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The clinical utility of biomarkers in asthma and COPD

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Biomarkers with potential utility in the diagnosis and prognosis of asthma and chronic obstructive pulmonary disease (COPD), and in monitoring the natural history of these diseases and the effect of therapeutic interventions, are being widely researched. This review critically describes the methodologies used for obtaining and analysing appropriate biofluid, tissue and exhaled breath samples for biomarker analysis. Currently measurements of sputum eosinophils and exhaled nitric oxide in asthmatics are the best established markers for disease activity and response to anti-inflammatory therapy. Circulating C-reactive protein (CRP) levels have been shown to predict risk of hospitalisation and death from COPD. Biomarker measurements in exhaled breath condensate are the least well-validated techniques. Other assessments in both conditions have potential value in clinical use but require further research and validation.

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Introduction

A biomarker has been defined as 'a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacologic responses to a therapeutic intervention' [1], which could include, for example, measures of lung function or lung imaging, although the term 'biomarker' historically refers to analytes in biological samples [2], and this review will confine itself to the consideration of markers assayed in biofluids, tissues and exhaled breath. However, any measurement that predicts a patient's disease state (a diagnostic or prognostic marker) or response to treatment (a clinical endpoint or surrogate for such a measure) can be called a biomarker. Asthma and chronic obstructive pulmonary disease (COPD) are the commonest non-infectious disorders of the airways and hence are major target diseases for novel therapeutic agents, and in recent years extensive research has gone into identifying and attempting to validate relevant diagnostic biomarkers and markers of disease activity and therapeutic response in these conditions.

General principles

Biofluids available to the respiratory investigator include blood and urine, sputum (spontaneous or induced) and broncho-alveolar lavage (which necessitates a bronchoscopy, also allowing biopsy material to be obtained). Analysis of exhaled gases and mediators in exhaled breath condensate allows non-invasive measurements of potential inflammatory markers. Figure 1 shows these biophases ranked in order of increasing invasiveness.

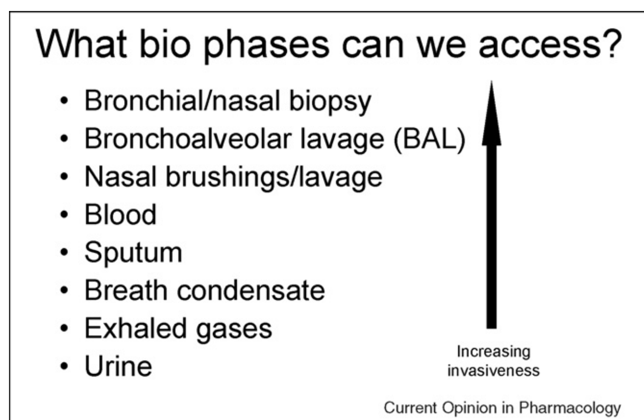
It is imperative that biomarker assays be validated in the appropriate biophase available for study before their use for diagnostic or therapeutic purposes. This may necessitate initial methodology studies to collect biofluids and develop the assay, validating the findings for example, by 'spiking in' known concentrations of the analyte [3]. Once the assay is validated, stored samples can be used to establish the reference range of the biomarker in the target population and its inter-subject variability (needed to power clinical trials appropriately). If samples can be taken sequentially over days or weeks, intra-subject variability of the biomarker over time can also be established.

Sputum methodology

Collection and analysis of sputum has become a commonly used non-invasive means of assessing airway inflammation in both COPD and asthma and is also of value in diagnostic assessment, particularly in asthma [4]. Sputum sampling reflects biofluid in the central airways rather than the lower and peripheral lungs [5] and is therefore especially suitable for monitoring COPD patients with a chronic bronchitis phenotype.

Some asthmatics and most chronic bronchitics can produce sputum spontaneously [6]. However, it has been shown that there is considerably lower cell viability in samples prepared from spontaneous sputum compared with sputum induced by the use of inhaled hypertonic saline [7,8], and hence induced sputum (IS) has become the major method in clinical use. Using IS also permits comparisons between patients and healthy age-matched and smoking-matched controls; reference values for differential cell counts in IS from healthy volunteers have been published [9]. Age matching is important, as it has been shown that the IS differential neutrophil count in healthy volunteers increases significantly with age [10]. Note that not all subjects (whether patients or healthy

Figure 1



Accessible biophases in asthma and COPD.

controls) necessarily produce a sample in response to the stimulus of inhaled saline (success rate is in the order of 80–90%), and the relevance of induced 'sputum' from healthy subjects who, by definition, are not sputum producers could be debated.

One potential drawback to the use of IS is that sputum induction itself induces a local inflammatory response, with a transient neutrophilia and longer lived eosinophilia, possibly due to local changes in osmolarity activating epithelial and mast cells, so it is recommended to avoid repeated inductions within a 48-h period [11]. There is a small risk of saline-induced bronchoconstriction due to the induction procedure, but this is not a major problem, and the risks appear to be acceptable [12].

