PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

ELSEVIER

World J Gastroenterol 2005;11(43):6833-6838
World Journal of Gastroenterology ISSN 1007-9327

• *RAPID COMMUNICATION* •

Effect of pegylated interferon alpha 2b plus ribavirin treatment on plasma transforming growth factor-β1, metalloproteinase-1, and tissue metalloproteinase inhibitor-1 in patients with chronic hepatitis C

Robert Flisiak, Jerzy Jaroszewicz, Tadeusz W Łapiński, Iwona Flisiak, Danuta Prokopowiczi

Robert Flisiak, Jerzy Jaroszewicz, Tadeusz W Łapiński, Danuta Prokopowicz, Department of Infectious Diseases, Medical University of Bialystok, Poland
Iwona Flisiak, Department of Dermatology and Venereology, Medical University of Bialystok, Poland
Correspondence to: Professor Robert Flisiak, Department of Infectious Diseases, Medical University of Bialystok, 15-540 Bialystok, Zurawia str., 14, Poland. flisiakr@priv.onet.pl
Telephone: +4885-7409481 Fax: +4885-7434613
Received: 2005-04-07 Accepted: 2005-05-12

Abstract

AIM: To evaluate the effect of antiviral treatment on plasma levels of transforming growth factor-β1 (TGF-β1), metalloproteinase 1 (MMP-1), and tissue inhibitor of metalloproteinase-1 (TIMP-1) in patients with chronic hepatitis C.

METHODS: TGF-β1, MMP-1, and TIMP-1 plasma concentrations were measured by an enzyme immunoassay in 28 patients, during 48 wk of treatment with pegylated interferon-alpha 2b (PEG-IFN-α2b) plus ribavirin (RBV) and after 24 wk of follow-up. Patients were divided into two groups: responders (R) and non-responders (NR) related to achieved sustained virologic response. Normal values were evaluated in plasma samples of 13 healthy volunteers.

RESULTS: Baseline plasma concentrations of TGF-β1 and TIMP-1 (30.9±3.7 and 1 506±61 ng/mL respectively) measured in all subjects significantly exceeded the normal values (TGF-β1: 18.3±1.6 ng/mL and TIMP-1: 1 102±67 ng/mL). In contrast, pretreatment MMP-1 mean level (6.5±0.9 ng/mL) was significantly lower than normal values (11.9±0.9 ng/mL). Response to the treatment was observed in 12 patients (43%). TGF-β1 mean concentration measured during the treatment phase decreased to the control level in both groups. However at wk 72, values of NR patients increased and became significantly higher than in R group. TIMP-1 concentrations in R group decreased during the treatment to the level similar to normal. In NR group, TIMP-1 remained significantly elevated during treatment and follow-up phase and significant difference between both groups was demonstrated at wk 48 and 72. MMP-1 levels were significantly decreased in both groups at baseline. Treatment caused rise of its concentration only in the R group, whereas values in NR group remained on the level similar to baseline. Statistically significant difference between groups was noted at wk 48 and 72.

CONCLUSION: These findings support the usefulness of TGF-β1, TIMP-1, and MMP-1 in the management of chronic hepatitis C. Elevated TIMP-1 and low MMP-1 plasma concentrations during antiviral therapy may indicate medication failure.

Key words: HCV; Hepatitis; Liver; Interferons; Fibrosis

Flisiak R, Jaroszewicz J, Lapinski TW, Flisiak I, Prokopowicz D. Effect of pegylated interferon alpha 2b plus ribavirin treatment on plasma transforming growth factor-β1, metalloproteinase-1, and tissue metalloproteinase inhibitor-1 in patients with chronic hepatitis C. *World J Gastroenterol* 2005; 11(43): 6833-6838
http://www.wjgnet.com/1007-9327/11/6833.asp

