Clinical validity of plasma and urinary desmosine as biomarkers for chronic obstructive pulmonary disease

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ORIGINAL ARTICLE

ABSTRACT

Background Although an increased concentration of degraded elastin products in patients with chronic obstructive pulmonary disease (COPD) has been reported for many years, its clinical validity and utility remain uncertain due to technical difficulties, small study groups and the unknown relationship between exacerbation and elastin degradation. The objectives of this study were to determine the validity of urinary and blood total desmosine/isodesmosine in patients with COPD and asthma and to evaluate their relationship to exacerbation status and lung function.

Methods Urinary and blood desmosine levels were measured using validated isotopic dilution liquid chromatography–tandem mass spectrometry methods.

Results 390 study participants were recruited from the following groups: healthy volunteers, stable asthma, stable and ‘during an exacerbation’ COPD. Compared with healthy non-smokers, we found increased urinary or blood desmosine levels in patients with COPD, but no differences in patients with asthma or healthy smokers. The elevation of urinary desmosine levels was associated with the exacerbation status in patients with COPD. Approximately 40% of patients with stable and ‘during an exacerbation’ COPD showed elevated blood desmosine levels. Blood desmosine levels were strongly associated with age and were negatively correlated with lung diffusing capacity for carbon monoxide.

Conclusion The results suggest that urinary desmosine levels are raised by exacerbations of COPD whereas blood desmosine levels are elevated in a subgroup of patients with stable COPD and reduced lung diffusing capacity. The authors speculate that a raised blood desmosine level may identify patients with increased elastin degradation suitable for targeted therapy. Future prospective studies are required to investigate this hypothesis.

INTRODUCTION

Pulmonary emphysema, the destruction of elastic tissue in alveolar septae of the lung, is known to affect 12–28% of patients with chronic obstructive pulmonary disease (COPD)1 and is thought to represent an end result of the proteinase–antiproteinase imbalance associated with aberrant inflammation.2 There is no early diagnostic tool available to stratify at-risk patients with elevated elastin degradation. Such a tool could be critical for the development of intervention strategies to prevent disease progression.

The analysis of turnover of mature elastin may offer promising potential as a surrogate marker for lung destruction and for testing the efficacy of agents with the potential to reduce elastin breakdown. Two isomeric pyridinium-based amino acids, desmosine (DES) and isodesmosine (IDS) resulting from the condensation of four lysine residues between elastin proteins by lysyl-oxidase, represent ideal biomarkers for monitoring elastin turnover because these special cross-links are only found in mature elastin in mammals.3 The release of these two compounds and DES-containing or IDS-containing peptides into body fluids has been used to estimate levels of elastin degradation in diseases such as COPD.4 Despite being one of the most promising biomarkers linking directly to the pathophysiology, the clinical validity and utility of urine and blood total DES/IDS (uDES and bDES) as biomarkers for COPD remains unproven. The major reasons are issues related to analytical validity (including sensitivity, specificity, inaccuracy) of assays, small study groups,4 and the lack of large longitudinal studies to determine the predictive power of uDES and bDES for patients’ clinical outcome.

Key messages

What is the key question?

Are urinary and plasma desmosine useful biomarkers of disease in chronic obstructive pulmonary disease (COPD) and asthma, taking account of smoking and exacerbation status?

What is the bottom line?

Plasma desmosine concentrations are elevated in patients with stable COPD but reduced lung diffusing capacity. Urinary desmosine concentrations are raised during exacerbations of COPD.

Why read on?

This study using improved validity of analytical assays and substantial patient numbers describes the value of urinary and plasma desmosine as a biomarker of disease activity in COPD.
Analytical specificity has been one of the key problems for methodologies such as immunoassays,\textsuperscript{5–8} capillary electrophoresis,\textsuperscript{9–12} electrokinetic chromatography,\textsuperscript{13} and to a lesser degree, high-performance liquid chromatography isotope dilution.\textsuperscript{14–17} Liquid chromatography—tandem mass spectrometry (LC–MS/MS) methods are believed to provide the best specificity and sensitivity. However, mass spectrometry is not intrinsically quantitative—often matrix compounds in the complex samples interact with the analytes in the MS ion source resulting in variability that adversely impacts data quality. To address this issue, our laboratory has developed and analytically validated reliable isotope dilution LC–MS/MS methods for DES/IDS measurement.\textsuperscript{18}

Using these validated methods, the primary aim of this study was to examine the clinical validity of uDES and bDES as phenotyping biomarkers for COPD. The secondary goals were to investigate the relationships of uDES and bDES levels to smoking status, disease entity, severity, exacerbations and lung function.

