



CME review

Noninvasive evaluation of airway inflammation in patients with severe asthma

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Introduction

Asthma is a chronic inflammatory disease of the airways, characterized by airflow obstruction and bronchial hyper-responsiveness. In most patients, asthma is well controlled using inhaled steroids with or without long-acting bronchodilators. However, a small percentage of asthmatic patients (approximately 5%–10%) remain symptomatic despite extensive reevaluation of diagnosis and management for an observation period of at least 6 months. This clinical condition has been defined as severe refractory asthma (SRA).¹ The term SRA was used for the first time in 1956 to describe asthma that was difficult to control despite treatment with systemic corticosteroids.² It was not until 2000 that a workgroup from the American Thoracic Society (ATS) suggested a comprehensive definition for SRA, based on 2 major criteria (continuous high-dose inhaled corticosteroids or oral corticosteroids for >50% of the previous year) and 7 minor criteria regarding aspects of lung function, exacerbations, disease stability, and use of additional controller medications, after poor adherence to treatment and untreated comorbidities have been excluded.³ Current Global Initiative for Asthma and NAEPP guidelines have implemented an approach to asthma management based on asthma control rather than asthma severity in patients treated with controller therapy. However, many studies describe groups of asthmatic patients undergoing treatment with severity classifications. Although the diagnosis of SRA according to the established ATS criteria is relatively straightforward, it remains a heterogeneous disease that can be subdivided on the basis of different clinical, etiologic, pathologic, or inflammatory characteristics.

The indirect assessment of airway inflammation through symptoms and spirometry is problematic and, even among specialists, frequently inaccurate. Bronchial biopsies or bronchoalveolar lavage are too invasive to be performed repetitively. In the last 20 years, noninvasive methods for assessing airway inflammation have been developed and include induced sputum, exhaled nitric oxide, and exhaled breath condensate (EBC). Interest in these techniques is rapidly growing, and accumulating evidence suggests that, even though they are currently used as a research tool, they have the potential to become validated methods that may provide valuable information in the daily clinical setting.

The aim of the present review is to summarize the major clinical studies that have used noninvasive techniques for the evaluation of airway inflammation in SRA. A MEDLINE search was conducted using, but not limited to, the following keywords: *severe refractory asthma, sputum induction, exhaled breath condensate, fraction of exhaled nitric oxide, markers in exhaled air, exhaled biomarkers, and assessment of airway inflammation*. Manual searches were also performed, and all articles retrieved from references of other articles were additionally examined. Finally, we selected those articles that addressed the topic more adequately.

Sputum Induction

In the 1990s after the standardization of the method of sputum induction by Pin et al,⁴ sputum induction became a particularly promising technique, providing information on both cellular and molecular aspects of airway inflammation. Thereafter, an impressively large number of articles, including a comprehensive review on sputum methods by the European Respiratory Society (ERS) Task Force, were published.^{5,6} Sputum induction is considered to be a safe, well-validated method of sputum processing. It also has the advantage of phenotyping patients with SRA through both sputum cell counts and inflammatory mediators in the supernatants, providing a rationale for specific therapies.

Differential Cell Counts

Generally, asthma has been categorized into 4 subgroups based on the prevalence of sputum cell counts: eosinophilic asthma (>3%

eosinophils), neutrophilic asthma (>60% neutrophils), mixed granulocytic asthma (>3% eosinophils and >60% neutrophils), and paucigranulocytic asthma (<3% eosinophils and <60% neutrophils).⁷ Persistent eosinophilia in asthmatic patients has been suggested to be a predictor of poor control, increased severity, and worse lung function regardless of treatment.⁸ However, the value of sputum eosinophil counts in monitoring asthma severity remains a matter of debate. Some studies have reported that sputum eosinophilia is correlated with the degree of airflow obstruction and clinical symptoms,⁴ whereas others report a poor association among sputum eosinophilia, asthma severity, bronchial hyper-responsiveness, and quality of life.⁹

Several studies have found that sputum eosinophilia usually predicts a clinical response to steroid treatment in asthmatic patients.¹⁰ Persistent sputum eosinophilia in patients with “difficult-to-treat asthma” should raise the possibility of undertreatment or nonadherence with corticosteroid medication.¹¹ Moreover, Romagnoli et al reported that eosinophils and eosinophilic mediators, including eosinophilic cationic protein, leukotriene (LT) E₄, and RANTES (Regulated on Activation, Normal T-Expressed, and Secreted), were increased in patients with poorly controlled asthma independently from the degree of severity. Thus, it was suggested that airways eosinophilia is associated with the level of control and not with the severity of chronic asthma.¹² Possible mechanisms for the persistence of sputum eosinophilia despite treatment with inhaled corticosteroids are as follows: undertreatment, an overactive intense inflammation, the presence of inflammation in small airways, and corticosteroid resistance due to reduced glucocorticoid receptor binding affinity.