INTRODUCTION

Transforming growth factor-β1 (TGF-β1) is considered as a pivotal inducer of liver fibrosis acting through activation of hepatic stellate cells (HSCs) and their transformation to myofibroblasts, which are the main source of extracellular matrix (ECM) proteins[1,2]. Moreover, TGF-β1 stimulates the production of tissue inhibitor of TIMP-1 that inhibits MMP activity. This effect is responsible for the inhibition of ECM protein breakdown and its accumulation[3]. TGF-β1 inhibits DNA synthesis serving as a terminator of regenerative cell proliferation and induces apoptosis of hepatocytes[4]. Additionally, TGF-β1 may inhibit stellate cell apoptosis and promote their survival, at least in part as a result of anti-apoptotic effect of TIMP-1[5,6]. On the other hand, TGF-β1 exerts regulatory, mostly immunosuppressive effects on the immune system and as demonstrated recently can also suppress hepatitis C virus (HCV) replication[7,8]. Since HCV infection is related to an immune response, cell proliferation and fibrosis as well as modulation of TGF-β1 can affect the course of chronic hepatitis C. As demonstrated recently, HCV core and

nonstructural proteins regulate biological functions in HSC and increase the secretion of TGF-β1 and the expression of ECM proteins in both HSCs and parenchymal hepatic cells[9,10]. The possible role of TGF-β1, TIMP-1, and MMP-1 as predictive biomarkers of chronic hepatitis activity and progression is supported by recent clinical studies[11-19]. These studies demonstrated association with hepatic function impairment or fibrosis, and only few evaluated possible effects of antiviral treatment on growth factors, but they did not include possible metalloproteinase involvement[20,21].

We undertook this study to evaluate the effect of pegylated interferon-α2b plus ribavirin (PEG-IFN-α2b/RBV) treatment on plasma TGF-β1, TIMP-1, and MMP-1 levels in patients with chronic hepatitis C.

MATERIALS AND METHODS

Patients

Ethical approval for the study was obtained from the Bioethical Committee of the Medical University of Bialystok. Informed consent was obtained from 28 patients (8 females and 20 males, mean age 49±12 years) with chronic hepatitis C, who were included into the protocol of PEG-IFN-α2b (Pegintron™, Schering-Plough) and RBV (Rebetol™, Schering-Plough) treatment. All patients had proven chronic hepatitis C through the presence of anti-HCV antibodies with elevated ALT activities demonstrated at least twice during a 6-mo observation period. Additionally, the disease activity was confirmed by the presence of viral replication and liver biopsy (Hepafix System, Braun, Melsungen, Germany). Patients with HBV infection and a history of alcohol abuse or psychiatric disorders were excluded from the study. Patients received combination therapy with weekly doses of 100 μg PEG-IFN-α2b administered subcutaneously and RBV administered orally at daily doses of 1 000 or 1 200 mg/d based on body weight <75 or ≥75 kg, respectively. The total duration of treatment was 48 wk. Liver biopsy was performed before and after antiviral therapy. Patients were divided into two groups related to sustained virologic response (SVR), defined as undetectable HCV RNA, 24 wk after the end of therapy. Patients who achieved SVR were included into the responder group (R) and those without SVR into non-responder group (NR). Paraffin-embedded biopsy specimens were stained and evaluated using the scoring system according to Scheuer[22]. TGF-β1, TIMP-1, and MMP-1 plasma concentrations were measured at baseline, 24 and 48 wk after treatment and additionally 24 wk after the termination of the treatment (wk 72). Serum liver function tests and scored histological changes were investigated for the possible correlation with TGF-β1, TIMP-1, and MMP-1. Normal values of TGF-β1, TIMP-1, and MMP-1 were collected from 13 healthy volunteers (5 females and 7 males , mean age: 48± 6 years).

Methods

Venous blood for plasma TGF-β1, TIMP-1, and MMP-1 was collected on ice using tubes with EDTA. Samples for TGF-β1 were immediately activated with acetic acid and urea and assayed with ELISA using recombinant human TGF-β soluble receptor Type II (TbR-II) as a solid phase precoated onto a microplate (Quantikine®, R&D Systems Inc., Minneapolis, USA) as described previously[23]. TIMP-1 and MMP-1 were assayed by the two-site ELISA sandwich technique (Amersham Pharmacia Biotech, Little Chalfont, Buckinghamshire, UK) using specific antibodies as a solid phase. MMP-1 assay recognized total human MMP-1, namely free and complexed with TIMP-1. TIMP-1 assay recognized total human TIMP-1, including free and complexed with any of the metalloproteinases bound to the solid phase. TIMP-1 or MMP-1 bound to the solid phase was detected by peroxidase-labeled antibodies. There was no cross-reactivity between TIMP-1 and MMP-1 in these assays. Alanine and aspartate aminotransferase (ALT and AST) activity and bilirubin concentration were measured in serum using a Cobas Mira instrument (Roche).

