

CONCISE COMMUNICATIONS

YKL-40, a Matrix Protein of Specific Granules in Neutrophils, Is Elevated in Serum of Patients with Community-Acquired Pneumonia Requiring Hospitalization

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The serum concentration of YKL-40, a matrix protein of specific granules in neutrophils, was determined by RIA in 90 patients hospitalized with pneumonia of suspected bacterial origin. Of these, 64 were followed prospectively during antibiotic treatment with blood samples taken on day 0 (on admission and the start of treatment) and on days 1, 3, 5, 7, 10, and 21. Serum YKL-40 at admission was increased in patients with *Streptococcus pneumoniae* pneumonia (median, 893 $\mu\text{g/L}$; 95% confidence interval [CI], 704–1560), compared with healthy subjects (median, 102 $\mu\text{g/L}$; 95% CI, 64–247 $\mu\text{g/L}$; $P < .001$) and in patients with pneumonia of unknown etiology (median, 448 $\mu\text{g/L}$; 95% CI, 334–700; $P < .05$). Peak YKL-40 serum values were observed on day 1 and thereafter declined steeply to almost normal by day 3. During the first 10 days, there was a close relation between serum YKL-40 and markers of specific granules of neutrophils (serum lactoferrin and neutrophil gelatinase-associated lipocalin), which suggests that serum YKL-40 reflects exocytosis of specific granules of neutrophils in persons with acute bacterial pneumonia.

YKL-40 is a matrix protein in the specific granules of human neutrophils isolated from the blood of healthy subjects [1]. Proteins located in specific granules are thought to be released during migration in tissues and when needed for oxygen-dependent or -independent bactericidal activity at the inflammatory focus [2]. YKL-40 is a member of the 18-glycosylhydrolase family [3–9], a protein family that also includes chitinases and chitinase-related proteins. The protein was termed YKL-40 from its molecular weight (40 kDa) and the one letter code for its three N-terminal amino acids, tyrosine,

lysine, and leucine. It is also called human cartilage glycoprotein-39 [4].

YKL-40 is a lectin that binds chitin [9] and heparin [6], but it has no chitinase activity [4, 9]. The physiologic function of YKL-40 is unknown; however, the pattern of its expression in normal and disease states suggests that it plays a role in the degradation of extracellular matrix or in tissue inflammation [1, 3–12]. YKL-40 is also synthesized by activated macrophages [7–9] and by articular chondrocytes and synovial cells from persons with rheumatoid arthritis [3, 4].

Persons with active rheumatoid arthritis have increased levels of YKL-40 in serum and synovial fluid [5], and YKL-40 has been suggested as an autoantigen in rheumatoid arthritis that serves as a target for the immune response [11]. The gene for YKL-40 has been sequenced [7], but little is known about the regulation of YKL-40. Transforming growth factor β reduces YKL-40 mRNA in chondrocytes to barely detectable levels [4]; however, the expression is not influenced by insulin-like growth factor 1 [4] or by interleukin (IL)–1 and tumor necrosis factor α in synovial cells [3]. This study evaluated circulating YKL-40 levels measured sequentially in the course of acute community-acquired pneumonia of suspected bacterial etiology.

Materials and Methods

Patients. The study was composed of 90 patients (45 men and 45 women, aged 20–95 years) admitted to Odense University Hos-

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

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pital from February 1996 to July 1997 who fulfilled the following inclusion criteria: a history of cough or sputum production or pleuritic chest pain or dyspnea, rectal temperature $>37.9^{\circ}\text{C}$, chest radiograph showing infiltrative changes of the lung, total leukocytes $>10.0 \times 10^9/\text{L}$ (normal range, $4.0\text{--}10.0 \times 10^9/\text{L}$), and/or serum C-reactive protein (CRP) $>40 \text{ mg/L}$ (normal range, $<10 \text{ mg/L}$). The exclusion criteria were treatment with oral or intravenous glucocorticoid in the 2 weeks preceding hospitalization, known cancer or liver disease, joint replacement surgery within the preceding 6 months or major surgery within the preceding 3 months, fibroproliferative diseases, diseases of growth and abnormal development, pregnancy, or inability to give informed consent. Persons with mild asthma or mild chronic obstructive lung disease were included in the study ($n = 20$). Twenty of the 90 patients had received antibiotics for 1–10 days before admission to the hospital. All study subjects had blood and sputum cultures and chest radiographs plus the following serologic tests when relevant: *Legionella* antibody titer, immunofluorescence test, and urinary antigen test; *Mycoplasma pneumoniae* antibody titer; cold agglutinin titer; and *Chlamydia* complement fixation test.

