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ORIGINAL ARTICLE

YKL-40, CCL18 and SP-D predict mortality in patients hospitalized with community-acquired pneumonia

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ABSTRACT

Background and objective: **The aim of this study was to investigate the prognostic value of four biomarkers, YKL-40, chemokine (C-C motif) ligand 18 (CCL18), surfactant protein-D (SP-D) and CA 15-3, in patients admitted with community-acquired pneumonia (CAP). These markers have been studied extensively in chronic pulmonary disease, but in acute pulmonary disease their prognostic value is unknown.**

Methods: **A total of 289 adult patients who were hospitalized with CAP and participated in a randomized controlled trial were enrolled. Biomarker levels were measured on the day of admission. Intensive care unit admission, 30-day, 1-year and long-term mortality (median follow-up of 5.4 years, interquartile range (IQR): 4.7–6.1) were recorded as outcomes.**

Results: **Median YKL-40 and CCL18 levels were significantly higher and levels of SP-D were significantly lower in CAP patients compared to healthy controls. Significantly higher YKL-40, CCL18 and SP-D levels were found in patients classified in pneumonia severity index classes 4–5 and with a CURB-65 score ≥2 compared to patients with less severe pneumonia. Furthermore, these three markers were significant predictors for long-term mortality in multivariate analysis and compared with C-reactive protein and procalcitonin level on admission, area under the curves were higher for 30-day, 1-year and long-term mortality. CA 15-3 levels were less predictive.**

Conclusion: **YKL-40, CCL18 and SP-D levels were higher in patients with more severe pneumonia, possibly reflecting the extent of pulmonary inflammation. Of these, YKL-40 most significantly predicts mortality for CAP.**

Clinical trial registration: NCT00471640 at ClinicalTrials.gov

Key words: biomarkers, chemokine (C-C motif) ligand 18 protein, human, mortality, pneumonia, YKL-40 protein, human.

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SUMMARY AT A GLANCE

The prognostic value of YKL-40, chemokine (C-C motif) ligand 18 (CCL18) and surfactant protein-D (SP-D) has been investigated in chronic pulmonary diseases, but their value is largely unknown in acute illness. This study shows that these markers are increased in patients with severe community-acquired pneumonia. YKL-40 predicts both short-term and long-term mortality.

Abbreviations: A/H1N1, Influenza A/ hemagglutinin 1 neuraminidase 1; AUC, area under the curve; CA 15-3, cancer antigen 15-3; CAP, community-acquired pneumonia; CCL18, chemokine (C-C motif) ligand 18; COPD, chronic obstructive pulmonary disease; CREST, calcinosis, Raynaud phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasia; CRP, C-reactive protein; CURB-65, Confusion, BUN, respiratory rate, blood pressura, age above 65; DL_{CO}, diffusing capacity of the lung for carbon monoxide; DM, diabetes mellitus; HR, hazard ratio; i.v., intravenous; ICU, intensive care unit; IPF, idiopathic pulmonary fibrosis; IQR, interquartile range; MIP-4, macrophage inflammatory protein-4; MW, molecular weight; PSI, pneumonia severity index; ROC, receiver operator curve; RR, blood pressure; SP-D, surfactant protein-D; WBC, white blood cell.

INTRODUCTION

Community-acquired pneumonia (CAP) is, together with influenza, the leading cause of death due to infectious disease worldwide.[1] A high percentage of patients with CAP require hospital admission mainly for i.v. antibiotics or physiological support such as restoration of fluid balance or oxygen supply. To decide which patients have to be treated in-hospital and to assess the severity of CAP, the pneumonia severity index (PSI) and CURB-65 scores may be used.[2,3] Furthermore, several biomarkers have been investigated for their use in risk assessment, such as pro-adrenomedullin, pro-atrial natriuretic peptide and procalcitonin.[4–6] Research on the prognostic value of the more lung-specific inflammatory markers YKL-40, chemokine (C-C motif) ligand 18 (CCL18), surfactant protein-D (SP-D) and CA 15-3,

however, is scarce. These markers reflect lung damage more directly, possibly giving more accurate information on the severity of CAP.

YKL-40 (also termed human cartilage glycoprotein-39) is a matrix protein expressed in specific granules of neutrophils. It is a member of the 18-glycosyl hydrolase family and named YKL-40 because of its MW of 40 kDa and the single letter codes of the three N-terminal amino acids.[7] The specific physiological function of YKL-40 at present is unknown. However, the sites of expression suggest a role in facilitating cell migration through the extracellular matrix and in tissue remodelling at sites of inflammation. In pulmonary diseases such as in COPD and obstructive sleep apnoea syndrome as well as in sepsis, correlations between YKL-40 level and disease severity have been found.[8–11] In idiopathic pulmonary fibrosis (IPF), a fatal diffuse parenchymal lung disease and other interstitial pneumonias, YKL-40 levels are associated with worse prognosis in some but not all studies.[12,13] One previous study investigated the prognostic value of YKL-40 level in patients hospitalized with CAP and found correlations with infection severity.[14] However, there are no studies on the relation between YKL-40 level and mortality in CAP.

