

Serum YKL-40, a Potential New Marker of Disease Activity in Patients with Inflammatory Bowel Disease

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Background: YKL-40 is secreted by macrophages and neutrophils and is a growth factor for vascular endothelial cells and fibroblasts. Elevated serum concentrations of YKL-40 are found in patients with diseases characterized by inflammation or ongoing fibrosis. The aim of this study was to seek association between serum YKL-40 in patients with ulcerative colitis (UC) and Crohn disease (CD) and clinical disease activity. **Methods:** One-hundred-and-sixty-four patients with UC and 173 patients with CD were studied. The Simple Clinical Colitis Activity Index (SCCAI) and the Harvey-Bradshaw (H-B) score were used to assess disease activity. Serum YKL-40 (determined by ELISA) was related to C-reactive protein (CRP) and disease activity. **Results:** In patients with UC, the median serum YKL-40 rose with increasing disease activity, and patients with severe active disease had higher serum YKL-40 (median 59 µg/L (95% CI: 26–258 µg/L), $P < 0.001$) than patients with inactive UC (33 µg/L (19–163)) and age-matched controls (43 µg/L (20–124)). Patients with severe active CD had higher serum YKL-40 (59 µg/L (21–654), $P < 0.001$) than age-matched controls, but not higher than inactive CD patients (43 µg/L (17–306)). Serum YKL-40 was elevated in 41% of the patients with severe UC, in 10% with inactive UC, in 46% with severe CD and in 30% with inactive CD. Serum YKL-40 correlated with SCCAI in UC patients but not with H-B score in CD patients. In both patient groups, low correlations were found between serum YKL-40 and CRP, albumin and leucocytes. **Conclusions:** Serum YKL-40 is elevated in patients with active IBD and may be complementary to inflammatory markers and clinical characteristics in the assessment of disease activity.

Key words: Crohn disease; inflammation; serum YKL-40; ulcerative colitis

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Inflammatory bowel disease (IBD), the precise aetiology of which remains unknown, comprises mainly two forms of chronic relapsing intestinal inflammation—ulcerative colitis (UC) and Crohn disease (CD). Hallmarks of intestinal inflammation in IBD include a mononuclear cell and neutrophil infiltrate and mucosal ulceration with remodelling of the extracellular matrix (ECM). In both acutely and chronically inflamed intestines, healing of the damaged wall requires reconstruction of the tissue framework and remodelling of ECM components. Fibrosis is a non-specific result of the chronic inflammation that is observed clinically in both CD and UC (1, 2). The level and distribution of inflammatory cell infiltrates may determine the clinical outcome in IBD, including the increased submucosal collagen observed in UC (1) and transmural fibrostenosis, and the obstruction that frequently complicates CD (3). The main serological markers used to assess disease activity in IBD are C-reactive protein (CRP), erythrocyte sedimentation rate (ESR) and orosomucoid. These markers are unspecific and not produced locally in the inflamed intestinal tissue, but instead in the liver in response to increased cytokine

production. New informative serological markers that might identify high-risk patients of severe disease activity and ongoing fibrosis are therefore needed.

YKL-40 (also named human cartilage glycoprotein-39 (HC gp-39)) (4, 5) is a member of family 18 glycosyl hydrolases (4–8). The precise biological function of YKL-40 is unknown, but it has been reported that it has growth factor activity for fibroblasts (9), chondrocytes, synovial cells (10) and acts as a chemoattractant for endothelial cells. It stimulates migration of these cells at a level comparable to that achieved by basic fibroblast growth factor (11). YKL-40 also modulates vascular endothelial cell morphology by promoting the formation of branching tubules, indicating that it may function in angiogenesis by stimulating the migration and reorganization of vascular endothelial cells (11). YKL-40 works synergistically with IGF-1 in stimulating the growth of the fibroblasts and is effective in a concentration range similar to IGF-1 (9).

