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Urinary desmosine as a biomarker in acute lung injury

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Abstract

Acute lung injury (ALI) is a complex disorder associated with an acute inflammatory response thought to contribute to tissue injury. Desmosine, a cross-linking amino acid present in elastin, is released during matrix degradation and cleared by the kidney. Results from animal models and human disease studies have suggested that ALI is associated with the release of desmosine, resulting in increased urinary desmosine. A radioimmunoassay was used to monitor urinary desmosine levels over 10 days in ten patients with ALI. The concentration of desmosine was measured with and without acid hydrolysis. Baseline urinary desmosine was increased in two of ten patients. The concentration of desmosine at baseline did not appear to be related to age, gender, neutrophil elastase (NE)/α_1-antiprotease complex concentration or P_aO_2/F_iO_2 ratio. No meaningful changes in desmosine levels were noted after removal from mechanical ventilation. Baseline desmosine concentrations did not appear to correlate with the risk of death. The limited sensitivity, predictive correlations and dynamic modulation would suggest that urine desmosine has a limited role as a biomarker for ALI. Hydrolysis of urine samples appears necessary for optimal measurement of urine desmosine.

Keywords: *Desmosine, acute lung injury, acute respiratory distress syndrome, neutrophil elastase*

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Introduction

Acute lung injury (ALI) is a complex disorder characterized by acute inflammation and increased permeability of the pulmonary vasculature (Bernard et al. 1994, Ware & Matthay 2000). Most commonly, it is associated with sepsis syndrome, aspiration, pneumonia or trauma. Several non-invasive biomarkers have been proposed for clinical use, including P_aO_2/F_iO_2, Von Willebrand Factor, surfactant proteins and various cytokines. At this point, however, there are no well-characterized prognostic biomarkers capable of predicting the outcome of the disease (Martin & Moss 2003, Ware 2005). Neutrophils and neutrophil elastase (NE) have been implicated in the pathogenesis of ALI and are believed to play a key role in the endothelial injury and

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increased vascular permeability characteristic of ALI (Smedly et al. 1986, Petty 1991, Carden et al. 1998).

Evidence that neutrophils and NE play a role in ALI comes from both animal models and clinical studies. Endotracheal administration of elastase in experimental animals induces lung pathology similar to that seen in human acute respiratory distress syndrome (Janoff et al. 1979). Animal models have also demonstrated that neutrophil depletion or treatment with an NE inhibitor can attenuate the severity of experimental ALI (Heflin & Brigham 1981, Kawabata et al. 2000). In clinical studies, neutrophils are the predominant inflammatory cell in bronchoalveolar lavage fluid (BALF) obtained from patients with ALI (Cochrane et al. 1983, Idell et al. 1985, Weiland et al. 1986, Suter et al. 1992). Furthermore, both the neutrophil count and the concentration of total NE in BALF correlate with the severity of lung dysfunction (Idell et al. 1985, Weiland et al. 1986). BALF and plasma NE levels also appear to correlate with the subsequent development of ALI in patients at risk (Suter et al. 1992, Donnelly et al. 1995).

Degradation of elastin by NE is associated with the release of peptides containing the cross-linking amino acids desmosine and isodesmosine. Administration of elastase by bronchoalveolar lavage in experimental animal models induces ALI and a transient rise in alveolar, blood and urinary desmosine (Goldstein & Starcher 1978, Janoff et al. 1983, Starcher & Peterson 1999). Increased urinary desmosine levels have been demonstrated in patients with ALI and in other conditions associated with lung injury, including exacerbations of cystic fibrosis and chronic lung disease (Starcher et al. 1995, Bode et al. 2000). There is some evidence that the excretion of desmosine may show diurnal variation, particularly in women (Stone et al. 1998). These data suggest that measurement of urinary desmosine may provide an index of *in vivo* NE activity, particularly in patients with ALI, and serve as a useful biomarker for ALI. Published information on desmosine levels during the clinical course of ALI in adult patients (Idell et al. 1989, Tenholder et al. 1991) is limited to two studies.

A number of analytical approaches for the measurement of urinary desmosine have been described (Gunja-Smith & Boucek 1981, Watanabe et al. 1989, Starcher et al. 1995, Stone et al. 1998, Bode et al. 2000). Several of these assays are not amenable to high-throughput analysis. The present authors developed and validated a radio-immunoassay (RIA) for urinary desmosine and assessed the utility of the assay and desmosine as a biomarker of ALI in a small cohort of patients with ALI.