CCL18, also known as macrophage inflammatory protein-4 (MIP-4), is a potential regulatory cytokine, produced mainly by dendritic cells, monocytes and alveolar macrophages during pro-inflammatory conditions.[15] CCL18 stimulates collagen production and can also be induced by fibroblasts, which may indicate a role in aberrant tissue repair.[16] CCL18 is involved in attracting naive T-cells, T-regulatory cells, T-helper 2 cells, dendritic cells, basophils and B-cells.[15,17,18] In various chronic pulmonary diseases, including IPF [19] and COPD,[20,21] CCL18 levels have been found to be elevated.

SP-D is a collagenous C-type lectin synthesized by lung epithelial cells. Its function is to decrease surface tension at the air–blood interface but it also plays a role in pulmonary host defence.[22,23] SP-D in CAP has been investigated in three studies, once in critically ill patients suffering from A/H1N1 infection and twice in all-cause pneumonia patients.[24–26] CA 15-3, also known as mucin-1 and Krebs von de Lungen-6, is a glycoprotein also produced by lung epithelial cells. CA 15-3 is elevated in some patients with IPF, among others.[27]

In chronic pulmonary diseases, these biomarkers levels have been extensively investigated, as indicated above. However, in acute pulmonary disease, such as CAP, the prognostic value of these markers is unknown. Because all four markers are produced by lung cells, we hypothesized that elevated levels might be found in more severe CAP due to pulmonary damage. We therefore studied the prognostic value of these biomarkers on short-term and long-term outcomes in a cohort of patients hospitalized with CAP.

METHODS

Patients

Adult patients with CAP who participated in a randomized control trial (NCT00471640) conducted in the St Antonius Hospital in Nieuwegein and the Gelderse Vallei Hospital in Ede (both teaching hospitals in The Netherlands) were enrolled. This trial, performed between November 2007 and September 2010, evaluated the effect of 5 mg dexamethasone i.v. as adjunctive therapy in CAP. Therefore, patients with an indication for corticosteroid treatment, such as patients with a COPD exacerbation but also patients directly admitted to the intensive care unit (ICU), were excluded.[28] Further inclusion and exclusion criteria can be found in Appendix S1 (Supplementary Information). Extensive medical microbiology assessment was performed (more information can be found in Appendix S1, Supplementary Information). Co-morbidities were recorded for each patient and baseline PSI and CURB-65 scores were calculated.[2] ICU admission during hospitalization, in-hospital mortality and 30-day mortality were documented for each patient. Long-term mortality was assessed by contacting the general practitioner of each participant and a double check was performed through the hospital record by business intelligence software on 1 November 2014. When possible, outpatient lung function testing was performed including measurement of diffusing capacity of the lung for carbon monoxide (DL_{CO}). The study was approved by the Medical Ethical Committees of the St Antonius Hospital and the Gelderse Vallei Hospital.

A control group comprised 20 healthy subjects, 11 males and 9 females, with a mean age of 60.9 years (SD = 3.7).

Measurement of biomarkers in blood

Blood was sampled and stored on the day of admission before study medication (dexamethasone or placebo) was given and again 30 days after admission. YKL-40, CCL18, SP-D and CA 15-3 concentrations were determined by two separate duplex bead-based immunoassays (R&D Systems, Minneapolis, MN, USA) in accordance with the manufacturer's instructions. Biomarker concentrations were measured on a Bio-Plex System 100 (Bio-Rad, Hercules, CA, USA). Details of the protocol are given in Appendix S1 (Supplementary Information).

Data analysis

Overall, descriptives were stated as *n* (%), mean (SD) or median (interquartile range (IQR)), as appropriate. The Mann–Whitney U test was used to calculate differences in serum concentration among different categories for, respectively, PSI severity, DL_{CO} and ICU admission. Univariate Cox regression analysis was used with log-normally transformed variables to calculate differences in serum concentration for mortality outcome parameters.

Using receiver operator curve (ROC) analysis, the cut-off value with the highest sensitivity and specificity was calculated for the biomarkers on the day of admission, with 30-day mortality as state variable. Differences in baseline characteristics between high (above cut-off value) and low (below cut-off value) biomarker level on admission were compared using the independent sample T-test, chi-square test, Fisher's exact test or Mann–Whitney U test, where appropriate. For this

analysis, a *P*-value <0.0025 was considered significant, using the Bonferroni correction for multiple testing with 20 variables. Cox regression analysis with log-normally transformed variables was used to analyse 30-day, 1-year and long-term mortality, by implementing PSI score and baseline characteristics with a *P*-value <0.10 which are not used to calculate PSI score, in the model. All variables were entered in the multivariate model at once. Two sensitivity analyses were performed: (i) excluding patients with COPD, because biomarkers are known to correlate with COPD severity and (ii) using CURB-65 score instead of PSI score. Area under the curves (AUCs) were calculated for the biomarkers C-reactive protein (CRP) and procalcitonin level on admission.