Study design. Blood samples were taken from all patients on admission (day 0). Sixty-four patients were followed prospectively for up to 21 days, with serial blood sampling on days 0, 1, 3, 5, 7, 10, and 21. The patients were treated with antibiotics for ≥ 7 days (penicillin G, 53; penicillin V, 2; ampicillin, 6; pivampicillin, 1; and erythromycin, 2). Antibiotics were initially given intravenously and then changed to oral medication once the temperature had become normal. The time between the start of antibiotic treatment and the collection of the serum samples (day 0) did not exceed 12 h. Fifty-three patients completed the first week, and 47 completed the 3-week study period. Seventeen patients dropped out: death ($n = 2$; supposed pulmonary embolism and pneumonia, 1 each), treatment with glucocorticoid ($n = 4$), transfer to another hospital ($n = 1$), leaving the country for vacation ($n = 2$), and not wanting to provide follow-up blood samples ($n = 8$).

Biochemical analysis. Serum CRP was analyzed by turbidimetry. The total leukocyte count and differential count were determined by routine methods. Serum YKL-40 was determined by RIA [5]. The median serum concentration of YKL-40 in healthy adults ($n = 260$, aged 18–79 years) was $102 \mu\text{g/L}$. The upper normal value was defined as the 95th percentile, which was $247 \mu\text{g/L}$ [12]. Lactoferrin, neutrophil gelatinase-associated lipocalin (NGAL), and myeloperoxidase (MPO) in serum were determined by ELISA as described elsewhere [13–15].

Statistical analysis. The statistical analysis was done with SPSS (Chicago) software and CIA (London). Results are given as median and range unless otherwise stated. Confidence intervals (CIs), given for the median of a certain variable, were calculated at the 95% level. Comparison between groups was done by the nonparametric Mann-Whitney test for unpaired differences. Temporal differences within groups were tested by Wilcoxon matched-pairs signed rank sum test. Correlation analysis was based on the Spearman ρ test. $P < .05$ was considered significant.

Results

A specific bacterial pneumonia etiology was identified in 32 patients (36%): 22 were infected with *Streptococcus pneumoniae*,

5 with *Haemophilus influenzae*, 1 with *Klebsiella pneumoniae*, and 4 had atypical pneumonia. At the time of hospitalization (day 0), serum YKL-40, serum CRP, and polymorphonuclear neutrophil (PMN) counts did not differ between untreated patients and those treated with antibiotics prior to hospital admission. Serum YKL-40 did not change significantly within the first 12 h after initiation of antibiotics.

Serum YKL-40. On admission (day 0), patients infected with *S. pneumoniae* had significantly ($P < .001$) increased serum YKL-40 (median, $893 \mu\text{g/L}$; 95% CI, 704–1560) compared with healthy subjects (median, $102 \mu\text{g/L}$; 95% CI, 64–247). Patients with *S. pneumoniae* bacteremia tended to have higher serum YKL-40 levels (median, $1080 \mu\text{g/L}$; range, 176–9000; $n = 15$) than those with a positive sputum culture only (median, $704 \mu\text{g/L}$; range, 118–1880; $n = 7$). The highest serum YKL-40 level (9000 $\mu\text{g/L}$) was observed in a patient with *S. pneumoniae* pneumonia with complicating empyema. Serum YKL-40 in patients with pneumonia of unknown etiology (median, $448 \mu\text{g/L}$; 95% CI, 334–700) was elevated compared with normal subjects ($P < .05$) but lower than in persons infected with *S. pneumoniae* ($P < .05$). Serum YKL-40 was normal or only slightly elevated in persons with atypical pneumonia and *H. influenzae* pneumonia (median, $204 \mu\text{g/L}$; 95% CI, 112–512; $n = 9$). The single patient with *K. pneumoniae* had serum YKL-40 of $1590 \mu\text{g/L}$.