Comparisons of spontaneously produced sputum with induced sputum from COPD and asthmatic patients have generally shown no differences in total or differential cell counts or concentrations of inflammatory mediators [7,8,13]; one COPD study found a lower purulence score with induced compared with spontaneous sputum, possibly due to the dilutional effect of the inhaled saline [13]. Repeatability of cell counts and soluble mediator assays in IS from patients with asthma [14] and COPD [15] is acceptable, although the variability is sufficient to suggest that studies of sputum inflammatory makers may easily be underpowered.

The induction, collection, processing and analysis of sputum may be carried out differently in different research centres. To attempt to address this problem the recommendations of an initiative to develop a series of harmonised and standardised procedures for sputum induction and processing have been published [16•].

Sputum is inhomogeneous and may be contaminated with saliva. Some groups physically pick out the 'plugs' for further processing, while some process whole sputum. Although 'plug-picking' minimises squamous-cell contamination, the discarded fluid will include some of the soluble phase of the sputum and some epithelial lining fluid, which may contain inflammatory mediators. Hence analysis of whole sputum is an accepted alternative [17]. Sputum can be homogenised by physical methods such as ultracentrifugation [18] or ultrasonication[19], but these have the drawback that the cellular content of the sample may be disrupted.

Mucolytic agents such as dithiothreitol (DTT) or dithioerythritol (DTE) are commonly employed in sputum processing, in order to homogenise the sputum sample by breaking the disulfide bonds in the mucin molecules, allowing cells to be released from the matrix [20] and making the quantification and characterisation of sputum cell content more reliable [17]. However, the reducing and protein-denaturing effects of these agents may affect biomarkers of interest and interfere with their detection [17,19]. Studies have found that processing of IS from asthmatics with DTT increased measurable concentrations of eosinophil cationic protein (ECP) but lowered concentrations of myeloperoxidase (MPO) and eosinophil peroxidase (EPO) [17]. Similarly, using DTT to homogenise sputum from patients with chronic bronchitis or bronchiectasis lowered the detectable concentrations of tumour necrosis factor alpha (TNFα), leukotriene B4 (LTB_4) and MPO [21]. Several methods have been developed to try to overcome these effects, including addition of bovine serum albumin (BSA) to the sputum sample [22], dialysing the sample to elute off the DTT [23] (which runs the risk of dialysing off other analytes of interest, including therapeutic drugs), and taking a separate portion of the unprocessed sputum from that to be used for cell counts and using ultracentrifugation, ultrasonication or solubilisation with phosphate buffered saline (PBS) [24] to obtain an homogenous sample for analysis; a universally agreed method for measurement of soluble mediators in sputum is yet to be established.

Bronchoalveolar lavage and bronchial biopsy

Bronchoalveolar lavage (BAL) samples the more peripheral airways and alveoli. Guidelines on methodology are available [25,26•]. The major limitation of the technique is that it is invasive; however, it is generally well tolerated and has been shown to be safe in subjects with airway obstruction whose FEV1 is >60% predicted, and can be performed in patients with worse lung function with appropriate precautions. Bronchospasm, mild fever and transient asymptomatic pulmonary infiltrates are occasional complications [27]. Overdosage of topical anaesthetic used during the procedure has very rarely proved fatal [28]. Other limitations of the method are that the procedure itself is mildly pro-inflammatory, limiting

the frequency with which it can be repeated, and that a variable dilution factor for the sample is introduced; it has been estimated that the acellular constituents may be diluted 20–60-fold. Several components of BAL fluid have been studied as potential internal dilution standards; at present none is generally accepted, although urea is probably the most frequently used [29,30]. A task force report on the measurement of BAL acellular components has been published [31]. Advantages of BAL are that it is also possible to take bronchial epithelial brushings and biopsies during the procedure and that the BAL sample probably reflects events lower in the bronchial tree than does sputum; although it has been proposed that the late fraction of induced sputum (L-IS) samples the more distal lung compared with the early fraction (E-IS) [32]. In a comparative study E-IS showed a significantly higher neutrophil count than L-IS, with a significant correlation between E-IS and BAL eosinophil counts; there were no correlations between E-IS or L-IS and BAL for any other cell types, nor between E-IS/L-IS and submucosal inflammatory cell counts from bronchial biopsy [33].

Neutrophil counts have been shown to be higher in sputum from asthmatics and COPD patients than in BAL; conversely, macrophage and lymphocyte numbers were higher in BAL than sputum [5,34]. However, eosinophil numbers [33] and markers of eosinophilic inflammation have been shown to correlate well between BAL and IS in COPD, as have interleukin (IL)-8 levels [5].

