

Changes in Collagen Turnover in Early Acute Respiratory Distress Syndrome

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Pulmonary fibrosis is a well-recognized feature of acute respiratory distress syndrome (ARDS). Using immunoassays of bronchoalveolar lavage (BAL), fluid we investigated the synthesis of type I procollagen (PICP) and type I/II collagen degradation products (COL2-3/4C_{short} neoepitope) in patients with ARDS, acute lung injury (ALI), subjects with risk factors for ARDS (At Risk), and healthy/ventilated control subjects. PICP was measured by ELISA as a marker of type I procollagen synthesis. COL2-3/4C_{short} neoepitope was measured by an inhibition ELISA as a marker of collagenase degradation of type I/II collagen. BAL was performed initially within 48 h of ventilation (Day 1) and then subsequently on Day 4. Dilution of epithelial lining fluid (ELF) was corrected for by plasma urea comparison. Increased PICP levels were observed in the ELF from ARDS and ALI subjects on Day 1 compared with subjects At Risk (median values, 124.9 and 95.0 ng/ml versus 38.0 ng/ml, respectively, $p < 0.0005$). By contrast, the levels of COL2-3/4C_{short} neoepitope were significantly reduced in the subjects with ARDS versus the At Risk subjects (13.22 ng/ml versus 32.33 ng/ml, $p < 0.0005$). This translated into a greatly increased PICP:COL2-3/4C_{short} ratio in the subjects with ARDS ($p < 0.0001$). There was a significant decline in the PICP level in the subjects with ARDS between Days 1 and 4 ($n = 15$, $p < 0.05$). Linear regression analysis showed a significant association between PICP and lung injury score in the subjects with ARDS ($p = 0.01$). Our data suggests an early shift in balance between type I collagen synthesis and degradation by collagenase. The resultant increase in type I collagen would favor matrix deposition and the development of pulmonary fibrosis in the lungs of subjects with ARDS. Armstrong L, Thickett DR, Mansell JP, Ionescu M, Hoyle E, Billinghamurst RC, Poole AR, Millar AB. Changes in collagen turnover in early acute respiratory distress syndrome.

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Lung injury may be considered to be a continuum of increasing disease severity from acute lung injury (ALI) to acute respiratory distress syndrome (ARDS), arising from a number of direct and indirect initiating events, including sepsis, pancreatitis, and trauma (1). The predisposing insult is thought to induce an inflammatory cascade that subsequently leads to lung injury. Pulmonary fibrosis is a well-recognized event in this inflammatory response, which contributes to the unremitting respiratory failure and death in a significant proportion of patients with ARDS (2). A major feature of pulmonary fibrosis, in addition to the influx of inflammatory cells, is an overall increase in the synthesis and deposition of a collagen-rich ex-

tracellular matrix. Postmortem analysis of lungs from patients with ARDS who have died as a direct consequence of respiratory failure has revealed increased collagen content when compared with normal lungs (3). There are 18 different species of collagen, and types I and III fibrillar collagens predominate in the interstitium of both healthy and fibrotic lungs (4). Bronchoalveolar lavage (BAL) analysis has shown increased presence of fibrogenic cytokines in ARDS such as transforming growth factor- α (5) and tumor necrosis factor (6). Immunohistochemical analysis of lung tissue from patients with ARDS has revealed increased deposition of both type I and type III collagens (7, 8). Collagen turnover is a dynamic process in the lung, necessary to maintain normal architecture. We hypothesized that an imbalance between synthesis and degradation may contribute to the net accumulation of type I collagen previously described in ARDS (7).

Fibrillar collagens are secreted as soluble precursors (bearing large extension propeptides at both amino and carboxy termini) that self-associate to form an insoluble fibril. This is dependent upon the extracellular removal of the propeptides. These propeptides are soluble proteins and can therefore be sampled with relative ease by BAL. Therefore they are good

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markers of collagen synthesis without resorting to invasive tissue sampling. Various groups have measured the C-terminal propeptide (PICP) as a marker of type I collagen synthesis in the lung (9). Mature type I collagen has been shown to be increased with respect to type III in lung biopsies taken from four patients with ARDS of greater than 10 days' duration (7). Although the propeptide of type III collagen has been found to be significantly associated with mortality (10), PICP has not previously been measured in ARDS.

Type I collagen is degraded in the lung by the activity of collagenase(s) into three-quarter and one-quarter fragments. This cleavage results in the generation of a carboxy terminal sequence on the three-quarter fragment, which can be detected by a specific antibody and measured by immunoassay (11). Quantitation of this neoepitope provides an indication of the degree of collagen degradation by collagenase occurring in the lung.