Data were analysed with SPSS statistical software for Windows, version 22.0 (IBM Corp., Armonk, N.Y, USA). Figures were drawn with Graph Path Software (La Jolla, California, USA). For all analyses, except for the baseline comparisons, a *P*-value <0.05 was considered statistically significant.

RESULTS

Patients

In 289 of 304 (95.1%) patients, samples were available for measurement of biomarkers on admission and these patients could be included in our analysis. In 158 of 289 (54.7%) patients, DL_{CO} on day 30 was available. Sixteen patients (5.5%) were admitted to the ICU after admission. For long-term mortality, median follow-up was 5.4 years (IQR: 4.7–6.1). Eighteen patients (6.2%) died within 30 days, 50 (17.3%) within 1 year and 95 (32.9%) within the long-term follow-up period. Additional baseline characteristics are presented in Table 1. An overview of all causative pathogens is given in Table S1 (Supplementary Information).

Median levels of the biomarkers in healthy controls were 23 ng/mL (IQR: 13–37) for YKL-40 and 38 ng/mL (IQR: 28–51) for CCL18. This was significantly lower compared to levels in CAP patients: YKL-40 189 ng/mL (IQR: 67–456, $P < 0.001$) and CCL18 77 ng/mL (IQR: 43–123, $P < 0.001$). For SP-D, median levels in healthy controls were significantly higher compared to levels in CAP patients: 9.8 ng/mL (IQR: 5.9–14.3) versus 6.3 ng/mL (IQR: 3.0–11.0), $P = 0.03$. CA 15-3 levels did not significantly differ between CAP patients and healthy controls: 23.8 U/mL (IQR: 14.0–37.5) versus 19.5 U/mL (IQR: 11.8–25.7), $P = 0.08$.

Prognostic value of YKL-40

Higher YKL-40 levels were found in patients classified in PSI classes 4–5 and CURB-65 score ≥2 and also in patients who were admitted to the ICU. We found a significant correlation between YKL-40 and PSI score (Pearson's r = 0.41, $P < 0.001$). Patients with DL_{CO}% predicted value <70% on day 30 had significantly higher YKL-40 levels. Furthermore, in univariate analysis, 30-day, 1-year and long-term survivors had lower YKL-40 levels compared to patients who died (Fig. 1A).

The optimal cut-off value of YKL-40 to predict 30-day mortality was calculated to be 280 ng/mL (sensitivity: 0.89, specificity: 0.66). Table 1 shows baseline characteristics of patients with high YKL-40 versus low YKL-40 level on admission. Patients with high YKL-40 levels were older and had more often chronic renal failure, lower systemic blood pressure, higher PSI and CURB-65 scores, higher CRP, higher white blood cell count and higher procalcitonin levels on admission. Figure 2A shows univariate long-term survival curve for high and low YKL-40 level on admission.

Hazard ratios (HRs) of univariate regression analysis for 30-day, 1-year and long-term mortality are given in Table S2 (Supplementary Information). In multivariate analysis, YKL-40 level was a significant predictor for 30-day mortality and both YKL-40 level and PSI classes 4–5 were significant predictors for 1-year and long-term mortality (Table 2).

Prognostic value of CCL18

Patients in PSI classes 4–5 and CURB-65 score ≥2 had significantly higher CCL18 levels. We found a significant correlation between CCL18 and PSI score (Pearson's r = 0.30, $P < 0.001$). 30-Day, 1-year and long-term mortality were significantly associated with higher CCL18 level on admission. Figure 1B shows CCL18 levels for all outcome measures.

For 30-day mortality, a cut-off value of 90 ng/mL was calculated (sensitivity: 0.78, specificity: 0.62). Table 1 shows baseline characteristics of patients with low and high CCL18 levels on admission. Patients with high CCL18 levels on admission were older, were more often nursing home residents and had higher PSI and CURB-65 scores and procalcitonin levels on admission. Figure 2B shows univariate long-term survival curve for patients with high and low CCL18 levels on admission.

HRs of univariate analyses are shown in Table S2 (Supplementary Information). In multivariate analysis, CCL18 level, PSI classes 4–5 and smoking all remained significantly associated with long-term mortality (Table 2).

Prognostic value of SP-D

Higher SP-D levels were found in patients classified in PSI classes 4–5 and CURB-65 score ≥2. We found a significant correlation between SP-D and PSI score (Pearson's r = 0.17, $P = 0.004$). Figure 1C shows SP-D levels for all outcome measures.

The optimal cut-off value to predict 30-day mortality was calculated to be 10 ng/mL (sensitivity: 0.67, specificity: 0.77). Patients with high SP-D levels had lower body temperature on admission. In univariate and multivariate analyses, SP-D and PSI classes 4–5 were significantly associated with 30-day, 1-year and long-term mortality (see Table S2, Supplementary Information, for univariate results and Table 2 for the multivariate results).

