

REVIEW

Multi-dimensional phenotyping: towards a new taxonomy for airway disease

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Summary

All the real knowledge which we possess, depends on methods by which we distinguish the similar from the dissimilar. The greater the number of natural distinctions this method comprehends the clearer becomes our idea of things. The more numerous the objects which employ our attention the more difficult it becomes to form such a method and the more necessary. [1].

Classification is a fundamental part of medicine. Diseases are often categorized according to pre-20th century descriptions and concepts of disease based on symptoms, signs and functional abnormalities rather than on underlying pathogenesis. Where the aetiology of disease has been revealed (for example in the infectious diseases) a more precise classification has become possible, but in the chronic inflammatory diseases, and in the inflammatory airway diseases in particular, where pathogenesis has been stubbornly difficult to elucidate, we still use broad descriptive terms such as asthma and chronic obstructive pulmonary disease, which defy precise definition because they encompass a wide spectrum of presentations and physiological and cellular abnormalities. It is our contention that these broad-brush terms have outlived their usefulness and that we should be looking to create a new taxonomy of airway disease—a taxonomy that more closely reflects the spectrum of phenotypes that are encompassed within the term airway inflammatory diseases, and that gives full recognition to late 20th and 21st century insights into the disordered physiology and cell biology that characterizes these conditions in the expectation that these will map more closely to both aetiology and response to treatment. Development of this taxonomy will require a much more complete and sophisticated correlation of the many variables that make up a condition than has been usual to employ in an approach that encompasses multi-dimensional phenotyping and uses complex statistical tools such as cluster analysis.

Introduction

Asthma is a condition characterized by variable airflow obstruction, airway hyper-responsiveness (AHR) and airway inflammation (although only generally defined by the first two of these), which in its classical presentation is readily recognized and defined. However, it is also a condition that in the clinic is heterogeneous in its appearance. The situation is further complicated by asthma being just one of a number of conditions in which there is a disorder of the conducting (as opposed to the gas exchange) compartment of the lung. The most familiar of these conditions is smoking-related chronic obstructive pulmonary disease (COPD); but there are many others such as bronchiectasis, viral wheeze, eosinophilic bronchitis and obliterative bronchiolitis [2]. These conditions, while each having distinctive features, share overlapping clinical features and pathophysiology. The relationship between these conditions is illustrated schematically in Fig. 1.

Diagnostic terms such as asthma, COPD and chronic bronchitis were originally coined to categorize a clinical presentation that appeared to comprise a more or less distinct disease process [3]. These labels were affixed in a subjective manner onto relatively non-specific symptoms and signs such as cough, wheeze and breathlessness, and in some cases physiological abnormalities. As medical science progressed and our knowledge of the objective and more precisely measurable physiological, pathological and molecular abnormalities associated with disease processes became known, the general approach to airway disease has been to try and match these abnormalities to the original disease classification rather than start with the objective abnormalities themselves as the basis for classification. Disease classification is robust when it represents a discrete entity, when the label accurately maps to the underlying disorder. The label can then be used as a shorthand for standardizing disease management and research into pathogenesis and drug development. For example, tuberculosis is a disease in which there is a clearly defined cause to which all the abnormal clinical features and pathophysiology can be traced. Similarly with cystic fibrosis. In each case, we are dealing with a single disease process. It is instructive to note that even in these coherent and clearly circumscribed diseases, there is a wide variation in clinical

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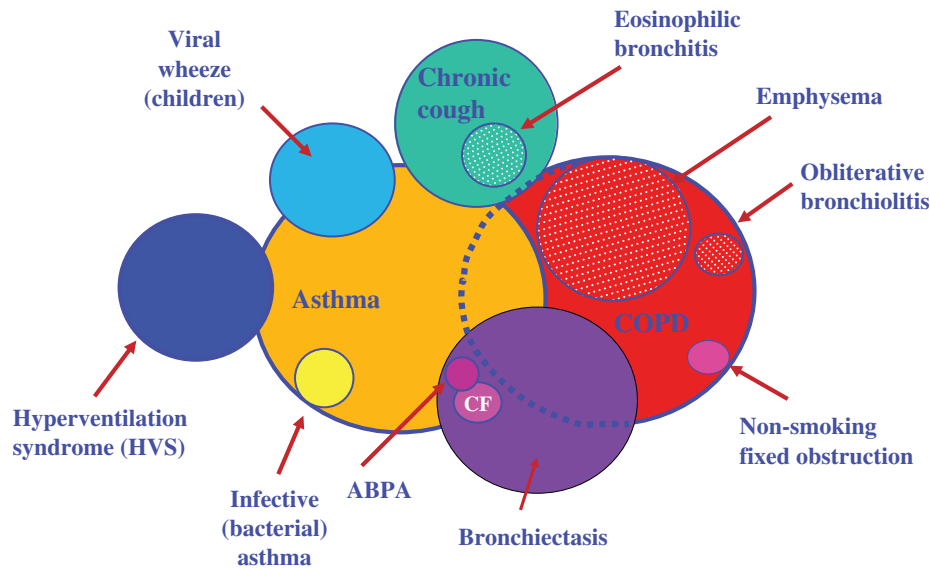


