# High Serum Concentration of YKL-40 Is Associated with Short Survival in Patients with Acute Myeloid Leukemia

Olav J. Bergmann,[1,3,5] Julia S. Johansen,[2] Tobias W. Klausen,[1] Anne K. Mylin,[1] Jørgen S. Kristensen,[5] Eigil Kjeldsen,[4] and Hans E. Johnsen[1,6]

**Abstract**

**Purpose:** YKL-40 is secreted by cancer cells, macrophages, and neutrophils. It may be a growth or differentiation factor, play a role in angiogenesis, or protect against apoptosis. High serum YKL-40 is associated with poor prognosis in solid carcinomas. The aim was to examine serum YKL-40 in patients with acute myeloid leukemia (AML).

**Experimental Design:** YKL-40 was measured by ELISA in serum from 77 patients recently diagnosed with AML before and during the first month of chemotherapy.

**Results:** Forty (52%) of the AML patients had elevated serum YKL-40 (compared with age-matched healthy subjects) and their survival was shorter than in patients with normal serum YKL-40 (median, 128 days; interquartile range, 18-629 days versus 386 days; interquartile range, 180-901; $P = 0.018$ Mann-Whitney test). Univariate analysis of serum YKL-40 (logarithmically transformed and treated as a continuous covariate) showed significant association with survival within the first month after start of chemotherapy [hazard ratio (HR), 1.7; 95% confidence interval (CI), 1.2-2.4; $P = 0.002$], first 12 months (HR, 1.6; 95% CI, 1.2-2.0; $P = 0.0002$), and overall survival (HR, 1.3; 95% CI, 1.1-1.6; $P = 0.003$). Multivariate Cox analysis showed that serum YKL-40 was an independent prognostic variable for survival (first month: HR, 1.7; $P = 0.011$; 12 months: HR, 1.6; $P = 0.0002$; overall survival: HR, 1.4; $P = 0.002$). High serum YKL-40 at start of chemotherapy was a risk factor for pneumonia within the first month, and serum YKL-40 increased ($P = 0.002$) at time of pneumonia and was unchanged in patients without infections.

**Conclusions:** Serum YKL-40 is a prognostic biomarker of survival in AML patients. Its role in AML and infections needs to be determined.

Acute myeloid leukemia (AML) is a malignant disorder of the blood characterized by impaired differentiation of hematopoietic precursor cells, resulting in abnormal accumulation of immature precursors and suppression of growth and maturation of cells involved in normal hematopoiesis (1). It is a clonal disease with a highly heterogeneous biology (2, 3). A number of genes encoding the transcription factors are involved in the recurring chromosomal translocations seen in AML and its variants may arise because the translocations result in significant alterations in regulatory processes controlling the growth and differentiation programs of the malignant cell (1–3). The prognosis for AML patients is very variable, ranging from survival of a few days to cure. Clinical outcome can be partly predicted by age, cytogenetic findings, and serum lactate dehydrogenase at the time of diagnosis (1, 4, 5). However, the prognosis of an individual AML patient cannot yet be estimated accurately. It is therefore important to identify new biomarkers in AML patients for the prediction of prognosis and treatment response, detection of relapse, and monitoring for minimal residual disease of AML.

YKL-40,[7] a phylogenetically highly conserved heparin- and chitin-binding lectin without chitinase activity, is a member of the "mammalian chitinase-like proteins" (6–9). The gene for human YKL-40 (10, 11) is localized on chromosome 1 and the crystal structure of human YKL-40 has been described (12, 13). The site and mode of binding of YKL-40 to cell surface receptors is unknown. Microarray gene analyses have identified the human YKL-40 gene to be one of the most overexpressed genes in glioblastoma multiforme (14, 15), papillary thyroid carcinoma (16), and extracellular myxoid chondrosarcoma (17). YKL-40 is secreted *in vitro* by cancer cell lines (18–20). Treatment with phorbol 12-myristate 13-acetate of human tumor cell lines that originate from immature cells of the monocytic differentiation lineage corresponding to monoblasts (U937, THP-1) and

[7] YKL-40 is also named human cartilage glycoprotein-39 (HC gp-39), 38-kDa heparin binding glycoprotein (Gp38k), Chitinase-3-like-1 protein (CHI3L1), breast-regressing protein 39 kDa (brp-39), and Chondrex.

myeloblasts (HL-60) induce differentiation of monocytes into an adherent macrophage-like cell type and an increase in YKL-40 expression (10, 21, 22). In normal bone marrow, the myelocyte-metamyelocyte express YKL-40 protein and it is stored in the specific granules of neutrophil granulocytes and released from fully activated cells (23). YKL-40 is also expressed by macrophages *in vitro* during the late stage of differentiation (9–11), *in vivo* by macrophages in tissues with inflammation (24), and by peritumoral macrophages (25).

YKL-40 is a growth factor for fibroblasts and chondrocytes, and acts synergistically with insulin-like growth factor-I (26, 27). YKL-40 initiates mitogen-activated protein kinase and phosphoinositide-3-kinase signaling cascades in fibroblasts leading to the phosphorylation of both the extracellular signal-regulated kinase-1/2 mitogen-activated protein kinase and protein kinase B (AKT)–mediated signaling cascades (26, 27), which are associated with the control of mitogenesis. The phosphoinositide-3-kinase pathway, and in particular, the phosphorylation of AKT, is strongly associated with cell survival. Up-regulated YKL-40 expression is found in a human glioblastoma cell line by genotoxic and microenvironmental stress (e.g., hypoxia, ionizing radiation; ref. 20), and human astrocytes transfected with YKL-40 had increased resistance to radiation and increased invasion capacity *in vitro* (15). This suggests that YKL-40 plays a role in the malignant phenotype as a cellular survival factor. Furthermore, YKL-40 modulates vascular endothelial cell morphology by promoting the formation of branching tubules, indicating that YKL-40 has a role in angiogenesis by stimulating the migration and reorganization of vascular endothelial cells (28). YKL-40 also acts as a chemoattractant for endothelial cells, stimulates their migration and promotes the migration and adhesion of vascular smooth muscle cells (28, 29).