Prognostic value of CA 15-3

CA 15-3 levels proved to be of less predictive value compared to the other three biomarkers. Data analysis is given in Tables S2, S3 and Appendix S2 (Supplementary Information).

Table 1 Baseline characteristics and outcomes of the 289 patients hospitalized with community-acquired pneumonia

	All patients (*n* = 289)	YKL-40 < 280 (*n* = 181)	YKL-40 > 280 (*n* = 108)	CCL18 < 90 (*n* = 169)	CCL18 > 90 (*n* = 120)
Male sex (%)	161 (55.7)	105 (58.0)	56 (51.9)	91 (53.8)	70 (58.3)
Age (year) (SD)	64.0 (18.4)	59.5 (18.7)	71.5 (15.4)**	60.3 (18.0)	69.3 (17.8)**
Caucasian (%)	284 (98.3)	178 (98.3)	106 (98.1)	165 (97.6)	120 (99.2)
Nursing home (%)	15 (5.2)	6 (3.3)	9 (8.4)*	3 (1.8)	12 (10.1)**
Smoking (%)[†]	77 (26.6)	54 (32.1)	23 (23.0)	53 (34.2)	24 (21.2)*
Co-morbidities (%)					
Chronic renal failure	27 (9.3)	6 (3.3)	21 (19.4)**	11 (6.5)	16 (13.3)*
Diabetes mellitus	41 (14.2)	18 (9.9)	23 (21.3)*	22 (13.0)	19 (15.8)
Liver disease	2 (0.7)	0	2 (1.9)	0	2 (1.7)
Neoplastic disease	19 (6.6)	7 (3.9)	12 (11.1)*	8 (4.7)	11 (9.2)
Chronic heart failure	47 (16.3)	26 (14.4)	21 (19.4)	22 (13.0)	25 (20.8)*
COPD	32 (11.1)	17 (9.4)	15 (13.9)	19 (11.2)	13 (10.8)
PSI class (%)					
Classes 1–3	151 (52.2)	122 (67.4)	29 (26.9)**	103 (60.9)	48 (40.0)**
Classes 4–5	138 (47.8)	59 (32.6)	79 (73.1)**	66 (39.1)	72 (60.0)**
CURB-65 (%)[‡]					
<2	128 (44.0)	102 (60.0)	26 (25.7)**	94 (58.4)	34 (30.9)**
≥2	144 (49.5)	68 (40.0)	75 (74.3)**	67 (41.6)	76 (69.1)**
Blood pressure					
Systolic (SD) (mm Hg)	132 (22)	135 (21)	126 (23)**	133 (21)	130 (23)
Diastolic (SD) (mm Hg)	74 (12)	77 (12)	69 (12)**	76 (11)	72 (13)*
Temperature (SD) (°C)	38.2 (1.1)	38.3 (1.1)	38.1 (1.0)	38.3 (1.1)	38.0 (1.0)*
Laboratory parameters					
CRP (IQR) (mg/L)	214 (96–329)	158 (74–289)	270 (131–386)**	209 (88–324)	229 (111–341)
WBC count (IQR) ($\times 10^9$/L)	10.6 (8.0–13.7)	9.9 (7.6–12.8)	12.0 (8.7–14.5)**	10.1 (7.5–13.4)	11.4 (8.8–14.9)*
Procalcitonin[§]	0.51 (0.15–3.05)	0.27 (0.10–1.22)	2.35 (0.43–8.70)**	0.36 (0.11–1.97)	1.28 (0.21–5.51)**
Days ill before admission (IQR)	4.5 (3–7)	5 (3–7)	4 (2–6)	4 (3–6.5)	5 (3–7)
Pretreated with antibiotics at home	80 (27.7)	61 (33.7)	19 (17.6)*	46 (27.2)	34 (28.3)
Dexamethasone (%)[¶]	144 (49.8)	90 (49.7)	54 (50.0)	88 (52.1)	56 (46.7)

Data are presented as *n* (%), mean (SD) or median (IQR).

*Indicates a *P*-value < 0.10 and therefore will be included in Cox regression analysis; **significant result after Bonferroni correction for multiple testing, a *P*-value < 0.0025.

[†]21 missings.

[‡]19 missings.

[§]16 missings.

[¶]Dexamethasone was given to 50% of the patients in the original randomized, placebo-controlled clinical trial.

CCL18; chemokine (C-C motif) ligand 18; CRP, C-reactive protein; CURB-65, Confusion, BUN, respiratory rate, blood pressura, age above 65; IQR, interquartile range; PSI, pneumonia severity index; SD, standard deviation; WBC, white blood cell.

Sensitivity analysis

HRs of YKL-40 and SP-D remained significant excluding patients with COPD from multivariate analysis. For CCL18, HR of long-term mortality remained increased but was not significant anymore. All HRs are shown in Tables S4–S6 (Supplementary Information).