Statistical analysis

Values were expressed as mean±SE. The significance of the difference was calculated by two-tailed Student's *t* test. For correlation analysis, the Pearson's product moment correlation was performed. $P<0.05$ was considered statistically significant.

RESULTS

Plasma concentrations of TGF-β1 and TIMP-1 measured before PEG-IFN-α2b/RBV treatment (mean: 30.9±3.7 and 1 506±61 ng/mL respectively) significantly exceeded the normal values (18.3±1.6 and 1 102±67 ng/mL respectively). Treatment resulted in a significant decrease of TGF-β1 by wk 24, and its further decline at the end of the treatment as well as 24 wk after its completion to the level similar to normal (Table 1). TIMP-1 plasma mean concentration also decreased, but did not differ significantly from baseline. Moreover, it remained on the level significantly exceeding controls during treatment and follow-up period (Table 1). Mean MMP-1 baseline level (6.5±0.9 ng/mL) was significantly lower than normal (11.9±0.9 ng/mL) but increased during the treatment. After treatment, its level still remained lower than normal but the difference was not significant (Table 1). There was a significant positive correlation between TIMP-1 and aminotransferases as well as between TGF-β1 and AST at baseline (Table 2). A significant correlation was also demonstrated between baseline TGF-β1 or TIMP-1 concentrations and scored fibrosis in pre-treatment liver biopsy specimens (Table 3). No association was

Table 1 Plasma concentrations of TGF-β1, TIMP-1, and MMP-1 during treatment (mean±SE)

	Controls	Weeks after starting treatment			
		0	24	48	72
TGF-β1 (ng/mL)	18.3±1.6	30.9±3.7[a]	21.2±2.8[c]	17.9±2.0[c]	21.0±2.5[c]
TIMP-1 (ng/mL)	1 102±67	1 506±61[a]	1 372±70[a]	1 389±51[a]	1410±66[a]
MMP-1 (ng/mL)	11.9±0.9	6.5±0.9[a]	7.2±1.5[a]	7.5±2.6	8.0±1.6

[a]$P<0.05$ *vs* normal, [c]$P<0.05$ *vs* baseline.

Table 2 Correlation expressed by r-value between biochemical indices of liver injury and TGF-β1, TIMP-1, or MMP-1 in chronic hepatitis C patients before treatment

	Bilirubin	ALT	AST
TGF-β1 (ng/mL)	0.240	0.163	0.388[a]
TIMP-1 (ng/mL)	0.023	0.393[a]	0.370[a]
MMP-1 (ng/mL)	–0.192	–0.130	–0.299

[a]$P<0.05$ biochemical indices *vs* TGF-β1, TIMP-1, and MMP-1.

Table 3 Correlation expressed by r-value between scored histological picture and TGF-β1, TIMP-1, or MMP-1 in chronic hepatitis C patients before treatmen

	Inflammation		Fibrosis
	Portal	Lobular	
TGF-β1 (ng/mL)	0.088	–0.132	0.495[a]
TIMP-1 (ng/mL)	0.091	0.326	0.404[a]
MMP-1 (ng/mL)	0.229	0.360	–0.018

[a]$P<0.05$ histological score *vs* TGF-β1, TIMP-1, and MMP-1

demonstrated between MMP-1 and biochemical or histological signs of liver injury (Tables 2 and 3).

SVR was observed in 12 among 28 patients (43%). Evaluation of baseline liver function tests showed no statistically significant differences between R and NR groups (Table 4). Treatment did not affect bilirubin levels in both groups. Responders demonstrated a significant decrease of ALT and AST activities during the treatment and follow-up. Decline of aminotransferases activity in NR group was only temporal and rose to values significantly higher than in R group after discontinuation of the treatment (Table 4). As demonstrated in Figure 1, scored values of histologic changes were similar before the treatment. There were no significant differences between biopsies preformed before and after the treatment in NR group. In contrast, responders demonstrated improvement after the treatment; however, statistically significant difference between score values at wk 0 and 48 was noted only in respect to portal inflammation.

