

The reclassification of asthma based on subphenotypes

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Purpose of review

Despite asthma being widely regarded as a heterogeneous disease, a consensus for an accurate system of classification has not been agreed. Recent studies have suggested that the recognition of subphenotypes of asthma based on the pattern of airway inflammation may be particularly useful in increasing our understanding of the disease. The present review discusses the important literature in this field, placing current work in its historical context.

Recent findings

The use of noninvasive markers of airway inflammation has suggested the presence of four distinct phenotypes: eosinophilic, neutrophilic, mixed inflammatory and paucigranulocytic asthma. Recent studies suggest that these subgroups may differ in their aetiology, immunopathology and response to treatment. Several studies have focused on refractory asthma as a distinct phenotype with evidence of a more distal pattern of airway inflammation and of upregulation of the tumour necrosis factor- α axis. Finally, novel treatment approaches targeted at specific patterns of airway inflammation are emerging, making an appreciation of subphenotypes particularly relevant.

Summary

The present review will discuss limitations to current classification systems, identify key current studies based on identifying inflammatory subphenotypes and provide suggestions for a novel approach that may further improve our understanding in this area.

Keywords

asthma, classification, inflammation, phenotypes, refractory

Introduction

Asthma has long been recognized as a heterogeneous disease. Recognition of specific subphenotypes may further our understanding of pathophysiology, treatment response, prognosis and the underlying genetic basis for the disease. An accurate and comprehensive system of classification therefore remains an important priority for asthma research, despite the effort of many clinicians over several generations.

Historical perspective

In the early twentieth century, Rackemann [1] proposed classifying asthma into 'extrinsic' and 'intrinsic asthma' based on aetiology, a concept that remains widely used. Additional asthma phenotypes based on causation have been subsequently identified, including aspirin-sensitive and occupational asthma. In the 1970s, Turner-Warwick [2] described subgroups of patients with asthma characterized by differing patterns of airflow obstruction including 'brittle asthma', 'irreversible asthma' and 'the morning dipper'. Ayres and colleagues [3] later recognized two distinct subgroups of brittle asthma based on the nature of exacerbations while the concept of 'irreversible' asthma persists with the observation of accelerated decline in lung function leading to fixed airflow obstruction in a minority of patients [4]. Asthma management guidelines tend to categorize asthma according to disease severity, usually based on the amount of treatment required to achieve control of symptoms and lung function [5–6]. Such an approach may be limited by the fact that access to healthcare along with patient and physician behaviours contribute to medication use as well as the underlying severity of the disease.

Alternative methods of classifying asthma based on the clinical presentation of the disease have been proposed. A subgroup of patients who appear to be at risk of more severe and frequent exacerbations has been described [7•]. There is, however, evidence to suggest that this pattern of exacerbations relates to the nature and extent of underlying airway inflammation [8,9••] indicating that quantitation of the inflammatory response is required to tease out these phenotypes. A distinction based on the age of onset of disease has also been suggested, but studies confined to children have also shown marked clinical and inflammatory heterogeneity [10], limiting the value of this approach.

More recently, classification of asthma according to the nature of the underlying airway inflammation has been

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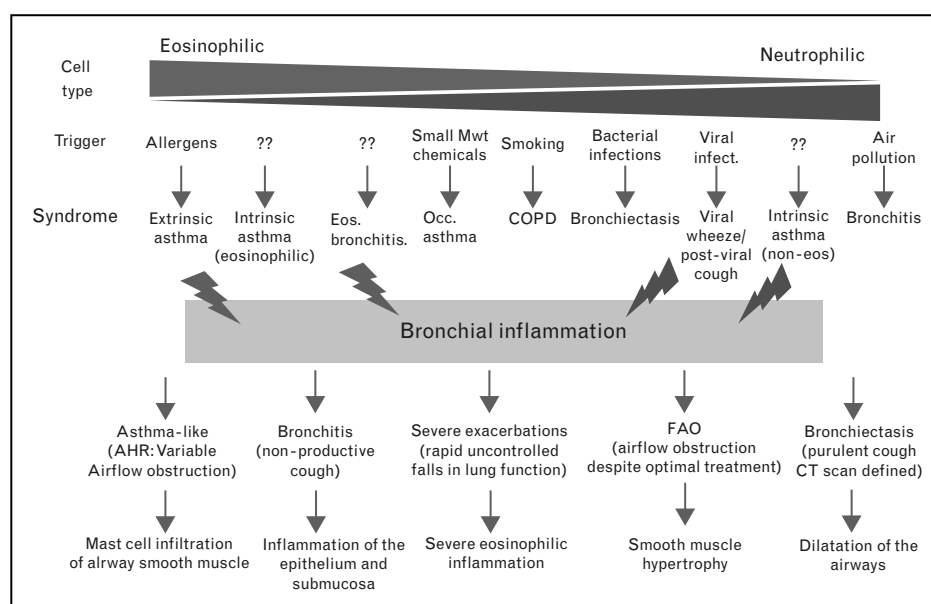
Abbreviations