When multivariate analysis was performed using CURB-65 score ≥2 rather than PSI classes 4–5, similar HRs for the biomarkers were found (see Table S7, Supplementary Information).

Prognostic value of YKL-40 and CCL18 levels combined

Combining YKL-40 and CCL18 gave the highest predictive value. Sixty-six (22.8%) patients had both high YKL-40 and high CCL18 levels and 127 patients (43.9%) had both low YKL-40 and low CCL18 levels. Long-term survival curve in Figure 2C shows a higher mortality in patients with both biomarkers increased compared to one or none of the markers increased.

Comparison of AUCs with other biomarkers

The AUCs of YKL-40, CCL18 and SP-D were higher compared to AUCs of CRP and procalcitonin for 30-day, 1-year and long-term mortality. Figure 3 shows all AUCs for 30-day mortality.

Markers 30 days after admission

In 209 of 291 (71.8%) patients, biomarkers could be determined in serum samples obtained at least 30 days after admission. High levels of YKL-40 and CCL18 were significantly associated with both 1-year or long-term

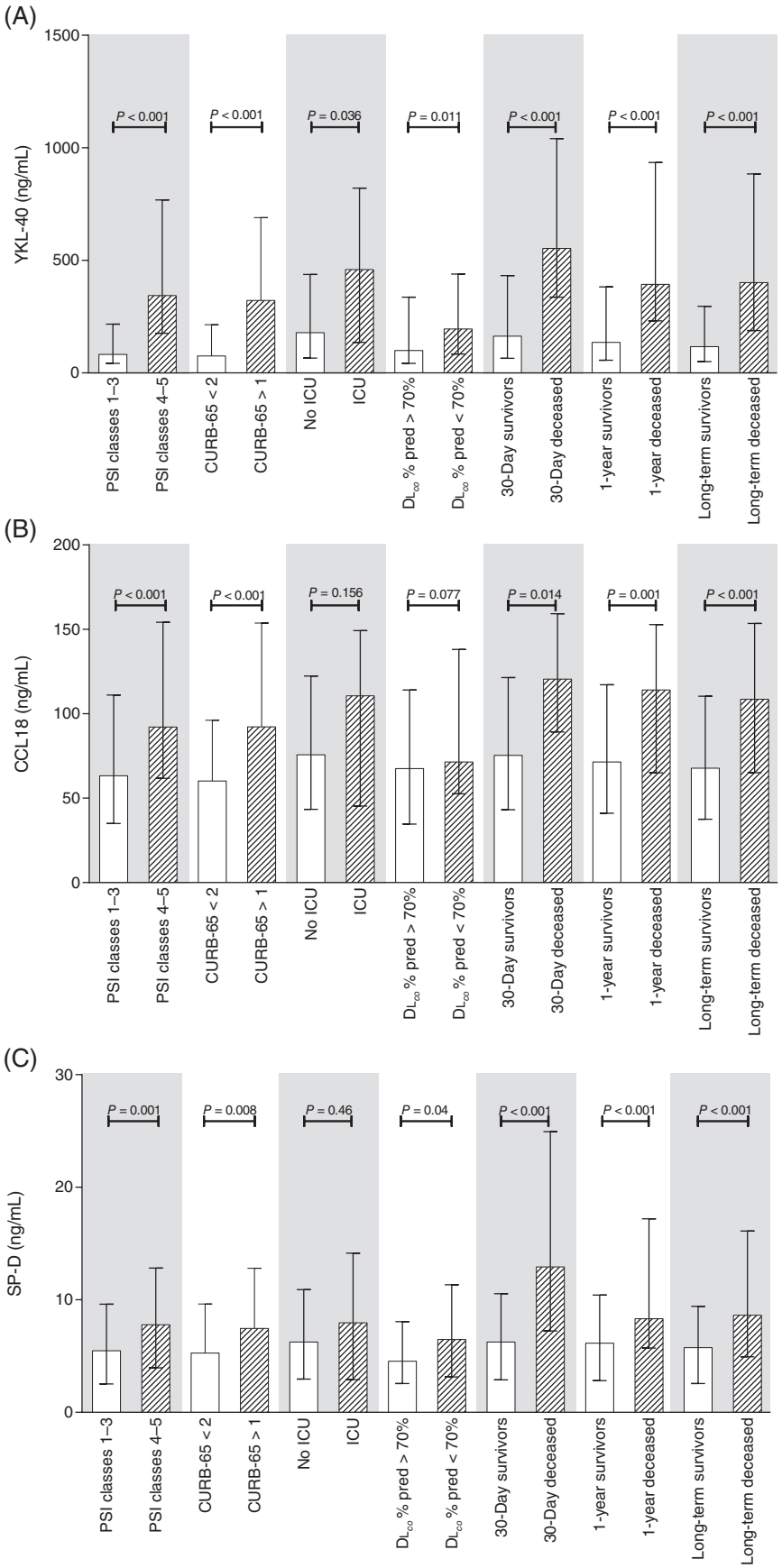

Figure 1 (A) YKL-40 level, (B) chemokine (C-C motif) ligand 18 (CCL18) and (C) surfactant protein-D (SP-D) level on admission stratified according to respectively pneumonia severity index (PSI) score, CURB-65 score, intensive care unit (ICU) admission, percentage predicted diffusing capacity of the lung for carbon monoxide (DL_{CO}% predicted) below 70%, and 30-day, 1-year and long-term mortality.