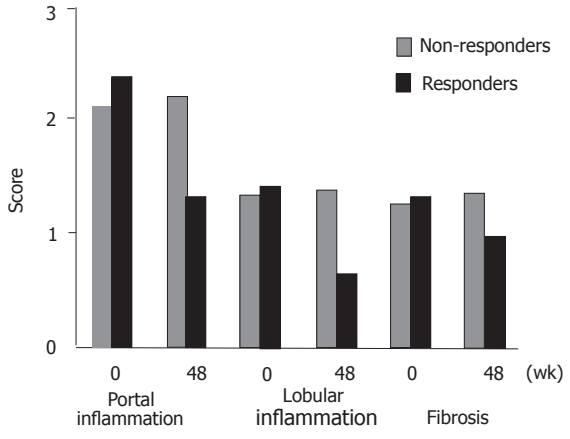

Figure 1 Mean score values of histologic changes in liver biopsy specimens before (wk 0) and after (wk 48) treatment. Statistically significant difference is indicated with arrows.

Table 4 Values of biochemical indices of liver injury during treatment (0, 24, and 48 wk) and 24 wk after its completion (wk 72) in both groups

		Weeks after the beginning of treatment			
		0	24	48	72
Bilirubin	Non-responders	1±0.1	1±0.1	0.7±0.1	0.9±0.1
(mg%)	Responders	1.1±0.1	1.1±0.1	0.9±0.1	1.0±0.1
ALT(U/L)	Non-responders	101±15	40±6[c]	55±20	98±18[a]
	Responders	96±13	35±9[c]	24±5[c]	22±2[c]
AST(U/L)	Non-responders	58±8	30±4[c]	34±6[a,c]	64±10[a]
	Responders	48±6	26±3[c]	20±1[c]	19±2[c]

[a]$P<0.05$ responders *vs* non-responders, [c]$P<0.05$ *vs* baseline.

There were no statistically significant differences in TGF-β1, TIMP-1, and MMP-1 concentrations between R and NR groups at the baseline and 24 wk after the treatment. As demonstrated in Figure 2A, TGF-β1 mean concentration decreased to the control level during treatment in both groups. However, 24 wk after the treatment (wk 72), values in NR patients increased (23.2± 2.3 ng/mL) and became significantly higher than those in R group (18.6±3.7 ng/mL). As shown in Figure 2B, mean concentration of TIMP-1 decreased during the treatment only in R group and there were no statistically significant differences in comparison with controls at wk 24, 48, and 72. In contrast, TIMP-1 concentration in NR group remained significantly elevated (above 1 500 ng/mL) during treatment and follow-up (Figure 2B). Significant difference between both groups was demonstrated at wk 48 and 72. As shown in Figure 2C, MMP-1 levels were significantly decreased in both groups at baseline. Treatment caused rise of its concentration only in the R group, whereas the values in NR group remained on the level similar to baseline. Statistically significant difference between groups was noted at wk 48 and 72 (Figure 2C).

DISCUSSION

The effect of TGF-β1 on liver fibrosis is at least in part related to stimulation of TIMP-1 that affects MMP activity and is responsible for inhibition of ECM protein breakdown[3]. The pivotal role of TGF-β1 in fibrogenesis is initially proved in transgenic mice with overexpression of TGF-β1, causing increase of its plasma levels up to 700 ng/mL and a marked upregulation of TIMP-1 gene expression[24-26]. Recent studies demonstrated that HCV proteins can stimulate secretion of TGF-β1 and production of ECM proteins by HSCs[9,10]. On the other hand, Murata *et al*[8] showed that TGF-β suppresses viral HCV-RNA replication and can affect the mechanism of liver disease caused by HCV. Chronic liver injury leading to fibrosis displays diminished ECM degradation mainly through TIMP induction following MMP inhibition[3]. As demonstrated recently, TIMP-1 recombinant plasmid has inhibitory effects on the production of types I and III collagens secreted by activated rHSCs *in vitro*[27].