Materials and methods

Preparation of the radiolabelled tracer

The Organic Synthesis Lab, Eli Lilly and Co. (Indianapolis, IN, USA), prepared the tracer. A total of 100 µl 0.1 M phosphate buffer was added to a vial of Desmosine Bolton-Hunter (Elastin Products, Owensville, MO, USA). The solution was swirled for several minutes to dissolve the material and was transferred to a 1.5-ml Eppendorf tube for the reaction. To a vial charged with an Iodobead (Pierce, Rockford, IL, USA), approximately 1000 µCi Na[^{125}I] were added and vortexed. After incubating at room temperature for 10 min, the solution was diluted to 1.0 ml with water and chromatographed by reverse-phase HPLC. Mobile phase A was 0.1% TFA/H_2O; mobile phase B was 0.1% TFA/acetonitrile. A gradient was set beginning with 0% B and increasing over 50 min to 90% B. Fractions were collected at 30-s intervals while

monitoring the radioactivity of the eluate. Fractions corresponding to peaks of radioactivity were then tested in the RIA. Those fractions that exhibited the greatest immunoreactivity in the assay were pooled. Pooled tracer was filtered using a 0.2-μm filter to remove any aggregates that may have been formed in the presence of organic reagents. This material was then used as the tracer.

Preparation of standards

As desmosine is normally present at a baseline level in normal urine, it was important to remove endogenous desmosine from the urine to be used for calibration standards. Desmosine was removed by adding 2 g activated charcoal (Sigma Chemical Co., St Louis, MO, USA), to 100 ml urine and mixing at room temperature for 1 h. The urine was centrifuged to pellet the charcoal. The supernatant was poured into a clean tube and another 2 g activated charcoal were added. The tube was again mixed for 1 h at room temperature and centrifuged. The supernatant was passed through a 0.45-μm filter.

Using purified desmosine standard, control samples were made at 20, 100 and 500 $ngml^{-1}$ in the charcoal-stripped urine (CSU) using independent dilutions. Additional control samples were prepared in assay buffer at 3, 5, 25, 100, 250 and 500 ng ml^{-1}. Controls were aliquoted and stored at $-70^{\circ}C$ until use.

Experimental procedure

To compare the clinical significance of acid hydrolysis, urine samples were split and analysed after either a 1:4 dilution in 1.0 M Hepes/phosphate buffered saline (PBS) or after undergoing acid hydrolysis. Acid hydrolysis was performed by adding 100 μl 6 N hydrochloric acid to 100 μl undiluted urine (3 N hydrochloric acid final concentration). Following incubation for 18–24 h at about 95°C, the sample was dried in a vacuum centrifuge for 75 min. A total of 400 μl 1.0 M Hepes/PBS was added to reconstitute and dilute the hydrolysed sample to the equivalent dilution of the unhydrolysed urine sample.

The RIA was a modification of the assay described by Starcher & Peterson (1999). Standards (#D866, Elastin Products) controls and test samples (diluted, non-hydrolysed urine or hydrolysed urine) were assayed in triplicate in a total volume of 525 μl. Each tube contained 400 μl tracer, 100 μl affinity purified rabbit anti-desmosine antibody dilution (#DS890, Elastin Products) and 25 μl sample/standard/control. The tubes were incubated 16–24 h at 4°C. Following incubation, 100 μl cold 1% BGG (bovine gamma globulin) and 1.0ml cold 20% PEG were added to each tube. The tubes were vortexed and placed at 4°C for about 20 min. Following centrifugation, the supernatant was decanted by inversion, and the tube containing the pellet was placed on a gamma counter for measurement.

Assay validation

RIA validation experiments were conducted according to current biomarker recommendations (Findlay et al. 2000). Inter- and intra-assay precision were determined for spiked calibration samples in assay buffer, CSU and hydrolysed CSU. Validation experiments were performed by measuring each concentration level four times in triplicate across eight separate assays over 4 days using two analysts. For each

concentration level, the *a priori* criteria for method acceptance for accuracy were $\pm 25\%$ of the nominal concentration for samples in assay buffer. Precision acceptance was based on a CV $<25\%$ for CSU and hydrolysed CSU.

Random urine samples were collected from 40 healthy adult volunteers (Bioreclamation, Inc., East Meadow, New York, NY, USA). The samples were analysed after dilution and after acid hydrolysis. A normal range was determined by calculating the mean ± 2 SD.

Clinical validation

An uncontrolled observational study of patients with ALI was designed to determine the time course of urinary desmosine excretion. The protocol was approved and performed according to the guidelines of the institutional review board at the Cleveland Clinic Foundation. The study was performed and informed consent was obtained according to the ethical principles of the Declaration of Helsinki (World Medical Association 2001).

