THE COPD BIOMARKERS PIPELINE
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Elastin Degradation: An Effective Biomarker in COPD

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Background

Chronic obstructive pulmonary disease (COPD) is well recognized as one of the major health problems in the world (1,2). In the United States, it has moved up to the third leading cause of death (3). The disease has been defined as progressive and not fully reversible (2). Existing therapies offer benefits. Therapies to reverse its progressive course have had limited success. There is clear recognition that new and effective therapies are needed in this disease. New drug development is costly and fraught with failure so mechanisms must be sought to facilitate this development. The development of biomarkers of COPD is one such strategy.

There are several "definitions" of biomarkers. The US Food and Drug Administration operationalizes biomarkers as a reliable measure that can be used in at least one of three contexts to facilitate drug development: subject stratification, dose ranging and as outcome measures (4). The National Institutes of Health (NIH) defines biomarkers as the "characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathological processes or pharmacological responses to a therapeutic intervention"(5).

Another criterion suggested for a biomarker that would be an outcome measure is (6) "a strong independent consistent association between the surrogate end point and the clinical endpoint" and "evidence from randomized control trials that improvements in the surrogate endpoint with a drug consistently leads to improvement in the target (clinical) endpoint."

At the present time, according to the FDA: "With the exception of lung function tests, there are no well validated biomarkers or surrogate endpoints that can be used to establish efficacy for a drug for COPD"[7] The following review is the first in a series of brief reviews to be published in the *Journal of COPD* that will summarize potential biomarkers that may be used to facilitate development of novel treatments in COPD. This review brings together the evidence for matrix elastin degradation products as effective biomarkers for COPD.

Elastin is synthesized in the extra-cellular space by elastin synthesizing cells which secrete the soluble precursor, monomer, tropoelastin, which is then cross linked mainly by two amino acids, desmosine and isodesmosine (Dl), which are derived by post-translational modification of lysyl residues (8). Cross-linking transfers the soluble tropoelastin to the insoluble cross linked mature elastin fiber. DI occur only in mature elastin and their presence in body fluids is an indicator of degradation of mature elastic fibers (9).

Elastin occurs mainly in lung, blood vessels and skin. Elastin is present in bronchi and lung parenchyma. In the alveolar regions of the lung, elastin fibers are deployed around alveolar ducts, the openings of alveoli and extensions into alveolar septa (10). Dissolution of elastic fibers in the latter regions leads to tissue loss, altered alveolar structure and emphysema. In

COPD, elastic fibers appear disrupted, fenestrated and distorted (11).

Some turnover of mature elastin occurs in normal adults as indicated by low but detectable levels of DI in plasma and urine of normal subjects. DI has not been detected in induced sputum in normal subjects (12), which suggests that bronchial and lower airway elastin is quite stable.

COPD research was greatly advanced by the discovery of genetically determined alpha-1 antitrypsin deficiency (AATD) (13). The severe deficiency of this protein in blood and tissue causes development of pulmonary emphysema at a young age. Studies of alpha-1 antitrypsin deficiency (AATD) brought focus to the tissue damaging effects of neutrophil elastase, which is synthesized and secreted by neutrophils and is a powerful chemical degrader of elastin (14). In addition, there exist other elastin degrading proteases such as metalloproteases which occur mainly in macrophages and are not inhibited by AAT but are by tissue inhibitors of metallproteases (TIMPs1-4). There are also cystine and aspartic proteases, which are active at acidic pH and act mainly intracellularly (15).

The low measurable levels of DI in normal subjects in urine and plasma suggest that the proteases and antiproteases maintain a balance so that body turnover of elastin is consistently low. However, when there is reduction in the inhibitory capacity by factors such as genetic deficiency or oxidants from smoking, which reduces inhibitory activity (16), the proteases increase elastin degradation in all body sites. Levels of DI in plasma and urine increase above normal and DI should be detectable in sputum and bronchoalveolar lavage fluid (BAL). Similarly, if there is an increase in inhibitory capacity or lessening of inflammatory stimuli, which produce elastases, levels of DI in urine, plasma, BAL and sputum will be reduced.

Biomarker Measurements *in vivo*

The detection and measurement of DI as a means of study of elastin degradation was recognized in past years (17–19). Since DI are present in body fluids in extremely low concentrations, their precise and specific measurement has been a challenge. The earlier methods were based on immunological techniques. Starcher developed a radio immunoassay for desmosine (20), which preceded the development of an ELISA for measurements in plasma and urine (21–23). A refinement in methods utilized isotope dilution to increase accuracy of measurements in urine (23).

Very significant insights were gained from measurements of DI in urine and plasma by these methods applied to COPD (19). It was demonstrated that smokers with COPD excreted more desmosine in urine than never-smokers (24). Stone et al showed that smokers with normal lung function and patients with COPD excreted more urinary desmosine than healthy never-smokers (25). Smokers who stopped smoking had elevated excretion of desmosine but lower levels of excretion than continuing smokers. Gottlieb et al also showed that smokers with a rapid decline in lung function in the normative aging study had higher levels of urinary desmosine and in urinary desmosine excretion than those with slow decline in lung function (26).

