Variability in Lung Collagen Amounts After Prolonged Support of Acute Respiratory Failure


In order to identify correlates of fibrosis in acute respiratory failure, autopsies were performed on 16 patients who died after an episode of more than two weeks' duration, and the left lower lung lobe was obtained for biochemical measurements. The average amount of lobar collagen in one group of patients was nearly three times that in another group. Both groups had been mechanically ventilated for the same length of time and could not be distinguished by primary diagnosis. However, the group with increased collagen had received nearly twice the level of positive end-expiratory pressure and had received an inspired oxygen fraction of more than 0.4 for twice as long as the normal collagen group. Examination of complications and other aspects of therapy did not reveal clearcut differences between the groups. Examination of the same clinical data in a group of patients who had survived an episode of similar duration indicated that survivors differed from the high collagen group in inspired oxygen levels.

Pulmonary fibrosis is a chronic interstitial lung disease with a variety of causes, many of which are unknown. Pulmonary fibrosis also occurs in the adult respiratory distress syndrome (ARDS). A previous study of nine patients who died after two weeks of severe ARDS showed an increase of total lung collagen biochemically as well as varying degrees of histologic fibrosis. While these findings might indicate that fibrosis was a cause of death, other patients in whom open lung biopsy specimens demonstrated fibrosis, later recovered. In the present study, in addition to patients with increased collagen, several patients who died after two weeks of ARDS did not have increased collagen in their lungs. We have compared the two groups in order to determine whether collagen accumulation occurs secondary to either severe or specific injuries, as a result of therapy, or secondary to additional complications arising during the ARDS episode. The two groups have also been compared to survivors of ARDS episodes of similar duration.

Materials and Methods

Clinical Parameters

All studies were performed after informed consent to a protocol approved by the Institutional Review Board of the University of Texas Health Science Center. Criteria for the diagnosis of ARDS were hypoxemia (PaO₂ <60 mm Hg) despite increased FiO₂, bilateral radiographic infiltrates; and low or normal pulmonary artery wedge pressures (Paw <18 mm Hg). All patients were intubated and placed on mechanical ventilation with volume ventilators for periods up to 39 days. Varying levels of positive end-expiratory pressure (PEEP) and FiO₂ were used in order to maintain a PaO₂ of at least 60 mm Hg. Blood gas measurements and determinations of peak airway pressure and dynamic compliance were made daily if possible.

Chest roentgenograms were retrospectively reviewed and graded for severity of pulmonary infiltrates1 without regard to knowledge of biochemical findings. Each lung was divided into upper and lower zones and the presence or absence of infiltrates in each zone noted. If none was seen, a score of 0 was assigned to that zone. An interstitial infiltrate was graded as 1 and an alveolar infiltrate as 2. Thus, diffuse bilateral alveolar infiltrates were assigned a total score of 8. All radiologic scores were totaled and a mean value derived to give a single value to that patient's hospital course.

Pathologic Studies

In all instances except one medical-legal case, permission for autopsy was obtained from the next of kin for the transthoracic injection of phosphate buffered 4 percent formaldehyde-1 percent glutaraldehyde (4CF-1G)/Cardiogreen fixative into the right lung immediately after death. At autopsy, the green-tinted fixed areas were removed for ultrastructural and light microscopic studies. In addition, sections were taken from all lobes. The routine autopsy blocks were studied at a later time and combined with those blocks obtained specifically for the present study. Thus, a range of 5 to 11 blocks was available for histologic evaluation with the exception of one medical-legal case in which only three blocks were available for study. Following fixation in 4CF-1G, specimens were embedded in paraplast and sectioned at 4 micra. Sections were stained with hematoxylin-eosin, Verhoeff/Masson connective tissue stain, and Snoek's reticulum stain. An assessment of the tissue was done utilizing a grading system of 0-3 for the presence of histologic fibrosis within the lung, 0 being normal, grades 1, 2, and 3, respectively, being mild, moderate, and marked deposition of connective tissue fibers. Slides were read without knowledge of the results of biochemical assays.
Biochemical Analyses