Patients 18 years of age or older with ALI on invasive mechanical ventilation for 48 h or less were eligible for enrolment. ALI was defined using the American–European consensus conference definition of ALI (Bernard et al. 1994). Patients must have had a $P_aO_2/F_iO_2 \leq 300$ mmHg on invasive mechanical ventilation regardless of their level of positive end expiratory pressure (PEEP). Patients must have had bilateral pulmonary infiltrates not explained by effusions, masses or atelectasis. There also must have been no evidence of congestive heart failure either clinically or, if measured, the pulmonary capillary wedge pressure must have been ≤ 18 mmHg. Patients who were moribund and not expected to survive at least 24 h or those whose family or physician were not committed to aggressive support for at least 72 h were excluded from participation in the study. Other exclusion criteria included patients with oliguric acute or chronic renal failure, severe pre-existing heart, liver or lung disease and those with uncontrolled malignancies.

During the first 10 days after enrolment, the following parameters collected as part of routine patient management were recorded: mechanical ventilation status (on or off invasive mechanical ventilation), P_aO_2 (mmHg), F_iO_2 and serum creatinine. All patients were followed to assess 28-day survival status.

A blood sample was obtained after enrolment in the study and shipped to Covance Central Laboratory Service (CCLS, Indianapolis, IN, USA) for the assessment of NE/α_1-antitrypsin complex using a commercially available assay with an established normal range. Fresh spot urines were obtained four times daily for the first 72 h after enrolment and then twice daily for the subsequent 7 days. The urine samples were prepared in aliquots, frozen and shipped to CCLS for analysis of urinary creatinine and to the Laboratory for Experimental Medicine, Eli Lilly and Co., for analysis of urinary desmosine.

Results

Assay characteristics

StatLIA (Brendan Scientific, Grosse Pointe Farms, MI, USA), a commercially available software program, was used to fit RIA binding data by a weighted 4/5 parameter logistic model. The desmosine concentrations in the samples were

estimated by interpolation of a standard curve of desmosine that ranged in concentration from 0.665 to 9500 pmol ml^{-1} in buffer. Under the conditions of performance of the assay, the maximum binding was about 50%.

A method for the removal of desmosine was evaluated to permit assessment of potential matrix effect and preparation of control samples with a known concentration of desmosine in urine matrix. The utility of charcoal stripping urine to remove desmosine was tested in two ways. First, desmosine tracer was added to urine. Charcoal stripping was able to remove >90% of the radioactivity from the sample. The second test involved spiking desmosine into both assay buffer and urine, measuring desmosine in an undiluted sample, extracting with charcoal then measuring desmosine a second time in an undiluted sample. For buffer, charcoal stripping completely removed up to the highest concentration of desmosine added (250 ng ml^{-1}). For the urine component of this experiment, urine previously charcoal stripped was spiked with 50–100 ng ml^{-1} desmosine. The sample was then charcoal stripped and analysed for desmosine. Unlike the buffer sample, 7–12 ng ml^{-1} desmosine were measured after charcoal extraction of the spiked urine sample; this concentration was similar to the measured concentration after the initial charcoal extraction. This residual component is defined as a matrix effect. Its effect was largely removed by dilution of the sample in assay buffer to at least a 1:4 dilution. Consequently, all patient samples were analysed at a dilution of 1:4 (or greater).

Desmosine was spiked into assay buffer to determine the accuracy of the assay. Based on the accuracy and precision data, the validated range of the assay was 5.7–950 pmol ml^{-1}. The assay demonstrated robust dilutional linearity in a urine matrix and thus samples with values >950 pmol ml^{-1} could be further diluted and analysed, permitting measurement of desmosine concentrations in patient samples up to 38 000 pmol ml^{-1}. The pre-study validation samples were used as control samples during the analysis of the study samples. During pre-study validation, the precision

Table I. Baseline characteristics and survival status of ALI patients.

Patient number	Gender	Age (years)	Aetiology	28-Day survivor?	P/F ratio[§]	Baseline desmosine[⋆]	Baseline NE[¶]
1	female	63	*Escherichia coli* urinary tract infection	yes	57.0	545.6	207
2	male	80	pneumonia	yes	77.8	214.8	163
3	male	74	pyelonephritis	yes	148.0	164.0	197
4	male	78	pneumonia	yes	49.0	110.8	200
5	male	39	pneumocystis pneumonia	no	223.3	363.7	161
6	male	78	urolithiasis with urosepsis	yes	146.7	117.5	412
7	male	36	pancreatitis secondary to hypertriglyceridemia	no	58.8	95.4	106
8	female	48	*Escherichia coli* pneumonia	yes	121.7	100.5	574
9	male	60	aspiration pneumonia	yes	62.0	138.2	39
10	female	77	pneumonia	no	48.9	190.8	88
Mean		63.3			99.3	204.1	214.7
Normal						42.0–216.4	<86

[§]Ratio of P_aO_2 to F_iO_2 (mmHg).
[⋆]Urine desmosine is expressed as pmol mg^{-1} urine creatinine.
[¶]Plasma neutrophil elastase/α_1-antiprotease complex ($\mu g\ l^{-1}$).