METHODS

Study participants

Participants were recruited from the hospital respiratory clinics and from general practices in Glasgow (Glasgow COPD and Asthma Biomarker study; group 1) and Dundee (group 2). Potentially eligible patients were identified by scrutiny of hospital records, from general practice asthma clinic registers, by advertisements in local papers and from computerised general practice records. For group 1, 506 patients were screened for the study and 204 met the required inclusion and exclusion criteria. All study participants gave their written informed consent and the original study was approved by the local ethics committees and Institutional Review Board.

Participants were recruited from the following groups:

- **Stable COPD**: age range 40–80 years and duration of COPD ≥6 months; symptoms of cough and breathlessness, history of smoking over 10 pack years; forced expiratory volume in 1 s (FEV1)/forced vital capacity (FVC) post bronchodilator <70%; FEV1 50–80% predicted with reversibility to 2.5 mg salbutamol of <200 ml; exacerbation free for 4 weeks and no chest infection for 2 weeks.
- **COPD during an exacerbation**: age range 40–80 years and duration of COPD ≥6 months; symptoms of cough and breathlessness, current or past history of smoking >10 pack years; all participants were hospitalised because of the exacerbation.
- **Stable asthma**: age range 18–75 years and duration of asthma ≥6 months; symptoms of episodic wheezing chest tightness and/or dyspnoea (see online supplementary information for further details); on stable medication for 4 weeks and no exacerbation for 4 weeks or chest infection for 2 weeks; definition of smokers, ≥10 pack years and currently smoking at least give cigarettes per day.

Healthy controls (smokers and non-smokers): age range 18–80 years; no known respiratory disease or recent (in last 2 weeks) chest infection; normal spirometry (FEV1/FVC >80%; FEV1 > 80% predicted); no chronic respiratory symptoms; no known exposure to passive smoke in the 4 h prior to performing the tests in healthy non-smokers based on history and further examination by serum cotinine level (median (IQR): Q1–Q3)=0.9 (0.3–1.1) ng/ml).

Sample collection

The majority of participants (group 1) had spot urine, blood and sputum samples collected during two visits to establish the stability of uDES and bDES levels in each group. The remaining participants (group 2) had spot urine and sputum or blood samples collected (see online supplementary information for further details).

**uDES, bDES and sputum matrix metalloproteinase-9 activity assays**

uDES and urinary creatinine assays were carried out using validated isotope dilution LC–MS/MS methods.\textsuperscript{18} Similarly bDES concentration was analysed using a modified assay with 10 times the lower limit of quantification. The uDES levels were expressed as uDES concentration normalised to urinary creatinine concentration according to previous reports.\textsuperscript{4} The matrix metalloproteinase-9 (MMP-9) activity kit was purchased from R&D systems (Abingdon, UK) and has been validated for use in sputum supernatant. The assay performance can be found in supplementary online table E1.

**Lung function measurements**

Spirometry was performed to American Thoracic Society guidelines. Transfer factor of the lung for carbon monoxide (TLCO%) was measured using the body box technique (Zan500 Body Plethysmography, nSpire Health Ltd, Hertford, UK) and corrected for haemoglobin and carboxy haemoglobin in patients with stable asthma and COPD.

Data analysis

Peak area under the curve for DES/IDS and D$	extsubscript{2}$-DES, calibration curve, SD, coefficient of variation (CV) and % bias were generated by LCquann (ThermoFisher, Hemel Hempstead, UK). Data normality was examined using the Shapiro-Wilk test to determine the use of parametric or non-parametric tests. Two sample t test, Mann–Whitney U test, one-way analysis of variance (ANOVA), Kruskal–Wallis ANOVA and Spearman correlation were performed using Prism 5.04 or SPSS V.17.0 as appropriate.

