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The imbalance of peripheral interleukin-18 and transforming growth factor-β1 levels in patients with cirrhosis and esophageal varices

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ABSTRACT

Introduction: The presence of esophageal varices in liver cirrhosis indicates clinically significant portal hypertension (PH), that results from structural and dynamic changes in the liver and systemic circulation including the activation of several fibrotic and inflammatory pathways. We assessed if interleukin-18 (IL-18) and transforming growth factor-β1 (TGF-β1) serum levels can be used as PH markers and reflect its severity.
Material and methods: IL-18 and TGF-β1 peripheral blood levels were analyzed in 83 cirrhotic patients with esophageal varices compared to healthy individuals, in relation to MELD and Child-Pugh scores, laboratory and Doppler ultrasound parameters, and non-selective beta-blocker therapy (NSBB).
Results: IL-18 concentration was significantly higher in cirrhotic patients, while TGF-β1 concentration was lower than in controls. MELD score correlated positively with IL-18 levels and negatively with TGF-β1 levels. IL-18 levels correlated positively with bilirubin, INR, ALT and AST levels, and negatively with albumin levels and erythrocyte count. TGF-β1 levels correlated positively with platelet count, leukocyte, and erythrocyte count, and negatively with bilirubin levels and prothrombin time. Moreover, significant correlations were found: between IL and 18 levels and portal, mesenteric superior, and splenic vein velocity, and between TGF-β1 levels and splenic vein diameter and spleen size. In a subgroup of patients, IL-18 levels significantly decreased after NSBB.
Conclusion: The observed imbalance of peripheral IL-18 and TGF-β1 levels indicates clinically significant PH associated with the presence of esophageal varices in cirrhosis. The correlation of IL-18 levels with liver failure indicators and decrease with NSBB suggest an important role of IL-18 in disease progression and its potential use as noninvasive test for PH assessment.

1. Introduction

Esophageal varices are the most relevant portal-systemic collaterals that develop in patients with cirrhosis as a consequence of portal hypertension (PH). High portal pressure and variceal diameter are the major determinants of variceal hemorrhage, which is associated with high mortality. The risk of varices and variceal bleeding increases with the stage of liver failure [1–3].

PH results from both increased intrahepatic resistance and increased portal blood inflow. The increased intrahepatic resistance is caused by structural changes due to fibrosis and formation of regenerative nodules, and dynamic changes associated with active contraction of endothelial cells and hepatic stellate cells (HSCs). The increased portal venous inflow results from mesenteric arterial vasodilation and worsens PH despite the development of portal-systemic collaterals. In cirrhosis, HSCs, endothelial cells, and Kupffer cells (KCs) undergo reprogramming leading to their structural and functional changes thereby, contributing further to matrix depositions and causing an imbalance between

Abbreviations: PH, portal hypertension; HSCs, hepatic stellate cells; KCs, Kupffer cells; NO, nitric oxide; ET-1, endothelin-1; TGF-β1, transforming growth factor-β1; IL-1β, interleukin-1β; TNF- α, tumor necrosis factor-α; IL-18, interleukin-18; MELD, Model for End Stage Liver Disease; HBV, hepatitis B virus; HCV, hepatitis C virus; TRL4, toll-like receptor 4; LPS, lipopolysaccharide

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vasodilators and vasoconstrictors. Endothelial dysfunction occurs both within the liver and in systemic circulation. Several factors can modulate the processes described above such as nitric oxide (NO), endothelin-1 (ET-1), transforming growth factor β (TGF-β), and cytokines [1–4]. We have previously shown that patients with cirrhosis have significantly higher ET-1 levels and lower TGF-β1 levels in peripheral blood that correlated with their hepatic levels, liver failure indicators and hepatic venous pressure gradient. However, that study assessed early stages of pH by including only patients with esophageal varices without bleeding [5].

There is growing evidence that systemic inflammation and immune system dysfunction due to bacterial translocation from the gut play an important role in the worsening of liver function and PH. Numerous cytokines are involved in these processes and include interleukin-1β (IL-1β), interleukin-6, interleekin-8, interleukin-17, tumor necrosis factor-α (TNF- α), and CXC family [6–12]. In addition, inflammasomes which are multiprotein complexes that can sense and recognize various exogenous and endogenous danger signals and eventually activate interleukin (IL)-1β and IL-18, play an important role in the initiation and progression of chronic liver disease [12]. Recently, interleukin-18 (IL-18), an important member of the IL-1 family, was found to be increased in patients with alcoholic hepatitis, hepatitis C virus, and cirrhosis as well as in other diseases [13–18]. IL-18 is released by several cells including macrophages, Kupffer cells (KCs), and monocytes. It participates in TNF-α and IFN-γ expression, T cell and vascular endothelial cell stimulation, and nitric oxide and chemokine production. Its unique property is the induction of FasFAs ligand-mediated liver injury [18,19]. IL-18 as pro-inflammatory and immunoregulatory factor may play an important role in systemic inflammation, and immune and endothelial dysfunction observed in patients with cirrhosis and complications of PH.

In this paper, we postulate that imbalances in the systemic levels of IL-18 and TGF- β1 may serve as non-invasive tests to predict PH in cirrhosis at levels associated with the presence of esophageal varices. We analyzed IL-18 and TGF- β1 levels in a larger group of patients with cirrhosis and esophageal varices in relation to previous episodes of variceal bleeding, liver failure and portal hypertension indices. Early recognition of inappropriate changes of the immune system may be essential for overcoming complications such as development of esophageal varices and variceal bleeding thereby delaying disease progression, and reducing mortality.

