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Serum Level of YKL-40 is Elevated in Patients with Streptococcus pneumoniae Bacteremia and is Associated with the Outcome of the Disease

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YKL-40 is secreted by activated macrophages and neutrophils. Elevated serum concentrations of YKL-40 are found in patients with diseases characterized by inflammation or ongoing fibrosis. The aim of this study was to evaluate serum YKL-40 levels in patients with Streptococcus pneumoniae bacteremia and to correlate these levels with clinical findings and outcomes. YKL-40 was determined by ELISA and 89 patients were included in the study. Serum YKL-40 levels were significantly higher in patients with S. pneumoniae bacteremia (median 342 μg/l; range 20–20,400 μg/l) than in age-matched healthy subjects (44 μg/l; 20–184; $p < 0.001$). Serum YKL-40 levels were related to the severity of the infection, with significantly higher serum YKL-40 levels being observed in patients who needed hemodialysis ($p < 0.001$), pharmacological treatment of hypotension ($p < 0.001$) and mechanical ventilation ($p = 0.003$) compared to those in patients who did not need this supportive treatment. Nineteen patients died and these patients had significantly higher serum YKL-40 levels (980 μg/l; 88–20,400 μg/l) than those of survivors (256 μg/l; 20–9,100 μg/l; $p < 0.001$). Serum YKL-40 level was an independent prognostic factor of survival in logistic multivariate regression analysis ($p = 0.002$). In conclusion, high serum levels of YKL-40 indicated a poorer prognosis for patients with S. pneumoniae bacteremia.

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INTRODUCTION

YKL-40 is a 40 kDa heparin- and chitin-binding lectin (1, 2), with no chitinase activity (2–4), and is secreted from activated neutrophils (5) and macrophages (6, 7). The exact physiological function of YKL-40 is not known; however, it has recently been reported that YKL-40 is a growth factor for connective tissue cells (8). The pattern of YKL-40 expression in normal and disease states suggests that it may play a role in inflammatory processes and in remodeling the extracellular matrix. High serum concentrations of YKL-40 are correlated with morbidity from such varied diseases as active rheumatoid arthritis (9, 10) and ongoing hepatic fibrosis (11). A high serum YKL-40 concentration is also a prognostic marker of a fatal outcome in patients with recurrent breast cancer and colorectal cancer (12, 13). The importance, or usefulness, of serum YKL-40 concentration as a prognostic marker of morbidity and mortality in infectious diseases has not previously been investigated.

Streptococcus pneumoniae is a major cause of community-acquired pneumonia, bacteremia, meningitis and acute otitis media. The incidence of invasive pneumococcal disease is highest among young children and the elderly, varying from 14 to 600 per 100,000 infants and from 20 to 190 per 100,000 adults aged > 65 y (14). The reason for this variation, with the exception of geographical differences, is not known. About one-third of adults with invasive pneumococcal disease have no recognized risk factors. The mortality varies between 10% and 40% and is mainly dependent on the age of the patient, with the highest mortality being observed in the elderly (15).

The aim of the present study was to investigate whether serum YKL-40 level is related to morbidity and outcome in patients with pneumococcal bacteremia.

MATERIALS AND METHODS

Patients

Between October 1999 and June 2000, adults (≥ 18 y) admitted to 1 of 5 university hospitals (Aalborg, Aarhus, Odense, Hvidovre and Rigshospitalet) in Denmark with S. pneumoniae bacteremia were included in the study. Clinical data during the time of admission were collected prospectively and blood samples were drawn for the determination of serum YKL-40 protein concentrations after obtaining informed consent from the patients. Determination of an APACHE score or any other "severity score" was not performed in this study. Blood samples were taken within 24–48 h of a positive blood culture. Mortality was defined as dead at the time of discharge from the hospital. Meningitis was diagnosed by means of the clinical presentation and the presence of > 5 leukocytes/μl in spinal fluid. The presence of an infiltrate on X-ray of the thorax was needed for diagnosing pneumonia. All patients received appropriate antibiotic treatment, either from the time of admission or from when the blood culture became positive.

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Healthy controls

Serum samples from 230 healthy volunteers (125 females, 105 males; median age 51 y; range 20–79 y) were used as controls. The study was approved by the local ethical committees.

Biochemical analysis

Serum concentrations of YKL-40 were determined by ELISA (Quidel, Mountain View, CA) (10). The YKL-40 assay is a sandwich enzyme immunoassay in a microtiter stripwell format. The Fab fragment of a monoclonal anti-YKL-40 antibody conjugated to biotin binds to streptavidin on the strip and captures YKL-40 in a standard, control or sample. A polyclonal anti-YKL-40 antibody conjugated to alkaline phosphatase binds to the captured YKL-40. Bound enzyme activity is detected using p-nitrophenyl phosphate as substrate. The inter- and intra-assay variations were 5.4% and 3.8%, respectively and the detection limit of the assay was 20 µg/l. White blood cell (WBC) count and levels of CRP, hemoglobin and thrombocytes were determined routinely.

