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Connective tissue growth factor (CTGF/CCN2) in serum is an indicator of fibrogenic progression and malignant transformation in patients with chronic hepatitis B infection

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ABSTRACT

Still a challenging medical problem is the non-invasive monitoring of patients with a variety of chronic liver diseases being on risk to develop fibrosis, cirrhosis, and, finally, primary liver cell carcinoma. Previously, we have shown that CTGF/CCN2, a down-stream mediator of TGF-β, in serum might be a promising non-invasive biomarker of fibrosis, which is extended in the following study to cirrhosis and liver cell carcinoma.

Healthy individuals (n = 56), as well as fibrotic (n = 77), cirrhotic (n = 17), and HCC-patients (n = 72) with chronic hepatitis B (HBV) infection, clinically, biochemically and histopathologically well characterized and classified, were included for the measurements of CTGF-concentrations in serum using a newly developed CTGF-enzyme immunoassay.

A statistical significant increase of the mean serum CTGF-concentrations was associated with different stages of fibrosis, ranging from 15.9 μg/L (S0), 20.3 μg/L (S1/2) to 36.9 μg/L (S3/4). The highest CTGF-concentrations were measured in cirrhotic patients (43.6 μg/L), compared to healthy subjects (17.7 μg/L), followed by a decrease in cirrhotic HCC-patients (38.5 μg/L; $p = 0.001$). Of note, HCC patients without underlying cirrhosis (n = 8) had CTGF levels (13.5 ± 13.2 μg/L) comparable to those in healthy controls. No statistical relation between CTGF levels and parameters of liver injury (e.g. AST, ALT) was noticed, but CTGF levels are correlated negatively with serum albumin levels ($p = 0.007$) and platelet counts ($p = 0.0032$), respectively. The latter was negatively correlated with the stage of fibrosis ($p = 0.025$). In HCC patients, CTGF concentrations decreased with tumor progression and size, with lower levels in TNM stage II (30.5 μg/L) and stage III (33.6 μg/L) compared to TNM stage I (41.6 μg/L).

Our data suggest a valuable diagnostic impact of CTGF in serum for the follow-up of patients suffering from chronic liver diseases developing fibrosis, cirrhosis and finally HCC. CTGF serum levels in HCC are most likely due to underlying fibrosis/cirrhosis but not due to malignancy per se.

1. Introduction

Chronic liver diseases are the fifth most frequent cause of death in the European Union and the United States, as they entail multiple risks, such as portal hypertension, ascites, spontaneous bacterial peritonitis, hepatorenal and hepatopulmonary syndromes, hepatic encephalopathy and, of course, hepatocellular carcinoma (HCC) [1].

Liver fibrosis, and ultimately liver cirrhosis, are the common end-stage of all chronic liver diseases. At the beginning of fibrogenesis stands a chronic inflammatory condition. But it is not the virus- or toxin-induced hepatocellular damage that primarily causes tissue-destruction and the formation of granulation tissue, but the activation of immunocompetent cells (e.g. Kupffer-cells) and the release of proinflammatory cytokines, such as tumor necrosis factor α (TNF-α), interleukin (IL)-6 and IL-12. These mediators and the accumulation of potentially toxic free fatty acids generate highly reactive oxygen species (ROS), which

Abbreviations: AFP, alpha-fetoprotein; ALT, alanine aminotransferase; APRI, ALT-platelet-ratio index; AST, aspartate aminotransferase; BMP, bone morphogenetic proteins; CLD, chronic liver diseases; CTGF, connective tissue growth factor; ECM, extracellular matrix; HAV, hepatitis A virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HEV, hepatitis E virus; HIV, human immunodeficiency virus; HCC, hepatocellular carcinoma; HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B e antigen; HBcAb, hepatitis B core antibody; anti-HCV, anti-hepatitis C virus antibody; PLT, platelets, thrombocytes; TGF-β, transforming growth factor type β; TNM, tumor, nodes (lymph nodes), metastasis.