The most important factor affecting TGF-β1 measurement in human beings is from platelets which are an important source of this cytokine[28]. The Quantikine ELISA System is recommended because

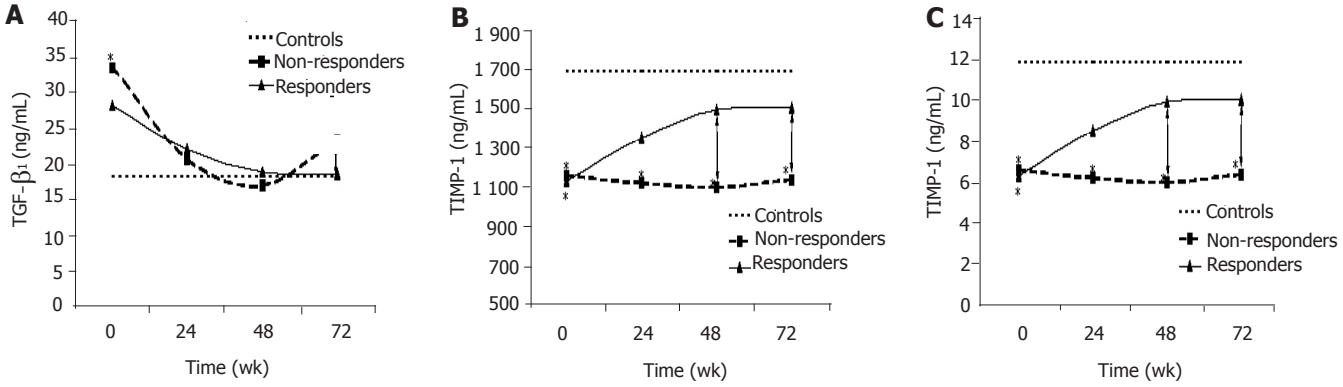

Figure 2 Mean TGF-β1 (**A**), TIMP-1 (**B**), and MMP-1 (**C**) plasma concentrations before and during IFN-α plus RBV therapy as well as 24 wk after its completion (wk 72) in respect to the treatment efficacy. Statistical significance in comparison to normal values is indicated with asterisks and between groups with arrows.

of quick and simple activation with acid and urea that disrupt the majority of TGF-β1 complexes. Mean plasma concentration of TGF-β1 measured in our healthy controls with this method is consistent with the range from more than 20 studies reviewed by Grainger *et al*[28]. According to our previous research, TGF-β1 and TIMP-1 correlate with the degree of liver insufficiency, hepatocyte injury and degree of fibrosis in human beings with liver cirrhosis and chronic viral hepatitis[12,23,29]. Association between TGF-β1 mRNA in liver specimens and fibrogenic activity in chronic hepatitis is demonstrated for the first time by Castilla *et al*[30]. Ten years after the association between circulating or tissue TGF-β and liver fibrosis in HCV infection has been confirmed by Kanzler *et al*[13]. As demonstrated by Yoo *et al*[31] and Lee *et al*[32], HBV antigens also stimulate TGF-β1 synthesis. According to Neuman *et al*[20,33], serum TNF-α reflects the progression of inflammation, whereas TGF-β reflects the degree of fibrosis in HCV patients. A similar relationship has been demonstrated with respect to primary biliary cirrhosis and alcoholic liver disease[34]. Our previous study showed that a positive predictive value of TGF-β1 plasma levels exceeding the upper normal range reaches 96% for liver cirrhosis[23]. According to Boeker *et al*[11] measurement of plasma TIMP-1 detects cirrhosis with 100% sensitivity but a lower specificity. Lichtinghagen *et al*[14] demonstrated that MMP-1 mRNA expression increases steadily with fibrosis progression during the course of chronic hepatitis C. Walsh *et al*[17] who studied liver histology in patients with chronic hepatitis C have underlined the high sensitivity of TIMP-1 and TIMP-2 in detecting advanced liver disease. According to Nie *et al*[35], there is a significant correlation between circulating and liver levels of TIMP-1 in cirrhotics, indicating that its measurement in plasma may be useful in fibrosis management. These observations indicate the usefulness of both TGF-β1 and TIMP-1 as possible early non-invasive biomarkers for liver fibrosis.