Lung tissue can be obtained during bronchoscopy by bronchial brushings (which provide samples of superficial epithelium), endobronchial biopsies (which yield epithelium and submucosa) and transbronchial biopies (which can provide full-thickness samples of the bronchial wall and adjacent lung parenchyma). Endobronchial biopsy is the safer and more commonly performed technique. Three to six tissue biopsy samples can be obtained at different levels in the bronchial tree (e.g. segmental/subsegmental carinae, main carina) during a single bronchoscopy [25].

There is a lack of correlation between sputum or BAL inflammatory cell counts and biopsy data [5,33], suggesting that cellular inflammation in the airway lumen and in the submucosa may be distinct. However, biopsies give reliable histopathological information on the inflammatory state within the bronchial tissue that is independent of factors that may affect sputum and BAL samples, such as processing and dilutional issues [35]. Bronchial biopsies are particularly valuable for assessing effects on tissue resident cells such as the alveolar macrophage, fibroblasts, airway smooth muscle cells, mucus glands and lymphocytes. A detailed review of methods of processing bronchial biopsies is available [36•].

Histopathology can be used as an endpoint in clinical trials, and data have been published on reproducibility and variability of biopsy sampling in COPD that can guide sample sizing [37].

Although bronchoscopic approaches are of considerable interest for studying disease pathology and its modification by treatment, such invasive methodology is currently unlikely to be widely used in assessing therapeutic efficacy unless its predictive value proves substantially better than other less invasive assessments.

Exhaled biomarkers

Analysis of exhaled breath is a potentially valuable non-invasive method for the measurement and monitoring of inflammation in the respiratory tract. The measurement of exhaled nitric oxide (eNO) is the most advanced in terms of robustness, reproducibility and standardisation of measurement. Recommendations for the standardised measurement of eNO in adults and children have been published [38•]. The usefulness of measuring other exhaled gases, and mediators in exhaled breath condensate, is still under investigation [39].

Exhaled NO levels are generally considered to be independent of gender and age [39], although values in the elderly (median age 72 years) have been found to be higher than in young adults (median age 24 years) [40]. Values in Asian and Black children are approximately twice as high as in Caucasian children and also vary to a lesser extent with age and height, but not weight or BMI [41]. Both exhaled and nasal NO are considerably reduced in subjects with the rare condition of primary ciliary dyskinesia (PCD), compared with healthy subjects and patients with non-PCD bronchiectasis, or cystic fibrosis, and can be used as a screening tool for PCD [42]. eNO is reduced by smoking and alcohol and transiently reduced by physical exertion and sputum induction [39]. Administration of L-arginine, the substrate for NO synthetase, increases eNO, as do respiratory infection and air pollution with ozone, chlorine or NO itself [39]. Caffeine has variously been reported to increase, reduce and have no effect on eNO levels [43]. Levels of nasal NO are very much higher than eNO, and care must be taken to avoid nasal NO contamination when measuring eNO. Nonetheless, in general eNO has been shown to be a reproducible technique in both healthy and asthmatic subjects, and free from diurnal variation [44], making it a very attractive method for assessing airway inflammation in asthma. In COPD eNO is still very much a research tool, whose usefulness is hampered by the effects of smoking, and published data are conflicting as to the status of exhaled NO in COPD relative to healthy control subjects; levels of eNO have been found to be the same in healthy subjects and those with stable COPD, but reduced in smokers and elevated in unstable COPD [45]; whereas in recent publications eNO levels were shown to be raised in both smoking and ex-smoking COPD patients relative to healthy non-smokers [46,47].

Single constant flow exhaled NO measurement cannot distinguish between the sources of NO in the lung. It has been demonstrated that if exhaled NO is measured at multiple expired flow rates, it can be portioned into NO from the alveolar compartment and NO from the bronchial compartment. Mathematical models to calculate these two fractions of eNO have been described by several groups (reviewed in [48]). Using a two-compartment model as a research tool it has been demonstrated that NO is elevated in the alveolar compartment of COPD patients and correlated with disease severity [49]. However, further studies are required to validate this measurement as a tool for monitoring inflammation in COPD.

Exhaled carbon monoxide (CO) can be readily and reproducibly measured [50]. It is normal or minimally increased in mild asthma, but higher levels are found in severe disease [51]; there is some association with airway hyper-responsiveness [52]. eCO levels are higher in smokers than non-smokers and can be used to determine smoking habit [53].

Exhaled breath condensate (EBC) measurements involve collecting the warm breath of subjects onto cooled tubes so that the moisture condenses and can be collected. This condensate can then be analysed for both volatile and non-volatile inflammatory markers and mediators. In principle, numerous potential biomarkers could be measured in this fashion, and the technique is non-invasive, allowing repeated samples to be collected and can be used even with small children [54•]. However, there are several unresolved problems with this methodology. The concentrations of many of the analytes are very low, often at or below the lower detection limit of the assay; contamination by nasal air or saliva, and variable dilution during the collection process can also affect the results [54•]. It has recently been shown that the major proteins in EBC are in fact contaminant skin keratins [55]. Recent publications have also indicated that the condensate collection device and the condenser coatings can influence measurement of biomarkers in EBC [56,57]. Currently the technique remains a promising research tool, with the potential for monitoring the inflammatory state in the airways and the effect of novel therapeutic agents; however, more sensitive methods for measuring analytes and standardisation of the collection methodology are needed.