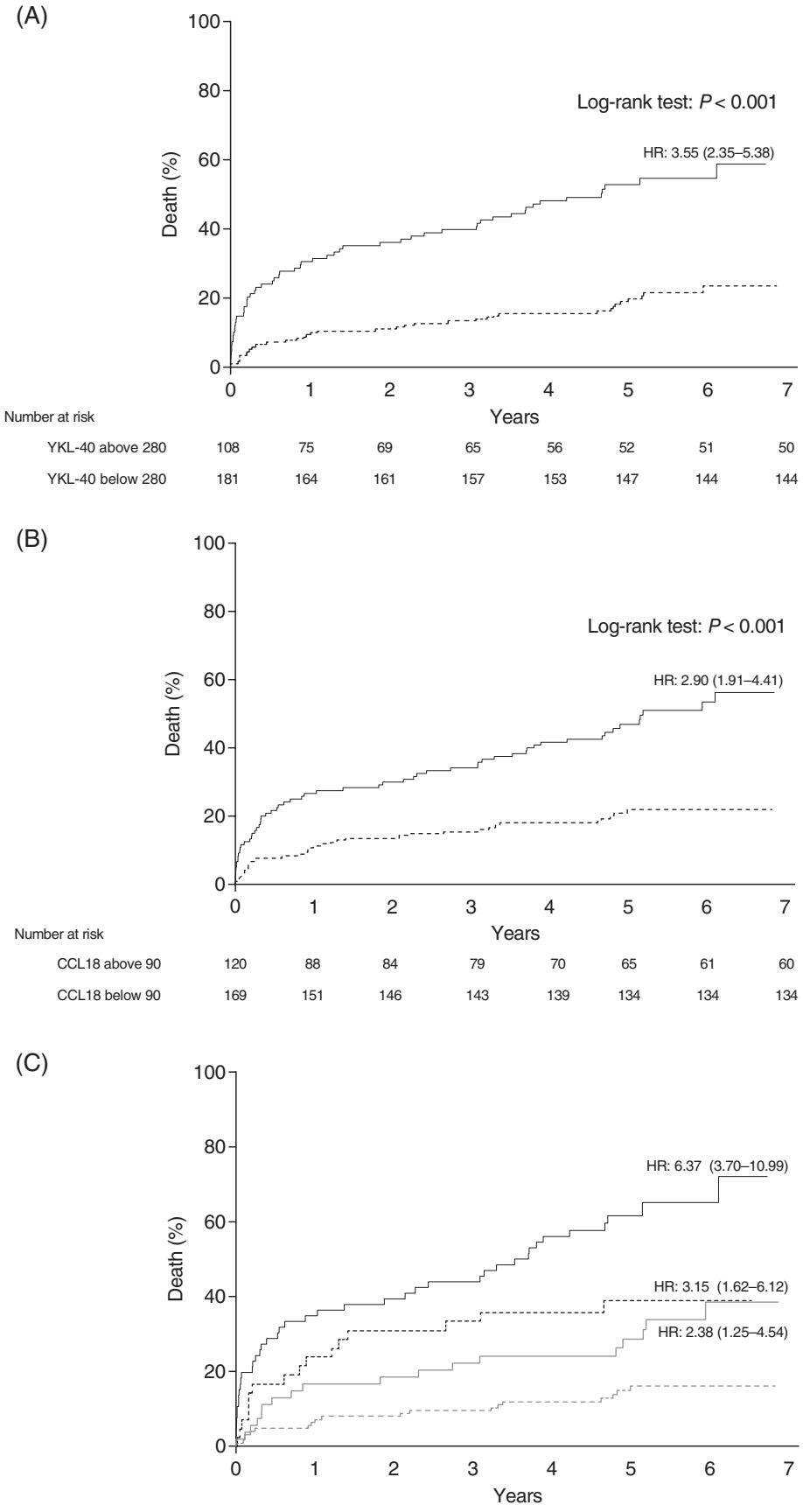

Figure 2 Survival curves for (A) high and low YKL-40 level on admission (——, YKL-40 above 280; - - - -, YKL-40 below 280), (B) high and low chemokine (C-C motif) ligand 18 (CCL18) level on admission (——, CCL18 above 90; - - - -, CCL18 below 90) and (C) YKL-40 and CCL18 levels combined (——, both high; - - - -, YKL-40 above 280; ——, CCL18 above 90; - - - -, both low). Hazard ratios (HRs) are given with 95% confidence intervals, calculated with univariate Cox regression analysis. *P*-value of Log-rank test for difference between both high and both low markers on admission was <0.001.

