Overview of Serum Interleukin-18 (IL-18) Levels in Liver Cirrhosis Patients and Their Correlation to Hepatic Encephalopathy

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ABSTRACT

Background: *The inflammatory process has an important role in the pathophysiology of hepatic encephalopathy (HE) in liver cirrhosis. Interleukin-18 (IL-18) is a key mediator who plays a role in neuroinflamation processes that can lead to symptoms of HE. This study aimed to determine serum IL-18 levels in liver cirrhosis patients and to assess the association of serum IL-18 levels with HE.*

Method: *A total of 52 subjects (32 patients with liver cirrhosis and 20 healthy controls) were enrolled in this study. Thirty two (32) patients with liver cirrhosis will be assessed for HE based on West-Haven criteria. All subjects were examined for serum IL-18 levels which is measured by ELISA method. We performed a comparative analysis between serum IL-18 levels of liver cirrhosis patients and healthy controls, a correlation analysis between serum IL-18 levels and HE, and a comparative analysis of serum IL-18 levels among degrees of HE.*

Results: *Mean serum IL-18 levels in the liver cirrhosis group were 688.5 ± 674.3 pg/mL, and in the healthy controls group were 163.9 ± 100 pg/mL with $p = 0.01$ ($p < 0.05$). There was a significant correlation between IL-18 and HE ($r = 0.85$; $p = 0.00$). Serum IL-18 levels in covert and overt HE groups were significantly higher than those without HE ($p < 0.05$).*

Conclusion: *Serum IL-18 levels were significantly higher in liver cirrhosis patients than in healthy controls. There was a positive correlation between IL-18 and HE. Serum IL-18 levels in liver cirrhosis patients with HE were significantly higher than those without HE.*

Keywords: *interleukin-18, liver cirrhosis, hepatic encephalopathy*

ABSTRAK

Latar belakang: *Proses inflamasi memiliki peran penting dalam patofisiologi terjadinya ensefalopati hepatik (EH) pada sirosis hati**.** Interleukin-18 (IL-18) merupakan mediator kunci yang berperan dalam proses neuroinflamasi yang dapat menimbulkan gejala EH. Penelitian ini bertujuan mengetahui gambaran kadar IL-18 serum pada pasien sirosis hati, menilai hubungan kadar IL-18 serum dengan EH dan menilai perbedaan kadar serum IL-18 antara pasien sirosis hati tanpa EH dan dengan EH.*

Metode: *Sebanyak 52 subjek (32 pasien sirosis hati dan 20 subjek sehat sebagai kontrol) terlibat dalam penelitian ini. Tiga puluh dua (32) pasien sirosis hati akan dinilai derajat EH berdasarkan kriteria West-Haven. Seluruh subjek penelitian diperiksa kadar IL-18 serum dengan metode ELISA. Dilakukan analisis perbandingan*

antara kadar IL-18 serum pasien sirosis hati dengan subjek sehat, analisis korelasi antara kadar IL-18 serum dengan derajat EH, dan analisis perbandingan kadar serum IL-18 antara derajat EH.

Hasil: *Rata-rata kadar IL-18 serum pada kelompok sirosis hati sebesar 688.5 ± 674.3 pg/mL, dan kelompok kontrol sehat sebesar 163.9 ± 100 pg/mL dengan nilai p sebesar 0.01 ($p < 0.05$). Hasil analisis korelasi antara kadar IL-18 serum dengan EH didapatkan koefisien korelasi sebesar 0.85 dengan nilai p sebesar 0.00 ($p < 0.05$). Kadar serum IL-18 kelompok EH covert dan EH overt lebih tinggi dan berbeda bermakna dibandingkan dengan tanpa EH ($p < 0.05$)*

Simpulan: *Kadar IL-18 serum pada pasien sirosis hati lebih tinggi bila dibandingkan dengan subjek sehat, terdapat hubungan positif antara kadar IL-18 serum dengan EH pada pasien sirosis hati, dan kadar IL-18 serum pada pasien sirosis hati dengan EH lebih tinggi dibandingkan dengan tanpa EH.*