Several studies of patients with solid tumors have shown that serum YKL-40 is elevated in some patients with primary or metastatic carcinoma of the breast (30, 31), colorectal (32), ovary (33–35), lung (36), prostate (37), kidney and glioblastoma (14). Interestingly, the studies showed that high serum YKL-40 was related to short recurrence-free interval and short overall survival, and that high serum YKL-40 was an independent prognostic variable of poor prognosis (30–37). A high serum YKL-40 in patients with first recurrence of breast cancer predicted less responsiveness to anthracycline therapy (30). In patients radically operated for colorectal cancer a high serum YKL-40 postoperatively increased the risk of recurrence or death within the following 6 months by 6.9- and 8.5-fold (38). Immunohistochemical analysis of biopsies of glioblastoma has shown that YKL-40 is a diagnostic marker for histologic subtypes (39).

This is the first study of serum YKL-40 in patients with hematologic malignancies. We report data on serum YKL-40 in patients with AML with regard to the prognostic effect on survival and as treatment monitoring during the first month of chemotherapy.

## Patients and Methods

*Study design.* From March 1990 to May 1993, 77 Danish Caucasoid patients (>18 years of age) recently diagnosed with AML and admitted to the Department of Hematology, Aarhus University Hospital (Aarhus, Denmark), were included in a prospective and consecutive study. The patients were given follow-ups, with blood samples taken weekly during the first 28 days after the beginning of chemotherapy, day 1 being the day when treatment was initiated. The remission induction chemotherapy consisted of either a 7-day course of cytarabine (100 mg/m$^2$ daily i.v.) plus a 3-day course of aclarubicin (75 mg/m$^2$ daily i.v., $n$ = 57), or a 5-day course of cytarabine (100 mg/m$^2$ daily i.v.) plus a 2-day course of aclarubicin (75 mg/m$^2$ daily i.v., $n$ = 19). One patient was treated with a 7-day course of cytarabine (100 mg/m$^2$ daily i.v.) plus a 3-day course of mitoxantrone (12 mg/m$^2$ daily i.v.). The patients were classified according to the French-American-British (FAB) classification (40): 1 patient was classified as $M_0$, 22 as $M_1$, 22 as $M_2$, 0 as $M_3$, 18 as $M_4$, 8 as $M_5$, 4 as $M_6$, 1 as $M_7$, and 1 unknown. Complete remission after 4 weeks was defined by a bone marrow with normal hematopoiesis of all cell lines, <5% blast cells, and peripheral blood with at least $1.5 \times 10^9$/L neutrophils and $100 \times 10^9$/L platelets. Therapeutic failures were classified as persistent leukemia or early death (i.e., within the first month). Survival was defined as the interval from the day of starting chemotherapy and the time of death. Results from the study group have been previously published (41, 42). The study protocol was approved by the local ethics committee, and informed consent was obtained from each subject.

All patients were examined by the same investigator (O.J. Bergmann). Septicemia and pneumonia were chosen as indicators of severe infection. Septicemia was defined as the presence of at least one positive blood culture, except in the case of *Staphylococcus aureus*, coagulase-negative staphylococci, *Corynebacterium* spp., and *Bacillus* spp., which were only considered indicative of septicemia when cultured from at least two separate specimens of blood. The diagnosis of pneumonia included the presence of an infiltrate on chest X-ray. Oral temperature (Craftemp, Astra Tech, Mölndal, Sweden) was measured twice daily by nurses. Fever was defined as oral temperature >36.5°C in the morning or >37.0°C in the evening. Empirical broad-spectrum antibacterial treatment was initiated when the oral temperature was ≥38.0°C for >2 hours, and always when there were clinical signs of infection. Forty patients received acyclovir prophylaxis (41, 42). Antibacterial or antifungal prophylaxis, HEPA-filtered air, or cytokines were not used. Erythrocyte sedimentation rate (ESR), hematologic variables, serum creatinine, liver enzymes, serum lactate dehydrogenase, and serum albumin were monitored on day 1 and thereafter twice weekly.

*Healthy controls.* The reference range of serum YKL-40 was determined in 245 healthy subjects (134 females and 111 males; median age, 49; range, 18-79 years). These subjects were all healthy, were not on medication, and had no signs or clinical symptoms of cancer, joint, liver, metabolic or endocrine diseases. The study was approved by the local ethics committee and written informed consent was obtained from each subject.

*Cytogenetics.* Cytogenetic analyses were available from 65 (84%) of the patients and done according to standard protocols. Cytogenetic data were classified according to the International System for Human Cytogenetic Nomenclature. Patients were classified into three subgroups based on cytogenetics (43): the group associated with a favorable prognosis ($n$ = 3) included AML patients with t(8;21), t(15;17), or inv(16); the adverse prognosis group ($n$ = 11) contained AML patients with aberrations of chromosomes 5 or 7, deletion of 5q or with a complex aberrant karyotype; the intermediate prognosis group ($n$ = 51) included AML patients with other karyotype aberrations as well as a normal karyotype ($n$ = 39).

*Serum YKL-40 analysis.* Serum samples were collected on day 1, i.e., immediately before the start of treatment, and thereafter on days 8, 15, 22, and 29. Samples were stored at −80°C until analysis for YKL-40. Samples from each patient were analyzed in the same assay without knowledge of the clinical, biochemical, or survival data. Serum concentrations of YKL-40 were determined by a commercial two-site, sandwich-type ELISA (Quidel, San Diego, CA; ref. 44) using streptavidin-coated microplate wells, a biotinylated-Fab monoclonal capture antibody, and an alkaline phosphatase–labeled polyclonal detection antibody. The sensitivity of the ELISA was 10 μg/L. The intra- and interassay coefficient of variation were <3.6% and <7.1%, respectively.