Use of a high performance capillary electrophoresis separation method (27) showed that patients with COPD during exacerbations excreted more desmosine than patients with stable COPD. Thus these early studies demonstrated differences in urinary excretion of desmosine where healthy non-smokers had the lowest levels of excretion, followed by increases in excretion in smokers with normal lung function, followed by increases in excretion in patients with stable COPD, followed by increases in patients with COPD during exacerbations, followed by increases in excretion of COPD patients with AATD.

The acceptance of desmosine as a biomarker in COPD was limited by methodologic concerns related to quantification by immunological techniques or spectrographic methods that had limited specificity and sensitivity for measuring the DI molecules. The existing methods led to variable quantitative results from different laboratories and clinical settings. An additional concern was: the ultimate objective to measure the degradation of mature elastin in situ and not include precursor elastin molecules or other sources that could confound the analysis.

To improve the specificity and sensitivity of measurements, Ma and colleagues developed an analytical method using high performance liquid chromatography (HPLC) followed by electrospray ionization mass spectrometry (12). DI are then measured by specific molecular ion mass-to-charge ratio, which is 526. Using this method it was possible to show the presence of DI in sputum for the first time, which is an index of elastin degradation in the lung *per se*. Using this method, results show significant increases in DI in plasma and sputum in patients with COPD compared with non-smoking controls (28). The highest levels of DI were observed in COPD patients with homozygous AATD, as would be expected since emphysema is a significant structural abnormality in AATD.

The HPLC mass spectrometer (MS) analytical method allows detection of a free, unconjugated form of DI which is detected without acid hydrolysis of urine. The free component is 12–28% of the total DI in urine in normals but is increased to 37–50% in patients with COPD (15,31). This increase may be the result of previously reported increases in the content of intracellular neutrophil elastase in COPD patients (29).

Ma et al. further improved the analytical technique for detecting and measuring DI in body fluids (30) by using tandem mass spectrometry (MS/MS) that detects reaction ion M/Z-481 and M/Z-395 which results from collision reactions of molecular ion M/Z-526 and a confirmation of molecular specificity and accuracy

of measurement. Other investigators have published results using HPLC-MMS technology showing quantitative results in plasma and urine similar to those in our laboratory (31). Using the HPLC-MS/MS technology it was possible to show a decrease of levels of DI in plasma, urine and sputum and the free component of DI in urine in COPD patients receiving 2 months of therapy with tiotropium (32). This is a demonstration of the use of this biomarker to show a possible therapeutic effect in COPD, which may be due to anti-inflammatory effects of tiotropium (33).

DI have also been used as biomarkers of elastin injury and degradation in mice exposed to tobacco smoke. Levels of DI in bronchoalveolar lavage fluid of animals developing pulmonary emphysema from tobacco smoke exposure are initially elevated and are reduced by a therapeutic intervention of Hyaluronan Aerosol (34).

Two newer studies add data showing the value of desmosine related to longer term clinical aspects of COPD. Fregonese et al. (35) studied 11 ex-smokers with ZZ-homozygous AATD related COPD followed over 14 months (not on augmentation therapy) with repeated measurements of DI in plasma and urine at 6 month intervals. DI levels significantly increased in both plasma and urine with time. There was also a significant reduction in gas transfer measurements. This study demonstrates the ability of DI to correlate with advancing disease.

Also, measurements of DI were carried out by Lindberg et al. (36) in a population of patients drawn from the Swedish Twin Registry in which urine samples were obtained from 349 subjects and plasma samples from 318 subjects. Analysis for DI was carried out by HPLC-MS/MS. Subjects had pulmonary function measurements. Approximately 1/3 of subjects had a diagnosis of COPD. Urinary desmosine values were significantly correlated with all lung function measurements after adjustments for age, gender, height, BMI and smoking. Concentrations of plasma desmosine were significantly correlated with FEV_1 and D_{LCO}. This study provides data on the largest population of COPD patients studied thus far with measurements of DI in urine and plasma.

In summary, the amino acids, DI, specific products of body elastin degradation, have had a long history of development as biomarkers in COPD. At this time they fulfill most of the desirable features of such an agent:

1) Elastin is a recognized and vulnerable target in the pathogenesis of COPD;
2) Accurate measurements of low concentrations of DI are possible in all body fluids, including sputum as well as plasma, urine and BAL;
3) DI measurements are uniformly increased in body fluids in COPD with highest levels in patients with AATD;
4) Studies demonstrate DI as biomarkers responding to therapy and showing progress of severity in AATD;
5) Correction factors of DI levels for age, gender and smoking history are available for large or small population studies.

Future studies should address correlations of DI levels with chest computed tomography (CT) evidence of loss of lung mass at various phases of the natural history of COPD. Studies of DI levels in the earliest phases of COPD related to smoking or to AATD are needed since the earliest detection of COPD may have the greatest prospect of successful therapies. DI holds particular promise as a biomarker in COPD as it is likely to reflect disease activity, and thus may serve as a measure in individuals with progressive disease and as a measure of the effect of disease-modifying therapy.

Declaration of Interest

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