YKL-40 (Chitinase-3-Like Protein 1) Serum Levels in Aortic Stenosis

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BACKGROUND: Identification of novel biomarkers could provide prognostic information and improve risk stratification in patients with aortic stenosis (AS). YKL-40 (chitinase-3-like protein 1), a protein involved in atherogenesis, is upregulated in human calcific aortic valves. We hypothesized that circulating YKL-40 would be elevated and associated with the degree of AS severity and outcome in patients with symptomatic AS.

METHODS: Plasma YKL-40 was analyzed in 2 AS populations, one severe AS (n=572) with outcome measures and one with mixed severity (n=67). YKL-40 expression in calcified valves and in an experimental pressure overload model was assessed.

RESULTS: We found (1) patients with AS had upregulated circulating YKL-40 compared with healthy controls (median 109 versus 34 ng/mL, $P<0.001$), but levels were not related to the degree of AS severity. (2) High YKL-40 levels (quartile 4) were associated with long-term (median follow-up 4.7 years) all-cause mortality (adjusted hazard ratio, 1.93 [95% CI, 1.37–2.73], $P<0.001$). (3) YKL-40 protein expression in human calcifi valves co-localized with its putative receptor IL-13rα2 in close proximity to valve interstitial cells. (4) Myocardial YKL-40 increased in experimental pressure overload (6-fold in decompensated versus sham mice).

CONCLUSIONS: YKL-40 levels were elevated in AS and associated with mortality but not with other metrics of disease severity including the degree of AS severity. Despite scientific rationale for its role in AS, the clinical utility of circulating YKL-40 as a biomarker is limited.

REGISTRATION: URL: https://www.clinicaltrials.gov; Unique identifier: NCT01794832.

Key Words: aortic valve stenosis ■ biomarkers ■ inflammation ■ prognosis ■ survival analysis

Aortic valve stenosis (AS) is the most common cause of left ventricular (LV) outflow obstruction and aortic valve replacement (AVR) surgery. It is the third most common cardiovascular disease after coronary artery dis- ease and hypertension.[1,2] Due to changing demographics with an increasing elderly population, the burden of AS is predicted to increase.[3,4] Progression of AS is an active process involving macrophage-driven inflammation, osteogenesis, and remodeling of the extracellular matrix comparable to, but also distinct from, atherogenesis.[1,5]

YKL-40 (chitinase-3-like protein 1) is a plasma glycoprotein and a member of the mammalian chitinase-like protein, secreted by several cell-types. In the presence of inflammation, activated macrophages and neutrophils are the most important cellular sources of YKL-40.[6] The precise function of YKL-40 has not been established, but it plays a role in inflammation, fibrosis, and extracellular matrix remodeling.[7] Bossé et al[8] identified YKL-40 as one of the most upregulated mRNAs in human calcific aortic valves compared with healthy aortic valves,

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WHAT IS NEW?

- Biomarkers that can monitor progression, severity, and prognosis of aortic stenosis are needed improve risk stratification and decision making regarding timing of aortic valve area and follow-up.
- YKL-40 expression has been shown to be markedly upregulated in calcified aortic valves.
- We found that circulating YKL-40 was not correlated with aortic stenosis severity but was associated with poor prognosis.

WHAT ARE THE CLINICAL IMPLICATIONS?

- Circulating YKL-40 does not give useful information regarding severity of aortic stenosis and cannot contribute to decision making.
- High circulating YKL-40 or an increase in levels over time may carry important prognostic information.

Nonstandard Abbreviations and Acronyms

AS	aortic valve stenosis
AVA	aortic valve area
AVR	aortic valve replacement
HR	hazard ratio
hsCRP	high sensitivity C-reactive protein
hsTnT	high-sensitive troponin T
LV	left ventricular
MACE	major adverse cardiovascular events
NT-proBNP	N-terminal pro-B-type natriuretic peptide

but data on circulating levels of YKL-40 in AS patients are lacking.

Use of biomarkers could improve risk stratification in AS and aid clinical decision-making by providing prognostic information. Recent studies using NT-proBNP (N-terminal pro-B-type natriuretic peptide) and hsTnT (high–sensitive troponin T) demonstrated the value of biomarkers for evaluating patients with AS.[9,10] Several studies revealed increased circulating YKL-40 in patients with atherosclerosis and heart failure and showed that high YKL-40 levels confer risk of long-term adverse outcome.[6,11,12] Based on the enhanced YKL-40 mRNA levels in aortic calcified valves and similarities between AS and atherosclerosis, we hypothesized that circulating levels of YKL-40 may also be upregulated and associated with outcome (ie, all-cause mortality) in patients with severe symptomatic AS. To test these hypotheses, we evaluated if (1) circulating YKL-40 levels were increased in patients with AS versus healthy controls, correlated with hemodynamic measures, severity of AS, or mortality; (2) YKL-40 was expressed in calcified valves; and (3) myocardial YKL-40 was upregulated in an experimental AS mouse model using aortic banding.

