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Serum levels of YKL-40 and PIIINP as prognostic markers in patients with alcoholic liver disease

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***Background/Aims*: YKL-40 (growth factor) and PIIINP (N-terminal propeptide of Type III procollagen) are potential markers of liver fibrosis. The aim was to evaluate the prognostic value of serum YKL-40 and PIIINP levels in patients with alcoholic liver disease.**

***Methods*: Three hundred and seventy patients with alcoholic liver disease were studied in a trial of malotilate with a median follow-up period of 470 days; 75 patients died; 336 patients had a liver biopsy on entry. Serum levels of YKL-40 and PIIINP were determined by radioimmunoassay (RIA).**

***Results*: Serum YKL-40 and PIIINP were elevated in the patients compared to controls. Patients with steatosis or no fibrosis had the lowest serum levels of YKL-40 and PIIINP, whereas patients with alcoholic hepatitis and/or cirrhosis had the highest levels. Serum YKL-40 was associated with the presence of fibrosis, and serum PIIINP was also associated with the different grades of fibrosis. Patients with elevated serum YKL-40 or PIIINP had shorter survival than patients with normal serum levels of YKL-40 ($P < 0.0001$) or PIIINP ($P = 0.044$). High degree of fibrosis predicted shorter survival ($P = 0.004$).**

***Conclusions*: Serum levels of YKL-40 and PIIINP are elevated in alcoholic patients, related to the presence of liver fibrosis and may provide prognostic information.**

***Keywords*: YKL-40; Human cartilage glycoprotein; PIIINP; N-terminal propeptide of Type III procollagen; Alcoholic liver disease; Liver fibrosis; Prognostic marker**

1. Introduction

Alcoholic liver disease covers a wide spectrum of morphological features from minimal lesions such as steatosis to more advanced, and prognostically more serious lesions like alcoholic hepatitis and liver fibrosis/cirrhosis [1,2]. Alcohol can lead to progressive perivenous injury, impaired functioning of the hepatocytes, and loss of the endothelial cell pores [3,4]. The fibrogenic stimulus transforms the quiescent hepatic stellate cells (HSCs) into myofibroblast-like cells producing extracellular matrix (ECM) constituents consisting of fibril-forming collagens and matrix glycoconjugates. The increase in ECM deposited in the space of Disse leads to hepatic fibrosis and cirrhosis [4].

It has been suggested that markers of fibrosis might be useful in the follow-up of patients with alcoholic liver disease, because of their proposed ability to identify patients with increased or progressive collagen synthesis [5,6]. Clinical and routine biochemical characteristics of patients with liver disease can provide useful, but rather imprecise,

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information on prognosis, and new markers of survival are needed [7–9]. Potential markers of liver fibrosis and fibrogenesis are YKL-40 and PIIINP levels. YKL-40, a member of the glycohydrolase family 18 [10], is a growth factor of connective tissue cells and endothelial cells [11–13]. PIIINP is the aminoterminal propeptide of Type III procollagen, the cleavage product during the conversion of procollagen III into collagen III [14], which is a component of the ECM in liver fibrosis [15]. Several studies have shown that patients with liver fibrosis and alcoholic cirrhosis have increased serum levels of these two markers [15–24].

The aim of this study was to evaluate the serum levels of YKL-40 and PIIINP in patients with alcoholic liver disease in relation to the degree of liver fibrosis and survival.

2. Materials and methods

2.1. Patients

The study population consisted of patients from a prospective randomised multicentre placebo-controlled trial of the effect of an antifibrogenetic drug, malotilate (diisopropyl 1,3-dithiol-2-ylidenemalonate) on the survival of patients with alcoholic liver disease. Of the 407 patients enrolled in the multicentre trial, 68 patients were lost to follow-up but alive at the end of the study. Three hundred and seventy had complete follow-up data and entered the present study. The patients consisted of 277 men and 93 women (median age 49 years; range 23–79 years) from seven European centres [8]. The patients had various degrees of alcoholic liver disease and had a daily alcohol intake of 80 g or more over the preceding 4 years. Inclusion and exclusion criteria are described elsewhere [8]. During treatment, the patients were followed up prospectively with clinical and biochemical check-ups every 3 months for the first year and then every 6 months until the end of the study or death. Clinical and biochemical characteristics of the patients are summarised in Table 1. The study was approved by the local ethics committees of the hospitals involved. Briefly, the results of this trial showed a slightly better, but not significant, survival rate in patients receiving a medium dose of malotilate 750 mg/day compared with patients receiving placebo or the highest malotilate dose, 1500 mg/day.