MMP-9 matrix metalloproteinase-9
TNF tumour necrosis factor

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Figure 1 Schematic diagram illustrating the heterogeneity of airways disease in terms of triggers, pattern of airway inflammation, associated diseases, airway physiology and the specific underlying pathological abnormality

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suggested [11,12,13**] (Fig. 1). Importantly, new evidence is emerging to suggest that the identification of such inflammatory phenotypes may help to guide the management of asthma for individual patients. The present review aims to discuss the evidence provided by recent studies that may help to reclassify asthma, concentrating on inflammatory phenotypes and on studies that support the use of phenotype-specific asthma management. Limitations to our current classification systems will be outlined and suggestions provided for a novel approach that may enhance our understanding in this area.

Eosinophilic asthma

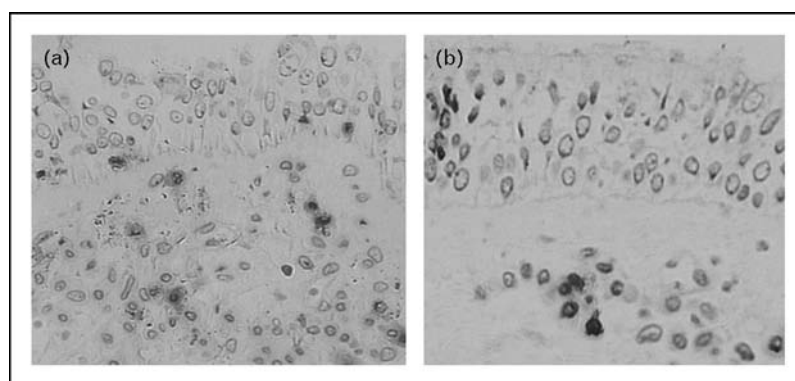
Early bronchoscopy studies examining the immunopathology of patients with mild asthma suggested that eosinophilic airway inflammation was the characteristic abnormality [14–16]. This was thought to be the case in both atopic and nonatopic asthma [17–20], aspirin-sensitive asthma [21] and occupational asthma [22]. On the whole, these studies reported a broad correlation between clinical asthma severity and the degree of airway eosinophilia, particularly when the eosinophils appear activated [14,23]. The development of the technique of sputum induction [24,25*] which has allowed the characterization of more diverse patient populations has revealed a complex relationship between eosinophilic inflammation and other asthma markers. Studies comparing the correlation between sputum eosinophilia and lung function [26,27] have been inconsistent and airway hyperresponsiveness and eosinophilia appear to be independent components of the asthma phenotype [28]. In contrast, eosinophilic airway inflammation appears to be

much more closely related to the risk of severe asthma exacerbations [8,9**,29].