METHODS AND MATERIALS

Patients

The data from this study are available on request from the corresponding author (Dr Ueland). We determined circulating plasma YKL-40 in 2 populations of consecutively enrolled patients with AS, who had been evaluated for AVR, according to current guidelines. Inclusion/exclusion criteria and details on AVR and outcome data in these populations are show in Figure I and Table I in the Data Supplement, while baseline demographics are presented in Table 1. Population 1 consisted of 2 cohorts with AS obtained from our tertiary center (Oslo University Hospital Rikshospitalet, Norway) on 2 occasions. Cohort 1: One hundred thirty-six patients evaluated between May 2005 and January 2007. This was a cross-sectional design, and patients were followed for a mean 4.3 years, and data on all-cause mortality was obtained. Cohort 2: Four hundred thirty-six patients evaluated between May 2010 and March 2013. A second blood sample was obtained after 1-year from 319 patients in this cohort. Patients were followed for a mean 4.0 years, and data on all-cause mortality was obtained (see next paragraph). In addition, major adverse cardiovascular events (MACE) were a prespecifi outcome in this cohort (see defi of MACE below). A second population (population 2; URL: https://www.clinicaltrials.gov; Unique identifi NCT03422770) consisted of 62 patients (Table 1) with confi AS evaluated for AVR surgery or just followed at the out-patient clinic. These were consecutively enrolled between January and November 2018 at St Olav's Hospital, Trondheim, Norway. This population was not followed for outcome. AS severity in both populations was classifi according to guidelines.[13,14]

For comparison, 76 healthy controls without AS (no AS) sampled between November 2009 and November 2010, with no history of chronic disease or use of any medications and normal basic clinical chemistry data (Table 1) were used.

For evaluation of *YKL*-40 levels in AS and associations with AS severity (ie, aortic valve area [AVA] and aortic mean gradient), we combined both population 1 and population 2. For evaluation of outcome, population 1 was used for assessing all-cause mortality and cohort 2 of population 1 was used for accessing MACE, since these data were available only in the indicated populations and cohorts.

Informed consent was obtained from each study subject. The study protocols were approved by the regional committee for ethics in medicine, including approval from local hospitals and complied with the Declaration of Helsinki.

Mortality and MACE

For population 1, we retrieved complete all–cause mortality data from the Norwegian National Cause of Death Registry and the causes of death was obtained from the Norwegian Cause of Death Registry. For cohort 2 of population 1, we also reviewed the patients' medical records from their operating and local hospitals to acquire data regarding all adverse clinical events during the year following study inclusion. The composite end point of MACE comprised the time from baseline to diagnosis of transient ischemic attack, stroke, myocardial infarction, all–cause death or hospitalization for cardiovascular cause (endocarditis, myocarditis, heart failure, atrial fibrillation; Table I in the Data Supplement). To ensure completeness of data, we reviewed the official clinical coding system for diagnosis and procedures used in Norway (International Statistical

Table 1. Control and Patient Characteristics

	No AS	AS
Variables	n=76	n=639
Mean age, y	66±8	74±11*
Male sex, n (%)	44 (57)	366 (57)
Body mass index, kg/m^2	25.0±3.3	26.0±4.3
NYHA class III/IV	---	264 (47)
AVR	---	448 (78)
Severity AS, mild/moderate/ severe, n	---	10/38/591
Medical history, n (%)		
Hypertension	0 (0)	262 (41)*
Atrial fibrillation, all types	0 (0)	137 (21)*
Diabetes mellitus type I and II	0 (0)	63 (10)*
Coronary artery disease	0 (0)	129 (20)*
Medication, n (%)		
β-blocker	0 (0)	274 (43)*
ACEi/ARB	0 (0)	241 (38)*
Warfarin	0 (0)	111 (17)*
Statin	0 (0)	314 (49)*
Hemodynamics		
LVEF, %	---	56±11
Cardiac index, L/(min·m^2)	---	2.6±0.6
IVS, cm	---	1.0±0.2
LVID, cm	---	4.9±0.8
LVPW, cm	---	1.1±0.2
E/A ratio	---	1.0±0.5
Aortic mean gradient, mmHg	---	53±19
Aortic valve area, cm^2	---	0.7±0.3
Biochemistry		
Creatinine, µmol/L	75±14	85±31†
eGFR, mL/min	87±14	74±3*
NT-proBNP, ng/L median (IQR)	8 (4-13)	847 (313-2143)*
Hs-TnT, ng/mL median (IQR)	---	13 (10-24)
Hs-CRP, mg/L	1.2 (0.8-2.2)	1.8 (0.8-4.9)‡

Categorical data is presented as n (%) while continuous data is given as mean±SD or median and 25th/75th percentile depending on distribution. Full data sets were not available for all measures. ACEi indicates angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; AS, aortic valve stenosis; AVR, aortic valve replacement; E/A ratio, ratio of the early (E) to late (A) ventricular filling velocities; eGFR, estimated glomerular filtration rate (Cockcroft–Gault formula); hs-CRP, high-sensitivity C-reactive protein; hs-TnT, high-sensitivity troponin T; IQR, interquartile range; IVS, intraventricular septum; LVEF, left ventricular ejection fraction; LVID, left ventricular internal diameter; LVPW, left ventricular posterior wall; NT-proBNP, N-terminal pro-B-type natriuretic peptide; and NYHA, New York Heart Association.

