version of ACIs are effective. Kwang-Ha Yoo and co-workers went one step further and modified the standard ACT, producing a Korean version that reflected local cultural background and also proved to be efficient[11].

The value of ACIs in clinical practice needs to be demonstrated[14]. In a clinical trial setting the five-item version ACQ-5 appears to be more responsive to changes in asthma control than the standard GINA defined asthma control categories, and is preferred in most asthma medication clinical trials (e.g., SMART)[114]. Recently, investigators were able to predict future asthma exacerbations using the ACQ, with each 1-point increase being associated with a 50% increased risk of exacerbation in the following 2 weeks, and proposed its in both clinical practice and trials[120].

The need for using ACIs is more evident in severe asthma. Lower levels of asthma control are associated with a growing risk of severe asthma associated events, ranging from unscheduled office visits to severe exacerbations that require emergency department management or hospitalization. A validated ACI may help clinicians to identify patients with poor asthma control requiring intervention in order to avoid severe asthma related complications[115].

Investigators have shown that patients with severe asthma who experience near fatal attacks have abnormal respiratory control mechanisms such as reduced chemosensitivity to hypoxia and blunted perception of dyspnoea (both at rest and at the end point of various forms of exercise)[116-7]. A validated ACI may be a valuable predictive tool for the physician attending such patients.

The importance of ACIs in the monitoring of severe asthma in children has been documented. The C-ACT was found to be better than objective parameters in identifying young Chinese children with uncontrolled asthma at the lower levels of control, who were at risk for severe asthma events[118-9]. The use of a second cut-off point of 12 in the C-ACT can identify children with the lowest level of control, who are at risk for more serious outcomes[119].

Lopes and colleagues implemented principal components factor analysis to show that FeNO, an asthma control questionnaire (ACQ) and FEV_1 may have complementary roles in the evaluation of asthma control [22].

Physicians who are responsible for the management of severe asthma should always confirm the diagnosis, confirm compliance and investigate comorbidities that may aggravate asthma[14]. Apart from the validated ACI, questions that measure adherence to medication regimes (e.g., ICS), comorbidities (e.g., allergic rhinitis) and behavioural and environmental factors (e.g., smoking) may reveal causes of poor asthma control, the modification of which will help in the management of severe asthma. Poor asthma control has been shown to be associated with self-reported rhinitis, smoking and poor medication adherence, and the identification of such factors through a self-report questionnaire can be introduced into daily clinical practice[121]. At present there is a lack of objective measurements of adherence to medication and asthma management plans [123].

CONCLUSIONS - FUTURE PERSPECTIVES

Non-invasive assessment of severe asthma permits better understanding, diagnosis and treatment of this difficult entity. The ease of measuring FeNO makes it an attractive, non-invasive biomarker, and it is already proving to be useful in both diagnosis o asthma and monitoring of compliance with ICS therapy[124]. To evaluate its role as an aid to the improvement of asthma control, further studies are needed in carefully selected populations that are likely to benefit. Monitoring of FeNO may be useful in the early detection of exacerbations of asthma so that appropriate intervention may be instituted earlier, and it may also be a useful way of measuring corticosteroid resistance, as FeNO responds rapidly (i.e., within several hours) to ICS therapy. Corticosteroid resistance is an important feature in severe asthma and in patients with asthma who smoke. In the future, forms of treatment that target corticosteroid resistance may be developed, in which case quantifying this defect will become important. The introduction of hand-held monitoring devices has extended the measurement of FeNO to the primary care setting and home monitoring, although the present expense of these devices may preclude their widespread use. In the future, it may be possible to develop devices that are cheaper, smaller, and readily available for home monitoring[124].

Partitioning exhaled NO provides further information, particularly about inflammation in the lung periphery, and may be used in the future to study the effects of new forms of treatment of peripheral inflammation in severe asthma. Several new kinds of treatment at present under development for severe asthma, and this technique provides a non-invasive method of monitoring their

anti-inflammatory profile[124].

Looking to the future, phenotypic characterization will undoubtedly become a crucial step in the management of severe asthma, together with the delineation of the pathophysiological and inflammatory mechanisms for each phenotype. This kind of integrative approach will need the help of all the non-invasive methods described above, and will offer better prospects of developing the target specific treatments that will be effective in specific phenotypes of severe asthma.

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