The pattern of YKL-40 expression in normal and disease states also suggests that the protein plays a role in inflammatory processes and in remodelling the extracellular

matrix. YKL-40 is secreted *in vitro* by activated neutrophil granulocytes (12) and macrophages during late states of differentiation (8, 13, 14). *In vivo*, the YKL-40 protein is expressed by macrophages in inflamed synovial membranes (15–17) and atherosclerotic plaques (18) and by giant cells and macrophages in the media of arteritic vessels of patients with giant cell arteritis (19). YKL-40 is also produced by chondrocytes (4, 17, 20) and cancer cells (21, 22). Moderate to significant elevated serum concentrations of YKL-40 are found in patients with a variety of diseases characterized by inflammation and tissue remodelling, such as active rheumatoid arthritis (17, 23–25), giant cell arteritis (19), bacterial infections (26–28), cancer (29, 30) and liver fibrosis (31, 32).

In a small study of 24 patients with IBD, the plasma level of YKL-40 was elevated compared to normals (25). Interestingly, YKL-40-derived peptides may be a target of the T-cell-mediated immune response in IBD (33) and rheumatoid arthritis (33–36). YKL-40 derived peptides that are predicted to bind to DRB1*0401 with the aid of a DRB1*0401 peptide-binding motif are selectively recognized by peripheral blood mononuclear cells (PBMC) from patients with rheumatoid arthritis and IBD, and induce a proliferative response of PBMC (33).

The aim of the present study was to determine serum YKL-40 concentrations in patients with UC and CD and to seek associations with clinical assessment of disease activity and serum CRP.

Methods

Patients

Three-hundred-and-thirty-seven consecutive patients with a diagnosis of IBD based on standard criteria (37) were studied; 164 had UC while 173 had CD. The patients were seen in the outpatient clinic or were hospitalized because of severe disease activity at the Dept. of Gastroenterology, Hvidovre Hospital during the period October 2000 to May 2001. Patient characteristics and clinical features are given in Table I. The patients were treated according to a strategy using systemic 5-aminosalicylic acid (5-ASA) as maintenance treatment and topical 5-ASA at relapsing episodes (5-ASA p.o.: UC $n = 96$, CD $n = 86$; 5-ASA supp: UC $n = 31$, CD $n = 4$). In flare-up periods, systemic steroids were used and tapered down during the following 2–3 months (low-dose steroid (0.5 mg/kg p.o.): UC $n = 11$, CD $n = 20$; high-dose steroid (1 mg/kg p.o.): UC $n = 8$, CD $n = 9$). Steroid-dependent and steroid-resistant IBD patients were treated with azathioprine (UC $n = 10$, CD $n = 32$). Patients with CD who developed fistulas or were steroid-dependent or -resistant were treated with infliximab ($n = 16$). Cyclosporin A was given to one steroid-resistant UC patient. The existing endoscopic or radiological localization of disease extent was not assessed.

The study was performed in accordance with the Helsinki II declaration, and the research protocol was approved by the

local ethics committee. The patients were informed about the study verbally and in writing, and all gave their written consent.

Clinical outcomes

Clinical assessment of disease activity was evaluated in the UC patients using the Simple Clinical Colitis Activity Index (SCCAI) (38) and in the CD patients using the Harvey-Bradshaw (H-B) index (39). The SCCAI and H-B indices are symptom-based, with ranges of 0–19 and 1–30, respectively. The indices consist of a 5-point score based on: in UC (SCCAI) 1) number of stools daily, 2) number of stools nightly, 3) blood in stools, 4) general well-being, and 5) extraintestinal manifestations; in CD (H-B) 1) number of stools daily, 2) abdominal mass, 3) abdominal pain, 4) general well-being, and 5) extraintestinal manifestations. As no definition has been outlined in the literature concerning inactive, mild to moderate and severe active disease, we defined disease activity as follows: a disease activity score of ≥ 1 in SCCAI was considered as active disease, with a score of 1–4 = mild to moderate disease and a score ≥ 5 = severe active disease (38–40). A disease activity score of ≥ 5 on the H-B index was considered as active disease, with a score of 5–8 = mild to moderate disease and of > 8 = severe active disease (41).