2.2. Healthy controls

The normal range of serum YKL-40 and PIIINP was determined in 237 healthy volunteers (107 men and 130 women; median age 51 years; range 23–79 years). They were not taking any medicine, and had no clinical signs or symptoms of cancer or joint, liver, metabolic, or hormonal diseases [25]. Gender has no effect on serum YKL-40 levels [25]. The median serum YKL-40 level was 103 μg/l (upper 95% confidence limit = 249 μg/l) and the median serum PIIINP level was 2.9 μg/l (upper 95% confidence limit = 4.3 μg/l).

2.3. Biochemical evaluation

Blood samples were taken between 8 a.m. and 2 p.m. non-fasting. The blood samples were allowed to clot at room temperature and then centrifuged at 2000 × *g* for 10 min within 3 h from blood sampling. The serum samples were stored at −20°C until analysed. Routine biochemical tests (serum creatinine, immunoglobulin (Ig) M, alanine aminotransferase, alkaline phosphatases, bilirubin, albumin, and coagulation factors 2, 7, and 10) were measured by routine methods. Serum YKL-40 was determined by an in-house radioimmunoassay (RIA) with rabbit antibody raised against human YKL-40 [26]. Purified human YKL-40 was used for standard and tracer. The tracer was prepared by the Iodogen method (Pierce and Warriner, Chester Ltd, UK) and antibodybound and free ^{125}I-labelled YKL-40 were separated by use of Sac-cel (a donkey anti-rabbit antibody-coated cellulose suspension; Wellcome Diagnostics Ltd, UK). The intra- and interassay coefficients of variation were <6.5 and <12%, and the detection limit was 20 μg/l. Serum PIIINP was measured by RIA (Orion Diagnostica, Espoo, Finland) as described elsewhere [14]. The intra- and interassay coefficients of variation were <4.5 and <5.5%, and the detection limit was 0.2 μg/l.

2.4. Histological evaluation

The diagnosis of alcoholic liver disease was histologically verified in 336 patients within 6 months of the time of the first blood sample collection. Liver biopsy was contra-indicated in the remaining 34 patients, and their diagnosis was based on accepted biochemical and clinical criteria. The liver biopsy was performed percutaneously according to the Menghini technique, with a needle diameter of 1.6 mm. The biopsies were fixed in neutral formalin, embedded in paraffin, cut into 5 μm thick sections,

Table 1
Clinical and biochemical characteristics of 370 patients with alcoholic liver disease

Variable	Median (range) or count (%)
Age (years)	49 (23–79)
Gender (male)	277 (75%)
Cirrhosis (present)	188 (56%)
Encephalopathy (present)	33 (9%)
Ascites (present)	110 (30%)
Excessive alcohol intake (years)	15 (0–45)
Observation time (days)	470 (1–1544)
Serum creatinine (mmol/l, 0.05–0.12)	0.08 (0.03–0.26)
Serum IgM (μmol/l, 0.3–3.7)	1.9 (0.3–14.2)
Serum alanine aminotransferase (U/l, 10–40)	43 (5–460)
Serum alkaline phosphatase (U/l, 50–275)	291 (16–1215)
Serum bilirubin (μmol/l, 2–17)	16 (2–530)
Serum albumin (μmol/l, 540–800)	529 (214–743)
Coagulation factors 2,7, and 10 (arbitrary units, 0.7–1.3)	0.79 (0.01–1.92)
Serum YKL-40 (μg/l, upper 95% confidence limit 249)	350* (20–5550)
Serum PIIINP (μg/l, upper 95% confidence limit 4.3)	10.1* (1.4–117.5)

Units and reference intervals in parentheses.
Mann–Whitney's test vs. controls: *$P < 0.001$.

stained, and then evaluated by a single pathologist, who graded the biopsies semiquantitatively for histological variables (Appendix A) [27].

2.5. Statistics

The statistical analysis was performed with SigmaStat (SPSS Inc, Chicago IL, USA), SAS (logistic regressions; SAS Institute, Cary, NC, USA), and SPSS (prognostic analysis). Variables are summarised as median and range. Comparison between groups was performed by the non-parametric Mann–Whitney rank sum test, and when more than two groups were compared, the Kruskal–Wallis test was used. Correlation analysis was based on the Spearman rho test. When evaluating the value of the blood markers in predicting the degree of fibrosis, we used logistic regression for varying thresholds, since a proportional odds model for ordinal data did not fit the data. In the logistic regressions, the serum YKL-40 and PIIINP levels were logarithmically transformed when used as covariates, since this gave the strongest relation and the best protection against influence from extreme observations. The prognostic significance of serum YKL-40 and PIIINP levels was studied with the log rank test to compare the Kaplan–Meier survival curves of the subgroups defined according to the level of serum YKL-40 and PIIINP. Cox multiple regression analysis [28] was used to evaluate the additional prognostic influence of serum YKL-40 and PIIINP levels together with the variables previously found to have independent prognostic information on these patients [7,8,29], i.e. age, gender, years of high alcohol intake (>80 g alcohol per day), creatinine, IgM, alkaline phosphatases, coagulation factors 2, 7, and 10, and grade of fibrosis. The final model was achieved by backward elimination stratified according to therapy. P values less than 0.05 were considered to be statistically significant.