2. Material and methods

2.1. Patients

A total of 83 patients with liver cirrhosis and esophageal varices hospitalized in the Department of Gastroenterology and Internal Medicine, Medical University of Bialystok, Poland, were recruited to this study. The diagnosis of cirrhosis was made based on clinical data, laboratory tests, imaging data, and/or liver biopsy (if available). We excluded patients with hepatocellular carcinoma or other malignances, portal vein thrombosis, severe cardiac or chronic kidney disease, active infections, or a history of antibiotic therapy in the previous 4 weeks. The presence of esophageal varices was confirmed by endoscopy performed by experienced endoscopists. The study protocol was approved by the local ethics committee. All patients provided informed written consent prior to inclusion in the study.

2.2. Clinical, laboratory, and Doppler ultrasound parameters

The following data was collected: age, gender, etiology of liver disease, previous episodes of variceal bleeding, and routine laboratory tests such as erythrocyte count, leukocyte count, platelet count, bilirubin, prothrombin time, albumin, creatinine, alanine aminotransferase, aspartate aminotransferase, and gamma-glutamyltransferase. Child-Pugh score and Model for End-Stage Liver Disease (MELD) score, as previously described, were used to assess the severity of liver disease [20,21]. The stages of severity based on Child-Pugh score were classified as: Child A (score of 5–6), B (score of 7–9), and C (score of ≥ 10). The following Doppler ultrasound parameters (Simens Elegra, Siemens Medical Systems, Inc, Ultrasound Group) were analyzed: spleen length and width, left and right liver lobe lengths, portal vein diameter, splenic vein diameter, superior mesenteric vein diameter, portal vein velocity, splenic vein velocity, and mesenteric vein velocity.

2.3. Biochemical parameters

Blood levels of IL-18 and TGF-β1 were measured in 83 patients with cirrhosis and 25 healthy individuals as the control group. Blood samples were collected from the peripheral vein after inclusion to the study and stored at −80 °C before analysis. Measurements of IL-18 (Human IL-18 Elisa Kit, MBL, CO, LTD) and TGF-β1 (Quantitative Human TGF- β1 Immunoassays, R&D Systems, Minneapolis, MN) were performed according to the manufacturers' instructions.

2.4. Treatment

In addition, changes of peripheral IL-18 levels after 4 to 6 months of non-selective beta-blocker therapy (NSBB) were assessed in a subgroup of 39 patients with esophageal varices without previous episodes of variceal bleeding.

2.5. Statistical analysis

Statistical analysis was performed using STATISTICA 12.0. Results are presented as median and interquartile range (IQR), and relative frequencies (%). For comparison of variables the following tests were used where appropriate: Wilcoxon matched-pairs singed ranks test, Mann-Whitney U-tests or ANOVA. The Spearman rank-correlation test was used for assessment of correlations. All p values are reported are two-sided and p values less than 0.05 were considered as significant.

3. Results

3.1. Patients

The study population consisted of 83 patients (65 M/18F) with liver cirrhosis with a median age of 52.5 years (IQR: 44.0-59.0). Among all the causes of liver cirrhosis, alcoholic liver disease was the main cause (n = 53; 63.9%). A viral etiology was found in 15 patients (18.1%) and 4 patients had a mixed alcoholic and viral etiology of liver disease (Supplementary Table 1). There were 33 patients (39.8%) with Child-Pugh A class, 28 patients (33.7%) with Child-Pugh B class, and 22 patients (26.5%) with Child-Pugh C class. All patients had esophageal varices, including 42 patients (50.6%) without a history of previous bleeding and 41 patients (49.4%) with a history of variceal bleeding. Patients with a history of variceal bleeding had higher Child-Pugh and MELD scores, bilirubin levels, and lower albumin levels, erythrocyte counts, and platelet counts (Table 1).

3.2. Peripheral serum concentrations of IL-18 and TGF-β1 are differ in patients with liver cirrhosis

The serum concentration of IL-18 in patients with cirrhosis was compared with that of healthy controls. Median IL-18 levels were significantly higher in patients with cirrhosis compared to normal controls [506.25 (IQR: 364.50-609.95) vs 242.10 (IQR: 194.35-283.37) pg/mL; $p < 0.0001$]. Patients with alcoholic liver cirrhosis had significantly higher levels of IL-18 than patients with non-alcoholic liver cirrhosis [537.10 (407.85–637.20) vs 402.28 (294.95–591.55) pg/mL, $p = 0.0344$].

Table 1
Characteristics of patients with liver cirrhosis and varices esophagi with and without a history of variceal bleeding.