(%CV) of the assay for unhydrolysed urine ranged from 3.8 to 7.8% for intra-assay and from 6.6 to 20.8% for inter-assay. For hydrolysed urine, the precision ranged from 3.9 to 6.8% and from 5.1 to 15.3%. During analysis of the study samples, the inter-assay precision for unhydrolysed urine ranged from 7.4 to 9.3%. For hydrolysed urine, the inter-assay precision ranged from 10.2 to 18.5%. Due to the presence of a urine matrix effect that was not additive in nature, accuracy was calculated in assay buffer instead of in a urine matrix.

To determine the specificity of the RIA, isodesmosine, elastin fragments and pyrodinoline were tested in the assay to determine if they competed for the tracer. None of the substances tested showed >1.5% cross-reactivity.

Long-term stability of stored urine samples was assessed after 6 and 12 months of storage at −70°C. At 6 months, the measured desmosine concentration was 96% of the original value, while at 12 months, the measured concentration was 83% of the original value. Freeze–thaw recovery was robust with 95% recovery rate observed after four freeze thaw cycles.

Normal range

Urine samples from 40 disease-free adult volunteers were assayed both unhydrolysed and after acid hydrolysis to determine the normal range of urinary desmosine. The desmosine concentration in hydrolysed samples ranged from 60.7 to 238.2 pmol mg^{-1} Cr, with a mean of 129.2 pmol mg^{-1} Cr. The desmosine concentration in unhydrolysed samples ranged from 25.4 to 90.8 pmol mg^{-1} Cr. Hydrolysed urine from males had a mean desmosine concentration of 131.8 compared with 49.0 pmol mg^{-1} Cr in unhydrolysed urine. Hydrolysed urine from females had a mean desmosine concentration of 129.9 compared with 48.9 pmol mg^{-1} Cr in unhydrolysed urine. These results indicate that there is no significant difference between male and female urinary desmosine concentrations. The normal range for desmosine was established as 42.0–216.4 pmol mg^{-1} Cr for hydrolysed samples and 17.6–79.6 pmol mg^{-1} Cr for unhydrolysed samples.

Clinical results

Ten patients with ALI were enrolled in the study. No patients withdrew from the study. Nine patients had all 26 urine samples collected, while one expired on the fifth day of the study. Seven of the ten patients survived to day 28. A summary of baseline patient characteristics is shown in Table I. Nine of the ten patients had an infectious aetiology of ALI with six of these nine having a pulmonary source of infection.

At baseline (i.e. the first sample collected after enrolment into the study), the mean urine concentration of desmosine (expressed as a ratio to urine creatinine to normalize for urine concentration) was near the upper limit of normal (Table I). Only three of ten or two of ten patients had a urine concentration of unhydrolysed desmosine or hydrolysed desmosine, respectively, above the upper limit of normal. However, baseline NE/α_1-antiprotease complex concentrations were abnormally elevated in nine of ten patients. As shown in Figure 1, the concentration of desmosine did not show much day-to-day variation over the course of the study and there was no evidence of significant diurnal variation in the concentration of desmosine. Urine desmosine