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expose the hepatocyte to an oxidative stress, which, primarily via peroxidation of membrane lipids and DNA damage, leads to hepatocellular injury. In the meantime, it comes to an activation of mesenchymal cells, resulting in an increased synthesis and interstitial deposition of extracellular matrix components [2]. These mesenchymal cells, hepatic stellate cells (HSC), also known as Ito cells, are pericytes found in the perisinusoidal space of the liver also known as the space of Disse. Following liver injury, HSC undergo "activation" which connotes a transition from quiescent vitamin A-rich cells into proliferative, fibrogenic, and contractile myofibroblasts (MFB). This pathway has long been, and probably still is, considered as the "canonical" pathway in the pathogenic understanding of liver fibrogenesis. The major phenotypic changes after activation include proliferation, contractility, fibrogenesis, matrix degradation, chemotaxis, retinoid loss, and white blood cell chemoattraction [2].

In the western countries, most frequent causes of chronic liver failure are of nutritive-toxic origin: chronic alcohol abuse, followed by virus hepatitides. Hereditary causes (e.g. hemochromatosis or Morbus Wilson), autoimmune processes (e.g. primary biliary cirrhosis, primary sclerosing cholangitis or autoimmune hepatitis), metabolic disorders, venous obstruction/liver congestion are in the minority.

More than 50% of all patients with complicated liver cirrhosis die within the first 17 years following diagnosis, mostly from HCC. In more than 90% of all cases, the HCC develops within a cirrhotic liver. Therefore, attenuation of the fibrogenic process can significantly lower morbidity [1].

This urgently requires reliable tools for early diagnosis and continuous monitoring of patients at risk [3]. Due to its highly invasive nature and serious analytical limitations the histological evaluation of liver biopsy specimens is no longer recommended for this purpose [4]. As an alternative, non-invasive procedures like liver elastography to measure the increasing stiffness of the tissue due to accumulating extracellular matrix (ECM) [5], various imaging methods [6], and multi-parametric biochemical scores [7–9] have been developed. About 20 of these algorithms, mostly based on routine biochemical and hematological parameters, are presently recommended for the follow-up of patients at risk to develop fibrosis, cirrhosis and, finally, primary hepatocellular carcinoma (HCC). Previously, we have shown that the diagnostic value of multi-parametric panels is limited due to analytical imprecision and globally unstandardized methods for the measurement of biochemical routine parameters [10]. Thus, both the comparability and reproducibility of grading the activity and staging the extent of fibrotic tissue transition might scatter considerably between the various investigators, which hamper their large-scale application and comparison. We therefore have focused our efforts on finding a single biochemical parameter, which per se is directly involved in the pathogenesis of liver inflammation and fibrogenesis.

CYR61-CTGF-NOV (CCN) 2/connective tissue growth factor (CTGF), a member of the CCN superfamily of secreted, cysteine-rich glycoproteins, has been implicated in the pathogenesis of hepatic fibrosis and is currently suggested to be an important downstream amplifier of the effects of the profibrogenic master cytokine transforming growth factor (TGF)-β [11–13]. Its molecular mechanism of action is still not known in detail, but it very likely strengthens the binding of TGFβ1 to its cognate receptors. Its crucial role in fibrogenesis is documented by strong upregulation in fibrotic liver tissue, and even more importantly by recent studies, in which knock-down of CCN2/CTGF by siRNA leads to substantial attenuation of experimental liver fibrosis (summarized in [14]). We were among the first to identify that hepatocytes (PC) substantially synthesize CCN2/CTGF in cell culture and in injured liver, and that CCN2/CTGF is sensitively up-regulated by TGFβ1 [15].

Significant increases of CTGF in serum/plasma of patients with fibrogenic CLD were shown by us previously using an in-house immunoassay for CTGF [16,17]. Thus, there is good evidence for CTGF as a diagnostic relevant fibrogenic master switch in fibrotic CLD [18].

In the present study we evaluated a new commercial ELISA for CTGF which is based on our previous assay to measure CTGF concentrations in the serum of patients with various stages of developing fibrotic liver diseases and, for the first time, HCC. The data suggest CTGF in serum as a promising single-type biochemical parameter for the diagnosis and follow-up of patients with CLD.