**RESULTS**

**Clinical characteristics of study groups**

We investigated uDES and bDES levels in a total of 390 participants in two study groups: group 1 comprised five subgroups, including 26 healthy non-smokers, 20 healthy smokers, 53 stable asthma non-smokers, 56 stable asthma smokers, and 55 patients with stable COPD (table 1); group 2 comprised two subgroups including 81 healthy volunteers and 105 patients with COPD at the time of an exacerbation (table 2). In the asthma and stable COPD groups, patients with different severities (ie, mild, moderate and severe) were equally distributed so that the relationship between DES levels and increasing disease severity can be investigated. The average age of patients with COPD in group 1 and group 2 (urine and sputum) was significantly older compared with the other groups (p<0.001, table 1 and 2). There was a higher percentage of men in the healthy volunteers in the blood samples of group 2.

**uDES levels in patients with stable asthma, stable and ‘during an exacerbation’ COPD and healthy volunteers**

In group 1, there was no significant difference in uDES levels between smokers and non-smokers in both healthy volunteers and patients with asthma (figure 1A and table 1). In addition, we did not observe a difference between healthy volunteers and patients with asthma (figure 1A and table 1). uDES levels were not associated with serum cotinine levels in healthy volunteers (r=0.02, Spearman correlation).
Table 1  Demographic and desmosine data for group 1 consisting of healthy volunteers and patients with stable chronic obstructive pulmonary disease (COPD) and asthma

| Sample type       | Group 1                                                                 |
|-------------------|------------------------------------------------------------------------|
|                   | Blood, urine and sputum                                                |
|                   | Healthy volunteers: non-smokers                                        |
|                   | Healthy volunteers: smokers                                            |
|                   | Asthma: non-smokers                                                   |
|                   | Asthma: smokers                                                       |
|                   | Stable COPD                                                           |
| Number of participants | 26 20 53 56 53                                                           |
| Gender (M/F)       | 8/18 9/11 22/31 27/29 23/30                                             |
| Smoking status     | 0/0/26 20/0/0 0/0/53 56/0/53 28/25/0                                    |
| Age (years)        | 51±13 52±9 46±12 47±10 65±7*                                           |
| Body mass index    | 27 (22−28) 27 (21−30) 28 (25−36) 26 (23−30) 27 (23−30)                 |
| FEV1 (% predicted) | 106 (95−115) 93 (88−100) 79 (70−91))† 78 (58−93)† 62 (44−77)†           |
| uDES (ng/mg creatinine) | 9 (8−13) 11 (9−15) 8 (6−10) 10 (7−13) 11 (9−15)**                    |
| bDES (ng/ml)       | 0.21 (0.19−0.24) 0.23 (0.19−0.26) 0.21 (0.19−0.25) 0.22 (0.19−0.25) 0.29 (0.24−0.35)* |

Data are shown as median (IQR) or mean ± SD based on whether data were normally distributed. Kruskal–Wallis analysis of variance (ANOVA) with Dunn’s method for pairwise multiple comparison or one-way ANOVA with Tukey’s method was used. *p<0.001, versus other four groups. †p<0.05, versus healthy non-smokers and smokers. **p<0.001 versus non-smokers and smokers with asthma. ***p<0.01 versus non-smokers with asthma.

In COPD groups, patients with stable COPD excreted a ~57% higher level of uDES compared with non-smokers with asthma (p<0.01, group 1) but no difference was found between the stable COPD group and healthy non-smokers or smokers (table 1). Similar results were found at a subsequent visit (see online supplementary table E2). Patients with ‘during an exacerbation’ COPD, however, had significantly higher uDES levels compared with other subgroups (p<0.001, figure 1A and table 2). No significant difference was found between non-smokers, current smokers and ex-smokers in the ‘during an exacerbation’ COPD group (p=0.11; see online supplementary table E5). There was no correlation between uDES levels and age, gender or body mass index (data not shown). These results suggest that the elevation of uDES levels only occurs in COPD at the time of an exacerbation, whereas during clinical stable disease, the uDES levels remain normal.