Table 2 Prognostic effect of YKL-40, CCL18 and SP-D level on admission for short-term and long-term mortality in multivariate Cox regression analysis

	30-Day mortality		1-Year mortality		Long-term mortality	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
YKL-40	2.15 (1.26–3.68)	0.005	1.66 (1.21–2.28)	0.002	1.60 (1.28–2.01)	<0.001
PSI classes 4–5	3.75 (0.79–17.74)	0.10	4.95 (1.99–12.32)	0.001	3.99 (2.23–7.14)	<0.001
DM	0.41 (0.09–1.83)	0.24	1.10 (0.53–2.31)	0.80	1.25 (0.75–2.08)	0.40
RR diastolic	1.00 (0.96–1.04)	0.92	1.01 (0.98–1.03)	0.49	1.00 (0.98–1.01)	0.68
CRP	0.79 (0.50–1.27)	0.33	0.74 (0.57–0.96)	0.02	0.77 (0.64–0.93)	0.01
WBC count	0.68 (0.26–1.77)	0.43	0.53 (0.27–0.98)	0.04	0.84 (0.52–1.37)	0.48
Procalcitonin	0.95 (0.73–1.24)	0.70	0.95 (0.81–1.11)	0.50	0.90 (0.80–1.00)	0.06
Antibiotic use	0.43 (0.10–1.90)	0.26	0.81 (0.38–1.73)	0.59	1.14 (0.71–1.85)	0.59
CCL18	1.43 (0.71–2.91)	0.32	1.38 (0.90–2.12)	0.14	1.40 (1.04–1.88)	0.03
PSI classes 4–5	6.25 (1.38–28.30)	0.02	6.86 (2.83–16.68)	<0.001	5.32 (3.06–9.26)	<0.001
Smoking	0.46 (0.10–2.05)	0.31	0.52 (0.22–1.26)	0.15	0.43 (0.23–0.81)	0.008
RR diastolic	1.00 (0.97–1.04)	0.94	1.01 (0.99–1.04)	0.33	1.00 (0.98–1.01)	0.71
WBC count	0.85 (0.31–2.31)	0.74	0.69 (0.37–1.28)	0.24	1.17 (0.73–1.89)	0.52
Procalcitonin	1.12 (0.87–1.44)	0.39	1.00 (0.86–1.17)	0.97	0.94 (0.84–1.06)	0.31
SP-D	2.21 (1.36–3.59)	0.001	1.57 (1.12–2.19)	0.01	1.58 (1.23–2.03)	<0.001
PSI classes 4–5	5.30 (1.16–24.28)	0.03	5.69 (2.51–12.90)	<0.001	5.22 (3.08–8.84)	<0.001
Smoking	0.63 (0.13–2.97)	0.56	0.56 (0.23–1.37)	0.20	0.45 (0.24–0.84)	0.01
WBC count	1.07 (0.65–1.78)	0.78	0.86 (0.65–1.12)	0.25	0.96 (0.79–1.16)	0.65
Antibiotics at home	0.34 (0.08–1.52)	0.16	0.61 (0.29–1.28)	0.19	0.87 (0.53–1.41)	0.56

For long-term mortality, median follow-up was 5.4 years (IQR 4.7–6.1).

CCL18, chemokine (C-C motif) ligand 18; CI, confidence interval; CRP, C-reactive protein; DM, diabetes mellitus; HR, hazard ratio; IQR, interquartile range; PSI, pneumonia severity index; RR, blood pressure; SP-D, surfactant protein-D; WBC, white blood cell.

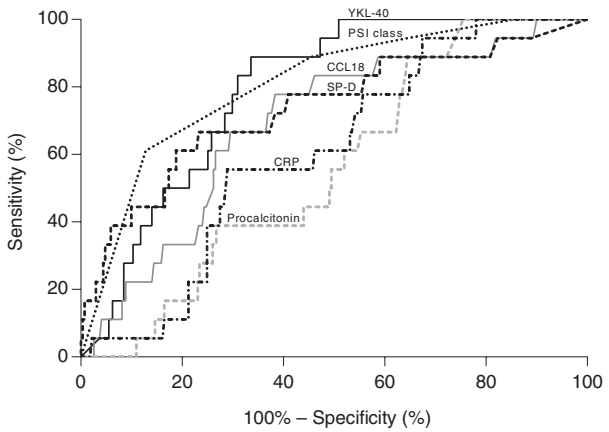

Figure 3 Area under the curves (AUCs) for 30-day mortality of YKL-40, chemokine (C-C motif) ligand 18 (CCL18), surfactant protein-D (SP-D), pneumonia severity index (PSI) classes, C-reactive protein (CRP) and procalcitonin level on admission. AUC of the five PSI classes was 0.81 (95% confidence interval (CI): 0.71–0.91), of YKL-40 0.79 (95% CI: 0.72–0.86), of CCL18 0.69 (95% CI: 0.57–0.80), of SP-D 0.74 (95% CI: 0.60–0.87), of (low) CRP 0.61 (95% CI: 0.50–0.71) and of procalcitonin 0.59 (95% CI: 0.48–0.71).

mortality, whereas SP-D and CA 15-3 were not (see Table S8, Supplementary Information).