	Cirrhosis and esophageal varices		
	Variceal bleeding (–)	Variceal bleeding (+)	p
Age, (IQR)	54.29 (10.27)	54.27 (13.91)	
MELD score, mean (SD)	9.42 (3.59)	13.50 (5.04)	*< 0.0001*
Child-Pugh score, mean (SD)	6.98 (1.97)	8.82 (2.61)	*0.0018*
Heart rate, mean (SD)	72.88 (8.38)	73.78 (13.21)	0.9892
Systolic arterial blood pressure, mean (SD)	120.71 (10.45)	122.73 (15.06)	0.7477
Mean arterial blood pressure, mean (SD)	91.76 (5.92)	90.73 (7.74)	0.1162
Laboratory parameters, mean (SD)			
Alanine aminotransferase (IU/L)	52.31 (41.01)	70.58 (71.20)	0.0979
Aspartate aminotransferase (IU/L)	38.10 (26.78)	35.21 (26.38)	0.7457
Gamma-glutamyltransferase (IU/L)	188.28 (244.97)	161.72 (156.09)	0.7493
Bilirubin (mg/dL)	1.82 (2.41)	2.38 (1.52)	0.0034
Prothrombin time (s)	14.26 (2.13)	16.53 (2.61)	*< 0.0001*
Creatinine (mg/dL)	0.78 (0.14)	0.87 (0.41)	0.7528
Albumin (g/dL)	3.71 (0.58)	3.10 (0.50)	*< 0.0001*
Leukocyte (10^3/μL)	5.42 (2.35)	6.03 (2.91)	0.8863
Erythrocyte (10^6/μL)	4.26 (0.65)	3.42 (0.64)	*< 0.0001*
Platelet count (10^3/μL)	150.08 (74.19)	96.13 (45.60)	*0.0002*

MELD – Model of End Stage Liver Disease score, SD – standard deviation, (+) with a history of variceal bleeding, (-) without a history of variceal bleeding.

We have previously shown that patients with cirrhosis and varices esophagi without history of variceal bleeding, when compared to healthy controls had significantly lower peripheral levels of TGF-β1 (5). In this study, which included a larger group of patients with varices esophagi without and with a history of bleeding, we confirmed that patients with cirrhosis had significantly lower concentrations of TGF-β1 than healthy controls [19.02 (IQR: 13.28-29.79) vs 41.06 (IQR: 36.97-48.88); $p < 0.0001$]. The median levels of TGF-β1 did not significantly differ considering the etiology of liver disease.

3.3. Peripheral serum levels of IL-18 and TGF-β1 correlate with severity of cirrhosis

The levels of IL-18 positively correlated with MELD ($R = 0.4500$, $p < 0.0001$) and Child-Pugh scores ($R = 0.3942$, $p = 0.0003$). The median concentration of IL-18 in patients with Child-Pugh C score [581.95 (IQR: 541.55–833.95) pg/mL] was higher than in those with Child-Pugh B score [496.45 (IQR: 341.82-654.50) pg/mL] or Child-Pugh A score [414.10 (IQR: 336.15-516.05) pg/mL] ($p = 0.004$) (Fig. 1A). Moreover, patients with Child-Pugh A score had significantly higher IL-18 concentration than normal controls ($p < 0.0001$). In addition, compared to controls, IL-18 concentration was significantly increased in both patients with esophageal varices without previous episodes of variceal bleeding and patients after variceal bleeding (Fig. 2A).

Previously, we showed that peripheral levels of TGF- β1 and endothelin-1 correlated with severity of cirrhosis in patients with cirrhosis and esophageal varices who have never presented with bleeding and that levels of TGF- β1 correlated with the HVPG value (5). In the current study, we confirmed that TGF-β1 levels negatively correlated with the MELD score ($R = -0.3145$; $p = 0.0057$). Median TGF-β1 concentration was significantly higher in patients with Child-Pugh A score than in those with Child-Pugh B score and slightly higher than in patients with Child-Pugh C score [24.08 (IQR: 13.91-32.85) vs 17.60 (IQR: 11.67-21.30) vs 17.35 (IQR: 12.80-30.55) pg/mL, $p = 0.012$] (Fig. 1B). In addition, patients with a history of variceal bleeding had significantly lower peripheral TGF-β1 concentration compared to patients without a history of variceal bleeding (Fig. 2B). Among patients with esophageal varices without previous episodes of bleeding, lower TGF-β1 concentration was observed in patients with large varices than in patients with medium or small varices [13.26 (12.03–13.52) vs 24.26 (14.29–31.06) vs 31.41 (343.85–591.55), $p = 0.0069$]. There were no significant differences in median IL-18 concentration when considering the varices size. There was an inverse, significant correlation between peripheral levels of IL-18 and TGF-β1 ($R = -0.3476$; $p = 0.0002$).

3.4. Peripheral serum levels of IL-18 and TGF-β1 in relation to laboratory tests

Peripheral levels of IL-18 correlated positively with bilirubin levels. In addition, we found several significant, although weak positive correlations between IL and 18 levels and prothrombin time, alanine aminotransferase levels, and aspartate aminotransferase levels, and negative correlations between IL and 18 and albumin levels and erythrocyte counts (Table 2).

We found a strong positive correlation between the peripheral levels of TGF-β1 and platelet count, leukocyte, and erythrocyte counts. Moreover, TGF-β1 levels correlated negatively with bilirubin levels and prothrombin time (Table 2).

3.5. Correlation of IL-18 and TGF-β1 levels with Doppler ultrasound parameters

Next, we analyzed the correlation of IL-18 with Doppler ultrasound parameters and found several negative correlations with portal vein velocity, splenic vein velocity, and mesenteric superior vein velocity (Table 2).

Levels of TGF-β1 negatively correlated with splenic vein diameter, spleen length, and spleen width, and positively with right liver lobe diameter (Table 2).

