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REVIEW ARTICLE

Use of Biomarkers in Chronic Obstructive Pulmonary Disease: Clinical Implications

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ABSTRACT

Chronic obstructive pulmonary disease (COPD) is characterised by airflow limitation, which may be progressive and leads to considerable morbidity and mortality. Aside from lung function measurements, there are no biomarkers that are routinely used clinically in the care of patients with COPD. Biomarker is commonly 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". Discovery and implementation of biomarkers may enhance the precision of COPD diagnosis, assessment of its risk and severity, response to therapy, and predict progression, enabling personalised health in COPD. In this review, we summarise recent advances in COPD biomarkers and discuss their clinical implications. **(BRN Rev. 2018;4:84-107)**

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INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is an inflammatory lung disease, which has a significant systemic component that contributes to its overall morbidity and mortality. The burden[1] and mortality[2] of COPD is increasing, but many COPD patients remain under- or undiagnosed[3]. COPD is a major public health problem worldwide. Identification and implementation of biomarkers to diagnose, predict and prognose COPD patients would enable precision health and improve health outcomes of COPD patients. However, to date, other than lung function measurements, there are no widely used clinical biomarkers to guide management of COPD patients.

Although there is no universally accepted definition of a biomarker, the National Institutes of Health (NIH) Biomarkers Definitions Working Group defines biomarker as a "characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to therapeutic interventions"[4]. In theory, an ideal biomarker is one that is safe, accurate, inexpensive, easy to measure, modifiable with therapy, and actionable. Similar to drug development, biomarker development requires a robust pipeline including discovery, validation, assay migration, optimization and clinical implementation that involves in most cases a stringent randomised controlled trial demonstrating improvement of patient outcomes in a cost-effective manner[5,6]. Based on their putative roles in the clinic, biomarkers may be categorised as prognostic, predictive, or response markers[7]. The potential applications of these biomarkers in COPD are shown in figure 1.

Biomarkers can be ascertained in any tissue but ultimately must reflect the pathogenic process of the disease in question. Common sources for biomarker discovery in COPD include blood, sputum, saliva, exhaled condensates, urine, and lung tissues obtained by surgical or bronchoscopic procedures. A biomarker may be a single measurement or consist of multiple components that are individually measured and then integrated together using sophisticated statistical or network analysis; it may be derived based on *a priori* knowledge of pathophysiology (i.e. candidate biomarker approach) or in an unbiased (unsupervised) fashion using genome-wide analysis.

BLOOD BIOMARKERS

To date, most of the biomarker efforts in COPD have relied on blood as the source of biomarker discovery. Blood is a very attractive source of biomarkers because it is easy and safe to obtain, and blood tests (once fully developed from the original biomarker work) are often highly reproducible and accurate. The downside of blood biomarkers is that the biomarker signal may not accurately reflect the disease process in the lungs or may be so weak (i.e. have high signal-to-noise ratio) that it cannot be deployed for patient care. Nevertheless, given the advantages of blood sampling, there has been considerable interest and progress in ascertaining blood-based biomarkers. These biomarkers are summarised in table 1 and table 2 and discussed below.

Cellular biomarkers in blood

One of the most common biomarkers that have been evaluated to date has been total white