This study had three main objectives. Firstly, to measure total PICP, and compare it with the levels of the cleavage neoepitope in the BAL fluid derived from subjects with ARDS, ALI, subjects with predisposing risk factors for ARDS (At Risk) and ventilated control (VC) subjects within 48 h of ventilation. The combination of these assays provides an opportunity to assess the balance between type I collagen synthesis and degradation in these subjects. Secondly, by analyzing sequential samples, we wished to determine how these markers change over the course of disease. Finally, we wished to determine whether these markers had any prognostic value in terms of disease severity and mortality.

METHODS

Subjects

We studied 76 patients in the following groups associated with the development of ARDS/ALI: pneumonia ($n = 23$), gastric aspiration ($n = 19$), massive blood transfusion ($n = 5$), pancreatitis ($n = 4$), emergency aortic aneurysm repair ($n = 5$), and abdominal sepsis ($n = 20$). Patients were studied on admission to the Intensive Therapy Unit (ITU), Southmead Hospital, Bristol. Lung injury was defined according to the American-European consensus (12). Patients with bilateral infiltrates on chest radiograph and a $\text{Pa}_{\text{O}_2}:\text{F}_{\text{I}_{\text{O}_2}}$ ratio < 300 mm Hg were defined as having ALI ($n = 10$), whereas patients with a $\text{Pa}_{\text{O}_2}:\text{F}_{\text{I}_{\text{O}_2}}$ ratio < 200 mm Hg were defined as having ARDS ($n = 44$). Patients with predisposing risk factors for ARDS/ALI, but without lung injury, were classified as "At Risk" ($n = 22$). Eight patients ventilated post-operatively for elective abdominal aortic aneurysm repair (without cardiopulmonary bypass) were included as ventilated control (VC) subjects. We also recruited six nonsmoking volunteers as healthy controls (HC) (see Table 1).

The first bronchoscopy with BAL was performed on all patients within 48 h of arrival to the ITU (Day 1). Subsequent BAL was performed on 15 patients with ARDS and six patients at risk of ARDS on Day 4. A third BAL was performed on three patients with ARDS on Day 12. All the subjects were mechanically ventilated at the time

of bronchoscopy, with the exception of the healthy control subjects. The study was approved by the Southmead NHS Trust Ethics Committee.

Bronchoalveolar Lavage

Each bronchoscopy was performed through an indwelling endotracheal tube, with the exception of HC. BAL was performed in the right middle lobe. Eight 20-ml aliquots of phosphate-buffered isotonic saline were instilled and gently aspirated into a siliconized bottle kept on ice. The chilled BAL fluid was strained through a single layer of coarse gauze to remove mucus clumps and then centrifuged at $400 \times g$ for 10 min to recover cells. The resultant cell-free fluid was stored at -70°C until analysis.

Measurement of PICP

The concentration of the C-terminal propeptide of type I procollagen (PICP), which reflects the biosynthesis of type I procollagen molecules (13), was measured by using a commercially available ELISA (Metra Biosystems, Oxford, UK). The Prolagen-C assay is a sandwich immunoassay in a microtitre plate format using an anti-PICP monoclonal antibody coated to the plate. A rabbit anti-PICP antiserum, a goat anti-rabbit alkaline phosphatase conjugate, and a *p*-nitrophenol phosphate substrate allow for the quantification of PICP. Undiluted BAL samples (100 μl) were added to each well of the microtiter plate as instructed by the manufacturer. Developed plates were read at 405 nm using a microtiter plate reader (Multiskan MCC/340; Labsystems, Helsinki, Finland).

Inhibition ELISA for the Determination of COL2-3/4C_{short} Neoepitope

The ELISA assay recognizes the same primary cleavage site in types I and II collagens that is produced by eukaryotic interstitial collagenases. In degenerate osteoarthritic articular cartilage, use of this assay has demonstrated increased cleavage of type II collagen by collagenase (11). The assay utilizes a rabbit polyclonal antipeptide antibody that reacts with the carboxyterminus (neoepitope) of the TC^A (3/4) piece generated by mammalian collagenases. The ELISA employs the immunizing peptide bound to the assay well. Antibody binding is measured by a secondary antibody labeled with alkaline phosphatase that generates a colored reaction product. Binding of the antibody to the immobilized peptide can be inhibited by the same peptide in solution (as the standard curve) or by the neoepitope in a sample to be assayed. By reference to the standard curve, the cleavage epitope can be measured in the BAL sample. Immulon 2 plates (Dynatech Laboratories, Alexandria, VA) were coated with 50 μl /well of peptide epitope conjugated to keyhole limpet hemocyanin (KLH) (100 ng/ml in PBS at pH 7.2) and incubated overnight at 4°C . In preincubation plates (polypropylene; Costar, Cambridge, MA), 50 μl /well of rabbit polyclonal antiserum (affinity purified) was diluted 1:150 (in PBS, 1% BSA, 0.1% Tween 20 at pH 7.2) and added to 50 μl /well undiluted BAL samples or 50 μl /well of standards (dilutions of the COL2-3/4C_{short} peptide only in PBS, 1% BSA at pH 7.2). Four nonspecific binding wells each contained 50 μl PBS, 1% BSA, 0.1% Tween 20, and 50 μl PBS, 1% BSA. Binding of goat antirabbit IgG was measured using an alkaline phosphatase substrate and NAPDH (ELISA Amplification System; Gibco, Grand Island, NY, BRL), which was added to each well. After 15 min at room temperature, 50 μl of amplifier solution (alcohol dehydrogenase and diaphorase; Gibco, BRL) was added to each well. After a final 15-min incubation at room temperature, the color development was halted with 50 μl of 0.3 M H_2SO_4 and the absorbance measured at 490 nm on a platereader. Results are expressed as nmol/ml neoepitope.