Biomarkers in blood and urine

In general, laboratories are more used to handling and performing analyses on blood plasma and serum, and urine, than, for example, sputum or exhaled breath, and there are more accepted and established assay methods available. Nonetheless the same principles apply; for each biomarker a specific assay needs to be developed and validated in the appropriate biofluid, and normal reference levels and variability within and between subjects and over time should be established. Although both asthma and COPD are generally thought of as disorders of the respiratory system, both may have systemic components (particularly COPD) that make evaluation of biomarkers outside the lung compartment worthwhile.

Biomarkers in asthma: (1) sputum

The eosinophil is the key cell type studied in asthmatic sputum. Eosinophilia is seen in up to 80% of steroid-naive asthmatics, and eosinophils and eosinophil cationic protein (ECP) in sputum from asthmatic patients are significantly elevated compared with healthy control subjects [58,59]. Sputum eosinophil numbers correlate with asthma severity, airway hyper-reactivity (AHR), peak flow rate variability and daily asthma symptom scores [60]. Increase in the eosinophil count following inhaled allergen challenge correlates with the magnitude of the late asthmatic response and with airway hyper-reactivity [61], and severe exacerbations of asthma or withdrawal of corticosteroid therapy are followed by rapid, reversible increase in sputum eosinophil counts [62]. The changes in sputum eosinophil number during an exacerbation are related to the severity of airway obstruction [63]. In children sputum eosinophils and ECP levels are positively related to the frequency of asthma episodes [64].

During asthma exacerbations sputum levels of mediators associated with eosinophil recruitment (interleukin-5, eotaxin) also increase. Treatment of the exacerbations with oral corticosteroids reduced sputum eosinophil and IL-5 levels but had no effect on eotaxin levels [65].

The neutrophil has also been shown to play a role in asthmatic inflammation, particularly in severe asthma [66]. Sputum neutrophils, and levels of their product MPO correlate with PEF variability and daily symptom scores [67]. In patients with moderate, stable asthma, rapid withdrawal of inhaled corticosteroids (ICS) led to an exacerbation that was preceded by an increase in sputum IL-8 concentration and neutrophil numbers, contrasting with the situation when ICS are slowly reduced, when an increase in eosinophil numbers has been reported [68].

Recently TNFα has been found to correlate even more strongly with AHR in severe asthma than do eosinophil and ECP levels [69]. A study with an anti-TNFα therapy, etanercept, in patients with severe asthma produced improvements in FEV_1 and AHR but did not affect sputum eosinophil levels [70], suggesting that AHR is unrelated to eosinophilic inflammation and that sputum TNFα instead may be a useful predictive marker for improvement in AHR.

Can any of these sputum biomarkers of inflammation be used to predict therapeutic efficacy in asthma? High

sputum eosinophil levels predicted recurrence of asthma symptoms and an exacerbation after withdrawal of ICS therapy [71], and it has subsequently been shown that adjusting therapy to normalise sputum eosinophil numbers results in improved control of exacerbation frequency and reduced hospital admissions, without the need for additional anti-inflammatory treatment [72•].

Biomarkers in asthma: (2) broncho-alveolar lavage and biopsy findings

BAL fluid from asthmatic patients shows increased amounts of inflammatory mediators, including eotaxin [73] and the CCR4 chemokine receptor ligand, MDC, levels of the latter correlating with AHR [74]. Segmental allergen challenge in asthmatic patients increases eosinophils, neutrophils and macrophages as well as lymphocytes, and there is a parallel increase in the CCR4 ligands MDC and TARC and the Th2 cytokines IL-5, IL-13 in BAL compared with pre-challenge levels [75,76].

Interestingly, BAL from patients with status asthmaticus contains large numbers of neutrophils, with an increase in neutrophil elastase (NE) levels, and elevated levels of both pro-inflammatory (IL-1β, IL-5, IL-6 and TNFα) and anti-inflammatory cytokines (IL-10, IL-1ra, sTNF receptors) together with large quantities of the chemokines monocyte chemotactic protein (MCP)-1, MIP-1α and RANTES, but no increases in eotaxin or MCP-3 [77,78].

Biopsy tissue from asthmatics contains increased numbers of eosinophils and lymphocytes compared with controls [79]. Mucosal eosinophil numbers show a significant correlation with lung function [80], but otherwise cellular infiltrate correlates poorly with disease activity, which shows a much closer relationship with airway smooth muscle hypertrophy and fibroblast number [81].