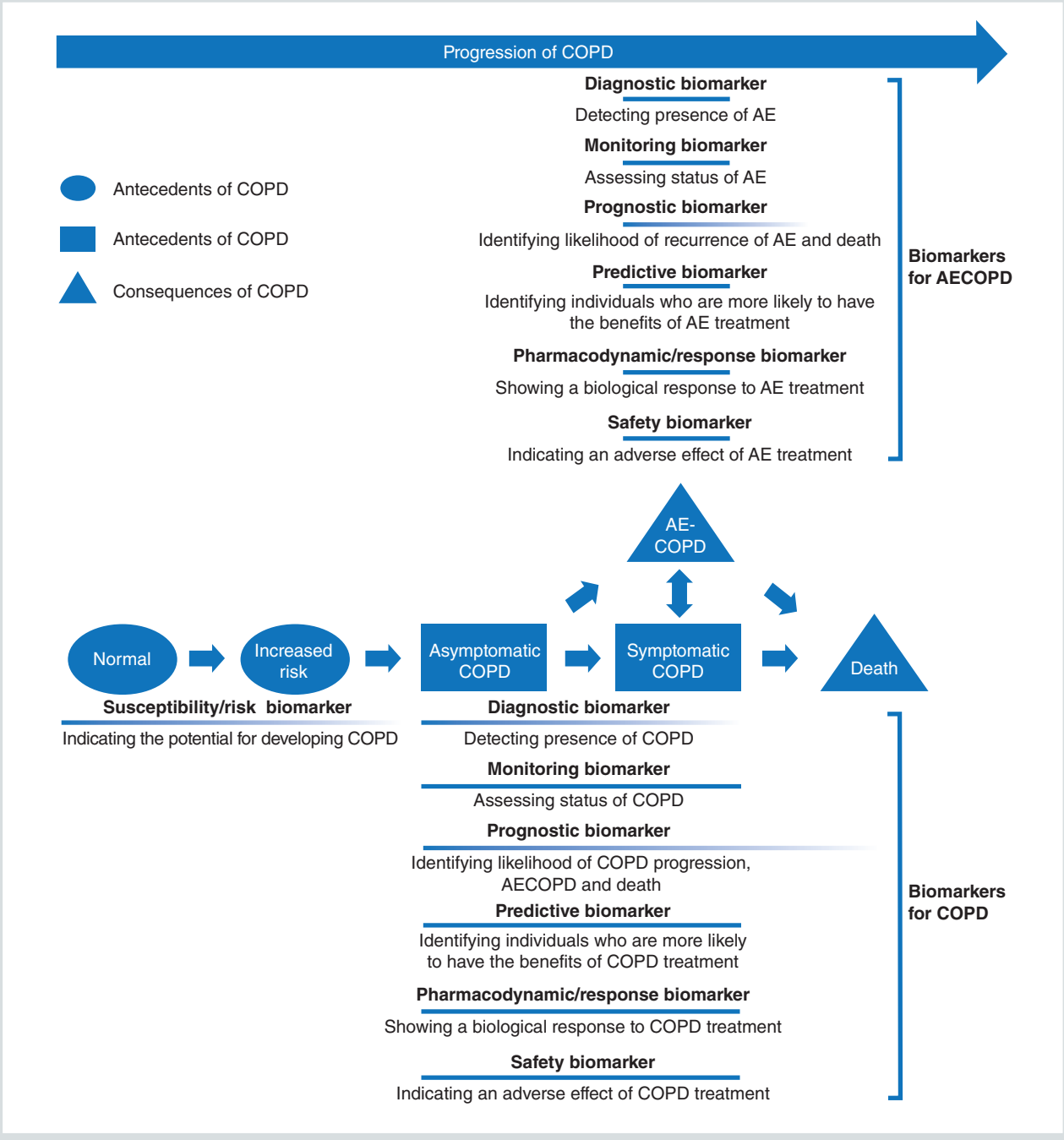

Figure 1. Categories of biomarkers according to the progression of chronic obstructive pulmonary disease (COPD). Schematic diagram of COPD is presented, and each bar of the categories of biomarkers means its utility according to the progression. To date, there is no diagnostic biomarker of COPD except spirometric indices. Monitoring biomarker is repeatedly measured to assess the status of the disease, and prognostic biomarker identifies the likelihood of a future clinical event. Predictive, pharmacodynamic/response and safety biomarker are related to treatment. Although acute exacerbation of COPD (AECOPD) is a clinical event that depends on COPD, except the susceptibility/risk biomarker for COPD, the categories of biomarkers an be stratified into those for COPD and those for AECOPD because diagnosis, prognosis and treatment are different between them. The prognostic biomarker of COPD could be considered as the susceptibility/risk biomarker for AECOPD.
AE: acute exacerbation.

Table 1. Cellular biomarkers in blood and their main findings according to the categories

	Susceptibility (future risk of COPD)	Diagnosis of COPD	Monitoring (severity of COPD: FEV_1 or presence of emphysema)	Prognosis (risk of mortality, rate of FEV_1 decline, or progression of emphysema)	Prognosis (risk of acute exacerbation)	Prognosis of acute exacerbation (recovery or mortality after AE)	Prediction of response to therapy
Total white blood cell count			Negative association with FEV_1 and FVC[8]	Positive association with mortality[10]	Positive association with future exacerbation[9]		
Eosinophil	Negative association with FEV_1[11]			Negative association with mortality[13,14] and progression of emphysema[12]	Positive association with severe exacerbation[15]	Eosinophilic phenotype of AE: a shorter length of hospitalization following systemic steroid[27] and a lower risk of bacterial presence[29]	Effects of ICS: reduced risk of pneumonia[16], unchanged bacterial load[17], short-term improvement of FEV_1[18], reduced rate of post-BD FEV_1 decline[19], and better prevention of AE[20-23]. Effects of anti-IL-5: reduced rate of moderate to severe AE[26]
Platelet						Thrombocytosis at admission: positive association with 1-year mortality and in-hospital mortality[30]	

AE: acute exacerbation; COPD: chronic obstructive pulmonary disease; FEV_1: forced expiratory volume in one second; ICS: inhaled corticosteroid; IL: interleukin; post-BD: post-bronchodilator

blood cell (WBC) count in blood. In COPD, the WBC count has been shown to be negatively associated with forced expiratory volume in one second (FEV_1) and forced vital capacity (FVC) at a cross-sectional level[8]. During acute exacerbations of COPD (AECOPD), WBC counts increase further[9]. WBC counts also predict 3-year mortality in COPD patients with or without adjustments for confounders[10]. However, owing to the relative poor resolution of the signal, WBC count cannot be used at an individual level to predict future risk of mortality, though at a population level it is a useful prognostic biomarker.

Blood eosinophil count is another commonly used biomarker in COPD. Similar to total WBC counts, at a population level, elevated peripheral eosinophil count is associated with reduced FEV_1 over 24 years of follow-up as demonstrated in the Vlagtwedde/Vlaardingen study, which evaluated 3,550 subjects between the ages of 15 and 35 years[11]. On the other hand, in the Evaluation of COPD Longitudinally to Identify

Table 2. Molecular biomarkers in blood and their main findings according to the categories

	Susceptibility (future risk of COPD)	Diagnosis of COPD	Monitoring (severity of COPD: FEV_1 or presence of emphysema)	Prognosis (risk of mortality, rate of FEV_1 decline, or progression of emphysema)	Prognosis (risk of acute exacerbation)	Diagnosis of acute exacerbation	Prediction of response to therapy	Comorbidity
Adiponectin			Positive association with emphysema on CT[33,34]	Positive association with rapid decline of FEV_1[32], no association with emphysema progression[34]				Inversed relation to cardiovascular disease and cardiovascular mortality[31]
Leptin			Negative association with FEV1[34]	Enhanced progression of emphysema[34]				
Leptin/adiponectin ratio			Negative association with FEV1[34]	Rapid decline of FEV_1[32], enhanced progression of emphysema[34]				
Aα-Val360			Lower FEV1 and worse emphysema on CT[115]	Rapid decline of FEV_1 and enhanced progression of emphysema[115]				
Alpha 1-antitrypsin (AAT)	Generically determined AAT deficiency is related to COPD with panacinar emphysema[116]	AAT was elevated in patients with COPD compared to healthy subjects[35]		Genetically lowered AAT was related to increased risk of emphysema and AE[36]	Increased plasma levels of AAT were associated with increased risk of AE[36]	AAT was elevated in acute exacerbation compared to stable state[35]		
Bilirubin		Negative association with presence of COPD[37]	Positive relation to post-BD FEV_1 in mild COPD[38]	Negative relation to FEV_1 decline[38]	Lower risk of AE[39]			Negative association with cardiac mortality[38]

(continued)

TABLE 2. Molecular biomarkers in blood and their main findings according to the categories *(Continued)*

	Susceptibility (future risk of COPD)	Diagnosis of COPD	Monitoring (severity of COPD: FEV_1 or presence of emphysema)	Prognosis (risk of mortality, rate of FEV_1 decline, or progression of emphysema)	Prognosis (risk of acute exacerbation)	Diagnosis of acute exacerbation	Prediction of response to therapy	Comorbidity
Brain natriuretic peptide (BNP) and amino-terminus of the prohormone BNP (NT-proBNP)				High mortality regardless of lung function[40]				Diagnosis of left heart involvement[41] and ischemic heart disease[42] at AE, and pulmonary hypertension[40]
Clara cell secretory protein (CC-16)		Negative association with presence of COPD[43]	Negative association with severity of COPD[43]	Negative relation to FEV_1 decline[44]				
Cholecalciferol (Vitamin D)				No association with lung function decline[45] and mortality[48]	No association with future risk of AE[47,48]			
C-reactive protein (CRP)		Elevated in COPD[50,51]		An accelerated decline in FEV_1 and increased mortality[52]		Elevated in AE compared to stable state[117]		Higher risk of cardiac infarction[118], progression of bronchial dysplasia[52]
Desmosine		Elevated in COPD[55]						Elevated in cardiovascular disease[55]
Fibrinogen		Elevated in COPD[51]		Increased risk of mortality[56,57]	Association with hospitalised AE[57]	Elevated in recent AE[59]		Remittent depressive symptoms in patients with high fibrinogen level[119]
Growth differentiation factor 11 (GDF11)		Decreased in COPD[60]	Inverse correlation with FEV_1[60]					