DISCUSSION

In this study, YKL-40, CCL18 and SP-D were significant predictors for both short-term outcomes and long-term mortality in patients hospitalized with CAP. Until now, prognostic value of mainly acute phase markers has been investigated. To the best of our knowledge, this is the first study that investigated the prognostic value of these biomarkers on short-term and long-term mortality in CAP.

YKL-40 is secreted mainly by neutrophils, CCL18 by dendritic cells, monocytes and macrophages and SP-D by lung epithelial cells.[7,15,29] This study shows higher levels of these markers in more severe pneumonia, possibly reflecting the degree of pulmonary inflammation. This could explain the prognostic value on short-term outcomes of these markers. Yet, long-term mortality is also predicted by these markers on admission. It is known that long-term mortality of patients who experienced a CAP episode is increased compared to patients who were never hospitalized with CAP.[30] The hypothesis that YKL-40 and CCL18 are biomarkers for mortality is supported by our finding that both markers, when measured 30 days after admission, were still associated with higher 1-year and long-term mortality. Another study investigated the prognostic value of YKL-40 levels in CAP. In agreement with our study, this study found significant correlations between YKL-40 levels on admission and pneumonia severity.[14]

Out of all 289 patients, one patient suffered from IPF and one patient had a history of CREST syndrome; both diseases are known to be associated in some studies with elevated YKL-40 and CCL18 levels and a reduced life expectancy in the case of IPF.[13] As far as we know, none of the other patients developed IPF during the follow-up period after the CAP episode.

Multivariate analysis showed a protective effect of smoking on long-term mortality. This remarkable finding is most likely caused by the exclusion of patients in need for corticosteroid therapy from the randomized controlled trial, such as patients with a COPD exacerbation. As a consequence, smokers with COPD were excluded from inclusion, while smokers without COPD (often younger patients or smokers with less pack-years) were included. This potential explanation is supported by the younger age of smokers compared to non-smokers (mean: 55.7, SD: 15.8 vs mean: 68.2, SD: 18.0).

YKL-40 level was higher in patients with a DL_{CO} <70% of expected 30 days after admission. Unfortunately, DL_{CO} was only available in 55% of patients. It would be interesting to investigate if DL_{CO} will remain lower in these patients, for example 1 year after the CAP episode.

This study has several strengths. Foremost, this is one of the first studies that investigated the prognostic value of four biomarkers in CAP. Furthermore, our study cohort is a large, well-defined CAP cohort, which enabled multivariate analyses. Lastly, follow-up was on average more than 4 years.

A limitation of this study is its retrospective character, although samples were collected before study medication was given. Furthermore, since cause of death was not reported we could not explore possible relationships between cause of death and specific biomarkers. Lastly, patients who were directly admitted to the ICU were excluded.

In conclusion, YKL-40, CCL18 and SP-D levels were higher in patients with more severe pneumonia, possibly reflecting the extent of pulmonary inflammation. Especially, YKL-40 predicts short-term and long-term mortality for CAP and therefore might be used for risk stratification in the future. The correlation between the increased levels of inflammatory biomarkers and outcomes in patients with CAP underscores the potential importance of inflammation control. These results need to be confirmed in an independent validation cohort, before they can be generalized.

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Supplementary Information

Additional supplementary information can be accessed via the *html* version of this article at the publisher's website.

Appendix S1 Materials and methods.

Appendix S2 Results.

Table S1 Main causing pathogen in 289 patients hospitalized with community-acquired pneumonia.

Table S2 Univariate analysis on the prognostic effect of the four biomarkers on admission for short-term and long-mortality.

Table S3 Multivariable analysis of the prognostic effect of CA 15-3 level on admission for short-term and long-term mortality.

Table S4 Sensitivity analysis on the prognostic effect of YKL-40 level on admission for mortality, excluding patients with COPD from multivariate Cox regression analysis.

Table S5 Sensitivity analysis on the prognostic effect of CCL18 level on admission for mortality, excluding patients with COPD from multivariate Cox regression analysis.

Table S6 Sensitivity analysis on the prognostic effect of surfactant protein-D (SP-D) level on admission for mortality, excluding patients with COPD from multivariate Cox regression analysis.

Table S7 Sensitivity analysis on the prognostic effect of the four biomarkers on admission for mortality, using CURB-65 score in multivariate Cox regression analysis.

Table S8 Univariate Cox regression analysis for 1-year and long-term mortality for biomarker levels measured during the outpatient visit 30 days after admission.