2. Materials and methods

2.1. Patients

A total of 222 serum samples, including patients with liver fibrosis (n = 77), liver cirrhosis (n = 17), HCC (n = 72), and healthy control subjects (n = 56), were collected in this study. Patients with HBV-infected liver cirrhosis and HBV-related HCC were recruited from the Eastern Hepatobiliary Hospital, Shanghai, China. All enrolled patients with HCC were diagnosed with histological confirmation, while liver cirrhosis was diagnosed by the physical condition of the patient and by imaging techniques. The HCC stage was classified according to the TNM-criteria [19] and the liver function was scored according to the Child–Turcotte–Pugh classification [20]. Patients with liver fibrosis suffering from chronic hepatitis B virus (HBV) infection were recruited from the first people's hospital, Shanghai Jiaotong University, China. All selected patients received liver biopsy directed by ultrasonography within 1 week after admission, using a needle with an internal diameter of 1.4 mm (G14, Quick-Cut; Hakko. Company, Japan). A minimum length of at least 1.0 cm of the liver biopsy and at least 6 portal tracts were required for diagnosis. Specimens were fixed in 10% formalin, embedded in paraffin, followed by hematoxylin-eosin (HE) staining and Masson's trichrome staining. Histological staging was blindly and independently determined by two pathologists using Scheuer's classification from stage 0 to stage 4 [21]. Moreover, patients with HAV, HCV, HEV, or HIV infection, alcoholic liver disease, autoimmune liver disease, and drug-related liver disease were excluded from the study. 56 cases of sex and age matched healthy subjects were recruited from Eastern Hepatobiliary Hospital, Shanghai, China and served as a control group. The study protocol was approved by the Chinese Ethics Committee of Human Resources, Eastern Hepatobiliary Hospital. Additionally, informed consent was obtained from all participants for the use of their blood in this study.

Table 1
Compilation of personal and laboratory data (mean ± SD) of the patient study and control groups (n = 225).

	n	Male(%)	Age	Bilirubin [μmol/L]	Albumin [g/L]	ALT [U/L]	AFP [μg/L]	PLT [$\times 10^9$/L]
Normal	56	35 (62.5)	50.93 ± 6.2	13.0 ± 4.8	47.6 ± 2.4	20.2 ± 9.8	3.4 ± 2.6	226 ± 43
Fibrosis stage (Scheuer)								
0	9	5 (55.6)	36.44 ± 8.9	17.4 ± 7.7	45.3 ± 5.5	54.1 ± 27.9	2.65 ± 1.4	180 ± 60
1	18	13 (72.2)	34.2 ± 5.9	21.6 ± 25.2	42.5 ± 2.9	174.3 ± 86.8	3.4 ± 0.1	186 ± 59
2	19	15 (78.9)	31.7 ± 11.1	32.4 ± 37.7	41.2 ± 4.1	348.3 ± 428.8	11.8 ± 10.1	198 ± 51
3	15	14 (93.3)	32.7 ± 6.6	49.3 ± 52.5	39.1 ± 4.3	328.8 ± 252.3	61.8 ± 78.6	184 ± 59
4	16	16 (100)	39.4 ± 9.6	40.0 ± 56.2	38.7 ± 7.0	253.6 ± 612.8	55.4 ± 83.4	118 ± 53
Cirrhosis	17	14 (82.4)	48.8 ± 8.5	35.7 ± 19.6	34.8 ± 7.3	158.3 ± 468.2	25.2 ± 37.9	71 ± 36
HCC	72	61 (84.7)	49.3 ± 10.5	15.4 ± 6.2	41.7 ± 4.1	44.6 ± 26.4	199.8 ± 311.2	157 ± 67

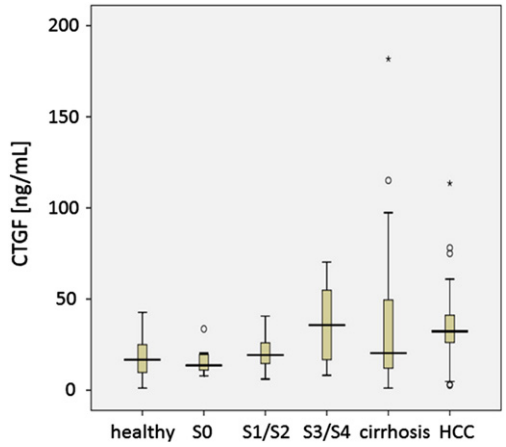

Fig. 1. Box plot diagram of the concentrations of CTGF in the serum of healthy individuals and patients with different stages of fibrosis (Scheuer score) (S0–S4), cirrhosis, and primary hepatocellular carcinoma (HCC).