bDES levels in patients with stable asthma, stable and exacerbated COPD and healthy volunteers

Similar to the findings on uDES, the bDES levels were no different among healthy smokers and non-smokers, smokers with asthma and non-smokers with asthma in group 1 (table 1 and figure 1B). bDES levels were not significantly correlated with serum cotinine levels in healthy volunteers (r=0.01, Spearman correlation). Interestingly, in contrast to little or no change in uDES levels, the bDES levels in these patients with stable COPD were significantly elevated compared with healthy non-smokers and smokers (p<0.001, figure 1B). The bDES levels between patients with stable and ‘during an exacerbation’ COPD were similar. Despite the distinct distribution of these two measurements according to the patient’s exacerbation status, the levels of uDES and bDES were found to be positively correlated in both stable (r=0.55, p<0.001, Spearman correlation).

Table 2  Demographic and desmosine data for group 2 consisting of healthy volunteers and patients with an exacerbation of chronic obstructive pulmonary disease (COPD)

| Sample type       | Group 2                                                                 |
|-------------------|------------------------------------------------------------------------|
|                   | Urine and sputum                                                      |
|                   | Healthy volunteers (HV2a)                                              |
|                   | Patients with ‘during an exacerbation’ COPD                           |
|                   | Blood                                                                  |
|                   | Healthy volunteers (HV2b)                                              |
|                   | Patients with ‘during an exacerbation’ COPD                           |
| Number of participants | 62 50* 19 102*                                                        |
| Gender (M/F)       | 24/38 24/26 18/1 43/59                                                |
| Smoking status     | 13/41/8/0 31/25/2 10/9/8/0 55/33/0/14                                  |
| Age (years)        | 22 (21−45) 69 (60−74)† 68 (65−73) 72 (66−79)                           |
| Body mass index    | 25±4 26±7 NA 26±7                                                     |
| FEV1 (% predicted) | 103±13 39±16† NA 47±18                                                |
| uDES (ng/mg creatinine) | 8 (6−10) 16 (14−22)†                                                  |
| bDES (ng/ml)       | — 0.7 (0.12−0.23) 0.30 (0.21−0.37)†                                   |

Data are shown as median (IQR) or mean ± SD. Note that healthy volunteers recruited for urine and sputum analysis (HV2a) were different from those for blood analysis (HV2b).

*p<0.001, versus healthy volunteers, Mann–Whitney test. †p<0.001, versus healthy non-smokers and smokers, Mann–Whitney test. 

Note that healthy volunteers recruited for urine and sputum analysis (HV2a) were different from those for blood analysis (HV2b).

A total of 47 patients with ‘during an exacerbation’ COPD were the same as those who had urine, sputum and blood collected.

bDES, blood desmosine; FEV1, forced expiratory volume in 1 s; uDES, urinary desmosine.
Correlation and ‘during an exacerbation’ COPD (r=0.38, p=0.01) (figure 1C). These results suggest that elevated bDES levels are specific to COPD and occur when patients are clinically stable or at the time of an exacerbation.

Increased bDES levels, however, were not observed in all patients with COPD (figure 1). Twenty-two of 52 (42%) patients with stable COPD showed an elevated bDES concentration based on a nominal cut-off of 0.30 ng/ml (defined by the upper limit of a 95% prediction interval for desmosine values in all healthy non-smokers) on at least one visit. Among these patients, 17 of 48 (35%) appeared to express elevated levels of bDES on both visits despite the intra-subject variability being significantly increased (see online supplementary figure E1). A similar observation was found in the ‘during an exacerbation’ COPD group in which 46 of 102 (45%) had values above the cut-off.