*P<0.001.

†P<0.01.

‡P<0.05.

Classification of Diseases and Related Health Problems; ICD–10), and read the journal texts of each medical record.

Echocardiography

Dimensional and velocity parameters were calculated as described previously and are detailed in the Data Supplement.[15]

Blood Sampling Protocol

Peripheral venous blood was drawn into pyrogen-free EDTA tubes. The tubes were immediately immersed in melting ice and centrifuged within 30 minutes at 2000*g* for 20 minutes to obtain platelet-poor plasma. All samples were stored at −80°C and thawed <3×.

Biochemistry

NT-proBNP, hsCRP (high sensitivity C-reactive protein), hsTnT, and standard biochemistry was assayed as described,[15] while plasma YKL-40 was measured by enzyme immune-assay (R&D Systems, Minneapolis, MN). See the Data Supplement for details.

Aortic Valve Sampling

For immunohistochemistry and immunofluorescence, aortic valve specimens were obtained from patients (n=4) undergoing elective AVR surgery with no history of other cardiac or concomitant disease (n=2) or macroscopic signs of heart disease (n=2) obtained from autopsies. Aortic valve specimens were immediately immersed in formalin and then embedded in paraffin. For mRNA analysis, aortic valve specimens were obtained from 16 patients undergoing elective AVR surgery and immediately flash frozen in liquid nitrogen and stored at −80°C.

Immunohistochemistry and Immunofluorescence

For immunohistochemistry, 5µ sections of paraffin-embedded aortic valves were deparaffinised in xylene and hydrated in alcohol series, and treated with 0.5% H_2O_2, followed by high-temperature antigen retrieval in citrate-buffer (pH 6), blocked with 0.5% BSA and then incubated with primary antibodies against human YKL-40 (polyclonal, 4815, QUIDEL, San Diego, CA; 1:100) or its putative receptor IL-13rα2 (Interleukin-13 receptor subunit alpha-2; polyclonal, AF146, R&D systems, Oxon, United Kingdom; 1:25) for 1 hour at room temperature. After washing, the slides were incubated for 30 minutes with peroxidase-conjugated secondary antibody (Impress-Vector, Vector Laboratories, Burlingame, CA), rinsed, and developed with chromogen for immunoperoxidase staining (DAB Plus, Vector Laboratories) for 7 minutes. The sections were counterstained with hematoxylin. Omission of the primary antibody was used as a negative control.

For immunofluorescence, 5-micron sections of paraffin-embedded aortic valves were deparaffinized in xylene, rehydrated in alcohol series and immersed in distilled water, followed by high-temperature antigen retrieval in citrate buffer (pH 6) and blocked with 0.5% BSA. The slides were stained with primary antibodies against YKL-40 (polyclonal, 4815, QUIDEL, San Diago, CA; 1:100), IL-13rα2 (polyclonal, AF146, R&D systems, Oxon, United Kingdom; 1:25) and vimentin (monoclonal, M0725, Clone V9, Dako, Glostrup, DK; 1.100) for 1 hour at room temperature and counterstained with Alexa Fluor F488 goat anti-rabbit IgG (1:500), Alexa Fluor donkey anti-goat IgG (1:500) or Alexa Fluor 568 goat anti-mouse or Rat on Mouse AP Polymer kit in combination with Warp Red Chromogen kit (Biocare Medical, San Francisco, CA), respectively. The slides were mounted with SlowFade Gold antifade reagent with DAPI

(Life Technologies, Carlsbad, CA). Images were taken by a Nikon Eclipse E400 microscope (Tokyo, Japan).

Mouse Model of Experimental LV Pressure Overload

The mouse models have been described in elsewhere[16,17] (see the Data Supplement for details).

mRNA Analysis

Total RNA from mouse LV, human aortic valve specimens, and cardiac cells was extracted and analyzed as described.[16] See the Data Supplement for details.

Statistics

Descriptive statistics are provided as frequencies and proportions for categorical variables, and as mean and SD or median and interquartile range as appropriate for continuous variables. Means and proportions were compared using χ^2 tests for categorical variables or *t* tests or the Kruskal Wallis test for continuous variables. For survival analysis, Kaplan-Meier analysis with log-rank test was performed to compare the number of events in quartiles of YKL-40). The importance of YKL-40 as a risk factor for all-cause mortality was investigated by multivariable Cox-regression with a predefined adjustment strategy as described previously.[15] More details on the statistical methods are given in the Data Supplement.