(continued)

Table 2. Molecular biomarkers in blood and their main findings according to the categories *(Continued)*

	Susceptibility (future risk of COPD)	Diagnosis of COPD	Monitoring (severity of COPD: FEV_1 or presence of emphysema)	Prognosis (risk of mortality, rate of FEV_1 decline, or progression of emphysema)	Prognosis (risk of acute exacerbation)	Diagnosis of acute exacerbation	Prediction of response to therapy	Comorbidity
Helicobacter pylori seropositivity			Association with reduced lung function[62]				Prevention of AE by prophylactic azithromycin[63]	
Interleukin-6 (IL-6)	Association of IL-6 SNP with a rapid decline of FEV_1[65]		Positive association with reduced FEV_1[66]			Higher in AE than in stable or convales-cent state[67] Elevated in patients with virus infection[68]		
Immunoglobulin G (IgG)					Association of lower IgG level with higher frequency of AE[69]			
Gamma-induced protein 10 (IP-10)						Diagnosis of AE due to human rhinovirus (HRV) infection[120]		
Leukocyte telomere length (LTL)		Association of shorter LTL with COPD[70,71]	Association of shorter LTL with lower FEV_1[82]	Association of shorter LTL with a higher risk of cancer and mortality[73]				
Monocyte chemotactic protein 1 (MCP-1)						Elevated in patients with virus infection[68]		

(continued)

TABLE 2. Molecular biomarkers in blood and their main findings according to the categories *(Continued)*

	Susceptibility (future risk of COPD)	Diagnosis of COPD	Monitoring (severity of COPD: FEV_1 or presence of emphysema)	Prognosis (risk of mortality, rate of FEV_1 decline, or progression of emphysema)	Prognosis (risk of acute exacerbation)	Diagnosis of acute exacerbation	Prediction of response to therapy	Comorbidity
Myeloperoxidase (MPO)				Association of elevated MPO with accelerated decline of FEV_1 and increased risk of cardiovascular mortality[121]				
Neutrophil gelatinase-associated lipocalin (NGAL)		Increased in COPD[122]						
Pulmonary and Activation-Regulated Cytokine/ Chemokine (C-C motif) ligand 18 (PARC/CCL18)		Increased in COPD[74,75]		Association of elevated PARC/CCL-18 with increased risk of mortality[74]	Increased in frequent exacerbator[75]			
Procalcitonin (PCT)							Aid to reduce antibiotics prescription during AE without deteriorated outcome[76]	
Symmetric dimethylarginine (SDMA)				No association with six years mortality[123]				
Sirtuin deacetylase (SIRT1)		Reduced in patients with COPD[124]	Association of increased SIRT1 with increased FEV_1 and 6MWT and decreased degree of emphysema on CT[124]		Negative correlation with frequency of AE_{124}			

(continued)

Table 2. Molecular biomarkers in blood and their main findings according to the categories *(Continued)*

	Susceptibility (future risk of COPD)	Diagnosis of COPD	Monitoring (severity of COPD: FEV_1 or presence of emphysema)	Prognosis (risk of mortality, rate of FEV_1 decline, or progression of emphysema)	Prognosis (risk of acute exacerbation)	Diagnosis of acute exacerbation	Prediction of response to therapy	Comorbidity
Pro-surfactant protein B (pro-SFTPB)			Association of increased plasma pro-SFTPB levels with reduced FEV_1 and increased emphysema on CT[125]	Association of Increased plasma pro-SFTPB levels with accelerated decline of FEV_1[125]				
Surfactant protein D (SP-D)		Elevated in COPD[79]	Association of higher levels of SP-D with less emphysema[80]		Association of high level of SP-D with an increased risk of exacerbations over the following 12 months[79]	Elevation during AE[81]		
Soluble receptor for advanced glycation end-products (sRAGE)		Reduced in patients with COPD[82]	Positive correlation with FEV_1[82] Negative association with degree of emphysema[83]	Negative association with rate of progression in emphysema[80]				
Soluble TNF receptor 75 (sTNFR75)					Positive association with risk of AE[126]		Declines of sTNFR75 with treatment were associated with the preventive effect of azathioprine[126]	
Tumor necrosis factor (TNF)-α						Elevated in patients with virus infection[68]		Association with depression and fatigue[127]

6MWT: 6-minute walking test;AE: acute exacerbation; COPD: chronic obstructive pulmonary disease; CT: computed tomography. FEV_1: forced expiratory volume in one second; post-BD: post-bronchodilator

Predictive Surrogate End-points (ECLIPSE) cohort, patients with persistently high blood eosinophils (≥ 2% of WBC) had more favourable clinical features at baseline including higher FEV_1, improved health status as reflected in lower St. George's Respiratory Questionnaire (SGRQ) scores, less intense symptoms as evidenced by lower modified Medical Research Council (mMRC) dyspnoea score and a lower rate of emphysema progression compared to those with persistently low or variable blood eosinophil count[12]. Consistent with these findings, in a pragmatic COPD cohort in Spain, patients with persistently high blood eosinophils (≥ 150/μL) demonstrated improved survival compared with those with low (< 150/μL) or variable blood eosinophil count[13]. In patients in the COPD History Assessment In SpaiN (CHAIN) cohort and the body-mass index, airflow Obstruction, Dyspnoea, Exercise performance (BODE) cohort, persistent blood eosinophilia (≥ 300 cells/μL) over two years was not a risk factor for acute exacerbation (AE) but was a significant predictor of improved survival[14]. In the Copenhagen General Population Study, COPD patients with blood eosinophilia (≥ 340 cells/μL) had a 1.76-fold increased risk of severe exacerbation compared to COPD patients without blood eosinophilia after adjusting for confounders[15]. It should be noted that blood eosinophils may not reflect lung tissue expression of eosinophils or airway eosinophilia[13], which may in part explain the heterogeneity in results across studies that have evaluated the relationship between peripheral eosinophils and COPD outcomes.