Noneosinophilic asthma

The use of induced sputum [11,30,31**], along with small bronchoscopy studies of patients with severe asthma [12], have clearly demonstrated that eosinophilic inflammation is not universally present (Fig. 2). In a study of 23 patients with asthma treated with as-required β_2 adrenoceptor agonists alone, nine had induced sputum eosinophil counts of <3%. The eosinophilic and noneosinophilic groups had similar lung function, peak flow variability and symptom severity but noneosinophilic patients were less likely to be atopic, less hyperresponsive and more likely to be current smokers [11]. We repeated this assessment in 259 patients with symptomatic asthma receiving treatment at steps 1–3 of the British Thoracic Society guidelines and found considerable heterogeneity in induced sputum cell counts, particularly in nonatopic patients. We identified a subgroup of 60 patients with a distinctive sputum cell profile with a sputum neutrophilia and a normal sputum eosinophil count; 35 of these patients were corticosteroid naïve. These subjects were older, predominantly female and more likely to be non-atopic but otherwise had similar clinical and physiological features to the group as a whole [30]. Previous studies have noted neutrophilic inflammation in some patients with severe asthma [32,33] and in small numbers of patients studied during asthma exacerbations [34]. Gibson and colleagues [35] reported that 59% of patients with persistent asthma taking high doses of inhaled corticosteroids had suppressed sputum eosinophil counts but evidence of neutrophilic inflammation. Wenzel *et al.*

Figure 2 Examples of submucosal staining for (a) eosinophils ($\times 100$) and (b) neutrophils ($\times 100$) from subjects with eosinophilic and noneosinophilic asthma respectively



[12] performed a bronchoscopy study of patients with severe asthma treated with high doses of inhaled and oral corticosteroids and described a subgroup with a predominant neutrophilic airway inflammation, absence of eosinophils and normal subreticular basement membrane thickness. Intense neutrophilic inflammation has also been demonstrated in patients ventilated for acute severe asthma [36] and in those who die suddenly of asthma [37]. It is not known whether this neutrophilic asthma phenotype is particularly associated with more severe disease, however, as the effects of corticosteroid treatment may be confounding.

Recent work has furthered our understanding of the 'noneosinophilic' phenotype. Simpson and colleagues [31^{••}] carefully assessed the inflammatory profile of 93 patients with asthma of varying severity and identified four distinct inflammatory phenotypes. The largest group (41% of patients) had eosinophilic inflammation but normal neutrophil counts. Twenty per cent of patients had an isolated sputum neutrophilia while a further 8% had increased levels of both eosinophils and neutrophils. Finally, 31% of patients were said to have 'paucigranulocytic asthma' with sputum cell counts within the normal range. Subjects with increased sputum neutrophils were older but the four groups were otherwise clinically similar. Repeated measurements in a proportion of patients suggested that the noneosinophilic phenotype remains stable over both the short (4 weeks) and longer term (5 years). The authors also studied the activity of proteolytic enzymes in induced sputum from the same patients, grouping those with mixed inflammation and neutrophilic inflammation together. They found that whereas the eosinophilic phenotype was associated with high levels of matrix metalloproteinase-9 (MMP-9) activity, neutrophilic patients had significantly higher neutrophil elastase activity with inactive MMP-9. Proteolytic enzyme activity in paucigranulocytic patients did not differ from healthy controls [38[•]]. It is possible that

this heterogeneity in inflammatory response could lead to different levels of repair and remodelling between phenotypes. This suggestion is supported by the finding of a negative correlation between sputum neutrophil count and FEV₁/FVC ratio [31^{••}], and the absence of collagen deposition in the subreticular basement membrane in neutrophilic patients [12].