Statistical analysis

The statistical analysis was performed using the SPSS software package (SPSS, Chicago, IL). The Mann–Whitney U-test was used for comparison of serum YKL-40 concentrations between survivors and non-survivors. The χ^2 test was used for comparison between different clinical parameters and Spearman's rank test was used for correlation between different parameters. Logistic regression analysis was applied for multivariate analysis. $p < 0.05$ was considered significant.

Table I. *Clinical data of 89 patients with S. pneumoniae bacteremia and their influence on mortality. Values shown indicate numbers of patients, with percentage in parentheses*

Paraneter	Survivors	Non-survivors	*p*
Number of patients	70 (79)	19 (21)	
Sex			
Female	40 (83)	8 (17)	
Male	30 (73)	11 (27)	NS
Age (y)			
18–60 (37)	29 (88)	4 (12)	
61–75 (39)	25 (71)	10 (29)	
>75 (24)	16 (76)	5 (24)	NS
Cerebral symptoms[a]			
No	50 (86)	8 (14)	
Yes	17 (61)	11 (49)	0.002
Mechanical ventilation[b]			
No	57 (83)	12 (17)	
Yes	11 (61)	7 (39)	0.032
Pharmacological treatment of hypotension[a]			
No	59 (69)	13 (31)	
Yes	8 (57)	6 (43)	0.018
Hemodialysis[b]			
No	64 (83)	13 (17)	
Yes	3 (33)	6 (66)	<0.001

[a] Clinical data were missing for 3 patients.
[b] Clinical data were missing for 2 patients.

RESULTS

Eighty-nine patients (48 females, 41 males; mean age 64 y; range 20–98 y) were included, 63% of whom ($n = 55$) had an underlying illness: malignancy ($n = 12$); chronic pulmonary disease ($n = 12$); alcoholism ($n = 12$); uncompensated cardiac disease ($n = 6$); diabetes ($n = 5$); and other chronic diseases ($n = 8$). Pneumonia (81%; $n = 72$) was the most common focus of infection. Thirteen patients (15%) had meningitis and 4 patients (4%) had an unknown focus of infection. The mortality rate of the 89 patients with pneumococcal bacteremia was 21% ($n = 19$), and 2 of the patients who died had meningitis.

The clinical parameters of prognostic importance in terms of death due to the infection were cerebral symptoms (confusion, unconsciousness) at time of admission ($p = 0.002$), the need for hemodialysis treatment ($p < 0.001$), mechanical ventilation ($p = 0.032$) and pharmacological treatment of hypotension ($p = 0.018$) (Table I). Underlying disease and advanced age were not found to be of significant importance in this study in terms of a fatal outcome of invasive pneumococcal infection.

Serum YKL-40

The patients with pneumococcal bacteremia had significantly higher serum concentrations of YKL-40 (median 342 µg/l; range 20–20,400 µg/l) compared to those of healthy age-matched controls (44 µg/l; 20–184 µg/l; $p < 0.001$) and 71% ($n = 63$) of the patients had a serum YKL-40 level above the upper limit of the normal range. The 19 patients who died from their infection had significantly higher serum YKL-40 concentrations (980 µg/l; 88–20,400 µg/l) compared to the survivors (256 µg/l; 20–9,100 µg/l; $p < 0.001$) (Fig. 1). These results were also reflected in the severity of the infection, with significantly higher serum YKL-40 concentrations being observed in the patients who needed hemodialysis, mechanical ventilation and pharmacological treatment of hypotension compared to those in patients who did not need this supportive treatment (Table II). Even though comorbidity was not associated with fatal outcome, higher levels of YKL-40 were found in patients with an underlying disease compared to those in previously healthy patients ($p = 0.046$) (Table II). There was no correlation between serum YKL-40 concentrations and the age of the patients. Values of other laboratory parameters, such

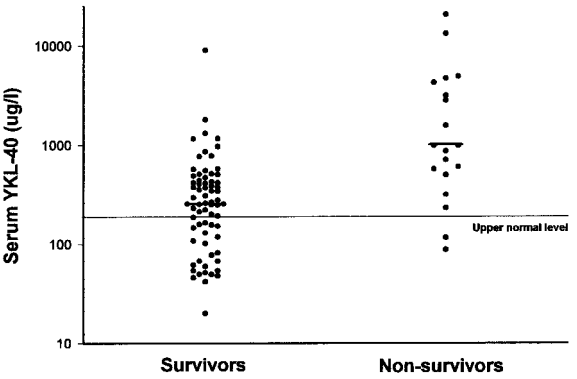

Fig. 1. Median serum concentrations of YKL-40 in 89 patients with pneumococcal bacteremia.