The course of serum YKL-40 and PIIINP was studied in overlapping groups each defined by a certain minimal duration of observation [7]. The total period of observation was divided into intervals according to scheduled follow-up and the course was analysed in groups of patients having values in all the intervals of each studied period. The patients with the shortest observation only contribute to the first interval, whereas patients with the longest observation contribute to all intervals. Thus the studied groups overlap, each group including patients with observation times equal to or greater than the period being investigated. So each individual curve is based on the same patients contributing to all its points from start to end and is, therefore, unbiased. By comparing the relative position of the various curves at any given time, the effect of the patient loss (from any cause) with time on the level of values can easily be seen [7].

3. Results

Table 1 shows the patients clinical and biochemical data. The serum YKL-40 and PIIINP levels in the patients with alcoholic liver disease were significantly higher than in healthy controls ($P < 0.001$). No significant differences were observed in serum YKL-40 or PIIINP, or any other biochemical and clinical parameters between patients treated with malotilate (750 and 1500 mg/day) and those receiving placebo (Kruskal–Wallis test, P = NS). The baseline serum levels of YKL-40 and PIIINP were related (rho = 0.27, $P < 0.001$). Higher values of serum YKL-40 and PIIINP were associated with more abnormal values of bilirubin, albumin, and coagulation factors 2,7, and 10 (bilirubin: PIIINP rho = 0.58, YKL-40 rho = 0.21; albumin: PIIINP rho = −0.54, YKL-40 rho = −0.41; coagulation factors 2,7, and 10: PIIINP rho = −0.53, YKL-40 rho = −0.15). Age was correlated to serum YKL-40 (rho = 0.25) but not to serum PIIINP levels.

3.1. Serum YKL-40 and PIIINP levels in relation to fibrosis and alcoholic hepatitis

Fig. 1a, b illustrates the serum YKL-40 and PIIINP levels in relation to the degree of liver fibrosis defined histologically in the 336 patients who had a liver biopsy performed. The serum YKL-40 level was above the 95% confidence limit of the levels in the controls in 26% of the patients with