The association among uDES levels, proteinuria and lung inflammation in patients with ‘during an exacerbation’ COPD

We postulated that the cause of higher urinary excretion of degraded elastin products in patients with ‘during an exacerbation’ COPD is due to compromised renal capillary endothelial permeability. Among factors occurring during COPD exacerbations, acute inflammation is known to affect renal capillary endothelial permeability leading to proteinuria or albuminuria. To investigate this possibility, we measured MMP-9 activity, a parameter known to be associated with acute airway inflammation, in the sputum samples taken from the same patients during the same visit. As expected, a much higher (on average 10-fold) sputum MMP-9 activity was observed in patients with ‘during an exacerbation’ COPD compared with healthy volunteers in group 2 (p<0.001, figure 2A) while patients with stable COPD only showed a moderate 36% increase compared with healthy volunteers in group 1 (p=0.05, figure 2A). The increased sputum MMP-9 activity correlated with the uDES concentration (figure 2B). The results support the hypothesis that many patients with ‘during an exacerbation’ COPD had an acute inflammatory response. In line with this hypothesis, urinary protein concentration in these patients was also found to be significantly higher (p<0.001, compared with healthy volunteers and patients with stable COPD, figure 2C), whereas there were no differences among the stable COPD and other subgroups in group 1 (p=0.7–1.0). In addition, we found that urinary protein concentration in all groups was highly correlated with non-normalised uDES level (r=0.79, p<0.0001, Spearman correlation, figure 2C). When the normalisation of uDES is based on urinary protein concentration (instead of creatinine as normally performed), no difference between healthy volunteers and patients with ‘during an exacerbation’ COPD was observed (p=0.97, figure 2D).

Intra-subject variability of uDES and bDES levels

Intra-subject variability in uDES and bDES levels between the two visits over a period of 6–8 weeks was assessed using CV and Bland Altman plot for each participant. The intra-subject variability of bDES was relatively smaller (average CV=6–12%) compared with uDES (41–50%) in healthy volunteers and patients with COPD (see online supplementary figure E1). The Bland-Altman plots suggest that the bDES levels were stable between visits and that the limits of agreement may be different (in absolute terms) between the groups.

Association of bDES with clinical variables

The potential correlations of bDES with clinical variables (age, gender, smoking pack years, blood cotinine levels, FEV1, etc.) and uDES were assessed using Spearman correlation. The correlation coefficient and p values expressed as r, p for Spearman correlation were 0.53, <0.001; 0.18, 0.33; 0.50, <0.001; and 0.38, 0.01 for healthy non-smokers, healthy smokers, patients with stable COPD, and patients with ‘during an exacerbation’ COPD, respectively.
FEV1/FVC and transfer factor corrected for haemoglobin and carboxy haemoglobin (TLCO%COHb) were examined. Age and TLCO%COHb were the only two variables significantly associated with bDES levels. bDES levels were positively correlated with age in healthy volunteers (r = 0.42, p = 0.02, Spearman correlation), in patients with stable COPD (r = 0.54, p < 0.001) and patients with ‘during an exacerbation’ COPD. A significant correlation was found between these two measurements when all participants were included (r = 0.57, p = 0.001, Spearman correlation). The un-normalised uDES level was highly correlated with urinary protein concentration (p < 0.001, r = 0.78, 0.63, 0.49 for healthy volunteers (group 2, HV2a), patients with stable COPD and patients with ‘during an exacerbation’ COPD, respectively). Urinary protein concentration was significantly elevated in patients with ‘during an exacerbation’ COPD in group 2 (p < 0.001 vs healthy volunteers, Mann-Whitney U test). No difference was observed between healthy volunteers and patients with ‘during an exacerbation’ COPD in group 2 when uDES levels were normalised to urinary protein levels; p = 0.97, two sample t test. The top boundary of the bar indicates the mean and the error bar indicates the SD.

