almost all be similarly categorised using the LLN or other criteria. The differences that we would find relate to the mild category. In the BOLD study, GOLD stage 1 was not included in the overall estimates,[9] although others have shown that people in this category have increased morbidity and mortality.[10 11] While mild disease may be more "treatable" it may also be part of the spectrum of "normal". It may also be true that early evidence of disease may be more important as an indicator of non-respiratory disease, such as cardiovascular disease. Furthermore, in mild to moderate disease the recommended interventions are based on treating symptoms, whereas in severe to very severe disease they are based on both treating symptoms and preventing exacerbations.

To answer the question posed in the title, I do not believe that the use of statistics and mathematical "norms" is the best way to diagnose and classify disease. If everybody fails, nobody passes (but the tests and the teaching need to be critically evaluated). I continue to believe that a disease classification scheme that is easy to remember (such as the fixed FEV_1/FVC ratio) and to teach others remains useful. I also strongly believe that interventions need to be based on factors other than lung function, particularly in mild to moderate disease. I also support continuing to evaluate this problem by focusing on outcomes and not simply mathematical distributions of data in populations.

Competing interests: DMM has received research grants from GlaxoSmithKline, Pfizer and Novartis, and serves as a consultant to GlaxoSmithKline, Pfizer, Boehringer-Ingelheim, Astra-Zeneca, Dey, Sepracor and Novartis.

Thorax 2008;**63**:1031–1032.
doi:10.1136/thx.2008.100081

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COPD and biomarkers: the search goes on

Gerard M Turino

As understanding of cellular and molecular mechanisms underlying disease pathogenesis advances, the opportunities increase to identify specific compounds or molecules which are altered by the disease process or appear de novo. These markers of the pathological process have the potential advantage of indices which are indicative of the existing state or change and can be available non-invasively.[1]

In this issue of *Thorax* there is a report of the use of Clara cell secretory protein-16 (CC-16, CC-10 or uteroglobulin) as a biomarker for epithelial cell dysfunction (***see page 1058***).[2] CC-16 is a member of the secretoglobin family of secreted disulfide-bridged dimeric proteins.[3] It is secreted by non-ciliated Clara cells which reside in respiratory bronchi and by non-ciliated columnar cells of the large and small airways.[4 5] CC-16 also occurs in the epithelial cells of the nose and the urogenital tract of men and women.[5] There is evidence, however, that serum levels of CC-16 are largely the result of secretion by cells of the respiratory tract rather than the cells of the urogenital tract.[6] Serum levels of CC-16 rise following acute exposure to smoke, chlorine and lipopolysaccharide; in patients with asthma, obliterative bronchiolitis and smokers the serum CC-16 levels are low.[7] There is an extensive literature on CC-16 levels in serum and bronchoalveolar lavage fluid in normal individuals, experimental animals and individuals exposed to atmospheric pollutants, as well as asthma.[7] The exact function of CC-16 is not known, but it may play a role in reducing inflammation in airways.[8]

The processes which control serum levels of CC-16 are: (1) the rate of synthesis of CC-16 by Clara cells and secretion into the alveolar fluid; (2) the rate of diffusion from alveolar fluid into the capillary blood, which is influenced by leakiness of the pulmonary epithelial barrier; and (3) renal clearance of CC-16. In normal individuals there is variation as a function of gender, age, body mass index, circadian rhythm, ethnicity, temperature, humidity, pulmonary infection and exposure to allergens.[7]

The ECLIPSE study, a 3-year longitudinal multicentre study of patients with chronic obstructive pulmonary disease (COPD), provided serum for evaluation of the usefulness of CC-16 as a biomarker to identify characterising clinical features of the disease.[9] In this trial of 1888 individuals with COPD, 296 smoking controls with no airflow obstruction and 201 non-smoking controls, there were significant differences between the mean CC-16 levels in current and former smokers with no airflow obstruction. There were also significant differences in mean CC-16 levels between current and former smokers with no airflow obstruction and non-smoking controls. The serum CC-16 levels were significantly reduced in 1888 current and former smokers with COPD compared with 296 current and former smokers without airflow obstruction.

A strength of this study is the documentation of serum CC-16 levels in this well-characterised cohort of a large number of patients with COPD, with detailed smoking histories, pulmonary function testing and CT scans of the chest.

