

REVIEW

The investigation of severe asthma to define phenotypes

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Summary

Severe asthmatics often exhibit poor control despite high doses of inhaled corticosteroids with or without systemic corticosteroids and suffer from persistent symptoms and/or recurrent exacerbations. Five to ten percentage of the asthmatic population falls within this category. Patients with severe asthma are a heterogeneous group and should be investigated to confirm the diagnosis, identify comorbidities, exclude alternative diagnoses, together with an evaluation of treatment adherence and side-effects from medications. Optimization of asthma medications and monitoring the control and pattern of asthma usually takes place over a period of 6 months. In patients with confirmed severe refractory asthma, further evaluation is needed in terms of detailed lung function, of airway and lung structure using high resolution computed tomographic scanning, and of airway inflammatory processes and biomarkers using induced sputum or bronchial biopsies. Patients with severe asthma are best investigated and managed with a multidisciplinary team. Severe asthma consists of different phenotypes that need defining. Investigation of severe asthma should bring into the open the various characteristics of the disease that could point to particular phenotype. Inclusion of investigations based on transcriptomics and proteomics should expand, improve classification and understanding of severe asthma, with the ultimate hope of finding more effective treatments and a step towards personalized medicine.

Keywords asthma phenotypes, biomarkers, exhaled nitric oxide, severe asthma, sputum eosinophils

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Introduction

Whilst the majority of patients with asthma can be well controlled with inhaled corticosteroids and long-acting β_2 -agonists, between 5% and 10% of asthmatics have severe refractory disease. It is estimated that severe asthma costs the NHS over £680 million per year, which is over 50% of the total available NHS resources for asthma of £1 billion [1, 2]. The considerable morbidity and cost of this particular type of asthma has led to a special focus on severe asthma, with regard to diagnosis and further understanding the condition in the hope of rationalizing treatment approaches and finding newer effective treatments.

Definition of severe asthma

Asthma that becomes difficult-to-control has been denoted by various terms including severe asthma, prob-

lematic asthma, poorly controlled asthma or therapy-resistant asthma. A European Respiratory Society (ERS) Task Force in 1999 defined severe asthma as asthma that remains uncontrolled despite high dose inhaled corticosteroids with or without systemic corticosteroids, with uncontrolled defined as persistent symptoms or recurrent exacerbations [3]. The American Thoracic Society (ATS) definition which used similar criteria of treatment needs with high dose inhaled or systemic corticosteroids was more precise with uncontrolled asthma defined as having two of seven criteria that characterizes control namely (i) the requirement for a controller medication in addition to inhaled corticosteroids, e.g. long-acting β -agonist, theophylline, or leukotriene antagonist, (ii) asthma symptoms requiring short-acting β -agonist use on a daily or near daily basis, (iii) persistent airway obstruction ($FEV_1 < 80\%$ predicted; diurnal PEF variability $>20\%$), (iv) one or more urgent care visits for asthma per year, (v) three or more oral steroid 'bursts'

per year, (vi) prompt deterioration with $\leq 25\%$ reduction in oral or inhaled corticosteroid dose, and (vii) near fatal asthma event in the past [4].

A more recent definition from the World Health Organisation (WHO) workshop of a uniform and global definition of severe asthma identified the components of asthma severity not only as the level of control defined by current clinical control and/or exacerbations and by the level of treatment prescribed, but also by responsiveness to treatment and by future risk [5]. This workshop classified severe asthma into three types based on experience: (i) untreated severe asthma, a condition most common in countries where asthma treatments cannot be afforded by the patient (ii) difficult-to-treat severe asthma, which may be so because of poor adherence to treatments or the asthma is inadequately treated (iii) treatment-resistant asthma, which comprised asthma for which control is not achieved despite the best and highest treatments available, and also of asthma that can ultimately be controlled, but at the highest dose of treatments.

In yet another definition, that forms the basis of recruitment of a European cohort, the Innovative Medicines Initiative (IMI) definition, the term 'problematic asthma' is used as a term to cover 'poorly-controlled asthma', which could be divided into two groups, namely (i) difficult asthma, a group of asthmatics where the asthma is difficult because of problems with adherence to treatments or inadequate treatments, and (ii) severe refractory asthma being those with poorly controlled asthma, frequent exacerbations despite good adherence and high intensity treatments and treatment of comorbidities [6]. This definition reflects the issue that it is necessary to exclude the first group of patients from being labelled as severe refractory asthma.

These definitions of severity differ from previous asthma guidelines, which did not have a category for severe asthma. However, more recent guidelines such as those set out by the Global Initiative for Asthma (GINA) and the National Asthma Education and Prevention Program (NAEPP) have recognized severe asthma on a similar basis of uncontrolled asthma despite high dose treatments with one or more controller therapies. Intrinsic in the definition of severe asthma is the loss of control and occurrence of exacerbations, both of which are concepts that have been recently refined [7].

Systematic assessment of severe asthma

A systematic evaluation of difficult-to-control and severe asthma is clearly of value. One of the first studies evaluating difficult-to-control asthma (defined as patients requiring greater than 10 mg of prednisolone every other day for at least three consecutive months per year) in a secondary care centre was performed in

1984, and showed the value of this approach. It found that 31 of 42 patients were judged to be no longer difficult-to-control following systematic assessment [8]. Two or more contributing factors to difficult-to-control asthma were identified in 80% of the subjects. Treatment of gastrooesophageal reflux (GORD) and the addition of inhaled corticosteroids (ICS) were the most helpful therapeutic interventions. Therefore, in this small cohort, only 11 of 42 difficult-to-control asthmatics were deemed severe.