2.2. Serum CTGF detection and routine laboratory test

Blood was collected using a standard protocol and serum separated by centrifugation at 3000 rpm for 10 min, and then stored at −80 °C. The following laboratory parameters were measured: serum albumin, total bilirubin, alanine aminotransferase (ALT), and aspartate aminotransferase (AST). Platelets (PLT, thrombocyte) were counted in anticoagulated citrate buffered blood. Routine biochemical tests, including bilirubin, albumin, ALT, AST, and ALT were performed using standard methods and matched reagents (Hitachi 7600 Analyzer, Hitachi, Tokyo, Japan; Wako diagnostic reagents, Wako Pure Chemical Industries Ltd., Osaka, Japan). Serological investigation of viral hepatitis (HBsAg, HBsAb, HBeAg, HBeAb, HBcAb, and anti-HCV) was performed by immunological methods (Roche E170, Switzerland). Platelet counting was done using a Sysmex XE-2100 hematological analyzer (Sysmex, Japan).

Serum CTGF concentrations were measured using a newly developed commercially available solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle following the instructions of the supplier (DRG Instruments GmbH, Marburg, Germany, www.drg-diagnostics.de). The dynamic assay range is determined to be between 7.8 and 500 ng/mL. All measurements were performed in duplicate by the Department of Laboratory Medicine, Eastern Hepatobiliary Surgery Hospital, Shanghai, China.

2.3. Statistical analysis

All quantitative variables are expressed as mean values ± SD (standard deviation) unless stated otherwise. Quantitative variables were compared with Student *t*-tests in two groups, one-way ANOVA analysis and multiple comparisons among more than two groups. Pearson coefficients of correlation (Spearman coefficients of correlation were calculated for ordinal categorical variables) and their associated probabilities (*p* values) were used to evaluate correlations between parameters. All statistical *p* values were two-tailed, and $p < 0.05$ was considered to be statistically significant. Statistical analysis was performed using SPSS11.0 software (SPSS, Chicago, USA). The diagnostic value of CTGF in serum was assessed by calculation of the area under the receiver operating characteristic (ROC) curve (AUC). Diagnostic accuracy was determined by specificity, sensitivity, and positive and negative predictive values for the chosen cut-off values.

Table 2a
Concentrations of CTGF in the serum (mean ± SD) of patients with different stages of fibrosis (Scheuer score).

Fibrosis stage (Scheuer)	n	CTGF [ng/mL]
0	9	15.9 ± 8.0
1	18	21.2 ± 9.4
2	19	19.4 ± 8.3
3	15	26.4 ± 20.6
4	16	46.9 ± 12.6***

*** $p < 0.001$ vs. other stages.

Table 2b
Concentrations of CTGF in the serum (mean ± SD) of patients with HCC and different TNM-stages.

		n	CTGF [ng/mL]	*p*
TNM	I	46	41.5 ± 47.0	$p = 0.680$
	II	5	30.5 ± 12.1	
	III	21	33.6 ± 23.7	
Cirrhosis present	No	8	13.5 ± 13.2	$p = 0.059$
	Yes	64	41.6 ± 41.0	

3. Results

3.1. Composition of the study cohort and biochemical parameters

Clinically and biochemically healthy, roughly age and gender matched persons (n = 56) served as a control population for patients with HBV-related liver fibrosis, cirrhosis, and primary HCC, respectively. Table 1 summarizes the epidemiological data of control subjects and patients, i.e. number, age, and gender. The degree of liver fibrosis was staged from stages 0 to 4. The biochemical parameters, i.e. total bilirubin, albumin, ALT-activities, and alpha-fetoprotein (AFP) were, as expected, within the reference range for control subjects but significantly pathologic for patients with fibrosis, cirrhosis, and HCC (Table 1). Depending on the stage of fibrosis AFP-levels increased significantly being lower in patients with cirrhosis but highly elevated in those with HCC. Platelet numbers were significantly lower in cirrhotic subjects and slightly reduced in patients with fibrosis stage 4 (Table 1).