Fig. 1. Schematic diagram illustrating the overlapping relationship between syndromes characterized by disordered airway function. For example, the asthma label includes some but not all children with viral-induced wheeze, subjects with cough as their predominant symptom, subjects with significant smoking history and a degree of fixed airflow obstruction [chronic obstructive pulmonary disease (COPD)], who have features such as steroid responsiveness, eosinophilic airway inflammation and marked reversibility, more associated with an asthma phenotype, and patients with bronchiectasis, a subset of whom will have allergic bronchopulmonary aspergillosis (ABPA) and/or cystic fibrosis (CF). The degree of overlap represents approximately the degree to which a proportion of patients with any one syndrome has features of another.

features and pathophysiology. Until we understand the precise cause of a disease process it is difficult to know whether a disease label represents variations on a theme or a number of different themes grouped together, and this is particularly true of the chronic inflammatory airway diseases.

Does this matter? When it comes to disease classification there have always been 'lumpers' and 'splitters': those who prefer to recognize commonality in disease processes and those who prefer to emphasize complexity. The lumpers risk denying the insight that accurate classification brings to understanding the disease, whereas the splitters can end up counting angels on a pin if their system of classification is not grounded in pathogenesis. The accuracy of disease classification would matter less if it was well recognized that disease labels in inflammatory airway disease are imprecise; however, this is often forgotten and there are circular debates and sclerotic restrictions about which phenotype, for example reversibility, belongs to which label. One concept that underpins this paper is that we should start with the phenotype (preferably defined in objectively measurable terms) and try to explain it in terms of pathophysiology without too many prior assumptions about which disease label it belongs to. Of course, a given phenotype, for example, fixed airflow obstruction (FAO) or eosinophilic airway inflammation, in one individual may not have the same pathogenesis as the same phenotype in another individual. We should therefore try to describe the multiple phenotypes that comprise airway disease in an as precise and detailed way as possible. Comparing and contrasting closely related phenotypes may then be very useful in providing insights into the pathophysiology of the defining variable. Factors that have discouraged dealing with phenotypic complexity in airway disease include a reluctance to measure it, the influence of drug development and marketing and the ascendancy of animal models in approaches to understanding pathogenesis.

Measuring complexity

You can only manage complexity if you measure the variables that comprise it. If classification of airway disease is to be based on a clustering of objective measures of disordered physiology and cell biology as well as clinical features, then these variables have to be regularly measured. However, even such basic measurements as reversibility are not routinely practised in the UK and measurement of AHR or airway inflammation (two of the current defining features of asthma) is not part of the standard work of airway disease in most UK hospitals. Indeed, it is an article of faith by some centres not to measure these variables at all [4]. Very few centres, if any, currently include other measurements such as airway pathology, *in vitro* steroid responsiveness or genetic background in their patient assessment. This is partly to do with lack of resources and partly to do with the absence of any obvious usefulness in terms of diagnosis or management, but unless we have the measurements we cannot properly address phenotypic complexity. Another difficulty is that in any one clinic population there will be relatively few patients representing each phenotype, which means that information from several centres will need to be pooled if a more relevant system of classification is to be developed. This will require the creation of collaborating networks of respiratory centres carrying out a range of standardized investigations.

Drug development and marketing

It is difficult to do human research on multiple phenotypes. Studying a full spectrum of disease will inevitably increase the background noise and require more patients to appropriately power the study so that subgroup analysis can be undertaken. It is good science to try and study uniform and well-defined

populations. However, it cannot be assumed that findings in one phenotype, mild atopic asthma for example, in the majority of bronchoscopy studies, or markedly reversibly airway disease in the majority of drug trials in asthma, inform other 'asthma' phenotypes. The markedly reversible phenotype, which is the stock in trade of clinical trials, is in fact relatively uncommon, being present in only about 30% of asthmatic patients attending a secondary care clinic and even less in a primary care population [5]. The regulatory agencies are complicit in this process. By setting up a restrictive set of benchmarks to regulate clinical trials they limit the freedom of the pharmaceutical industry to explore phenotypic diversity. The emphasis on reversibility as a defining factor of asthma and irreversibility as a defining factor of COPD (which in neither case is very sensitive or specific) is an example of this [6, 7]. It is possible that long-acting β_2 -agonists only benefit the subset of patients with marked reversibility (the group which has been studied in clinical trials), yet they are being marketed as a treatment for all asthmatics. Another disadvantage in not addressing complexity in drug development is that the outcome measures chosen for the study might not reflect the actions of the drug. Thus, anti-IL-5 has been judged against its effects on symptoms and measures of variable airflow obstruction, whereas its effects may be much more closely related to exacerbations [8]. Pharmaceutical companies also have an understandable vested interest in their drug being prescribed to everyone for whom they obtain a licence. Although in most clinical trials any given drug benefits only a proportion of patients, from a marketing perspective companies generally have limited interest in distinguishing between responsive and unresponsive sub-phenotypes as this will both limit the market for their drug and make it difficult to generate the simple messages necessary to sell their products. This is not to criticize the pharmaceutical industry for their marketing strategy. They require drugs with a large market to recoup the costs of drug development. It is generally less profitable and more challenging to target drugs specifically to phenotypes that perhaps represent only 10% or 20% of their target group, especially if it requires a special test to identify that population [9].