Blood eosinophils have also been considered as a response biomarker in COPD. A meta-analysis of ten clinical trials that evaluated the clinical effects of inhaled corticosteroids (ICS) or combination of ICS and long-acting beta-agonists (ICS/LABA) in COPD demonstrated that patients with blood eosinophil counts of less than 2% of total WBC at pre-randomisation had a higher risk of pneumonia than those with eosinophil counts of 2% or more[16]. In a clinical trial of ICS/LABA versus LABA to evaluate the change of sputum bacterial load, patients who were treated with ICS and had a lower baseline sputum (≤ 2%) or blood eosinophil (≤ 2%) showed increased bacterial load after one year of treatment with ICS compared with those without these traits[17]. COPD patients with high blood eosinophils (> 260/μL) and high plasma periostin (> 23 ng/mL) levels demonstrated greater improvements in FEV_1 (> 12% and 200 mL increase from baseline) with a 3-month treatment of ICS/LABA compared with those COPD patients who did not have these features[18]. In the Inhaled Steroids in Obstructive Lung Disease in Europe (ISOLDE) study, which evaluated the effects of ICS for three years, COPD patients with blood eosinophilia ≥ 2% had a slower rate of post-bronchodilator (post-BD) FEV_1 decline by 33.9 mL/year with fluticasone propionate versus placebo[19]. Recently, *post hoc* analyses of studies comparing ICS/LABA and LABA demonstrated that increased eosinophil count in blood was associated with improved responses to ICS/LABA[20-22] and poorer responses to ICS withdrawal[23]. In contrast, in the Effect of Indacaterol Glycopyronium versus Fluticasone Salmeterol on COPD Exacerbations (FLAME) study, which compared the effects of LABA combined with long-acting muscarinic antagonist (LAMA) versus ICS/LABA, baseline blood eosinophil count did not modify the effects of LABA/LAMA or ICS/LABA on the rates of COPD exacerbations. However, it is notable that the FLAME study excluded patients who demonstrated a blood

eosinophil count of > 600 cells/μL[24]. Benralizumab is an anti-interleukin 5 (IL-5) receptor α monoclonal antibody that reduces exacerbation in patients with eosinophilic asthma. In moderate-to-severe COPD patients who demonstrated sputum eosinophilia ≥ 3% and had at least one AE in the previous year, there was a trend towards increased (beneficial) response to benralizumab among patients with blood eosinophilia ≥ 200 or ≥ 300 cells/μL[25]. Mepolizumab is a humanised anti-IL-5 monoclonal antibody that has been approved by the Food and Drug Administration (FDA) for the treatment of severe eosinophilic asthma. In COPD patients who had blood eosinophils ≥ 150 cells/μL at screening or ≥ 300 cells/μL at any point in the previous year and had a history of moderate to severe AECOPD in the year prior to randomisation, treatment with mepolizumab reduced the rate of moderate-to-severe AECOPD compared with placebo[26]. Nevertheless, given the cost of these biologics and the availability of cheaper alternates (e.g., inhaler-based therapy, azithromycin or roflumilast), the role of these targeted anti-eosinophilic therapy in COPD remains uncertain.

Eosinophil has also been evaluated as a possible response biomarker for systemic corticosteroid treatment. In hospitalised AECOPD patients, those who demonstrated an increased eosinophil count in peripheral blood ≥ 200 cells/μL or ≥ 2% of total WBC had lower levels of c-reactive protein (CRP) at admission and experienced shorter lengths of hospitalization following oral corticosteroid treatment than those without this feature[27]. Aaron et al.[28] showed in 81 patients with COPD that treatment with prednisone (40 mg/d for 10 days) was less likely to fail compared with treatment with etanercept, a tumour necrosis factor α (TNF-α) antagonist, in patients whose blood eosinophils were 2% or greater at the time of AECOPD (22% versus 50%; p = 0.08). In contrast, there were no differences in the treatment failure rates between the two treatment groups in patients whose peripheral eosinophil counts were less than 2%[28].

Eosinophilic phenotyping may also assist in identifying the aetiology of AECOPD. In the Acute Exacerbation and Respiratory InfectionS in COPD (AERIS) cohort, which consisted of patients with moderate to very severe COPD, those who had high blood eosinophils (≥ 2%) at baseline demonstrated persistent eosinophilia during AE (and were labelled eosinophilic phenotype). The patients with an eosinophilic phenotype had a lower rate of bacterial infection/colonization in sputum samples during exacerbations compared to those who had a non-eosinophilic phenotype[29].

However, there are several important limitations to the implementation of peripheral eosinophil count as a response biomarker in COPD. First, although at a population level (or in large therapeutic trials), increased peripheral eosinophil count is associated with improved responses to ICS to prevent exacerbations or oral corticosteroids to prevent treatment failures during AECOPD and may be useful for predicting responses to anti-IL5 therapy, its relatively signal-to-noise ratio makes it difficult to apply this biomarker to predict therapeutic responses at an individual level. Second, there is no consensus on the optimal cut-off that should be used to determine who should and should not receive steroid-based therapy. The cut-offs used to define eosinophilia have varied across different studies. Moreover, many cut-offs that have been employed have been at a level that would be considered "normal" (e.g., 300 cells/uL).

Until these issues can be resolved, peripheral eosinophil cannot be adopted widely in clinical practice for patient care.

Although less extensively studied than peripheral WBC count or blood eosinophils, platelet counts have also been evaluated as biomarkers in COPD. Thrombocytosis over 400×10^9 cells/mm^3 at admission to hospital for AECOPD has been associated with increased in-hospital and 1 year-mortality. The use of antiplatelet medications including clopidogrel and aspirin has been associated with lower 1-year mortality in the same study[30].

Molecular biomarkers in blood

Adiponectin and leptin are adipokines that are secreted mainly by adipose tissue and associated with inflammation and nutrition. Serum adiponectin concentrations have been shown to be related to respiratory mortality[31], increased bronchial reactivity[31], an accelerated decline in lung function[31,32], and emphysema on computed tomography (CT)[33,34]. Plasma leptin and the leptin/adiponectin ratio were also associated with reduced FEV_1 and accelerated progression of emphysema on CT[34]. However, the associations of adipokines with a decline of FEV_1 or progression of emphysema have been discordant among the studies[32,34].

Alpha-1 antitrypsin (AAT) is an inhibitor of neutrophil elastase. Blood levels of AAT can be used to detect AAT deficiency, which is responsible for COPD in a select number of patients. However, because AAT is an acute phase reactant, it may be elevated during AECOPD[35], and increased plasma levels of AAT have been associated with increased risk of AECOPD[36].

Bilirubin has anti-oxidant, anti-inflammatory and anti-proliferative properties. After adjusting for other health indicators, a 0.1-mg/dL increase in serum bilirubin levels has been associated with a 6% decrease in the risk of COPD[37]. In mild COPD, bilirubin has been positively related to post-BD FEV_1 and negatively related to the annual decline in FEV_1 and risk of death from coronary heart disease following adjustments for baseline demographics, smoking, lung function[38]. Higher bilirubin has also been associated with lower hazard for AECOPD in the MACROlide azithromycin to prevent COPD exacerbations (MACRO) Study[39].

Brain natriuretic peptide (BNP) and amino-terminus of the prohormone BNP (NT-proBNP) are biomarkers for mechanical stress in the cardiomyocytes related to volume overload and are also raised in the presence of pulmonary hypertension. Elevated BNP (> 75 pg/ml) has been associated with significant pulmonary hypertension as measured by right heart catheterization and poor survival regardless of the severity of lung function impairment or extent of hypoxemia[40]. An NT-proBNP level of less than 1,000 pg/ml has been shown to be useful in ruling out left ventricular systolic dysfunction in AECOPD[41] and increased NT-proBNP at exacerbation has been noted in patients with ischemic heart disease[42].

