

Short Communication

Diurnal, Weekly, and Long-Time Variation in Serum Concentrations of YKL-40 in Healthy Subjects

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Abstract

Serum YKL-40 is a potential biomarker of prognosis in cancer patients, but assessment of serum YKL-40 requires knowledge of its normal variation. In this study, we evaluated diurnal, weekly, and long-term variation in serum YKL-40 in healthy subjects using a commercial ELISA. The intra-assay coefficient of variation was $\leq 5.0\%$ and interassay $\leq 10.2\%$. Systematic changes in diurnal measurements of serum YKL-40 could not be shown. Physical exercise for 20 min had no effect on serum YKL-40. The within-subject coefficient of variation, including variation over time and inter-assay, was 28.8% and 30.2% over a period of 2 and 3 years, and the intraclass correlation coefficients were

72.4% and 72.2%, indicating reasonable reliability of serum YKL-40 measurements. The 95% confidence limits for the difference between two measurements (same subject), including interassay variation, were a 52% reduction and a 109% increase in serum YKL-40. These studies show that relatively small variation is found in serum YKL-40 in healthy subjects. However, a single measurement of serum YKL-40 from an individual may not have a prognostic value, and serum YKL-40 alone cannot be a good biomarker for cancer because serum YKL-40 can be elevated in patients with other diseases characterized by inflammation and tissue remodeling. (Cancer Epidemiol Biomarkers Prev 2008;17(10):2603–8)

Introduction

YKL-40 is produced by cancer cells and tumor-associated macrophages (1). Serum YKL-40 is elevated compared with healthy subjects in many patients with primary or metastatic carcinoma of the breast (2), colorectal (3), ovary (4, 5), lung (6), prostate (7), kidney (8), endometrial (9), cervical (10), and head and neck (11), and in patients with glioblastoma (12, 13), melanoma (14, 15), acute myeloid leukemia (16), and multiple myeloma (17). Figure 1 shows the distribution of serum YKL-40 in patients with different types of cancer, showing the range, median, and the percentage with elevated levels. In these studies, high serum YKL-40 was an independent prognostic biomarker of short recurrence- or progression-free interval and short overall survival. This was found in patients with local or metastatic cancer, at time of first cancer diagnosis and at time of relapse, and serum YKL-40 was independent of other biomarkers

when tested in multivariate Cox analysis (e.g., estrogen receptor status, HER2, CEA, CA125, and LDH; refs. 1-17).

However, serum YKL-40 is not specific for cancer. Elevated serum YKL-40 is found in patients with diseases characterized by acute or chronic inflammation and high remodeling of the extracellular matrix as found in, for example, rheumatoid arthritis, inflammatory bowel disease, asthma, and liver fibrosis (18, 19).

Normal variation in a circulating biomarker may affect the interpretation and usefulness of a biomarker in patients with cancer. The aims of the present study were to determine the diurnal-, short-term, and long-term variations in serum YKL-40 in healthy subjects.

Materials and Methods

Reference Interval. Serum was collected from 245 healthy subjects (women-men, 134:111; median age, 49 years; range, 18-79).

Diurnal Variation. Serum was collected 7 times during a 24-h period (day 1: 10 a.m., 1 p.m., 4 p.m., 7 p.m., 10 p.m.; day 2: 7 a.m., 10 a.m.) from 16 healthy subjects (10:6; 48 years; range, 32-66).

Day-to-Day Variation over 3 Weeks. Serum was collected at 8 a.m., 5 times during a 3-week period (day 1, 2, 8, 15, and 22), from 38 subjects recruited from the hospital staff (21:17; 41 years; range, 22-66). At day 8, samples were also collected at 2 p.m.

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Note: H.J. Nielsen is Danish Cancer Society Professor of Surgical Oncology.

Quidel had no role in (a) the design of the study, (b) the data collection, analysis, and interpretation, and (c) the preparation of the manuscript, and had no rights to approve, delay, or disapprove of publication of the work.

