Connective tissue responses in acute community-acquired pneumonia

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Abstract Circulating connective tissue components including the aminoterminal propeptides of type III collagen (PIIINP), type I collagen (PINP) and hyaluronan were determined in patients hospitalised for pneumonia of suspected bacterial origin. Ninety patients were included, 64 of these were followed prospectively for up to 21 days after initiation of therapy. Serum PIIINP was determined by RIA, s-PINP by ELISA, and s-hyaluronan by a radiometric assay. S-PIIINP rose significantly above the zero value within 24 h in both pneumococcal pneumonia (T₀: 5.3 µg/l, 95% CI: 2.7–8.1 µg/l vs. T₁: 6.7 µg/l, 95% CI: 3.8–9.1, P < 0.01) and in pneumonia of unknown aetiology (T₀: 4.0 µg/l, 95% CI: 3.6–4.8 vs. T₁: 4.5 µg/l, 95% CI: 3.8–5.1, P < 0.05) followed by a gradual decline. At T₁, S-PIIINP was higher in pneumococcal pneumonia compared with pneumonia of unknown aetiology (P < 0.05). By contrast, s-PINP tended to decline within 24 h in both pneumococcal pneumonia (T₁: 30 µg/l, 95% CI: 23–40, ns) and in pneumonia of unknown aetiology (T₁: 32 µg/l, 95% CI: 22–42, ns) followed by a steady increase. The PINP antigen size distribution remained constant throughout the follow-up period. S-hyaluronan in pneumococcal pneumonia paralleled s-PIIINP reaching a peak value on day 1 (121 µg/l, 95% CI: 65–191, P = 0.38). There was a positive correlation between s-PIIINP and C-reactive protein (CRP). The study demonstrates, that community-acquired pneumonia elicits a differentiated mesenchymal response, which is turned down in response to successful antibiotic therapy. © 2003 Published by Elsevier Science Ltd.

Keywords Procollagen peptide; Pneumonia; Connective tissue; Hyaluronan; Acute phase response.

INTRODUCTION

Bacterial pneumonia is a leading cause of hospitalisation with a mortality ranging from 4% to 36% (1,2). The assessment of inflammatory activity and prognosis relies on clinical findings combined with the extent of pulmonary opacities on X-ray and acute phase reactants (1,3). However, acute phase proteins are only indirectly coupled to the inflammatory process, because they are synthesised at sites topographically separated from the inflammatory focus (4). This implies, that they may not provide an optimal reflection of inflammatory activity as reported in e.g. systemic lupus erythematosus (5). From a pathophysiological point of view, biomarkers, which are directly related to the inflammatory process, would appear to possess inherent advantages. Extracellular matrix proteins are abundant in lung tissue with collagen accounting for approximately 20% of the dry weight (6). Besides its scaffolding role, the connective tissue serves both as a site and a target for inflammatory cells and mediators of inflammation in infectious and non-infectious lung inflammation. In a broad spectrum of fibroproliferative conditions and bone diseases, the serum concentration of procollagen peptides, which are cleaved off in a stoichiometric manner following secretion of procollagen into the extracellular space, has been shown to be reliable indicators of the biosynthetic rate of their parent collagen types, collagen I and III in particular (7–13). This also applies to hyaluronan in serum (14–16). Studies in chronic fibrosing lung diseases have yielded conflicting results (17–19). In one previous cross-sectional study of pneumonia, s-PIIINP was increased (20). The purposes of the present prospective study were (1) to investigate if pneumonia of suspected bacterial origin elicits a mesenchymal response detectable as changes in the serum concentration of the aminoterminal propeptides of collagen III (PIIINP), I (PINP) and hyaluronan and...
to study the relation between these components and conventional markers of inflammation.

**METHODS**

**Patients and study design**

The study comprised 90 patients with acute community-acquired pneumonia. A total of 90 patients were included, 45 men and 45 women, aged 20–95 years. The patients fulfilled the following criteria: A history of cough or sputum production or pleuritic chest pain or dyspnoea, rectal temperature above 37.9°C, chest X-ray showing infiltrative lung changes, a total white blood cell (WBC) count above 10.0×10⁹/l (normal range: 4.0×10⁹–10.0×10⁹/l) and/or serum C-reactive protein (CRP) exceeding 40 mg/l (normal range: <10 mg/l). Patients treated with oral or intravenous glucocorticoids within 2 weeks before admission were excluded as were patients with malignancies, chronic renal and hepatic disease, other fibroproliferative diseases, abnormalities of growth and development, major surgery within 3 months, pregnancy and inability to give informed consent. Twenty patients with mild asthma or mild chronic obstructive lung disease were included. Blood cultures, sputum culture and chest X-ray was done on all patients and when considered during the course. Blood samples were taken from all patients on admission (day 0).