Clara cell secretory protein (CC-16) is a marker of club cell toxicity and its expression in lung and blood is reduced in patients with COPD[43]. In patients with mild-to-moderate COPD, decreased serum CC16 levels have been associated with accelerated decline in FEV_1 after adjusting for confounders including age, sex, race, smoking status, airway reactivity, body mass index, and baseline FEV_1[44].

Baseline plasma 25-hydroxyvitamin D levels were not shown to be predictive of lung function decline in the Lung Health Study (LHS) cohort[45], whereas in another healthy male smoker cohort, baseline vitamin D deficiency (< 20 ng/mL) was associated with lower lung function and accelerated decline in FEV_1[46]. Baseline 25-hydroxyvitamin D (25[OH]D) levels have not been shown to relate to subsequent risk of AECOPD[47,48] or mortality[48]. Baseline 25(OH)D has not been associated with the changes in FEV_1 after four weeks of ICS treatment[49].

C-reactive protein (CRP) is an acute phase protein, which rises during acute infectious, inflammatory or neoplastic processes. Serum CRP levels have been shown to be significantly higher (on average) in patients with COPD than in non-smoking or smoking control subjects[50]. A meta-analysis of 5 studies showed that CRP was higher in patients with COPD than in normal controls[51]. In mild-to-moderate COPD, CRP levels have also been associated with accelerated decline in FEV_1, as well as all-cause, cardiovascular, and cancer-specific causes of mortality[52]. In patients with a smoking history of 30 or more pack-years and who demonstrated dysplastic lesions larger than 1.2 mm on bronchoscopic biopsies, plasma CRP > 0.5 mg/L was associated with increased odds for progression of disease than those with CRP ≤ 0.5 mg/L[52]. CRP may also predict treatment failure to therapy. In data from 152 patients of the placebo arm of a randomised trial of amoxicillin/clavulanate for exacerbations of mild-to-moderate COPD, the probability of failure without antibiotics was highest in the presence of sputum purulence and high CRP concentration (≥ 40 mg/L)[53].

Desmosine is a biomarker of elastin degradation that is a major feature of emphysema and increased arterial stiffness[54]. Plasma desmosine has been shown to be elevated in patients with COPD compared to control subjects, and also increased in COPD patients who also have concomitant cardiovascular diseases. Elevated plasma desmosine has been associated with all-cause mortality but not with emphysema[55].

Fibrinogen is an acute phase reactant and has been qualified by FDA as a prognostic biomarker for exacerbations and all-cause mortality in patients with COPD. Plasma fibrinogen, on average, has been shown to be higher in patients with COPD than in normal controls[51] and positively associated with mortality[10,56]. In a pooled analysis of 6,376 COPD patients from five studies, high plasma fibrinogen levels (> 350 mg/dL) were associated with an increased risk of hospitalised exacerbations within 12 months (in four studies) of blood draw and death within 36 months (in five studies) of blood draw[57]. Despite this, in an analysis that used Mendelian randomisation, genetically increased levels of fibrinogen were not significantly related to the risk of AECOPD, suggesting that fibrinogen may not be causally involved in this process[58]. In the ECLIPSE cohort, fibrinogen was the most repeatable biomarker among those that relate to AECOPD[59].

Growth differentiation factor 11 (GDF11) belongs to the transforming growth factor β superfamily and is a circulating protein that may retard the aging process. The levels of plasma GDF11 have been shown to be significantly decreased in patients with COPD compared with controls and inversely related to FEV_1[60]. GDF11 could be a predictive biomarker for patients with COPD who might benefit from GDF11 supplementation therapy[61].

Seropositivity of *Helicobacter pylori*, as defined by *Helicobacter pylori* immunoglobulin G concentrations above 18 DU/mL, has been associated with reduced lung function and increased risk of cardiovascular mortality in patients with mild to moderate COPD[62]. In the Azithromycin for the Prevention of Exacerbations of COPD (MACRO) study, azithromycin was most effective in reducing the risk of exacerbation in those who were seropositive to *Helicobacter pylori*[63]. *Helicobacter pylori* seropositivity may be a biomarker to predict the therapeutic effectiveness of azithromycin in the prevention of AECOPD.

IL-6 is an inflammatory cytokine which could reflect systemic inflammation and is produced by adipocytes, muscles, liver, and lungs[64]. Single nucleotide polymorphisms (SNP) in IL-6 have been associated with a rapid decline of FEV_1[65]. Serum IL-6 levels have also been associated with reduced FEV_1[66] and rises to even higher concentrations during acute exacerbations[67]. In hospitalised patients with AECOPD, blood levels of inflammatory cytokines including interleukin (IL)-6, TNF-α, and monocyte chemotactic protein 1 (MCP-1) have been shown to be elevated in patients with COPD and increase further with acute virus infections[68].

Serum immunoglobulin G (IgG) levels are promising predictive biomarkers for exacerbation in COPD. In a pooled analysis consisting of data from the MACRO study and the Prospective Randomized Placebo-Controlled Trial of Simvastatin in the Prevention of COPD Exacerbations (STATCOPE) study, approximately one in five patients with COPD had total IgG levels below the limit of normal for an adult (< 7.0 g/L) and, most importantly, these individuals had two times the risk of AECOPD compared with patients with normal serum IgG levels[69].

Leukocyte telomere length (LTL) is a biomarker of cellular senescence. Patients with COPD have on average shorter LTL and a higher rate of telomere attrition than control smokers or nonsmokers[70,71]. LTL has been associated with FEV_1 and FEV_1/FVC in patients with COPD[72]. In the LHS, those with reduced LTL had a higher risk of cancer and total mortality compared with those with normal LTL[73]. LTL could be a risk factor both for COPD and lung cancer.

Pulmonary and activation-regulated cytokine/chemokine (C-C motif) ligand 18 (PARC/CCL-18) is an inflammatory chemokine produced in the lung. Serum PARC/CCL-18 levels have been shown to be elevated in patients with COPD compared with smoking or non-smoking controls[74,75], which in turn has been associated with increased risk of mortality[74] and AECOPD requiring hospitalization in the previous 12 months[75].

In a meta-analysis of eight trials, procalcitonin (PCT)-based protocols could reduce antibiotic prescription and total antibiotic exposure without worsening clinical outcomes; however, these data should be interpreted cautiously as the overall quality of data was deemed low[76]. An observational study that used data from a geographical consortium of hospitals demonstrated that decisions to initiate antibiotics therapy or duration of antibiotics treatment on AECOPD were not impacted by PCT testing[77].