Importantly, ten Brinke et al¹³ reported that airway eosinophilia in patients with severe asthma is not a static phenomenon but an indicator of disease activity, suggesting that persistent airway eosinophilia is not associated with a specific phenotype in severe asthma. In another study, no difference in sputum eosinophils was observed in comparing severe, unstable asthma to severe, stable asthma.¹⁴

Because exacerbations have the greatest effect on patients' quality of life, morbidity, and health care use, their prevention seems to be the most important clinical outcome in the management of asthma. Green et al¹⁵ demonstrated that a treatment strategy aiming at normalization of sputum eosinophils resulted in a greater reduction of acute exacerbations and hospitalizations for asthma compared with the usual strategy of control of symptoms and pulmonary function in patients with moderate-to-severe asthma. Two studies reported an association between the risk of exacerbations and eosinophilic airway inflammation in patients with SRA. These studies also demonstrated that symptoms, fraction of exhaled nitric oxide (FeNO), and lung function can be dissociated from eosinophilic inflammation and may be improved with corticosteroid treatment through the modulation of the interaction between airway smooth muscle and infiltrating mast cells.^{16,17} A post hoc analysis of exacerbation frequency in patients with severe noneosinophilic and eosinophilic asthma revealed that, with standard management, exacerbation frequency was substantially lower in those with noneosinophilic asthma.¹⁵ There is a recent study of SRA that supports that blocking IL-5 with an anti-IL-5 monoclonal antibody could result in a reduction of exacerbations and in better quality of life in asthmatic patients with high eosinophil sputum cell counts despite oral corticosteroid treatment.¹⁷

Some asthmatic patients have severe persistent asthma in the absence of eosinophilic inflammation, and they may also experience exacerbations without an increase in eosinophilic inflammation.^{8,18,19} In asthmatic patients who have poor lung function despite very high doses of oral and inhaled corticosteroids, the most consistent cellular finding was the predominance of

neutrophils in their bronchoalveolar lavage and bronchial biopsy specimens.²⁰ Corticosteroids increase neutrophil survival by reducing apoptosis.²¹ Gibson et al²² used induced sputum to assess 56 patients with persistent asthma taking high doses of inhaled corticosteroids and found that 59% of them had suppressed sputum eosinophil counts with evidence of neutrophilic inflammation, suggesting that sputum neutrophilia in persistent asthma could represent a consequence of the disease or its treatment.

Persistent airflow limitation is often present in SRA. ten Brinke et al²³ found that the only independent factor associated with persistent airflow limitation in SRA is a sputum eosinophil percentage of 2% or higher. In contrast, a study by Shannon et al²⁴ that included patients with severe asthma found that combined increased sputum eosinophils and neutrophils defined patients with the most severe asthma as those with the lowest lung function, worse asthma control, more intense symptoms, most use of health care resources, and persistent inflammation despite usual treatment. Furthermore, Shaw et al²⁵ found that the sputum total neutrophil count but not the differential eosinophil count was associated with a lower postbronchodilator forced expiratory volume in 1 second (FEV₁), suggesting that neutrophilic airway inflammation contributes to the development of fixed airflow obstruction in asthma. The European Network for Understanding Mechanisms of Severe Asthma (ENFUMOSA) reported that persistent symptoms and abnormal lung function, despite high-dose regular use of controller and reliever medications, are accompanied by a component of irreversible airflow obstruction, neutrophilic inflammation, ongoing mediator release, and reduced association with atopy. The investigators of ENFUMOSA additionally suggested that severe asthma shares features with chronic obstructive pulmonary disease.²⁶ These findings are consistent with a substantial heterogeneity of severe asthma as assessed by induced sputum cell counts.

Inflammatory Mediators

Sputum supernatant has been used to measure a variety of inflammatory mediators. This can be useful in patients with severe asthma apart from sputum cell counts because it may yield additional information regarding inflammation and remodeling in asthmatic airways. Few data support the upregulation of specific chemoattractants, including IL-5, eotaxin, and other mediators that promote eosinophilic inflammation, in severe as opposed to milder forms of asthma.²⁷ In addition to T_H2 cytokines or chemokines, upregulation of T_H1 pathway elements has been observed in severe asthma, with small clinical studies suggesting that specific interventions involving tumor necrosis factor α may improve outcomes in severe asthma.²⁸ In accordance with this, interferon γ messenger RNA levels in sputum supernatant from patients with severe asthma were significantly higher compared with those in patients with mild asthma and healthy controls.²⁹ LTB₄, IL-8, IL-17, and macrophage inflammatory protein 1a are increased in severe asthma and can induce neutrophil chemotaxis and upregulation of endothelial adhesion molecules.³⁰ The results of a study demonstrated that patients with SRA had significantly higher induced sputum levels of osteopontin than those with milder forms of the disease and also a significant association between osteopontin and transforming growth factor β 1, IL-13, and cysteinyl leukotrienes (Cys-LTs) was detected.³¹ Moreover, both angiopoietin 1 and 2-induced sputum levels were higher in SRA compared with patients with moderate asthma and healthy individuals, and angiopoietin 2 was associated with the airway vascular permeability index, matrix metalloproteinase 2, and vascular endothelial growth factor in SRA.³²

Epidermal growth factor receptor, a marker of epithelial stress and damage, is also increased in proportion to disease severity.