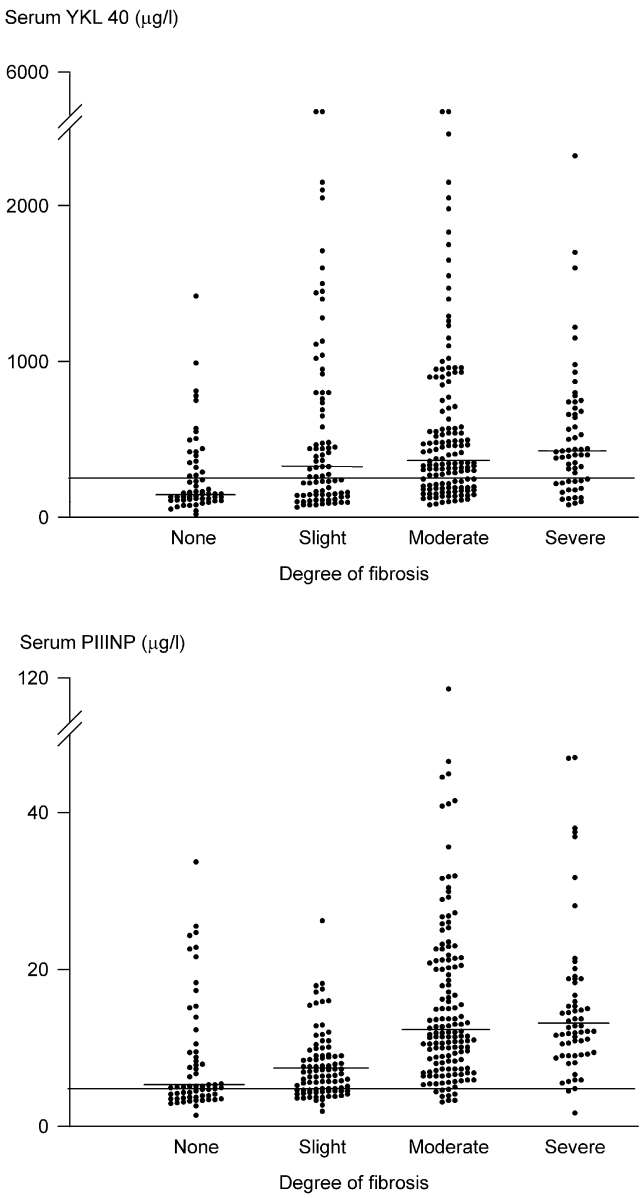

Fig. 1. Serum concentrations of YKL-40 (a) and PIIINP (b) in patients with alcoholic liver disease in relation to the degree of liver fibrosis. Serum YKL-40 median (range) in patients with no fibrosis: 150 μg/l (20–810) (n = 43); slight fibrosis: 325 μg/l (64–5550) (n = 88); moderate fibrosis: 388 μg/l (80–5550) (n = 146) and severe fibrosis: 420 μg/l (80–3200) (n = 59). Serum PIIINP median (range) in patients with no fibrosis: 4.5 μg/l (1.4–25.5); slight fibrosis: 6.9 μg/l (2.7–51.9); moderate fibrosis: 12.0 μg/l (3.1–117.5) and severe fibrosis: 12.8 μg/l (1.7–100.4). The horizontal line represents the upper 95% confidence limit of healthy controls (serum YKL-40: 249 μg/l; serum PIIINP: 4.3 μg/l).

Table 2
Odds ratio (OR) (95% confidence limit) for different grades of fibrosis, when the marker is doubled

Threshold	Log serum YKL-40		Log serum PIIINP	
Grade of fibrosis 0 vs. 1–3	1.92 (1.41–2.72)	$P < 0.0001$	2.96 (1.87–4.94)	$P < 0.0001$
Grade of fibrosis 0–1 vs. 2–3	1.17 (0.98–1.40)	$P = 0.09$	3.08 (2.29–4.24)	$P < 0.0001$
Grade of fibrosis 0–2 vs. 3	1.03 (0.83–1.28)	$P = 0.77$	1.47 (1.16–1.87)	$P = 0.002$

The logistic regressions for thresholds grade 0 vs. 1–3, grades 0–1 vs. 2–3, and grades 0–2 vs. 3, respectively, with the logarithmically transformed serum YKL-40 and PIIINP levels as covariates.

no fibrosis (grade 0), in 58% with slight fibrosis (grade 1), in 70% with moderate fibrosis (grade 2), and in 71% with severe fibrosis (grade 3). The corresponding percentages of patients with increased serum PIIINP were 51 (grade 0), 84 (grade 1), 96 (grade 2), and 98% (grade 3). Serum YKL-40 and PIIINP levels were significantly higher (Mann–Whitney's rank sum test, $P < 0.001$) in all groups, as compared to the levels in the controls, and higher ($P < 0.001$) in patients with different grades of fibrosis, as compared to patients with no fibrosis or controls. There was no significant difference in the serum YKL-40 of the three groups with fibrosis (Kruskal–Wallis test, $P = 0.41$), whereas a significant difference in serum PIIINP was found between the group with grade 1 fibrosis and the groups with grades 2–3 fibrosis (Kruskal–Wallis test, $P < 0.001$). The logistic regression analysis for different thresholds of fibrosis (grades 0 vs. 1–3, 0–1 vs. 2–3, and 0–2 vs. 3, respectively) with the logarithmically transformed serum YKL-40 and PIIINP as covariates gave the same type of conclusion (Table 2). There was a statistical association between serum YKL-40 and presence of fibrosis. Serum PIIINP was also to some degree statistically associated with the different grades of fibrosis (grades 0–1 compared to grades 2–3 and to some extent also between grades 0–2 and grade 3).

Patients with simple steatosis (without fibrosis, cirrhosis, or alcoholic hepatitis) and patients with no fibrosis had a significantly higher level of serum YKL-40 (steatosis: median 150 μg/l, $P < 0.001$; no fibrosis: 150 μg/l, $P < 0.003$) and serum PIIINP (steatosis: 4.5 μg/l, $P < 0.001$; no fibrosis: 4.3 μg/l, $P < 0.001$) than the controls, but the level was lower than in the patients with fibrosis, cirrhosis, or alcoholic hepatitis ($P < 0.001$). Non-cirrhotic patients with alcoholic hepatitis had higher ($P < 0.001$) serum levels of YKL-40 and PIIINP than non-cirrhotic patients without alcoholic hepatitis (Fig. 2a, b left). There was no significant difference in the levels of serum YKL-40 in cirrhotic patients with alcoholic hepatitis or without alcoholic hepatitis, whereas the level of serum PIIINP was higher ($P < 0.001$) in the cirrhotic patients who also had alcoholic hepatitis (Fig. 2a, b right).