The long time coefficient of variation in serum YKL-40 was 5% in 30 healthy women (ages 24-62 years) who had serum samples collected five times with 7-day intervals and subsequently again after 3 years.[8]

*Statistics.* Statistical analysis were done with SPSS statistical software system (SPSS Inc., Chicago, IL; version 12.0). The duration of fever, antibiotic treatment, or leukopenia were expressed as the time with the particular finding in the percentage of the total time in which the patient was included in the study. Nonparametric tests (Mann-Whitney, Fisher's exact, and Wilcoxon tests) were used to compare pretreatment and within-treatment data. The Spearman correlation test was used. Two-sided $P < 0.05$ were considered statistically significant. A normal reference of serum YKL-40 was calculated on the log-transformed serum YKL-40 values of the healthy controls adjusting for age, and the 95% percentile was chosen as the cut-point. Survival curves were constructed using the Kaplan-Meier method and the log rank test was used to compare survival between groups. The Cox proportional hazards model, log-likelihood statistics, was applied for univariate analyses of covariates and for multivariate analysis. Significant variables with a $P < 0.05$ were included in the multivariate Cox analyses to identify variables of independent significance. When evaluating the value of serum YKL-40 at day 1 in predicting complete remission, pneumonia, or sepsis, a logistic regression of serum YKL-40 (logarithmically transformed) as covariate was used. Cumulative incidence estimates of pneumonia or septicemia were plotted as a graphic representation of the risk of pneumonia or septicemia during the first month of chemotherapy.

## Results

The median serum concentration of YKL-40 just before the start of chemotherapy in the 77 newly diagnosed AML patients was 116 μg/L (range 15-3,637) and was significantly higher compared with the levels in 245 healthy controls (43 μg/L, range 20-184; $P < 0.001$). The individual serum YKL-40 in the AML patients and controls are illustrated in Fig. 1. Forty (52%) of the AML patients had a serum YKL-40 level above the upper 95th percentile (age-corrected) of serum YKL-40 in the controls. Patients with elevated serum YKL-40 were indistinguishable from patients with normal serum YKL-40 with respect to age, sex, cytogenetics, and FAB subtypes of AML (Table 1).

All patients developed fever, in about 50% within the first week. More patients with elevated serum YKL-40 had fever at time of starting chemotherapy. The durations of temperature >37°C or >38°C, leukopenia, antibiotics, or antifungal treatment were similar in the two groups of patients (Table 1). Serum YKL-40 correlated at time of diagnosis with age (Spearman $\rho = 0.35$; $P = 0.002$), serum albumin ($\rho = -0.48$; $P < 0.001$), serum aspartate aminotransferase ($\rho = 0.24$; $P = 0.04$), serum lactate dehydrogenase ($\rho = 0.28$; $P = 0.02$), and serum alkaline phosphatase ($\rho = 0.39$; $P < 0.001$). No correlations were found with leukocytes, platelets, hemoglobin, ESR, creatinine, serum alanine aminotransferase, and serum bilirubin.

*Serum YKL-40 at day 1 in relation to treatment response and survival.* Thirty-eight (49%) of the patients achieved complete remission within the first 4 weeks. There was no difference in treatment response ($P = 0.7$) and overall survival ($P = 0.6$) between the different treatment regimens. There was no relationship between serum YKL-40 at day 1 (normal versus elevated) and effect of treatment within the first months (complete remission versus non-response; Fisher exact test, $P = 0.26$). The logistic regression for complete remission versus no response with serum YKL-40 (logarithmically transformed) at

day 1 as covariate showed borderline association with remission (odds ratio, 1.4; 95% confidence interval (CI), 1.0-1.9; $P = 0.060$ when serum YKL-40 is doubled for not achieving remission).

The patients were followed-up until death or up to >12 years. The median survival was 276 days (range 2-5,195 days). Seventeen (22%) patients died within the first month and 44 (57%) died within the first year. At the time of the last follow-up (December 2004) eight patients (10%) were still alive. The survival time for patients with elevated serum YKL-40 at day 1 (i.e., the day of start of treatment) was significantly shorter (median, 128 days; interquartile range, 18-629 days) compared with patients with normal serum YKL-40 (median, 386 days; interquartile range, 180-901; $P = 0.018$ Mann-Whitney test). Univariate analysis of serum YKL-40 (logarithmically transformed and treated as a continuous variable) at day 1 showed a significant association with survival within the first month and the first year after starting chemotherapy (1 month survival, hazard ratio (HR), 1.7; $P = 0.002$; 1 year survival: HR, 1.6; $P = 0.0002$) and with the overall survival (HR, 1.3; $P = 0.003$; Table 2). Fever at day 1 and elevated serum lactate dehydrogenase, bilirubin and liver enzymes were also significantly associated with short survival (Table 2). Multivariate Cox regression analysis showed that only serum YKL-40 (1 month survival: HR, 1.7, 95% CI, 1.1-2.2; $P = 0.011$; 1 year survival: HR, 1.6, 95% CI, 1.3-2.0; $P = 0.0002$; and overall survival: HR, 1.4, 95% CI, 1.1-1.7; $P = 0.002$), serum bilirubin (1 month survival: HR, 3.9, 95% CI, 1.2-13; $P = 0.048$), and fever at day 1 (1 year survival: HR, 2.3, 95% CI, 1.2-4.3; $P = 0.010$; and overall survival: HR, 1.9, 95% CI, 1.1-3.2; $P = 0.029$) were independent prognostic variables of survival. Figure 2A and B illustrate the first month and first year survival plots when the patients were grouped by elevated or normal serum YKL-40 at start of chemotherapy.
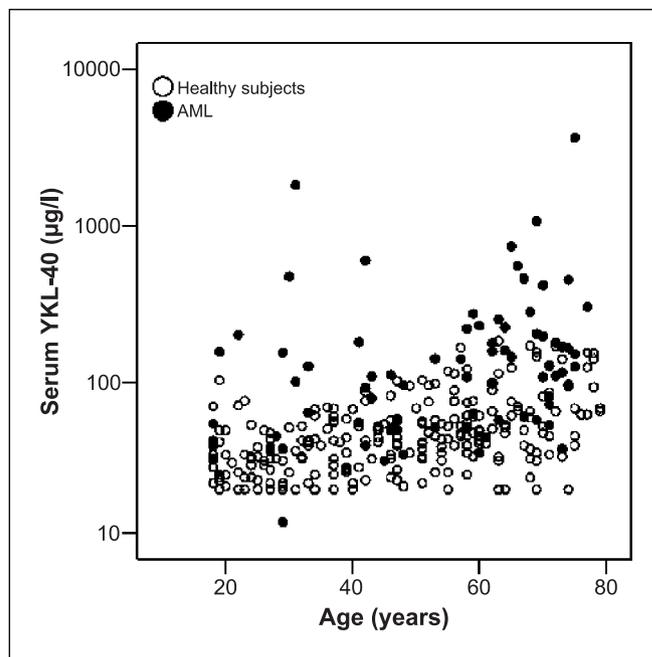