Epidermal growth factor receptor expression in the bronchial epithelium correlates with IL-8 because epidermal growth factor stimulates IL-8 release and enhances tumor necrosis factor α -induced IL-8 release from the epithelium, thereby linking it to neutrophilic inflammation in severe asthma.³³ High levels of sputum IL-5 and granulocyte-macrophage colony-stimulating factor were found in patients with severe asthma with frequent exacerbations but not persistent bronchoconstriction, whereas sputum IL-8 levels were particularly elevated in patients with severe asthma and persistent bronchoconstriction. These findings suggest that different phenotypes of severe asthma are associated with different types of inflammatory mediators, however with great heterogeneity.³⁴

Exhaled Nitric Oxide

Nitric oxide is the most extensively studied noninvasive exhaled marker of airway inflammation. It is produced in the airways by the conversion of L-arginine to L-citrulline, which is catalyzed by enzymes known as nitric oxide synthases.³⁵ The levels of nitric oxide can be measured repeatedly in exhaled breath using a range of commercially available analyzers, including smaller less costly devices that have become available in the past few years. Recommendations outlining standardized procedures for adequate FeNO measurement have been published and recently updated by a task force of the ATS and the ERS.³⁶

Association With Inflammatory Cells

FeNO has been primarily related to eosinophilic inflammation in asthma and has been prospectively used to guide asthma treatment, with conflicting results.³⁷ A few studies have investigated FeNO levels in SRA. Most of them did not reveal any significant difference in the levels of FeNO between patients with severe asthma, patients with moderate asthma,³⁸ and healthy controls.^{39,40} Using different selection criteria for severe asthma in their study design, Lemiere et al³⁸ reported a median value for FeNO of 13.2 ppb, whereas Silkoff et al³⁹ reported a median value of 28.25 ppb. In a previous study of our group, we found increased FeNO levels in SRA (43 ppb) compared with patients with moderate asthma (25 ppb), although with a wide range of values.⁴¹ Among SRA patients, different cutoff values of FeNO identified those with a predominance of eosinophils in the bronchial airways (>19 ppb) and those with mixed cellular profile (<19 ppb). SRA patients with sputum eosinophilia had elevated FeNO levels despite high-dose corticosteroid treatment, whereas SRA patients with sputum neutrophilia had low FeNO levels regardless of the concomitant presence of sputum eosinophils.⁴² These differences might be attributed to different selection criteria for SRA patients used within studies or different phenotypes of SRA due to cigarette smoking or exacerbation frequency. However, when Silkoff et al³⁹ classified SRA according to eosinophil counts in airway tissue biopsy or bronchoalveolar lavage fluid specimens, the eosinophilia-positive SRA group had significantly higher median FeNO levels compared with the eosinophilia-negative SRA group, the patients with mild-moderate asthma, and the healthy controls. These results are in agreement with previous findings that reported a close relationship between increased FeNO levels and eosinophilic inflammation,⁴² and it is important that this linkage is retained in SRA despite treatment with high-dose inhaled and/or oral corticosteroids.^{39,41}

Clinical Predictive Value

A few studies have challenged the predictive value of FeNO for specific clinical, inflammatory, and lung function characteristics. Patients with an FeNO of 20 ppb or higher and FEV₁ percentages within normal limits had a 3.1-fold risk of accelerated decline in

lung function in the following 5 years, suggesting that FeNO could be used as a predictor of accelerated decline in lung function in patients with difficult-to-treat asthma but with normal FEV₁ values.⁴³ Another study concluded that in patients with severe asthma, high FeNO levels (>35 ppb) could identify an at-risk phenotype that appeared to be more severe than patients with severe asthma and low FeNO because the elevated FeNO group demonstrated greater airway reactivity, greater degree of airflow limitation, higher eosinophilic sputum cell count, and more frequent emergency department use and intensive care unit admissions.⁴⁴ Another study described 5 different groups of patients with asthma who differed in clinical, physiologic, and inflammatory parameters. The variables that determined the assignment to each of the 5 SRA subgroups were prebronchodilator and postbronchodilator FEV₁ percentage, age of asthma onset and the duration of the disease, medication used (inhaled corticosteroids or β -agonists), and sex. FeNO levels were similar in all SRA groups.⁴² Gelb et al⁴⁵ suggested recently that increased FeNO during outpatient exacerbation in patients with moderate-to-severe asthma taking inhaled corticosteroids and long-acting β_2 -agonists who are not receiving maintenance oral corticosteroids does not provide clinically useful therapeutic information regarding the clinical and spirometric response to oral corticosteroids.