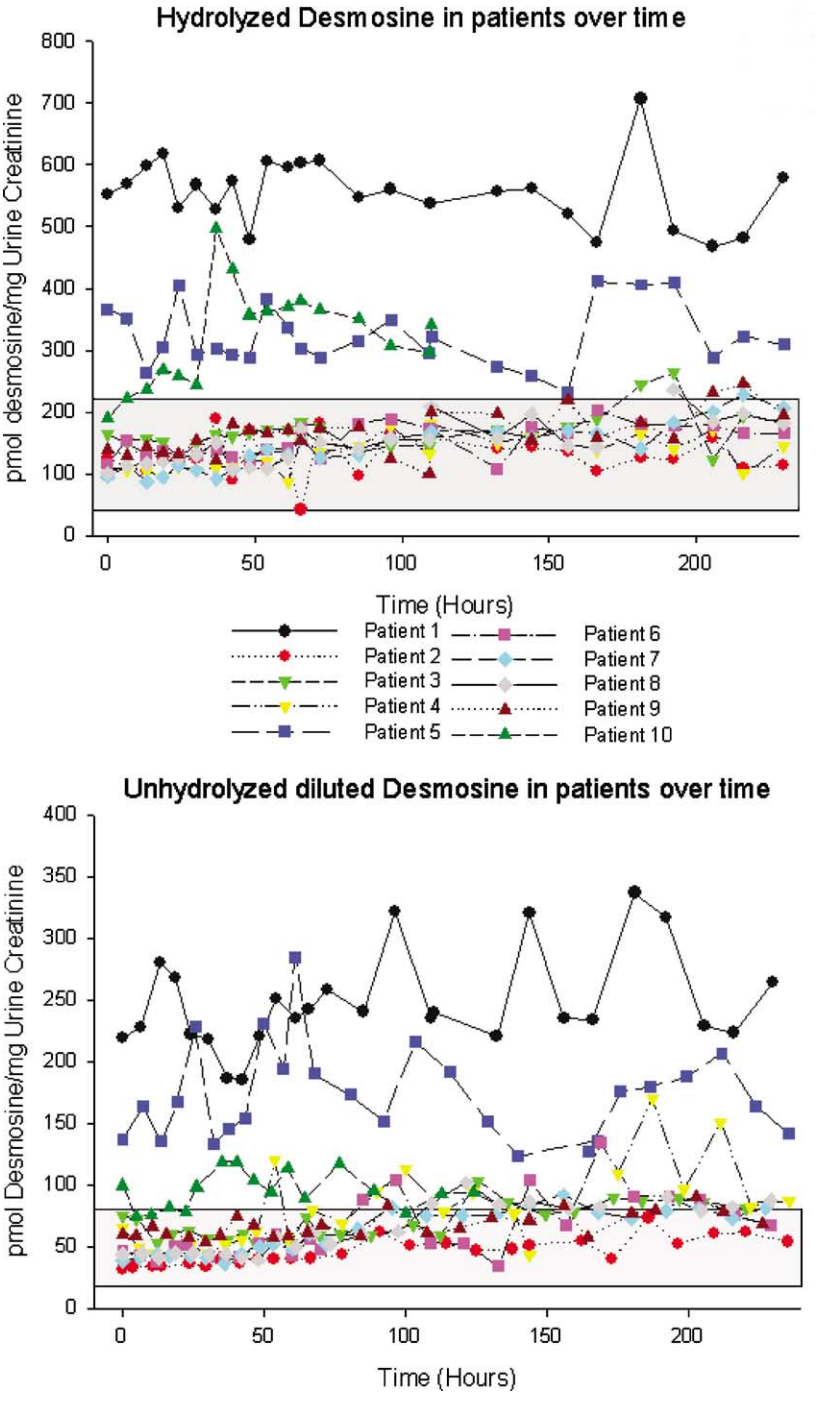

Figure 1. Time course for urine desmosine excretion for patients 1–10. Results are for desmosine (pmol mg^{-1} creatinine) after hydrolysis or dilution of urine. The normal range is shaded. Values tend to remain fairly stable without evidence of significant diurnal variation.

concentrations did not show any spikes or decreases that clearly correlated with clinical events.

To assess further the relationship of desmosine to clinical status, the baseline urine desmosine concentrations were also compared with baseline P_aO_2/F_iO_2 ratio ($r^2 = 0.008$, $p = 0.785$), baseline NE/α_1-antiprotease complex concentration ($r^2 = 0.019$, $p = 0.681$) and 28-day mortality ($r^2 = 0.001$, $p = 0.892$). Neither of these biomarkers or the clinical endpoint showed any correlation to the baseline urine desmosine concentration.

Discussion

The validation of the assay demonstrated that the dynamic range and precision of the assay were adequate to determine meaningful changes in desmosine concentrations in urine samples from patients with ALI. The acceptability criteria were based on Findlay et al. (2000), who provide guidance for immunoassay validation. Some criteria were adjusted to accommodate the presence of endogenous analyte and matrix effect.

A persistent small signal after charcoal stripping was observed in some, but not all, undiluted urine samples. This indicates that there was a matrix effect that did not appear to be due to residual endogenous desmosine. Several potential contributing factors to this matrix effect were considered, including the presence of divalent cations and urea. However, a specific contributing factor could not be identified. Dilution of the urine sample in buffer to at least a 1:4 dilution largely reduced, but did not completely eliminate, the matrix effect. This matrix effect added about 10–20 pmol ml^{-1} to the measured desmosine value of undiluted urine samples at the bottom range of the assay. However, at the higher levels of desmosine (>200 pmol ml^{-1}) generally seen in this study, the matrix effect was either not present or could not be distinguished from the variability of the assay. Some form of background subtraction was considered for inclusion in the calculation of desmosine concentration. However, given the heterogeneity of urine samples in ALI patients with multiple organ failure and renal insufficiency, the matrix effect would likely vary greatly between individuals. Subtracting an average background would therefore not be representative and obtaining backgrounds for individual samples was not feasible.