As part of the autopsy protocol, the left lower lobe was routinely taken for biochemical analysis. The wet weight was determined and samples were taken either immediately or after the lung had been frozen. The pleura was removed and subpleural parenchymal samples were taken from several regions of the lobe. Initially, six samples of 1 g each were taken for analyses. Later, four samples of 10 g each were taken in order to sample a larger portion of the lobe. Both sampling techniques yielded similar final results. The samples were homogenized in 0.5 M acetic acid<sup>1</sup> and aliquots were taken for analysis of dry weight, total protein by the Lowry method<sup>4</sup>, DNA by the method of Burton<sup>5</sup>, elastin by the method of Naun and Morgan<sup>7</sup>, and collagen by hydroxyproline determinations<sup>8,9</sup> as described in previous studies on these components of lung tissue<sup>8,9</sup>. From the estimates of the amount of components in the samples, values for the entire left lower lobe were calculated as done previously<sup>10</sup>.

Solubilization of collagen with 0.5 M acetic acid was carried out by published methods<sup>10</sup>.

Statistical Analyses

Statistical tests used were regression analysis, analysis of variance and Student's t-test using standard programs on the University of Texas Health Science Center DEC-20 computer. A p value of less than 0.05 was considered significant.

Results

Collagen determinations were made on tissue obtained from the left lower lobe in 33 patients. In 17 patients ventilated for less than 14 days (Fig. 1), the mean collagen content of the left lower lobe was 3.98 ± 1.68 g (X ± SD). This value was in agreement with amounts for normal lungs<sup>1,10</sup>. The 16 patients who were ventilated for periods greater than 14 days (Fig 1) could be arbitrarily divided into two groups—an increased collagen group and a normal collagen group. A collagen content of 7 g per left lower lobe was used to divide these groups. By this definition, one of 17 (6 percent) patients ventilated for less than 14 days demonstrated increased collagen compared to six of 16 (38 percent) of patients ventilated for more than 14 days. The division of 14 days was chosen because previous data suggested an increase in lobar collagen content occurred commonly after that time<sup>4</sup>. The sex, age, and clinical diagnosis leading to the development of ARDS in patients ventilated for more than 14 days are listed in Table 1. None of these 16 patients had preexisting lung disease. Radiologic scores as an index of severity<sup>2</sup> were the same in both groups of patients when averaged for their entire hospital course (6.7 ± 1.1 for the increased collagen group vs. 6.5 ± 1.0 for the normal collagen group).

Mean values of several parameters, which were measured daily in most patients and averaged for the patient's hospital course, are shown in Table 2. Findings were compared among patients with normal collagen, increased collagen, and a group of eight patients with ARDS who also required ventilatory support for more than 14 days but survived their ARDS episode. Patients with increased collagen required an FIO<sub>2</sub> > 0.40 for 21 days, significantly greater than that for patients with normal collagen (six days, p < 0.001) or surviving patients (12 days, p < 0.05). The mean FIO<sub>2</sub> was also significantly greater in the increased collagen group (p < 0.02). The number of days that PEEP was required was similar in all groups but the mean level of PEEP during those days in the increased collagen group (9.6 cm H<sub>2</sub>O) was significantly greater than that of the normal collagen group (5.7 cm H<sub>2</sub>O, p < 0.02). The mean level of PEEP in surviving patients (7.5 cm H<sub>2</sub>O) was not different from either of the other groups. Oxygenation as reflected by PaO<sub>2</sub>/FIO<sub>2</sub> was significantly worse in the increased collagen group than in the normal collagen group (p < 0.01).

The relationship between the mean level of PEEP and lobar collagen content was examined further by

![Collagen Content in ARDS Autopsies](http://journal.publications.chestnet.org/pdffileaccess.ashx?url=/data/journals/chest/21409/)

**Figure 1.** Collagen content of left lower lobe in ARF. The left lower lobe was sampled and analyzed for collagen as described in Methods. Each point represents the total left lower lobe collagen for one patient dying after the indicated duration of ARF.
regression analysis in the 16 patients ventilated for more than 14 days. By this analysis, increasing levels of PEEP were significantly associated with increasing lobar collagen content, according to the relationship: lobar collagen (g) = 0.61 PEEP (cm H\textsubscript{2}O) + 1.71 (r = 0.55, p < 0.03). A highly significant association was also found between days of FiO\textsubscript{2} > 0.4 and lobar collagen content (lobar collagen [g] = 0.33 FiO\textsubscript{2} > 0.4 [days] + 2.23; r = .79, p < 0.001).