**Fig. 1.** Individual serum YKL-40 levels according to age in 77 patients with AML (●) at diagnosis and just before chemotherapy and in 245 healthy subjects (○). The *y*-axis is a logarithmic scale.

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[8] Personal observation.

**Table 1.** Demographic characteristics and clinical outcome of the 77 AML patients according to normal ($n = 37$) or elevated ($n = 40$) serum YKL-40 before start of chemotherapy

| Characteristics | Normal serum YKL-40 | High serum YKL-40 | P |
|---|---|---|---|
| Age (y)* | 58 (18-75) | 63 (19-77) | 0.61[‖] |
| Sex (male/female)[†] | 20/17 | 22/18 | 1.0[‖] |
| Cytogenetic type, favorable/intermediate/adverse prognosis[†] | 3/23/6 | 0/28/5 | 0.20[‡][‖] |
| AML FAB[§] type M$_2$, M$_3$, M$_4$ vs. other[†] | 15/22 | 25/15 | 0.07[‖] |
| AML FAB[§] type M$_4$, M$_5$ vs. other[†] | 13/24 | 13/27 | 0.81[‖] |
| Fever at day 1 ($n$, %)[†] | 6 (16%) | 16 (40%) | 0.025 |
| Proportion (%) of study period with ›37.0°C* | 57 (7-100) | 70 (0-100) | 0.067[‖] |
| Proportion (%) of study period with ›38.0°C* | 27 (0-89) | 38 (0-100) | 0.28[‖] |
| Proportion (%) of study period with leucopenia (‹1.0 × 10$^9$/L)* | 50 (0-89) | 50 (0-100) | 0.36[‖] |
| Proportion (%) of study period on antibacterial antibiotics* | 50 (14-82) | 60 (0-100) | 0.14[‖] |
| Antifungal treatment ($n$, %)[†] | 21 (57) | 18 (45) | 0.37[‖] |

*Values are median (range) and Mann-Whitney test.
[†]Values and Fisher's exact test.
[‡]Intermediate versus adverse prognosis group.
[§]FAB classification.
[‖]Not significant.

Twenty-six patients had fever and/or were treated with antibiotics at day 1. These patients had significantly elevated serum YKL-40 at day 1 (median, 150 µg/L; range, 35-3,637 µg/L) compared with patients without fever and no treatment with antibiotics at day 1 (median, 94 µg/L; range, 15-557 µg/L; $P$ = 0.016, $n$ = 51). Univariate Cox analysis of serum YKL-40 (logarithmically transformed and treated as a continuous variable) in the group of patients with fever and/or treatment with antibiotics at day 1 showed significant association with survival after starting chemotherapy (1 month survival: HR, 1.7;

**Table 2.** Univariate Cox Analysis of 1-month survival, 12-months survival, and overall survival after diagnosis of AML

