Difference in serum procalcitonin levels between decompensated liver cirrhosis patients with and without bacterial infection

Rino Alvani Gani, Oska Mesanti, Marcellus Simadibrata, Suhendro, Irsan Hasan, Andri Sanityoso, C. Rinaldi A. Lesmana

Abstract

***Background*: Decompensated liver cirrhosis may increase procalcitonin (PCT) levels in patients without bacterial infection. Previous studies have not provided conclusive results about the difference in serum PCT levels caused by specific liver decompensation and bacterial infection.**

***Objective*: To examine the role of PCT in assisting the diagnosis of bacterial infection in decompensated liver cirrhosis patients.**

***Methods*: A cross-sectional study on decompensated liver cirrhosis patients who were outpatients and admitted to Cipto Mangunkusumo Hospital, Jakarta, was conducted between December 2015 until May 2016. Procalcitonin levels were examined and bacterial infection was identified using standard criteria for each type of infection suspected. Analysis was performed to determine the difference in PCT levels between patients with and without bacterial infection, and to obtain the cutoff point of PCT for bacterial infection diagnosis using the receiver operating characteristic (ROC) curve.**

***Results*: There were 38 patients with decompensated liver cirrhosis, 16 (42.1%) with bacterial infection, and 22 (57.9%) without bacterial infection. Patients with bacterial infection (3.607±0.643 ng/ml) had significantly higher PCT levels than those without bacterial infection (0.738±1.185 ng/ml). The level of PCT for bacterial infection in decompensated liver cirrhosis had an area under the ROC curve of 0.933 (CI 0.853-1.014). The cutoff point of PCT for bacterial infection diagnosis in decompensated liver cirrhosis patients was 2.79 ng/ml, with a sensitivity of 87.5% and specificity of 86.4%.**

***Conclusion*: The PCT levels of decompensated liver cirrhosis patients with bacterial infection were higher than those of patients without bacterial infection. The cutoff point of PCT for bacterial infection diagnosis in decompensated liver cirrhosis patients was 2.79 ng/ml.**

Key words: Procalcitonin, decompensated, bacterial infection.

Introduction

Bacterial infection is one of the most common acute complications in liver cirrhosis patients. The global prevalence of bacterial infection in hospitalized liver cirrhosis patients varies between 25% and 47%. (1) These data do not differ greatly from the infection data for Jakarta, which indicate that 37.4% of hospitalized liver cirrhosis patients have bacterial infection. (2) We also know that gram-negative bacteria cause 64% of infection cases. (3) Approximately 33% of acute-on-chronic liver failure (ACLF) cases occur because of bacterial infection. (3) It is also responsible for 43.5% of cumulative mortality in liver cirrhosis patients. (4)

Immune system dysfunction and systemic inflammation are the two major mechanisms in the pathophysiology of liver cirrhosis. The process starts with the damage of liver parenchyma through damage-associated molecular patterns, which then activate the immune system, causing sterile systemic inflammation. This process is followed by the development of pathogen-associated molecular patterns, which occur through the stimulation of bacterial translocation and its products (lipopolysaccharide [LPS] and methylated DNA) and further activate the immune system, worsening the systemic inflammation. This pathophysiological process continues until the exhaustion and loss of immune response to pathogens, leading to bacterial infection and progression into a decompensated state or ACLF. (5)

From Hepatobiliary Division, Internal Medicine Department, Faculty of Medicine University of Indonesia, Cipto Mangunkusumo Hospital, Jakarta, Indonesia (Rino Alvani Gani, Oska Mesanti, Marcellus Simadibrata, Suhendro, Irsan Hasan, Andri Sanityoso, and C. Rinaldi A. Lesmana).