Although FeNO levels are thought to be reduced by corticosteroid therapy, high FeNO levels in SRA justify a need for further investigation. One study demonstrated that there was no significant difference in inhaled corticosteroid dose between the high and low FeNO SRA groups. However, the SRA group with high FeNO had the highest proportion of oral corticosteroid use and patients were much more likely to be taking theophylline daily. Accordingly, it was suggested that the high FeNO SRA group may have a relative resistance to corticosteroid therapy, whereas low FeNO levels in SRA patients may be related to either less nitric oxide synthesis or greater oxidative consumption in the airways.⁴⁴

Exhaled Breath Condensate

Collection of EBC is another method of sampling the respiratory tract that can be repeated with short intervals between sampling, enabling the method to be used in longitudinal studies. Despite some methodologic problems mainly related to dilution of droplets in EBC, its noninvasive nature gives the opportunity for repeated measurements in the same person and provides valuable information for the assessment of airway inflammation. The recent ERS/ATS recommendations have made suggestions toward the standardization of the procedure,⁴⁶ and there is emerging evidence that parameters obtained from EBC reflect changes in the airway lining fluid.⁴⁷

EBC pH

Measurement of pH with electrodes after deaeration for the removal of carbon dioxide with an inert gas (eg, argon or helium) is a technically validated EBC measurement in the published literature.⁴⁸ Measurement of EBC pH is a simple, robust, and reproducible marker of asthma. In healthy individuals, EBC pH has a mean value of 7.7, with a reference range of 7.4 to 8.8.⁴⁹ There is also evidence that EBC pH is significantly correlated with the number of eosinophils in induced sputum in patients with moderate asthma, as well as with parameters expressing oxidative stress and nitric oxide metabolism in EBC.^{48,50} Regarding the EBC pH levels in SRA, there are only a few published articles reporting that they are significantly lower compared with the levels in patients with moderate asthma and healthy controls (mean [SD], 7.22 [0.08] vs 7.36 [0.06] vs 7.72 [0.1]). Yet, in the same study no significant association was detected between EBC pH and sputum eosinophils or other inflammatory cell types in SRA patients; therefore, it was

concluded that EBC pH has no predictive value for the identification of the predominant sputum inflammatory cell type.⁴¹

Another study, including a large cohort of 250 patients with SRA and 291 patients with nonsevere asthma, concluded that EBC pH did not differ between asthmatic patients and controls and, furthermore, that patients with severe asthma tended to have a slightly higher pH compared with patients with nonsevere asthma. The study also revealed that in the overall study population, low pH values were significantly correlated with gastrointestinal reflux disease symptoms, race other than white, history of pneumonia, allergic symptoms without cold or flu, acute or recurrent sinusitis, nasal polyps, absence of corticosteroid treatment, anti-IgE therapy, more severe allergic symptoms, high body mass index, low baseline prebronchodilator FEV₁, and low Asthma Quality of Life Questionnaire.⁵¹

Nitric Oxide–Related Products

Nitric oxide is a free radical and may react with oxygen to yield nitrogen oxide or with superoxide anion to yield peroxynitrite, a highly reactive substance.⁵² Thus, nitric oxide synthesis and release in the respiratory system have been assessed indirectly by quantifying nitrite and nitrate, nitrotyrosine, and S-nitrosothiol in EBC. High S-nitrosothiol and nitrite levels have been found in patients with severe asthma compared with patients with mild asthma and controls.⁵³ Other studies revealed that EBC nitrite and nitrate levels were significantly higher in patients with asthma compared with healthy controls, and they presented significant associations with the underlying severity, EBC levels of hydrogen peroxide, and lung function impairment.⁵⁴ EBC nitrotyrosine levels were lower in patients with moderate and severe asthma treated with inhaled or oral corticosteroids compared with patients with untreated mild asthma and controls, indicating that corticosteroid treatment may inhibit oxidative stress induced by inflammation in the airways.⁵⁵

Arachidonic Acid Metabolites (Leukotrienes and Isoprostanes)

Leukotrienes are classified into 2 classes: LTB₄ and Cys-LTs (eg, LTC₄, LTD₄, and LTE₄). Cys-LT levels have been found to be significantly elevated in asthma, and 2 previous studies have reported increased EBC levels of Cys-LTs and LTB₄ in patients with SRA and moderate asthma compared with patients with mild asthma and healthy controls.^{55,56} However, Cys-LTs have not been found to be correlated with FEV₁ in patients with severe asthma.⁵⁵

8-Isoprostane, a prostaglandin F(2 α) isomer, is a marker of oxidative stress that is known to be implicated in the airway inflammatory process in asthmatic patients. 8-Isoprostane levels were found to be significantly elevated in patients with SRA compared with patients with mild or moderate asthma and healthy controls. They were also strongly correlated with exhaled Cys-LTs levels in SRA.⁵⁷