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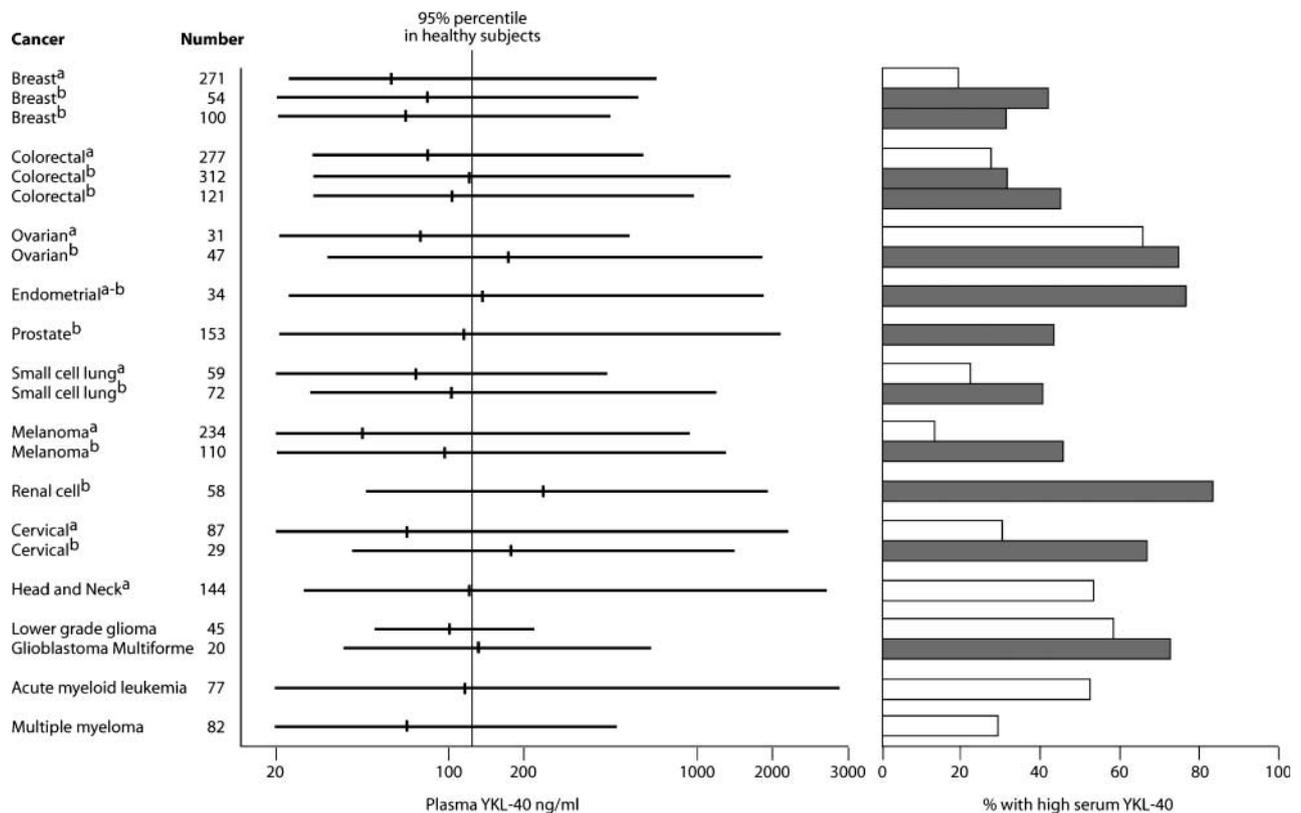


Figure 1. The distribution of serum YKL-40 in 13 cancer diseases. *Left*, the range and median serum YKL-40 levels. *Right*, the percentages of patients with elevated serum YKL-40 (compared with levels in healthy age- and gender-adjusted subjects; the upper limit defined as the 95th percentile). *a*, localized disease; *b*, regional or distant metastasis (1-17).

Week-to-Week Variation over 2 Years. Serum was collected from 23 subjects recruited from the hospital staff (14:9; 42 years; range, 31-66) at 8 a.m., 5 times during a 3-week period (day 1, 2, 8, 15, and 22), and repeated 6, 12, and 24 months later.