RESULTS

Characteristics of the Study Groups

Table 1 shows demographics of patients with AS (population 1 and 2) and healthy controls (no AS). Comparing demographics between the patients and controls (Table 1) revealed a heathy control population that was younger, had better kidney function and as expected markedly lower NT-proBNP levels than patients with AS.

YKL-40 Levels in Patients With AS

Figure 1A shows the range of YKL-40 values in all patients with AS divided into quartiles. Patients with AS, on average, had higher YLK-40 levels than control patients, adjusted for age, gender, and body mass index (Figure 1B, $P<0.001$). Further adjustment for estimated glomerular filtration rate and NT-proBNP had no impact on this difference ($P<0.001$ using all covariates). Receiver operating characteristic analysis revealed good discrimination of AS from controls (area under the curve=0.89, Figure 1B) and a cutoff of 51 ng/mL gave high sensitivity (0.97) and moderate specificity (0.79). YKL-40 was inferior to NT-proBNP (area under the curve=0.93) but superior to hsCRP (area under the curve=0.60) for detection of AS.

Figure 1C shows YKL-40 levels in all patients with AS according to severity. No significant differences in YKL-40 between mild, moderate, and severe AS were detected. As shown in Figure 1D, YKL-40 correlated modestly with AVA ($r=-0.20$, $P<0.001$), but not with aortic mean gradient in AS ($r=0.06$, $P=0.11$).

Table II in the Data Supplement shows correlations between YKL-40 and demographic features in patients with AS. YKL-40 correlated with multiple variables including comorbidities, medications, and biochemistry, and multivariable linear regression revealed advancing age and New York Heart Association class, history of hypertension, warfarin use and high hsCRP and hsTnT levels as the strongest predictors of YKL-40 level.

YKL-40 and Outcome

As stated in methods, Table I and Figure I in the Data Supplement show details on outcome measures, including which populations they were available in, follow-up times and details on MACE. Data on all-cause mortality were available in population 1 (n=572), while MACE data were available in cohort 2 of population 1 (n=436).

During a mean follow-up of 4.2 years (range, 0.1–6.8 years) in population 1, 170 (30%) patients died; 78 (46%) in the nonsurgical group and 92 in the AVR (54%) group. Figure 2A shows an association between the concentration of YKL-40 and all-cause mortality, especially in those with the highest YKL-40 levels (Q4). When evaluating associations between YKL-40 alone and all-cause mortality in cox regression, we included AVR status, since this had a large effect on mortality. Thus adjusting for AVR gave a hazard ratio (HR) for all-cause mortality of 2.47 (95% CI, 1.81–3.37) for Q4.

During the first year of follow-up, 22% of the patients in cohort 2 of population 1 experienced MACE, mostly due to hospitalization for cardiovascular causes (Table I in the Data Supplement). As depicted in Figure 2B, increasing YKL-40 levels were associated with MACE. This was mainly driven by patients experiencing MI, who had higher YKL-40 levels (mean 368 ng/mL) than patients experienced TIA/stroke (mean 134 ng/mL), were hospitalized for cardiovascular causes (mean 207 ng/mL) or who died within the first year (mean 211 ng/mL). Comparing Q4 of YKL-40 with the lower quartiles, adjusting for AVR, this gave a HR of 1.82 (1.19–2.78; Table 2). Cox models for All-Cause mortality and MACE using YKL-40 as a continuous variable with HR expressed per SD change in log YKL-40 gave similar results (Table 2).

For multivariable analysis we used a predefined strategy established and reported for cohort 2.[15] Adjustment variables were in addition to AVR status: age at inclusion, gender, estimated glomerular filtration rate), New York Heart Association class, left ventricular ejection fraction, NT-proBNP, hsTnT, hsCRP, AVA, symptoms (symptomatic or asymptomatic). Figure 3 shows all adjustment variables included in this model with adjustment for AVR alone (due