Hydrogen Peroxide

Oxidants can cause airway inflammation and airway hyperresponsiveness, which are major characteristics of asthma. Patients with asthma have increased reactive oxygen species production by macrophages, eosinophils, and neutrophils that leads to increased production of hydrogen peroxide. Elevated levels of hydrogen peroxide have been reported in corticosteroid-naïve asthmatic patients, sometimes being 26-fold higher than in healthy controls. Hydrogen peroxide levels are influenced by the underlying disease severity, the smoking habit, and the presence of symptoms in patients with unstable asthma, whereas atopy did not affect hydrogen peroxide.⁵⁸ The EBC hydrogen peroxide levels in patients with mild persistent asthma were significantly higher than those in patients with mild intermittent, moderate persistent, or severe

Table 1

Major findings from studies evaluating noninvasive measurements of airway inflammation in severe refractory asthma

Parameter	Sputum cells	Sputum mediators	FeNO	pH EBC	EBC mediators
Compared with mild-moderate asthma	↑ Eosinophils ^{4,8,12} ↑ Neutrophils ^{19–21}	↑ IL-5 ^{26,33} ↑ Eotaxin ²⁶ ↑ TNF- α ²⁷ ↑ IFN- γ mRNA ²⁸ ↑ IL-8 ^{29,33} ↑ LTB4, IL-17, MIP-1a ²⁹ ↑ EGFR ³² ↑ OPN ³⁰ ↑ Ang-1, Ang-2 ³¹	↑ ^{38,40} ↔ ³⁷	↓ ⁴⁰ ↔ or ↑ ⁵⁰	↑ Cys-LTs ⁵⁴ ↑ LTB4 ⁵⁴ ↑ 8-isoprostane ⁵⁵ ↔ or ↓ hydrogen peroxide ⁵³ ↑ RS-NOS and NO ₂ ^{–52}
Exacerbation	↑ Eosinophils ^{15–17}				
Relation to reduced lung function	↑ Eosinophils ²³ ↑ Neutrophils ^{25,26} ↑ Eosinophils and neutrophils ²⁴		↑ ^{42,43}		
Relation between inflammatory indices			↑ and ↑ Eosinophils ^{38–41}		
Effect of treatment	ICS ↓ eosinophils ^{10,11}				

Abbreviations: Ang, angiopoietin; Cys-LT, cysteinyl leukotriene; EGFR, epidermal growth factor receptor; IFN- γ , interferon γ ; IL, interleukin; LT, leukotriene; MIP-1a, macrophage inflammatory protein 1a; mRNA, messenger RNA; NO₂[–], nitrite; OPN, osteopontin; RS-NOS, S-nitrosothiol; TNF- α , tumor necrosis factor α . Symbols: ↑, increased; ↓, decreased; ↔, no change; +, positive relation; –, negative relation.

persistent asthma, whereas no significant differences were found among the latter 3 disease severity groups.⁵⁴

Discussion

Bronchial asthma is a common health problem with enormous medical and economic effects. However, it has been estimated that most asthma expenditure is consumed by only a few asthmatic patients who experience more severe disease.²⁷ The critical component of asthma care is a rapid, specific assessment of the patient's illness. The severe asthmatic is easily recognized, based on symptom frequency, impairment of lung function, and treatment requirement for control of asthma symptoms, but a comprehensive evaluation, including the confirmation of an accurate diagnosis of asthma, the assessment of adherence to treatment, the control of environmental exposure, and the management of comorbidities, preferably after proper follow-up in the setting of an asthma specialist, is required.¹ Objective measures of airflow limitation are definitely necessary to confirm the presence of bronchial obstruction and evaluate its magnitude. Physicians' subjective estimates of obstruction, including those of chest specialists, are often grossly inaccurate and cannot be solely relied on. More recently, quality-of-life measures together with noninvasive inflammatory markers have emerged as additional potential parameters for better evaluation (Table 1).

The categorization of asthma into 4 subgroups based on the prevalence of sputum cell count (eosinophilic, neutrophilic, mixed granulocytic, and paucigranulocytic asthma) is intriguing because knowledge of sputum inflammatory phenotype seems to relate to treatment response, mechanistic pathways involved in disease pathogenesis, and future disease risk. Persistent eosinophilia has been suggested to be a predictor of poor asthma control regardless of treatment; however, the value of sputum eosinophil counts in monitoring asthma severity still remains a matter of debate.⁸ Moreover, airway eosinophilia in severe asthma is not a static phenomenon but an indicator of disease activity, and accordingly it is not associated with a specific phenotype in severe asthma.¹³ IL-5 is important for eosinophil maturation, activation, and recruitment in the bronchial mucosa of asthmatic patients. Two recent studies reported a reduction in exacerbations in asthmatic patients with persistent sputum eosinophilia and symptoms despite treatment with oral corticosteroids using an anti-IL-5 monoclonal antibody.^{17,59} Sputum neutrophilia occurs in persistent asthma and may represent either an effect of the disease or of the treatment regimens.²² Sputum total neutrophil count has been associated with a lower postbronchodilator FEV₁, suggesting that neutrophilic

airway inflammation contributes to the development of fixed airflow obstruction in asthma.²⁵