These findings suggest a relationship between aspects of therapy and collagen accumulation in the lungs. In order to determine if increased PEEP levels and increased requirements for oxygen were the result of more severe lung injury in the increased collagen group, a number of analyses was performed, some shown in Table 3. Using mean values for the first four days, the increased collagen group required higher FiO\textsubscript{2} (p < 0.05). The level of PEEP utilized (8.1 vs 4.5 cm H\textsubscript{2}O) approached significance (p = 0.058), while the other parameters were not significantly different in the two groups. Similar, slight differences between the early findings in the two groups were present when compared for other days or combinations of days. Overall, these findings of slight differences early suggest that the group of patients who later demonstrated increased collagen may have sustained a more severe initial lung injury, but the difference between these and patients with normal collagen was small.

The complications which occurred in these 16 non-surviving patients are shown in Table 4. Multiple organ failures, diagnosed according to predetermined criteria, occurred commonly in both groups of patients. Pneumonia diagnosed by histologic criteria at autopsy\textsuperscript{a} and other sites of infection were similarly frequent in both groups.

In addition to significant differences in total lobar collagen, the two groups had significantly different wet weights, dry weights, elastin, and total protein amounts in their left lower lobes (Table 5). Determination of the collagen to dry weight ratio in the multiple tissue samples taken indicated that the collagen concentration was also greater in the high collagen group (Table 5), and that multiple samples from individual lungs did not vary excessively. Extraction of selected samples with 0.5 M acetic acid\textsuperscript{a} indicated that less than 5 percent of the total hydroxyproline was acid-soluble.

Histologic findings revealed a spectrum of pathologic lesions and a gradation of connective tissue deposition. No patient had normal histologic findings. Those patients with less severe fibrotic changes

### Table 1—Age, Sex, and Diagnosis of ARF Patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age</th>
<th>Diagnosis</th>
<th>Patient</th>
<th>Sex</th>
<th>Age</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1</td>
<td>M</td>
<td>54</td>
<td>Sepsis</td>
<td>N1</td>
<td>M</td>
<td>64</td>
<td>Aspiration</td>
</tr>
<tr>
<td>H2</td>
<td>M</td>
<td>64</td>
<td>Gastrointestinal hemorrhage</td>
<td>N2</td>
<td>F</td>
<td>65</td>
<td>Sepsis</td>
</tr>
<tr>
<td>H3</td>
<td>M</td>
<td>18</td>
<td>Pancreatitis/sepsis</td>
<td>N3</td>
<td>M</td>
<td>67</td>
<td>Sepsis</td>
</tr>
<tr>
<td>H4</td>
<td>M</td>
<td>55</td>
<td>Multiple trauma</td>
<td>N4</td>
<td>M</td>
<td>28</td>
<td>Pulmonary contusion</td>
</tr>
<tr>
<td>H5</td>
<td>M</td>
<td>72</td>
<td>Pneumonia due to Legionella pneumophila</td>
<td>N5</td>
<td>F</td>
<td>78</td>
<td>Heat stroke</td>
</tr>
<tr>
<td>H6</td>
<td>M</td>
<td>40</td>
<td>Lung abscess</td>
<td>N6</td>
<td>M</td>
<td>25</td>
<td>Multiple trauma</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>N7</td>
<td>F</td>
<td>55</td>
<td>Cancer, sepsis</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>N8</td>
<td>M</td>
<td>48</td>
<td>Cirrhosis</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>N9</td>
<td>M</td>
<td>57</td>
<td>Leukemia</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td>N10</td>
<td>M</td>
<td>52</td>
<td>Aspiration, sepsis</td>
</tr>
</tbody>
</table>