DISCUSSION

In the current study, we evaluated the clinical validity of bDES and uDES as biomarkers for COPD in the largest study of its kind to date, using validated isotope dilution LC-MS/MS methods. With the advantages in analytical validity of assays and study size, we demonstrated that the elevation of bDES and uDES levels was specific to COPD (but not asthma or smoking status); approximately 40% of patients had elevated bDES levels in stable COPD and during exacerbation groups; the association between bDES levels and decreased diffusing capacity of the lung was also found in smokers with asthma but did not reach statistical significance in non-smokers with asthma (p = 0.21 and 0.55, respectively, age controlled). We did not observe a significant correlation between FEV1 (% predicted) and bDES in patients with stable or ‘during an exacerbation’ COPD (see online supplementary figure E2). The data suggest that bDES levels may represent disease activity at the time rather than disease severity as assessed by baseline FEV1.
The relationship between blood desmosine (bDES) and the diffusing capacity for carbon monoxide (transfer factor of the lung for carbon monoxide corrected for haemoglobin and carboxy haemoglobin (TLCO % predicted COHb)) was negatively associated with bDES levels in patients with stable chronic obstructive pulmonary disease (COPD) (r = -0.31, p = 0.02, partial correlation (Spearman) controlled for age). In the non-smokers and smokers with asthma, the negative association was not significant (p = 0.21 and 0.55).

Figure 4 The relationship between blood desmosine (bDES) and the diffusing capacity for carbon monoxide (transfer factor of the lung for carbon monoxide corrected for haemoglobin and carboxy haemoglobin (TLCO % predicted COHb)) was negatively associated with bDES levels in patients with stable chronic obstructive pulmonary disease (COPD) (r = -0.31, p = 0.02, partial correlation (Spearman) controlled for age). In the non-smokers and smokers with asthma, the negative association was not significant (p = 0.21 and 0.55).

In summary, this study has demonstrated that the elevation of uDES or bDES levels was only significant in patients with COPD, but not in those with asthma or healthy smokers. The elevation of uDES level, however, was only observed during an exacerbation of COPD but not in stable COPD. We found that blood desmosine levels are elevated in a subgroup of approximately 40% of patients with stable COPD. We speculate that a raised blood desmosine level may identify patients with increased elastin degradation suitable for targeted therapy. Future prospective studies are required to investigate this hypothesis.

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Patient consent Obtained.

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Contributors Conception and design: JH, RC, GF, SM, JB, CP, DM, NT; analysis and interpretation: JH, RC, AB, CG, OA, PR, CW, MM, CM, NS, DM, NT; drafting the manuscript for important content: JH, RC, NT.