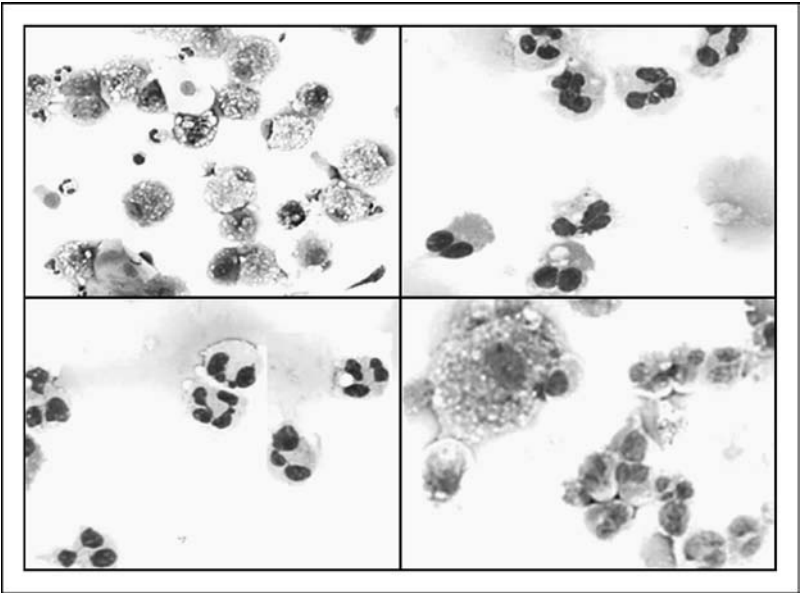
Whether the distinct inflammatory phenotypes occur as a result of different aetiological pathways remains unclear, although recent studies may provide some support for this concept. In neutrophilic asthma there is evidence that the innate immune system is more activated with increased expression in sputum of the receptors TLR2, TLR4 and CD14, and the pro-inflammatory cytokines IL-8 and IL-1 β in association with higher levels of endotoxin [39^{••}]. Furthermore, subjects with eosinophilic asthma were found to produce significantly higher concentrations of sputum IL-4 than patients with noneosinophilic asthma or healthy controls, suggesting a Th2 cytokine predominance. In addition, the eosinophil group produced lower levels of TNF- α than the noneosinophilic group, suggesting that treatment with agents inhibiting the TNF- α pathway may not be helpful in this group [40]. In this study, patients with neutrophilic inflammation were equally distributed between the eosinophilic and non-eosinophilic groups, highlighting the lack of consistency between studies in the characterization of phenotypes. We would suggest that future studies should consider stratification into 'eosinophilic', 'neutrophilic', 'mixed inflammatory' and 'paucigranulocytic' as suggested by Gibson and colleagues [31^{••}] (Fig. 3) [25].

Refractory asthma as a distinct inflammatory phenotype

A small proportion of patients with asthma have 'refractory' disease, which remains difficult to control despite usual treatment (Table 1) [41]. A greater understanding of the inflammatory phenotype would be particularly

Figure 3 Sputum cytopspins from different subjects with asthma illustrate the heterogeneity of the airway inflammation

In the upper left panel the predominant cells are macrophages with a normal neutrophil and eosinophil count, this cytopspin cannot be distinguished from a sample from a healthy control [paucigranulocytic asthma ($\times 100$)]; upper right panel combined neutrophilic and eosinophilic inflammation ($\times 400$), lower left neutrophilic inflammation ($\times 400$), lower right eosinophilic inflammation ($\times 400$) [25].



helpful as this group of patients experience considerable morbidity and consume substantial healthcare resources. While eosinophilic and neutrophilic inflammation have both been described in patients with severe asthma as already discussed, it is possible that some pathological features are characteristic of a ‘refractory phenotype’. We measured the alveolar nitric oxide (NO) concentration in patients with asthma across the spectrum of severity [42^{••}]. Alveolar NO concentrations were significantly higher in patients with refractory disease and were reduced by treatment with oral but not inhaled corticosteroids, suggesting that asthma refractory to standard treatment may be characterized by distal airway inflam-

mation. The finding of intense alveolar inflammation in 20 subjects dying of fatal asthma supports this [43[•]]. We have also shown that refractory asthma is associated with an upregulation of the tumour necrosis factor (TNF)- α axis, as demonstrated by increased expression of membrane-bound TNF- α , TNF- α receptor 1, and TNF- α converting enzyme by peripheral blood monocytes. The same patients had significantly improved measures of asthma control following a placebo controlled double-blind crossover study of treatment with soluble TNF- α receptor etanercept (Fig. 4) [44^{••}]. This is consistent with the finding of increased mucosal TNF- α expression in severe asthma compared with mild asthma [45[•]]. Thus