Animal models

Animal models by definition are based on identifying one or two key abnormalities involved in a disease process and then recreating these in an animal under carefully controlled conditions. The extent to which the model relates to the same abnormality in humans is always debateable, but however good it is in that respect it clearly cannot model the phenotypic complexity of real life disease. However, animal models are attractive because of their relative consistency and simplicity, the ability to elegantly manipulate the model to explore biological mechanisms and to test new compounds in a definitive manner. The archetypal model in the field of airway inflammation is the mouse model of asthma in which mice are sensitized in a T-helper type 2 (Th2) manner with an antigen (usually ovalbumin) and the animal is then challenged to generate a pulmonary eosinophilia and increased lung resistance to bronchoconstricting stimulus. This recreates two

supposed hallmarks of asthma, Th2-driven eosinophilic inflammation and AHR [10, 11]. The extent to which AHR in the mouse is related to AHR in humans or even the extent to which asthma is a Th2 disease remains questionable, but few would argue that the mouse is satisfactorily able to model the complex set of phenotypes that are grouped under the term asthma. There is nonetheless a seductive tendency to think of the mouse model as being important in its own right and believe that by showing something in the mouse you have necessarily discovered something about asthma.

Phenotypic complexity in airway disease

Airway disease is composed of many domains or *dimensions*. For example, these include the environmental triggers and context in which they occur (timing, dose, duration of exposure), clinical features including co-existent diseases, physiology, pathology, immunology, cell biology, genetic background and response to treatment. A particularly important dimension, although one where it is difficult to get good data, is time: for example in the evolution of viral wheeze in children [12]. Each of these *dimensions* contain *variables* that can range from the tens for physiology [forced expiratory volume in 1 s (FEV₁), forced vital capacity (FVC), peak expiratory flow (PEF), total lung carbon monoxide gas transfer (TLCO), etc.] to tens of thousands for single-nucleotide polymorphisms. It is therefore not surprising that the host response to an insult (whether exogenous or endogenous) is complex and multi-faceted. A fundamental problem is deciding which of the combination of factors that create a clinically distinguishable phenotype are sufficiently closely related to represent variations on a single disease process and which are sufficiently distant to be recognized as different diseases. It is also difficult to know which variables within a phenotype are central to the disease process and which are epiphenomena. As indicated in Fig. 1, there are a number of conditions associated with airway inflammation that are characterized by a common set of symptoms, signs and physiological abnormalities. The atopic state and therefore the influence of allergy on airway inflammation is imposed on top of this picture, with each of the diseases containing some patients with specific IgE to common allergens and some without. In each case, atopy may be one of the inflammatory triggers although in no case including asthma is it a necessary one. Atopy is therefore one of the key variables to enter into a cluster analysis. A central question is the relationship between the inflammatory stimulus, the pattern of inflammation that results from it and the response of the target organ to that inflammatory stimulus. This is shown in Fig. 2, which illustrates how a number of different insults can lead to a pattern of inflammation that ranges between highly eosinophilic and neutrophilic in nature. Presumably, this relates to the different degrees to which innate immune responses or Th1 vs. Th2 adaptive immune responses are involved. Out of this black box of inflammation comes different physiological responses depending on host susceptibility and the nature and severity of the inflammation. In some cases, the physiological response is strongly dependent on the nature of the inflammatory stimulus. For example, the response to acute allergen challenge in atopic