3.2. Elevated concentrations of connective tissue growth factor (CTGF) in the serum of patients with liver disease

Compared with the mean serum CTGF-concentrations of control subjects (17.7 ± 10.7 ng/mL), concentrations were significantly increased in HBV-infected patients with near end-stage fibrosis (stages 3/4; 36.9 ± 19.7 ng/mL), cirrhosis (43.6 ± 48.5 ng/mL), and HCC (38.5 ng/mL), respectively (Fig. 1). Compared to healthy subjects, serum CTGF-concentrations of patients with no (stage 0; 15.9 ± 8.0 ng/mL) and slight to moderate fibrosis (stages 1/2; 20.3 ± 8.8 ng/mL) were not or only insignificantly elevated (Table 2a). In patients with HCC, CTGF-concentrations were not significantly associated with TNM-stages ($p = 0.680$), but patients with progressive disease (TNM II and III) displayed markingly lower CTGF levels compared to those with a small tumor (TNM I) (Table 2b). Of particular interest, CTGF-concentrations in HCC-patients with cirrhosis (41.6 ng/mL) were markingly higher than in those HCC-patients without underlying liver cirrhosis (13.5 ng/mL)

Table 3
Statistical correlation of the serum concentrations of CTGF with routine laboratory parameters of patients with all S-stages of liver fibrosis. TBIL, total bilirubin; ALB, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; PLT, platelet count; CTGF, connective tissue growth factor; APRI, AST to platelet count ratio.

		TBIL	ALB	ALT	AST	PLT	CTGF	APRI
S-stage	r	0.162	−0.318	0.167	0.264	−0.343	0.480	0.385
	p	0.181	0.007	0.149	0.025	0.003	<0.001	<0.001
		TBIL	ALB	ALT	AST	PLT		
CTGF	*r*	0.227	−0.203	−0.034	0.010	−0.255		
	p	0.059	0.091	0.768	0.936	0.032		

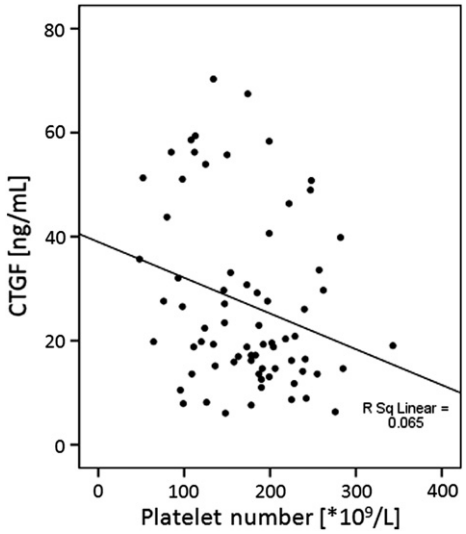

Fig. 2. Statistical correlation of CTGF serum concentrations with platelet count (PLT) of patients with histologically proven liver fibrosis.

(Table 2b). Thus, as could be expected, no statistical correlation between the serum concentrations of CTGF and AFP was found.

3.3. Statistical relation between CTGF concentration in serum and various routine biochemical parameters and diagnostic power of CTGF

Possible statistic correlations of the biochemical routine parameters of liver injury (AST, ALT), cholestasis (total bilirubin), and liver synthesis capacity (albumin, platelet count) with serum CTGF concentrations were tested. In fibrotic patients, the elevation of CTGF in serum was significantly correlated ($p < 0.001$) with the fibrotic stage (Table 3). Also the platelet count ($p = 0.003$) (Fig. 2) was inversely and significantly correlated with the stage of fibrosis (Table 3). It should be emphasized that AFP-levels and tumor size did not show any correlation with CTGF in serum; even high levels of AFP were frequently associated with moderate CTGF-elevations (not shown). Importantly, patients with HCC without underlying cirrhosis/fibrosis mostly had CTGF values within the normal range. Thus, liver cell malignancy per se obviously is not determining the level of CTGF in serum.