In the reports of the ERS Task Force and the ATS Workshop on severe asthma 15 years later [3, 4], it was emphasized that patients with severe asthma should undergo investigations to confirm the diagnosis of asthma, to evaluate and treat confounding or exacerbating factors, and to optimize the 'standard' asthma pharmacotherapy. In addition, these reports also emphasized an integrated approach to define clinical phenotypes, evaluate risk factors, understand pathophysiology and find novel therapies. This is an essential exercise as much more needs to be known about severe asthma.

More recent cohorts have found that a systematic assessment identifies alternative diagnoses, comorbid conditions and addresses compliance with prescribed therapy [9, 10]. Heaney et al. found that 39 of 73 patients who underwent a systematic assessment achieved good symptom control following the identification and management of alternative diagnoses or comorbidities whilst the remaining 34 were considered to have treatment-resistant asthma [9]. Of 100 patients investigated for difficult asthma at the Royal Brompton Hospital, 12% had been misdiagnosed as asthma with COPD being the most common condition in six cases, followed by single cases of $\alpha 1$ -antitrypsin-deficient emphysema, cystic fibrosis, cardiomyopathy, obliterative bronchiolitis, respiratory muscle incoordination and vocal cord dysfunction with anxiety syndrome [10].

The investigations that patients with difficult-to-treat severe asthma patients should undergo include (i) those associated with confirmation of the diagnosis of asthma and exclusion of other diagnoses (ii) those needed to assess disease severity, including biomarkers (iii) those associated with the determination of risk factors, comorbidities, and factors that increase asthma severity (iv) a temporal assessment of the pattern of disease (Table 1).

What would be the ideal way of managing such patients?

Whilst the majority of patients with asthma are managed within a primary respiratory care settings, those with uncontrolled asthma are increasingly being referred to secondary care level or to specialized asthma clinics in secondary or tertiary care set-ups. A systematic assessment of severe asthma entails a series of

Table 1. Suggested investigations incorporated in a systematic assessment

	Reason:
Blood tests	
Full blood count, blood urea and electrolytes, liver function tests	General screen, peripheral blood eosinophil count
Inflammatory markers (C-reactive protein, erythrocyte sedimentation rate)	Intercurrent infection
Brain natriuretic peptide	Evidence of pulmonary hypertension or heart failure
Total serum IgE and specific IgE to common aeroallergens with additional tests according to history	Classifying atopic asthma, evidence of ABPA and identification of allergic triggers
Autoimmune profile (including antineutrophil cytoplasmic antibody)	To look for associated autoimmune conditions, such as Churg Strauss syndrome
Serum prednisolone and cortisol levels (2h post dose)	Assess adherence to medications – alongside checking prescription records
Theophylline assay (4–6h post dose)	Look for evidence of immune deficiency
IgG to pneumovax and haemophilus	Steroid induced diabetes/glucose intolerance
Fasting glucose and glycated haemoglobin (HbA1c)	
Pulmonary function tests	
Peak flow chart	Confirmation of asthma diagnosis and to establish reversibility, control and identification of type 1 or type 2 respiratory failure
Spirometry	
Lung volumes and gas transfer	
End capillary or arterial blood gas	
Bronchodilator reversibility	
Histamine/Methacholine PC ₂₀	
Skin prick testing	
To common aeroallergens: house dust mite, grass pollen, cat and dog dander, aspergillus fumigatus, cladosporium, alternaria, silver birch, mugwort	Classifying atopic asthma and identification of allergic triggers
Sputum analysis	
Culture and drug sensitivities	Identify any infections
Cell differential	Eosinophil and neutrophil percentage to help with diagnosis, response to corticosteroid treatment and phenotype patients
Radiology	
Chest radiograph	
High resolution computed tomography of thorax	Looking for changes consistent with asthma such as bronchial wall thickening, but additionally may identify alternative or coexistent diagnoses such as fibrotic lung disease or bronchiectasis
Computed tomography of sinuses	Usually requested following a formal ENT review
Dual energy X-ray absorptiometry scan	Particularly important for patients on maintenance oral steroids or requiring regular courses
Specialty review	
Ear-nose-throat	
Gastroenterology	
Psychiatry	
Health psychology	
Physiotherapy	
Immunology	
Additional tests	
Exhaled nitric oxide	May help identify eosinophilic associated airway inflammation
24h pH studies	Objective evidence of acid reflux
Sleep studies	Look for evidence of OSA, obesity related hypoventilation and other sleep disordered breathing problems
Transthoracic echocardiogram	Evidence of heart failure and pulmonary hypertension
Bronchoscopy	Usually performed for research purposes but can be useful in particular cases
Prednisolone absorption studies	In patients where prednisolone/cortisol levels are not clear

investigations, usually conducted at a tertiary centre with a special interest in severe asthma [11]. This assessment should be facilitated through a dedicated multidisciplinary difficult asthma service, by a team experienced in the assessment and management of difficult asthma [12]. At present, centres differ in the exact way that they investigate patients with severe asthma and there is no standardized protocol. These issues were discussed by an expert panel at the Consensus on Difficult Asthma Consortium UK (CODAC-UK) in 2005 [13] and a set of quality indicators for the definition and investigation of difficult asthma were identified. However, there has not been a widespread uptake of such a consensus driven approach. It is the opinion of the authors that this should be set-up, and that a minimum set of investigations be agreed as part of the management of patients with difficult-to-treat asthma.