Data Analysis

Collagen values in the BAL fluid were adjusted for dilution effects by comparing BAL and plasma urea values, as previously described (14). Thus, samples are described as concentration per milliliter of epithelial lining fluid (ELF). The uncorrected data were very similar to the corrected data, but there was a small number of patients (both ARDS and At Risk) with levels of both PICP and neoepitope about 50 times higher than the median values. There was no significant difference in the volumes of ELF calculated to be contained in the BAL. The data, presented as median values, were compared using Mann-Whitney U

TABLE 1
PATIENTS' CHARACTERISTICS AND SEVERITY OF LUNG INJURY

Patient Group	M/F	Survived/Died	Age Range (median)	Median LIS	Median $\text{Pa}_{\text{O}_2}:\text{F}_{\text{I}_{\text{O}_2}}$
ARDS	30/14	20/24	18–93 (62)	2.5	127
ALI	5/5	9/1	39–85 (59)	1.9	241
At Risk	14/8	14/8	29–78 (68)	1.15	280
VC	5/3	8/0	34–77 (70)	N/A	N/A
HC	3/3	N/A	18–27 (20)	N/A	N/A

Definition of abbreviation: LIS = Lung Injury Score.

analysis for unpaired samples, and Wilcoxon's test for paired samples on Minitab for Windows. A p value less than 0.05 was regarded as statistically significant.

RESULTS

PICP is Elevated in the ARDS and ALI Groups Compared with At Risk and Control Subjects

The median levels of PICP in the ELF derived from subjects with ARDS or ALI on Day 1 were 124.9 and 95.0 ng/ml, respectively, compared with 38.0 ng/ml for the At Risk group (Figure 1). Median PICP levels detected in ELF derived from VC and HC were 37.2 and 33.5 ng/ml, respectively. PICP levels in the ARDS versus both the At Risk and the VC groups were significantly different ($p = 0.0005$, $p = 0.001$, respectively).

Generation of the COL2-3/4C_{short} Neopeptide is Reduced in ARDS and ALI Compared with At Risk

There was significantly less COL2-3/4C_{short} neopeptide present in the subjects with ARDS than in the At Risk subjects (median values, 13.22 nmol/ml versus 32.33 nmol/ml, $p = 0.0005$). The neopeptide level in the At Risk group was also significantly higher than that in the ALI group (median, 15.75 nmol/ml, $p = 0.03$) (Figure 2).

Ratio of PICP:COL2-3/4C_{short} Is Elevated in the ARDS/ALI Groups when Compared with the At Risk and VC Subjects

The ratio of PICP to neopeptide was significantly greater in the subjects with ARDS when compared with the At Risk subjects (median ratio, 11.35 versus 1.23; $p = 0.0001$) (Figure 3). Similarly, the subjects with ALI had a significantly higher ratio than the At Risk group (median ALI ratio 6.47, $p = 0.044$).

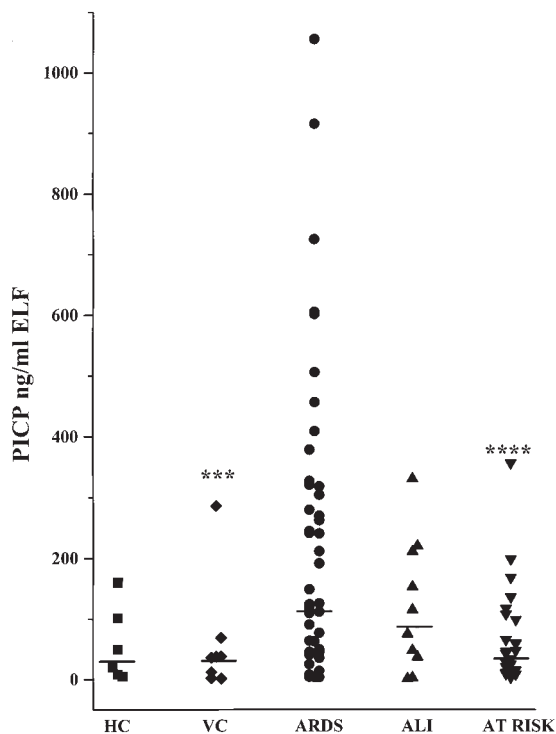


Figure 1. PICP detected in the ELF by ELISA. The horizontal bars denote median values. *** $p < 0.001$ VC versus ARDS; **** $p = 0.0005$ At Risk versus ARDS.