3.6. Levels of IL-18 after non-selective beta-blocker therapy

We have previously shown that the treatment with NSBB led to the increase of peripheral ET-1 concentration, while TGF-β1 concentration decreased (5). In this study we assessed peripheral IL-18 concentration in a subgroup of 38 patients with esophageal varices without previous episodes of bleeding before and after 4 to 6 months of NSBB therapy and found a significant decrease of IL-18 after therapy [510.13 (343.85–609.15) vs 439.18 (342.40–574.45), $p = 0.03612$].

4. Discussion

This study assessed peripheral levels of IL-18 and TGF-β1 in patients with cirrhosis and esophageal varices with and without a history of variceal bleeding in relation to liver failure and PH indicators. In our previous study, we found significantly lower TGF-β1 levels and higher ET-1 levels in peripheral blood of patients with cirrhosis that correlated with their hepatic levels, liver failure indicators, as well as the hepatic venous pressure gradient. However, in that study we assessed only patients with esophageal varices without bleeding episodes [5]. In this study, the inclusion of a larger and more heterogenous group of cirrhotic patients allowed us to observe lower TGF-β1 concentrations in both groups of patients (with esophageal varices with and without previous episodes of variceal bleeding). The first group had the lowest factor concentration. The detected low TGF-β1 levels in peripheral blood may be due to advanced cirrhotic changes. In the study by Weng at al., TGF-β1 signaling was not detectable in a subgroup of patients with more advanced fibrosis (stage ≥ 3) [22]. In contrast, compared to healthy controls, patients with cirrhosis had significantly increased levels of IL-18, including patients with esophageal varices with and without a history of variceal bleeding. Moreover, its levels were

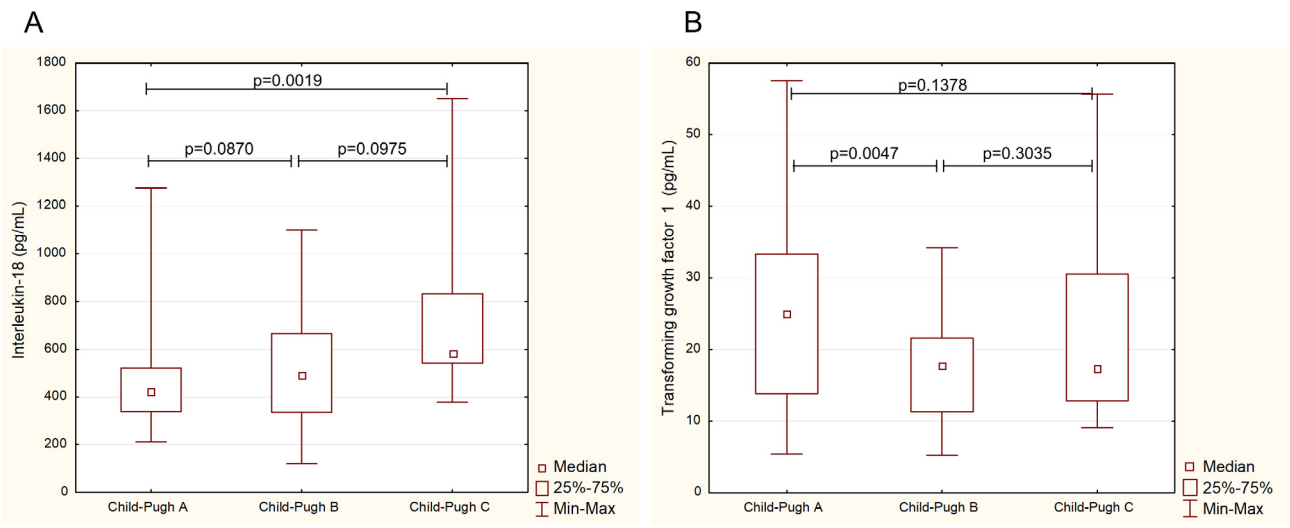

Fig. 1. The concentration (pg/mL) of interleukin-18 (A) and transforming growth factor-β1 (B) in relation to Child-Pugh score.

significantly higher in patients with alcoholic liver disease than in patients with non-alcoholic liver disease. Ludwiczek et al. also observed elevated IL-18 plasma levels in patients with cirrhosis. In addition, they found elevated levels of IL-18 binding protein – an antagonist of IL-18 and suggested that it may be not sufficient to counteract the overwhelming proinflammatory response in end stage liver disease. The levels of IL-18 and its binding protein correlated with laboratory parameters of liver injury and inflammation. Their levels were elevated increased with the disease progression, but independently of the etiology of chronic liver disease [14]. In contrast, Weng et al. first found that the etiology of liver damage may impart different cytokines as a driving force of fibrogenesis suggesting different signaling pathways. They observed TGF-β1 predominance in hepatitis B virus (HBV) related fibrogenesis and steatohepatitis (including alcoholic fatty liver disease) and IL-13 predominance in chronic hepatitis C virus (HCV) infection [22].

The importance of IL-18 in liver diseases was also suggested by other authors. Increased circulating levels of IL-18 and its binding protein were found in alcoholic hepatitis and correlated with hepatitis severity [13]. In another study, polymorphism of the IL-18 promoter regions was suggested to be associated with different outcomes of HBV infection [23]. Sharma et al. observed elevated IL-18 levels in patients with HCV infection that correlated with disease severity and increased IL-18 mRNA expression in peripheral blood mononuclear cells [15]. In our study, the highest IL-18 concentration and the lowest TGF-β1 concentration were observed in patients with Child-Pugh C score. However, significant changes in factors' levels, compared to healthy controls, were detected in earlier stages – in patients with Child-Pugh A score. In addition, peripheral levels of factor correlated with liver failure indicators including MELD and Child-Pugh scores, results from several laboratory tests and Doppler ultrasound parameters. These results suggest their potential use as markers of cirrhosis with the ability to detect early PH stages and to monitor PH therapy. We have previously shown that treatment with NSBB therapy that reduces portal pressure in patients with esophageal varices influences the levels of TGF-β and ET-1 [5]. In the current study, in a subgroup of the patients, NSBB therapy significantly reduced IL-18 levels.