This systematic assessment may be achieved over a period of 4–6 months of observation, following a series of coordinated investigations accompanying a detailed history and examination, followed by 6–8 weekly follow-up on an outpatient basis. This process is detailed in Fig. 1. The evaluation and treatment of patients with severe asthma may benefit from a multidisciplinary approach. Specialist asthma nurses, physiotherapist, dieticians, otorhinolaryngology clinic, health psychologists and psychiatrists can all play an important role. Patient education, evaluation of inhaler technique and creating an action plan should also form an integral part.

The importance of improving our understanding of the condition we have now defined as severe asthma

can be addressed alongside the systematic assessment. Some centres in the UK are already involved in the British Thoracic Society Difficult Asthma Network, which has a national registry, and others are contributing to more research-driven projects such as the 'Unbiased Biomarkers for the Prediction of Respiratory Disease Outcomes' (U-BIOPRED) study. Some of the investigations undertaken for severe asthma are at the interface between for clinical need and for research needs. In addition, for many of the investigations proposed, the cost-benefit implications remain unclear. With the current state of knowledge, a pragmatic view must be taken with regard to the clinical objective of the investigation being recommended, whilst recognizing the fact that patients with severe asthma should be investigated under research protocols so that such information can be used to improve our understanding of this disease.

The diagnosis of asthma in a patient with 'difficult-to-control' asthma

Confirming the diagnosis of asthma may be challenging given that a range of asthma treatments may have been used that could mask asthma biomarkers. Diagnostic errors are not unusual and reflect the fact that the diagnosis of asthma is commonly made on the basis of symptoms of wheeze and cough, and possibly on the pattern of these symptoms and their response to asthma therapies. Diagnoses that could mimic asthma should be excluded, as listed in Table 2.

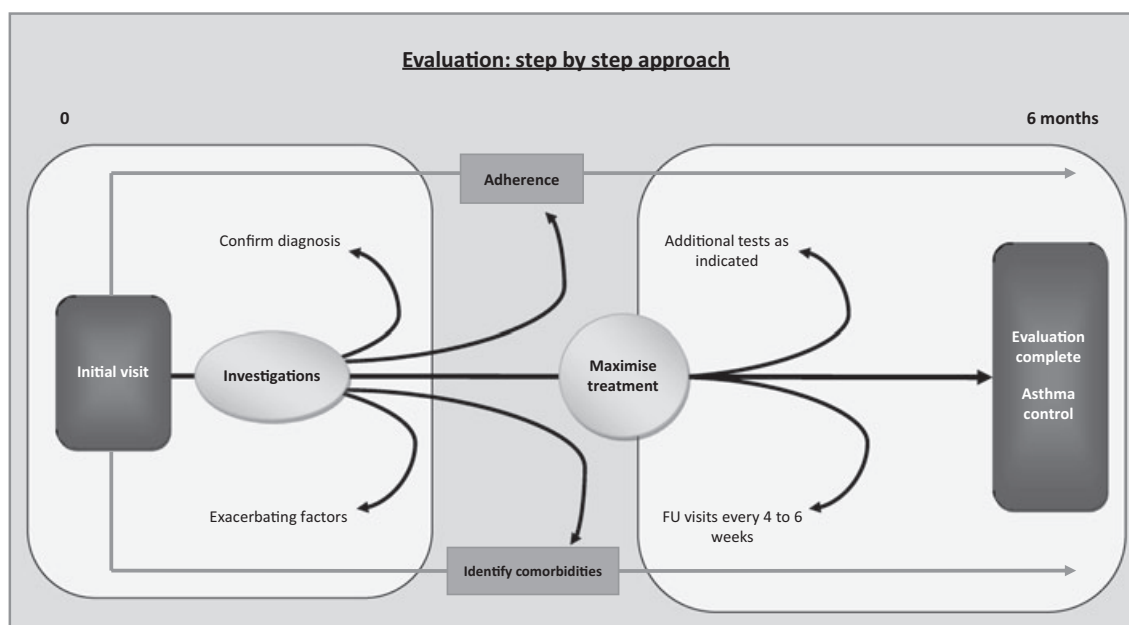


Fig. 1. Evaluation of severe asthma over the first 6 months.

Table 2. Alternative diagnoses and comorbidities in severe asthma

	May mimic asthma	Comorbidities
Dysfunctional breathing	×	×
Chronic obstructive pulmonary disease	×	
α 1-antitrypsin deficiency	×	
Allergic bronchopulmonary aspergillosis	×	
Churg–Strauss syndrome	×	
Obliterative bronchiolitis	×	
Heart failure	×	
Cardiomyopathy	×	
Eosinophilic syndromes	×	
Vocal cord dysfunction	×	×
Bronchiectasis	×	×
Cystic fibrosis	×	
Inhaled foreign body	×	
Large airway stenosis	×	
Lung cancer	×	
Sarcoidosis	×	
Pulmonary fibrosis	×	
Chronic cough syndromes	×	×
Adverse drug reaction e.g. angiotensin converting enzyme inhibitor associated cough	×	
Pulmonary emboli	×	
Rhinitis		×
Nasal polyps		×
Obstructive sleep apnoea		×
Recurrent respiratory infections		×
Laryngospasm	×	×
Psychiatric disorders		×