Biomarkers in asthma: (3) exhaled breath

Exhaled NO is increased in untreated asthmatic patients compared with healthy controls, and decreases with corticosteroid therapy; eNO concentration correlates with the degree of eosinophilic inflammation present, particularly in atopic asthmatics [82]. eNO levels have also been shown to correlate with AHR, bronchodilator reversibility, allergen skin prick test positivity, serum IgE level and eosinophil count [83].

A recent clinical trial compared mild-to-moderate asthmatics who were randomised to have their inhaled steroid dose adjusted either in line with international guidelines, or based on measurements of eNO; both groups achieved similar control of their disease, but the group whose dosing was adjusted according to eNO level concluded the study on an average dose of inhaled steroid ~40% lower than the group that was managed conventionally [84]. Baseline measures of eNO have also been demonstrated to predict asthmatic patients most at the risk of having an exacerbation [85].

Biomarkers in asthma: (4) blood

Asthmatic patients have increased circulating numbers of eosinophils compared with healthy controls; the blood count tends to correlate with disease severity and AHR. There is an associated increase in eosinophil activation markers (ECP and EPO [86]. During exacerbations there is a rise in circulating eosinophils and their progenitor cells [87]. Treatment with corticosteroids, in the stable state or during an exacerbation, leads to a fall in eosinophil counts [87]. Other circulating markers that have been found to be raised in asthmatics include eotaxin (a chemokine that selectively recruits eosinophils) [88], IL-4 and IL-5, and the CCR4 ligand TARC [89]. Plasma concentrations of MCP-4 are elevated in asthmatics and further increased during an asthma exacerbation [90].

Although superficially attractive, the use of circulating eosinophil counts as a biomarker of clinical efficacy is debatable, since treatment with an anti-IL-5 blocking antibody (mepolizumab) completely inhibited circulating eosinophil numbers but had no effect on clinical parameters, probably because only a modest decrease in tissue eosinophils in the lung was achieved [91•,92].

Total blood IgE levels are elevated in some but not all asthmatics, and correlate with asthma severity [93]; treatment with a specific anti-IgE antibody (omalizumab) had no effect on AHR in mild-to-moderate asthmatics [94] but was associated with a modest reduction in exacerbations of severe asthma [95].

Biomarkers in COPD: (1) sputum

Neutrophils are the predominant cell type in sputum samples from stable COPD patients [5] and are present in significantly higher numbers than in asthmatic or healthy controls [5,96–98]. The neutrophil count correlates with the degree of airway obstruction and rate of decline of FEV1 [99], but not with the degree of emphysema as measured by high resolution computed tomography (HRCT) [100]. Inflammatory mediators involved in neutrophil recruitment (e.g. IL-8, Groα, LT-B4, NE, MCP-1, and human neutrophil lipocalin (HNL) have all been reported to be significantly elevated in sputum from stable COPD patients, as has the activated neutrophil product MPO [5,97,98,100–103]. Levels of IL-8 and MPO correlate negatively with FEV1 [101,103]. Increased sputum concentrations of matrix metalloproteases (MMP) 8, 9, and 12 have also been reported in COPD [104].

During exacerbations of COPD there is an increase in markers of neutrophilic inflammation including TNFα, IL-8, IL-6 and MPO [105,106]. Exacerbating patients with severe COPD have higher sputum levels of IL-6,

IL-8, and TNFα than exacerbating patients with mild-to-moderate disease; sputum IL-6 and IL-8 levels correlated negatively with FEV1 values [107]. When the exacerbation is associated with a bacterial infection there is a significantly enhanced neutrophilic inflammation compared with patients without an associated bacterial infection [108,109]. This heightened neutrophilic inflammation resolves rapidly with antibiotic therapy [110].

COPD patients with lower lobe bronchiectasis (as determined by HRCT) have higher levels of inflammatory cytokines in sputum that are associated with the degree of lower lobe bacterial colonisation, and experience more severe exacerbations of COPD and a longer recovery time than COPD patients without radiographic evidence of bronchiectasis [111].

Although more closely associated with asthma, eosinophil counts have also been shown to be raised in the sputum of stable COPD patients compared with healthy controls [5,112,113], and to be negatively correlated with lung function [112]. Eosinophil activation products such as ECP are also elevated [5]. Sputum eosinophil counts have been reported to increase during exacerbations of chronic bronchitis, compared with stable disease [114]. A factor analysis approach concluded that neutrophilic and eosinophilic inflammation in sputum are independent factors in the pathophysiology of stable COPD [115]; further work from this group has found that COPD patients with a chronic bronchitis phenotype have higher percentage eosinophil counts in sputum (and lower eosinophil counts in bronchial biopsies) than COPD patients without chronic bronchitis [113].

Lastly, CD8+ T lymphocytes have been reported to be increased in COPD sputum compared with healthy non-smokers, and CD4+ T cells to be reduced [116].