Sputum induction is the only noninvasive measure of airway inflammation that has a proven role in the management of moderate-to-severe asthma to date.⁶⁰ Regular monitoring of airway inflammation in this disease group may be required for optimal treatment and in particular in those patients with discordance between airway inflammation and symptom expression. The inclusion of sputum induction in the management of such asthmatic patients may be cost-effective. Economic analysis reveals that the health care–related savings as a consequence of the reduction in asthma exacerbations outweighs the cost of sputum induction and processing.¹⁵ Sputum induction requires laboratory support, but all of the equipment is available in a routine pathology laboratory and minimal training is required. Therefore, sputum induction should be available as a routine test in specialist centers to evaluate and treat patients with severe or difficult-to-treat asthma.

FeNO has primarily been related to eosinophilic inflammation in asthma, and its levels can be measured using commercially available analyzers and small devices. Most studies assessing FeNO in severe asthma did not reveal any significant difference between patients with severe and mild or moderate asthma. However, the identification of increased FeNO levels in optimally treated patients with severe asthma provides evidence for the presence of persistent eosinophilic airway inflammation, with possible implications for intensification of anti-inflammatory treatment.³⁷ Moreover, recent evidence suggests that different cutoff values of FeNO could identify those SRA patients with predominant eosinophilia and those with mixed cellular profile.^{39,41}

As far as EBC pH levels in SRA are concerned, only a few published articles have shown that they are significantly lower compared with those in patients with moderate asthma and healthy controls, yet no significant association was detected between EBC pH and sputum eosinophils.⁴¹ Moreover, elevated 8-isoprostane levels were found in patients with SRA compared with patients with mild or moderate asthma and healthy controls.⁵⁷

Asthma is a heterogeneous syndrome with differences in terms of the mechanisms and the response to therapy. Accordingly, it seems crucial to identify the different disease phenotypes within the range of this syndrome. The ideal approach is to try to identify specific biomarkers that are related to a particular phenotype and its underlying mechanisms. Possibly, the evaluation of multiple biologic markers, such as FeNO and sputum eosinophils, may provide a cumulative index for definition of asthma severity in the future as suggested by a recent study.⁴⁴

The widespread application of noninvasive evaluation of airway inflammation in severe asthma has provided insight into the relationship between airway function and airway inflammation. It has also provided a new tool for better assessment of the underlying mechanisms in severe asthma and possible new treatments or better treatment effects in particular phenotypes of severe asthma.