How does one ‘confirm’ the diagnosis of asthma? This is likely to be relatively straightforward in many cases on the basis and pattern of symptoms. In some cohorts, the diagnosis of asthma is only accepted with evidence of either bronchial hyperresponsiveness or of a bronchodilator response to β_2 -agonists of $\geq 12\%$ increase in FEV₁ or of evidence of spontaneous variation in peak expiratory flow of $\geq 20\%$. However, patients with severe asthma may not manifest any of these features that characterize asthma, because of medications that may normalize these features, the lack of bronchodilator response or the presence of irreversible airflow obstruction, which may be a feature of severe asthma. This particular group of patients may form a particular type of ‘severe asthma’ that needs to be investigated further.

Comorbidities and other factors contributing to poor asthma control

Comorbid conditions can influence asthma control and/or interfere with the therapeutic effect of treatments and are therefore important factors to identify and treat [14]. A list of comorbidities associated with asthma is

shown in Table 2. In severe asthma, the prevalence of certain comorbidities is considered to be high with a detrimental role on asthma control. Two previous cases series noted that co-existing disorders with asthma-like symptoms were found in 19% [10] and 34% [9] of patients with difficult asthma. In the Severe Asthma Research Program (SARP) project, there was an increase in GORD symptoms, aspirin intolerance, and reported history of sinusitis and pneumonia compared with non-severe asthma [15]. In a cohort of severe asthma patients with recurrent exacerbations, the reported comorbidities were severe nasal sinus disease, GORD, recurrent respiratory infections, psychological dysfunction and obstructive sleep apnoea [16], although this was not seen in a cohort of severe asthma with chronic airflow obstruction [17].

Fungal sensitization and allergic bronchopulmonary aspergillosis (ABPA)

Approximately two-thirds of asthmatic patients are atopic, demonstrating skin reactivity to common allergens [18]. Severe asthmatics have been reported to have a higher prevalence of atopy compared with other asthmatics [19] whilst sensitization to fungal allergens [20] and mould [21] may be associated with severe asthma attacks that require hospitalization. Sensitization to *Aspergillus fumigatus* in severe asthma is associated with bronchiectasis and increased airflow obstruction [22]. There appears to be a spectrum of fungal allergic disease encompassing ABPA and a group of patients with fungal sensitization who do not meet the criteria for ABPA. The term severe asthma with fungal sensitization (SAFS) has been used to define this phenotype of severe asthma [23, 24] and up to 25% of patients with ‘persistent’ asthma fall within this group [25]. One study reported that 5.6% of asthmatics seen at a referral clinic had ABPA [26] whilst another found that this rose to 14.3% in steroid dependent asthmatics [27]. However, the exact prevalence of ABPA in severe asthmatics remains unclear. The identification of patients with SAFS and ABPA may have therapeutic implications with the potential use of anti-fungal therapies, such as itraconazole and voriconazole in addition to glucocorticoids. The use of anti-IgE therapy (omalizumab) is additionally of interest in this group and awaiting further definitive clinical trials.

Rhinosinusitis and nasal polyps

Rhinosinusitis often coexists with asthma and the two may be part of the same disease spectrum [28]. More than 70% of participants recruited to two large asthma clinical trials reported symptoms of rhinosinusitis [29]. In a cohort of 382 severe asthma patients, 111 patients

(29%) had documented perennial rhinitis, 140 (36.6%) had seasonal rhinitis whilst 51 (13.4%) had a history of nasal polyposis [30]. The relationship between asthma and chronic rhinosinusitis is not explained by atopy alone and fungal exposure and nasal *Staphylococcus aureus* colonization may have a role in the pathophysiology [31]. Nasal polyps are associated with increased airway obstruction, increased inflammatory cells and a reduction in asthma control [32]. Rhinitis is associated with asthma severity and asthmatic patients with rhinitis are more likely to require emergency assessment for acute asthma and demonstrate a decreased asthma-related quality of life [33]. Two large retrospective studies found that the treatment of allergic rhinitis with topical steroid therapy resulted in improved asthma control [34]. However, other studies have failed to demonstrate this trend, and there is little data regarding severe asthma [35, 36].

Vocal cord dysfunction (VCD) and laryngospasm

Vocal cord dysfunction (VCD) is characterized by episodes of paradoxical inspiratory and/or expiratory adduction of the true and/or false cords [37]. The gold standard for VCD is laryngoscopic demonstration of paradoxical vocal cord movement (PVCMD). However, patients may have normal VC function between attacks and the diagnosis of VCD can be difficult. Patients may be misdiagnosed with asthma when they have VCD and additionally patients with asthma may have co-existent VCD making their symptoms worse and contributing to inappropriately high levels of asthma medications [38]. A recent study looking at the use of dynamic 320-slice computerized tomography of the larynx in difficult-to-treat asthmatics found that vocal cord movement was abnormal with excessive narrowing in 23 of 46 (50%) of patients with asthma. In nine patients (19%) this was classed as severe (abnormal >50% of inspiration or expiration time) [39]. Between 4% and 10% of patients referred to difficult asthma clinics have been diagnosed as having VCD [9, 38]. Therapeutic measures, such as speech and language therapy and in some selected cases, botulinum toxin injection, can be beneficial.