Biochemical methods

Blood samples were centrifuged at 2000g for 10 min, and serum was separated within 2 h and stored at -80°C until analysis was performed. Haemoglobin (normal range 7–11 mmol/L), total leucocytes (normal range $3\text{--}9 \times 10^9/\text{L}$), serum albumin (normal range 39.6–51.1 g/L) and serum CRP (normal < 20 mg/L) were determined by routine methods. Serum YKL-40 concentrations were determined using a two-site, sandwich-type enzyme-linked immunosorbent assay (YKL-40 ELISA, Quidel Corporation, Santa Clara, Calif., USA) (23) with streptavidin-coated microplate wells, a biotinylated-Fab monoclonal capture antibody, and an alkaline phosphatase-labelled polyclonal detection antibody. The source of the YKL-40 antigen used for standards and

Table I. Baseline demographics and clinical data

	Ulcerative colitis	Crohn disease
No. patients	164	173
Female/male	84/80	100/73
Age (years)	36 (15–84)	35 (18–92)
Disease duration (years)	5 (0–36)	6 (0–41)
Disease score*	2 (0–12)	4 (0–30)
Serum CRP (mg/L)	8 (8–244)	12 (8–280)
Serum albumin (g/L)	41.2 (19.0–49.8)	39.7 (21.2–48.8)
Haemoglobin (mmol/L)	8.5 (5.0–10.5)	8.3 (5.0–10.3)
Leucocytes ($10^9/\text{L}$)	7.1 (3.4–22.2)	8.3 (3.5–26.9)

Values are medians (range).

*Simple Clinical Colitis Activity Index (SCCAI) for patients with ulcerative colitis and Harvey-Bradshaw (H-B) score for patients with Crohn disease.

production of antibodies was purified from the conditioned medium of the human osteosarcoma cell line MG63. Sensitivity of the assay was 10 µg/L and the intra- and inter-assay variation coefficients were <3.6% and <5.3%, respectively. The median serum YKL-40 in 245 healthy adults (aged 18–79 years) is 43 µg/L. The upper limit (defined as the 90th percentile) of the serum concentration of YKL-40 is 59 µg/L in healthy controls aged 18–39 years (*n* = 91), 94 µg/L in controls aged 40–59 years (*n* = 86), and 152 µg/L in controls aged 60–79 years (*n* = 68). Further details about these healthy adults are described elsewhere (42).

Statistical analysis

The statistical analyses were performed with SigmaStat (SPSS Inc. Chicago, IL, USA). Results are given as median and 95% confidence interval (CI). Comparison between groups was performed using the non-parametric Mann-Whitney rank sum test. Correlation analyses between the different parameters were calculated using the Spearman test and *P* values less than 0.05 were considered to be significant.

Results

Fig. 1 illustrates the individual serum concentrations of YKL-40 in the UC (Fig. 1A) and CD (Fig. 1B) patients and in the healthy controls according to age. There was a significant correlation between serum YKL-40 concentration and age in the UC patients (Spearman's rho = 0.38, *P* < 0.001) and in the CD patients (rho = 0.36, *P* < 0.001). Serum YKL-40 levels could not discriminate between UC and CD patients. The median serum YKL-40 level in the UC patients was 44 µg/L (95% CI: 19–197 µg/L) and in the CD patients 54 µg/L (95% CI: 19–264 µg/L) and not different from each other or from age-matched controls (43 µg/L (95% CI: 20–124 µg/L)). There was no effect of gender on serum YKL-40 levels in the IBD patients.

Effect of disease activity on serum YKL-40 levels in IBD patients

Fig. 2 illustrates box plots of serum YKL-40 levels in patients with IBD according to disease activity. In patients with UC, the median serum YKL-40 level rose with increasing disease activity (Fig. 2a). Patients with inactive UC disease (SCCAI = 0, *n* = 61) had a median serum YKL-40 level of 33 µg/L (95% CI: 19–163 µg/L); those with mild/moderate UC (SCCAI = 1–4, *n* = 52) had a median serum YKL-40 level of 46 µg/L (95% CI: 14–203 µg/L); and those with severe UC (SCCAI = 5–12, *n* = 51) had a median serum YKL-40 level of 59 µg/L (95% CI: 26–258 µg/L). Serum YKL-40 levels for UC patients with severe disease were significantly higher (*P* < 0.001) than in patients with inactive disease and control patients, but not higher compared with patients with mild/moderate disease. Serum YKL-40 levels in patients with inactive UC did not differ from patients with mild/moderate disease (*P* = 0.059) and controls. Serum CRP