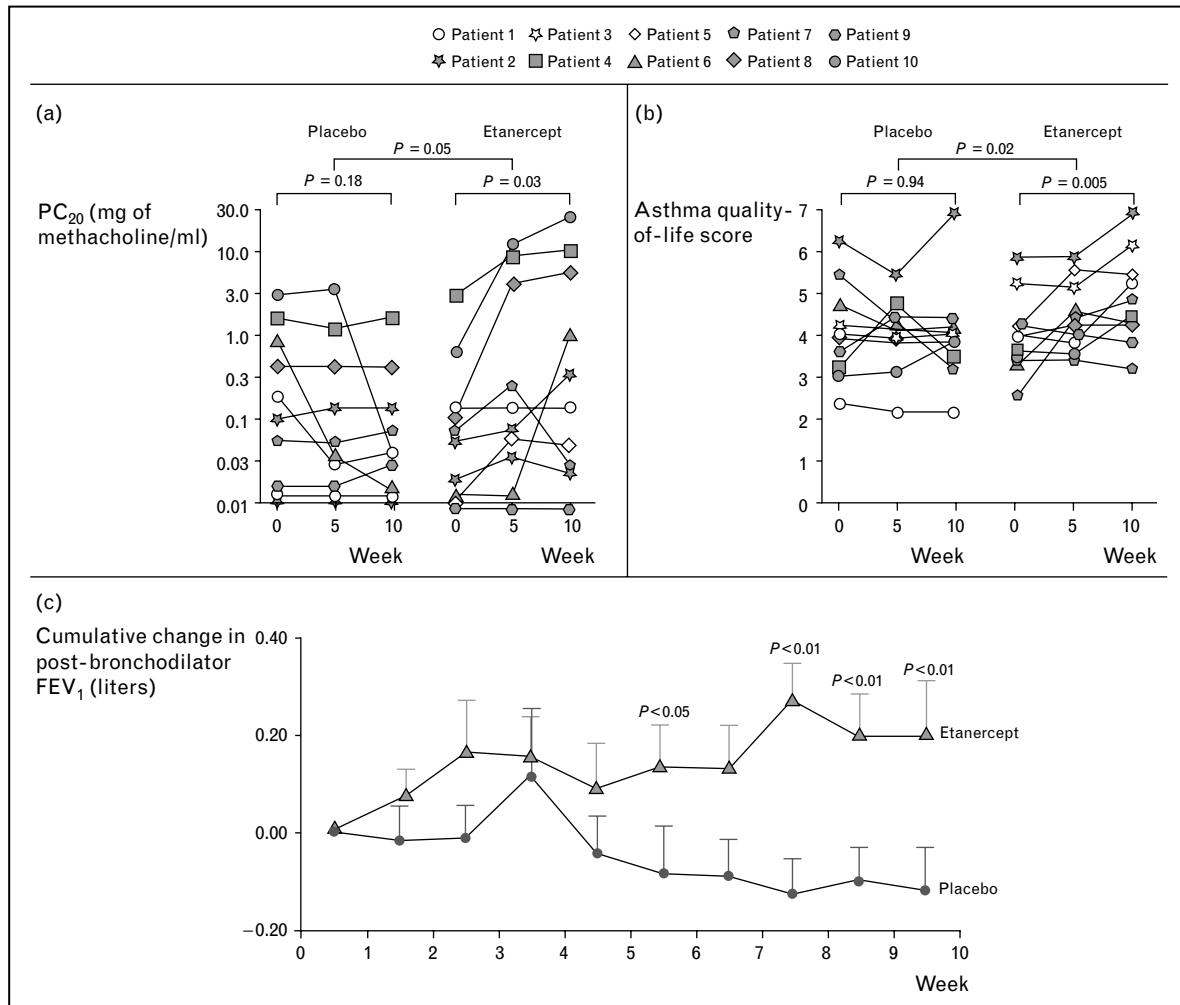
Table 1 American Thoracic Society definition for refractory asthma^a

Major characteristics		
Drug	Dose (μ g/day)	Dose (puffs/day)
a. Beclomethasone dipropionate	>1260	>40 puffs (42 μ g/inhalation >20 puffs (84 μ g/inhalation
b. Budesonide	>1200	>6 puffs
c. Flunisolide	>2000	>8 puffs
d. Fluticasone propionate	>880	>8 puffs (110 μ g), >4 puffs (220 μ g)
e. Triamcinolone acetanide	>2000	>20 puffs
Minor characteristics		
1. Requirement for daily treatment with a controller medication in addition to inhaled corticosteroids, e.g. long-acting agonist, theophylline, or leukotriene antagonist		
2. Asthma symptoms requiring short-acting agonist use on a daily or near daily basis		
3. Persistent airway obstruction (FEV1 < 80% predicted; diurnal PEF variability > 20%)		
4. One or more urgent care visits for asthma per year		
5. Three or more oral steroid ‘bursts’ per year		
6. Prompt deterioration with 25% reduction in oral or inhaled corticosteroid dose		
7. Near fatal asthma event in the past		

In order to achieve control to a level of mild-moderate persistent asthma: (1) treatment with continuous or near continuous (50% of year) oral corticosteroids; (2) requirement for treatment with high-dose inhaled corticosteroids. Reproduced with permission [41].