Associated with VCD is the condition that is sometimes labelled as laryngeal spasm which can present with an acute episode of upper airway obstruction and which can lead to collapse of the patient from apnoea and respiratory failure. Such patients may be intubated and ventilated, but usually for a very short period as there is little resistance to airflow in the lower airways. This condition is not well recognized and often these patients are treated with asthma medications including oral corticosteroids without effect. It probably represents an extreme form of vocal cord dysfunction, mimicking asthma attacks to some extent.

Gastro-oesophageal reflux disease (GORD)

Gastro-oesophageal reflux disease has been linked with cough, dyspnoea and wheezing. Acid reflux has been suggested as a cause of bronchoconstriction via microaspiration and additionally through indirect mechanisms, such as a vagally-mediated response to acid on the upper airways or oesophagus [40]. GORD is a common finding in severe asthma [41] and may be compounded by the use of oral corticosteroids. A meta-analysis of 28 studies found that GORD symptoms were reported in 59.2% of asthmatic patients [42]. A total of 158 patients from a cohort of 382 severe asthmatics (41.4%) had a history of GORD [30]. However, the direction of causality remains unclear.

Proton pump inhibition therapy in adults with symptomatic GORD and severe asthma has been associated with reduced exacerbations and an improved quality of life [43]. In addition, some studies have demonstrated improved FEV₁ and PEF rates with PPI use [44]. However, these improvements were small and unlikely to be of clinical significance. Patients who experience a benefit to their asthma control should continue on PPIs long-term whilst those who have an unsuccessful trial should continue to undergo further investigations, such as 24 pH monitoring and potentially a review with a gastroenterologist. In severe asthmatics with asymptomatic GORD the use of PPI's does not appear to lead to improved asthma outcomes [45].

Obstructive sleep apnoea (OSA)

Asthmatic patients report a higher prevalence of snoring and witnessed apnoeas compared with healthy controls [46] and OSA correlates with poor asthma control [16]. Factors underlying mechanisms include weight gain, adipokine imbalance, systemic inflammation (increased cytokine expression – in particular IL-6 and TNF- α), oxidative stress, lung mechanics, GORD, atopy and allergic rhinitis [47]. OSA is more prevalent in severe asthma compared with mild to moderate disease [48], which may be related more to obesity than the underlying obstructive airways disease [49]. Continuous positive airway pressure (CPAP) can lead to improved asthma quality of life [50], improved spirometry and lower bronchodilator requirements [51].

Psychiatric disorders

Anxiety, depression and panic disorders are more prevalent in asthmatic patients compared to healthy controls [52–54] although no correlation appears to exist for schizophrenia, bipolar disorder or personality disorders [14, 55]. There is a strong bidirectional association between uncontrolled asthma and psychiatric disorders

[56]. In addition, psychological comorbidities may reduce treatment adherence, follow-up appointments and compromise asthma control [14]. Frequent and high doses of systemic glucocorticoid therapy used in severe asthmatics may cause neurotoxicity and lead to cognitive impairments [57].

Patients with severe asthma exhibit greater psychological distress and difficulty coping with their disease compared with patients with moderate asthma [58]. Fifty-nine percent of patients (33 of 56) who underwent a consultant psychiatric review as part of their severe asthma assessment had a psychiatric component to their symptoms, of which just under a third were noted to have a major component [10]. Some studies found that cognitive behavioural therapy in asthma led to improved quality of life [59]. However, there is a paucity of data regarding psychological intervention in severe asthma.

Therapy-induced comorbidities

Many severe asthmatics will have received frequent high dose steroid courses for exacerbations and some may be on long-term maintenance steroids. The potential side effects encountered are listed in Table 3. The risk of vertebral and hip fractures occurs soon after commencing treatment and can occur with relatively small doses, such as 2.5–7.5 mg/day [60]. These conditions may be identified through a combination of assessments, including blood tests, optician assessment and use of dual energy X-ray absorptiometry scanning.

Adherence to medication

Poor adherence with prescribed medication is recognized as a significant factor in asthma, with adherence

rates ranging from 30% to 70% [61, 62]. One of the major issues relates to how adherence can be assessed. Direct questioning, checking pharmacy records to establish the amount and frequency of medications dispensed, and the use of monitored inhalers have been used. Prednisolone and cortisol level assays in patients on long-term prednisolone can also be utilised, although this way of assessing adherence is yet to be validated. Using this method, only 50% of patients (9 of 18) with severe asthma who were prescribed more than 15 mg/day were found to be compliant with prescribed oral steroid therapy [10]. Another study found that 23 of 51 patients (45%) who were prescribed oral steroids were found to be non-adherent based on plasma prednisolone and cortisol assay levels. Patients found to have poor adherence with inhaled therapy were more likely to be female, have a lower generic quality of life and had an increased risk of hospital admission [63]. The same study found that adherence to inhaled therapy can also be poor with 63 (35%) of 182 patients completing 50% or fewer of their inhaled medication prescriptions whilst 57 (88%) acknowledged low adherence following initial denial [63]. The reasons behind poor adherence are complex, but may be related to patient's fears of side-effects or of poor perceived benefits from the therapies, particularly if they have refractory asthma. However, this is an area that needs more attention [64].