Clinical validation

The patient population included in this study represented varying degrees of ALI as assessed by the degree of hypoxaemia and the 30% mortality rate at 28 days (Table I). The baseline NE/α_1-antiprotease complex was elevated at baseline in nine of ten patients, which is consistent with a systemic inflammatory response. The aetiology of ALI varied between individuals, but it represented the common causes of this syndrome. Overall, the group included in this study appeared to be a representative population of ALI patients.

The sampling scheme, in which urine samples were collected frequently in the first few days after mechanical ventilation, was intended to monitor closely any changes that might take place in these initial stages of ALI. This collection scheme was based in part on a sheep lung-injury model in which it was demonstrated that much of the desmosine released following injury occurred with the first 48 h, with levels returning

to baseline after 72 h (Janoff et al. 1983). Patients with ALI did not demonstrate this type of pattern. At baseline, within 48 h of initiation of mechanical ventilatory support, desmosine was still within the normal range in eight of ten patients and there was little change over the following 10 days. Thus, the animal model did not predict the clinical findings. In the animal model, the onset of lung injury was sudden and time limited. In contrast, in the clinical setting, the onset of the injury may not be as easily identified and may not be as rapid or time limited. Rather, lung injury in ALI patients may build up over many hours and resolve over several days. This may result in a slower release of desmosine over an extended time, which is consistent with what was observed in this cohort of patients. It is possible that the maximal excretion of desmosine may have been missed. Patients were enrolled only after they had already been diagnosed with ALI and on mechanical ventilation. If patients at risk were to be examined for developing ALI, it is possible that earlier in the course of their disease urine desmosine excretion might be greater and predictive of those who would go on to develop ALI.

Several investigators have evaluated changes in desmosine in other disease states including scleroderma (Brinckmann et al. 2001), COPD (chronic obstructive pulmonary disease) (Bode et al. 2000, Cocci et al. 2002), Marfans's syndrome (Gunja-Smith & Boucek 1981), cystic fibrosis (Starcher et al. 1995, Bode et al. 2000), cirrhosis (Velebny et al. 1983, Afdhal et al. 1997) and severe burns (Yu et al. 1981), but there are little data on desmosine as a marker of lung injury in ALI. The analytical methods of measuring desmosine have varied greatly, including RIA (King et al. 1980, Janoff et al. 1983), HPLC (Cumiskey et al. 1995), isotope dilution (Stone et al. 1991), amino acid analysis (Gunja-Smith & Boucek 1981), ELISA (Watanabe et al. 1989) and, more recently, mass spectrometry (Ma et al. 2003). Of note, the data reported seem to be as variable as the methods used to measure desmosine. Units have been reported in anything from mg desmosine dl^{-1} of urine to pmol desmosine/24 h. While changes documented with a single method may be real, it has been difficult to correlate the results between the various methods used.

The pretreatment of the sample can greatly affect the final apparent concentration. Watanabe et al. (1989) show that when column purification of the urine sample was used, the binding capacity was greatly affected by the salt concentration and pH of the sample. Variability of these components in critical care patients with renal damage can be great. At least one researcher (Viglio et al. 1998) has treated the sample with activated charcoal to decolorize it. The present authors observed that use of activated charcoal would actually remove endogenous desmosine from urine; extended treatment (2 h) was sufficient to remove nearly all of the desmosine.

There have been two studies of desmosine levels in humans with ARDS (Idell et al. 1989, Tenholder et al. 1991). Tenholder et al. carried out a careful study including 12 patients with ARDS (acute respiratory distress syndrome), 12 patients with cardiogenic pulmonary oedema and a group of 17 critically ill patients with non-pulmonary oedema. They showed a statistically significant difference between the desmonsine concentration in the ARDS and CPE (cardiogenic pulmonary edema) groups when desmosine was normalized with serum creatinine. This determination was based on an average of all collected samples, but there is no indication about how many samples were collected, when in the course of the disease they were collected or what was the range of desmosine concentrations in the individual samples. The present data when normalized to serum creatinine are consistent with Tenholder et al.'s CPE patient data

(0.07 ± 0.04 (urine desmosine ($\mu g\ ml^{-1}$)/serum creatinine ($mg\ dl^{-1}$)) versus our observation of 0.06 ± 0.05), but much lower than the desmosine concentration Tenholder et al. observed in patients with ARDS (0.78 ± 0.28). The present authors did not collect serum creatinine on their normal range data, so it is unknown if these results are outside normal values.

Idell et al. (1989) examined desmosine concentrations in BALF of ARDS patients. They observed no difference in the desmosine concentration in BALF of 20 ARDS patients when compared with a control group. The urine desmosine findings in the present study correlate well with this observation. These two studies suggest that if neutrophils have a role in increased elastin destruction in ALI, the rate of destruction may be slower and/or lower than observed in animal models, resulting in gradual excretion into the urine. In line with this was the lack of evidence of a sudden increase in desmosine in association with rapid change in the clinical parameters in patients with ALI.