Table 1 shows the demographic and initial clinical characteristics of the subjects in the longitudinal study. Figure 1 illustrates the changes in serum YKL-40 in persons with pneumonia caused by *S. pneumoniae* and pneumonia of unknown etiology during treatment with antibiotics and at follow-up on day 21. After peaking on day 1, serum YKL-40 declined rapidly and significantly ($P < .01$), to reach the upper limit of the normal range (95th percentile, $247 \mu\text{g/L}$) by 3 days in patients with pneumonia of unknown etiology and by 7 days in patients with *S. pneumoniae* pneumonia. At the time of follow-up (day 21), serum YKL-40 was still above the upper normal level in 16 of the 47 study patients. However, serum YKL-40 only exceeded $500 \mu\text{g/L}$ in 4 patients, all of whom had pneumonia of unknown etiology. Patients with *H. influenzae* or atypical pneumonia had normal or slightly elevated values throughout the study period.

Serum CRP and PMN. Initial serum CRP and PMN counts were significantly elevated ($P < .001$) in patients with all types of pneumonia. These parameters did not differ between patients with *S. pneumoniae* pneumonia and those with pneumonia of unknown etiology (table 1). Serum CRP peaked on day 1 after initiation of antibiotics in patients with pneumonia of unknown etiology (significantly higher than the initial value, $P < .05$; figure 1). Serum CRP subsequently declined and reached the upper limit of the normal range on day 10. In patients with *S. pneumoniae* pneumonia, serum CRP declined steadily from day 0 to reach the normal range on day 21. In both groups, PMN decreased after 1 day of antibiotic therapy and reached the upper limit of the normal range at days 3–5.

Markers of neutrophil granules. Serum lactoferrin and

Table 1. Initial demographic, clinical, and laboratory data for patients with different types of pneumonia who participated in the follow-up study.

	Unknown etiology	<i>Streptococcus pneumoniae</i>	Atypical pneumonia	<i>Haemophilus influenzae</i>	Normal range
No. of patients (men/women)	41 (22/19)	14 (7/7)	4 (0/4)	4 (2/2)	
Age, years	60 (21–95)	58 (20–90)	66 (46–80)	63 (31–68)	
Duration of symptoms, days	3 (1/2–7)	2.5 (1/2–7)	7 (4–7)	7 (2–7)	
Temp. on admission, °C	39 (37.6 ^a –40.4)	39.2 (37.4 ^b –41.3)	39 (38.9–39.3)	39.1 (37.6 ^c –39.5)	
No. of patients on antibiotics ^d	6 (for 1/2–6 days)	1 (for 1 day)	1 (for 3 days)	2 (for 5–6 days)	
Serum YKL-40, µg/L	448 ^c (108–2820)	812 (118–3120)	306 (104–512)	176 (112–680)	64–247
Serum CRP, mg/L	158 (10–565)	290 (23–421)	237 (194–254)	166 (56–348)	0–10
White blood cells × 10 ⁹ /L	14.9 (6.2–43.6)	15.2 (3.6–26.3)	12.4 (8.4–15.5)	12.4 (7.7–17.6)	4–10
PMN in blood × 10 ⁹ /L	12.4 (4.3–41.9)	13.9 (2.4–24.4)	10.8 (9.1–13.2)	9.5 (5.6–13.9)	1.8–7.5

NOTE. Values are median (range) except as noted. CRP, C-reactive protein; PMN, polymorphonuclear neutrophils.

^a Temperature <38°C was recorded for 1 patient who had received an antipyretic drug shortly before admission.

^b Temperatures <38°C were recorded for 2 patients who had received an antipyretic drug shortly before admission.

^c For 1 patient the axillary temperature was accepted because rectal temperature could not be obtained.

^d Patients who had received antibiotics before admission.

^e $P < .05$ compared to patients with *S. pneumoniae* pneumonia (Mann-Whitney's rank sum test).

NGAL (markers of specific granules) and serum MPO (marker of azurophil granules) were determined in 11 patients with *S. pneumoniae* pneumonia. All three markers were highest on day 0 (1011, 366, and 465 µg/L, respectively); after 3–5 days of treatment, the concentrations had decreased significantly ($P < .05$ –.01). In 6 of the 11 patients, the temporal course of serum YKL-40 was parallel to that of serum lactoferrin and serum NGAL; in 4 others, the curves were nearly parallel. In only 1 patient did serum YKL-40 change in a manner that was completely different from serum lactoferrin and NGAL. By contrast, the curves reflecting YKL-40 and PMN counts were parallel or nearly parallel in only 4 patients. The temporal course of serum YKL-40 was parallel to that of MPO in 3 patients and nearly parallel to MPO in 3 others.