In this study, we confirmed the association between the degree of hepatocyte injury or liver fibrosis and plasma TGF-β1 or TIMP-1 levels in patients with chronic hepatitis C. As the levels of TGF-β1 showed a similar behavior in both groups during therapy, it is unclear whether its decrease is a direct effect of medication on the expression or an effect caused by HCV inhibition. However, measurement carried out 24 wk after treatment demonstrated an association with treatment efficiency. Similar effects on plasma TGF-β1 have been observed by Castilla *et al*[30] and Neuman *et al*[20] and in our previous study of chronic hepatitis B[29]. TIMP-1 and MMP-1 concentrations demonstrated significant differences between groups at the end of the treatment and after 24 wk of follow-up. Since plasma TIMP-1 and MMP-1 remained on the baseline level in non-responder group only, lack of their normalization should be considered as a possible indicator of ineffective antiviral therapy. Results of the present study are in accordance with our previous findings, demonstrating the strong association between TGF-β1 or TIMP-1 plasma levels and scored hepatic fibrosis evaluated in biopsy specimens of patients with chronic hepatitis B and C[12]. Since the findings of increased TGF-β1 and TIMP-1 are accompanied with an elevation in plasma carboxyterminal cross-linked telopeptide of type 1 procollagen (ICTP), indicating type I collagen degradation, collagenolytic mechanisms precede TGF-β1/TIMP-1 dependent stimulation of liver fibrosis[12]. Low MMP-1 plasma levels before the treatment in the present study are consistent with this observation as well as in accordance with Murawaki *et al*[15] who demonstrated a decrease in MMP-1 concentration during histological progression of chronic hepatitis. Moreover, significantly decreased baseline plasma MMP-1 followed by an increase during treatment supports the role of TGF-β1/TIMP-1 dependent mechanism of liver fibrosis in patients with active chronic hepatitis C. Similar effects on MMP-1 and TIMP-1 in patients with chronic hepatitis C have been observed by Ninomiya *et al*[16] who showed improvement of liver histology after treatment with IFN-α alone. Downregulation of the mechanism causing an increase of MMP-1 activity should be considered as the probable reason for this effect. As we demonstrated recently, treatment of chronic hepatitis B with lamivudine affects TGF-β1, TIMP-1, and MMP-1 plasma levels in a similar way and this mechanism should be recognized as an effect of response to the antiviral treatment,

irrespective of the etiology[29].

Results of this study support the role of TIMP-1 and MMP-1 balance in the TGF-β1 dependent mechanism of liver fibrosis related to HCV infection. Association between hepatic injury and antiviral treatment efficacy suggests their possible usefulness in chronic hepatitis C management. Elevated TIMP-1 and low MMP-1 plasma concentrations during antiviral therapy may indicate medication failure.