Variation over 3 Years. Serum was collected between 8 and 10 a.m., 5 times during a 4-week period (day 1, 8, 15, 22, and 29), from 30 healthy women (48 years; range, 24-62), and repeated 3 years later in 21 of the subjects.

Variation after Exercise. Serum was collected before physical exercise, immediately after a biphasic 25-min exercise program using an ergometer bicycle, and 1 and 3 h postexercise from 14 healthy subjects (10:4; 50 years; range, 35-64).

The healthy subjects included in the present study had no medical history, did not experience any symptoms, had no signs of disease, and were not taking any medicine.

Ethics. The studies were approved by the regional scientific ethical committee and carried out in accordance with the Declaration of Helsinki. The subjects were informed about the studies verbally and in writing, and all gave their written informed consent. All were informed that they could stop the study at any time.

YKL-40 ELISA. Proper handling of blood samples are important to minimize changes in serum YKL-40 that are

not related to disease processes but represent methodologic variability (1, 18, 20). Blood samples were allowed to clot at room temperature, centrifuged within 0.5 to 2 h at minimum $2,500 \times g$ for 10 min, and serum was stored at -80°C until analysis. Serum YKL-40 was determined in duplicates by a commercial two-site, sandwich-type ELISA (Quidel Corporation) using streptavidin-coated microplate wells, a biotinylated-Fab monoclonal capture antibody, and an alkaline phosphatase-labeled polyclonal detection antibody (20). The recovery of the ELISA was 102% and detection limit $20 \mu\text{g/L}$ (18, 20). The intra-assay coefficient of variation was $\leq 5.0\%$ and interassay coefficients of variation $\leq 10.2\%$.⁷ Samples from each subject were analyzed on the same ELISA plate.

Statistical Analysis. Descriptive statistics for serum YKL-40 were presented by the median or the geometric mean, coefficient of variation, and 95% confidence interval, and range. The distribution of serum YKL-40 is skewed, and therefore, the log transform (natural) is used for statistical estimation. The reference interval was estimated using linear regression with YKL-40 on the log scale. The variations in serum YKL-40 analyzed over time (variability during 24 h and over 3 weeks, 6 months,

⁷ Personal observation.

12 months, 24 months, and 3 years) were given by the coefficient of variation and compared with the intra- and interassay coefficient of variation of the YKL-40 ELISA. The variance components for within-subjects, between subjects, and between rounds were estimated assuming a random effects model with YKL-40 log transformed (multiplicative model) and presented by the coefficient of variation of the geometric means (21). The 95% confidence limits for the difference between two measurements of YKL-40 in an individual were calculated on the log scale and back transformed. The relative homogeneity between subjects compared with the total variation was estimated by the intraclass correlation coefficient. Serum YKL-40 in the analysis of diurnal long-term variation and physical activity were analyzed using a general linear model with repeated measures. *P* values <5% were considered significant. *P* values for multiple testing were corrected using the Bonferroni Correction. All statistical calculations were done using SAS (9.1, SAS Institute).

Results

In healthy subjects, the median serum YKL-40 was 43 $\mu\text{g/L}$ (range, 20-184 $\mu\text{g/L}$; 5-95% interval, 20-124), and no difference between men and women ($P = 0.54$). Serum YKL-40 increased with age ($r = 0.45$; $P < 0.0001$). A normal reference interval for serum YKL-40 adjusted for age and gender was constructed by linear regression with serum YKL-40 as the dependent variable (log transformed) and age and gender as the explanatory variables. The upper limit was defined as the 95th percentile for given age and gender. The intersubject coefficient of variation adjusted for age was 45%.

Figure 2A illustrates the individual diurnal variation in serum YKL-40 at 7 time points during 24 h. The mean serum YKL-40 increased 23% from 10 a.m. to 10 p.m. ($P = 0.01$), however nonsignificant when corrected for multiple testing. No other significant differences were observed. No changes in serum YKL-40 were found after 25 min of bicycling ($P > 0.08$; linear model).