Several factors contribute to the pathogenesis, progression and severity of pH including fibrosis, endothelial dysfunction, angiogenesis, inflammation and immune dysfunction [6–12]. The main feature of immune dysfunction in cirrhosis is the alteration in the toll-like receptor 4 (TLR4) signaling pathway caused by lipopolysaccharides

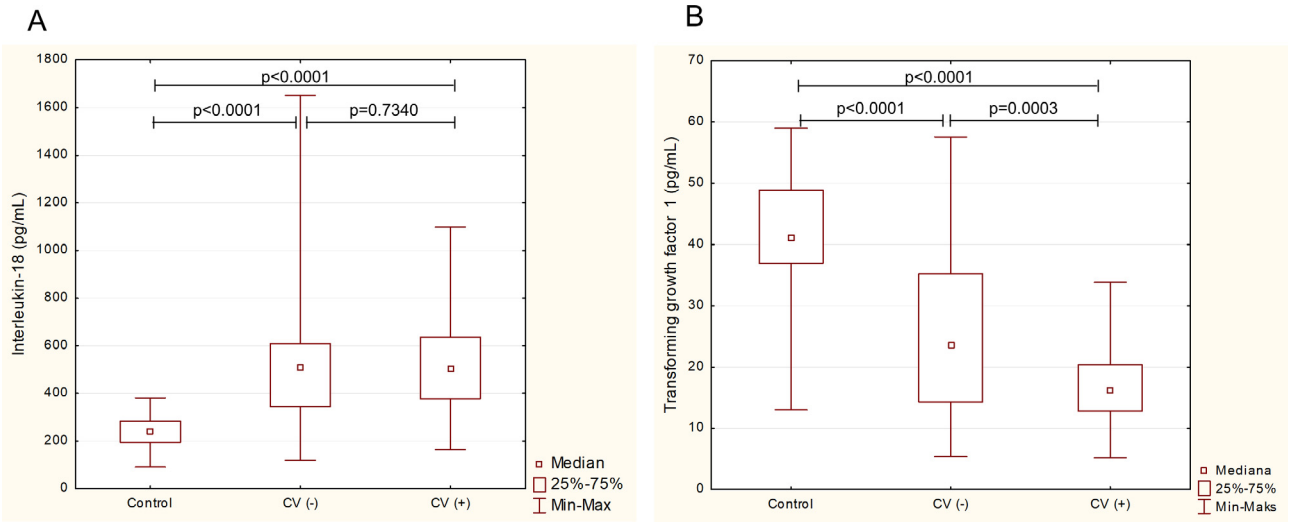

Fig. 2. The concentration (pg/mL) of interleukin-18 (A) and transforming growth factor-β1 (B) in cirrhotic patients with esophageal varices without and with a history of bleeding and in healthy controls. CV (-) – patients with cirrhosis and esophageal varices without a history of variceal bleeding, CV (+) – patients with cirrhosis and esophageal varices with a history of variceal bleeding.

Table 2
The correlation of interleukin-18 (IL-18) and transforming growth factor-β1 (TGF- β1) levels with laboratory tests and Doppler ultrasound parameters.

Rs, p value	IL-18 (pg/mL)	TGF-β1 (pg/mL)
Laboratory tests		
Alanine aminotransferase (IU/L)	*0.3624, 0.0008*	−0.0087, 0.9383
Aspartate aminotransferase (IU/L)	*0.2984, 0.0068*	0.0305, 0.7866
Gamma-glutamyltransferase (IU/L)	0.1959, 0.1016	0.0826, 0.4934
Bilirubin (mg/dL)	*0.4633, < 0.0001*	*−0.2749, 0.0130*
PT (sec)	*0.2218, 0.0452*	*−0.3964, < 0.0001*
Creatinine (mg/dL)	−0.1394, 0.2144	0.1080, 0.3374
Albumin (g/dL)	*−0.2440, 0.0303*	−0.2080, 0.2101
Leukocyte (10^3/μL)	0.1664, 0.1353	*0.4726, < 0.0001*
Erythrocyte (10^6/μL)	*−0.2969, 0.0068*	*0.4481, < 0.0001*
Platelet count (10^3/μL)	−0.1976, 0.0753	*0.7860, < 0.0001*
Doppler Ultrasound parameters		
Spleen length (mm)	−0.0413, 0.7977	*−0.6225, < 0.0001*
Spleen width (mm)	0.0156, 0.9229	*−0.4965, 0.0001*
Liver left lobe length (mm)	0.2208, 0.1709	−0.1335, 0.4113
Liver right lobe length (mm)	0.0700, 0.6677	*0.03485, 0.0276*
Portal vein diameter (mm)	0.2064, 0.1898	−0.2875, 0.0649
Splenic vein diameter (mm)	−0.006, 0.9889	*−0.6212, < 0.0001*
Mesenteric vein diameter (mm)	0.2337, 0.1363	−0.3014, 0.0524
Portal vein velocity (cm/s)	*−0.3200, 0.0388*	0.1426, 0.3678
Splenic vein velocity (cm/s)	*−0.3644, 0.0192*	−0,1235, 0.4417
Mesenteric vein velocity (cm/s)	*−0.3328, 0.0359*	0.0133, 0.9349

IL-18 – interleukin-18, TGF- β1 – transforming growth factor-β1, Rs – Spearman's rank correlation coefficient.