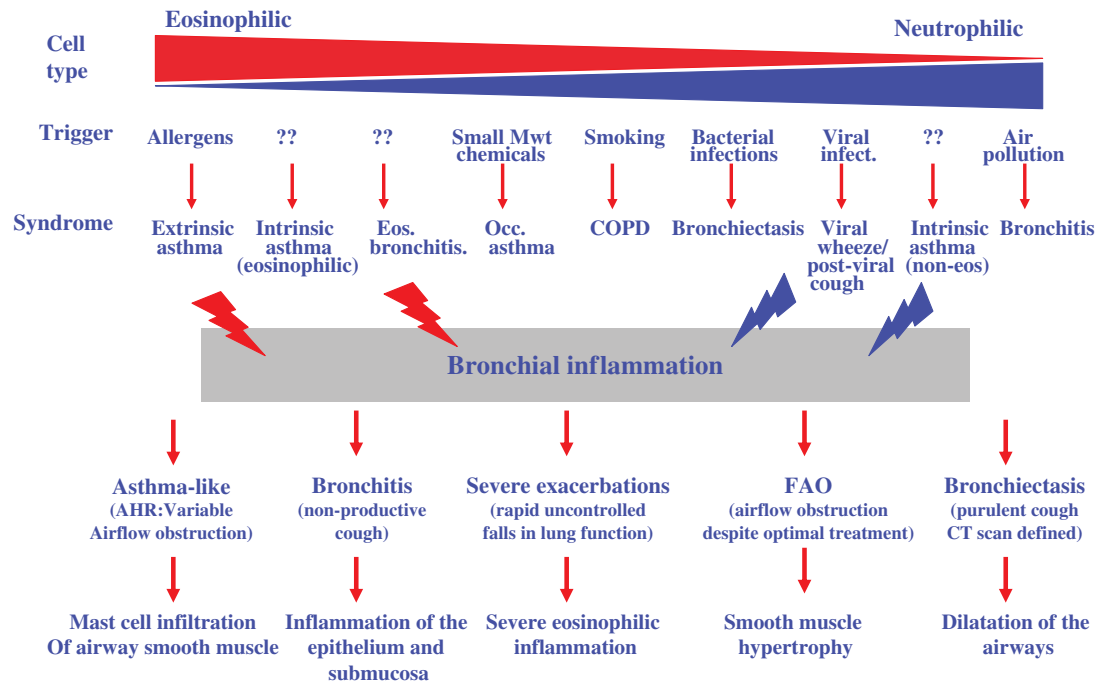


Fig. 2. Schematic diagram illustrating the complex relationship between various triggers of airway inflammation, the pattern of inflammation generated, the diseases they are associated with, the outcomes in terms of disordered airway physiology and the associated specific pathological abnormality. Thus, there are a wide range of environmental stimuli that can cause airway inflammation of variable type, severity and persistence. Allergens are an important group of stimuli that generally cause an eosinophilic inflammatory process in individuals with a T-helper type 2/specific IgE background. At the other end of the spectrum, exposure to high levels of air pollutants such as ozone generally causes a more neutrophilic pattern of inflammation presumably mediated by innate rather than specific immune processes. The inflammatory process generated then leads to various physiological outcomes depending on the nature of the stimulus and the host response. Generally speaking, these fall into five major categories: an asthma-like phenotype of variable airflow obstruction and wheeze, a bronchitic phenotype of non-productive cough, a severe exacerbation phenotype characterized by rapid and uncontrolled falls in lung function, a fixed airflow obstruction phenotype (airflow obstruction despite optimal treatment) and a bronchiectasis phenotype of productive cough and dilated airways on a computed tomography scan. Each phenotype is associated with a more or less specific pathological abnormality, which gives a clue to pathogenesis. The more we know about the link between phenotype and pathology the closer we are to defining a specific disease process, which in turn will aid understanding of pathogenesis and aetiology. The best example of this is where infiltration of the airway smooth muscle (ASM) with mast cells is closely and specifically associated with the asthma phenotype. Thus, 'classical asthma' may be more precisely defined as a condition of variable airflow obstruction, airway hyper-responsiveness and mast cell infiltration of the ASM. Although particular triggers are associated with particular patterns of inflammation and clinical-physiological outcomes (e.g. allergens and asthma), this is by no means absolute or clear cut.

subjects generally produces an immediate and delayed response with eosinophilic inflammation. However, even in this standardized and controlled setting responses between individuals are very variable in terms of the severity of the inflammatory response, the degree of airflow obstruction and the effect on AHR [13]. The response to chronic allergen exposure in a natural setting (for example cat owners sensitized to cat allergens) is even more variable with responses ranging from eosinophilic airway inflammation without symptoms, a classical asthma response of cough wheeze and variable airflow obstruction, a phenotype of generally asymptomatic eosinophilic inflammation punctuated by severe exacerbations or a more COPD-like phenotype with a significant degree of FAO. The degree of eosinophilic inflammation in asthma, at least as measured by induced sputum, is also very variable [14]. The inflammatory stimulus of tobacco smoke can produce classical COPD with FAO, a phenotype of recurrent episodes of purulent sputum production with computed tomography (CT) scan evidence of bronchiectasis (chronic bronchitis), or an asthma-like phenotype of variable airflow obstruction and AHR. Recurrent episodes of bacterial infection associated with bronchiectasis can sometimes be associated with a clearly asthmatic-like pattern even in the absence of allergic bronchopulmonary aspergillosis (ABPA). What is far from

clear is whether the common physiological abnormalities such as variable airflow obstruction, AHR and FAO, when they are associated with different inflammatory stimuli (e.g. allergen exposure or tobacco smoke), have the same pathogenesis. The fact that asthmatic smokers are more at risk of developing FAO suggests there might be common pathways; but there are also clear differences in the immunopathology of allergen-associated and tobacco smoke-induced airflow obstruction [15–18].