3.2. Serum YKL-40 and PIIINP levels in relation to survival

The patients were followed for median 470 days (range 1–1544) or until death. During the follow-up time, 75

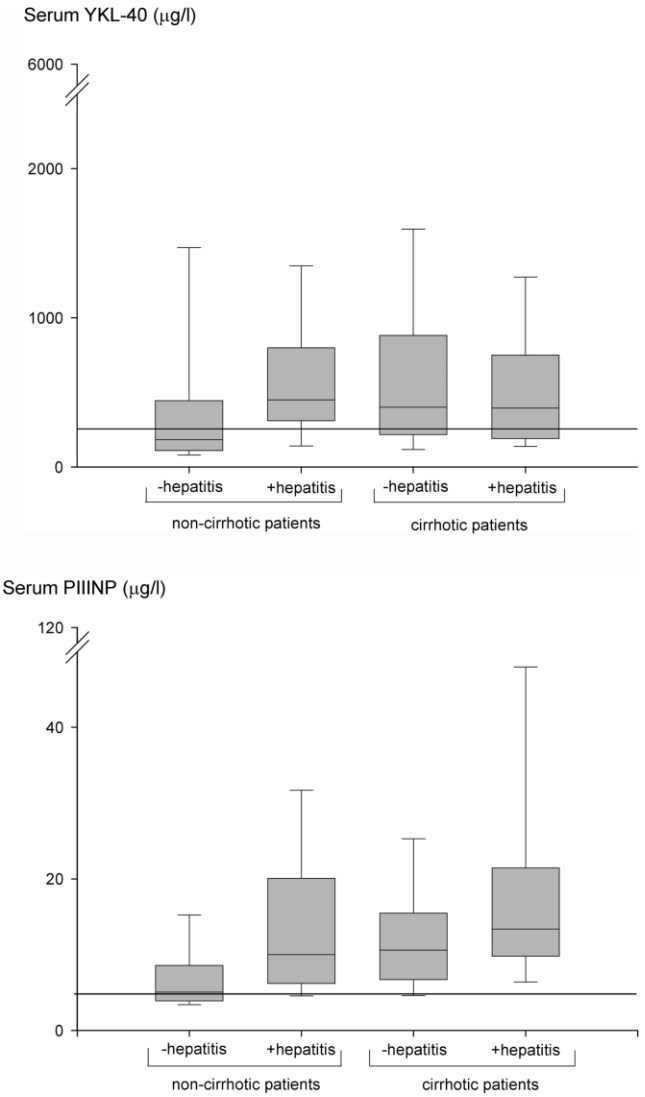

Fig. 2. Serum YKL-40 (a) and PIIINP (b) in patients with different histological variables in the liver biopsy illustrated by a standard box plot. Median (range) of serum YKL-40 and PIIINP levels in non-cirrhotic patients with alcoholic hepatitis ($n = 45$) was 450 μg/l (100–2550) and 10 μg/l (3.8–52.9); non-cirrhotic patients without alcoholic hepatitis ($n = 102$): 185 μg/l (20–5550) and 5.1 μg/l (1.4–25.5); cirrhotic patients with alcoholic hepatitis ($n = 118$): 395 μg/l (95–3250) and 13.4 μg/l (4.4–117.5); cirrhotic patients without alcoholic hepatitis ($n = 70$): 400 μg/l (80–5550) and 10.6 μg/l (1.7–100.4). The horizontal line represents the upper 95% confidence limit of healthy controls (serum YKL-40: 249 μg/l; serum PIIINP: 4.3 μg/l).

patients died. To investigate the association between survival and the serum levels of YKL-40 and PIIINP, respectively, the patients were divided into three groups of equal size, according to their values of serum YKL-40 and PIIINP. Patients with moderate or highly elevated serum YKL-40 and PIIINP were significantly associated with equally poor prognosis (YKL-40: $P < 0.0001$; PIIINP: $P < 0.01$) (figures not shown). These results justified a regrouping of patients into two groups with either normal or elevated serum values of YKL-40 and PIIINP. The Kaplan–Meier plots are shown in Fig. 3a, b. Elevated serum YKL-40 (i.e. >249 μg/l) predicted a shorter survival for alcoholic patients than for patients with normal serum YKL-40 (relative risk, RR = 4.24, 95% confidence interval, CI: 2.18–8.26, $P < 0.0001$). Patients with elevated serum PIIINP (i.e. >4.3 μg/l) also had a shorter survival than patients with normal serum PIIINP (RR = 3.32, 95% CI: 1.05–10.53, $P = 0.042$). To investigate the association between the grade of fibrosis and survival, patients were divided into four groups according to the grade of fibrosis. A high degree of fibrosis predicted a shorter survival ($P = 0.004$). The most significant difference in survival was seen between the patients with fibrosis grades 0–2 and grade 3 (RR = 2.34, 95% CI: 1.37–3.97, $P = 0.002$). The Kaplan–Meier plot is shown in Fig. 3c.