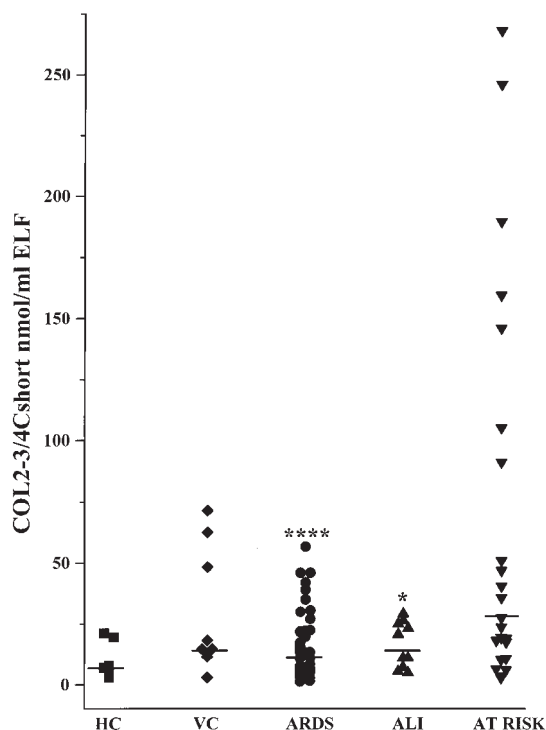


Figure 2. COL2-3/4C_{short} detected in the ELF. * $p < 0.05$ ALI versus At Risk; **** $p = 0.0005$ ARDS versus At Risk.

PICP Synthesis Peaks Early in the Course of ARDS and Declines over Time

Fifteen subjects with ARDS had an additional BAL on Day 4, and three subjects with ARDS had a third BAL on Day 12.

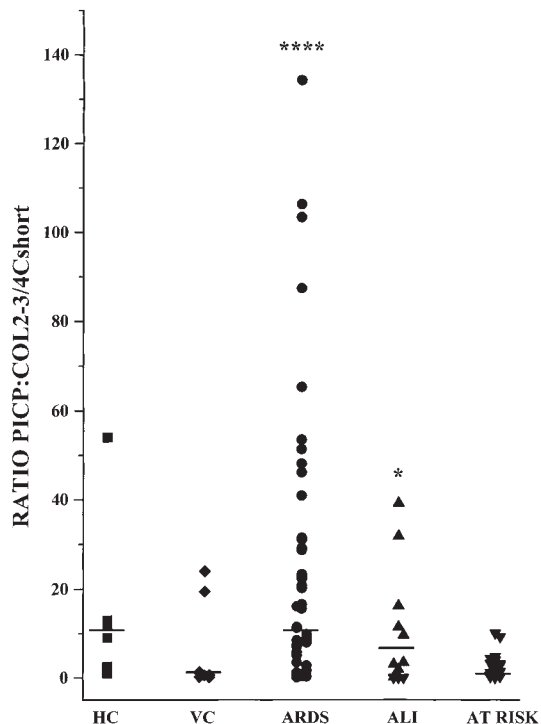


Figure 3. Ratio of PICP:COL2-3/4C_{short} in the ELF. * $p < 0.05$ ALI versus At Risk; **** $p < 0.0001$ ARDS versus At Risk.

The median PICP value in the ELF derived from these subjects with ARDS was 240.8 ng/ml on Day 1. This declined significantly to 108.5 ng/ml by Day 4, $p = 0.03$ (Figure 4, *left panel*). This decrease was observed in 14 of 15 subjects with ARDS studied. The three patients who had a BAL on Day 12 demonstrated a further decline in PICP levels, to a median of 22.3 ng/ml. In contrast, the PICP levels in the ELF derived from subjects At Risk of ARDS significantly increased, from a median of 5.31 ng/ml on Day 1 to 76.4 ng/ml on Day 4 ($p = 0.04$) (data not shown). This increase was observed in six of six At Risk subjects studied.