Non-invasive inflammatory biomarkers

Fractional exhaled Nitric Oxide (Fe_{NO})

Although Fe_{NO} has been proposed to be a useful way to monitor asthma and prevent exacerbations, randomized controlled trials in patients with mild to moderate asthma have not supported this [65–67]. The role of Fe_{NO} in the investigation and follow-up of patients with severe asthma remains unclear.

In a group of difficult to treat asthmatics, levels of Fe_{NO} correlated with symptoms and rescue β -agonists but not with lung function [68]. In patients with severe asthma with persistent tissue eosinophilia despite oral corticosteroid therapy, Fe_{NO} correlates with eosinophilic inflammation [69]. In the SARP study, high levels of Fe_{NO} identified severe asthma patients characterized by the greatest airflow obstruction and hyperinflation and most frequent utilization of emergency care [70]. However, other studies have found that Fe_{NO} does not distinguish between asthma severity and levels exhibit broad heterogeneity in severe asthma [70–73]. This trend may be partly determined by differences in atopy and corticosteroid therapy.

The utility of using Fe_{NO} to monitor response to treatment has not been supported by studies of

Table 3. Steroid induced side-effects

Bone:	Cardiovascular:
Osteopenia/osteoporosis	Hypertension
Avascular necrosis	Hypercholesterolaemia
Skin and soft tissue:	Atherosclerosis
Purpura, striae	Psychiatric:
Hirsutism/hypertrichosis	Insomnia
Acne	Dysphoria
Cushingoid appearance	Psychosis
Eye:	Endocrine and renal:
Cataracts	Diabetes mellitus
Glaucoma	Adrenal suppression
Gastrointestinal and hepatic:	Fluid shifts and electrolyte imbalance
GORD	Other:
Peptic ulcer disease	Myopathy
Gastritis	Increased infection risk
Steatohepatitis	Intrauterine growth retardation
Pancreatitis	Amenorrhoea and infertility

mepolizumab in steroid-resistant eosinophilia, where mepolizumab-induced reduction of sputum eosinophilia was not accompanied by reductions in Fe_{NO} [74, 75]. In a longitudinal study of severe asthmatics over 5 years, patients with raised Fe_{NO} levels and normal lung function at baseline had an increased risk of accelerated decline in lung function compared with those with normal Fe_{NO} levels [76].

Raised Fe_{NO} in severe asthma may be related to more uncontrolled asthma and a greater risk of lung function decline. However, further studies are needed to determine the potential use of Fe_{NO} in severe asthma.

Sputum inflammatory cells

Both spontaneous and induced sputum have similar cellular and molecular characteristics in asthma. Sputum eosinophilia is a common finding in asthma, exhibited by 60–80% of patients with symptomatic untreated asthma and up to a half of patients taking inhaled corticosteroids [77, 78]. The sensitivity of sputum eosinophilia in diagnosing asthma (defined as consistent symptoms with objective evidence of abnormal variable airflow obstruction) is about 80% [79]. However, there is no convincing data that patients with severe asthma have higher levels compared with moderate asthmatics [71, 72, 80–82].

Some studies have shown that the use of induced sputum eosinophil count to titrate therapy can result in a lower exacerbation frequency without the need to increase treatment overall [83, 84]. However, whilst sputum eosinophilia has been proposed as a good marker of response to corticosteroid therapy in moderate asthma [84], there is often little suppression of eosinophils in severe asthma. This may reflect corticosteroid insensitivity and potentially may be overcome by the use of anti IL-5 therapy [75, 85].

A combined increase in sputum eosinophils and neutrophils has been reported in severe asthma [80, 82, 86]. This appears to be associated with reduced lung function, worse asthma control, increased symptoms and higher healthcare utilization [80]. The higher sputum neutrophil count in severe asthma [82, 87] may be associated with corticosteroid therapy, particularly systemic use [88, 89]. The use of sputum neutrophils as a marker of corticosteroid therapy requires further validation.

Airway neutrophils in severe asthma appear to be in an activated state with release of myeloperoxidase and leukotriene B₄ [90], and there may be the possibility that these represent innate immune responses to an altered airway microbiome that has been described in asthmatic airways [91]. Indeed, increased presence of bacterial infections has been reported in sputum samples of patients with severe asthma [92].

Investigations

Fibreoptic bronchoscopy

Fibreoptic bronchoscopy remains a procedure for research purposes, with examination of mucosal biopsies and of bronchoalveolar lavage cells and obtaining lung cells, such as epithelial cells, airway smooth muscle cells and macrophages, for *in vitro* studies. Alveolar macrophages have been used to demonstrate the presence of corticosteroid resistance [93]. Provided that criteria for bronchoscopic procedures are followed, this procedure has a high safety record when performed in specialized centres [94]. It is sometimes performed for diagnostic indications in patients with difficult-to-control asthma looking for clues that support a diagnosis of asthma, such as the presence of submucosal eosinophilic inflammation, or for any other alternative diagnoses that could mimic asthma.

Using endobronchial biopsies, both low and high eosinophil counts associated with neutrophilic severe asthma have been described; the high eosinophil counts being associated with more severe disease [95]. In a biopsy study of patients with severe asthma compared with non-severe asthma, no significant differences were noted in eosinophils, neutrophils, CD3⁺ T cells and mast cells between the groups, with counts ranging from zero to high levels [72]. These results raise the issue of the relationship between eosinophils and neutrophils in the different airway compartments, such as sputum, biopsy and bronchoalveolar lavage fluid. In this study, the only positive correlation found was between the percentage of eosinophils in bronchoalveolar lavage fluid and that in sputum [72]. Severe asthmatics display increased subbasement membrane thickening, and increased airway smooth muscle mass compared with non-severe asthmatics, a reflection of the excessive airway wall remodelling [72, 96, 97].