Kata kunci: *interleukin-18, sirosis hati, ensefalopati hepatik*

INTRODUCTION

Hepatic encephalopathy (HE) is a neuropsychiatric syndrome associated with chronic and acute liver dysfunction, with clinical spectrum of neuropsychological disorders range from mild (subclinical) to coma.[1] The prevalence rate of HE is quite high, in a mild/ subclinical clinical spectrum, the prevalence of HE ranging about 30-88% in patients with liver cirrhosis.[2] Over the last 30 years, knowledge of HE has not changed much and seems to be largely ignored. This is seen with therapy that still aims to decrease the production of ammonia levels or increase its metabolism through the hepatic-urea cycle.[3]

The central role of ammonia in the pathogenesis of HE remains undeniable, but there has been some evidence that serum ammonia levels may be normal in 30% of patients with overt HE,[4] and serum ammonia levels may increase in 69% of patients without any HE signs and symptoms.[5] This suggests that there are other factors in the pathogenesis of HE. The inflammatory process appears to be a key factor for HE, in addition to disturbed gastrointestinal tract disorders which lead to microbiota dysbiosis, overgrowth of bacteria, bacterial translocation, systemic endotoxaemia and immune dysfunction.[1] Shawcross shows that patients with liver cirrhosis with hyperamonia due to amino acid oral administration to sepsis conditions, there are increases in proinflammatory mediators such as TNF-α, IL-1β and IL-6, and poor neuropsychiatric examination results. This is different than patients with hyperamonia without an inflammatory condition.[7] This suggests that hyperamonia alone is not enough to make patients with liver cirrhosis fall into HE.

One of the key mediators involved in neuroinflammatory processes is IL-18.[8] The IL-18 bond with its receptors in the central nervous system in addition to their role in neuroinflammatory processes may also affect individual behavior, as occurs in liver cirrhosis patients with HE.[9] Ludwiczek et al shows significant plasma IL-18 level increase in patients with liver cirrhosis compared with healthy controls. The IL-18 plasma level increases in parallel with the progression of liver cirrhosis.[10] However, its relationship to the stage of HE has not been clearly defined. In Indonesia alone, no study have shown correlation between serum levels of IL-18 and HE, therefore this study aims to know the serum IL-18 level in liver cirrhosis patients, assess the association of serum IL-18 with HE and assess the difference in serum IL- 18 between liver cirrhosis patients without HE and with HE.

METHOD

This study was cross-sectional observational. The population of this study were all patients of liver cirrhosis in Dr. Saiful Anwar General Hospital, Malang. The sample of this study was liver cirrhosis patients who had excluded and met inclusion criteria. Patients are given an explanation of the intent and purpose of the study, then asked to sign an informed consent if they are willing to take part in the study. Technique of sampling by consecutive sampling. The inclusion criteria of this study include: (1) Male or female patients aged 18-79 years; (2) Liver cirrhosis patients with or without hepatic encephalopathy. Exclusion criteria for the study included: (1) Patients with hepatocellular carcinoma; (2) Liver cirrhosis patients with stroke and its sequelae; (3) Hypoglycemia.

The healthy subjects will be used as controls to compare serum IL-18 levels in healthy subjects and patients with liver cirrhosis. The healthy subjects will be matched by age and gender with liver cirrhosis patients. Subjects said to be healthy after anamnesis, physical examination and laboratory test showed normal results. Blood samples were taken by

phlebotomy of venous blood as much as 3 mL and inserted into vacutainer tube without ethylene diamine tetra acetate. The blood is centrifuged for 10 minutes to obtain serum. The obtained serum is stored at -80 °C until it is used for enzyme-linked immunosorbent assay (ELISA) examination. IL-18 examination using ELISA rapid with Human IL-18 serum Elabscience kit.

This study was conducted between May 2017 and October 2017 in Gastroentero-hepatology Polyclinic, Internal Medicine inpatient ward, Dr. Saiful Anwar General Hospital Malang, and Biomedical Laboratory, Faculty of Medicine, Universitas Brawijaya Malang.

Statistical analysis with numerical comparative tests will be used to see differences between serum IL-18 levels in cirrhotic patients with healthy control and to determine the ratio of serum IL-18 levels between groups of HE stages. The correlation test will be used to analyze the association of serum IL-18 levels with hepatic encephalopathy. Statistical analysis of data using statistical products and service solutions (SPSS) program statistics, version 17 for windows.