Generation of the COL2-3/4C_{short} Neopeptide Peaks Early in the Course of ARDS and Declines over Time

Eleven of 15 subjects with ARDS showed a decline in neopeptide at Day 4 compared with Day 1 (Figure 4, *middle panel*). However, the median value on Day 1 increased from 6.89 nmol/ml to 10.11 nmol/ml by Day 4. There was a significant decline in the PICP:COL2-3/4C_{short} ratio from a median of 22.89 to 13.87 ($p = 0.008$) (Figure 4, *right panel*). This decline was observed in 14 of 15 subjects. There was no significant increase or decrease in neopeptide levels in the six subjects At Risk (data not shown).

Relationship between PICP on Day 1 and Mortality in Subjects with ARDS

The mortality in the ARDS group was 54.5%, compared with 10.0% mortality in the ALI group, and 50% mortality in those patients At Risk of ARDS. The median value for PICP in the subjects who survived was 116.0 ng/ml compared with 217.9 ng/ml in nonsurvivors ($p = 0.05$) (Figure 5, *left panel*). All seven patients with ARDS who had a PICP value in excess of 400 ng/ml were nonsurvivors. We explored any possible relationship between collagen markers and the origin of lung in-

jury. Subjects were subgrouped into those who developed ARDS as a result of a direct pulmonary insult (pneumonia, gastric aspiration) and those who had a remote systemic insult (abdominal sepsis, pancreatitis, blood transfusion). The PICP values were comparable in subjects who had developed ARDS as a result of direct lung injury and those who had a remote systemic insult (data not shown).

Relationship between COL2-3/4C_{short} Neopeptide on Day 1 and Mortality in Subjects with ARDS

The median values for neopeptide in the ELF derived from ARDS survivors and nonsurvivors were 12.06 and 17.67 nmol/ml and were not significantly different (Figure 5, *right panel*). However, all six subjects with ARDS with a neopeptide level in excess of 31 nmol/ml ELF were nonsurvivors. Only one of six of these patients had a PICP level in excess of 400 ng/ml. There was no difference in the levels of neopeptide between patients with direct versus indirect lung injury (data not shown). There was no difference in the ratio of PICP:COL2-3/4C_{short} between direct and indirect lung injury groups, or ARDS survivors versus nonsurvivors (data not shown).

PICP Levels in the ELF Correlate Positively with Lung Injury Score (LIS)

Linear regression analysis revealed a weak but significant correlation between PICP detected in the ELF of subjects with ARDS and ALI, and the LIS ($r^2 = 0.146$, $p = 0.014$). There was no significant correlation between neopeptide levels or PICP:COL2-3/4C_{short} ratio and LIS. There was no clear association between collagen content and duration of ventilation/length of ITU stay in our study. However, most ARDS nonsurvivors died within the first few days of admission. We found no association between cellularity/BAL neutrophils and col-