In summary, the present paper developed and stringently validated an RIA to measure desmosine in human urine samples. The assay was then used to measure serial urine samples from patients with ALI. Urine desmosine levels ranged from the upper normal range to moderately increased. There was little correlation between baseline desmosine and either P_aO_2/F_iO_2 ratio, plasma concentration of NE/α_1-antiprotease complex or 28-day mortality. The assay did not show significant variation over time despite clinical changes. At this time, it does not appear that urinary desmosine is a very informative biomarker in patients with ALI.

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References

Afdhal N, Keaveny A, Cohen S, Nunes D, Maldonado N, O'Brien M, Stone P. 1997. Urinary assays for desmosine and hydroxylysylpyridinoline in the detection of cirrhosis. Journal of Hepatology 27:993–1002.

Bernard G, Artigas A, Brigham K, Carlet J, Hudson L, Lamy M, Legall J, Morris A, Spragg, R. 1994. The American–European Consensus Conference on ARDS. Definitions, mechanisms, relevant outcomes, and clinical trial coordination. American Journal of Respiratory and Critical Care Medicine 149:818–824.

Bode D, Pagani E, Cumiskey W, Von Roemeling R, Hamel L, Silver P. 2000. Comparison of urinary desmosine excretion in patients with chronic obstructive pulmonary disease or cystic fibrosis. Pulmonary Pharmacology and Therapy 13:175–180.

Brinckmann J, Neess C, Gaber Y, Sobh IH, Notbohm H, Hunzelmann N, Fietzek P, Muller P, Risteli J, Gebker R, Scharffetter-Kochanek K. 2001. Different pattern of collagen cross-links in two sclerotic skin diseases: lipodermatosclerosis and circumscribed scleroderma. Journal of Investigative in Dermatology 117:269–273.

Carden D, Xiao F, Moak C, Willis B, Robinson-Jackson S, Alexander S. 1998. Neotrophil elastase promotes lung microvascular injury and proteolysis of endothelial cadherins. American Journal of Physiology 275:H385–H392.

Cocci F, Miniati M, Monti S, Cavarra E, Gambelli F, Battolla L, Lucattelli M, Lungarella G. 2002. Urinary desmosine excretion is inversely correlated with the extent of emphysema in patients with chronic obstructive pulmonary disease. International Journal of Biochemistry and Cell Biology 34:594–604.

Cochrane D, Spragg R, Revek S, Cohen A, McGuire W. 1983. The presence of neutrophil elastase and evidence of oxidation activity in bronchoalveolar lavage fluid of patients with adult respiratory distress syndrome. American Reviews of Respiratory Disease S25–S27.

Cumiskey W, Pagani E, Bode D. 1995. Enrichment and analysis of desmosine and isodesmosine in biological fluids. Journal of Chromatography B: Biomedical and Science Applications 668:199–207.

Donnelly S, MacGregor I, Zamani A, Gordon M, Robertson C, Steedman D, Little K, Haslett C. 1995. Plasma elastase levels and the development of the adult respiratory distress syndrome. American Journal of Respiratory and Critical Care Medicine 151:1428–1433.

Findlay J, Smith W, Lee J, Nordblom G, Das I, DeSilva B, Khan M, Bowsher R. 2000. Validation of immunoassays for bioanalysis: a pharmaceutical industry perspective. Journal of Pharmacology and Biomedical Analysis 21:1249–1273.

Goldstein R, Starcher B. 1978. Urinary excretion of elastin peptides containing desmosine after intertracheal injection of elastase in hamsters. Journal of Clinical Investigation 61:1286–1290.

Gunja-Smith Z, Boucek R. 1981. Desmosines in human urine. Biochemistry Journal 193:915–918.

Heflin AJ, Brigham K. 1981. Prevention by granulocyte depletion of increased vascular permeability of sheep lung following endotoxemia. Journal of Clinical Investigation 68:1253–1260.

Idell S, Kucich U, Fein A, Kueppers F, James H, Walsh P, Weinbaum G, Colman R, Cohen A. 1985. Neutrophil elastase-releasing factors in bronchoaveolar lavage from patients with adult respiratory distress syndrome. American Reviews on Respiratory Disease 132:1098–1105.

Idell S, Thrall R, Maunder R, Martin T, McLarty J, Scott M, Starcher B. 1989. Bronchoalveolar lavage desmosine in bleomycin-induced lung injury in marmosets and patients with adult respiratory distress syndrome. Experimental Lung Research 15:739–753.