Biomarkers in COPD: (2) BAL and biopsy

In contrast to sputum, the predominant cell type in BAL is the macrophage [5,104], presumably reflecting the more distal airway sampling using this procedure; lymphocytes are also present in somewhat greater numbers than in sputum. Absolute numbers of macrophages have been reported to be both elevated [117] or reduced [118] in COPD BAL compared with healthy controls, but expressed as a percentage of total BAL cell counts there seems to be no difference between patients and healthy subjects [5,118]. Similarly, absolute lymphocyte numbers have been reported to be higher [118], the same [119] and lower [117] in COPD compared with controls, but the same when expressed as a percentage count [118]. However, the percentage of CD8+ T lymphocytes is significantly higher, and that of CD4+ T cells significantly lower, in COPD (and healthy smokers) compared with healthy non-smokers [119], similar to the findings in sputum [116], and this was also shown in epithelial tissue from bronchial brushings [119]. Neutrophils and eosinophils have generally been shown to be increased in COPD BAL compared with non-smoking controls (both as absolute and percent cell counts) [5,117,118], and mast cell numbers have also been reported to be increased [117].

Markers of neutrophilic and eosinophilic inflammation (including IL-8, IL-6, TNFα, MPO, eotaxin-1, and ECP) are increased in BAL fluid from patients with COPD compared with BAL fluid from healthy non-smoking and smoking controls [5,118–121], subjects with bacterial colonisation having the highest levels of neutrophil and eosinophil activation products [118,120]. BAL neutrophil markers [118], and both BAL and epithelial cell CD8+ T cell numbers [119] were inversely associated with FEV1, while ECP and eotaxin-1 levels were positively associated with bronchodilator response and the extent of radiographic emphysema [121].

Bronchial biopsy studies in stable COPD have generally shown an increased infiltration of macrophages and CD8+ T cells [104]; eosinophils are increased [5,122], mainly in the lamina propria rather than the epithelium [122], but with lower counts in COPD patients with chronic bronchitis [113]. There is a further increase in tissue eosinophilia during exacerbations [114], together with eotaxin and its receptor, CCR3 [123]. Interestingly, despite the prominent neutrophilia seen in the airway lumen in COPD, neutrophils are only modestly increased in the tissues in stable mild-to-moderate disease [122,124,125], being present in increased numbers in severe disease [124] and during exacerbations [125], when there is also an increase in expression of the neutrophil chemoattractants ENA-78 (CXCL-5) and IL-8, and the receptors CXCR-1 and CXCR-2 [125].

Biomarkers in COPD: (3) exhaled breath

COPD patients (both current and ex-smokers) have higher eNO levels than healthy controls [126,46], although EBC concentrations of nitrates and nitrites (NOx), which are NO metabolites, are the same in COPD patients and controls [46]. Levels of eNO are higher in unstable disease and during acute exacerbations [127] and correlate negatively with lung function [128,129]. Patients with high sputum eosinophil counts have higher levels of exhaled NO [130], and it has been suggested that monitoring eNO might be a useful marker of COPD patients who would benefit from corticosteroid therapy and might have a better bronchodilator response [130]. Findings to date have been equivocal; no difference was found in eNO or EBC NOx levels between steroid-naive COPD patients and those on steroid therapy [46], and of two therapeutic trials of ICS, one showed a reduction in eNO [131] and one did not [132].

Although exhaled carbon monoxide is simple to measure, and is slightly increased in COPD compared with healthy

smokers, the signal is small and confounded by the effects of environmental CO concentrations and passive smoking, so has not proved a useful measure in clinical practice [104].

Exhaled ethane (a marker of oxidative stress) in COPD patients has been shown to correlate with FEV_1 and to reduce after treatment with corticosteroids [133].

Levels of EBC 8-isoprostane (another marker of oxidative stress) are raised in COPD patients (current smokers and ex-smokers) compared with healthy smokers and healthy non-smokers [134] and also increase acutely after cigarette smoking in healthy subjects. Concentrations of EBC 8-isoprostane (and LTB4) have also been found to increase during acute exacerbations of COPD and to decrease with antibiotic therapy [135], but the effects of steroid therapy are not known. The aldehyde, malondialdehyde, which is generated as a result of lipid peroxidation, has been measured in EBC and shown to distinguish COPD subjects from healthy smokers [136].

Several inflammatory mediators have been reported to be elevated in EBC from COPD patients, including IL-6 (which correlates with number of cigarettes smoked daily, and with lung function), LTB4, and prostaglandin (PG)E2 [137–139]. Concentrations of LTB4 and PGE2 appear to be similar in steroid-treated and steroid-naive patients [139].

Biomarkers in COPD: (4) blood

Although primarily a chronic inflammatory disorder of the lungs, COPD is now recognised to have a systemic inflammatory component [140] and to be associated with an increased risk of cardiovascular disorders, lung and other cancers, and cachexia, although to what extent some of these reflect inflammatory overspill from the lung or additional effects of smoking (the main cause of COPD) is unclear.