Surfactant protein D (SP-D) is produced by alveolar type II cells and has an immunomodulatory role which is essential to host defenses. Several SNPs in surfactant protein–D (SFTPD)

have been associated with increased susceptibility to COPD in some but not all cohorts[78]. SP-D is elevated in patients with COPD compared to those without COPD[79]. Higher plasma levels of SP-D have been associated with less emphysema[80] but with an increased risk of exacerbations[79]. Serum SP-D levels are also increased in patients during exacerbations[81].

Soluble receptor for advanced glycation end-products (sRAGE) may be a biomarker of the underlying inflammatory process of COPD. sRAGE levels are generally lower in patients with COPD than in controls and are positively correlated with FEV_1[82]. Furthermore, higher sRAGE levels have been associated with less emphysema[80,83] and reduced rate of emphysema progression over time[80].

Multi-component biomarkers in blood

Biomarker panels consisting of composite signatures are now beginning to emerge as potential blood tests for clinical application. For instance, a higher fibronectin to CRP ratio (> 150) has been related to increased risk of all-cause mortality[84]. When WBC count, CRP, IL-6, and fibrinogen have been assessed, aggregate score based on all of these components was more predictive of all-cause mortality than individual assays in COPD patients[85]. This also held true for assessing other endpoints such as the risk of myocardial infarction, heart failure, diabetes mellitus, lung cancer, and pneumonia[86,87]. However, some clinical endpoints, which may not be sensitive to inflammation, such as depression, are not responsive to biomarkers of inflammation singly or in aggregate[88].

Another "panel" of biomarkers is the concurrent measurement of CRP and NT-proBNP. Together they have a higher performance as measured by receiver operating characteristics (ROC) area under the curve (AUC) than CRP alone in diagnosing AECOPD that require hospitalisation[89]. Similarly, combination of two cardiac markers (high-sensitivity troponin I and copeptin) showed better performance in predicting 30 day mortality than individual components[89].

Other combinations of biomarkers including CC-16, sRAGE, fibrinogen, CRP, and SP-D have been evaluated in COPD and some have shown associations with COPD outcomes including AECOPD, disease progression, and mortality[90]. Although in general this is true, reproducibility of these combinatorial biomarkers has been problematic. Other approaches to combinatorial biomarker discovery are to use a multiplex protein array[91-93] and to combine biomarkers to clinical parameters[10,94,95]. In one study, investigators measured 143 serum biomarkers using a multiplex immunoassay platform. Of these, 24 proteins demonstrated significant relationships with FEV_1, diffusing capacity of carbon monoxide (DL_{CO}), 6-minute walk test (6MWT), BODE index or risk of exacerbations[93].

SPUTUM BIOMARKERS

Sputum samples may better reflect the inflammatory process in the airways of COPD than blood samples. However, to properly

collect high quality sputum samples for interrogation, centres must have considerable expertise and resources for sputum induction, processing and evaluation. Another disadvantage is that inflammatory biomarkers measured in sputum generally have lower repeatability than similar molecules measured in blood. Additionally, investigators should be aware that there may also be significant variation in biomarker levels between spontaneously expectorated and induced sputum from the same individual[96]. Sputum biomarkers are summarised according to their category in table 3 and briefly discussed below.

In a cohort of 148 COPD patients with a follow-up of 2.91 years, a high neutrophil count and high sputum IL-6 levels were found to be associated with rapid FEV_1 decline[67]. In the ECLIPSE study, there was a weak association between percentage of neutrophils in sputum and FEV_1% predicted and SGRQ[97]. In the Subpopulations and Intermediate Outcomes in COPD Study (SPIROMICS) cohort, the high sputum eosinophil group (≥ 1.25%) had lower pre- and post-BD FEV_1%, higher emphysema score and more gas trapping on CT scans than those without these features. They were also more likely to experience corticosteroid-requiring AECOPD events than those who had low percentages of eosinophils in their sputum[98].

Mucin is a macromolecule that consists of mucus and a protein backbone, which together forms a barrier to pathogen invasion in the airways[99]. In patients with severe COPD, mucosal occlusion of small airways is the single best predictor on histology for mortality[100]. Total mucin concentrations have also been associated with severity of airflow obstruction as indexed by Global Initiative for Chronic Obstructive Lung Disease (GOLD) grades (1 versus 2) and are higher in those who experience frequent exacerbations. Moreover, total mucin concentrations are significantly higher in COPD patients who have either classically defined or SGRQ-defined chronic bronchitis compared with those who do not have chronic bronchitic features regardless of the presence of emphysema[101].

OMICS BIOMARKERS

Omics approaches have been emerging to accelerate the discovery and development of new biomarkers in COPD (Fig. 2). One of the major advantages of this approach is the ability to ascertain truly novel biomarkers using "hypothesis-free" experiments. Large-scale genome-wide association studies (GWAS) is one such example. In a meta-analysis of four large COPD cohorts, a significant hit was identified on the locus of chromosome 19q13. These SNPs have also been associated with COPD, pre-bronchodilator (preBD) FEV_1, and severe COPD in a separate cohort[102]. Using GWAS data, investigators found that a genetic risk score based on 95 variants that was predictive of COPD[103]. There are other examples: for instance, using exome sequencing to test coding genetic variants, IL-27 variant that regulates expression of mitochondrial Tu translation elongation factor (TUFM) was found to be significantly associated with COPD[104]. By applying a weighted gene co-expression network analysis (WGCNA) to peripheral blood transcriptome, two modules enriched in IL-8 and IL-10 pathway were found to be negatively associated with COPD and one module enriched in DNA transcription

TABLE 3. Biomarkers in sputum and their main findings according to the categories

	Diagnosis of COPD	Monitoring (severity of COPD: FEV_1 or presence of emphysema)	Prognosis (risk of mortality, rate of FEV_1 decline, or progression of emphysema)	Prognosis (risk of acute exacerbation)	Diagnosis of acute exacerbation	Comorbidity
Neutrophil		Weak association with FEV_1[97]	Association with rapid decline of FEV_1[67]			
Eosinophil		Association of high sputum eosinophil with low FEV_1 and high emphysema and air trapping[98]		Positive association with corticosteroid-requiring AE[98]		
Defensin	Higher in patients with COPD than those with asthma and healthy controls[128]					
Matrix metalloproteinase-2 (MMP-2, gelatinase A) and prostaglandin E2 (PGE2)	Higher in COPD[129]	Inverse correlation with FEV_1[129]				
Mucin		Association with airflow obstruction[101]		Higher in patients with exacerbations[101]		Higher in patients with chronic bronchitis[101]
Neutrophil gelatinase-associated lipocalin (NGAL)	Higher in patients with ACO than in those with asthma or COPD alone[130]					
Elafin and secretory leukoprotease inhibitor (SLPI)					Association of lower SLPI with subsequent bacterial infection after rhinovirus infection[131]	
Sulfatase modifying factor-1 (SUMF1)	Lower in patients with COPD[132]					

ACO: asthma-COPD overlap; AE: acute exacerbation; COPD: chronic obstructive pulmonary disease; FEV_1: forced expiratory volume in one second.

and translation was positively associated with COPD[105]. Whole exome sequencing revealed that rs10859974 in CCDC38 could be a variant which attenuates lung function decline related to smoking[106]. Gene expression profile signatures in sputum and blood cells that have included B3GNT, LAF4, and ARHGEF10 have been associated with frequent exacerbations[107].