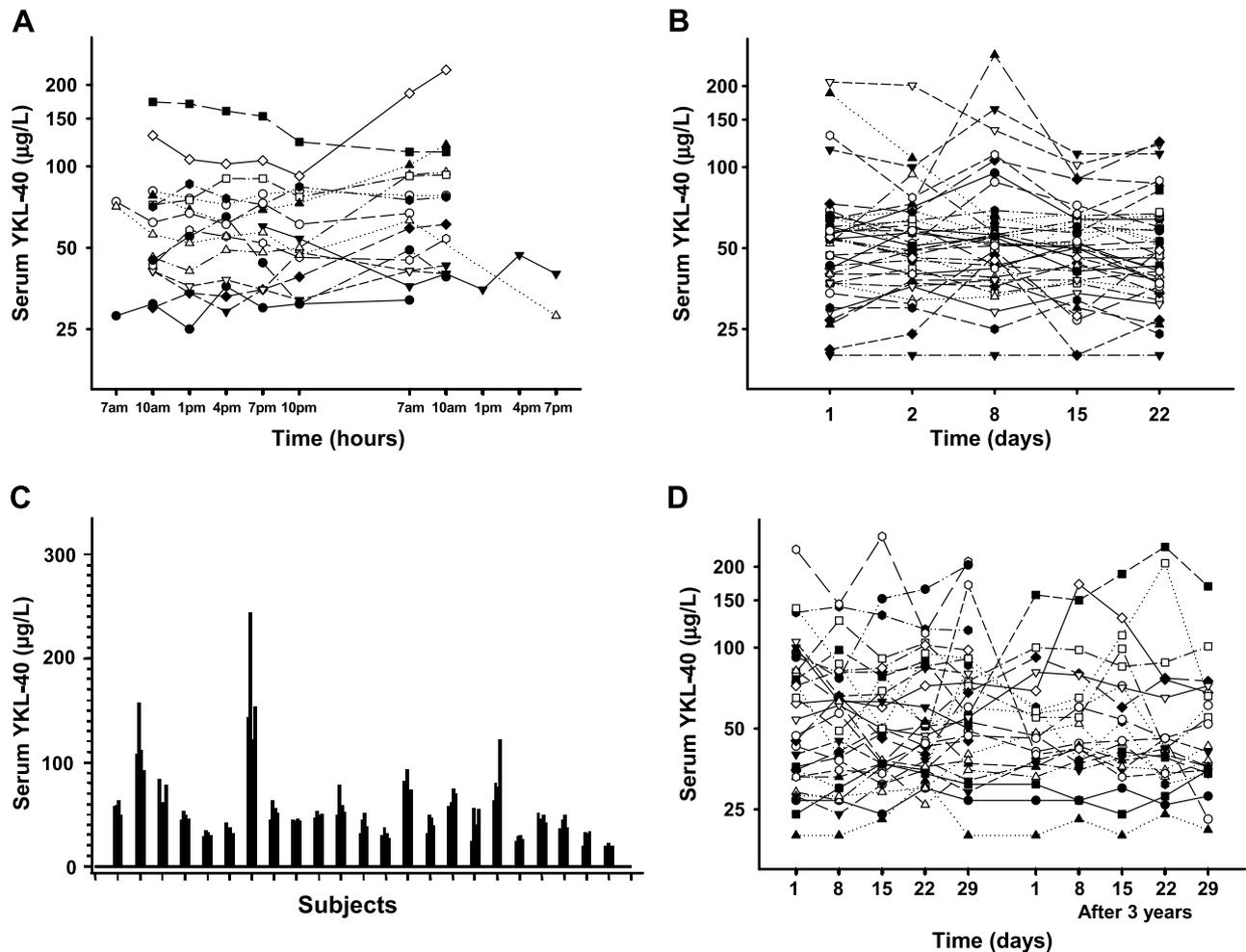


Figure 2. A. Individual diurnal variation in serum concentrations of YKL-40 in 16 healthy subjects. B. Individual variation in serum YKL-40 levels of 38 healthy subjects over a period of 3 wk. C. The median serum YKL-40 level for 23 individuals over 3 wk, available in each of 4 rounds (each bar is the median of one round for each subject). D. Individual serum YKL-40 levels of 30 healthy women sampled over 4 wk and repeated 3 y later for 21 of the women.