RESULTS

There were 52 subjects were enrolled during this study that consist of 32 patients with liver cirrhosis and 20 people as healthy control. We had performed HE assessment to 32 cirrhotic patients based on the West Haven criteria. It was obtained that 6 patients without symptoms of HE; 5 patients with minimal HE; 6 patients with stage 1 HE; 3 patients with stage 2 HE; 8 patients with stage 3 HE; and 4 patients with stage 4 HE. Characteristics of subjects are shown in Table 1.

Table 1. Characteristics of liver cirrhotic patients and healthy controls

Characteristics	Group		p
	Liver cirrhosis (n = 32)	Control (n = 20)	
Sex			0.45
Male	24 (75%)	13 (65%)	
Female	8 (25%)	7 (35%)	
Age	49.5 ± 8.2	48.4 ± 12	0.70
Cause of liver Cirrhosis			
Hepatitis B	19 (59.4%)	-	-
Hepatitis C	8 (25%)	-	-
Non-B Non-C	5 (15.6%)	-	-
Stage of hepatic encephalopathy (HE)			
Without HE	6 (18.8%)	-	-
Minimal HE	5 (15.6%)	-	-
Stage 1	6 (18.8%)	-	-
Stage 2	3 (9.4%)	-	-
Stage 3	8 (25%)	-	-
Stage 4	4 (12.5%)	-	-
Child-turcotte-pugh (CTP)			
A	8 (25%)	-	-
B	5 (15.6%)	-	-
C	19 (59.4%)	-	-
Hb (mg/dL)	9.8 ± 2.2	14.1 ± 0.5	0.00*
Leukosit (/µL)	11211.9 ± 7486	7538 ± 1405.5	0.14
Trombosit (/µL)	98000 ± 40452.3	342350 ± 79898.1	0.00*
AST (U/L)	55.8 ± 27.4	20.2 ± 7.5	0.00*
ALT (U/L)	39.6 ± 18.4	20.1 ± 12.3	0.00*
Ureum (mg/dL)	76.2 ± 69.5	29.8 ± 1.6	0.01*
Creatinin (mg/dL)	1.8 ± 1.9	0.8 ± 0.1	0.04*
Natrium (mmol/L)	128.1 ± 7.8	140 ± 3.5	0.00*
Kalium (mmol/L)	4.5 ± 1	3.9 ± 0.3	0.08
Cloride (mmol/L)	102.8 ± 7.7	98 ± 1.7	0.00*
Random blood glucose (mg/dL)	128.9 ± 26.3	103 ± 16	0.06

*statistically significant ($p < 0.05$) with Mann-Whitney test; Hb: hemoglobin; AST: aspartate aminotransferase; ALT: alanine aminotransferase

Serum IL-18 test results between the liver cirrhosis group and healthy control group can be seen in Figure 2.

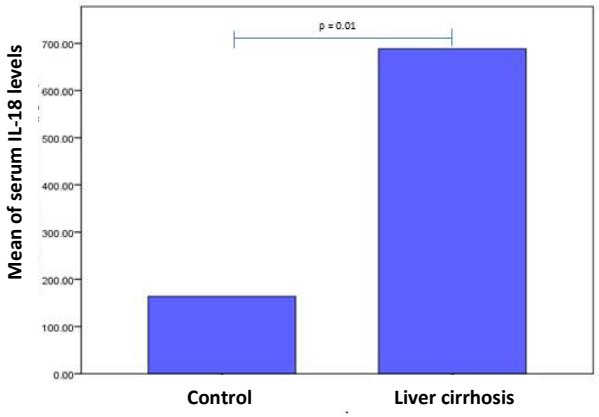

Figure 2. Differences in serum IL-18 levels between liver cirrhosis group and healthy control group.

Figure 2 shows that the mean serum IL-18 levels in the liver cirrhosis group were 688.5 ± 674.3 pg/mL, and the healthy control group of 163.9 ± 100 pg/mL. By using Mann-Whitney test, p is 0.01 ($p < 0.05$). It was proved that the mean serum IL-18 levels in the liver cirrhosis group were higher and significantly different with the healthy control group. The results of this analysis prove that there is elevated serum IL-18 level in patients with liver cirrhosis.