Cox multiple regression analysis (Table 3), including variables earlier found to contain prognostic information about survival in alcoholic patients, showed that years of high alcohol intake, serum creatinine, IgM, alkaline phosphatase, and coagulation factors 2, 7, 10 had independent prognostic significance, whereas serum YKL-40 was borderline significant ($P = 0.069$), and serum PIIINP, grade of fibrosis, and treatment had no independent prognostic value.

3.3. Follow-up values of serum YKL-40 and PIIINP

The course of the mean values of serum YKL-40 and PIIINP over time is illustrated in Fig. 4a, b. As most had incomplete follow-up data, the patients were divided into overlapping groups with complete data in each period of observation (every 3 months). The curves show that serum YKL-40 levels varied very little over time, whereas serum PIIINP levels decreased during the study period in all groups. Furthermore, it appears that the patients with the shortest observation time also had the highest values. This is in harmony with the Kaplan–Meier curves for this variable.

4. Discussion

In vitro studies have shown that YKL-40 is a growth factor of fibroblasts [13], chondrocytes, and synovial cells [12] and is a potent migration factor for endothelial cells [11]. Haemodynamic studies have shown that YKL-40 is released from the hepatosplanchnic area [17]

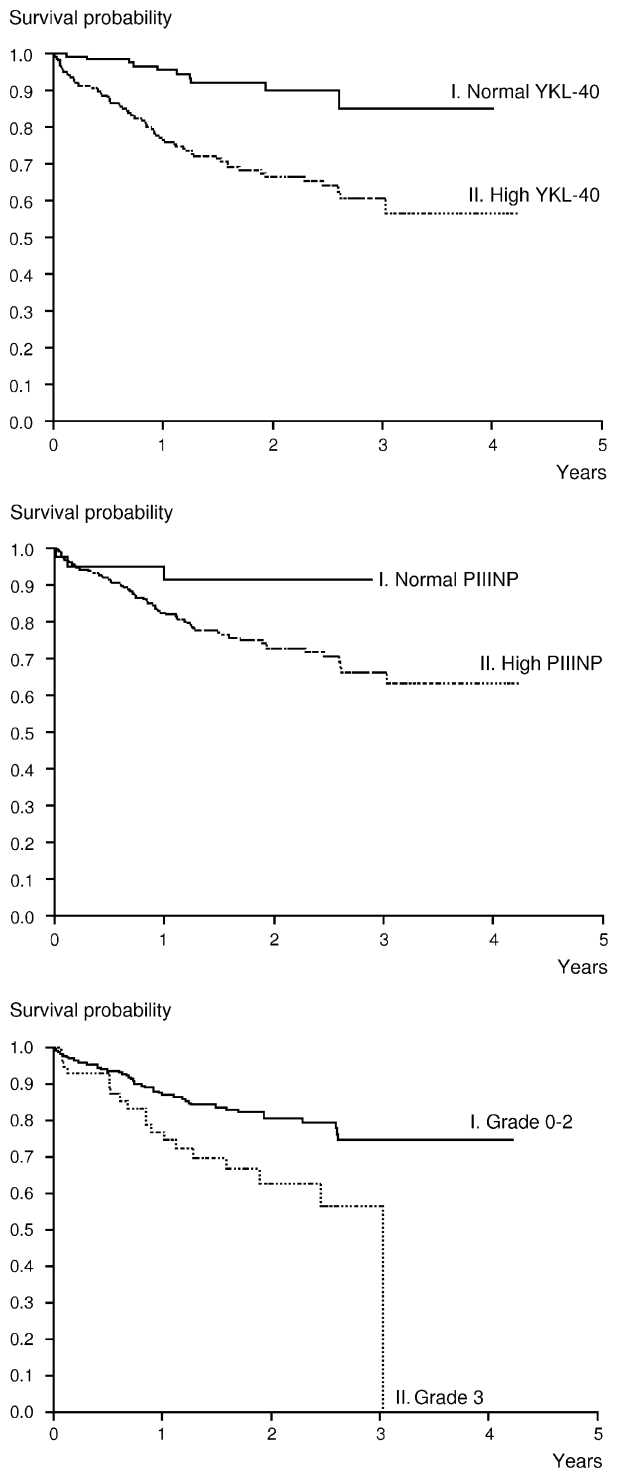

Fig. 3. Survival curves in groups of patients with alcoholic liver disease according to elevated vs. normal levels of serum YKL-40 (a), elevated vs. normal levels of serum PIIINP (b) and to different grades of fibrosis (c). The cut-off limit used is the 95% confidence limit of healthy controls. The strata are: (a) (I) Patients with normal serum YKL-40 (≤249 μg/l, $n = 136$) and (II) patients with elevated serum YKL-40 (>249 μg/l, $n = 222$). (b) (I) Patients with normal serum PIIINP (≤4.3 μg/l, $n = 44$) and (II) patients with elevated serum PIIINP (>4.3 μg/l, $n = 312$). (c) (I) Patients with grades 0–2 fibrosis ($n = 266$) and (II) patients with grade 3 fibrosis ($n = 59$).