High resolution computed tomograms (HRCT) of thorax

High resolution computed tomograms of thorax has become a common place investigation in patients with severe asthma. It may detect associated conditions such as allergic bronchopulmonary aspergillosis, and others that may mimic asthma including hypersensitivity pneumonitis and eosinophilic pneumonia. HRCT scans are usually assessed qualitatively, although it is now possible to accurately measure large airway size and thickness. In addition, small airways can be assessed – reflected as changes in lung parenchyma, and the degree of air trapping. Bronchial wall dilatation and thickening have been described in asthma, and some studies indicate that these changes are related to severity of asthma and longer disease duration [98–101]. The

significance of this degree of bronchial wall dilatation and thickening remains unclear. Bronchiectasis may be a comorbidity, or a reflection of the airway wall remodelling changes associated with severe asthma, or perhaps both. In the Leicester study of their difficult asthma cohort, 62% and 40% of patients had bronchial wall thickening and bronchiectasis respectively. Other features noted were central bronchiectasis consistent with ABPA in up to 0.5%, ground glass shadowing in 45.3%, air trapping in 7.6% and emphysema in 8.1%; 20% of scans were considered normal [102].

Quantitative studies of HRCT scans in asthmatics of all severities have reported a correlation between airway wall thickness and submembrane basement membrane thickness on biopsy [103, 104]. Aysola et al. also showed a correlation between % wall area or % wall thickness and the subbasement membrane thickness in severe asthma [100]. How these measurements of airway wall thickness and luminal diameter relate to spirometric measurements, airway hyperresponsiveness and symptoms are unclear although in several small studies airway wall thickness correlated with airflow limitation, airway hyperresponsiveness and air trapping [100, 105, 106]. Using the right upper lobe apical segmental bronchus, Gupta et al. reported that % wall area was increased in severe asthma, but there was a decrease in lumen area expressed per unit of body surface area; the % wall area was associated with airflow limitation and persistent neutrophilic inflammation [107].

The amount of air trapping can be measured in various ways including measurements of lung density of lung cross-sectional area during inspiration and expiration, and this has been shown to be increased in asthmatics, particularly with airflow obstruction [108–111]. A significant relationship between air trapping, measured by a quantitative analysis, and asthma severity as defined by ATS criteria has been reported [109]. Those with significant air trapping had a greater likelihood of having a history of asthma-related hospitalizations, intensive care unit visits and/or mechanical ventilation compared with subjects without air trapping. This indicated that the degree of air trapping could be identifying a group at risk of severe exacerbations of asthma.

Lung function

One of the main features of severe asthma is airflow limitation which is likely to be due to airway smooth muscle contraction, mucosal airway oedema and mucus accumulation. However, it is likely the contribution of each of these components can vary from patient to patient. Spirometric measurements of FEV₁, FVC and FEV₁/FVC ratio are most commonly used. Airflow

limitation is considered to be reversible in asthma, but in large proportion of patients with severe asthma, up to 78% in the SARP cohort, have chronic fixed airflow obstruction; although this was also a feature of patients classified as moderate asthma [15]. In addition, pharmacological responsiveness to β -adrenergic agents may also be impaired. In a detailed analysis of lung function of the SARP cohort, airway obstruction was partitioned into components of air trapping and airflow limitation [112]. Severe asthma patients showed prominent air trapping, confirmed by the residual volume (RV) to total lung capacity ratio (TLC), and increases in TLC and functional reserve capacity. This air trapping was found to be a characteristic feature of severe asthma. The degree of airflow obstruction described in this SARP population has also been studied in other cohorts and linked also to the degree of air trapping, but additionally has been linked to the degree of airway wall remodelling and inflammation. Assessment of the small airways remains difficult, but lung function tests indicate early closure of airways and air trapping may be secondary to small airways obstruction. Thus, the reduced FVC and increased RV to TLC ratio found in severe asthma suggest airways obstruction [112], as supported also by radiological measurement of air trapping [109]. Use of the forced oscillation technique may differentiate between airway and tissue resistance [113].

Exacerbations as episodes of reversible airflow obstruction

In the SARP study, 54% of severe asthmatics needed three or more oral bursts of CS therapy to treat exacerbations in the past year, and these illustrate the acute reversible episodes of airflow obstruction in these patients. Use of self-recorded peak expiratory flow rate and more recently spirometric monitoring may confirm the degree of severity of these exacerbations [114]. The characterization of fluctuations in airway function may provide a quantitative basis for objective risk prediction of asthma episodes and also for evaluating the effectiveness of therapy. Chronic exacerbators with severe asthma have been shown to be associated with comorbid factors, such as severe sinus disease, gastroesophageal reflux, recurrent respiratory infections, psychological dysfunction and obstructive sleep apnoea [16]. Asthmatics with a history of frequent exacerbations have increased closing volume capacities between exacerbations [115], and in those with a history of intensive care visits or of mechanical ventilation, air trapping is more likely to be present on HRCT [109]. One particular form of exacerbation which remains to be clarified is the 'brittle' exacerbation which occurs very rapidly in a patient who usually has normal lung function [116].