| Covariate | 1-month survival | | 12-months survival | | Overall survival | |
|---|---|---|---|---|---|---|
| | HR (95% CI) | P | HR (95% CI) | P | HR (95% CI) | P |
| Age (≤60 vs. ›60 y) | 1.1 (0.4-3.0) | 0.80 | 1.1 (0.6-2.1) | 0.66 | 1.5 (0.9-2.3) | 0.13 |
| Sex | 1.1 (0.4-2.9) | 0.84 | 0.7 (0.4-1.4) | 0.33 | 0.8 (0.5-1.2) | 0.26 |
| Cytogenetics (F vs. I vs. A) | — | 0.53 | — | 0.43 | — | 0.10 |
| Cytogenetics (A vs. other) | 0.9 (0.2-4.3) | 0.93 | 1.6 (0.7-3.4) | 0.29 | 1.7 (0.9-3.3) | 0.13 |
| AML FAB type [M2, (M3), M4 vs. other] | 1.4 (0.5-3.7) | 0.50 | 1.1 (0.7-2.1) | 0.58 | 1.1 (0.7-1.8) | 0.66 |
| AML FAB type (M4, M5 vs. other) | 1.4 (0.5-3.7) | 0.51 | 1.4 (0.8-2.6) | 0.29 | 1.4 (0.8-2.3) | 0.22 |
| Fever at day 1 (yes vs. no) | 2.7 (1.1-7.1) | 0.044 | 2.3 (1.2-4.2) | 0.010 | 1.8 (1.1-3.1) | 0.036 |
| Serum YKL-40* | 1.7 (1.2-2.4) | 0.002 | 1.6 (1.2-2.0) | 0.0002 | 1.3 (1.1-1.6) | 0.003 |
| ESR[†] | 1.0 (1.0-1.0) | 0.40 | 1.0 (1.0-1.0) | 0.57 | 1.0 (1.0-1.0) | 0.97 |
| Leukocytes count* | 1.1 (0.9-1.3) | 0.28 | 1.1 (1.0-1.2) | 0.096 | 1.1 (1.0-1.2) | 0.059 |
| Hemoglobin[†] | 0.5 (0.3-1.1) | 0.063 | 0.7 (0.5-1.1) | 0.14 | 0.7 (0.5-1.0) | 0.054 |
| Platelet* | 0.9 (0.6-1.3) | 0.71 | 1.0 (0.8-1.3) | 0.99 | 1.2 (1.0-1.5) | 0.062 |
| Serum creatinine[†] | 1.0 (1.0-1.0) | 0.98 | 1.0 (1.0-1.0) | 0.13 | 1.0 (1.0-1.0) | 0.40 |
| Serum albumin[†] | 1.0 (1.0-1.0) | 0.17 | 1.0 (1.0-1.0) | 0.15 | 1.0 (1.0-1.0) | 0.45 |
| Serum bilirubin (normal vs. high) | 5.4 (1.7-17) | 0.013 | 1.9 (0.7-5.4) | 0.25 | 2.2 (1.0-5.2) | 0.094 |
| Serum alanine aminotransferase* | 1.5 (1.0-2.2) | 0.041 | 1.2 (1.0-1.6) | 0.11 | 1.1 (0.9-1.3) | 0.61 |
| Serum aspartate aminotransferase* | 1.5 (1.0-2.0) | 0.037 | 1.3 (1.0-1.6) | 0.042 | 1.1 (0.9-1.4) | 0.27 |
| Serum alkaline phosphatase* | 1.7 (1.1-2.7) | 0.026 | 1.4 (1.1-1.9) | 0.018 | 1.3 (1.0-1.7) | 0.032 |
| Serum lactate dehydrogenase* | 1.2 (0.9-1.7) | 0.24 | 1.2 (1.0-1.5) | 0.039 | 1.1 (1.0-1.4) | 0.17 |

NOTE: Age, sex, cytogenetic prognostic groups [favorable (F), intermediate (I) and adverse (A)], AML FAB types, fever at day 1, and serum bilirubin were treated as categorical variables. Seventeen deaths within the first month, 44 deaths within the first year, and 69 deaths during the follow-up until December 2004 ($n$ = 77).
*Leukocyte counts, platelets, serum YKL-40, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, and lactate dehydrogenase were log-transformed and treated as continuous variables.
[†]Hemoglobin, serum albumin, creatinine, and ESR were treated as continuous variables.

$P = 0.015$; 1 year survival: HR, 1.7; $P = 0.001$; and overall survival: HR, 1.7; $P = 0.001$). This was not found in the group of patients ($n = 51$) without fever and/or no treatment with antibiotics at day 1 (Table 3).

Univariate Cox analysis of serum YKL-40 at day 1 in the 51 patients without pneumonia during the study (days 1-28) showed significant association with survival after starting chemotherapy (1 month survival: HR, 1.6; $P = 0.009$; 1 year survival: HR, 1.4; $P = 0.006$; and overall survival: HR, 1.3; $P = 0.016$; Table 3). In the 26 patients with pneumonia during the study period, serum YKL-40 at day 1 was also associated with

1-year survival (HR, 2.3; $P = 0.006$) but not with 1-month and overall survival (Table 3).

Univariate Cox analysis of serum YKL-40 at day 1 in the 39 patients without pneumonia and/or septicemia during the study (days 1-28) showed significant association with survival after starting chemotherapy (1 month survival: HR, 1.6; $P = 0.009$; 1 year survival: HR, 1.5; $P = 0.006$; and overall survival: HR, 1.4; $P = 0.005$; Table 3). In the 38 patients with pneumonia and/or septicemia during the study period, serum YKL-40 at day 1 was also associated with 1-year survival (HR, 1.6; $P = 0.022$) but not with 1-month and overall survival (Table 3).

The five patients with both high serum YKL-40 and cytogenetic adverse prognosis (Table 1) had the poorest median survival (47 days, and all five patients were dead within 51 days) contrasting a median survival of 399 days for patients with normal serum YKL-40 and cytogenetic intermediate prognosis. The three patients with favorable prognosis had normal serum YKL-40.