(LPS), foreign toxic agents (ethanol), and damaged hepatocyte-derived TLR ligands [8,24]. Stimulation of TLR4 signaling pathways leads to the activation of HSC, KCs, and endothelial cells resulting in fibrosis, pro-inflammatory cytokine production and angiogenesis [4,6,8]. Attenuation or inhibition of the LPS/TLR4 pathway by either intestinal decontamination (rifaximin) or the use of TLR4 mutant mice showed a significant reduction of HSC activation, angiogenesis, portal hypertension and fibrosis [25]. In addition, changes in TLR expression in response to various stimuli were shown in hepatic cells and peripheral blood mononuclear cells [8,26]. KCs, sinusoidal endothelial cells, and monocytes are hyporesponsive or have tolerance to LPS [8,26]. In our study, the detected imbalance between the levels of IL-18 – a potent proinflammatory and immunoregulative cytokine, and TGF-β – a profibrotic and anti-inflammatory factor, indicates the importance of inflammation as the main driving force in the pathogenesis of pH complications such as esophageal varices and variceal bleeding. IL-18 and TGF-β1 can link signaling pathways between HSC, KCs, immune cells, and bacterial translocation with their imbalance leading to disease progression. It is known that TGF-β1 is released by HSC and contributes to liver fibrosis [27]. In addition, TGF-β1 is predominantly expressed in the immune system by several cells such as macrophages and T cells and is believed to be an important pleiotropic cytokine with potent anti-inflammatory and immunoregulatory properties [27–29]. Intereleukin-18 is released by many cells such as monocytes, KCs, activated macrophages, and dendritic cells. HCV infection stimulates IL-18 and IL-1β secretion in circulatory and resident liver macrophages [30]. In the mouse model of concavalian A induced liver fibrosis, IL-18 administration worsened fibrosis while anti-IL-18 antibodies injections blocked fibrosis [31]. IL-18 is a caspase-1 dependent cytokine and has pro-fibrotic effects through TGF-β1 stimulation in wound healing or TLR4 expression, independent of TGF-β1, in renal tubular epithelial cells [32,33]. IL-18 can contribute to inflammation, endothelial dysfunction and angiogenesis in portal hypertension. IL-18 plays an important role in T-cell activation and regulation of the Th1 response by modulating production of INF-gamma. It may induce macrophages to produce TNF-α and NO [34]. Increased expression of IL-18 receptor was found in vascular endothelium within atherosclerotic lesions [35]. IL-18 upregulates endothelial cell adhesion molecules and induces endothelial cell migration and angiogenesis [36,37]. It was suggested that in rheumatoid arthritis, IL-18 has direct and indirect effects on angiogenesis by inducing angiogenic mediators including vascular endothelial growth factor (VEGF) [17], which also plays a relevant role in sinusoidal remodeling and development of portal-systemic collaterals in PH [2–4].

Our study has limitations. The aim of our study was to assess the levels of factors in patients with esophageal varices with and without a history of variceal bleeding whose presence indicates clinically significant PH. Therefore, we did not include patients with cirrhosis without esophageal varices. In contrast to our previous study, we did not analyze measurements of the hepatic venous pressure gradient [5] because they were available only for a small subgroup of patients included in the current study. The levels of IL-18 after NSBB therapy were only assessed in a subgroup of patients with esophageal varices without previous episodes of bleeding. In addition, we did not analyze of IL-18 binding protein. However, its elevated levels were showed in patients with liver diseases by other authors [11–13]. We did not examine the IL-18 and TGF -β1 receptors that can be differently activated with the progression of portal hypertension. To our knowledge this is the first study that examined prospectively both IL-18 and TGF-β1 concentrations in patients with cirrhosis and esophageal varices and showed an imbalance of their concentrations at early stages of portal hypertension – in patients with esophageal varices without a history of bleeding. While the median concentration of TGF-β1 was lower in our patients with a history of bleeding than in patients who have never bled, the median IL-18 concentration was elevated in both groups of patients with and without variceal bleeding. These observations suggest an important role of IL-18 at both compensated and decompensated stages of the disease and a possible role of IL-18 as triggering factor of liver decompensations. The imbalance of IL-18 and TGF -β1 levels, and their receptors may cause disease progression and decompensation and may indicate patients who are at risk of developing varices and patients with a higher risk of bleeding. However, such study needs a large number of patients to be recruited and a few years of follow-up.

In conclusion, the imbalance between the levels of IL-18 and TGF-β1 reflects clinically significant portal hypertension in cirrhosis at levels connected with the presence of esophageal varices. High IL-18 levels and low TGF-β1levels in peripheral blood that correlate with liver failure and PH indices suggest their potential use in non-invasive assessment of portal hypertension. The factors' imbalance, predominance of IL-18 levels and their reduction with non-selective beta blocker therapy indicate an important role of IL-18 in the pathogenesis of portal hypertension associated with systemic inflammation, endothelial dysfunction and angiogenesis, and may be an important target for development of new treatment strategies.. Detection of inappropriate changes in systemic circulation may be essential for overcoming complications such as development of esophageal varices, delaying disease progression, and reducing mortality.