### Table 2—Clinical Parameters of ARF Patients*

<table>
<thead>
<tr>
<th></th>
<th>Nonsurvivors</th>
<th>Survivors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Increased Collagen</td>
<td>Normal Collagen</td>
</tr>
<tr>
<td>n</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>50.5±19.2</td>
<td>53.7±16.6</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>63±7</td>
<td>74±13</td>
</tr>
<tr>
<td>FiO\textsubscript{2} &gt; 0.4 (days)</td>
<td>21±6</td>
<td>6±5</td>
</tr>
<tr>
<td>FiO\textsubscript{2}</td>
<td>.50±.05</td>
<td>.41±.04</td>
</tr>
<tr>
<td>PEEP (days)</td>
<td>15.5±6.7</td>
<td>15.9±12.6</td>
</tr>
<tr>
<td>PEEP (cm)</td>
<td>9.6±3.5</td>
<td>5.7±2.5</td>
</tr>
<tr>
<td>Days on ventilator</td>
<td>34±9</td>
<td>27±10</td>
</tr>
<tr>
<td>PaO\textsubscript{2}/FiO\textsubscript{2} (mm Hg)</td>
<td>171.6±44.2</td>
<td>231.8±35.8</td>
</tr>
</tbody>
</table>

*Values for p and r are given and discussed in the text.
showed primarily an exudative phase of diffuse alveolar
damage. Occasionally, focal areas were seen to be
undergoing early fibroplasia. Patients ranked as showing
more fibrosis had varying degrees of collagen
deposition. The fibrotic process was seen both within
the alveolar walls and the alveolar spaces. Patients with
increased collagen biochemically were not distinguish-
able from the normal collagen groups by histologic
grading of fibrosis; the mean value for the normal
group was 2.0 (moderate fibrosis), while the high group
had a value of 2.3, which was not significantly different.
However, three of four patients with grade 3 histologic
fibrosis (severe) had a lobar collagen content exceeding
7 g compared to three of 11 graded as moderate (grade 2)
fibrosis.

**DISCUSSION**

The study of Zapol and co-workers indicated that
pulmonary fibrosis was present in ARDS patients who
died after two weeks of the syndrome. The fibrosis was
evidenced by increased collagen, both histologically and
biochemically. All patients had been ventilated for
several days at an FIO₂ of 0.5 or greater. The data in this
report indicate that not all patients dying with ARDS of
more than two weeks duration have increased levels of
collagen. Increased collagen was associated with
higher distending pressures exerted by PEEP, and by
longer duration of increased oxygen exposure
(FIO₂>0.4) (Table 2). Comparison of the two groups of
nonsurvivors during the first four days of the episode
indicated the group differences were becoming appar-
ent in respect to mean PEEP, FIO₂, and PAO₂/FIO₂
(Table 3). Whether high O₂ and high PEEP were
needed to treat a more severe lung injury and caused
the collagen increase or reflected the lung's reduced
compliance secondary to the presence of increased

| Table 3—Comparison of Clinical Parameters During First Four Days of ARDS Episode |
|---------------------------------|-----------------|-----------------|-----|
| Parameter                      | Increased       | Normal          | p   |
| FIO₂                           | .51 ± .08       | .43 ± .07       | .046|
| PEEP (cm H₂O)                  | 8.1 ± 3.0       | 4.3 ± 3.6       | .588|
| PAO₂/FIO₂ (mm Hg)              | 171 ± 53        | 226 ± 62        | .094|
| Peak airway pressure (cm H₂O)  | 40 ± 6          | 43 ± 7          | .526|
| Dynamic compliance (ml/cm H₂O) | 29 ± 7          | 25 ± 4          | .216|
| Radiographic score              | 6.2 ± 1.6       | 6.1 ± 1.8       | .930|

**Table 4—Complications Occurring in 16 Nonsurviving Patients Who Required Ventilation for More Than 14 Days**

<table>
<thead>
<tr>
<th>Complication</th>
<th>Increased Collagen</th>
<th>Normal Collagen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barotrauma</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Multiple organ failure</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>Pneumonia at autopsy</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Other infections at autopsy</td>
<td>2</td>
<td>6</td>
</tr>
</tbody>
</table>

connective tissue cannot be determined at present.
Analysis of dynamic compliance data did not indicate a
statistically significant difference between the two
groups either over the course of the entire episode or
during the last four days of the episode when such a
difference might most likely be expected (data not shown).
Analysis of primary diagnosis (Table 1), secondary
diagnosis, complications in other organ systems, and
the presence of infections (Table 4) did not differenti-
ate the two groups of nonsurvivors. Analysis of
clinical data did not prove the hypotheses that the
normal collagen group had died of something other
than their lung injury or that the increased collagen
group had a more severe lung injury requiring more
intense treatment. The survivors were significantly
different from the increased collagen group, but not
from the normal group, with respect to days FIO₂>0.4.
Survivors were intermediate between the two groups
of nonsurvivors with regard to PEEP levels.