Finally, the statistical criteria defining the diagnostic power of serum CTGF for detecting advanced stages of fibrosis (S3/S4) alone and fibrosis (S3/S4) together with cirrhosis were calculated by plotting a Receiver Operating Characteristic (ROC) curve and calculating the area under the curve (AUC) (Fig. 3a, b). We found slightly better AUCs for the detection of fibrosis (0.78) than for the detection of fibrosis plus cirrhosis together (0.73). Diagnostic sensitivities, specificities, positive and negative predictive values, and accuracies were calculated using two cut-off values optimally selected with the ROC curves and are displayed in Tables 4a and 4b.

Table 4a
Diagnostic criteria of the serum CTGF concentrations for diagnosing advanced fibrosis (S3/S4) using a cut-off value optimally selected with the ROC curves.

Cut off value	Sensitivity (%)	Specificity (%)	Predictive value		Accuracy (%)
			Positive (%)	Negative (%)	
26 ng/mL	64.5	81.3	76.9	70.3	73.0

4. Discussion

Increased intercellular deposition of connective tissue, i.e. certain types of collagens, proteoglycans, structural glycoproteins, and of hyaluronan is a hallmark of liver fibrosis, which in turn is an important histological feature of liver cirrhosis [22,23]. The driving forces of fibrogenesis, i.e. the excess de novo generation and aberrant tissue deposition of ECM are necro-inflammatory processes underlying a broad variety of etiologically quite different CLDs [21]. We have focused this study on fibrotic, cirrhotic, and neoplastic liver diseases (HCC) due to chronic hepatitis B virus (HBV) infection, a hepatotropic viral infection that is a prevalent cause of CLD in many Asian countries such as China and Japan. Our data clearly show significant elevations of CTGF concentrations in the serum of patients with HBV-induced, histologically proven fibrotic and cirrhotic liver diseases. Furthermore, the increase of serum CTGF is correlated with the Scheuer score displaying normal values in stages 0/1 and gradually increasing concentrations from stage 2 to highly significant elevations in stage 4. A further increase of the median value was observed in the sera of cirrhotic patients (43.6 ± 48.4 ng/mL) but the individual CTGF concentrations in this cohort scatter strongly. Thus, our data in Fig. 1 and the ROC curves of Fig. 3 support the view of CTGF in serum as a biomarker of the fibrogenic activity, i.e. the formation of connective tissue in liver.

In patients with primary hepatocellular carcinoma (HCC) we observed a slight decrease of mean CTGF concentrations in serum

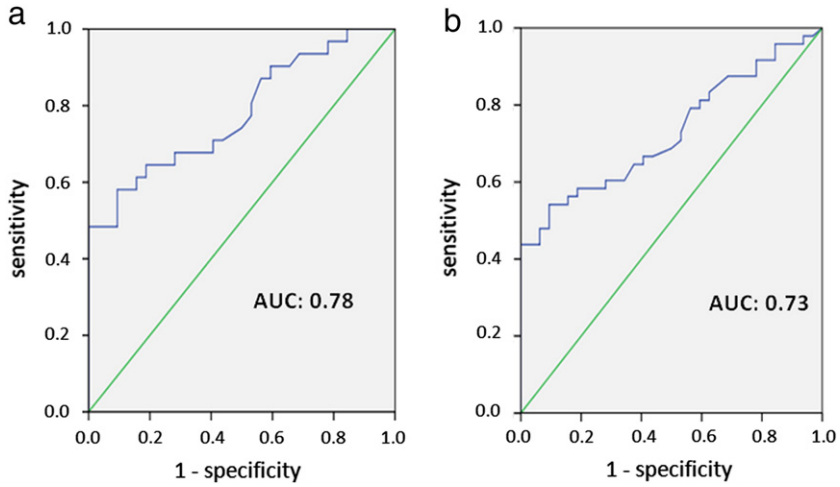

Fig. 3. Receiver Operating Characteristic (ROC) curves of serum CTGF for diagnosing advanced stages of fibrosis (S3/S4) (a) and of fibrosis (S3/S4) + cirrhosis (b), respectively referred to healthy controls. AUC, area under the curve.