Serum YKL-40 versus plasma YKL-40. In 25 patients, corresponding serum and plasma samples were available at day 0. A highly significant correlation was found between the serum and plasma levels of YKL-40 ($\rho = 0.9987$, $P < .001$). The serum/plasma ratio was 1.03. In 48 healthy subjects, a significant correlation was also found between serum and plasma YKL-40 levels ($\rho = 0.8963$, $P < .001$), and the serum/plasma ratio was 1.14. The plasma level of YKL-40 in healthy subjects was 116 µg/L ($n = 48$), and the neutrophil level of YKL-40 (by RIA) was 156 ng/10⁶ cells ($n = 7$, $SD \pm 27$). Assuming a mean hematocrit of 0.45 and a mean neutrophil count of 4.6×10^6 /mL, plasma YKL-40 concentrations were 9% of that in circulating neutrophils: [mean plasma concentration × (1 – hematocrit)]/[mean neutrophil concentration in 1 mL of blood × 100%] = {[116 × 0.55]/(156 × 4.6) × 100%}.

Discussion

We believe this study is the first to show that serum YKL-40 is increased in persons with acute community-acquired pneumonia requiring hospitalization. The highest levels were in persons with pneumococcal pneumonia, followed by persons with

pneumonia of unknown etiology. Patients with atypical or *H. influenzae* pneumonia had normal or only slightly increased YKL-40 levels. The reason for these differences between subsets of pneumonia cannot be determined from the present study. However, patients infected with *S. pneumoniae* were more seriously ill and had more widespread infiltrates on chest radiographs. This finding indicates that the magnitude of the increase in serum YKL-40 could be determined either by the extent of the infectious infiltrate or by a specific bacterial etiology.

Serum CRP and YKL-40 showed a partial parallelism during the course of antibiotic therapy. However, whereas YKL-40 peaked on day 1 after initiation of treatment and then declined rapidly to reach the normal range within 1 week, CRP declined more slowly. CRP is secreted by hepatocytes in response to proinflammatory mediators like IL-6 [16]. In contrast, YKL-40 is not secreted by hepatocytes [10]. Instead YKL-40 is secreted by activated macrophages [7–9] and released by exocytosis of specific neutrophil granules [1]. The parallel changes in serum YKL-40, serum lactoferrin, and NGAL (proteins present in the specific granules of neutrophils) in the first 10 days of antibiotic treatment in patients with pneumococcal pneumonia provides strong evidence that activated neutrophils are a principal source of elevated serum YKL-40 in the acute phase of bacterial pneumonia. Taken together, our findings indicate that serum YKL-40 reflects a different aspect of the inflammatory pulmonary process than conventional acute-phase proteins. This view is further supported by our recent finding that serum YKL-40 is not increased in persons with polymyalgia rheumatica. These persons have very high levels of serum CRP and high erythrocyte sedimentation rates (unpublished data).

The YKL-40 concentration did not differ between serum and plasma (~9% of that in circulating neutrophils). This contrasts with lactoferrin and NGAL, where the serum levels are much higher than in plasma [17] and where the plasma level is <1% of that in neutrophils. The high serum concentrations of these