Five major clinico-physiological phenotypes of airway disease

Taking the airway diseases detailed in Fig. 1 as a single group, we have identified five major clinico-physiological phenotypes. These are (a) spontaneous or bronchodilator-induced variable airflow obstruction with AHR (the classical asthma phenotype), (b) a bronchitic phenotype of non-productive cough, (c) severe exacerbations defined as marked, uncontrolled, relative rapid and bronchodilator resistant increases in airflow obstruction, (d) FAO defined as airflow obstruction persisting after optimal treatment with bronchodilators and systemic glucocorticoids and (e) bronchiectasis defined by CT

scan evidence of dilated airways. More than one of these features can be present in any one patient at one time or at different times during the course of their disease. Clearly, this is only a starting point and several other major phenotypes could be delineated: for example, viral wheeze in children or emphysema, which we have not included here as it is not an airway abnormality and is generally only seen in cigarette smokers. Comparison of the immunopathology of well-characterized and clearly demarcated examples of these phenotypes might give insights into the underlying pathophysiology. The paradigm for this approach was a comparison of the immunopathology of the classical mild asthma phenotype and the bronchitic phenotype exemplified by eosinophilic bronchitis defined as eosinophilic inflammation without AHR in which patients present with steroid responsive cough but no wheeze or shortness of breath [19]. The identification of the sole difference between the two conditions as mast cell infiltration of the airway smooth muscle suggested that AHR is caused by mast-cell-associated smooth muscle dysfunction and that eosinophils are not involved in the pathophysiology of this phenotype [20, 21]. It also suggested that the cough phenotype is associated with inflammation confined to the epithelial and superficial submucosal regions of the bronchi. If this were to hold true, in further studies the definition of asthma could then become 'variable airflow obstruction and airway hyper-responsive in the context of mast cell infiltration of the airway smooth muscle'. This is much more precise than that used by current guidelines. In contrast, there is increasing evidence that the severe exacerbation phenotype is closely associated with marked eosinophilic airway inflammation [22]. A study focusing on the immunopathology of FAO suggested in asthma but not tobacco-related disease that this was associated with smooth muscle proliferation and fibroblast proliferation [23]. Thus, as illustrated in Fig. 2, each physiological phenotype can be linked to a relatively specific pathological abnormality. This offers both an improved definition of disease and novel insights into the pathogenesis of that particular phenotype.

Heterogeneity of asthma

The above comments apply to airway disease taken as a whole; but even within the asthma label there is considerable heterogeneity [24]. This heterogeneity has always been recognized as exemplified by the many descriptive terms summarized in Table 1, which are based either on the pattern of clinical presentation (brittle, exercise induced, nocturnal, refractory) or on the suggested aetiology (extrinsic vs. intrinsic, occupational, aspirin induced) used to describe the disease [25–28]. The debate about whether asthma is a single disease or a syndrome consisting of several different diseases is a long-standing one, although no closer to being resolved now than 30 years ago. As a result, asthma defies an unequivocal definition, which creates a degree of nihilism in the approach to the diagnosis of the disease as well as complicating interpretation of genetic and epidemiological studies [29, 30]. In a severe (refractory) asthma population where the florid nature of the disease allows clear delineation of patterns of disease, several clinically and physiologically distinct phenotypes can be identified, which, to an extent, mirror those seen in the general

Table 1. Descriptive terms for asthma phenotypes

Category	Description	Comment
Clinical pattern	Chronic	Severity can be mild moderate or severe
	Acute severe Brittle Nocturnal Premenstrual Steroid resistant Cough-variant asthma	
Triggers	Extrinsic vs. intrinsic	Absence or presence of specific IgE to allergens
	Occupational	Small molecular weight chemicals
	Infective	Viruses, bacteria, fungi
	Aspirin sensitivity	NSAID
	Smoking	
	Exercise	
	Toxic exposures	

NSAID, non-steroidal anti-inflammatory drugs.

group of airway diseases [31] (Table 2). Imposed on the variability seen in these clinical and physiological hallmarks of airway disease are many other variables such as atopic status, total IgE, nasal polyposis, aspirin sensitivity, immunopathology, genetic background and response to treatment. Looked at in this way, the picture becomes very complicated. However, it is essential to address this complexity if we are to really understand airway disease, not least because it has major implications for optimal management as exemplified by the increasing evidence of a relationship between polymorphisms in the β_2 -adrenergic receptor and lung function [32]. One way to try and make sense of this complexity is to correlate all these variables in a way that will give clues as to how they associate with each other without prejudice to disease labels. Correlating variables is of course a standard analytical approach in medical research, but generally only two or three variables from one or two dimensions are correlated at any one time. Ideally, we would like to look at the association of tens if not hundreds of variables from all the different dimensions. One way to do this is to use cluster analysis.