Table 2. Serum IL-18 levels of liver cirrhosis patients with various degrees of hepatic encephalopathy

Stage of hepatic encephalopathy	Mean serum IL-18 levels (pg/mL)
Without hepatic encephalopathy (HE)	81.37 ± 47.68
Minimal hepatic encephalopathy (HE)	155.42 ± 103.35
Stage 1	354.02 ± 297.83
Stage 2	804.83 ± 561.61
Stage 3	1200 ± 629
Stage 4	1656.75 ± 33.75

From Table 2, the lowest average serum IL-18 levels were found in liver cirrhosis patients without HE, and this value continued to increase to the highest level in liver cirrhosis patients with a stage 4 HE. The results of analysis of correlation between serum IL-18 levels and HE in liver cirrhosis patients, it was obtained

correlation coefficient of 0.85 with p = 0.00 (p < 0.05). It was shown that there was a significant relationship between serum IL-18 levels and HE in liver cirrhosis patients. The correlation coefficient of 0.85 indicates a very strong association between serum IL-18 levels and HE in liver cirrhosis patients. The positive correlation coefficient indicates that elevated serum IL-18 levels will be followed by elevated HE degree, and vice versa. Correlation of serum IL-18 levels with HE levels in patients with liver cirrhosis is shown in Figure 3.

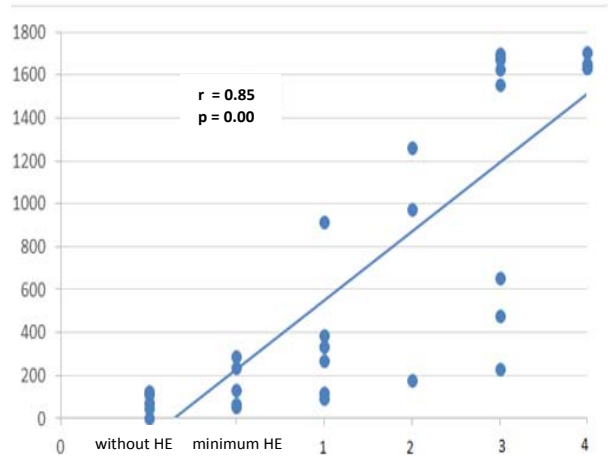

Figure 3. Correlation of serum IL-18 levels with hepatic encephalopathy (HE) degree in liver cirrhosis patients

Difference in test results of serum IL-18 levels based on the degree of HE in patients with liver cirrhosis are shown in Figure 4.

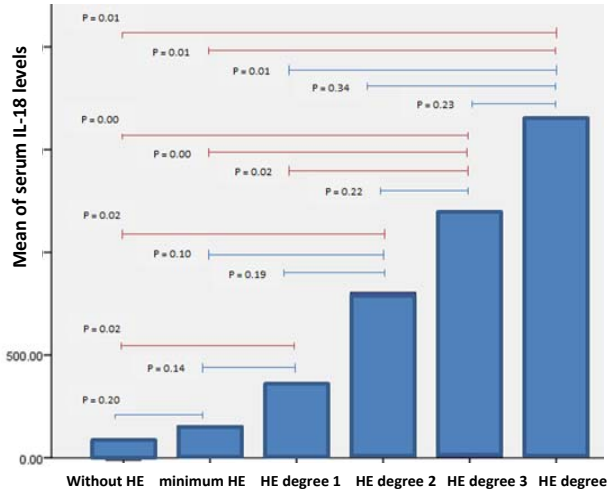

Figure 4. Comparison of serum IL-18 levels Based on hepatic encephalopathy (HE) degree in liver cirrhosis patients. Description: the red line indicates p < 0.05, blue line indicates p > 0.05

With the Mann-Whitney test, the difference in serum IL-18 levels in the liver cirrhosis patients between without HE and with minimum HE group, it was obtained p-value of 0.247 (p > 0.05) showed no significant difference. While compared to the groups of patients at 1-4 degrees, p was less than 0.05 (p < 0.05) indicating a significant increase in IL-18 levels.

Based on severity of symptom, HE can be divided into 2 groups ie covert HE (minimum HE and stage 1 HE) and overt HE (stage 2, 3 and 4 HE). To determine the difference of IL-18 levels between the groups, Kruskal-Wallis test and Mann-Whitney test were performed. The test results of different serum IL-18 levels based on the each stage HE group in liver cirrhosis patients are shown in Figure 5.