Janoff A, Chana A, Joel D, Susskind H, Laurent P, Yu S, Dearing R. 1983. Evaluation of the urinary desmosine radioimmunoassay as a monitor of lung injury after endobronchial elastase instillation in sheep. American Reviews on Respiratory Disease 128:545–551.

Janoff A, White R, Carp H, Dearing R, Lee D. 1979. Lung injury induced by leukocyte proteases. American Journal of Pathology 97:111–136.

Kawabata K, Hagio T, Matsumoto S, Nakao S, Orita S, Aze Y, Ohno H. 2000. Delayed neutrophil elastase inhibition prevents subsequent progression of acute lung injury induced by endotoxin inhalation in hamsters. American Journal of Respiratory and Critical Care Medicine 161:2013–2018.

King G, Mohan S, Starcher B. 1980. Radioimmunoassay for desmosine. Connective Tissue Research 7:263–270.

Ma S, Leiberman S, Turino GM, Lin YY. 2003. The detection and quantitation of free desmosine and isodesmosine in human urine and their peptide-bound forms in sputum. Proceedings of the National Academy of Sciences USA 100:12941–12943.

Martin G, Moss M. 2003. Crystal ball for acute lung injury prognosis: filled with surfactant? Critical Care Medicine 31:311–313.

Petty T. 1991. Protease mechanisms in the pathogenesis of acute lung injury. Annals of the New York Academy of Sciences 624:267–277.

Smedly L, Tonnesen M, Sandhaus R, Hasslett C, Guthrie L, Johnston R, Henson P, Worthen G. 1986. Neutrophil-mediated injury to endothelial cells. Enhancement by endotoxin and essential role of neutrophil elastase. Journal of Clinical Investigation 77:1233–1243.

Starcher B, Green M, Scott M. 1995. Measurement of urinary desmosine as an indicator of pulmonary disease. Respiration 62:252–257.

Starcher B, Peterson B. 1999. The kinetics of elastolysis: elastin catabolism during experimentally induced fibrosis. Experimental Lung Research 25:407–424.

Stone P, Beiser A, Gottlieb D. 1998. Circadian variation of urinary excretion of elastin and collagen crosslinks. Proceedings of the Society of Experimental Biology and Medicine 218:229–233.

Stone P, Bryan-Rhadfi J, Lucey E, Ciccolella D, Crombie G, Faris B, Snider G, Franzblau C. 1991. Measurement of urinary desmosine by isotope dilution and high performance liquid chromatography. American Reviews on Respiratory Disease 144:284–290.

Suter P, Suter S, Girardin E, Roux-Lombard P, Grau G, Dayer J. 1992. High bronchoalveolar levels of tumor necrosis factor and its inhibitors, interleukin-1, interferon, and elastase, in patients with adult respiratory distress syndrome after trauma, shock, or sepsis. American Reviews on Respiratory Disease 145:1016–1022.

Tenholder M, Rajagopol K, Phillips Y, Dillard T, Bennett L, Mundie T, Tellis C. 1991. Urinary desmosine excretion as a marker of lung injury in adult respiratory distress syndrome. Chest 100:1385–1390.

Velebny V, Kasafirek E, Kanta J. 1983. Desmosine and isodesmosine contents and elastase activity in normal and cirrhotic rat liver. Biochemistry Journal 214:1023–1025.

Viglio S, Zanaboni G, Luisetti M, Trisolini R, Grimm R, Cetta G, Iadarola P. 1998. Micellar electrokinetic chromatography for the determination of urinary desmosine and isodesmosine in patients affected by chronic obstructive pulmonary disease. Journal of Chromatography B: Biomedical and Science Applications 714:87–98.

Ware L. 2005. Prognostic determinants of acute respiratory distress syndrome in adults: impact on clinical trial design. Critical Care Medicine 33:S217–S222.

Ware L, Matthay M. 2000. The acute respiratory distress syndrome. New England Journal of Medicine 342:1334–1349.

Watanabe T, Ishimori K, Verplanke A, Matsuki H, Kasuga H. 1989. An enzyme-linked immunosorbent assay (ELISA) for the quantitation of urinary desmosine. Tokai Journal of Experimental and Clinical Medicine 14:347–356.

Weiland J, Davis W, Holter J, Mohammed J, Dorinsky P, Gadek J. 1986. Lung neutrophils in the adult respiratory syndrome. Clinical and pathophysiologic significance. American Reviews on Respiratory Disease 133:218–225.

World Medical Association. 2001. Declaration of Helsinki: Ethical principles for medical research involving human subjects. Bulletin of the World Health Organization 79:373–374.

Yu S, Janoff A, Harel S, Ayvazian V. 1981. Urinary excretion of desmosine in patients with severe burns. Metabolism 30:497–501.