Among the follow-up cohort, the duration of symptoms before admission averaged 3.5 days (0.5–7 days). The mean temperature on admission was 39.0°C (range 37.4°–41.3°C). Fifty-three (83%) had positive stethoscopic findings. The lag time between initiation of antibiotic treatment and the collection of serum samples did not exceed 12 h. Serial blood samples were obtained from 64 patients on days 1, 3, 5, 7, 10, and 21. All patients were treated with antibiotics for at least 7 days (penicillin V or G: 52 patients, ampicillin/pivampicillin: nine patients, erythromycin: four patients). Fifty-three and 47 patients completed 1 and 3 weeks follow-up, respectively. Two patients died and 15 dropped out for various reasons (21).

**Ethics**

The study was performed in accordance with the Helsinki II declaration. The local ethics committee approved the research protocol. The patients were informed about the study verbally and in writing and all gave their written consent. They were told that they could withdraw from the study at any time.

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*Including a few patients who had received antipyretics before admission.

**Biochemical analyses**

Sera for quantification of connective tissue components were stored at −80°C until analysed for serum PIIINP, PINP and serum hyaluronan. Hyaluronan was only measured consecutively in pneumococcal pneumonia.

PIIINP was determined by the PIIINP Radioimmunoassay Kit (Orion Diagnostica, Oulu, Finland) (22), which is an equilibrium type of assay. The normal range in our laboratory, based on healthy adult blood donors (aged 20–80 years), was 1.9–4.5 μg/l (5th and 95th percentiles, respectively) with a median value of 2.9 μg/l. No sex or age differences were found. The intra- and inter-assay coefficients of variation were 4.3% and 9.0%, respectively.

PINP was measured by means of ELISA (13), which recognises two molecular size variants of PINP antigen shown by size chromatography and corresponding to an apparent molecular mass of 30 and 100 kDa. These size variants have been demonstrated to be monomeric α1-chains of PINP and α1-chains in a trimeric structure (23). The ELISA system uses immunospecifically purified rabbit anti-PINP antibodies as both catcher and indicator antibodies. The intra- and inter-assay coefficient of variation was <5.0% and 5.4%, respectively. In 57 healthy, adult subjects (age 19–77 years, 19 women and 38 men) the serum concentration was 23–84 μg/l (5th and 95th percentiles, respectively) with a median value of 56 μg/l.

PINP size chromatography was carried out by applying 200 μl serum to a Superose 12 HR 10/30 column connected to a fast-performance liquid chromatography (FPLC) system (Pharmacia, Uppsala, Sweden). The chromatography was performed with PBS, pH 7.3 at a flow rate of 0.5 ml/min and 0.5 ml fractions were collected (13). Serum from two selected patients was subjected to size chromatography. One of the patients had rising s-PINP throughout the observation period (>300% rise from day 0 (35 μg/l) to day 21 (135 μg/l)) and the other patient had a constant, normal serum level around 40 μg/l throughout the course. Samples from days 0, 5, 10 and 21 were analysed in the patient with rising PINP levels and sera from days 0 and 21 were analysed in the other patient (Fig. 4). Molecular size chromatography in a healthy person has been reported previously (24).

Determination of hyaluronan was performed with the Pharmacia HA test (Pharmacia AB, S-75182 Uppsala, Sweden) as previously described (25). This radiometric assay is based on the use of a specific hyaluronan-binding protein, isolated from bovine cartilage. The normal range in healthy blood donors was 10–97 μg/l, median 28 μg/l. The inter-assay coefficient of variation was 8.3%. Serum CRP was measured by turbidimetry.

**Statistical analysis**

The statistical analysis was done with Statistical Package for the Social Science (SPSS®) Software and CIA
(London). Results are given as median and range unless otherwise stated. Confidence intervals (CI), given for the median of a certain variable, were calculated at the 95% level. Comparison between groups was performed by the Mann–Whitney test for unpaired differences. Temporal differences within groups were tested by means of Wilcoxon’s matched-pairs signed rank sum test. Correlation analysis was based on the Spearman rho test. P values less than 0.05 were considered to be significant.