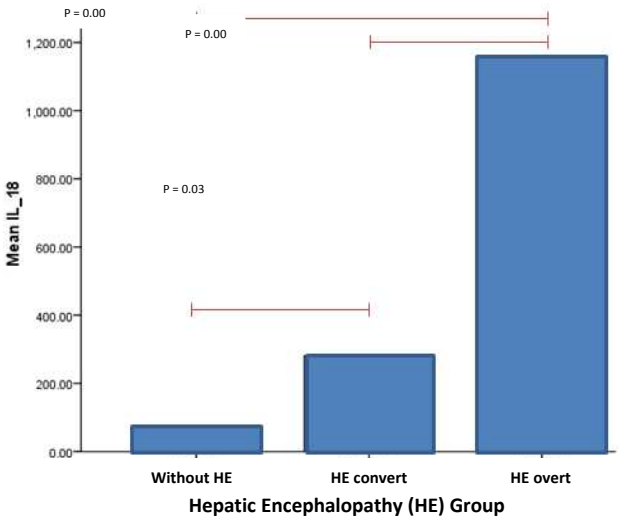

Figure 5. Differences in serum IL-18 levels between hepatic encephalopathy (HE) groups in liver cirrhosis patients. Description = The red line shows the value of p < 0.05

Based on Figure 5, there was a significant difference in serum IL-18 levels between group without HE and with covert HE (p = 0.03), group without HE with overt HE (p = 0.00), and covert HE with overt HE (p = 0.00).

DISCUSSION

Of the 32 patients with liver cirrhosis who were the subject of the study, 75% of the patients were male and 25% were female. In southern China, Xing Wang et al reported that most liver cirrhosis patients were men of 83.16% and 16.84% were female.[11] The study, carried out by Tambunan et al in Dr. Soedarso Pontianak during period January 2008 - December 2010 which also get the largest patient of cirrhosis hepatis is male equal to 69,6%.[12] The same thing reported by Dr. M. Djamil Padang General Hospital which in patients with liver cirrhosis, the percentage of male (65.8%) more than female.[13] Gender is thought to have a role in the occurrence of cirrhosis hepatis, men have different social environment and lifestyle than women, men generally have a greater chance of contact with viral

hepatitis and alcohol consumption.[14] The median age of patients with liver cirrhosis in this study was 49.5 ± 8.2 years. This is in accordance with the literature which states that the average age of sufferers most age group 30-59 years with peak around the age of 40-49 years.[15]

In our study, the most common cause of liver cirrhosis was hepatitis B virus infection of 59.4% followed by hepatitis C of 25%. In developed countries, the most common causes of liver cirrhosis are alcoholic liver disease and hepatitis C virus infection, whereas in Southeast Asia, the main causes of liver cirrhosis are hepatitis B and hepatitis C virus infection.[15,16]

From the results of this study, 18.8% (6 patients) had no symptoms of hepatic encephalopathy while 81.2% (26 patients) were accompanied by hepatic encephalopathy. Based on West Haven criteria, from 26 patients; 5 patients (15.6%) were found with minimal HE; 6 patients (18.8%) with stage 1 HE; 3 patients (9.4%) with stage 2 HE; 8 patients (25%) with stage 3 HE; and 4 patients (12.5%) with stage 4 degree HE. Thus, the outline of this study indicates that 34.3% of patients belong to covert HE and 47% of patients belong to overt HE. These findings are consistent with the literature which stated that HE prevalence in a mild/subclinical clinical spectrum varies between 30-88% in patients with liver cirrhosis, whereas overt HE occurs in about 45% of patients with liver cirrhosis.[2,17]

A total of 20 healthy subjects participated as controls in this study, including 7 women and 13 men with an average age of 48.4 ± 12 years. From the test results, the difference between liver cirrhosis group and control of gender characteristics and age was found p greater than 0.05 ($p > 0.05$) indicating that both groups had uniform or homogeneous gender and age characteristics. The uniformity of sex and age in this study is important because based on research conducted by Ferrucci et al showed that IL-18 levels differ by sex and age group.[18]

From the analysis, it was proved that the mean serum IL-18 levels in the liver cirrhosis group were higher and significantly different than the healthy control group. This is consistent with Ludwiczek et al's study which suggested that there is a significant difference in plasma IL-18 levels in liver cirrhosis patients when compared with healthy controls.[10]