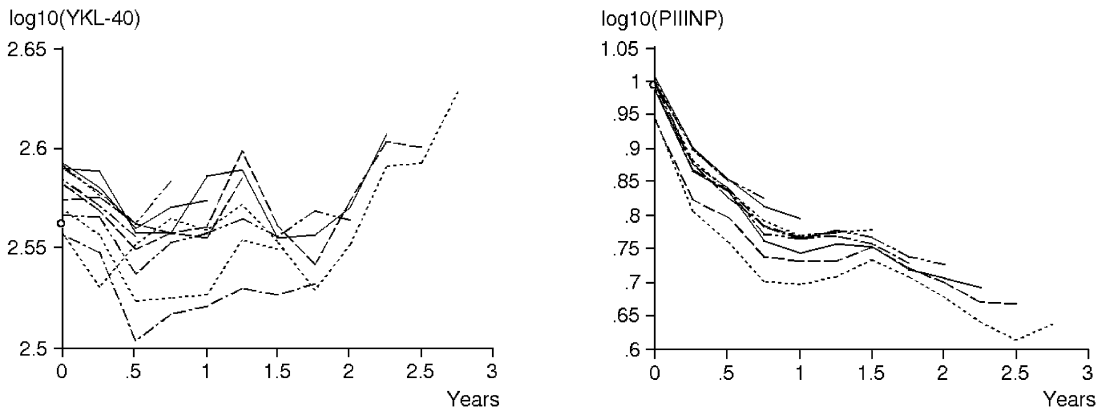

Fig. 4. Course of the mean values of serum YKL-40 (a) and PIIINP (b) over time in overlapping groups of patients with complete data in each period of observation. The logarithmic values of means in μg/l are given. The number of patients contributing to each curve is as follows: serum YKL-40: 0 months (o), 370; 3 months (- - -), 214; 6 months (— - —), 201; 9 months (— - - —), 174; 12 months (——), 155; 15 months (— — —), 138; 18 months (- - -), 123; 21 months (— - —), 106; 24 months (— - - —), 88; 27 months (——), 75; 30 months (— — —), 55; 33 months (- - -) 38; and serum PIIINP: 0 months (o), 368; 3 months (- - -), 212; 6 months (— - —), 199; 9 months (— - - —), 173; 12 months (——), 151; 15 months (— — —), 132; 18 months (- - -) 119; 21 months (— - —), 101; 24 months (— - - —), 81; 27 months (——), 70; 30 months (— — —), 49; 33 months (- - -), 30.

and immunohistochemical studies of liver biopsies have shown positive staining for YKL-40 in areas with fibrosis, particularly in areas with ongoing fibrogenesis [16,17]. We think that, YKL-40 is secreted by the HSCs [16], which is believed to be the principal effector cell in liver fibrogenesis [3,4]. The present large study of patients with alcoholic liver disease confirms the results of earlier smaller studies of alcoholic patients [16,17,21]. We found elevated serum levels of YKL-40 in patients with alcoholic liver diseases (steatosis, fibrosis, cirrhosis, and alcoholic hepatitis) as compared to healthy controls. The lowest serum levels of YKL-40 were found in patients with simple steatosis or no fibrosis, and the highest levels in patients with either alcoholic hepatitis or cirrhosis. The morphological features in alcoholic hepatitis include liver cell damage, inflammatory cell infiltrate of predominately neutrophils, and fibrosis [1]. YKL-40 can be secreted by macrophages [30] and by activated neutrophils [31]. The high serum level of YKL-40 in patients with alcoholic hepatitis is probably brought about by a secretion from both HSCs and inflammatory cells. The high serum YKL-40 levels in alcoholic patients with liver fibrosis is probably due to ongoing fibrogenesis with an increased amount of recruited HSCs producing ECM and growth factors. The serum level of YKL-40 is associated with the presence of fibrosis, and may, therefore, be used as a marker of fibrosis, but it cannot discriminate between the degrees of liver fibrosis. Interestingly, this is the first study to demonstrate a strong association between short-term survival and high serum levels of YKL-40 in patients with alcoholic liver disease. We also found that patients with severe fibrosis had a shorter time of survival than had patients with grades 0–2 fibrosis. However, multiple Cox regression analysis showed that serum YKL-40 and the degree of liver fibrosis were not significant independent prognostic variables of survival.