A disease biomarker should have: (1) high sensitivity, (2) high specificity, (3) biological relevance to the pathogenesis

Correspondence to: Dr G M Turino, James P Mara Center for Lung Disease, St Luke's-Roosevelt Hospital, 1000 Tenth Avenue, New York, NY 10019, USA; gmt1@columbia.edu

and (4) sufficient information so that changes in the concentration of the biomarkers can be clinically relevant.[10]

There are significant limitations to CC-16 as a marker of the disease components of COPD. In the cohorts studied, there was no correlation between the presence or severity of emphysema and the serum CC-16 level. Also, there was no correlation between the serum CC-16 level and the symptoms of chronic bronchitis. The use of inhaled corticosteroids or long-acting β agonists in the ECLIPSE cohort was not reflected in significant differences in serum CC-16 levels. We must be aware that, at present, it is not known if the serum CC-16 level is specific for COPD alone or only to exposure to tobacco smoke or ozone. Testing is required of CC-16 serum levels in other diseases of the lung. Also, studies are required in COPD to determine whether CC-16 can indicate disease progression or regression.

This report of CC-16 from the ECLIPSE study does provide baseline data on several patient cohorts in this large-scale study. Additional data are needed to establish whether CC-16 can reflect short-term or long-term progression or regression of COPD parameters of pulmonary function, clinical state or radiological indices of bronchial structure or, possibly, responses to treatment.

We need to establish where CC-16 might be considered in the spectrum of possible biomarkers of COPD. COPD is a systemic disease beyond the lung with inflammation as a significant contributor,[11] and markers of the inflammatory state such as C-reactive protein (CRP)[12 13] and interleukin 8 (IL-8)[14] have been found to be raised in COPD; significant increases in tumour necrosis factor α (TNFα) in COPD have also been reported.[15] However, these reflect augmentation of the systemic inflammatory state in COPD which can be influenced by co-morbid conditions in the cardiovascular system or metabolic co-morbidities. Thus, biomarkers of the pathological state of patients with COPD may be indicators of extrapulmonary processes as well as pulmonary pathology per se. CC-16 has the advantage that it is an indicator anchored to the Clara cells of the bronchial epithelium, which gives it potential relevance to the effects on the diseased lung. We need to separate biomarkers of COPD which reflect abnormalities in specific anatomical structures or biological functions of the lung per se from those such as CRP, IL-8 and TNFα which may reflect systemic pathology such as a heightened reactive state of inflammatory cells.

For decades the forced expiratory volume in 1 s (FEV_1) has been used as the indicator of severity of COPD, predictor of longevity and index of functional response to potential treatments.[16] However, as is well recognised, the variability of this measure in any single individual under study requires prolonged periods of observation and multiple measurements to establish significant results and therefore has limited usefulness as a timely indicator.

In several studies the plasma and urine levels of desmosine and isodesmosine have shown elevations as markers of above-normal elastin degradation in COPD.[17 18] Recent advances in the techniques of measurement of desmosine and isodesmosine have increased the specificity and sensitivity of quantification so that measurements can be made in sputum as well as plasma and urine.[19–21] Sputum measurements reflect changes in the lung matrix elastin rather than in non-pulmonary sources. Additional studies have shown a reduction in desmosine and isodesmosine levels in experimental animals exposed to smoke[22] and in patients with COPD treated with tiotropium.[23]

Any biomarker should have a relationship with the pathological process ongoing in the lung or bear some relationship to the physiological functions of the lung (such as air flow, gas exchange or pulmonary circulation) or to disease progression, regression and the impact of these changes on the patient's clinical state and quality of life.

Useful biomarkers in COPD which should not be overlooked are the cellular and cytokine components of sputum. In a study of 56 patients with chronic bronchitis studied over 4 years using physiological measures and CT densitometry, the results support a causative role for neutrophil inflammation and a predictive role in clinical practice.[24] A faster decline in lung function has also been correlated with sputum IL-6 levels, neutrophil count and plasma fibrinogen.[25 26]

When considering possible biomarkers for COPD, the presence of alpha-1 antitrypsin deficiency (ATTD) should be included since this neutrophil protease inhibitor deficiency and COPD are so closely linked. This association extends to heterozygote intermediate deficiencies as well as homozygous severe deficiencies and should continue to be ruled out routinely.[27] The development of COPD in the setting of severe ATTD is highly variable. It seems likely that modifier genes may interact with environmental factors to determine an individual's manifestations of lung disease. A study which focused on identifying risk factors for severe COPD found that sex, smoking, pneumonia and chronic bronchitis all had risk ratios of >2.[28] Such predictors could identify significant pathways for genetic modifiers of COPD in ATTD and possible non-ATTD COPD. More recent work has shown that IL-10 polymorphisms are associated with airflow obstruction in severe ATTD, attesting to the validity of searching for genetic determinants as distinguishing markers in COPD.[29]

Given the clinical complexity of COPD—which is really a syndrome with elements of bronchitis, airway hyperreactivity, pulmonary emphysema and an inflammatory state in variable proportions—it seems likely that multiple biomarkers will be required to characterise pathogenetic factors and their course over time. Biomarkers may be selected or designed to address specific questions of pathogenesis or treatment. Within the spectrum of biomarkers, CC-16 has the potential to indicate abnormal function of the Clara cell or Clara cell abnormalities in airway structure and function. However, its usefulness will be limited unless the measured levels of CC-16 can be shown to reflect the presence and severity of airway dysfunction, normalcy or injury to Clara cells and changes in these states with reasonable speed. To date, this has not been done. Biomarkers of the pathological state of patients with COPD may be indicators of extrapulmonary processes as well as pulmonary pathology per se. CC-16 has the advantage of being an indicator anchored to the Clara cells of the bronchial epithelium, which gives it a potential relevance to effects on the diseased lung.