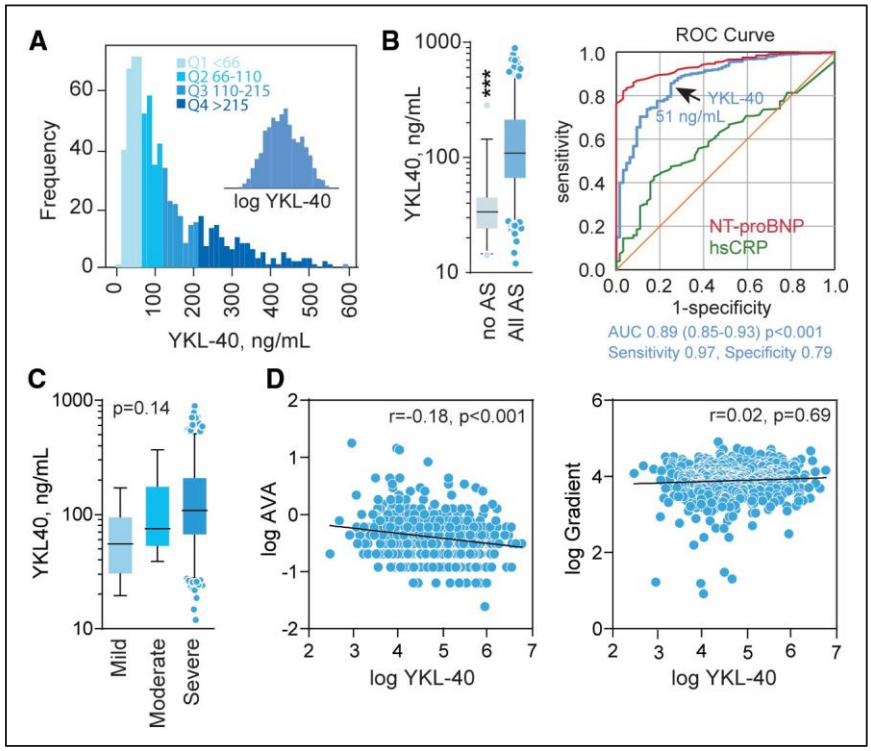

Figure 1. Plasma YKL-40 (chitinase-3-like protein 1) in patients with aortic stenosis (AS).
A, Distribution of plasma levels of YKL-40 in patients with AS (all patients, n=634). **B**, Plasma YKL-40 levels in patients with AS (population 1-2 combined) and in 76 healthy controls (No AS). *P<0.001 in adjusted analysis including age, sex, body mass index (BMI), estimated glomerular fi rate (eGFR), and NT-proBNP (N-terminal pro-B-type natriuretic peptide). Receiver operating characteristic (ROC) curve with area under the curve and sensitivity/specifi determined at the optimal cutoff for discrimination of AS from controls (YKL-40=51 ng/mL). **C**, Plasma YKL-40 levels according to severity in all patients with AS, using age, BMI, sex, eGFR, and NT-proBNP as covariates. Data are given as Tukey plot with whiskers at 2.5 and 97.5 percentiles in **B** and **C**. **D**, Correlation between YKL-40 and aortic valve area (AVA) and mean aortic gradient in all patients. hsCRP indicates high sensitivity C-reactive protein.

to its large impact on mortality) as well as with full multivariable adjustment. The association between Q4 YKL-40 and all-cause mortality was attenuated in the multivariable model, but remained significant and indicated a 1.9 times (P<0.001) higher risk of death with high YKL-40 levels (Table 2). This gave a c-index in the full model (with YKL-40: 0.76 and without: 0.74, and a difference of 0.012, P=0.056). Low left ventricular ejection fraction was also significantly associated with death in the fully adjusted model. Adjustment for other clinical factors eliminated the association between YKL-40 and MACE (Table 2).

YKL-40 During Follow-Up

In population 1, cohort 2, a second blood sample was obtained after 1-year follow-up in 318 patients with AS (Figure I in the Data Supplement), with subsequent clinical follow-up with a median time of 4.9 years. Of these, 66 patients died. The change in YKL-40 from baseline to follow-up was similar in AVR and non-AVR patients, and these were combined for further analysis. As shown in Figure 4A, a modest increase in YKL-40 was observed over time. Patients who had an increase in YKL-40 over time (ie, delta YKL40 >0 ng/mL, n=215, 68%) had poorer prognosis as evaluated by all-cause mortality (Figure 4B).

Presence of YKL-40 in Calcified Aortic Valves

To evaluate the presence of protein levels of YKL-40 and its putative receptor IL-13rα2[18] in calcifi aortic valves, we performed immunostaining of 4 aortic valves removed during AVR. Comparative immunohistochemical analyses were performed on aortic valves from persons with no medical history or macroscopic signs of heart disease (n=2) obtained from autopsies. YKL-40