Figure 2B shows the individual weekly changes in serum YKL-40 at 6 time points during a 3-week period (at 8 a.m. on days 1, 2, 8, 15, and 22). The median day-to-day coefficient of variation of serum YKL-40 for each subject was 16%. On day 8, samples were collected at 8 a.m. and 2 p.m., and serum YKL-40 increased slightly (47 versus 52 $\mu\text{g/L}$; 8% difference; $P < 0.0001$).

Figure 2C illustrates the individual variation in serum YKL-40 at 5 time points during a 3-week period (at 8 a.m. on days 1, 2, 8, 15, and 22; 1st round) and repeated after 6 months (2nd round), 12 months (3rd round), and 24 months (4th round). The median day-to-day coefficient of variation of serum YKL-40 for each subject was overall 16% (range, 0-92%), and 16% (0-63%; 1st round), 19% (5-92%; 2nd), 15% (0-64%; 3rd), and 21% (0-47%; 4th). No systematic increases or decreases were detected over the 4 rounds ($P = 0.09$). The estimates of the variance components using a random effects model with serum YKL-40 log transformed results in a within-subject coefficient of variation of 27.3% and a coefficient of variation over 24 months of 8.8%. The within-subject coefficient of variation, including the variation over time and interassay variation, was 30.2% over the 24-month period. The intraclass correlation coefficient over the 24 months was 72.4%. The estimated variation in serum YKL-40 within subjects, including interassay variation results in 95% confidence limits for the difference between two measurements on the same subject if the 2nd YKL-40 measurement is reduced by 52% or is increased by 109%, and differences of this magnitude could be considered as significant and not only a reflection of pre-analytic conditions, methodologic, and normal biological variability.

Figure 2D shows the individual weekly changes in serum YKL-40 at 5 time points during a month and subsequently again after 3 years. The median coefficient of variation in serum YKL-40 was 17% (1st round) and 13% (2nd round). In subjects analyzed in both rounds ($n = 21$), no changes in serum YKL-40 were observed between the 2 periods ($P = 0.37$, linear model). The estimates of the variance components using the random effects model with serum YKL-40 log transformed result in a within-subject coefficient of variation of 26.0% and coefficient of variation over 3 years of 7.3%. The within-subject coefficient of variation, including the variation over time and interassay variation, was 28.8%. The between subject variation, including within-subject variation and variation over time, was 54%. The intraclass correlation coefficient over 3 years was 72.2%, suggesting a relatively low within-subject variation compared with between subject variation.

Discussion

The present study shows that serum YKL-40 is stable in healthy subjects for short-term as well as long-term sampling periods of up to 3 years with a within-subject coefficient of variation of ~30%, including interassay variation. The between subject variation in serum YKL-40 was 45% in the study determining a normal reference interval and is similar to that found in the other studies of healthy subjects in the present study. The intraclass correlations of serum YKL-40 were 72.4%

and 72.2% over a period of 2 and 3 years, suggesting a relative low within-subject variation compared with between subject variations. The intraclass correlations found in the present study are similar to those found for other serologic markers; for example, Ockene et al. reported an intraclass correlation of 66% for high sensitive C-reactive protein (22). Nonetheless, regression dilution bias could pose a problem and necessitate correction in clinical studies (23). Small changes were found in serum YKL-40 in healthy subjects between 7 a.m. and 4 p.m., and light exercise had no effect on serum YKL-40.

The normal variation in serum YKL-40 is an important factor to be considered in clinical studies of patients with cancer using serum YKL-40 as a potential prognostic biomarker. A normal reference interval should be determined in each laboratory measuring serum YKL-40, and an elevated serum YKL-40 could be defined as >90th or 95th percentile of serum YKL-40 in healthy age-matched subjects.