There is substantial data implicating high levels of
oxygen as a cause of lung injury. In addition, high
distending pressures may contribute to the injury
pattern seen in immature lungs but this conclusion is
not universally accepted. In some experimental
systems with oxygen, both the histologic appearance
of fibrosis has occurred along with increased collagen
biochemically. In addition, previous studies of
ARDS noted increases in collagen both histologi-
and biochemically. Fibrosis correlated better
with the length of the ARDS episode and its accom-
pniring oxygen exposure rather than with other pa-
rameters such as the initial illness. What is different
in our study is that death occurred subsequent to
ARDS without increased collagen deposition in several
instances. Thus, death from ARDS is not dependent on
collagen accumulation. However, the histologic ap-
pearance of at least moderate fibrosis is a nearly
universal finding in ARDS.

The correlation of collagen levels with PEEP levels
was significant, although not as strong as with days
FIO₂>0.4. Little experimental data that would predict
such a finding exists. Rhythmic stretching of smooth
muscle cells stimulates collagen synthesis, but the
relationship of this observation to the effects of PEEP

**Table 5—Biochemical Contents of Left Lower Lobe**

<table>
<thead>
<tr>
<th>Component</th>
<th>Increased Collagen Group</th>
<th>Normal Collagen Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wet weight (g)</td>
<td>486 ± 127</td>
<td>310 ± 89*</td>
</tr>
<tr>
<td>Dry weight (g)</td>
<td>85.3 ± 20.1</td>
<td>53.5 ± 17.1*</td>
</tr>
<tr>
<td>DNA (g)</td>
<td>1.06 ± 0.36</td>
<td>0.81 ± 0.61</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>39.2 ± 15.9</td>
<td>23.6 ± 5.8*</td>
</tr>
<tr>
<td>Elastin (g)</td>
<td>2.35 ± 0.60</td>
<td>1.51 ± 0.39*</td>
</tr>
<tr>
<td>Collagen (g)</td>
<td>10.29 ± 1.96</td>
<td>3.54 ± 1.21*</td>
</tr>
<tr>
<td>Collagen/Dry weight (%)</td>
<td>12.1 ± 2.7</td>
<td>6.9 ± 2.0*</td>
</tr>
</tbody>
</table>

*p < 0.05 by two-tailed t-test.
on pulmonary interstitial cells is not direct. Previous work has been taken to indicate that all long-term ARDS involves collagen accumulation. Those ARDS patients correspond to our increased collagen group. One would expect, however, that treatment of ARDS with a proline analog such as one which has lessened lung collagen accumulation due to high oxygen exposure in experimental animals, might not be appropriate in patients who were not accumulating collagen in their lungs. Collagen accumulation is one possible effect of ARDS and not the primary cause of death. Studies in hamsters, in which collagen and elastin accumulation due to bleomycin was prevented by penicillamine, demonstrated that the physiologic changes induced by bleomycin were not altered by penicillamine. Thus, the deleterious physiologic aspects of fibrosis may occur without connective tissue accumulation. The use of penicillamine or proline analogs to prevent collagen accumulation in patients with ARDS would not be appropriate in all patients, but might be useful in some. It might be possible to predict which patients are likely to develop fibrosis based on clinical treatment parameters, especially days FlO₂>0.4 and mean PEEP.

Severity of injury is possibly related to fibrosis, but this could not be confirmed by our data. The type of injury does appear to be important for the development of fibrosis in animal models.

A grading system was used to assess connective tissue deposition histologically because the lungs had not been fixed with known inflation pressures. There was much variability in the correlation of biochemistry with pathology; the high and normal collagen groups determined biochemically were not separable by the histologic analysis. In this study, contributing influences to the difficulty in correlation could include the small size of the histologic samples in some of the cases, varying lesions such as pneumonia in different regions of the lung, and the use of only the left lower lobe for biochemical determinations in this protocol. Difficulty in correlating biochemistry with histology has been a problem in several earlier studies involving collagen levels in diseased lungs.

In conclusion, collagen accumulation does not occur in all patients dying with long-term ARDS, although at least moderate histologic fibrosis is present in all patients. Therapy appears to have a significant correlation with collagen accumulation, but the cause leading to the need for that therapy is not easily discernible.

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