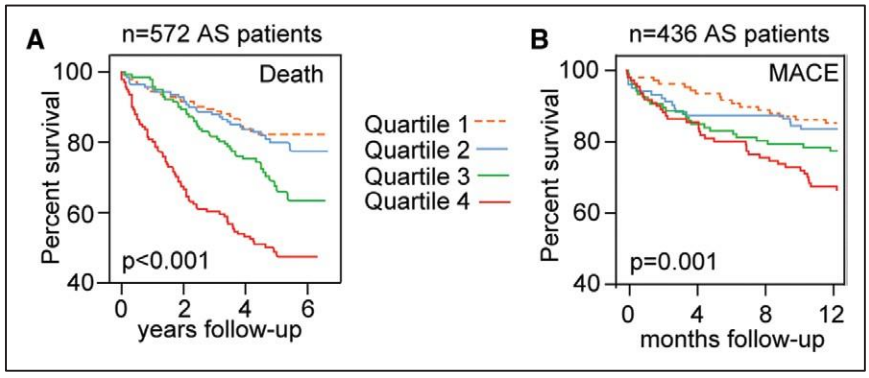

Figure 2. Association between plasma YKL-40 (chitinase-3-like protein 1) and outcome in patients with severe aortic stenosis (AS).
Kaplan-Meier curves showing the cumulative incidence of (**A**) all-cause mortality (n=170 deaths, median follow-up: 4.7 y) and (**B**) major adverse cardiovascular events (MACE, n=95) during the first year of follow-up according to quartiles of YKL-40. Quartile 1: <66 ng/mL, quartile 2: 66-110 ng/mL, quartile 3: 110-215 ng/mL, quartile 4: >215 ng/mL.

Table 2. Univariate and Multivariable Cox Models for YKL-40 (Chitinase-3-Like Protein 1) Given as Hazard Ratios (95% CI) and *P* Values, Evaluated as Dichotomized at Quartile 4 and as a Continuous Variable With HR Expressed per SD Change in Log YKL-40, and Association With All-Cause Mortality In Population 1 and MACE in Population 1 Cohort 2

	All-Cause Mortality		MACE	
	n=572, Events n=170 (30%)		n=436, Events n=95 (22%)	
	Univariate*	Multivariable	Univariate*	Multivariable
YKL-40 Q4	2.47 (1.81-3.37); *P* <0.001	1.93 (1.37-2.73); *P* <0.001	1.82 (1.19-2.78); *P*= 0.006	1.34 (0.84-2.12); *P*=0.22
YKL-40 log/SD	1.60 (1.37-1.88); *P* <0.001	1.35 (1.12-1.64); *P*= 0.002	1.42 (1.16-1.75); *P*= 0.001	1.12 (0.89-1.41); *P*=0.35

HR indicates hazard ratio; and MACE, major adverse cardiovascular events.

*Aortic valve replacement included in univariate analysis. Multivariable model: age, sex, aortic valve area, aortic valve replacement, estimated glomerular filtration rate, high-sensitivity C-reactive protein, high-sensitivity troponin T, left ventricular ejection fraction, N-terminal pro-B-type natriuretic peptide; New York Heart Association.

and the putative receptor IL13rα2 were present and more abundant in the calcified aortic valves compared with normal ones, although large heterogeneity was seen. For YKL-40 and IL-13rα2, a strong cell localization in fi cells was observed (Figure 5A and 5B). This was supported by immunofl staining showing co-staining with vimentin, a mesenchymal cell marker, of both YKL-40 (Figure 5C) and IL-13rα2 (Figure 5D) supporting the expression of YKL-40 and its receptor in valve interstitial cells.

In 16 patients (demographics in mean±SD or %: age 70±12, 50% women, AVA 0.70±0.19, aortic mean gradient 57±28, 44%≥ New York Heart Association 3), we determined mRNA levels of YKL-40 in aortic valves removed during AVR and protein level of YKL-40 in parallel plasma samples. However, no association was detected (r=0.28, P=0.30).

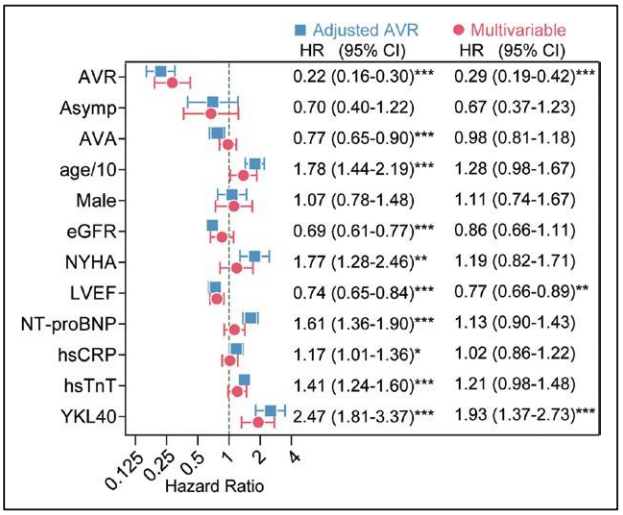

Figure 3. Multivariable Cox regression of YKL-40 (chitinase-3-like protein 1) and all-cause mortality in patients with symptomatic aortic stenosis.

Blue circles: hazard ratio (HR) and 95% CI adjusted for aortic valve replacement (AVR) status (n=572), red squares: HR and 95% CI in the fully adjusted multivariable model (n=496). AVA indicates aortic valve area; eGFR, estimated glomerular filtration rate; hsCRP, high-sensitivity C-reactive protein; hsTnT, high-sensitivity troponin T; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro-B-type natriuretic peptide; and NYHA, New York Heart Association. $^{*}P$<0.05, $^{**}P$<0.01, $^{***}P$<0.001.