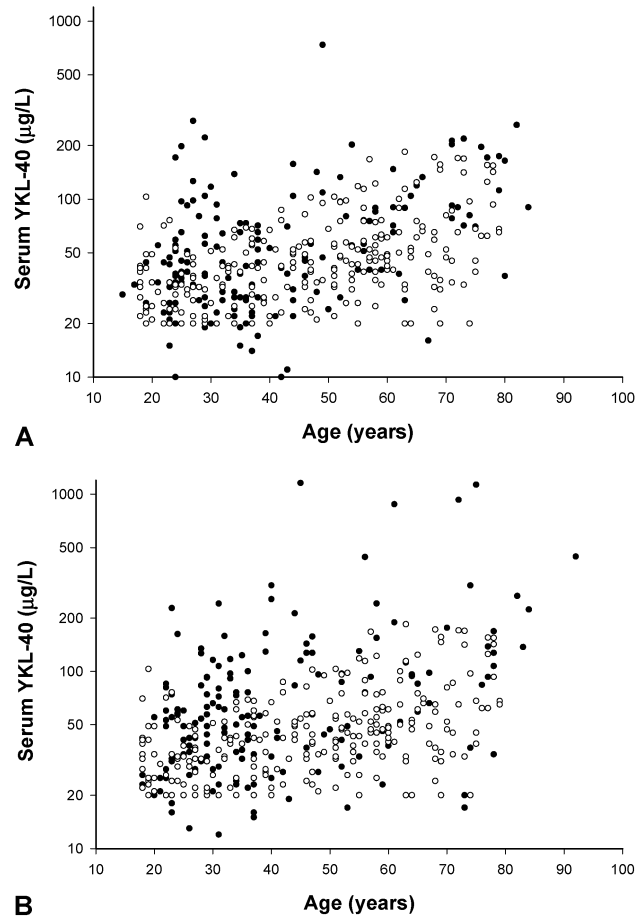


Fig. 1. Individual serum concentrations of YKL-40 in patients with ulcerative colitis (●) (Fig. 1A) and Crohn disease (●) (Fig. 1B) in relation to age and healthy age-matched controls (○).

was also significantly higher in patients with severe UC than in inactive patients (median 14 mg/L (95% CI: 8–110 mg/L) versus 8 mg/L (95% CI: 8–17 mg/L), *P* < 0.001). Serum YKL-40 was elevated (i.e. higher than the upper 90th confidence limit of age-matched controls) in 41% of the UC patients with severe disease activity compared to 27% of the UC patients with mild/moderate disease activity and 10% of the patients with inactive disease. The corresponding values for serum CRP were 32%, 12% and 2% (Fig. 3A). Ten of the 21 patients with severe active UC and elevated serum YKL-40 also had elevated serum CRP.

Forty-four percent of the patients with CD had active disease assessed by H-B index (i.e. score ≥5), but in patients with CD the median serum YKL-40 level did not rise with increasing disease activity (Fig. 2B). Patients with inactive CD (H-B < 5, *n* = 92) had a median serum YKL-40 level of 43 µg/L (95% CI: 17–306 µg/L), those with mild/moderate CD (H-B = 5–8, *n* = 34) a median serum YKL-40 level of 57 µg/L (95% CI: 16–157 µg/L) and those with severe CD (H-B > 8, *n* = 37) a median serum YKL-40 level of 59 µg/L (95% CI: 21–654 µg/L). Serum YKL-40 levels for CD