Table II. *Serum YKL-40 concentration in 89 patients with S. pneumoniae bacteremia in relation to clinical parameters*

Parameter[a]	YKL-40 level[b] (μg/l)	*p*
Sex		
Female (48)	265 (48–20,400)	
Male (41)	418 (20–13,150)	NS
Age (y)		
18–60 (33)	258 (46–13,150)	
61–75 (35)	344 (20–20,400)	
> 75 (21)	342 (42–4,260)	NS
Chronic disease[c]		
Yes (55)	388 (20–20,400)	
No (32)	253 (46–4,940)	0.046
Pneumonia[c]		
Yes (72)	343 (20–20,400)	
No (15)	254 (48–9,100)	NS
Meningitis[c]		
Yes (13)	158 (50–4,940)	
No (74)	343 (20–20,400)	NS
Cerebral symptoms[d]		
Yes (28)	459 (46–20,400)	
No (58)	254 (20–9,100)	0.033
Mechanical ventilation[c]		
Yes (18)	675 (54–20,400)	
No (69)	258 (20–4,260)	0.003
Pharmacological treatment of hypotension[d]		
Yes (14)	820 (54–20,400)	
No (72)	256 (20–4,260)	< 0.001
Hemodialysis[d]		
Yes (9)	1,560 (428–20,400)	
No (77)	258 (20–9,100)	< 0.001

[a] Numbers of patients are given in parentheses.
[b] Values shown are medians, with ranges in parentheses.
[c] Clinical data were missing for 2 patients.
[d] Clinical data were missing for 3 patients.

as WBC count and serum CRP level did not differ between survivors and non-survivors. However, there was a significant inverse correlation between YKL-40 concentration and WBC count ($\rho = -0.311$, $p = 0.018$; data not shown).

If a serum concentration of YKL-40 > 552 μg/l (3 times the highest value measured in the healthy controls) was used as a cut-off in a logistic multivariate regression analysis including significant risk factors for death (i.e. serum YKL-40 level, cerebral symptoms, mechanical ventilation, pharmacological treatment of hypotension and hemodialysis), a high serum YKL-40 level was found to be an independent predictor of death in patients with S. pneumoniae bacteremia ($p = 0.002$).

DISCUSSION

In this study we found that serum concentrations of YKL-40 were significantly higher in patients with S. pneumoniae bacteremia compared to those in healthy controls. Some patients had extremely high serum YKL-40 levels (up to 110 × higher than the upper limit of normal in 1 case), and interestingly our study showed that serum YKL-40 levels in patients with S. pneumoniae bacteremia were associated with both severity and fatal outcome of the disease. High levels of YKL-40 in serum predicted a poor prognosis in terms of survival from S. pneumoniae bacteremia. When the clinical characteristics were analyzed, mortality was also significantly associated with cerebral symptoms at admission, need for mechanical ventilation, pharmacological treatment of hypotension and hemodialysis. Correspondingly, serum concentrations of YKL-40 were significantly elevated in these severely ill patients compared to those of the bacteremic patients who did not need intensive supportive treatment. Multivariate regression analysis showed that serum YKL-40 level was an independent prognostic marker of survival in patients with S. pneumoniae bacteremia.

In accordance with our results, serum YKL-40 level has previously been shown to be elevated in patients with S. pneumoniae pneumonia, although no relation with outcome was reported (16). Nordenbaek et al. (16) found a decrease in serum YKL-40 concentration during antibiotic treatment but this was not investigated in the present study. However, previous studies have shown that serum YKL-40 level is related to poor prognosis in both recurrent breast cancer and colon cancer (12, 13). The physiological function of YKL-40 is still unknown, but in vivo studies have recently shown that YKL-40 is a growth factor for connective tissue cells (8). Thus, YKL-40 probably participates in inflammation and remodeling/degradation of the extracellular matrix/damaged tissue.

In the present study, a negative correlation between YKL-40 level and WBC count was found. This inverse relation is somewhat surprising as YKL-40 is secreted from activated neutrophils (5). However, macrophages resident in various tissues also secrete YKL-40 (17–19), and we have recently shown that YKL-40 is produced and detected in the spinal fluid in patients with meningitis (Østergaard et al., unpublished data).

In conclusion we found that patients with S. pneumoniae bacteremia have significantly higher serum levels of YKL-40 than healthy subjects and that high serum YKL-40 concentrations are associated with a poorer outcome. It is presently not known whether YKL-40 has any biological function in infectious diseases and further investigations into the pathophysiological importance of the YKL-40 protein are needed.

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