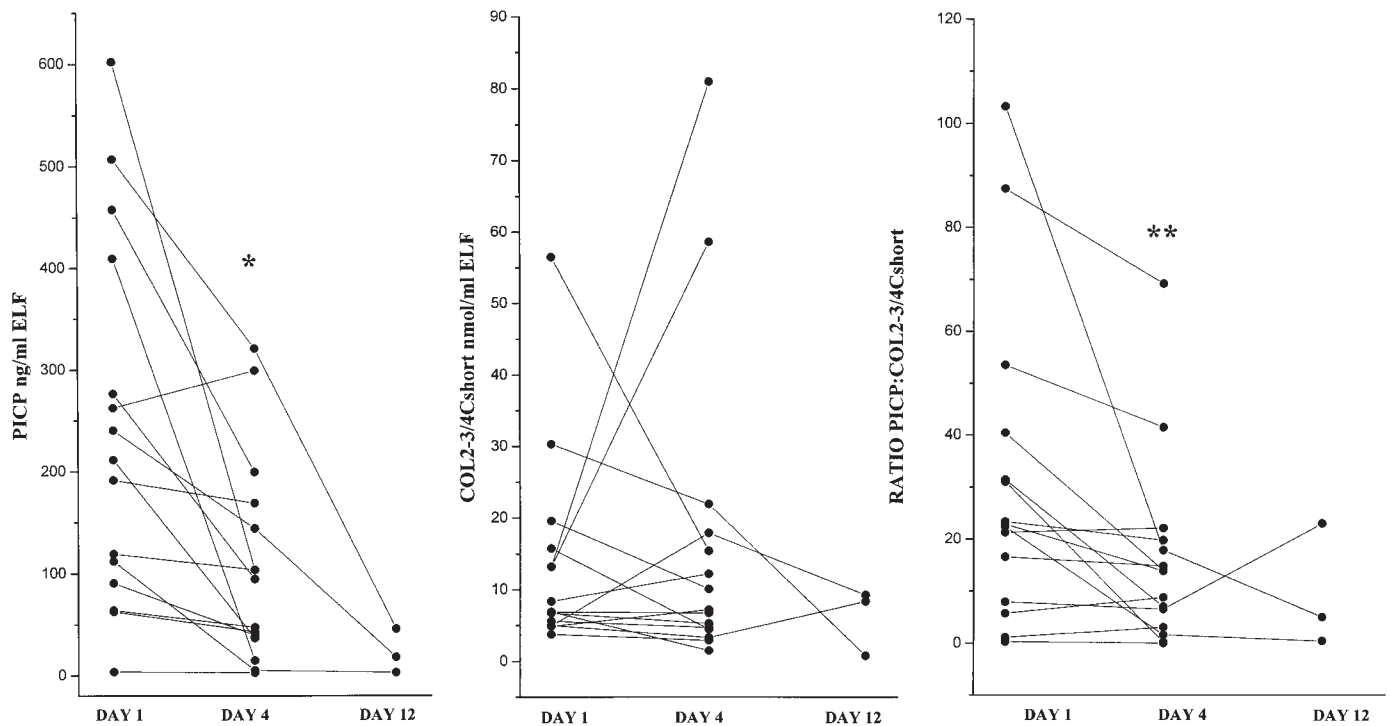


Figure 4. (*Left panel*) PICP detected in the ELF derived from subjects with ARDS at Days 1, 4, and 12. * $p < 0.05$ Day 1 versus Day 4. (*Middle panel*) COL2-3/4C_{short} detected in the ELF derived from subjects with ARDS at Days 1, 4, and 12. (*Right panel*) Ratio of PICP:COL2-3/4C_{short} derived from subjects with ARDS at Days 1, 4, and 12. ** $p < 0.01$ Day 1 versus Day 4.

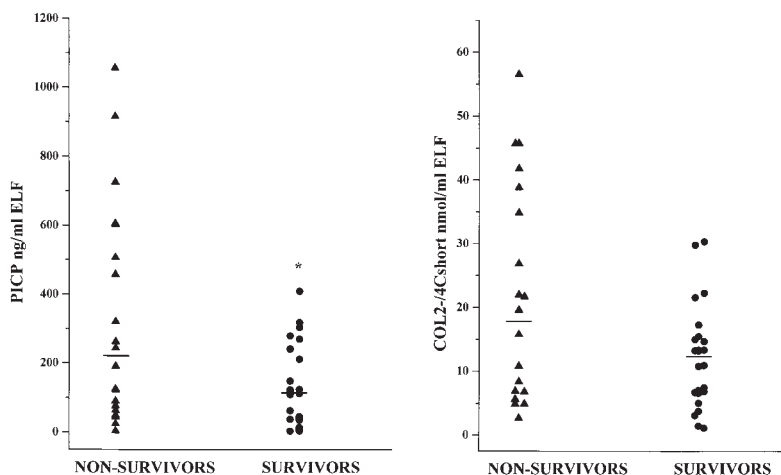


Figure 5. (Left panel) PICP detected in the ELF derived from nonsurvivors or survivors of ARDS. *p = 0.05. (Right panel) COL2-3/4C_{short} detected in the ELF derived from nonsurvivors or survivors of ARDS.

lagen markers and no significant difference in cellularity/BAL neutrophils between the ARDS/ALI/At Risk groups.

DISCUSSION

Collagen turnover in the lung is a dynamic process, necessary to maintain normal architecture. We have investigated this process in the context of lung injury. This study has shown that total PICP was increased in subjects with ARDS/ALI in comparison with At Risk subjects. By contrast, COL2-3/4C_{short}, a marker of collagen degradation, was decreased in ARDS compared with At Risk subjects. These data suggest the balance between synthesis and degradation in ARDS favors net type I collagen deposition.

Previous studies have found increased levels of insoluble type I collagen in postmortem lung sections from patients with ARDS (7). More recently, PICP has been investigated in a number of lung diseases as a marker of type I collagen synthesis because its presence in BAL fluid facilitates sampling by lavage without resorting to invasive biopsy (13, 15). Propeptides of collagen are released during fibril formation as a result of cleavage by specific extracellular N- and C-terminal proteinases. The C-propeptide of type III collagen (PIICP) has been studied by other groups in this context. However, this can also be liberated by degradation and inflammation, whereas PICP production is known to be directly associated with synthesis (13).