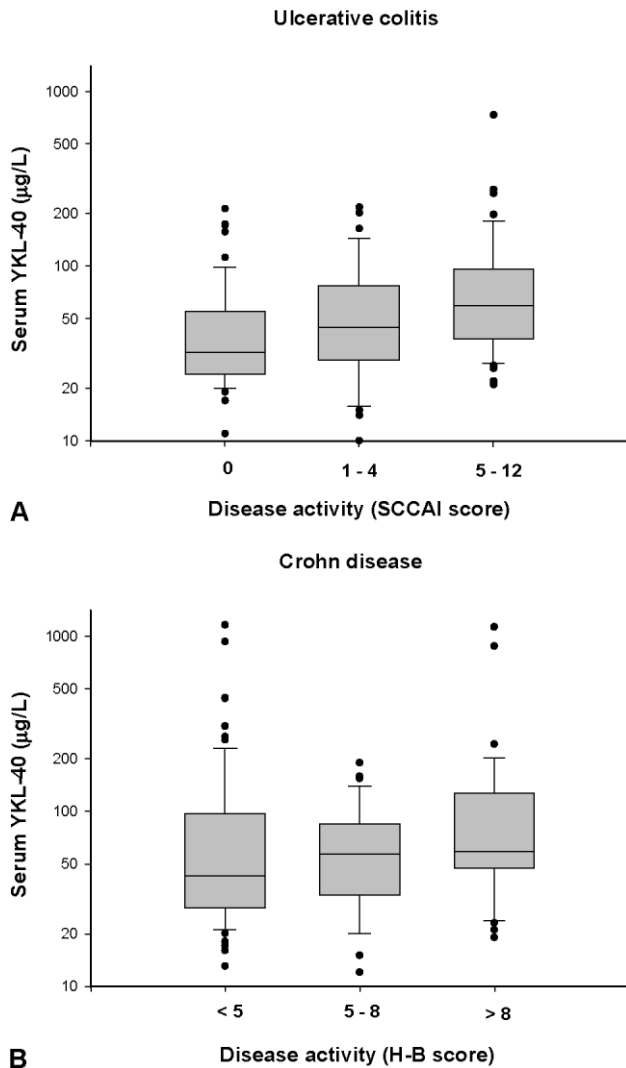


Fig. 2. Box and whiskers plot of serum YKL-40 levels in patients with ulcerative colitis (Fig. 2A) and Crohn disease (Fig. 2B) according to disease activity. Boxes indicate 25th, 50th, and 75th centiles. Whiskers indicate 5th and 95th centiles.

patients with severe disease activity were significantly higher ($P < 0.001$) than for age-matched controls, but not different from CD patients with inactive or mild/moderate disease activity. Serum YKL-40 levels in CD patients with inactive or mild/moderate disease activity were not different from controls. Serum CRP was significantly higher in patients with active CD than in patients with inactive CD (median 17 mg/L (95% CI: 8–187 mg/L) versus 10 mg/L (95% CI: 8–28 mg/L), $P = 0.002$). Serum YKL-40 was elevated (i.e. higher than the upper 90th confidence limit of age-matched controls) in 46% of the CD patients with severe disease activity, in 41% of those with mild/moderate CD and in 30% of the inactive CD patients. The corresponding values for serum CRP were 46%, 21% and 12% (Fig. 3B). Fourteen of the 31 patients with active CD and elevated serum YKL-40 also had elevated serum CRP.