Circulating neutrophil numbers are increased in smokers [141] and COPD patients [142], and their activation status in stable COPD patients (indicated by increased expression of the β_2-integrin unit CD11b/CD18) is also increased compared with healthy controls [142]. As with the findings in BAL and biopsy specimens, there is a significant increase in absolute number of circulating CD8+ T lymphocytes in both smoking and ex-smoking COPD patients compared with non-smoking controls, with a reversal of the normal CD4+/CD8+ ratio [119].

Several inflammatory markers and mediators have been shown to be increased in the plasma or serum of COPD patients. Circulating C-reactive protein (CRP) levels have been shown to be raised in the blood of stable COPD patients [143] and to predict prognosis in terms of hazard ratios for hospitalisation and death from COPD [144]. CRP values have been reported to increase further during acute exacerbations of COPD, irrespective of whether the exacerbation was associated with infection or not [145]; another study found that CRP levels are not increased in 50% of patients hospitalised with acute exacerbations, but are further elevated in subjects with significant sputum purulence [146]. However, no difference was shown in systemic CRP levels between frequent and infrequent exacerbators when matched by clinical status, lung function and sputum characteristics [147].

Cachexia and muscle wasting in COPD are associated with systemic inflammation and in particular with TNFα levels [148,149], although TNFα (and IL-6) are increased even in the absence of weight loss [150]. Systemic hypoxia has been proposed as the driver for activation of the TNFα system [151]. TNFα receptors are also raised in the circulation of COPD patients [143,152]. The percentage of CD8+ T cells in the blood producing TNFα and interferon-gamma (IFN-γ) is increased in COPD (both ex-smokers and current smokers) compared with smoking and non-smoking controls, while the percentage of CD4+ T cells producing Transforming Growth Factor-Beta (TGFβ) is reduced in both COPD and smoking controls compared with healthy non-smokers [119]. Elevated serum concentrations of IL-8, MMP-9, MCP-1 and vascular endothelial growth factor (VEGF) have also been reported in stable COPD patients compared with healthy controls [153].

Circulating levels of ECP, MPO and endothelin-1 (ET-1) are higher in stable COPD patients than controls and are further increased during exacerbations [154,155]. In the stable state, plasma ET-1 levels correlate inversely with lung function, while change in plasma ET-1 levels correlates with change in oxygen saturation. Serum leptin levels are raised in COPD patients compared with controls and show a relationship with severity of disease as assessed by FEV_1 and other systemic inflammatory markers [156].

During exacerbations of COPD fibrinogen and IL-6 levels in plasma have been shown to increase, with higher fibrinogen levels when the exacerbations are associated with purulent sputum [157,158]. Plasma fibrinogen levels correlate with accelerated lung function decline and an increased risk of hospitalisation [159]. ECP and the soluble IL-5 receptor-alpha (sIL-5Rα) are elevated in serum from exacerbating COPD patients compared with healthy controls, but only sIL-5Rα, and not ECP, is increased when the exacerbation is associated with a viral infection [160]. Recently blood levels of interferon-induced protein 10 (IP-10) and serum amyloid A (SAA) have been shown to be significantly increased during acute COPD exacerbations; SAA levels discriminated between pathogen and non-pathogen-associated episodes, being significantly higher in the former [161].

During treatment and recovery of COPD patients with exacerbations blood levels of the soluble decoy receptor for the pro-inflammatory cytokine IL-1β have been found to increase [143] as has total anti-oxidant capacity (TEAC), while concentrations of pro-inflammatory mediators decline [158].

Discussion and conclusions

The key potential value for biomarkers in disease is in diagnostic and prognostic indices, and as indicators of response to therapeutic interventions. This review concentrates on asthma and COPD and while detailed is by no means comprehensive. Among topics not discussed is the use of proof-of-mechanism biomarkers in drug discovery; for example, if developing a putative neutrophil elastase inhibitor it would clearly be essential to possess a validated assay for neutrophil elastase activity in an appropriate medium, for example, sputum or BAL.

It should be clear from the data reviewed that many of the techniques described are still at the exploratory stage, and not uncommonly published findings are equivocal or even contradictory. For some of the techniques described there is still a dearth of published data on values in healthy adults, without which cut-off points for defining abnormality in disease states cannot be established [162•].