Competing interests: None.

Thorax 2008;**63**:1032–1034.
doi:10.1136/thx.2008.105957

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RAGE: a biomarker for acute lung injury

Mark J D Griffiths,[1,2,3] Danny F McAuley[3,4,5]

Acute lung injury (ALI), and its more severe counterpart the acute respiratory distress syndrome (ARDS), are syndromes of acute respiratory failure associated with pulmonary oedema caused by increased permeability of the alveolar–capillary membrane. Many clinical scenarios are recognised as being associated with a high incidence of ALI, including the archetypal direct pulmonary and blood borne insults of pneumonia and severe sepsis, respectively. The internationally accepted diagnostic criteria[1] are non-specific to the point of including patients with relatively mild hypoxia and patients with lung pathology that may be different from the classical diffuse alveolar damage.[2] ALI is not uncommon but it is challenging to study, partly because the patients are heterogenous in the causes and severity of their illness. Furthermore, patients die *with* rather than *from* respiratory failure in the majority of cases.[3] These issues partly account for the fact that only one intervention has been shown to affect the survival of patients with ALI. The National Heart, Lung and Blood Institute (NHLBI) ARDS Network ARMA study,[4] arguably the most important trial in respiratory medicine in the last 20 years, demonstrated an approximately 10% survival advantage in favour of a ventilation strategy that limited tidal volume (6 ml/kg predicted body weight) and plateau pressure ($\leqslant$30 cm H_2O) compared with "standard" ventilatory parameters (12 ml/kg and $\leqslant$50 cm H_2O).

A biomarker is a clinical parameter that is measured with a view to providing information about a disease process, in this case ALI (box 1). Apart from informing the diagnostic process, biomarkers might be used to predict which patients at risk of ALI develop severe ARDS, which of these will develop pulmonary fibrosis requiring prolonged ventilatory support[5] and ultimately who dies. Soluble receptor of advanced glycation end-products (RAGE), the cleaved form of the receptor, measured in plasma has been proposed as a biomarker of type I alveolar cell injury. Plasma RAGE concentrations were elevated in samples from patients with ALI compared with healthy controls and patients with hydrostatic oedema.[6] In this issue of *Thorax*, Calfee and colleagues[7] from the NHLBI ARDS Network report the results of measuring soluble RAGE levels in plasma samples from 676 patients enrolled in the ARMA study, both at entry to the study and after 3 days of standard or protective ventilation ***(see page 1083)***. At entry, higher RAGE levels were associated with higher radiographic and physiological indices of ALI severity as well as the non-pulmonary Acute Physiology and Chronic Health Evaluation (APACHE 3) score.[7] These data suggest that RAGE may be a marker of disease severity but the potential predictive value of a raised plasma RAGE level needs to be tested in patients at risk of developing ALI in a prospective longitudinal study. Furthermore, in the group randomised to the "standard" mechanical ventilation but not the protective ventilation group, higher baseline RAGE was associated with increased mortality and fewer ventilator-free and organ failure-free days. Because ventilation using 6 ml/kg predicted body weight has become a standard of care,[8] this observation casts a shadow over the potential usefulness of RAGE although, as the authors state, such subgroup analyses should be viewed with caution.

In both groups plasma RAGE levels decreased 3 days after enrolment, but had fallen by 15% more in the protective ventilation group. Does this mean that RAGE joins the list of potential biomarkers of ventilator associated lung injury?[9] The

[1] Royal Brompton Hospital, AICU, London, UK; [2] Unit of Critical Care, National Heart and Lung Institute, Imperial College London, London, UK; [3] UK and Eire Acute Lung Injury Research Group; [4] Northern Ireland Regional Intensive Care Unit, Royal Victoria Hospital, Belfast, UK; [5] Respiratory Medicine Research Group, The Queen's University of Belfast, Belfast, UK

Correspondence to: Dr Mark Griffiths, Unit of Critical Care, Royal Brompton Hospital, Sydney Street, London SW3 6NP, UK; m.griffiths@imperial.ac.uk