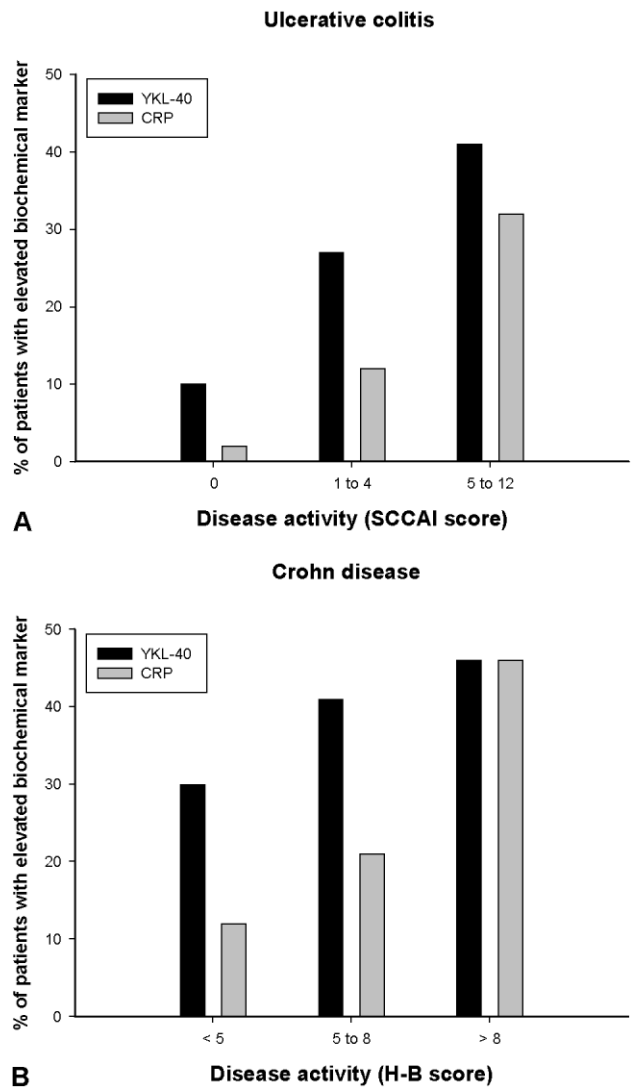


Fig. 3. Number (in percentage) of patients with elevated serum YKL-40 and CRP levels in patients with active or inactive ulcerative colitis (Fig. 3A) and Crohn disease (Fig. 3B).

Relationship between serum YKL-40 and extraintestinal complications

Twenty-eight CD patients had extraintestinal manifestations; 15 had arthralgia, 13 had extraintestinal manifestations such as erythema nodosum, mb. Sweet, uveitis, aphthous ulcerations and fistulas. Eighteen (64%) of the 28 CD patients with extraintestinal manifestations had elevated serum YKL-40. In the UC patients, 17 had extraintestinal manifestations. Only 3 (18%) of these patients had elevated serum YKL-40.

Relationship between serum YKL-40 and other markers of disease activity in IBD patients

Correlation coefficients between serum YKL-40 and other clinical and laboratory parameters of disease activity commonly measured in patients with UC and CD are given in Table II. Serum YKL-40 levels in UC patients correlated

Table II. Correlation between serum YKL-40 and CRP and clinical/biochemical parameters of disease activity

	Score	CRP	Leucocytes	Albumin	Haemoglobin
Ulcerative colitis					
Serum YKL-40	0.30***	0.40***	0.39***	-0.34***	-0.11
Serum CRP	0.41***	-	0.27***	-0.34***	-0.14
Crohn disease					
Serum YKL-40	0.14	0.25***	0.27***	-0.24**	-0.13
Serum CRP	0.29***	-	0.35***	-0.29***	-0.18

Values are Spearman's rho. ** $P < 0.01$, *** $P < 0.001$.

Score: Simple Clinical Colitis Activity Index (SCCAI) for patients with ulcerative colitis and Harvey-Bradshaw (H-B) score for patients with Crohn disease. CRP = C-reactive protein.

significantly with SCCAI, serum CRP, total leucocytes and inversely with serum albumin. In CD patients, serum YKL-40 correlated with serum CRP, total leucocytes and albumin, but no correlation with the H-B index. None of the correlation coefficients were high.

Discussion

The present study investigated serum YKL-40 levels in a large group of patients with IBD in relation to disease activity. We found that patients with active UC had higher serum YKL-40 levels than patients with inactive UC and age-matched healthy controls, whereas patients with inactive UC had normal serum YKL-40 levels. This is in accordance with the result of a small study of IBD patients (25). We also found low, but significant correlations in UC patients between serum YKL-40 levels and clinical disease activity score determined by SCCAI, serum CRP and albumin. Serum YKL-40 levels were elevated in more patients with active UC compared to the number of patients with elevated serum CRP. These results indicate that serum YKL-40 levels in UC patients reflect the inflammation of the disease. It is unknown whether serum YKL-40 is a more sensitive inflammatory marker in UC patients compared to serum CRP, but YKL-40 is probably produced locally in the inflamed bowel in contrast to CRP, which is produced in the liver in response to high IL-6 production.