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Declaration of interest

None.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cyto.2018.10.024.

References

[1] G. Garcia-Tsao, A. Albillos, Classification of cirrhosis: The clinical use of HVPG

measurements, Dis. Markers. 31 (2011) 121–128.
[2] A.J. Sanyal, J. Bosh, A. Blei, V. Arroyo, Portal Hypertension and its complications, Gastroenterology 134 (2008) 1715–1728.
[3] J. Bosch, A. Berzigotti, J.C. Garcia-Pagan, J.G. Abraldes, The management of portal hypertension: rational basis, available treatments and future options, J. Hepatol. 48 (2008) S68–S92.
[4] J. Bosch, R.J. Groszmann, V.H. Shah, Evolution in the understanding of the pathophysiological basis of portal hypertension: how changes in paradigm are leading to successful new treatments, J. Hepatol. 62 (2015) S121–S130.
[5] U. Wereszczynka-Siemiatkowska, A. Swidnicka-Siergiejko, A. Siemiatkowski, Z. Bondyra, J. Wasielica-Berger, B. Mroczko, J. Janica, A. Dabrowski, Endothelin 1 and transforming growth factor-β1 correlate with liver function and portal pressure in cirrhotic patients, Cytokine 76 (2015) 144–151.
[6] G. Mehta, T. Gustot, R.P. Mookerjee, J.C. Garcia-Pagan, M.B. Fallon, V.H. Shah, R. Moreau, R. Jalan, Inflammation and portal hypertension - the undiscovered country, J. Hepatol. 61 (2014) 155–163.
[7] A. Albillos, M. Lario, M. Álvarez-Mon, Cirrhosis-associated immune dysfunction: distinctive features and clinical relevance, J. Hepatol. 61 (2014) 1385–1396.
[8] N. Sipeki, P. Antal-Szalmas, P.L. Lakatos, M. Papp, Immune dysfunction in cirrhosis, World J. Gastroenterol. 20 (2014) 2564–2577.
[9] M. Dirchwolf, A.E. Ruf, Role of systemic inflammation in cirrhosis: From pathogenesis to prognosis, World J. Hepatol. 7 (2015) 1974–1981.
[10] M. Martínez-Esparza, M. Tristán-Manzano, A.J. Ruiz-Alcaraz, P. García-Peñarrubia, Inflammatory status in human hepatic cirrhosis, World J. Gastroenterol. 21 (2015) 522–541.
[11] M.G. Neuman, M. Yaakov, R.M. Nanau, E. Melzer, H. Mell, M. Opris, L. Cohen, S. Malnick, Alcoholic liver disease: role of cytokines, Biomolecules 5 (2015) 2023–2034.
[12] X. Wu, L. Dong, X. Lin, J. Li, Relevance of the NLRP3 inflammasome in the pathogenesis of chronic liver disease, Front Immunol. 8 (2017) 1728.
[13] L. Spahr, I. Garcia, S. Bresson-Hadni, L. Rubbia-Brandt, R. Guler, M. Olleros, Y. Chvatchko, A. Hadengue, Circulating concentrations of interleukin-18, interleukin-18 binding protein, and gamma interferon in patients with alcoholic hepatitis, Liver Int. 24 (2004) 582–587.
[14] O. Ludwiczek, A. Kaser, D. Novick, C.A. Dinarello, M. Rubinstein, W. Vogel, H. Tilg, Plasma levels of interleukin-18 and interleukin-18 binding protein are elevated in patients with chronic liver disease, J. Clin. Immunol. 22 (2002) 331–337.
[15] A. Sharma, A. Chakraborti, A. Das, R.K. Dhiman, Y. Chawla, Elevation of interleukin-18 in chronic hepatitis C: implications for hepatitis C virus pathogenesis, Immunology. 128 (2009) e514–e522.
[16] U. Wereszczynska-Siemiatkowska, B. Mroczko, A. Siemiatkowski, Serum profiles of interleukin-18 in different severity forms of human acute pancreatitis, Scand. J. Gastroenterol. 37 (2002) 1097–1102.
[17] M.V. Volin, A.E. Koch, Interleukin-18: a mediator of inflammation and angiogenesis in rheumatoid arthritis, J. Interferon Cytokine Res. 31 (2011) 745–751.
[18] C.A. Dinarello, D. Novick, S. Kim, G.G. Kaplanski, Interleukin-18 and IL-18 binding protein, Front Immunol. 4 (2013) 289.
[19] H. Tsutsui, K. Matsui, H. Okamura, K. Nakanishi, Pathophysiological roles of interleukin-18 in inflammatory liver diseases, Immunol. Rev. 174 (2000) 192–209.
[20] G. Garcia-Tsao, A.J. Sanyal, N.D. Grace, W. Carey, Prevention and management of gastroesophageal varices and variceal hemorrhage in cirrhosis. Practice guidelines committee of the American association for the study of liver diseases; practice parameters committee of the American college of gastroenterology, Hepatology 46 (2007) 922–938.