Table 4b
Diagnostic criteria of the serum CTGF concentrations for diagnosing advanced fibrosis (S3/S4) + cirrhosis using a cut-off value optimally selected with the ROC curves.

Cut off value	Sensitivity (%)	Specificity (%)	Predictive value		Accuracy (%)
			Positive (%)	Negative (%)	
26 ng/mL	64.6	62.5	72.1	54.1	63.8

(38.5 ± 39.8 ng/mL) but the median was higher than in cirrhotic patients (Fig. 1). Most important, those HCC-patients (n = 8), without underlying tissue fibrosis/cirrhosis displayed CTGF levels not different from the control group, which suggests that a neoplastic tissue per se does not contribute to elevated serum CTGF concentrations. Instead, higher serum CTGF concentrations in patients with HCC are most likely due to the active fibrogenic tissue matrix surrounding the tumor. We will address future studies to serum CTGF in HCC-patients with non-cirrhotic and non-fibrotic livers [24] in order to analyze this finding in some detail. But our findings suggest that patients with HCC in a non-fibrotic, non-cirrhotic, i.e. normal liver parenchyma present as a different entity of patients with chronic liver disease, both etiologically and clinically, than those with HCC and underlying liver fibrosis [24]. This suggestion is supported by the fact that there was no relevant statistical correlation between AFP and CTGF in serum. But even though it seems that CTGF in serum does not originate from the tumor tissue itself rather than from the surrounding fibrogenic matrix, the role of CTGF in tumor growth and/or metastatic dissemination and/or invasiveness in HCC still needs to be defined [25]. The most important transcriptional activator of CTGF is TGF-β and CTGF, in turn, acts as a down-stream mediator of this pleiotropic, profibrogenic master cytokine [26]. Indeed, most of the profibrogenic actions of TGF-β are mediated by CTGF, which consists of four modules, each with different functions and structural properties [26]. The transcriptional activation of the *CTGF* gene by TGF-β occurs primarily via the phospho-Smad2 (and 3) and ERK1/2 signaling pathways [27,28] involving also Ras/MEK/ERK and protein kinases C and A [29]. But also an intracrine signaling pathway of TGF-β leading to the activation of the *CTGF* promoter within cultured and in liver-injured hepatocytes has been described [30]. Supportive in promoting TGF-β actions, CTGF also attenuates the activity of the natural TGF-β antagonist BMP4 (by simultaneously enhancing the receptor binding and, hence, the function of TGF-β1) [31]. Thus, CTGF is shifting the equilibrium towards fibrogenesis, which underlines its major pathophysiological role in fibrogenic CLD. The important pathogenetic relevance of CTGF in fibrogenesis was shown by in situ hybridization of abundant CTGF transcripts in the fibrotic area of cirrhotic livers [32] but demonstrated also in vivo using a specific siRNA in experimental fibrosis [33]. In this study CTGF-silencing induced a sustained antifibrotic effect in the mouse model.

Even though TGF-β itself has a well-known pro-fibrogenic action, it is functionally and immunologically quite difficult to detect in body fluids such as blood due to binding to latent TGF-β-binding proteins (LTBPs) [34,35], alpha 2-macroglobulin [36] and other ligands. Thus, the measurement of CTGF generates important analytical advantages over TGF-β by simultaneously reflecting TGF-β activity [37]. Furthermore, in comparison to multiparametric algorithms of standard biochemical panels [38], we recognize significant advantages for a single biomarker for liver fibrosis with respect to analytical reliability and reproducibility [10], cost-effectiveness, and wide-spread standardization.

Based on these goals and the promising results presented in this study [16,17], a further large-scale evaluation of the diagnostic and prognostic power of CTGF in serum for monitoring fibrogenic reactions (not only) in CLD is recommended.

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