Characteristics and heterogeneity of severe asthma

Many cohorts using an investigative protocol have now been described [15, 30, 117, 118]. In the characterization of the SARP cohort, that comprised of patients recruited from eight centres in US and one centre in the UK, the 'average' severe asthma patient was characterized by abnormal lung function that is responsive to bronchodilators, by a history of sinopulmonary infections, persistent symptoms and increased health care utilization [15]. Additionally, the SARP cohort provided some insight into risk factors including oxidative stress and some genetic factors for the development of severe asthma; airway inflammation, remodelling and lung structure-function relationships; together with inflammatory biomarker profiles [119–121]. Other cohorts have also shown similar profiles and characteristics (Table 4). The BTS severe asthma cohort involving patients recruited from four UK specialist centres showed that there are significant differences in terms of the investigative profiles of these patients, which could have arisen from the different population mix and perhaps the referral pattern of these difficult-to-treat patients [30].

Factors that may increase the risk of developing severe asthma are race, atopic background, obesity and cigarette smoking. The SARP study indicated that high IgE levels were associated with more severe asthma in blacks but not in Caucasians and that the odds of having severe asthma more than doubled for black subjects with two or more family members with asthma [122]. This is supported by the recent report of a new susceptibility gene locus specifically associated to asthmatic individuals of African descent [123]. Obesity has been associated with more severe asthma and the fact that obesity antedates the diagnosis of asthma raises the possibility that it may contribute to the pathogenesis of asthma [124]. SARP data indicates that obesity should be considered as a comorbidity that affects asthma, particularly in early onset disease [125]. Similarly, environmental tobacco smoke exposure was associated with worse lung function, higher number of exacerbations,

more health care utilization and greater bronchial hyperreactivity [126]. Severe asthmatics who are cigarette smokers may develop corticosteroid insensitivity [127, 128].

Phenotyping towards personalized medicine

As the definition of severe asthma has relied on the non-response or partial response to asthma treatments particularly corticosteroid therapy, it is not surprising that the clinical presentation and natural history of severe asthma can be very varied. It is important to understand this variability in the context of severe asthma because certain types of asthma may respond better to certain specific therapies. Different types of severe asthma may represent different pathophysiological mechanisms to which certain specific therapies may work better. Different approaches are possible, and clinical phenotypes can be described based on observations and analysis of groups of patients either using biased or unbiased approaches.

To identify unique clinical phenotypes, an unsupervised hierarchical cluster analysis was performed on all adult SARP mild to severe patients, allowing grouping of patients based on similarities free from *a priori* bias. Using 34 qualitative and quantitative variables that included age of asthma onset and duration, sex, race, lung function, atopy and questionnaire data, five clusters were revealed that were differentiated through onset of asthma symptoms, baseline lung function, bronchodilator response and use of beta-agonist medication. Around 40% of these asthmatics had early onset asthma with an allergic background and mild to severe disease. Late onset, usually non-atopic female asthmatics with a reduced FEV₁ and frequent use of oral corticosteroids formed the rest of the clusters [129].

In another cluster analysis that included sputum eosinophilia as a measure of asthmatic inflammation, two clusters specific to severe refractory asthma were defined in relation to airway inflammation with eosinophils and symptom expression, with those that show concordance and those that do not [130]. On the basis of these two cluster analyses, it could be proposed that there are the following phenotypes of severe asthma: (i) an early onset severe asthma with an allergic background (ii) a late onset asthma with persistence of eosinophilic inflammation despite taking adequate corticosteroid therapy and (iii) a later onset asthma with very uncontrolled asthma particularly in females and associated with obesity, that is less eosinophilic.

Although these initial cluster analyses have been useful in confirming the existence of specific phenotypes, they still remain very descriptive. Phenotypic classification of asthma should be able to provide insights into pathogenesis, identify responders to asthma treatments

Table 4. Characteristics of severe asthma

Increased health care utilization
Atopic vs. non-atopic
Early onset vs. late onset
Obesity-associated
Female > Male
Cigarette smoke-associated
Chronic airflow obstruction
Recurrent exacerbations (≥ 3 per year)
Corticosteroid resistance
Impaired bronchodilator response
Eosinophilic vs. non-eosinophilic or neutrophilic

(particularly the specifically targeted therapies) and additionally enable the prediction of long-term prognosis and disease progression. This is where the contribution of biomarkers of asthma will become useful in adding different dimensions to the analysis of phenotypes. Linking clinical characteristics with biomarkers of inflammation, genetic analysis and imaging, should eventually provide a framework for a more comprehensive approach to subject characterization in severe asthma and potentially to stratified management. One of the recent initiatives that have taken an overall unbiased approach to hand-printing severe asthma is the U-BIOPRED project which is funded by the European Innovative Medicines Initiative [131]. The most innovative aspect of UBIOPRED is the application of comprehensive and unbiased functional genomics and

proteomics to provide a more integrated unbiased approach to increasing our understanding and definition of new disease phenotypes using a vast array of biomarkers. This will facilitate the development of novel diagnostic and therapeutic interventions targeting multiple components of the molecular and cellular pathways involved, which can be tested and validated in prospective clinical trials, and are a step towards personalized medicine for severe asthma.

Conflict of interest

KFC has received funds for research from GSK, traveling grants from Boehringer Ingelheim and Novartis, and consulting fees from GSK, Novartis and Gilead. DG reports no conflict of interest.

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