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REFERENCES

1. Agusti A, Calverley PM, Celli B, et al. Characterisation of COPD heterogeneity in the ECLIPSE cohort. Respir Res 2010;11:122.
2. Sakraifhanan A, Hanania NA, Kim V. Pathogenesis of emphysema: from the bench to the bedside. Proc Am Thorac Soc 2008;5:475–7.
3. Miao M, Bruce AE, Bhanji T, et al. Differential expression of two tropoelastin genes in zebrafish. Matrix Biol 2007;26:115–24.
4. Luissetti M, Ma S, Iadarola P, et al. Desmosine as a biomarker of elastin degradation in COPD: current status and future directions. Eur Respir J 2008;32:1146–57.
5. Cacci F, Minciardi M, Monti S, et al. Urinary desmosine excretion is inversely correlated with the extent of emphysema in patients with chronic obstructive pulmonary disease. Int J Biochem Cell Biol 2002;34:594–604.
6. Luissetti M, Sturani C, Sella D, et al. MR889, a neutrophil elastase inhibitor, in patients with chronic obstructive pulmonary disease: a double-blind, randomized, placebo-controlled clinical trial. Eur Respir J 1996;9:1482–6.
7. Mc Clintock DE, Starcher B, Eisner MD, et al. Higher urine desmosine levels are associated with mortality in patients with acute lung injury. Am J Physiol Lung Cell Mol Physiol 2006;291:L566–71.
8. Starcher B, Green M, Scott M. Measurement of urinary desmosine as an indicator of acute pulmonary disease. Respiration 1995;62:252–7.
9. Annovazzi L, Viglio S, Perani E, et al. Capillary electrophoresis with laser-induced fluorescence detection as a novel sensitive approach for the analysis of desmosines in real samples. Electrophoresis 2004;25:883–91.
10. Viglio S, Iadarola P, Lupi A, et al. MEKC of desmosine and isodesmosine in urine of smokers with and without chronic obstructive pulmonary disease. J Chromato B Biomed Sci Appl 1998;714:67–98.
11. Stone PJ, Konstan MW, Berger M, et al. Elastin and collagen degradation products in urine of patients with cystic fibrosis. Am J Respir Crit Care Med 1995;152:157–62.
12. Stolk J, Veldhuisen B, Annovazzi L, et al. Short-term variability of biomarkers of proteinase activity in patients with emphysema associated with type Z alpha-1-antitrypsin deficiency. Respir Res 2005;6:47.
13. Viglio S, Zanaboni G, Luissetti M, et al. Micellar electrokinetic chromatography for the determination of urinary desmosine and isodesmosine in patients affected by chronic obstructive pulmonary disease. J Chromato B Biomed Sci Appl 1998;714:67–98.
14. Stone PJ, Konstan MW, Berger M, et al. Elastin and collagen degradation products in urine of patients with cystic fibrosis. Am J Respir Crit Care Med 1995;152:157–62.
15. Stone PJ, Bryant J, Jue L, et al. Measurement of urinary desmosine by isotope dilution and high performance liquid chromatography. Correlation between elastase-induced air-space enlargement in the hamster and elevation of urinary desmosine. Am Rev Respir Dis 1991;144:284–90.
16. Cumiskey WR, Pogani ED, Bode DC. Enrichment and analysis of desmosine and isodesmosine in biological fluids. J Chromato B Biomed Sci Appl 1996;668:199–207.
17. Stone PJ, Gottlieb DJ, O’Connor GT, et al. Elastin and collagen degradation products in urine of smokers with and without chronic obstructive pulmonary disease. Am J Respir Crit Care Med 1995;152:157–62.
18. Albarbarawi O, Barton A, Lin Z, et al. Measurement of urinary total desmosine and isodesmosine using isotope-dilution liquid chromatography-tandem mass spectrometry. Anal Chem 2010;82:3745–50.
19. Cogo A, Giacca A, Legreni C, et al. Proteinuria in COPD patients with and without respiratory failure. Chest 2003;123:652–3; author reply 3.
20. Abid O, Sun Q, Sugimoto K, et al. Predictive value of microalbuminuria in medical ICU patients: results of a pilot study. Chest 2001;120:1984–8.
21. Warner RL, Bhagavathula N, Nerusu KC, et al. Matrix metalloproteinases in acute inflammation: induction of MMP-3 and MMP-9 in fibroblasts and epithelial cells following exposure to pro-inflammatory mediators in vitro. Exp Mol Pathol 2004;76:189–95.
22. Cohen AB, Girard W, McLarty J, et al. A controlled trial of colchicine to reduce the elastase load in the lungs of ex-cigarette smokers with chronic obstructive pulmonary disease. Am Rev Respir Dis 1991;143:1038–43.
23. Gottlieb DJ, Luissetti M, Stone PJ, et al. Short-term supplementation therapy does not affect elastin degradation in severe alpha(1)-antitrypsin deficiency. The American-Italian AATD Study Group. Am J Respir Crit Care Med 2000;162:2069–72.
24. Stoller JK, Rouham F, Brianty M, et al. Biochemical efficacy and safety of a new postol human plasma alpha(1)-antitrypsin, Resphin. Chest 2002;122:66–74.
25. Ma S, Lin YY, Turino GM. Measurements of desmosine and isodesmosine by mass spectrometry in COPD. Chest 2007;131:1363–71.
26. Ma S, Lin YY, Tartell L, et al. The effect of tiotropium therapy on markers of elastin degradation in COPD. Respir Res 2008;9:12.
27. Ma S, Lieberman S, Turino GM, et al. The detection and quantitation of free desmosine and isodesmosine in human urine and their peptide-bound forms in sputum. Proc Natl Acad Sci U S A 2003;100:12941–3.
28. Starcher B, Peterson B. The kinetics of elastolysis: elastin catabolism during experimentally induced fibrosis. Exp Lung Res 1999;25:407–24.
29. Armentano RL, Levenson J, Baro JS, et al. Assessment of elastin and collagen contribution to aortic elasticity in conscious dogs. Am J Physiol 1991;260:H1870–7.