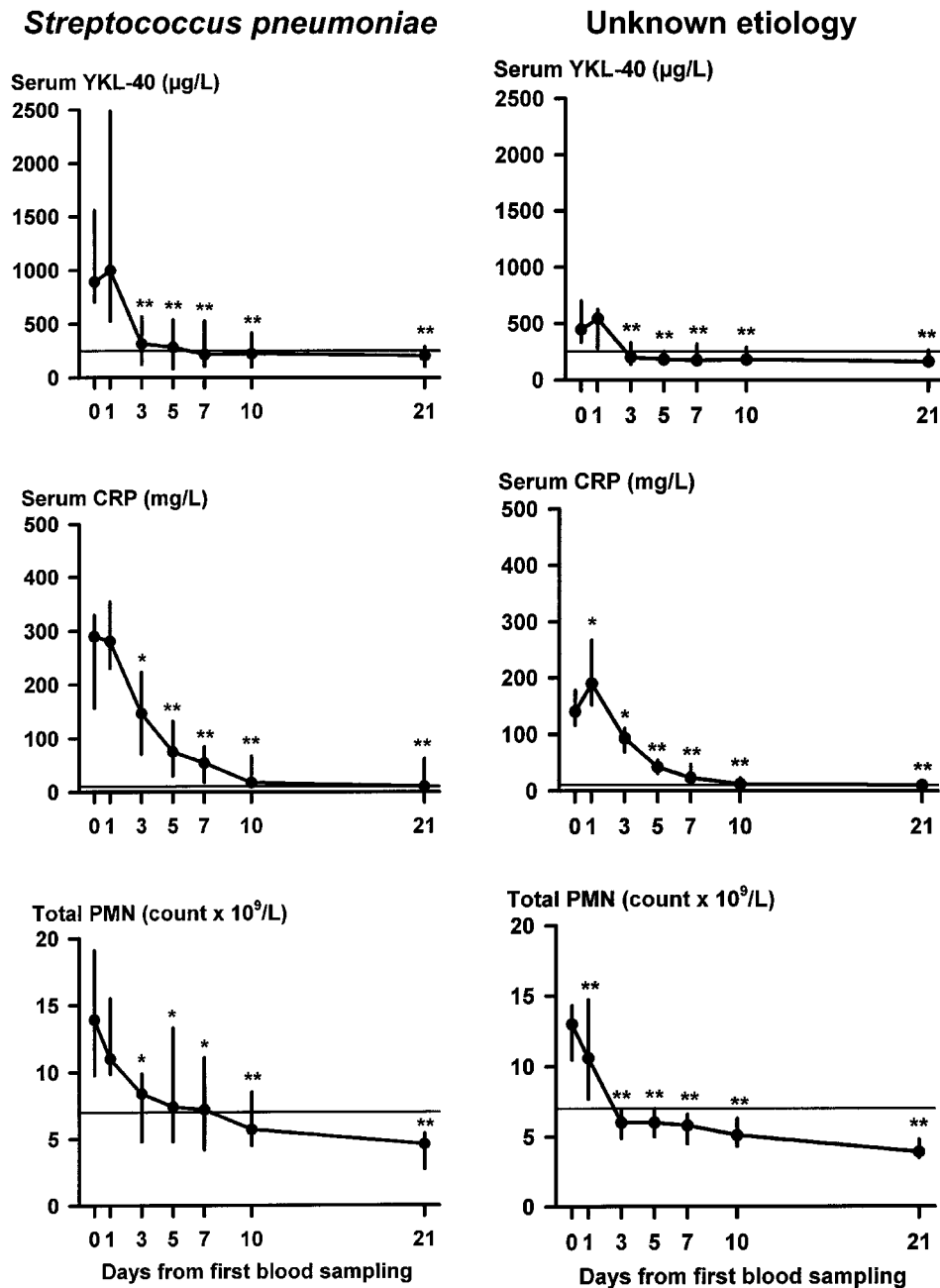


Figure 1. Temporal changes in serum YKL-40, serum C-reactive protein (CRP), and polymorphonuclear neutrophils (PMN) in sera of patients with *Streptococcus pneumoniae* pneumonia or with pneumonia of unknown etiology. Median values and 95% confidence intervals are given. Horizontal lines represent upper limit of normal range (YKL-40: 95th percentile, 247 µg/L; CRP, 10 mg/L; PMN, 7.5 × 10⁹/L). Wilcoxon test: *, P < .05; **, P < .01 vs. initial value.

proteins may be caused by degranulation during blood sampling [18]. Thus, we believe that the release of YKL-40 during coagulation contributes insignificantly to the levels recorded in serum. Furthermore, the relatively high plasma YKL-40 concentration compared with the level in neutrophils indicates that YKL-40 is not primarily derived from neutrophils undergoing degranulation during sample processing.

The function of YKL-40 is unknown. However, the pattern of its expression in normal and diseased tissue suggests that the protein plays a role in facilitating cell migration through the extracellular matrix and in tissue remodeling at sites of inflammation. This concept is supported by the localization of YKL-40 in the specific granules of neutrophils, where other matrix-degrading enzymes are stored as well [2].

In conclusion, serum YKL-40 concentrations were markedly increased in persons with acute bacterial pneumonia of different etiologies. Treatment with antibiotics led to normalization of serum YKL-40 within 1 week. The parallel courses of serum YKL-40 and markers of specific granules of PMN granulocytes indicate that the high serum content of YKL-40 in the acute phase of lung infection arises from activated neutrophils. This implies that YKL-40 may serve as a specific serologic marker of granulocyte function at the site of tissue inflammation as a supplement to conventional acute-phase proteins.

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