^a Definition of refractory asthma requires one or both major criteria and two minor criteria

Figure 4 Concentration of inhaled methacholine causing a 20% decrease (PC_{20}) in the forced expiratory volume (FEV_1) (a) and asthma quality-of-life scores (b) before, during and after 10 weeks of etanercept or placebo and the cumulative mean ($\pm$ SE) change in FEV_1 after the inhalation of 200 μ g of albuterol each week during the 10-week treatment trial (c)



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the identification of a characteristic refractory phenotype may identify specific therapeutic targets.

While 'refractory' asthma may be considered a discrete subphenotype with distinct pathophysiological abnormalities not seen in milder disease, severe asthma is particularly likely to be heterogeneous in its patterns of causation, symptoms, clinical airway inflammation and response to treatment [46*,47*]. Future studies aiming to characterize asthma phenotypes should be targeted at this severe population.

Phenotype specific asthma management

Reclassification of asthma is only worthwhile if it provides new insights about the aetiology of disease, helps to predict prognosis or if it identifies phenotypes which are more likely to respond to a particular treatment or management strategy. The development of novel therapeutic

agents for asthma provides an important stimulus for asthma phenotyping as the expense and specific nature of many of these drugs mean that only a minority of patients are likely to respond. The challenge is in identifying the responsive group.

Randomized controlled trials of the monoclonal anti-IgE antibody omalizumab have shown an improvement in asthma control with reduction in exacerbation frequency in patients with symptomatic atopic asthma who had at least one positive skin-prick test and elevated serum IgE levels [48–50]. Other than the presence of atopy, it is not known whether patients with a specific asthma phenotype are more likely to respond to treatment. One recent analysis of pooled data from seven studies of omalizumab suggested that patients with severe asthma characterized by persistent airflow obstruction, a need for high-dose inhaled corticosteroids and

frequent asthma exacerbations were more likely to benefit [51[•]], but further work linking response to clinical phenotype is required.

There is more evidence to suggest that the inflammatory phenotype predicts response to corticosteroids. The identification of an airway eosinophilia has been consistently associated with a favourable response to corticosteroid therapy in asthma [11,30,52[•]] and an increased risk of exacerbation following corticosteroid withdrawal [53]. Where patients have a sputum eosinophilia despite high-dose inhaled or oral corticosteroids, the use of intramuscular triamcinolone has been shown to control the inflammation and improve symptoms [54]. It is likely that the persistent inflammation can, at least in part, be explained by poor compliance with treatment. In contrast, noneosinophilic asthma is associated with a significantly poorer response to treatment with inhaled corticosteroids [11,30].

Recent studies have also supported a role for classification of asthma according to immunopathological phenotype in improving outcome using management strategies that aim to control airway inflammation. In a randomized controlled trial of 74 patients with moderate to severe asthma, we compared a management strategy targeted at reducing the induced sputum eosinophil count to traditional management based on symptoms and peak flow readings. Over 12 months, patients managed according to a strategy that aimed to keep the sputum eosinophil count <3% experienced significantly fewer severe asthma exacerbations and hospital admissions than patients treated by standard clinical guidelines (Fig. 5) [8]. A similarly designed multicentre study randomized 107 patients to sputum guided or clinically guided treatment. The sputum strategy delayed the occurrence of the first exacerbation and reduced exacerbation frequency but interestingly the benefit was largely confined to patients with more severe asthma and to the prevention of eosinophilic exacerbations

[9^{••}]. Thus, knowledge of the asthma phenotype may usefully predict the likely success of inflammation-targeted management in individual patients.