References

- [1] Bel EH, Sousa A, Fleming L, et al. Diagnosis and definition of severe refractory asthma: an international consensus statement from the Innovative Medicine Initiative (IMI). *Thorax*. 2011;66:910–917.
- [2] Louis R, Potmans V. Analysis of induced sputum in refractory asthma. *Presse Med*. 2008;37:155–159.
- [3] Proceedings of the ATS workshop on refractory asthma: current understanding, recommendations, and unanswered questions. American Thoracic Society. *Am J Respir Crit Care Med*. 2000;162:2341–2351.
- [4] Pin I, Gibson PG, Kolendowicz R, et al. Use of induced sputum cell counts to investigate airway inflammation in asthma. *Thorax*. 1992;47:25–29.
- [5] Djukanovic R, Sterk PJ, Fahy JV, et al. Standardised methodology of sputum induction and processing. *Eur Respir J Suppl*. 2002;37:1s–2s.
- [6] Efthimiadis A, Spanevello A, Hamid Q, et al. Methods of sputum processing for cell counts, immunocytochemistry and in situ hybridisation. *Eur Respir J Suppl*. 2002;37:19s–23s.
- [7] Simpson JL, Scott R, Boyle MJ, et al. Inflammatory subtypes in asthma: assessment and identification using induced sputum. *Respirology*. 2006;11:54–61.
- [8] Wenzel SE, Schwartz LB, Langmack EL, et al. Evidence that severe asthma can be divided pathologically into two inflammatory subtypes with distinct physiologic and clinical characteristics. *Am J Respir Crit Care Med*. 1999;160:1001–1008.
- [9] Louis R, Lau LC, Bron AO, et al. The relationship between airways inflammation and asthma severity. *Am J Respir Crit Care Med*. 2000;161:9–16.
- [10] Hargreave FE, Pizzichini E, Pizzichini M. Induced sputum examination. *J Allergy Clin Immunol*. 1998;101:569–570.
- [11] Jayaram L, Parameswaran K, Sears MR, et al. Induced sputum cell counts: their usefulness in clinical practice. *Eur Respir J*. 2000;16:150–158.
- [12] ten Brinke A, Zwinderman AH, Sterk PJ, et al. “Refractory” eosinophilic airway inflammation in severe asthma: effect of parenteral corticosteroids. *Am J Respir Crit Care Med*. 2004;170:601–605.
- [13] Romagnoli M, Vachier I, Tarodo de la Fuente P, et al. Eosinophilic inflammation in sputum of poorly controlled asthmatics. *Eur Respir J*. 2002;20:1370–1377.
- [14] Bartoli ML, Bacci E, Carnevali S, et al. Clinical assessment of asthma severity partially corresponds to sputum eosinophilic airway inflammation. *Respir Med*. 2004;98:184–193.
- [15] Green RH, Brightling CE, McKenna S, et al. Asthma exacerbations and sputum eosinophil counts: a randomised controlled trial. *Lancet*. 2002;360:1715–1721.
- [16] Haldar P, Pavord ID, Shaw DE, et al. Cluster analysis and clinical asthma phenotypes. *Am J Respir Crit Care Med*. 2008;178:218–224.
- [17] Haldar P, Brightling CE, Hargadon B, et al. Mepolizumab and exacerbations of refractory eosinophilic asthma. *N Engl J Med*. 2009;360:973–984.
- [18] Fahy JV, Kim KW, Liu J, et al. Prominent neutrophilic inflammation in sputum from subjects with asthma exacerbation. *J Allergy Clin Immunol*. 1995;95:843–852.
- [19] Pavord ID, Brightling CE, Woltmann G, et al. Non-eosinophilic corticosteroid unresponsive asthma. *Lancet*. 1999;353:2213–2214.
- [20] Wenzel SE, Szeffler SJ, Leung DY, et al. Bronchoscopic evaluation of severe asthma: persistent inflammation associated with high dose glucocorticoids. *Am J Respir Crit Care Med*. 1997;156:737–743.
- [21] Jatakanon A, Uasuf C, Maziak W, et al. Neutrophilic inflammation in severe persistent asthma. *Am J Respir Crit Care Med*. 1999;160:1532–1539.
- [22] Gibson PG, Simpson JL, Salto N. Heterogeneity of airway inflammation in persistent asthma: evidence of neutrophilic inflammation and increased sputum interleukin-8. *Chest*. 2001;119:1329–1336.
- [23] ten Brinke A, Zwinderman AH, Sterk PJ, et al. Factors associated with persistent airflow limitation in severe asthma. *Am J Respir Crit Care Med*. 2001;164:744–748.
- [24] Shannon J, Ernst P, Yamauchi Y, et al. Differences in airway cytokine profile in severe asthma compared to moderate asthma. *Chest*. 2008;133:420–426.
- [25] Shaw DE, Berry MA, Hargadon B, et al. Association between neutrophilic airway inflammation and airflow limitation in adults with asthma. *Chest*. 2007;132:1871–1875.
- [26] European Network for Understanding Mechanisms of Severe Asthma. The ENFUMOSA cross-sectional European multicentre study of the clinical phenotype of chronic severe asthma. *Eur Respir J*. 2003;22:470–477.
- [27] Chanez P, Wenzel SE, Anderson GP, et al. Severe asthma in adults: what are the important questions? *J Allergy Clin Immunol*. 2007;119:1337–1348.
- [28] Berry MA, Hargadon B, Shelley M, et al. Evidence of a role of tumor necrosis factor alpha in refractory asthma. *N Engl J Med*. 2006;354:697–708.
- [29] Truyen E, Coteur L, Dilissen E, et al. Evaluation of airway inflammation by quantitative Th1/Th2 cytokine mRNA measurement in sputum of asthma patients. *Thorax*. 2006;61:202–208.
- [30] Barczyk A, Pierzchala W, Sozanska E. Interleukin-17 in sputum correlates with airway hyperresponsiveness to methacholine. *Respir Med*. 2003;97:726–733.