PIIINP, the cleavage product of procollagen III into collagen III, is a component of the ECM deposited in the space of Disse and is produced by the HSCs as are all the other ECM components in the liver [32,33]. In the present study, we found that serum concentrations of PIIINP were elevated in patients with different alcoholic liver diseases. The lowest serum levels were found in patients with simple steatosis or no fibrosis and the highest levels in patients with cirrhosis in combination with alcoholic hepatitis. Serum levels of PIIINP were to some degree also associated with the different stages of fibrosis. These results agree with those of recent studies [5,15,18–24,34], but the present study is much larger. Patients with high serum PIIINP had a shorter

Table 3
Cox multiple regression-including serum YKL-40

Variable	Scoring	Regression coefficient	SE	*P*
Duration of alcohol intake	Years	0.036	0.012	0.002
Serum creatinine	mmol/l	11.23	2.883	0.0001
Serum IgM	μmol/l	0.138	0.069	0.045
Serum alkaline phosphatase	Log 10, U/l	1.683	0.686	0.014
Serum coag. factors 2,7,10	Log E (value in arb. units)	−1.355	0.309	<0.0001
Serum YKL-40	Normal: 0, elevated: 1 μg/l	0.663	0.36	0.069

time of survival than had patients with normal serum PIIINP, but the marker did not provide independent information on survival as reported by Oberti et al. [19]. We have in another study of patients with liver diseases [16] found a relation between serum YKL-40, PIIINP, and hyaluronan. Hyaluronan is probably best to identify patients with severe liver disease, but may be less precise in detecting early changes of tissue turnover [16,24].

The composition of fibrotic tissue varies depending on its maturity. Non-mature fibrotic tissue consists predominantly of Type III collagen, whereas mature fibrotic tissue has a lower collagen Type III:I ratio [35]. This may explain why serum PIIINP levels in the present study decrease with time, as the production of Type III collagen decreases and the production of Type I collagen (and smaller amounts of Types IV and V collagen [36]) increases. This is important information, if the marker is used as a follow-up marker of fibrosis. We presume that the stability of YKL-40 during the study period may be due to its function as a growth factor.

Patients with no histological sign of fibrosis and patients with simple steatosis had the lowest serum YKL-40 and PIIINP values, probably because of low fibrogenic activity. However, some of these patients actually had quite high serum levels of YKL-40 and PIIINP. This could be explained by sampling error, as the false negative biopsy rate in cirrhosis is approximately 20% [37]. Another assumption is that the elevated YKL-40 level in these patients is due to active reparative mechanisms. Nouchi et al. [18] found increased serum PIIINP levels during abstinence in alcoholics, which could be explained by reparative mechanisms or tissue healing. Unfortunately, it is not possible to distinguish between tissue undergoing healing and 'early' stages of fibrosis [35], but this problem might be solved by repeated measurement of the serological markers. We propose that the markers of fibrogenesis give a more dynamic picture of the hepatic fibrogenesis than does a liver biopsy. The markers can be repeated often, whereas the microscopic examination of the liver gives a static view and is encumbered with a certain risk of sampling error.

In conclusion, serum YKL-40 and PIIINP are elevated in patients with alcoholic liver disease and are both related to the presence of liver fibrosis. The exact function of YKL-40 in liver disease needs to be determined. Interestingly, patients with elevated serum YKL-40 and PIIINP have a poorer prognosis than have patients with normal levels. Determination of these two parameters may lead to identification of alcoholics at high risk of progression toward more serious liver disease and thus a less favourable prognosis.

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Appendix A. Histologic evaluation

The histological findings were assessed as follow [27]. The degree of fibrosis was estimated semi-quantitatively and graded on a scale of 0–3: 0, representing no increase in any part of the biopsy; 1, questionable/minimal increase, i.e. enhanced connective tissue staining in the central part of the lobule with a few coarse perisinusoidal fibers present, but with a preserved architecture; 2, moderate increase, i.e. a distinct occurrence of pericellular and perisinusoidal coarse fibers eventually accompanied by a moderate portal and periportal fibrosis, but with preserved lobular achitecture and 3, severe increase with bridging fibrosis and with extensive portal and periportal fibrosis.

Alcoholic hepatitis was graded on a scale of 0–1: 0, representing absence of steatosis, hepatocyte necrosis, and inflammatory infiltration with or without fibrosis; 1, presence of a combination of steatosis, hepatocyte necrosis, and inflammatory infiltration with or without fibrosis.

Appendix B. Relative risk calculation

Relative risc (RR) can be calculated as $\exp(b \times d) = \exp$ (regression coefficient × covariate difference (of the variable)).

Example, duration of alcohol intake: regression coefficient = 0.036 (Table 3), covariate difference = 10 years. RR = exp (0.036 × 10) = 1.43.

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