YKL-40 During Experimental Pressure Overload

To further examine the association between YKL-40 and AS, we examined the myocardial expression of YKL-40 and IL-13rα2 in an experimental model of myocardial pressure overload (ie, aorta banding), relevant to AS. As shown in Figure IIA in the Data Supplement, we found significant myocardial upregulation of YKL-40 in hearts with compensated and decompensated (significantly increased wet lung weight) LV hypertrophy 3 weeks following aorta banding as compared with sham-operated mice. In a separate experiment, we evaluated the cell specific expression of YKL-40 in sham-operated mice by measuring YKL-40 mRNA in 4 distinct cell populations within the heart (ie, cardiomyocytes, $CD45^{+}$ leukocytes, $CD31^{+}$ endothelial cells, and fibroblast-like cells; see Methods). YKL-40 mRNA levels were markedly higher in cardiac fibroblast-like cells, followed by leukocytes while expression was low in endothelial cells and cardiomyocytes (Figure IIB in the Data Supplement).

DISCUSSION

The present study evaluated YKL-40 in clinical and experimental AS. Our major findings were (1) we found enhanced plasma levels of YKL-40 in patients with AS compared with healthy controls; (2) YKL-40 protein expression in human calcific valves co-localized with its putative receptor IL-13rα2 in close proximity to valve interstitial cells; (3) mice with chronic pressure overload displayed elevated myocardial YKL-40 mRNA levels. (4) While high YKL-40 was associated with poor prognosis in patients with AS , circulating levels were not associated with the degree of AS severity. Thus, while patients with AS had enhanced plasma levels of YKL-40, we could not link YKL-40 levels to progression of disease, limiting its clinical utility as a biomarker in these patients.

Previously, Bossé et al[8] identified YKL-40 as one of the most upregulated mRNAs in human calcified aortic valves, prompting the current study. Indeed, we found support for enhanced YKL-40 expression in AS. Clinical AS was characterized by elevated YKL-40 levels in plasma compared with controls, and mice with chronic pressure overload, an experimental model with similarities

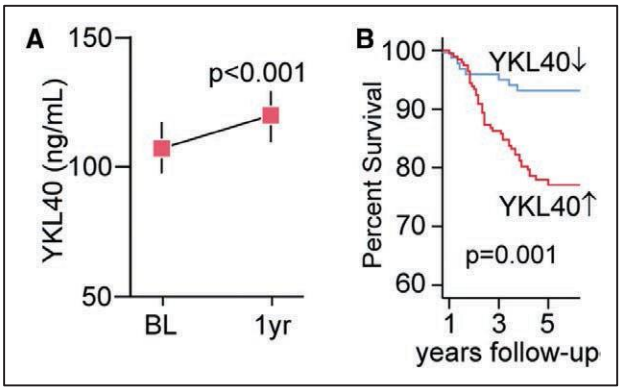

Figure 4. Plasma YKL-40 (chitinase-3-like protein 1) during follow-up in aortic stenosis (AS).
A, YKL-40 levels at baseline and 1-year follow-up in 318 patients with AS from population 1 cohort 2. Data are shown as back-transformed log YKL-40 levels and reflect geometric mean and 95% CI. **B**, Kaplan-Meier analysis of all-cause mortality (n=66, 21%, median follow-up 4.9 y) according to if patients increased (↑ n=205) or decreased (↓ n=113) YKL-40 during follow-up.

to AS, displayed elevated myocardial YKL-40 levels. Furthermore, in calcified valvular tissue from patients with AS, YKL-40 and its receptor co-localized in valve interstitial cells. However, a clinically useful biomarker in AS should reflect disease severity and potentially be used to determine the optimal timing of AVR. Further analysis revealed little support for a prominent role for YKL-40 in AS progression or clinical utility as a biomarker. Thus, while present in calcified aortic valves, YKL-40 mRNA levels did not correlated with systemic levels. Importantly, circulating YKL-40 correlated poorly with the degree of valvular stenosis as reflected by hemodynamic indices (ie, AVA and gradient) and could not distinguish patients with mild, moderate, or severe AS. Taken together, while our data support the upregulation of YKL-40 in AS, the enhanced levels do not seem to be related to the pathophysiology or progression of the disease, and argue against further studies in these patients.

YKL-40 seems to be a universal marker for infl - mation and extracellular matrix remodelling and is elevated in multiple disorders with an element of infl - mation and tissue remodelling,[7,19] including coronary artery disease.[11,20]

The elevated YKL-40 levels in our patients with AS compared with healthy controls, but lack of association with severity of AS may suggest the higher levels reflect an element of atherosclerosis or other underlying vascular conditions. Indeed, a range of demographic variables correlated with YKL-40 and could contribute to higher circulating levels including age, hsCRP, hypertension, hsTnT, and New York Heart Association class. The elevated myocardial YKL-40 mRNA levels we observed in experimental pressure overload, with high expression in cells involved in inflammation, fibrosis, and extracellular matrix remodeling, suggest that these tissues could contribute to elevated systemic levels. In our study, high YKL-40 levels were independently associated with all-cause mortality. We and others have shown that YKL-40 levels are elevated and associated with prognosis in heart failure,[11,12,21] a cause of death in unoperated AS.[22] Increased circulating YKL-40 in the general population is also associated with risk of death from multiple causes such as cardiovascular disease, cancer, and other chronic inflammatory diseases.[19,23] Thus, YKL-40 seems to be a quite nonspecific marker of poor prognosis, further limiting its clinical usefulness.