Cluster analysis

Cluster analysis is a group of multivariate analysis techniques 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 phenotypes. A range of clustering methods are available with techniques differing by the procedure used to measure the similarity between subjects and by the clustering algorithm applied; the clustering

Table 2. Clearly identifiable asthma phenotypes seen in a refractory asthma clinic based on a combination of clinical features, physiology and pattern of airway inflammation

Phenotype	Sub-phenotypes	Clinical characteristics	Treatment	Possible pathogenesis
Marked variability in airflow obstruction and AHR	Eosinophilic and pauci-eosinophilic	Always symptomatic; high requirement for β_2 -agonists	Bronchodilators particularly effective	Disorder of airway smooth muscle function
Inflammatory (eosinophilic)	Brittle and drifters	Symptomatic only during exacerbations; brittle patients have sudden falls in lung function whereas drifters more predictably deteriorate	Anti-inflammatory drugs (mainly glucocorticoids) most effective; systemic treatment required in severe cases	Marked eosinophilic inflammation sometimes associated with identifiable environmental trigger but often of unknown cause
Fixed airflow obstruction	Type 1 and 2 superimposed	Persistent airflow obstruction despite optimal treatment; chronic breathlessness major symptom	No specific treatment available	Structural remodelling of the airways particularly associated with increased airway smooth muscle
Non-eosinophilic	Neutrophilic and pauci-inflammatory	Otherwise typical asthma although poorly responsive to steroids; some have a bacterial trigger for exacerbations	Antibiotics in selected cases. Avoid overuse of glucocorticoids	Unknown
Asthmatic smokers	Asthmatic smokers and COPD with asthmatic features	Usually combine features of both asthma and COPD	Relatively unresponsive to steroids	Complex interaction between effects of tobacco and underlying asthma
Eosinophilic bronchiectasis	Allergic bronchopulmonary aspergillosis (ABPA) and non-ABPA	Usually a marked peripheral blood eosinophilia; exacerbations often complicated by infection as well as inflammation	Antibiotics and systemic steroids	Allergic reaction to fungal elements in some cases

AHR, airway hyper-responsiveness; COPD, chronic obstructive pulmonary disease; ABPA, ...

solution may differ according to the method. In addition, clustering techniques are generally viewed as heuristic in nature, rather than having a basis on formal statistical modelling. These caveats mean that cluster analysis is probably best seen as hypothesis generating rather than solving. Nevertheless, these techniques are particularly suited to the study of diseases that express considerable diversity and as such are ideally placed to address the multi-dimensional complexity evident in airway disease.

Cluster analysis is based on objective measurements of similarity, or 'distance' between subjects across a number of variables. The most commonly used measure is the Euclidean distance. To measure the similarity between two subjects, A and B, their scores for the first variable are taken and the difference calculated. Once this has been repeated for each available variable, the total differences are added up. As some differences will be positive (A having a bigger score than B) and some negative (A having a smaller score than B), each difference must be squared to prevent positive and negative differences from cancelling each other out. Once all the squared differences have been added up, the square root is calculated to revert back to the original unit of measurement and then divided by the number of variables to give the average Euclidean distance. If A and B are identical across all variables, the Euclidean distance will be zero; widely dissimilar cases will have large Euclidean distances, approaching 1. One difficulty is that variables observing wide scatter will heavily influence the result, as will variables that have a large absolute size. This is a particular issue where different variables have different units of measurement. To correct for this, scores should be first standardized, for example using Z-scores (actual score – mean score/standard deviation). Having obtained the measure of similarity between cases, hierarchical methods are usually applied to group cases based on their degree of similarity. At the outset, each individual case is seen as a cluster. Clusters are then merged in a stepwise way until there is finally just one cluster containing all cases. A number of methods may be used to merge clusters, the simplest being single linkage or 'nearest neighbour' where the two cases with the smallest Euclidean distance are merged to form a larger cluster. The case with the smallest Euclidean distance to either of the initial two cases is then merged with this larger cluster and so on until all cases have been merged into one final cluster. An example of a cluster analysis in a population of patients with airway disease is presented below. Here an alternative, more complex, method of linkage known as Ward's method was used. This method links the pair of clusters that produce the smallest variance in the merged cluster. Regardless of the precise method, a tree diagram or dendrogram is drawn to visualize the grouping of clusters.

Cluster analysis in airway disease

As a preliminary evaluation of the usefulness of cluster analysis, we have studied a heterogeneous population of 49 well-characterized adults with airway disease, 27 with a diagnosis of asthma and 22 with COPD. Asthma was defined as having symptoms consistent with the diagnosis of asthma and one or more of the following: a $>15\%$ increase in FEV₁

following 200 µg inhaled salbutamol, >20% within day variability in PEF assessed twice daily over 2 weeks, or a concentration of methacholine causing a 20% fall in FEV₁ (methacholine PC₂₀) of <8 mg/mL. All asthma patients were either never smokers or ex-smokers with a <10 pack year history. COPD was defined according to GOLD criteria (post-bronchodilator FEV₁ <80% of predicted or an FEV₁/FVC ratio of <0.7). Demographic data (including age, gender, pack year history), spirometry pre- and post-bronchodilator, serum total IgE and induced sputum eosinophil differential counts were available on all subjects. Age, gender, pack year history, FEV₁ percent predicted, FEV₁/FVC ratio, percent bronchodilator reversibility, total serum IgE and percent induced sputum eosinophils were entered into a hierarchical cluster analysis using Ward's method following standardization of variables by conversion to Z-scores (SPSS for Windows version 10.0). The resulting

