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# Increased YKL-40 expression in patients with carotid atherosclerosis

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ABSTRACT

*Objective:* We hypothesized a role for the inflammatory protein YKL-40 in atherogenesis and plaque destabilization based on its role in macrophage activation, tissue remodeling, and angiogenesis.
*Methods:* Serum YKL-40 levels were measured by enzyme immunoassay in 89 patients with carotid atherosclerosis and 20 healthy controls. Carotid expression of YKL-40 was examined by real time RT-PCR in 57 of the patients. Regulation and effect of YKL-40 were examined in THP-1 monocytes.
*Results:* Our main findings were: (1) serum YKL-40 levels were significantly elevated in patients with carotid atherosclerosis, with particularly high levels in those with symptomatic disease; (2) patients with recent ischemic symptoms (within 2 months) had higher YKL-40 mRNA levels in carotid plaque than other patients; (3) *in vitro*, the β-adrenergic receptor agonist isoproterenol, toll-like receptor (TLR) 2 and TLR4 agonists, and in particular releasate from activated platelets significantly increased the expression of YKL-40 in THP-1 monocytes and (4) *in vitro*, YKL-40 increased matrix metalloproteinase-9 expression and activity in THP-1 monocytes, involving activation of p38 mitogen-activated protein kinase.
*Conclusions:* Our findings suggest that YKL-40 might be a marker of plaque instability, potentially reflecting macrophage activation and matrix degradation within the atherosclerotic lesion.


## 1. Introduction

YKL-40, also called chitinase-3-like-1, is a 40 kDa heparin and chitin-binding glycoprotein without chitinase activity, expressed by macrophages in late phases of differentiation [1,2]. Under various inflammatory conditions, especially those characterized by tissue remodeling, other cellular sources of YKL-40 have also been reported such as vascular smooth muscle cells [3], neutrophils [4], and chondrocytes [5]. There are several reports of elevated serum YKL-40 levels in various inflammatory disorders such as type 2 diabetes mellitus [6], chronic obstructive pulmonary disease [7], inflammatory bowel disease [8–10], and rheumatoid arthritis [11], and YKL-40 has been suggested as a potential biomarker of inflammation and endothelial dysfunction [12]. However, although YKL-40 has been reported to play a role in macrophage activation, tissue remodeling, and angiogenesis, its role in physiological and pathophysiological processes is still unclear.

Atherosclerosis is a chronic disorder characterized by lipid accumulation and inflammatory responses. Until now, only a few reports on YKL-40 in atherosclerotic disorders have been published. Recently, Kucur et al. showed increased serum YKL-40 levels in patients with stable coronary artery disease (CAD) [13], and Boot et al. have previously reported expression of YKL-40 in human atherosclerotic lesions, primary located to macrophages [14]. However, the possible role of YKL-40 in atherosclerosis, and in particular its potential involvement in plaque destabilization, are far from clear. To further elucidate these issues, we examined the expression of YKL-40 in patients with asymptomatic and symptomatic carotid plaque, both systemically and within the lesion.

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**Table 1**
Baseline variables in patients according to symptomatic[a] and asymptomatic carotid plaques ($n = 89$).

| | Symptomatic plaques ($n = 54$) | Asymptomatic plaques ($n = 35$) | $p$ |
|---|---|---|---|
| Age (years) | 67 (46–83) | 67 (44–81) | 0.572 |
| Male sex[b] | 37 (68.5) | 23 (65.7) | 0.686 |
| Degree of stenoses (%) | 80 (60–95) | 80 (60–99) | 0.415 |
| Echolucent carotid plaque[b] | 19 (35.2) | 10 (28.6) | 0.440 |
| Ipsilateral ischemia on cerebral MRI[b] ($n = 47$) | 22 (78.6) ($n = 28$) | 15 (78.9) ($n = 19$) | 0.975 |
| Body mass index (kg/m$^2$) | 25.1 (20.4–35.5) | 26.8 (19–35.3) | 0.429 |
| Systolic blood pressure (mmHg) | 151 (110–200) | 150 (111–214) | 0.900 |
| Diastolic blood pressure (mmHg) | 80.00 (32–101) | 78 (49–102) | 0.183 |
| Coronary artery disease[b] | 26 (48.1) | 23 (65.7) | 0.106 |
| Statin treatment[b] | 46 (85.2) | 30 (85.7) | 0.843 |
| Aspirin treatment[b] | 46 (85.2) | 30 (85.7) | 0.975 |
| Clopidogrel treatment[b] | 18 (33.3) | 4 (11.4) | 0.018 |
| Combined aspirin/dipyramidole treatment[b] | 9 (16.7) | 4 (11.4) | 0.463 |
| Warfarin treatment[b] | 7 (13.0) | 4 (11.4) | 0.819 |
| Current smoking[b] | 29 (53.7) | 19 (54.3) | 0.902 |
| YKL-40 | 101.9 (26.3–663.5) | 72.9 (31.5–243.6) | 0.031 |
| Neopterin (nmol/l) | 8.7 (4.1–30.9) | 8.7 (5.7–60.3) | 0.667 |
| CRP (mg/l) | 4.0 (1–39) | 5 (0.6–55.0) | 0.784 |
| Total leukocyte count ($10^9$/l) | 8.3 (4.0–12.1) | 7.5 (3.8–11.8) | 0.114 |
| Cholesterol (mmol/l) ($n = 69$) | 4.4 (2.7–7.5) | 4.2 (2.8–7.5) | 0.229 |
| HDL cholesterol (mmol/l) ($n = 67$) | 1.3 (0.7–2.7) | 1.3 (0.8–2.6) | 0.944 |
| Triglycerides (mmol/l) ($n = 63$) | 1.3 (0.5–3.8) | 1.4 (0.6–3.5) | 0.411 |
| LDL ($n = 58$) | 2.6 (1.3–4.6) | 2.6 (1.5–5.1) | 0.127 |
| HbA1c (%) ($n = 77$) | 5.7 (4.4–9.5) | 5.8 (0.9–12.2) | 0.325 |
| β-Thromboglobulin (IU/ml) | 56.0 (26.7–325) | 55.2 (15.4–289) | 0.440 |
| Platelet count ($10^9$/l) | 276 (132–441) | 268 (168–386) | 0.989 |