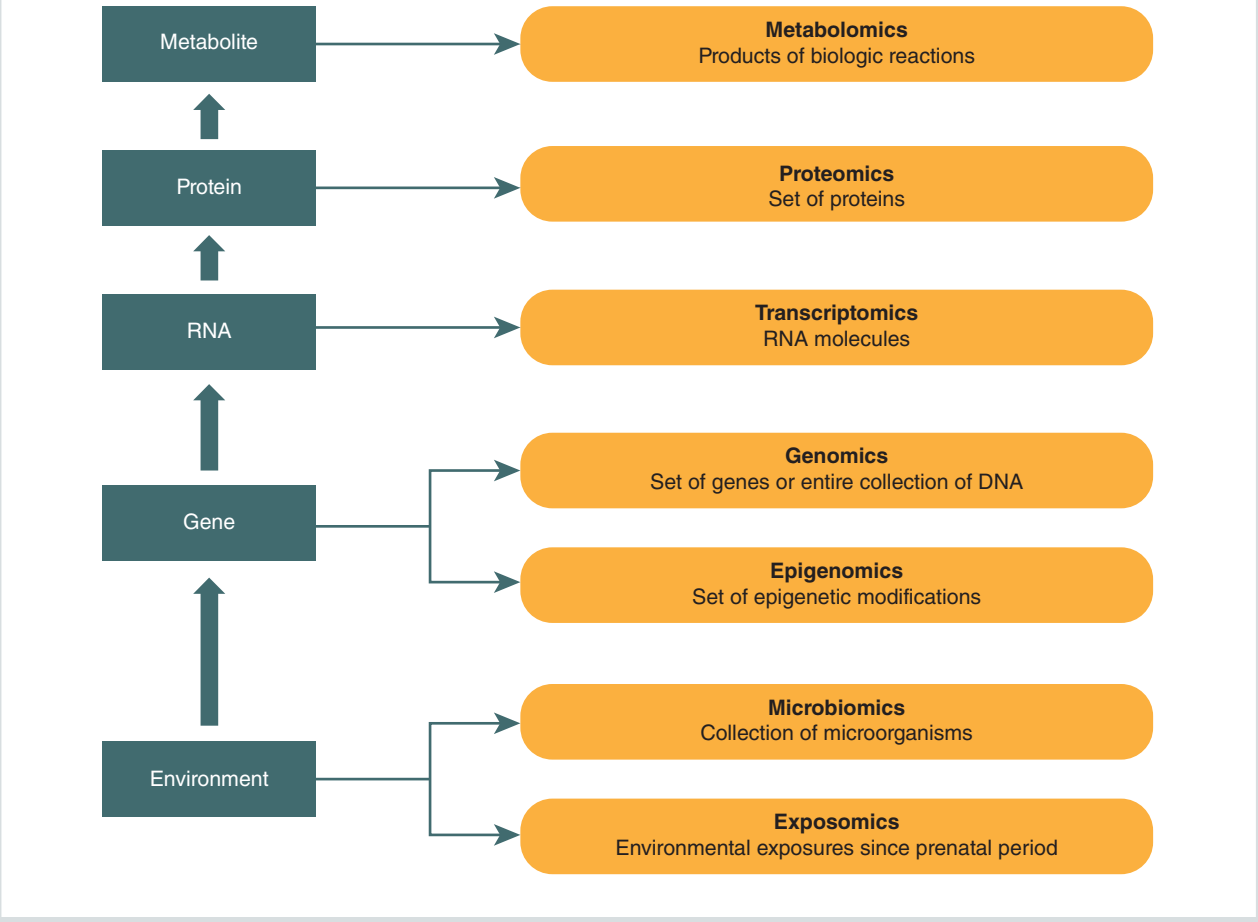

Figure 2. Example of biologic/pathological processes and corresponding kinds of omics.

At the protein level, mass spectrometry is now commonly used to discover novel biomarkers. In one study, using multiple reaction monitoring mass spectrometry, 129 blood proteins were compared in patients at the time of AECOPD versus convalescent states. Biomarker scores derived from five proteins (apolipoprotein A-IV, complement component C9, fibronectin, apolipoprotein C-II, lipopolysaccharide-binding protein) were differently expressed in blood in patients during AECOPD versus those in clinically stable conditions. The receiver operating characteristic cross-validation (CV)-AUC statistic was 0.73 in the discovery cohort, whereas the CV-AUC values were 0.77 and 0.79 in the replication cohort[108]. In the SPIROMICS and Genetic Epidemiology of COPD (COPDGene) cohorts, 90 blood proteins were measured by Myriad-RBM multiplex panel. A few biomarkers were replicable between these two cohorts but they added very little predictive value to clinical information for AECOPD[109]. When focusing on serum amino acid profiles, there were different patterns in COPD GOLD-4 versus former smoker controls; emphysema versus non-emphysema; cachexic versus non-cachexic[110]. Gas

chromatography and mass spectrometry (GC/MS) and electronic nose (eNose) identified that exhaled molecular profiles were associated with the type of inflammatory cells in mild to moderate COPD patients[111].

In human lung tissue samples, an increase of the Firmicutes (F) phylum in GOLD 4 patients compared to controls has been noted. However, the clinical relevance of flora changes in COPD lung remains unknown[112]. Cluster analysis of quantitative PCR in sequential sputum samples of COPD patients (stable, AECOPD, twice after AECOPD) built three clusters (high Gammaproteobacteria (G), high Firmicutes, balanced Gammaproteobacteria:Firmicutes) according to Gammaproteobacteria:Firmicutes ratio. High Gammaproteobacteria cluster had increased G:F ratio at AECOPD and decreased G:F ratio to baseline and the elevated ratio was related to high inflammatory markers and low FEV_1[113].

IMPLEMENTATION

Clinical implementation of biomarkers into routine clinical practice is extremely challenging. The biomarkers that have the best chance of clinical implementation are those with the following features: 1) strong performance characteristics (i.e. high sensitivity and high specificity for the endpoint in question). For example, biomarkers that have a potential for clinical translation should have a ROC AUC value of 0.7 or greater in multiple independent cohorts (Fig. 3); 2) can be easily migrated to a clinical platform where analytic validation needs to occur. This is particularly challenging for genomic biomarkers and ensemble biomarkers, which do not have obvious clinical platforms that can be used for assay development and deployment; 3) have a relatively rapid turnaround time for the assay. This is particularly important for assays that will be deployed in urgent clinics or emergency departments in which results are required within an hour or less of sample collection; 4) are relatively inexpensive. As COPD is a common disease, affecting one in four to five adults over the age of 40 years, health care payers may be hesitant to deploy very costly assays in their health care system; and most importantly 5) are actionable. Tests by themselves are not particularly useful. Only tests that modify management that improves health outcomes of patients with COPD are useful clinically. Thus, assay developers must ask the question whether the biomarkers in question are actionable and only those for which an affirmative answer can be provided should be moved along the translational pipeline[5,6].