***Changes in serum YKL-40 during the first month of chemotherapy in relation to infections.*** Fifty-six patients had not developed infections within the first week after starting chemotherapy and they had a significant decrease in serum YKL-40 (day 1: median, 112 μg/L; 95% CI, 34-473 μg/L, versus day 7: median, 95 μg/L; 95% CI, 28-268 μg/L; $P = 0.007$). Pneumonia occurred in 19 (25%) of the patients (4 of these also had septicemia) within the first month after starting chemotherapy. Cox regression for pneumonia versus no pneumonia (and no septicemia) with serum YKL-40 (logarithmically transformed) at day 1 as covariate showed no association with pneumonia ($P = 0.53$) when serum YKL-40 is doubled. Figure 3A illustrates that the cumulative incidence of pneumonia in the AML patients was higher in patients with elevated serum YKL-40 at start of chemotherapy compared with patients with normal serum YKL-40 (HR, 2.3; 95% CI, 1.0-5.2; $P = 0.04$). Furthermore, serum YKL-40 at the time of pneumonia was significantly higher (median, 302 μg/L; 95% CI, 201-484 μg/L) compared with the serum YKL-40 level at the time point preceding the infection (median, 166 μg/L; 95% CI, 113-213 μg/L; $P = 0.002$; Fig. 3B). Septicemia (without pneumonia) occurred in 14 (18%) patients within the first month and after starting treatment. The bacteriologic isolates comprised *Klebsiella pneumoniae* in four, *Escherichia coli* in three, *Pseudomonas aeruginosa* in two, *S. aureus* in one, coagulase-negative staphylococci in three, nonhemolytic streptococci in one, and hemolytic streptococci in one patient (one patient had two isolates). The Cox model for septicemia (and no pneumonia) versus no septicemia (and no pneumonia) with serum YKL-40 (logarithmically transformed) at day 1 as covariate showed no association with septicemia ($P = 0.18$) when serum YKL-40 is doubled. Figure 3C illustrates that the cumulative incidence of septicemia in AML patients was similar in patients with elevated and normal serum YKL-40 at start of chemotherapy ($P = 0.67$). Serum YKL-40 at the time of septicemia (and no pneumonia, median, 186 μg/L; 95% CI, 96-411 μg/L) was not significantly higher compared with serum YKL-40 at the time point preceding septicemia (86 μg/L; 95% CI, 61-176 μg/L; $P = 0.10$; Fig. 3D). Serum YKL-40 was unchanged in the group of patients without septicemia or pneumonia ($n = 25$) during the first month of chemotherapy (data not shown).
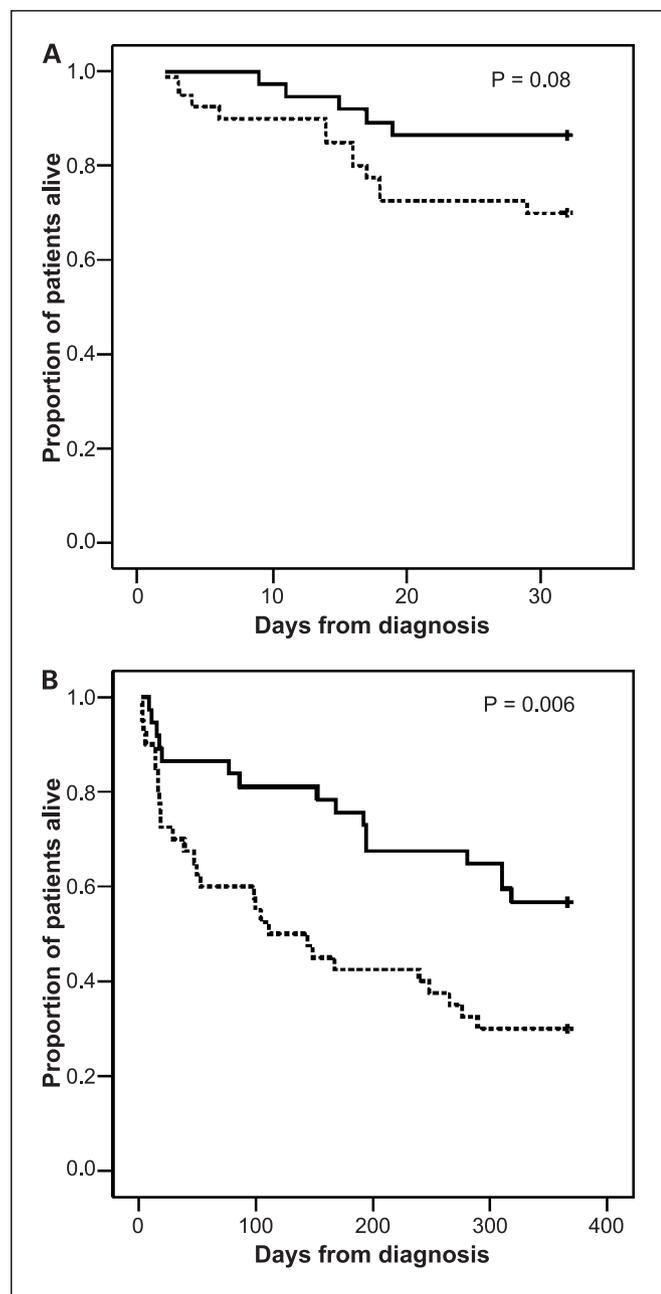


**Fig. 2.** Survival curves showing the association between serum YKL-40 and 1-month survival (*A*) and 12-months survival (*B*) in 77 patients with AML. Patients were dichotomized according to elevated (- - -, $n = 40$) versus normal (—, $n = 37$; age-adjusted) serum YKL-40 at diagnosis and just before chemotherapy. *P,* log rank test for equality of strata.

**Table 3.** Univariate Cox analysis of 1-month survival, 12-months survival, and overall survival after diagnosis of AML according to serum YKL-40 levels at day 1 and infections at different times

| Covariate (serum YKL-40 day 1)* | 1-month survival | | 12-months survival | | Overall survival | |
|---|---|---|---|---|---|---|
| | HR (95% CI) | *P* | HR (95% CI) | *P* | HR (95% CI) | *P* |
| Fever and/or antibiotics at day 1 | | | | | | |
| No (*n* = 51) | 1.3 (0.7-2.2) | 0.41 | 1.2 (0.9-1.7) | 0.27 | 1.1 (0.9-1.4) | 0.39 |
| Yes (*n* = 26) | 1.7 (1.1-2.5) | 0.015 | 1.7 (1.3-2.3) | 0.001 | 1.7 (1.2-2.2) | 0.001 |
| Pneumonia during the study | | | | | | |
| No (*n* = 51) | 1.6 (1.1-2.3) | 0.009 | 1.4 (1.1-1.8) | 0.006 | 1.3 (1.1-1.7) | 0.016 |
| Yes (*n* = 26) | 2.0 (0.7-5.5) | 0.15 | 2.3 (1.2-4.4) | 0.006 | 1.3 (0.9-1.8) | 0.11 |
| Pneumonia and/or septicemia during the study | | | | | | |
| No (*n* = 39) | 1.6 (1.2-2.3) | 0.009 | 1.5 (1.1-1.9) | 0.006 | 1.4 (1.1-1.8) | 0.005 |
| Yes (*n* = 38) | 1.5 (0.8-2.8) | 0.24 | 1.6 (1.0-2.5) | 0.022 | 1.2 (0.9-1.5) | 0.27 |

NOTE: Seventeen deaths within the first month, 44 deaths within the first year, and 69 deaths during the follow-up until December 2004 (*n* = 77).