[21] P.S. Kamath, W.R. Kim, Advanced liver disease study group, the model for end-stage liver disease (MELD), Hepatology. 45 (2007) 797–805.
[22] H.L. Weng, Y. Liu, J.L. Chen, T. Huang, L.J. Xu, P. Godoy, J.H. Hu, C. Zhou, F. Stickel, A. Marx, R.M. Bohle, V. Zimmer, F. Lammert, S. Mueller, M. Gigou, D. Samuel, P.R. Mertens, M.V. Singer, H.K. Seitz, S. Dooley, The etiology of liver damage imparts cytokines transforming growth factor beta1 or interleukin-13 as driving forces in fibrogenesis, Hepatology 50 (2009) 230–243.
[23] K.K. Migita, K. Sawakami-Kobayashi, Y. Maeda, K. Nakao, S. Kondoh, M. Sugiura, R. Kawasumi, O. Segawa, H. Tajima, M. Machida, M. Nakamura, K. Yano, S. Abiru, E.E. Kawasaki, H. Yatsuhashi, K. Eguchi, H. Ishibashi, Interleukin-18 promoter polymorphisms and the disease progression of Hepatitis B virus-related liver disease, Transl. Res. 153 (2009) 91–96.
[24] L. Li, L. Chen, L. Hu, Y. Liu, H.Y. Sun, J. Tang, Y.J. Hou, Y.X. Chang, Q.Q. Tu, G.S. Feng, F. Shen, M.C. Wu, H.Y. Wang, Nuclear factor high-mobility group box1 mediating the activation of Toll-like receptor 4 signaling in hepatocytes in the early stage of nonalcoholic fatty liver disease in mice, Hepatology 54 (2011) 1620–1630.
[25] Q. Zhu, L. Zou, K. Jagavelu, D.A. Simonetto, R.C. Huebert, Z.D. Jiang, H.L. DuPont, V.H. Shah, Intestinal decontamination inhibits TLR4 dependent fibronectin-mediated cross-talk between stellate cells and endothelial cells in liver fibrosis in mice, J. Hepatol. 65 (2012) 893–899.
[26] A. Uhrig, R. Banafsche, M. Kremer, S. Hegenbarth, A. Hamann, M. Neurath, G. Gerken, A.P.A. Limmer, Development and functional consequences of LPS tolerance in sinusoidal endothelial cells of the liver, J. Leukoc. Biol. 77 (2005) 626–633.
[27] A.M. Gressner, R. Weiskirchen, K. Breitkopf, S. Dooley, Roles of TGF-beta in hepatic fibrosis, Front Biosci. 7 (2002) d793–d807.
[28] I. Fabregat, J. Moreno-Càceres, A. Sánchez, S. Dooley, B. Dewidar, G. Giannelli, P. Ten Dijke, IT-LIVER Consortium, TGF-β signalling and liver disease, FEBS. J. 283 (2016) 2219–2232.
[29] A. Yoshimura, Y. Wakabayashi, T. Mori, Cellular and molecular basis for the regulation of inflammation by TGF-beta, J. Biochem. 147 (2010) 781–792.
[30] S. Shrivastava, A. Mukherjee, R. Ray, R.B. RB, Hepatitis C virus induces interleukin-1β (IL-1β)/IL-18 in circulatory and resident liver macrophages, J. Virol. 87 (2013) 12284–12290.
[31] Y.W. Tan, J.C. Wu, Y. Ye, L. Chen, P.L. Pai, Effects of interleukin-18 and anti-interleukin-18-mAb on experimental immunological liver fibrosis induced by repeatedly administrated concavalian A and its mechanism, Inf. Int. (Electronic Edition) 3 (2014) 161–172.
[32] H. Kämpfer, J. Paulukat, H. Mühl, C. Wetzler, J. Pfeilschifter, S. Frank, Lack of interferon-gamma production despite the presence of interleukin-18 during cutaneous wound healing, Mol. Med. 6 (2000) 1016–1027.
[33] K.K. Meldrum, H. Zhang, K.L. Hile, L.L. Moldower, Z. Dong, D.R. Meldrum, Profibrotic effect of interleukin-18 in HK-2 cells is dependent on stimulation of the Toll-like receptor 4 (TLR4) promoter and increased TLR4 expression, J. Biol. Chem. 287 (2012) 40391–40399.
[34] H. Tsutsui, K. Matsui, N. Kawada, Y. Hyodo, N. Hayashi, H. Okamura, K. Higashino, K. Nakanishi, IL-18 accounts for both TNF-alpha- and Fas ligand-mediated hepatotoxic pathways in endotoxin-induced liver injury in mice, J. Immunol. 159 (1997) 3961–3967.
[35] N. Gerdes, G.K. Sukhova, P. Libby, R.S. Reynolds, J.L. Young, U. Schönbeck, Expression of interleukin (IL)-18 and functional IL-18 receptor on human vascular endothelial cells, smooth muscle cells, and macrophages: implications for atherogenesis, J. Exp. Med. 195 (2002) 245–257.
[36] M.A. Amin, B.J. Rabquer, P.J. Mansfield, J.H. Ruth, H. Marotte, C.S. Haas, E.N. Reamer, A.E. Koch, Interleukin 18 induces angiogenesis in vitro and in vivo via Src and Jnk kinases, Ann. Rheum. Dis. 69 (2010) 2204–2212.
[37] C.C. Park, J.C. Morel, M.A. Amin, M.A. Connors, L.A. Harlow, A.E. Koch, Evidence of IL-18 as a novel angiogenic mediator, J. Immunol. 167 (2001) 1644–1653.