No blood biomarkers have been successfully implemented in clinics for the care of COPD patients. However, there are several in the pipeline that are promising. To facilitate translation, the Center for Drug Evaluation and Research (CDER) Biomarker Qualification Program has established some parameters for clinical implementation of biomarkers and for drug discovery (https://www.fda.gov/Drugs/DevelopmentApprovalProcess/DrugDevelopmentToolsQualificationProgram/BiomarkerQualificationProgram/ucm535383.htm). The COPD Biomarker Qualification Consortium has prioritised several novel biomarkers for development including plasma/serum sRAGE, desmosine, and blood eosinophils[114]. In sum, for successful clinical implementation, the biomarker must have strong performance characteristics, is reproducible, can be migrated

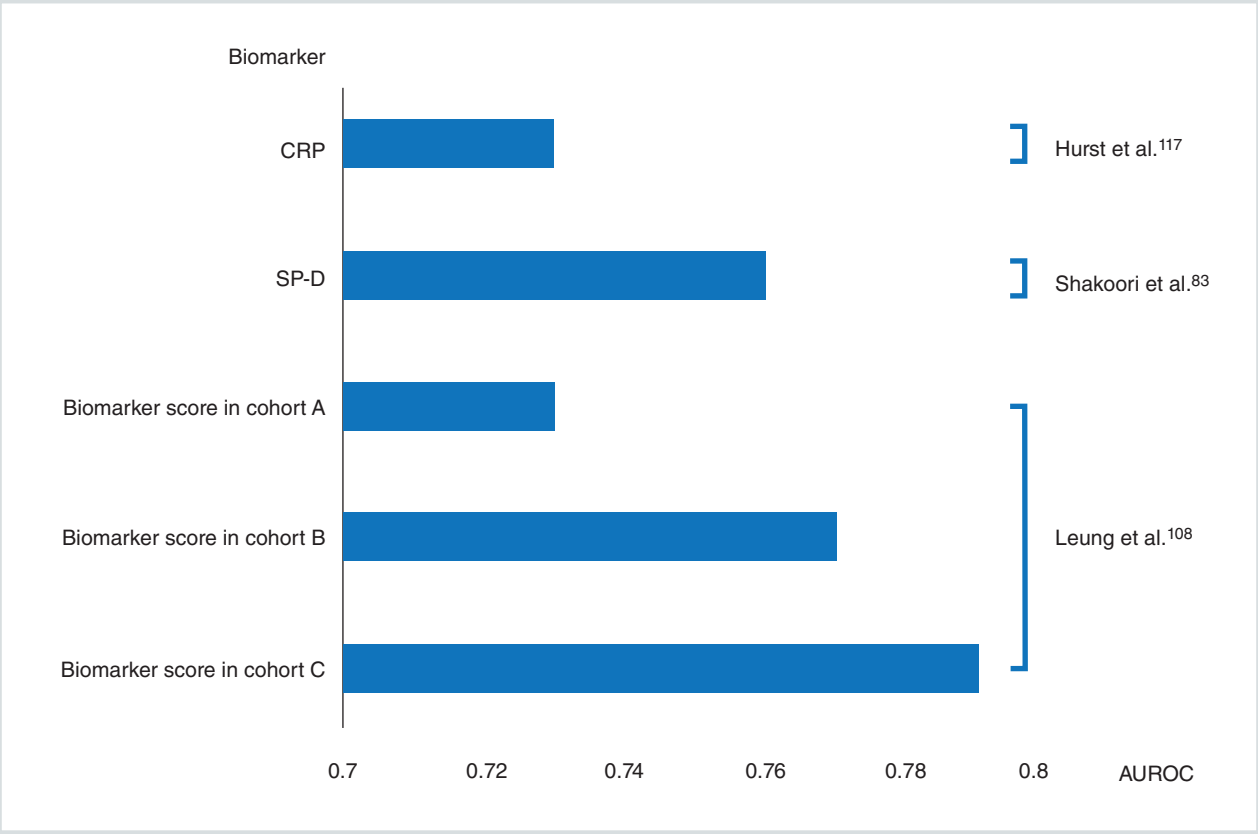

FIGURE 3. Comparison of area under the curve of the receiver operating characteristic for diagnosing acute exacerbation of chronic obstructive pulmonary disease. Biomarkers that have a potential for clinical usage should have an area under the curve of the receiver operating characteristic (ROC) values of 0.7 or greater in multiple independent cohorts *(reproduced with permission from Leung JM et al.[108])* CRP: C reactive protein; SP-D: surfactant protein D.

(or developed) onto a clinically accessible platform (e.g., mass spectrometry), and is actionable. In most cases, biomarker result should modify management, which improves health outcomes and/or reduces health care costs.

CONCLUSION

COPD is a disease that is rapidly growing in prevalence and as a major cause of morbidity and mortality throughout the world. Aside from lung function measurements, there are no established biomarkers to enhance and improve patient care and their outcomes. A majority of previous studies have been limited by small sample size, poor clinical phenotyping, low performance (low signal-to-noise ratio) and lack of reproducibility/validation. Notwithstanding, there are promising candidate biomarkers on the horizon. For mortality, plasma fibrinogen and CRP singly or in combination with each other or with other molecules is promising. For disease progression, as defined by rapid decline in FEV_1 or recurrent exacerbations, there are currently, no proteins or

genes which on their own have strong enough performance characteristics to be useful clinically. To this end, use of multi-omics and multi-stage (ensemble) approaches is likely to yield more promising results in the future. Over the next five to ten years, the omics revolution coupled with improved phenotyping of patients will enable discovery of novel biomarkers to guide therapeutic choices of COPD patients and improve their health outcomes.

AUTHOR'S CONTRIBUTIONS

All the authors contributed equally to the conception and writing of the manuscript.

CONFLICT OF INTEREST

Dr. Don D Sin has received research funding from AstraZeneca (AZ), Boehringer Ingelheim (BI) and Merck and has received honoraria for sitting on advisory boards of AZ, BI, Regeneron, Sanofi-Aventis and Novartis and for speaking engagements from AZ, BI, Novartis and is a holder of a Tier 1 Canada Research Chair in COPD. Dr Ji-Yong Moon and Dr. You Ji Cho have no potential conflict of interest.

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