*Serum YKL-40 levels at day 1 were log-transformed and treated as continuous variables.

## Discussion

This is the first report on the novel serum biomarker YKL-40 in patients with AML. We found that 52% of the patients at the time of diagnosis had elevated serum YKL-40 compared with healthy subjects, and these patients had the poorest prognosis. Interestingly, serum YKL-40 was independent of other prognostic factors, including cytogenetics, although the influence of cytogenetics might be underestimated due to the small number of patients in both the favorable and the adverse prognosis group. This finding is in line with previous studies of patients with different types of solid tumors (14, 30–38). The biological functions of YKL-40 in hematologic malignancies and solid carcinoma are unknown. It has been suggested that YKL-40 may play a role in the proliferation and differentiation of malignant cells, protect the cells from undergoing apoptosis, stimulate angiogenesis, and have an effect on extracellular tissue remodeling, although *in vivo* proof of this is yet to be obtained. *In vitro* studies of glioblastoma cell lines have shown that diverse types of stress resulted in YKL-40 mRNA and protein expression, suggesting an involvement of YKL-40 as a cellular survival factor (15, 20).

In the present study, it was also found that high serum YKL-40 in AML patients at the beginning of chemotherapy was a risk factor for pneumonia within the first month of chemotherapy. We examined the changes in serum YKL-40 weekly during the first 4 weeks of remission induction chemotherapy and observed significant increases (median 2-fold, and up to 8-fold) in serum YKL-40 in AML patients at the time of pneumonia. Septicemia was also followed by increases in serum YKL-40, although not statistically significant in the small number of patients. In the group of patients who did not develop pneumonia and/or septicemia during the first month of chemotherapy, the serum YKL-40 level at day 1 was still a prognostic marker of survival. In the group of patients who developed pneumonia and/or septicemia during the study period, the serum YKL-40 level at day 1 was only associated with 1-year survival.

YKL-40 can be regarded as an acute phase protein because its serum concentration increases by >25% following an inflammatory stimulus. It has been reported that plasma YKL-40 increased 5-fold at 24 hours after injection with endotoxin in healthy subjects (45) and that patients with *Streptococcus pneumoniae* pneumonia or septicemia had 8- to 10-fold higher serum YKL-40 compared with healthy subjects (46, 47). The biological functions of YKL-40 in infectious diseases are not clarified. YKL-40 may play a role in inflammatory and tissue remodeling processes or it could have a more direct function in fighting infections. It has been hypothesized that YKL-40 acts as an opsonin with a role in the immune response or as a chitin sensor, switching on innate defenses, helping to direct macrophages to the site of invasion, and to regulate the inflammatory response as a consequence of infection (12). It has recently been shown in interleukin (IL)-6 wild-type and IL-6 knock-out mice that YKL-40 is regulated by IL-6. However, in contrast to C-reactive protein (CRP) that is produced by hepatocytes in response to high circulating levels of IL-6 (48), no YKL-40 mRNA expression was found in the liver of IL-6 wild-type mice after injection with endotoxin. Instead increased YKL-40 mRNA expression was found in blood, lung, and adipose tissue. Injection with endotoxin in IL-6 knock-out mice did not increase YKL-40 mRNA expression in any tissues.[9,10]

We think that YKL-40 reflects other aspects of inflammation than serum CRP, the most used acute phase protein, although CRP is not produced locally by cells in areas with inflammation. Low correlations (Spearman $\rho$, 0.3-0.5) between serum YKL-40 and serum CRP levels are found in patients with rheumatoid arthritis (6, 44, 49), in patients with inflammatory bowel disease (50, 51) and in patients at the time of diagnosis of giant cell arteritis or polymyalgia (52). In patients with bacterial infections, serum YKL-40 peaked before serum CRP, and after treatment with antibiotics, serum YKL-40 reached reference range a few days earlier than serum CRP (46). Multivariate Cox regression analysis (including serum YKL-40, cerebral symptoms, mechanical ventilation, pharmacologic treatment of hypotension, and hemodialysis) showed that high serum YKL-40 in patients at the time of diagnosis of

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[9] Manuscript submitted for publication.