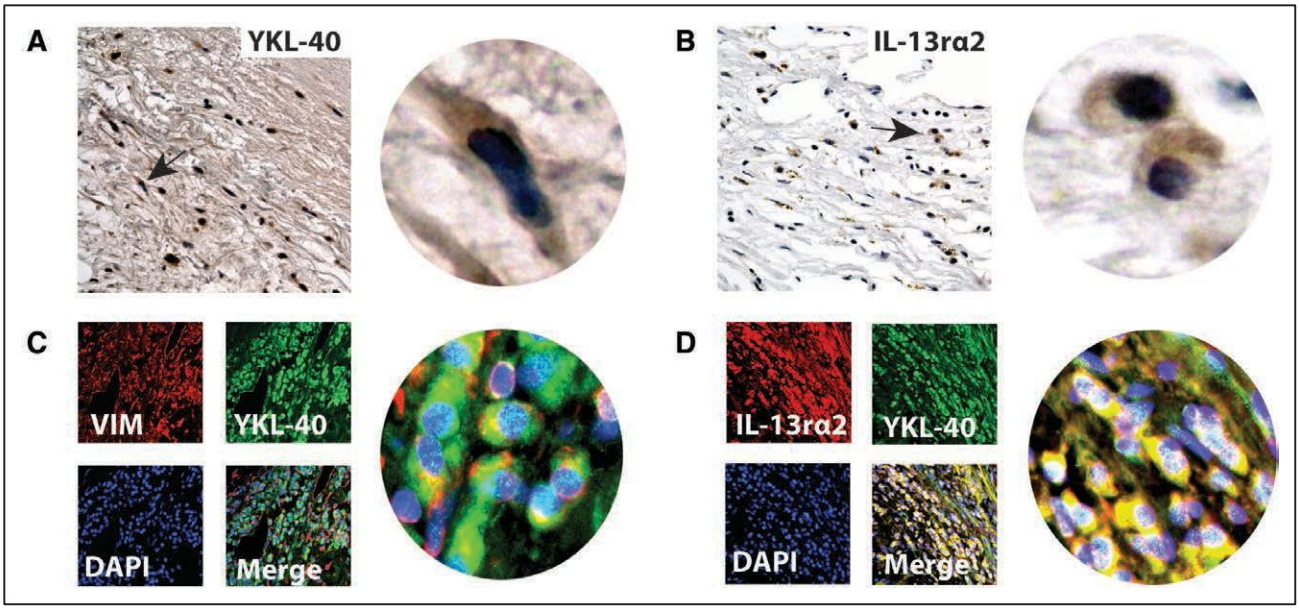

Figure 5. Localization of YKL-40 (chitinase-3-like protein 1) and its putative receptor IL-13rα2 (Interleukin-13 receptor subunit alpha-2) in calcified aortic valves.
Immunostaining of (**A**) YKL-40 and (**B**) IL-13rα2 in valves from patients with symptomatic aortic stenosis (representative images obtained with 40× objective), the magnifi circular image shows cells with positive staining. Co-staining of YKL-40 and (**C**) vimentin (a marker of VICs) and (**D**) IL-13rα2 in calcifi aortic valves indicating areas of co-localization. DAPI indicates 4',6-diamidino-2-phenylindole, Dilactate; and VIM, vimentin.

Limitations of our study include a relatively low number of patients in subgroup analyses. In particular, the number of patients with less severe AS in our study was limited, and a larger sample from patients at an earlier stage of AS with outcome data would provide more confidence. We also combined cohorts with varying data collection, different degrees of outcome ascertainment, and different duration of follow-up. Serial samples were available only in a small subset and particular caution needs to be taken with any conclusions drawn from those data. With experience, outcomes for TAVI have changed dramatically during the study period, for reasons we believe are unrelated to YKL-40 biology, so this cohort was not included in our analyses. Also, assessment of YKL-40 levels in blood from the coronary sinus and mRNA levels from myocardial tissue from patients with AS would give stronger information on the role and origin of YKL-40 in AS.

In conclusion, YKL-40 levels were elevated in patients with AS. Circulating YKL-40 is increased in multiple disorders and seems universally associated with adverse outcome. The lack of association between circulating levels and the degree of AS severity in this study indicates that YKL-40 has low clinical utility as a biomarker in patients with AS.

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