REFERENCES

1 **Knittel T**, Janneck T, Müller L, Fellmer P, Ramadori G. Transforming growth factor beta 1-regulated gene expression of Ito cells. *Hepatology* 1996; **24:** 352-360
2 **Williams EJ**, Gaça MD, Brigstock DR, Arthur MJ, Benyon RC. Increased expression of connective tissue growth factor in fibrotic human liver and in activated hepatic stellate cells. *J Hepatol* 2000; **32:** 754-761
3 **Knittel T**, Mehde M, Kobold D, Saile B, Dinter C, Ramadori G. Expression patterns of matrix metalloproteinases and their inhibitors in parenchymal and non-parenchymal cells of rat liver: regulation by TNF-alpha and TGF-beta1. *J Hepatol* 1999; **30:** 48-60
4 **Fausto N**, Liver regeneration. *J Hepatol* 2000; **32:** 19-31
5 **Saile B**, Matthes N, Knittel T, Ramadori G. Transforming growth factor beta and tumor necrosis factor alpha inhibit both apoptosis and proliferation of activated rat hepatic stellate cells. *Hepatology* 1999; **30:** 196-202
6 **Murphy FR**, Issa R, Zhou X, Ratnarajah S, Nagase H, Arthur MJ, Benyon C, Iredale JP. Inhibition of apoptosis of activated hepatic stellate cells by tissue inhibitor of metalloproteinase-1 is mediated via effects on matrix metalloproteinase inhibition: implications for reversibility of liver fibrosis. **J Biol Chem** 2002; **277:** 11069-11076
7 **Prud'homme GJ**, Piccirillo CA. The inhibitory effects of transforming growth factor-beta-1 (TGF-beta1) in autoimmune diseases. *J Autoimmun* 2000; **14:** 23-42
8 **Murata T**, Ohshima T, Yamaji M, Hosaka M, Miyanari Y, Hijikata M, Shimotohno K. Suppression of hepatitis C virus replicon by TGF-beta. *Virology* 2005; ***331:*** 407-417
9 **Bataller R**, Paik YH, Lindquist JN, Lemasters JJ, Brenner DA. Hepatitis C virus core and nonstructural proteins induce fibrogenic effects in hepatic stellate cells. *Gastroenterology* 2004; **126:** 529-540
10 **Taniguchi H**, Kato N, Otsuka M, Goto T, Yoshida H, Shiratori Y, Omata M. Hepatitis C virus core protein upregulates transforming growth factor-beta 1 transcription. *J Med Virol* 2004; **72:** 52-59
11 **Boeker KH**, Haberkorn CI, Michels D, Flemming P, Manns MP, Lichtinghagen R. Diagnostic potential of circulating TIMP-1 and MMP-2 as markers of liver fibrosis in patients with chronic hepatitis C. *Clin Chim Acta* 2002; **316:** 71-81
12 **Flisiak R**, Maxwell P, Prokopowicz D, Timms PM, Panasiuk A. Plasma tissue inhibitor of metalloproteinases-1 and transforming growth factor beta 1--possible non-invasive biomarkers of hepatic fibrosis in patients with chronic B and C hepatitis. *Hepatogastroenterology* 2002; **49:** 1369-1372
13 **Kanzler S**, Baumann M, Schirmacher P, Dries V, Bayer E, Gerken G, Dienes HP, Lohse AW. Prediction of progressive liver fibrosis in hepatitis C infection by serum and tissue levels of transforming growth factor-beta. *J Viral Hepat* 2001; **8:** 430-437
14 **Lichtinghagen R**, Bahr MJ, Wehmeier M, Michels D, Haberkorn CI, Arndt B, Flemming P, Manns MP, Boeker KH. Expression and coordinated regulation of matrix metalloproteinases in chronic hepatitis C and hepatitis C virus-induced liver cirrhosis. *Clin Sci* 2003; **105:** 373-382
15 **Murawaki Y**, Ikuta Y, Idobe Y, Kawasaki H. Serum matrix metalloproteinase-1 in patients with chronic viral hepatitis. *J Gastroenterol Hepatol* 1999; **14:** 138-145
16 **Ninomiya T**, Yoon S, Nagano H, Kumon Y, Seo Y, Kasuga M, Yano Y, Nakaji M, Hayashi Y. Significance of serum matrix metalloproteinases and their inhibitors on the antifibrogenetic effect of interferon-alfa in chronic hepatitis C patients. *Intervirology* 2001; **44:** 227-231
17 **Walsh KM**, Timms P, Campbell S, MacSween RN, Morris AJ. Plasma levels of matrix metalloproteinase-2 (MMP-2) and tissue inhibitors of metalloproteinases -1 and -2 (TIMP-1 and TIMP-2) as noninvasive markers of liver disease in chronic hepatitis C: comparison using ROC analysis. *Dig Dis Sci* 1999; **44:** 624-630
18 **Patel K**, Gordon SC, Jacobson I, Hézode C, Oh E, Smith KM, Pawlotsky JM, McHutchison JG. Evaluation of a panel of non-invasive serum markers to differentiate mild from moderate-to-advanced liver fibrosis in chronic hepatitis C patients. *J Hepatol* 2004; **41:** 935-942
19 **Leroy V**, Monier F, Bottari S, Trocme C, Sturm N, Hilleret MN, Morel F, Zarski JP. Circulating matrix metalloproteinases 1, 2, 9 and their inhibitors TIMP-1 and TIMP-2 as serum markers of liver fibrosis in patients with chronic hepatitis C: comparison with PIIINP and hyaluronic acid. *Am J Gastroenterol* 2004; **99:** 271-279
20 **Neuman MG**, Benhamou JP, Bourliere M, Ibrahim A, Malkiewicz I, Asselah T, Martinot-Peignoux M, Shear NH, Katz GG, Akremi R, Benali S, Boyer N, Lecomte L, Le Breton V, Le Guludec G, Marcellin P. Serum tumour necrosis factor-alpha and transforming growth factor-beta levels in chronic hepatitis C patients are immunomodulated by therapy. *Cytokine* 2002; **17:** 108-117
21 **Anatol P**, Robert F, Danuta P. Effect of interferon alpha2b plus ribavirin treatment on selected growth factors in respect to inflammation and fibrosis in chronic hepatitis C. *World J Gastroenterol* 2005; **11:** 1854-1858
22 **Scheuer PJ**. Classification of chronic viral hepatitis: a need for reassessment. *J Hepatol* 1991; **13:** 372-374
23 **Flisiak R**, Pytel-Krolczuk B, Prokopowicz D. Circulating transforming growth factor beta(1) as an indicator of hepatic function impairment in liver cirrhosis. *Cytokine* 2000; **12:** 677-681
24 **Kanzler S**, Lohse AW, Keil A, Henninger J, Dienes HP, Schirmacher P, Rose-John S, zum Büschenfelde KH, Blessing M. TGF-beta1 in liver fibrosis: an inducible transgenic mouse model to study liver fibrogenesis. *Am J Physiol* 1999; **276:** G1059-G1068
25 **Sanderson N**, Factor V, Nagy P, Kopp J, Kondaiah P, Wakefield L, Roberts AB, Sporn MB, Thorgeirsson SS. Hepatic expression of mature transforming growth factor beta 1 in transgenic mice results in multiple tissue lesions. *Proc Natl Acad Sci USA* 1995; **92:** 2572-2576
26 **Clouthier DE**, Comerford SA, Hammer RE. Hepatic fibrosis, glomerulosclerosis, and a lipodystrophy-like syndrome in PEPCK-TGF-beta1 transgenic mice. *J Clin Invest* 1997; **100:** 2697-2713
27 **Liu WB**, Yang CQ, Jiang W, Wang YQ, Guo JS, He BM, Wang JY. Inhibition on the production of collagen type I, III of activated hepatic stellate cells by antisense TIMP-1 recombinant plasmid. *World J Gastroenterol* 2003; **9:** 316-319
28 **Grainger DJ**, Mosedale DE, Metcalfe JC. TGF-beta in blood: a complex problem. *Cytokine Growth Factor Rev* 2000; **11:** 133-145
29 **Flisiak R**, Al-Kadasi H, Jaroszewicz J, Prokopowicz D, Flisiak I. Effect of lamivudine treatment on plasma levels of transforming growth factor beta1, tissue inhibitor of metalloproteinases-1 and metalloproteinase-1 in patients with chronic hepatitis B. *World J Gastroenterol* 2004; **10:** 2661-2665
30 **Castilla A**, Prieto J, Fausto N. Transforming growth factors beta 1 and alpha in chronic liver disease. Effects of interferon alfa therapy. *N Engl J Med* 1991; **324:** 933-940
31 **Yoo YD**, Ueda H, Park K, Flanders KC, Lee YI, Jay G, Kim SJ. Regulation of transforming growth factor-beta 1 expression