The results of this study indicate that there is an elevated serum IL-18 level in patients with liver cirrhosis. The high serum IL-18 concentration was associated with elevated plasma endotoxin or Lipopolysaccharide (LPS) levels of the gastrointestinal microbiota in liver cirrhosis patients.[19] Hanck et al demonstrated a significant increase in plasma LPS levels in liver cirrhosis patients when compared with healthy controls.[19] LPS plasma levels is also associated with increased expression of IL-18 genes by peripheral blood mononuclear cells.[19] In vitro study also showed that LPS stimulates expression of IL-18 by peripheral blood mononuclear cells.[20]

In addition to increased IL-18 expression by peripheral blood mononuclear cells due to LPS stimulation, high serum IL-18 levels in patients with liver cirrhosis reflect liver clearance failure on LPS caused by portosystemic shortcuts, phagocyte capacity defect of the liver reticuloendothelial system and decreased endotoxin binding capacity.[19,21]

This study has proved that there is a very strong association between serum IL-18 levels and hepatic encephalopathy in patients with liver cirrhosis with a positive correlation coefficient indicating elevated serum IL-18 levels will be followed by elevated HE stage. This is consistent with the Jain et al study which also showed a significant association between serum IL-18 levels and hepatic encephalopathy ($r = 0.76$; $p = 0.02$).[21] In addition to IL-18, Jain et al also proved a positive correlation between proinflammatory cytokines (TNF-α, IL-6), endotoxin serum with hepatic encephalopathy stage.[22]

This study shows that there is role of inflammation in the pathophysiology of hepatic encephalopathy in patients with liver cirrhosis. The underlying mechanisms of IL-18 in the pathophysiology of hepatic encephalopathy are evidenced by in vitro study of laboratory animals (rat) demonstrating that IL-18 destroys long-term potentiation and signal transduction mediated by N-methyl D-aspartate-receptor in the hippocampus.[23] Both processes are involved in learning and memory formation. This suggests that IL-18 may also cause changes that lead to decreased cognitive function.[24]

This study proves that IL-18 can differentiate between cirrhosis of the liver without hepatic encephalopathy, with covert and overt encephalopathy hepatic. Similarly, study conducted by Montoliu et al also demonstrated that significantly higher IL-18 levels in patients with liver cirrhosis with a minimum HE when compared with liver cirrhosis without HE.[24] IL-18 may be a good indicator for detecting covert HE.[24]

This study has shown that there is a role of inflammation in the pathophysiology of hepatic encephalopathy in patients with liver cirrhosis. The clinical consequence of the role of inflammation in the pathophysiology of hepatic encephalopathy is in

its treatment. Cauli et al in his study provided non-steroidal anti-inflammatory drug (NSAID) ibuprofen in rat-experimental animals with hepatic encephalopathy and resulted in decreased neuroinflammation and improved cognitive and motor abilities.[25] However, NSAID administration may induce gastropathy, and have secondary effects on damage kidney, so that other therapeutic modalities are required which able to eliminate neuroinflammation in patients with liver cirrhosis with hepatic encephalopathy.[26,27]

IL-18 is a potent protein kinase activator p38 mitogen-activated protein kinase (MAPK).[27] p38 MAPK is the meeting point of various signaling processes involved in inflammation. The activity of p38 increases in the brain cortex of mouse-fed animals with hepatic encephalopathy.[28,29] The role of p38 in inflammation and neuroinflammation makes it an attractive target for novel therapy.[29]

The limitation of this study is the number of samples lacking to test the diagnostic value of IL-18 in differentiating patients with liver cirrhosis without hepatic encephalopathy and with covert hepatic encephalopathy. In addition, this study did not observe changes in serum IL-18 levels when improving the symptoms of HE after patients received treatment.

CONCLUSION

From the results and analysis of the research, it can be concluded that serum IL-18 levels were significantly higher in liver cirrhosis patients than in healthy controls. There was a positive correlation between IL-18 and HE in liver cirrhosis patients and serum IL-18 levels in liver cirrhosis patients with HE were significantly higher than those without HE.

Further research with a larger sample is needed to test the serum IL-18 diagnostic value in distinguishing liver cirrhosis patients without hepatic encephalopathy and with covert hepatic encephalopathy. Serum IL-18 should be required after improvement of hepatic encephalopathy symptoms which will support IL-18 correlation with hepatic encephalopathy degree.

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