Values are median (range).
[a] Clinical symptoms include stroke, TIA or amaurosis fugax ipsilateral to the stenotic internal carotid artery within the last 6 months.
[b] Values are numbers (percentages), otherwise.

## 2. Materials and methods

### 2.1. Patients and controls

Between January 2004 and January 2008, all patients ($n = 89$) that were referred to Rikshospitalet, Oslo University Hospital, Oslo for surgery for documented high-grade internal carotid stenoses (≥70%), were screened for inclusion in the study. Exclusion criteria were concomitant inflammatory disease such as infection and autoimmune disorder, liver or kidney disease, and malignancies. None of the referred patients had any of these conditions, most probably reflecting a selection of patients before they were referred for surgery, rendering a total study population of 89 patients (Table 1). The carotid stenoses were diagnosed and classified by precerebral colour Duplex and CT angiography according to consensus criteria [15,16]. The study population included 60 (67%) men and 29 (33%) women, median age 67 years (range 44–83 years).

The patients were classified into two groups according to their plaque symptomatology. Fifty-four (61%) patients had suffered from clinical symptoms such as stroke, transitory ischemic attack (TIA) or amaurosis fugax ipsilateral to the stenotic internal carotid artery within the past 6 months and 35 (39%) patients were characterized as asymptomatic (symptoms more than 6 months ago or had never suffered from symptoms as outlined above) (Table 1). The asymptomatic carotid stenoses were detected during clinical examinations of patients with CAD, peripheral artery disease or stroke/TIA more than 6 months ago. The plaques were also divided into two groups depending on plaque echogenicity on ultrasound, and classified as echolucent or echogenic/heterogeneous [17,18].

All patients were screened for co-morbid atherosclerotic disorders based on disease history, clinical findings and ECG, and 55% of the patients had accompanying CAD (Table 1). Therapy with aspirin, clopidogrel, warfarin, and statins were frequent (Table 1).

For comparisons, blood samples were also collected from 20 healthy (based on self reporting) volunteers, recruited from the same population and comparable to the patients with regard to age (59 ± 6 years) and sex (5 women and 15 men). The protocol was approved by the regional ethics committee. Signed informed consent of participation in the study was obtained from all participants.

### 2.2. Blood sampling protocol

Peripheral venous blood was drawn into pyrogen-free tubes with no anticoagulant. The blood was allowed to clot for 1 h at room temperature, centrifuged at 2500 × *g* for 25 min, and serum was stored in multiple aliquots at −80 °C. The samples were thawed only once.

### 2.3. Carotid endarterectomy specimens

Atherosclerotic carotid plaques were retrieved from 56 of the patients (not different from the total study population) during carotid endarterectomy, washed in phosphate-buffered saline (PBS) and immediately frozen in liquid nitrogen and stored at −80 °C until RNA extraction or immunohistochemical analysis. In addition, a small number of plaques were fixed with formalin for immunofluorescence microscopy.

### 2.4. Cell culture

The human monocytic cell line THP-1 (American Type Culture Collection, Rockville, MD) was cultured for 4 days in RPMI 1640 (PAA Laboratories, Pasching, Austria) supplemented with 2.5% fetal bovine serum (Gibco, Paisley, UK) in the presence of tumor necrosis factor α (TNF-α, 5 ng/mL, R&D Systems; Minneapolis, MN), before further incubation with or without different YKL-40 concentrations (Quidel Corporation, Santa Clara, CA), interleukin (IL)-1β (1 ng/mL, R&D Systems), a toll-like receptor (TLR)4 agonist (lipopolysaccharide, LPS) from *Escherichia coli* 026:B6 (5 ng/mL, Sigma, St Louis, MO), a TLR2 agonist ($Pam_3Cys$, 10 μg/mL, Sigma), isoproterenol (20 μmol/L, Sigma), or platelet releasate (see below). In some experiments, two blockers of p38 mitogen-activated protein kinase (SB239063, 50 μmol/L; Alexis Biochemicals, Farm-