Although asthma and COPD tend to be thought of as discrete disease entities, both are common and hence may co-exist in an individual; in addition, the studies reviewed show clearly that there is a degree of overlap in disease biomarkers, for example, although neutrophilic inflammation is a hallmark of COPD, it is also seen in asthma, particularly in severe disease, and eosinophilic inflammation is seen in COPD (particularly in some exacerbations) as well as in asthma. One study attempted to discriminate between the two disorders by measuring serum and BAL levels of IL-8, secretory leukocyte protease inhibitor (SLPI), soluble intracellular adhesion molecule-1 (sICAM-1) and the acute phase protein sCD14; only BAL IL-8 was significantly higher in COPD than asthma [163]. Another group took consecutive outpatients with fixed airway obstruction and characterised them by lung function, AHR, eNO, sputum and BAL analysis, bronchial biopsy, and HRCT scans. Interestingly, patients with a history of COPD and those with a history of asthma had a similar degree of airway obstruction and AHR. However, the asthmatics had significantly more eosinophils in peripheral blood, sputum, BAL, and mucosa, and fewer neutrophils in sputum and BAL, and a higher CD4+/CD8+ ratio of T cells in the mucosa. They also had significantly higher eNO, lower residual volume and higher diffusing capacity, and greater reversibility to both bronchodilators and steroids, and a lower emphysema score on HRCT [96]. Conversely, the markers that appear to be most robustly elevated in individuals with COPD are serum or plasma levels of CRP, fibrinogen, leukocytes, and TNFα [164].

Eosinophil counts in induced sputum, and eNO, show the most promise as biomarkers of airway inflammation in asthma, for diagnosis and for therapeutic monitoring [59,72•,84], and for prediction of response to corticosteroid therapy [165]. eNO may also be useful in identifying asthmatic patients most at risk of an exacerbation [85].

For COPD, the value of biomarkers in clinical practice is less well defined. A comprehensive review of markers of COPD disease severity found that most measures differentiated only poorly between different stages of COPD; only sputum neutrophil count and IL-8 level, serum TNFα and CRP showed a trend to separation, as did the arterial oxygen tension [166]. Very recent work has shown significant increases in concentrations of both CXCR3 and CCR5 ligands in COPD sputum compared with controls, and levels of all these chemokines correlated negatively with FEV1 and FEV1/FVC ratio [167]. Recent data from the Framingham heart study population showed a clear relationship between the degree of systemic inflammation present and impairment of lung function [168]. A clinical trial of the phosphodiesterase type 4 (PDE4) inhibitor, cilomilast, is claimed to be the first pharmacological intervention in COPD to result in a reduction in tissue inflammatory cells, CD8+ T cells and macrophages; however, there were no changes in any sputum biomarkers, nor in FEV1 [169]. In a rather similar study to the one described above in asthma [72•], COPD patients were treated according to the British Thoracic Society guidelines, with one group additionally being managed with the aim of minimising eosinophilic inflammation in the airway by monitoring IS eosinophil counts. Although there was a significant 62% reduction in severe exacerbations in the additional intervention group, this was confined to subjects with a baseline eosinophilia of >3%, and there was no effect on mild or moderate exacerbation frequency [170]. A clinical trial of ICS in stable COPD resulted in a significant decrease in eNO, but no significant effect on FEV1 [171].

Breakdown products of connective tissue, such as hydroxyproline and desmosine have been proposed as appropriate markers of the excessive tissue breakdown assumed to be occurring during the development of emphysema, and hence as surrogate markers during clinical trials of protease inhibitors [172]. However, a recent study that showed a reduction in concentrations of sputum, plasma and urinary desmosine/isodesmosine in only 8 weeks actually used an anticholinergic bronchodilator as the therapeutic intervention [173]: since these agents have no known effects on the inflammatory process nor on tissue breakdown, this finding, although intriguing, only serves to confuse the picture.

Pharmacogenetic variation has been studied in asthma and (to a lesser extent) COPD, with findings suggesting that the response to several classes of drugs used in the management of airflow obstruction, including β2 adrenoceptor agonists, leukotriene antagonists and glucocorticosteroids, can be affected by genetic variation [174]. Recent studies have shown that individuals homozygous for the Arg16 β2 adrenoceptor polymorphism have a reduced bronchodilator response to albuterol (salbutamol) [175] although interestingly, not to the long-acting β2 agonists formoterol and salmeterol [176]. It is possible that pharmacogenetic screening may be needed to stratify study subjects in clinical trials for at least some drug targets in the future.

It is likely that in the future biomarkers will become increasingly required to aid in determining those patients who will benefit from a given drug therapy to improve risk and/or cost benefit, especially since it is becoming more widely considered that both COPD and asthma embrace heterogenous patient populations of mixed clinical, immunological and inflammatory phenotypes. For example, blood IgE levels are used as a biomarker to determine which patients in a severe asthma population will benefit from treatment with the anti-IgE therapy omalizumab.

In conclusion a number of tools and techniques exist for measuring biomarkers in asthma and COPD. In asthma some of these biomarkers such as sputum eosinophilia or exhaled NO have become incorporated into patient management and are also used for detecting anti-inflammatory effects in clinical trials with new potential therapies. However, the same level of confidence for use of biomarkers in COPD does not yet exist, and there is a need for both the pharmaceutical industry and those involved in patient care to identify biomarkers to predict clinical efficacy of novel therapies early in clinical development [177•], to enable better patient management, and to identify patients who will benefit from specific medications.

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