RESULTS

A specific bacterial diagnosis could be established in a total of 32 (36%) of the patients. Twenty-two (24%) had pneumococcal pneumonia, which compares well with previous reports, which however have yielded quite varying figures (26,27). Five were infected with Haemophilus influenzae (6%), one with Klebsiella pneumoniae and four had atypical pneumonia. In a total of 58 (64%), a bacterial aetiology could not be identified. Twenty of the patients (22%) had received antibiotics before admission, but s-PIIINP, s-PINP, s-hyaluronan, CRP and granulocyte counts did not differ between treated and untreated patients. In a preliminary study of 10 patients with bacterial pneumonia, s-PIIINP did not exhibit significant changes within the first 12 h after initiation of antibiotic treatment (personal observation).

Figure 1 illustrates the individual serum concentrations of s-PIIINP, s-PINP and s-hyaluronan in different subsets of pneumonia on admission (T₀). At T₀, median s-PIIINP for the whole group (4.5 μg/l, 95% CI: 3.8–4.9 μg/l) equalled the upper normal limit but with a considerable variation between individuals. Looking at the groups according to aetiology, PIIINP was initially slightly elevated in pneumococcal pneumonia (5.1 μg/l, 95% CI: 2.7–6.3) and atypical pneumonia but within the normal range in pneumonia of unknown aetiology (4.0 μg/l, 95% CI: 3.7–4.7) and H. influenzae infection. Figure 2 illustrates the temporal changes in s-PIIINP, s-PINP and s-hyaluronan in patients with pneumonia caused by Streptococcus pneumoniae and Fig. 3 shows the courses of s-PIIINP and s-PINP in pneumonia of unknown aetiology. Both figures illustrate serum values during treatment with antibiotics and at follow-up on day 21. In both groups, s-PIIINP rose significantly above zero level within 24 h after admission reaching peak values at 6.7 μg/l (95% CI: 3.8–9.1, P < 0.01) and 4.5 μg/l (95% CI: 3.8–5.1, P < 0.05), respectively. The peak value was significantly higher in S. pneumoniae pneumonia as compared with pneumonia of unknown aetiology (P < 0.05). During treatment with antibiotics, s-PIIINP declined significantly (P < 0.05) in patients with S. pneumoniae pneumonia, reaching the normal range by day 5. Throughout the course, median s-PIIINP remained within normal range in pneumonia of unknown aetiology.

On day 21, 10 of the patients (22%) still had elevated s-PIIINP, five of which had pneumococcal pneumonia, four pneumonia of unknown aetiology and one atypical pneumonia.

S-PINP at inclusion averaged 37 μg/l (95% CI: 32–42 μg/l). Median initial s-PINP was 35 μg/l (95% CI: 28–44) in patients with S. pneumoniae pneumonia and 40 μg/l (95% CI: 36–48) in patients with pneumonia of unknown aetiology (Fig. 1). In contrast to s-PIIINP, s-PINP showed a slight decline within the first 24 h of admission in patients with pneumococcal pneumonia (T₀: 31 μg/l, 95% CI: 26–37 vs. T₁: 30 μg/l, 95% CI: 23–40, ns) and the follow-up patients with pneumonia of unknown aetiology (T₀: 37 μg/l, 95% CI: 25–48 vs. T₁: 32 μg/l, 95% CI: 22–42 μg/l, ns). Thirty-one percent of the patients participating in the longitudinal study had s-PINP below the lower limit of the reference interval at T₁. S-PINP then...
gradually rose within the reference range without exceeding its upper normal limit (Figs. 2 and 3).

Size chromatography of PINP in the circulation of the two selected patients showed that the peak of the molecular weight fractions seen at $T_0$, $T_5$, $T_{10}$ and $T_{21}$ were identical to the peaks seen in healthy individuals (24) (Fig. 4).

S-hyaluronan at $T_0$ was 73 $\mu$g/l (95% CI: 58–88 $\mu$g/l). Patients with pneumococcal pneumonia had slightly higher s-hyaluronan (92 $\mu$g/l, 95% CI: 68–158) as compared with patients with pneumonia of unknown aetiology (69 $\mu$g/l, 95% CI: 49–88, $P<0.05$) (Fig. 1). S-hyaluronan was only measured consecutively in pneumococcal pneumonia where it paralleled s-PIIINP reaching a peak value on day 1 (121 $\mu$g/l, 95% CI: 65–191, $P=0.38$). Normal range was reached between days 1 and 3 (Fig. 2).