by the hepatitis B virus (HBV) X transactivator. Role in HBV pathogenesis. *J Clin Invest* 1996; **97:** 388-395

32 **Lee DK**, Park SH, Yi Y, Choi SG, Lee C, Parks WT, Cho H, de Caestecker MP, Shaul Y, Roberts AB, Kim SJ. The hepatitis B virus encoded oncoprotein pX amplifies TGF-beta family signaling through direct interaction with Smad4: potential mechanism of hepatitis B virus-induced liver fibrosis. *Genes Dev* 2001; **15:** 455-466

33 **Neuman MG**, Benhamou JP, Malkiewicz IM, Ibrahim A, Valla DC, Martinot-Peignoux M, Asselah T, Bourliere M, Katz GG, Shear NH, Marcellin P. Kinetics of serum cytokines reflect changes in the severity of chronic hepatitis C presenting minimal fibrosis. *J Viral Hepat* 2002; **9:** 134-140

34 **Neuman M**, Angulo P, Malkiewicz I, Jorgensen R, Shear N, Dickson ER, Haber J, Katz G, Lindor K. Tumor necrosis factor-alpha and transforming growth factor-beta reflect severity of liver damage in primary biliary cirrhosis. *J Gastroenterol Hepatol* 2002; **17:** 196-202

35 **Nie QH**, Cheng YQ, Xie YM, Zhou YX, Bai XG, Cao YZ. Methodologic research on TIMP-1, TIMP-2 detection as a new diagnostic index for hepatic fibrosis and its significance. *World J Gastroenterol* 2002; **8:** 282-287

Science Editor Wang XL and Guo SY **Language Editor** Elsevier HK