Measurement of increased synthesis alone does not give an indication of matrix deposition per se since a significant proportion of this newly synthesized collagen can be cleaved by specific metalloproteinases (the collagenases MMP-1, 8, and 13) (16). Metalloproteinases are increased in the lungs of patients with ARDS (14), but their presence doesn't necessarily reflect collagen degradation *in vivo*. The collagenases initially cleave the collagen molecule at a specific site approximately three quarters of the way from the N-terminal end of the molecule (17). The resulting denatured collagen α -chain fragments are susceptible to further digestion by gelatinases (MMP-2 and -9), which facilitate their elimination from the extracellular compartment. We have used the COL2-3/4C_{short} antibody, which detects this primary cleavage site, to assess collagen degradation by collagenases. The cleavage neopeptide COL2-3/4C_{short} is identical for both types I and II collagens because of the similar amino acid sequence at the C-terminal

region. Indeed, the antibody was first used to detect the neopeptide of type II collagen in the cartilage of osteoarthritic joints (11). However, experiments we have performed measuring type II collagen specifically have indicated that more than 90% of the neopeptide detected in the alveolar fluid is derived from type I collagen (unpublished findings).

This study has shown that subjects with ARDS/ALI had decreased levels of the degradation neopeptide compared with At Risk subjects. When the data were presented as a ratio of PICP:COL2-3/4C_{short} neopeptide there was a significant shift in favor of type I collagen synthesis in ARDS. However, we also observed that the highest levels of neopeptide in the ARDS group were nonsurvivors. A greatly enhanced rate of collagen turnover has previously been observed in patients with idiopathic pulmonary fibrosis (18), and our findings may suggest a similar pathology in patients with ARDS. Increased accumulation of degradation products in the lung may also be a consequence of severe epithelial injury in ARDS and increased activity of MMPs in nonsurvivors. Factors present in the lung that could affect the half-life of the neopeptide would also influence its detection, but the biologic processes involved have not been investigated.

One goal of this study was to determine how markers of collagen turnover change during the course of the disease. Using sequential samples from subjects with ARDS, we have shown that both collagen markers are elevated within 48 h, and then decline significantly. It has previously been reported that the fibroproliferative phase of ARDS commences 5 to 7 d after the onset of lung injury. However, the current study and a previous study that measured PIICP (10) have demonstrated that enhanced collagen formation may begin within the first 24 to 48 h of the predisposing insult. Further evidence for early fibroproliferation is provided by a recent study, which has shown upregulation of type I collagen mRNA expression in biopsy specimens within 2 h of cardiopulmonary bypass (19), a common predisposing risk factor for the development of ARDS. These findings may reflect the increased presence of fibrogenic cytokines such as tumor necrosis factor (20) and transforming growth factor- α (5) in the very early stages of acute lung injury.

A further aim of this study was to relate the expression of markers of collagen turnover to mortality. We found significantly higher PICP levels in nonsurvivors. We also found a significant correlation between PICP levels and lung injury

score. Although there was no significant association between neoepitope levels and mortality, all patients with ARDS with neoepitope levels in excess of 31 nmol/ml were nonsurvivors. This suggests that both these markers may be useful predictors of mortality, although a larger study would be required to confirm this.

In summary, we hypothesized that an imbalance between synthesis and degradation may contribute to the net accumulation of type I collagen in ARDS. We have demonstrated that synthesis of PICP is elevated in subjects with ARDS or ALI, and that this increased synthesis is associated with decreased collagen degradation by collagenase. We have observed a shift in the balance of collagen turnover towards net synthesis in subjects with ARDS and ALI, which is supported by previous reports of increased type I collagen deposition (7) and increased levels of denatured type I collagen in the BAL fluid (21) in ARDS. This profibrotic response occurs early in the course of disease and is associated with the severity of lung injury and mortality. We conclude that measurement of PICP and COL2-3/4C_{short} may prove to be useful in the assessment of lung injury in terms of both prognosis and potential intervention.