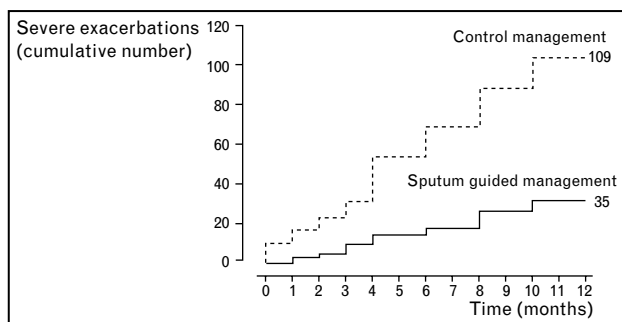
The practical limitations of sputum induction and processing have led to an interest in the use of exhaled NO in guiding asthma management. In a study of 97 patients comparing conventional management and management targeting exhaled NO, Smith and colleagues [55^{••}] found no significant reduction in exacerbation frequency in patients randomized to NO management, although the final mean daily dose of inhaled corticosteroid was lower in the NO group. We have confirmed these findings in a study of 118 patients managed in UK primary care in which patients treated according to NO measurements had similar rates of asthma exacerbations to patients treated in the traditional way [56[•]]. These findings suggest either that management strategies targeted at airway inflammation are not superior to clinical assessment in patients with milder disease or that exhaled NO does not accurately characterize the inflammatory phenotype.

Limitations of current classification

A number of limitations to our current systems of asthma classification remain. Whether based on aetiology, pattern of airflow obstruction or on the inflammatory phenotype, each method is largely descriptive and by necessity is focused on a single aspect of the disease. This represents a gross oversimplification, as asthma is a disease characterized by heterogeneity both between and within individuals. Indeed, the definition of asthma remains descriptive and includes a number of features including chronic inflammation of the airways, airway hyperresponsiveness, symptoms of wheeze, breathlessness, chest tightness and cough and variable airflow obstruction [6]. Each of these dimensions will be important to differing degrees between patients and will vary over time. This complexity has led to the recent suggestion that the term 'asthma' be abolished in favour of better characterization of the distinct disease processes that present as asthma [57^{••}]. One way to try to make sense of this complexity is to identify distinct patterns using a method that incorporates the many dimensions of the disease, for example by analysing large asthma populations using multivariate techniques.

Cluster analysis is one such multivariate statistical tool that seeks to organize information about variables so that heterogeneous groups of subjects can be classified into relatively homogeneous subgroups, or 'clusters'. The groupings are constructed such that the degree of association is strong between members of the same cluster and weak between members of different clusters. By including large numbers of variables, this approach may reveal associations between variables that were not previously evident and lead to the identification of distinct novel

Figure 5 Cumulative asthma exacerbations during a 12-month randomized trial of asthma management guided at controlling sputum eosinophilia compared with conventional management



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phenotypes. We have used the technique of cluster analysis to identify subphenotypes within a population of patients with severe asthma attending our clinic [58[•]]. We collected data on over 270 patients with severe asthma and analysed a large number of variables describing different aspects of the disease. Cluster analysis identified four distinct phenotypes: a population of patients with relatively well controlled symptoms and minimal airway inflammation; a group with early onset atopic asthma who had severe symptoms, persistent airway inflammation and markedly variable airflow obstruction; a third group of mainly women who had late onset asthma with marked symptoms but minimal eosinophilic inflammation, many whom were obese; and finally a group with a male preponderance who had late onset asthma characterized by persistent eosinophilic inflammation in the absence of symptoms [58[•]]. It is possible that the different management strategies may be appropriate for the distinct subgroups. Those with marked inflammation but minimal symptoms may particularly respond to a management strategy targeting sputum eosinophilia, for example, while patients whose symptoms relate closely to airway inflammation may be particularly suited to a treatment regime of inhaled budesonide in combination with formoterol for symptom relief [59[•]].

Conclusions

Novel methods of reclassifying asthma based on characterising the pattern of airway inflammation have already led to improved patient outcomes with the use of specifically targeted therapies and management strategies. Given the complex multidimensional nature of asthma, a number of questions remain. We suggest that multivariate analysis may transform our understanding in this field by recognizing distinct subphenotypes which are associated with a potentially worse prognosis but for which specific treatment approaches may be particularly effective.

References and recommended reading

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Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 113–114).

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