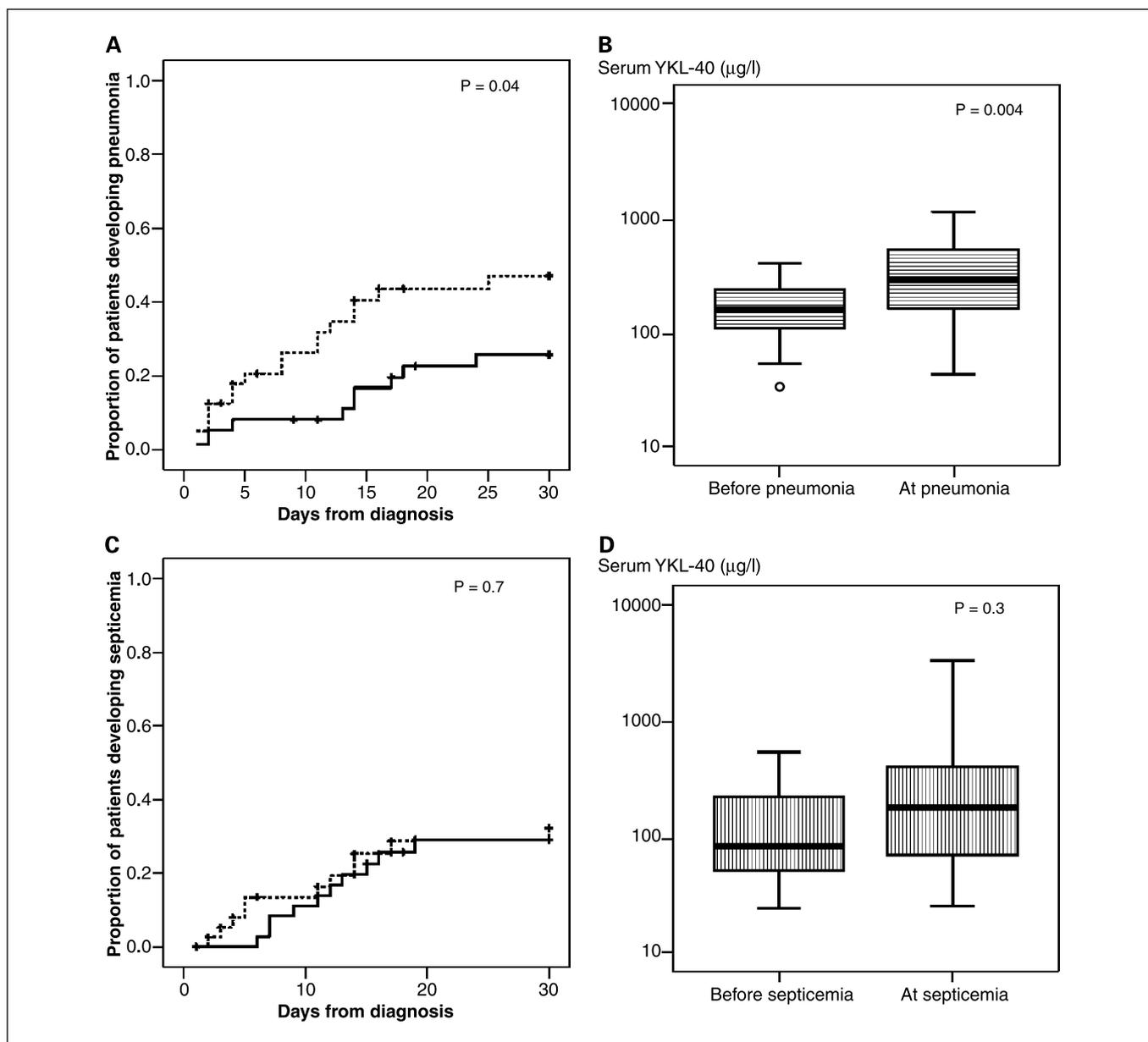[10] A.R. Nielsen et al., unpublished data.

**Fig. 3.** Cumulative incidence of pneumonia (*A*) and septicemia (*C*) in AML patients within the first month of chemotherapy according to elevated (- - -, *n* = 40) versus normal (—, *n* = 37; age adjusted) serum YKL-40 at diagnosis and just before chemotherapy. *P*, log rank test for equality of strata. Changes in serum YKL-40 (box plots) in 19 AML patients before and at the time of pneumonia (*B*) and in 14 AML patients before and at the time of septicemia (*D*) during the first 4 weeks of chemotherapy. The *y*-axis is a logarithmic scale. The edges of the box plots represent the first and third quartiles, the whiskers show the range within the 1.5 box length; (○) outliers.

*S. pneumoniae* septicemia was an independent prognostic marker of short survival (47). In the same patients serum CRP was not a prognostic marker of survival (47). Only 70% of active rheumatoid arthritis patients with elevated serum YKL-40 also had high ESR or serum CRP levels (49). Only 56% of patients with giant cell arteritis and signs of disease relapse (elevations in ESR and serum CRP) also had elevations in serum YKL-40, and serum YKL-40 was not correlated with serum CRP and ESR in these patients during prednisolone treatment (52). Serum CRP was not measured in the present study of AML patients. However, ESR, another acute phase reactant, was determined and not related to serum YKL-40 in the patients with AML.

YKL-40 expression is absent in normal human monocytes but is strongly induced *in vitro* during the late stages of macrophage differentiation (10, 11) and released from the specific granules of activated neutrophils (23). It is most likely that activated macrophages in the lung are the major source of the observed increase in serum YKL-40 in AML patients at the time of pneumonia during chemotherapy because the patients were leucopenic. Serial analysis of gene expression has shown 288-fold increased YKL-40 transcripts in monocytes stimulated with granulocyte-macrophage colony–stimulating factor, 182-fold in macrophage colony-stimulating factor–stimulated monocytes and 31-fold increased YKL-40 transcripts in the lipopolysaccharide-stimulated monocytes (53, 54).

*In vivo*, YKL-40 mRNA and protein are expressed by a subpopulation of macrophages in different tissues, such as inflamed synovial membranes from patients with rheumatoid arthritis (24), atherosclerotic plaques (55), arteritic vessels from patients with giant cell arteritis (52), sarcoid lesions from patients with pulmonary sarcoidosis (56), and by peritumoral macrophages in biopsies of small cell lung cancer (25). In patients with rheumatoid arthritis, YKL-40 is expressed by the CD16$^+$ monocytes with a dim expression of CD14 (24), a phenotype which can differentiate from classic CD14$^{++}$ monocytes by maturation *in vitro* and is a phenotype believed to be a more mature version of monocytes with properties of long-lived tissue macrophages, probably of the proinflammatory type. The blood count of CD14$^+$,CD16$^+$ monocytes is increased in numbers in patients with sepsis, tuberculosis, rheumatoid arthritis, and solid tumors (57). Using flow cytometry or immunohistochemistry, it would be interesting to examine which cells express YKL-40 in AML patients at the time of diagnosis and during infection.

We conclude that high serum YKL-40 is a new independent prognostic biomarker of short survival in patients with AML. Furthermore, high serum YKL-40 may also be a risk factor for developing pneumonia during the first months of chemotherapy. Studies are strongly needed to investigate the function of YKL-40 in AML and bacterial infections.

## Acknowledgments

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# High Serum Concentration of YKL-40 Is Associated with Short Survival in Patients with Acute Myeloid Leukemia

Olav J. Bergmann, Julia S. Johansen, Tobias W. Klausen, et al.

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