Briefly, CRP at $T_0$ was highly elevated in all categories of pneumonia. At day 1, patients with pneumococcal pneumonia had higher CRP levels compared with pneumonia of unknown aetiology ($P<0.05$). After day 1, CRP steadily declined in both groups reaching normal range on days 21 and 10, respectively. In both groups, elevated granulocyte counts normalised within 5 days after initiation of therapy.

Among the patients followed consecutively, there was significant correlations between s-PIIINP and conventional acute phase reactants like CRP ($P<0.01$), while
there was no correlation between s-PINP and CRP. Scattered correlation was demonstrated between s-PIIINP and s-PINP.

**DISCUSSION**

The present study demonstrates for the first time, that acute pneumonia acquired outside hospital is associated with a differentiated mesenchymal response involving collagen III in particular. The PIIINP response was highest in pneumococcal pneumonia as compared with pneumonia of unknown aetiology.

Increased s-PIIINP has been reported in one previous cross-sectional study of pneumonia (20). The origin of the increased s-PIIINP and s-hyaluronan cannot be determined from the present study. Although the concentration of PIIINP in bronchial lavage (BAL) fluid from patients with bacterial pneumonia did not exceed normal values (20), this observation should be interpreted with caution because PIIINP may have been degraded into smaller fragments by proteolytic enzymes in the BAL fluid. The sequential changes of s-PIIINP and hyaluronan in the present study are similar to the pattern reported during wound healing in experimental animals and humans, indicating that they reflect inflammation and repair in the affected lung tissue (8,28–33).

The relatively small rise in s-PIIINP in pneumonia may reflect that the structural damage to the lung is less severe as compared with fibrosing lung diseases like acute respiratory distress syndrome (ARDS), where significant increases in s-PIIINP have been reported (20,34). In various fibrosing lung diseases in which the mechanism behind the recruitment and activation of fibroblasts and the subsequent production of extracellular matrix is unknown, there have been conflicting reports on serum and BAL PIIINP content (17,19,35,36).

The PIIINP response was less pronounced in patients with pneumonia of unknown origin as compared with pneumococcal pneumonia. In this context, it should be noted that the most severely ill patients with the most extensive X-ray changes were found among the latter group. This suggests that s-PIIINP is determined by the extent of the infectious infiltrate rather than by the specific pathogen. This notion is favoured by animal experiments demonstrating a parallelism between s-PIIINP and collagen proliferation in subcutaneous granulomas in rats (8). Ten patients had persistently elevated s-PIIINP on day 21. Conceivably, this sustained connective tissue response represents extended repair and regeneration mechanisms at the site of lung inflammation.

In contrast to PIIINP and hyaluronan, the lowest s-PINP values were recorded within the first 24h in all kinds of pneumonia. Subsequently, s-PINP gradually increased within the normal reference area and had apparently not stabilised by the end of the observation period (Figs. 2 and 3). This observation is at variance with experience from wound healing studies, where the concentration of propeptides from collagens I and III are increased in serum as well as in wound fluid (29,37,38). Most of the PINP present in serum probably derives from the continuous turnover of skin and skeletal collagen (39,40). Most likely, the decreased PINP response to pneumonia serves to ensure that adequate energy supplies are directed to the trauma area at the cost of the household synthesis in remote and unaffected tissues (40). Since the duration of bed rest in this otherwise healthy patient cohort rarely exceeded a few days, decreased synthesis of bone collagen due to immobilisation probably was a contributing factor to the low PINP.

**FIG. 4.** Analysis of molecular weight fractions by size chromatography of serum from a patient with rising PINP during the course (No. 1) and a patient with a constantly normal serum level during the course (No. 2). Samples were analysed on days 0, 5, 10 and day 21 in the patient with rising PINP levels and at days 0 and 21 in the other patient.
values (41). Alternatively, the proportion of the different size variants of PINP could be altered in serum during the course of an infection. To study this issue, serum from two selected patients was subjected to size chromatography. This analysis showed that the distribution of PINP size variants remained stable throughout the course of the disease, and that the size variant profile did not differ from healthy controls (24) (Fig. 4).

In conclusion, this study has demonstrated that acute bacterial pneumonia elicits a transient mesenchymal reaction, which is gradually turned down in response to targeted antibiotic therapy and resolution of the infectious infiltrate. However, in uncomplicated bacterial pneumonia the clinical utility of these molecules as markers of disease activity is restricted due to the modest amplitude.

REFERENCES