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References

1. Fowler, A. A., R. F. Hamman, J. T. Good, K. N. Benson, M. Baird, D. J. Eberle, T. L. Petty, and T. M. Hyers. 1983. Adult respiratory distress syndrome: risk with common predispositions. *Ann. Intern. Med.* 98:593-597.
2. Clark, J. G., J. A. Milberg, K. P. Steinberg, and L. D. Hudson. 1995. Type III procollagen peptide in adult respiratory distress syndrome. Association of increased peptide levels in bronchoalveolar lavage with increased risk for death. *Ann. Intern. Med.* 122:17-23.
3. Zapol, W. M., R. L. Telstad, J. W. Coffey, I. Tsai, and R. A. Salvador. 1979. Pulmonary fibrosis in severe acute respiratory failure. *Am. Rev. Respir. Dis.* 119:547-554.
4. Madri, J. A., and H. Furthmayr. 1980. Collagen polymorphism in the lung: an immunochemical study of pulmonary fibrosis. *Hum. Pathol.* 11:353-366.
5. Madtes, D. K., G. Rubenfield, L. D. Klima, J. A. Milberg, K. P. Steinberg, T. R. Martin, G. Raghu, L. D. Hudson, and J. G. Clark. 1998. Elevated transforming growth factor- α levels in bronchoalveolar lavage fluid of patients with acute respiratory distress syndrome. *Am. J. Respir. Crit. Care Med.* 158:424-430.
6. Hyers T. M., S. M. Tricomi, P. A. Dettenmeier, and A. A. Fowler. 1991. Tumor necrosis factor levels in serum and bronchoalveolar lavage fluid of patients with the adult respiratory distress syndrome. *Am. Rev. Respir. Dis.* 144:268-271.
7. Last, J. A., A. D. Siefkin, and K. M. Reiser. 1983. Type I collagen content is increased in the lungs of patients with adult respiratory distress syndrome. *Thorax* 38:364-368.
8. Raghu, G., L. J. Striker, L. J. Hudson, and G. E. Striker. 1985. Extracellular matrix in normal and fibrotic human lungs. *Am. Rev. Respir. Dis.* 131:281-289.
9. Lammi, L., V. Kinnula, S. Lahde, J. Risteli, P. Paakko, E. Lakari, and L. Ryhanen. 1997. Propeptide levels of Type III and Type I procollagen in the serum and bronchoalveolar lavage fluid of patients with pulmonary sarcoidosis. *Eur. Respir. J.* 10:2725-2730.
10. Chesnutt, A. N., M. A. Matthay, F. A. Tibayan, and J. G. Clark. 1997. Early detection of Type III procollagen propeptide in acute lung injury. Pathogenic and prognostic significance. *Am. J. Respir. Crit. Care Med.* 156:840-845.
11. Billingham, R. C., L. Dahlberg, M. Ionescu, A. Reiner, R. Bourne, C. Rorabeck, P. Mitchell, J. Hambor, O. Diekmann, H. Tschesche, and A. R. Poole. 1997. Enhanced cleavage of Type II collagen by collagenases in osteoarthritic articular cartilage. *J. Clin. Invest.* 99:1534-1545.
12. Bernard, G. R., A. Artigas, K. L. Brigham, J. Carlet, K. Falke, L. Hudson, M. Lamy, J. R. Legall, A. Morris, and R. Spragg. 1994. The American-European consensus conference on ARDS. *Am. J. Respir. Crit. Care Med.* 149:818-824.
13. Risteli, J., and L. Risteli. 1995. Analysing connective tissue metabolites in human serum: biochemical, physiological and methodological aspects. *J. Hepatol.* 22:77-81.
14. Delclaux, C., M. D'Ortho, C. Delacourt, F. Lebargy, C. Brun-Buisson, L. Brochard, F. Lemaire, C. Lafuma, and A. Harf. 1997. Gelatinases in epithelial lining fluid of patients with adult respiratory distress syndrome. *Am. J. Physiol.* 272:L442-L451.
15. Lammi, L., L. Ryhanen, E. Lakari, J. Risteli, P. Paakko, K. Kahlos, S. Lahde, and V. Kinnula. 1999. Type III and type I procollagen markers in fibrosing alveolitis. *Am. J. Respir. Crit. Care Med.* 159:818-823.
16. Torii, K., K. Iida, Y. Miyazaki, S. Saga, Y. Kondoh, H. Taniguchi, F. Taki, K. Takagi, M. Matsuyama, and R. Suzuki. 1997. Higher concentrations of matrix metalloproteinases in bronchoalveolar lavage fluid of patients with adult respiratory distress syndrome. *Am. J. Respir. Crit. Care Med.* 155:43-46.
17. Harper, E. 1980. Collagenases. *Annu. Rev. Biochem.* 49:1063-1079.
18. Gadek, J. E., J. A. Kelman, G. Fells, S. E. Weinberger, A. L. Horwitz, H. Y. Reynolds, J. D. Fulmer, and R. G. Crystal. 1979. Collagenases in the lower respiratory tract of patients with idiopathic pulmonary fibrosis. *N. Engl. J. Med.* 301:737-742.
19. Deheinzelin, D., F. B. Jatene, P. H. Saldiva, and R. R. Brentani. 1997. Upregulation of collagen messenger RNA expression occurs immediately after lung damage. *Chest* 112:1184-1188.
20. Suter, P. M., S. Suter, E. Girardin, P. Roux-Lombard, G. E. Grau, and J. Dayer. 1992. High bronchoalveolar levels of tumour necrosis factor and its inhibitors, interleukin-1, interferon, and elastase, in patients with adult respiratory distress syndrome after trauma, shock or sepsis. *Am. Rev. Respir. Dis.* 145:1016-1022.
21. Farjanel, J., D. J. Hartmann, B. Guidet, L. Luquel, and G. Offenstadt. 1993. Four markers of collagen metabolism as possible indicators of disease in the adult respiratory distress syndrome. *Am. Rev. Respir. Dis.* 147:1091-1099.