Serum YKL-40 levels do not allow one to discriminate between UC and CD patients, but some discrepancies in serum YKL-40 levels were found between the two diseases. Interestingly, there was no relationship in CD patients between serum YKL-40 levels and disease activity measured by H-B score. Forty-six percent of the patients with active CD and 30% of those with quiescent or mild CD had elevated serum YKL-40 levels compared to controls, indicating that serum YKL-40 is also increased in clinically inactive CD patients. We are presently following these CD patients prospectively over 2 years in order to evaluate whether elevations in serum YKL-40 levels predict relapse in clinically inactive CD patients and if persisting elevated serum YKL-40 levels in CD patients may indicate persisting inflammation and ongoing fibrosis activity leading to clinical manifestations of fibrostenotic disease. Some of the circulat-

ing YKL-40 in CD patients may reflect ongoing fibrogenesis, since serum YKL-40 levels have been closely related to the degree of liver fibrosis (31, 32) and immunohistochemical analysis has shown YKL-40 production in areas with liver fibrosis (31). Furthermore, *in vitro* studies have shown that YKL-40 increases growth rates of fibroblast and works synergistically with IGF-I (9) and may therefore play a role in the pathological conditions leading to tissue fibrosis.

Although a relationship was found between serum YKL-40 and serum CRP in both UC and CD patients, many patients with elevated serum YKL-40 did not have elevated serum CRP and vice versa. This indicates that serum YKL-40 levels may reflect aspects of inflammation other than serum CRP. It has been shown that YKL-40 stimulates migration of vascular endothelial cells at a level comparable to that achieved by basic fibroblast growth factor and modulates vascular endothelial cell morphology by promoting the formation of branching tubules (11), indicating that YKL-40 may function in angiogenesis.

More than 60% of the CD patients with extraintestinal manifestations had elevated serum YKL-40 in contrast to only 3% of the UC patients with extraintestinal manifestations. The cellular source of the circulating YKL-40 levels in IBD patients is unknown. It is well documented that in addition to lymphocytes, monocytes/macrophages and leucocytes are activated and have a central function in the inflammatory process of IBD (43, 44). In IBD patients, YKL-40 is most likely secreted by monocytes/macrophages and leucocytes in the inflamed intestine. Immunohistochemical and *in situ* hybridization studies of YKL-40 expression in intestinal biopsies taken from IBD patients at time of sigmoidoscopy or operation are in progress in order to determine whether YKL-40 is expressed by these cells in areas with inflammation and whether YKL-40 is found in areas with ongoing fibrogenesis. Future studies should also determine if YKL-40 is an autoantigen in patients with IBD.

UC is a colonic disease with chronic inflammation of the intestinal submucosa and affection to the upper epithelium, with extension only possible to the colon. Whereas CD is characterized by a chronic panenteric transmural inflammation of the bowel, of which 50% of patients develop granulomas of Langerhans type (37), it is well known that one-third of CD patients will have a resection due to

fibrostenosis within the first year after the diagnosis of CD and 5% thereafter in the subsequent years (45). Studies have shown that the intestinal extracellular changes in response to chronic inflammation in both UC and CD are the development of fibrosis. The localization and intensity of the inflammatory infiltrate may be the reason for the different clinical outcome between these two diseases. Relapses are frequent in Crohn disease, occurring in 50% of unselected cohorts per year after diagnosis, and remain difficult to predict (46). The relapse risk is not homogeneously distributed among patients.

In conclusion, we found that serum YKL-40 is elevated in 40%–50% of UC and CD patients with active disease, and in 30% of patients with clinically inactive CD. Prospective studies of IBD patients are needed to evaluate whether serum YKL-40 levels can provide clinically useful information of disease activity.

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