- [31] Delimpoura V, Bakakos P, Tseliou E, et al. Increased levels of osteopontin in sputum supernatant in severe refractory asthma. *Thorax*. 2010;65:782–786.
- [32] Tseliou E, Bakakos P, Kostikas K, et al. Increased levels of angiopoietins 1 and 2 in sputum supernatant in severe refractory asthma. *Allergy*. 2010;67:396–402.
- [33] Hamilton LM, Torres-Lozano C, Puddicombe SM, et al. The role of the epidermal growth factor receptor in sustaining neutrophil inflammation in severe asthma. *Clin Exp Allergy*. 2003;33:233–240.
- [34] Dente FL, Carnevali S, Bartoli ML, et al. Profiles of proinflammatory cytokines in sputum from different groups of severe asthmatic patients. *Ann Allergy Asthma Immunol*. 2006;97:312–320.
- [35] Nathan C, Xie QW. Nitric oxide synthases: roles, tolls, and controls. *Cell*. 1994;78:915–918.
- [36] Silkoff PE, Erzurum SC, Lundberg JO, et al. ATS workshop proceedings: exhaled nitric oxide and nitric oxide oxidative metabolism in exhaled breath condensate. *Proc Am Thorac Soc*. 2006;3:131–145.
- [37] Shaw DE, Berry MA, Thomas M, et al. The use of exhaled nitric oxide to guide asthma management: a randomized controlled trial. *Am J Respir Crit Care Med*. 2007;176:231–237.
- [38] Lemiere C, Ernst P, Olivenstein R, et al. Airway inflammation assessed by invasive and noninvasive means in severe asthma: eosinophilic and non-eosinophilic phenotypes. *J Allergy Clin Immunol*. 2006;118:1033–1039.
- [39] Silkoff PE, Lent AM, Busacker AA, et al. Exhaled nitric oxide identifies the persistent eosinophilic phenotype in severe refractory asthma. *J Allergy Clin Immunol*. 2005;116:1249–1255.
- [40] Spanevello A, Confalonieri M, Sulotto F, et al. Induced sputum cellularity: reference values and distribution in normal volunteers. *Am J Respir Crit Care Med*. 2000;162:1172–1174.
- [41] Tseliou E, Bessa V, Hillas G, et al. Exhaled nitric oxide and exhaled breath condensate pH in severe refractory asthma. *Chest*. 2010;138:107–113.
- [42] Kharitonov SA, Barnes PJ. Exhaled markers of pulmonary disease. *Am J Respir Crit Care Med*. 2001;163:1693–1722.
- [43] van Veen IH, Ten Brinke A, Sterk PJ, et al. Exhaled nitric oxide predicts lung function decline in difficult-to-treat asthma. *Eur Respir J*. 2008;32:344–349.
- [44] Dweik RA, Sorkness RL, Wenzel S, et al. Use of exhaled nitric oxide measurement to identify a reactive, at-risk phenotype among patients with asthma. *Am J Respir Crit Care Med*. 2010;181:1033–1041.
- [45] Gelb AF, Moridzadeh R, Singh DH, et al. In moderate-to-severe asthma patients monitoring exhaled nitric oxide during exacerbation is not a good predictor of spirometric response to oral corticosteroid. *J Allergy Clin Immunol*. 2012;129:1491–1498.
- [46] Horvath I, Hunt J, Barnes PJ, et al. Exhaled breath condensate: methodological recommendations and unresolved questions. *Eur Respir J*. 2005;26:523–548.
- [47] Montuschi P. Exhaled breath condensate analysis in patients with COPD. *Clin Chim Acta*. 2005;356:22–34.
- [48] Kostikas K, Papatheodorou G, Ganas K, et al. pH in expired breath condensate of patients with inflammatory airway diseases. *Am J Respir Crit Care Med*. 2002;165:1364–1370.
- [49] Paget-Brown AO, Ngamtrakulpanit L, Smith A, et al. Normative data for pH of exhaled breath condensate. *Chest*. 2006;129:426–430.
- [50] Kostikas K, Koutsokera A, Papiris S, et al. Exhaled breath condensate in patients with asthma: implications for application in clinical practice. *Clin Exp Allergy*. 2008;38:557–565.
- [51] Liu L, Teague WG, Erzurum S, et al. Determinants of exhaled breath condensate pH in a large population with asthma. *Chest*. 2011;139:328–336.
- [52] Ricciardolo FL, Di Stefano A, Sabatini F, et al. Reactive nitrogen species in the respiratory tract. *Eur J Pharmacol*. 2006;533:240–252.
- [53] Corradi M, Montuschi P, Donnelly LE, et al. Increased nitrosothiols in exhaled breath condensate in inflammatory airway diseases. *Am J Respir Crit Care Med*. 2001;163:854–858.
- [54] Ueno T, Kataoka M, Hirano A, et al. Inflammatory markers in exhaled breath condensate from patients with asthma. *Respirology*. 2008;13:654–663.
- [55] Hanazawa T, Kharitonov SA, Barnes PJ. Increased nitrotyrosine in exhaled breath condensate of patients with asthma. *Am J Respir Crit Care Med*. 2000;162:1273–1276.
- [56] Montuschi P, Barnes PJ. Exhaled leukotrienes and prostaglandins in asthma. *J Allergy Clin Immunol*. 2002;109:615–620.
- [57] Samitas K, Chorianopoulos D, Vittorakis S, et al. Exhaled cysteinyl-leukotrienes and 8-isoprostane in patients with asthma and their relation to clinical severity. *Respir Med*. 2009;103:750–756.
- [58] Loukides S, Kontogianni K, Hillas G, et al. Exhaled breath condensate in asthma: from bench to bedside. *Curr Med Chem*. 2011;18:1432–1443.
- [59] Nair P, Pizzichini MM, Kjarsgaard M, et al. Mepolizumab for prednisone-dependent asthma with sputum eosinophilia. *N Engl J Med*. 2009;360:985–993.
- [60] Bakakos P, Schleif F, Alchanatis M, et al. Induced sputum in asthma: from bench to bedside. *Curr Med Chem*. 2011;18:1415–1422.