The present estimated variation in serum YKL-40 within healthy subjects, including interassay variation, suggests that an increase of >109% or a decrease of >52% in serum YKL-40 could be considered as significant and not only a reflection of pre-analytic conditions, methodologic, and normal biological variability. Some of the elevations in serum YKL-40 reported in cancer patients (1-17, 24-26) are not higher than could be explained by pre-analytic conditions and methodologic and normal biological variability, but in all types of cancer, some patients have much higher levels (Fig. 1). Like other biomarkers used in cancer patients (e.g., serum CEA, PSA, CA-125), a single measurement of serum YKL-40 may not have a prognostic value, but if elevated serum YKL-40 is confirmed by retesting at a later time point, this may have a clinical value. Serum YKL-40 alone cannot be a good biomarker for cancer, and it is very important to account for comorbidity because serum YKL-40 can be elevated in patients with nonmalignant diseases characterized by inflammation and tissue remodeling (18-20).

Serum YKL-40 may be useful for monitoring disease recurrence or progression in cancer patients after treatment (13, 14, 23-25). High serum YKL-40 during follow-up after curative operation for colorectal cancer (23) or stage I to II melanoma (14) were associated with short recurrence-free interval and short survival. During follow-up of patients operated for high-grade gliomas serum YKL-40 was lower in patients with no radiographic evidence of disease compared with patients with signs of disease, and high serum YKL-40 was associated with short survival (13). In patients with metastatic prostate cancer treated with endocrine therapy, an increase in serum YKL-40 during treatment was a predictor of short survival (24). During follow-up of patients with squamous cell carcinoma of the head and neck after radiotherapy, high serum YKL-40 predicted short survival (11). Serum YKL-40 decreased significant in patients with locally advanced breast cancer who responded to neoadjuvant chemotherapy (26). Future clinical cancer studies using serum YKL-40 to monitor disease progression may find the observed changes useful as preliminary "guidelines."

Mechanistically, the functions of YKL-40 in cancer diseases are unknown. YKL-40 may play a role in

cancer cell proliferation, differentiation, metastasis potential, protects against apoptosis, stimulates angiogenesis, and regulates extracellular tissue remodeling; however, *in vivo* proofs are needed (1). YKL-40 expression is up-regulated in glioblastoma cells following stress stimuli like serum depletion, hypoxia, ionizing radiation, and chemotherapy (27), and in vascular endothelial growth factor siRNA glioma, YKL-40 was one of the highest up-regulated genes, suggesting a role in regulating response of tumor cells to hypoxia (28). Furthermore, astrocytes transfected with YKL-40 had increased resistance to serum depletion and irradiation, and increased invasion potential (29). YKL-40 is a growth factor for fibroblasts, acts synergistically with insulin-like growth factor-I, is regulated by TNF- α and IL-6, and requires sustained activation of nuclear factor κ B. YKL-40 initiates mitogen-activated protein kinase and Phosphoinositide 3-kinase signaling cascades in fibroblasts, leading to the phosphorylation of extracellular signal-regulated kinase-1/2 mitogen-activated protein kinase and protein kinase B (AKT)-mediated signaling cascades associated with mitogenesis (1, 30, 31). YKL-40 also binds collagens; modulates type I collagen fibril formation (32); may have a role in proliferation, activation, and differentiation of fibroblasts or myofibroblasts surrounding cancer cells; and may influence the development of the desmoplastic fibroblast stroma, which is very abundant in several types of cancer. YKL-40 also acts as a chemoattractant for endothelial cells, stimulates their migration, and promotes migration and adhesion of vascular smooth muscle cells, suggesting a role in angiogenesis (33-35).

In conclusion, the present study could not show significant diurnal variation in serum YKL-40 nor an effect of physical exercise. A relatively low within-subject variation compared with between subject variation in serum YKL-40 was shown, suggesting that YKL-40 could be a reliable biomarker.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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