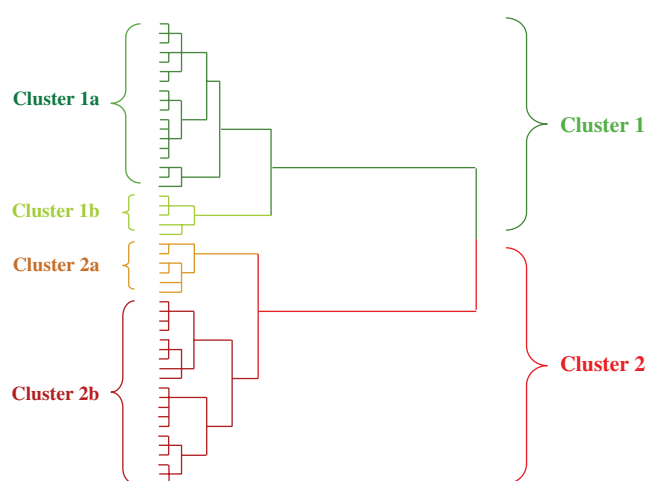


Fig. 3. Dendrogram illustrating the results of a cluster analysis of 49 patients with airway disease. Eight variables from patients recruited from one chronic obstructive pulmonary disease (COPD) and one asthma research study were entered into a database and cluster analysis undertaken. Each line represents an individual patient and the length of the horizontal lines represents the degree of similarity between individuals. Two major clusters corresponding to the COPD and asthma groups, with three exceptions as detailed in the text, can be seen. Four subgroups can also be identified with significant differences as detailed in Table 3.

dendrogram is given in Fig. 3. Inspection of the two cluster solution reveals that cluster 1 contains patients with a predominant diagnosis of COPD and cluster 2, mainly asthma patients. Three patients with a diagnosis of asthma were included in cluster 1. Further inspection of these cases demonstrated that each had a degree of FAO and thus could be reasonably classified as having COPD, although as a result of chronic asthma rather than cigarette smoking. Similar analysis of the two patients with COPD entered into cluster 2 revealed one patient with near-normal lung function not fitting the diagnostic criteria for COPD and a second patient with a high serum IgE and a sputum eosinophil count of 70%, who may reasonably be considered as having an asthma-like phenotype. Table 3 gives the comparison of the demographic and clinical data between the four clusters. Cluster 1a appears to describe a sub-population of older smokers with largely fixed airflow obstruction similar to that seen in 'classic' COPD, while cluster 1b includes a smaller population of patients whose other major abnormality appears to be markedly variable airflow obstruction. Cluster 2a contains patients with a predominantly eosinophilic inflammatory airway disease and cluster 2b appears to illustrate a population of largely never smokers with mild asthma. The adequacy of the four-cluster solution was evaluated using a discriminant function analysis using the available variables as predictors of group membership. This demonstrated that three significant functions accounted for 100% of the variance ($P < 0.001$): function 1 (FEV₁ percent predicted, FEV₁/FVC ratio and age) accounting for 52.2%, function 2 (total serum IgE and percent sputum eosinophils) accounting for 39.9% and function 3 (% bronchodilators reversibility and pack years smoked) accounting for 7.9% of the overall variance. Examination of the classification rates for the discriminant functions revealed excellent cluster separation with 95.9% correct placement of the cases into their respective clusters. Figure 4 demonstrates the variance between the four clusters according to functions 1 and 2.

These results, although preliminary, do suggest that this method of cluster analysis may be a valid and objective way to differentiate populations of well-characterized patients with airway disease into distinct subgroups. Further evaluation of the techniques will require similar analyses of larger

Table 3. Demographic and clinical data for the four clusters

Cluster	1a	1b	2a	2b	P-value
Asthma diagnosis	1	2	5	19	<0.001
COPD diagnosis	17	3	1	1	<0.001
Female: Male	5:13	1:4	2:4	12:8	0.148
Pack years smoked	50.4(7.8)	18.4(6.4)	12.3(10.5)	2.61(2.0)	<0.001
Sputum eosinophils %*	1.58(0.1)	6.92(1.9)	28.8(0.2)	1.95(0.1)	<0.001
% Bronchodilator reversibility	11.45(2.4)	36.5(5.0)	9.82(1.40)	9.28(2.0)	<0.001
Age in years	67.89(1.8)	59.80(8.6)	43.50(7.7)	48.20(3.1)	<0.001
FEV ₁ % predicted	52.7(2.4)	46.8(4.1)	77.7(5.8)	83.1(3.6)	<0.001
FEV ₁ /FVC ratio %	53.5(2.1)	50.2(2.9)	73.6(6.3)	72.0(1.7)	<0.001
IgE*	24.6(0.1)	371.54(0.3)	933.25(0.2)	52.5(0.1)	<0.001

Cluster 1a looks like classical COPD, 1b has features of an asthma/COPD overlap group, 2a is an asthma group with predominantly inflammatory pattern and high IgE and 2b an asthma group with minimal eosinophilic inflammation and low IgE.

FEV₁, forced expiratory volume in 1 s.AHR, airway hyper-responsiveness; COPD, chronic obstructive pulmonary disease.

Mean (SEM).

*Geometric mean (log SEM).

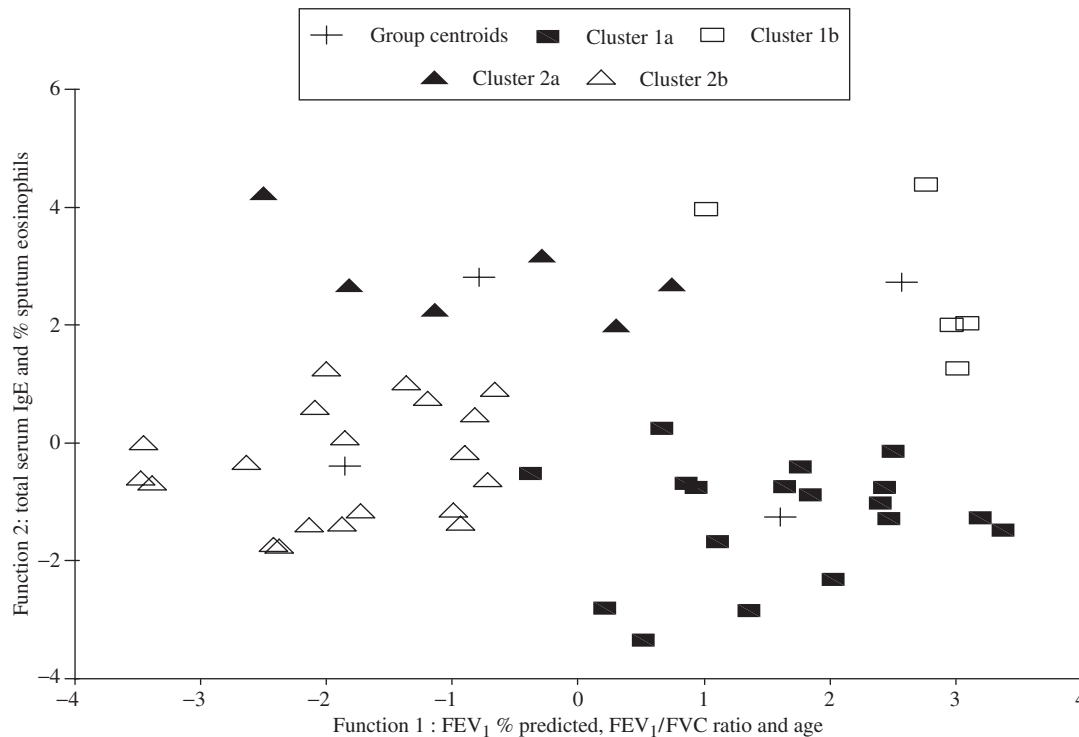


Fig. 4. Discriminant function analysis demonstrating variance between clusters according to clustering variables. This is another way of visually representing the degree of clustering within the four groups and the reliance for proximity on the variables contained within functions 1 and 2.

populations including patients with a wider range of airway diseases, meaningful correlation between cluster membership and novel variables such as treatment outcomes or immunopathological characteristics and prospective studies of differences in prognosis between members of different clusters.

Summary

The present system of classifying airway disease based largely on 19th century concepts of disease is outmoded and fails to recognize both phenotypic complexity within a given disease label and the pathophysiological links between physiological and cellular abnormalities common to different disease labels. The central idea proposed in this paper is that the current system of classification inhibits the development of new ideas about the pathophysiology of airway disease, distorts drug development and leads to sub-optimal disease management. We suggest that the creation of a new system of classification based on objectively measurable variables across several clinical, pathological and physiological dimensions and including longitudinal data should be developed in a manner that addresses phenotypic complexity and maps the phenotype to the underlying pathophysiology. We propose that sophisticated statistical tools such as cluster analysis are required to do this and we have presented some preliminary data to show how such an approach might be undertaken. Effective promotion of this approach will require sharing of standardized data between several centres to overcome the problem of small numbers of each phenotype being available to any one centre.

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This paper is the result of many discussions between members of the Institute for Lung Health regarding the pathogenesis of airway disease. The concept of multi-dimensional phenotyping in this context originated with M. S. R.G. undertook the cluster analysis.

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