Address for correspondence:
Rino Alvani Gani
Hepatobiliary Division, Internal Medicine Department, Faculty of Medicine University of Indonesia, Cipto Mangunkusumo Hospital, Jakarta
J. Pangeran Diponegoro 71, 10430, Jakarta Pusat, Indonesia
Tel: 021-31900924
Email: personaly@yahoo.com

The typical clinical manifestations of bacterial infection, such as fever and leukocytosis, are observed only in approximately half of liver cirrhosis patients, and systemic inflammatory response syndrome (SIRS) can be found in 10%-30% of decompensated liver cirrhosis patients without any evidence of bacterial infection. (6) Some clinical variables of sepsis often mimic the manifestation of the acute complications of liver cirrhosis such as variceal bleeding, hepatic encephalopathy, hypoalbuminemia, ascites progression, and the worsening of liver and kidney function. (5,6) These acute complications can sometimes be atypical signs of bacterial infection in a liver cirrhosis patient. (6) These various dilemmas necessitate identifying a specific and sensitive marker to help diagnose bacterial infection in liver cirrhosis patients.

Procalcitonin (PCT) is a potential marker for bacterial infection that is more accurate than C-reactive protein (CRP) and leucocytes. Microbial cultures that provide definite proof of bacterial infection also have some shortcomings, such as delayed diagnosis, suboptimal sensitivity, and low specificity due to contamination. (7) PCT is produced by numerous cells and body tissues as a response to endotoxin (LPS) and the release of proinflammatory mediators such as interleukin (IL)-1β, tumor necrosis factor-α (TNF-α), and IL-6. Thus, the production of PCT is closely related to the advancement and severity of infection, and is not affected by liver parenchyma damage. (7) A study compared PCT, CRP, IL-6, and TNF-α in liver cirrhosis patients, revealing that PCT has the best diagnostic ability among these markers. (8)

Several studies have indicated that serum PCT cutoff values of more than 0.5 ng/ml, (6) 0.58 ng/ml, (9) and 0.75 ng/ml (10) are markers for bacterial infection. However, another study found that approximately 46% of liver cirrhosis patients with acute alcoholic hepatitis exhibited a PCT level of more than 0.5 ng/ml even without bacterial infection. This was also observed in 31% of acute viral hepatitis patients. (10) PCT levels of more than 0.5 ng/ml can be observed in liver cirrhosis patients with or without bacterial infection, particularly in a decompensated state. For diagnosing bacterial infection in decompensated liver cirrhosis patients, PCT may have a specific cutoff value above 0.5 ng/ml. Until now, no studies have been conducted in Indonesia that could establish the difference between the serum PCT levels of patients with bacteria-infected decompensated liver cirrhosis and those of uninfected patients.

This study addressed the role of the serum PCT level in diagnosing bacterial infection in liver cirrhosis patients in order to improve the health care quality for patients in Indonesia. In addition, this study analyzed the average difference of serum PCT levels between decompensated liver cirrhosis patients with and without bacterial infection, and established the cutoff value of the serum PCT level for diagnosing bacterial infection in decompensated liver cirrhosis patients.

Research methods

Design, time, and place

This was a cross-sectional study and was conducted in the clinic and adult ward of Cipto Mangunkusumo Hospital, Jakarta, Indonesia, from December 2015 until May 2016.

Research subjects and sample

The target population in this study comprised adult patients with decompensated liver cirrhosis. The accessible population comprised decompensated liver cirrhosis patients in Cipto Mangunkusumo Hospital, whether in the clinic or in the adult ward, from December 2015 to May 2016. The research subject was the accessible population that fulfilled the research criteria. The sample magnitude was estimated to be 38 based on the calculation conducted.

Research criteria

Decompensated liver cirrhosis patients with and without bacterial infection who agreed to participate by signing the informed consent form were eligible for inclusion in this study. The exclusion criteria were receiving antibiotic therapy within 7 days prior to the sample being taken and any form of malignancy.

Variables

The independent variable in this study was the serum PCT level, and the dependent variable was the bacterial infection status of patients with decompensated liver cirrhosis.

Research procedures

The patients that were recruited had been diagnosed with liver cirrhosis and then underwent a series of supporting examinations to identify their Child-Turcotte-Pugh (CTP) scores. Subsequently, the patient's serum PCT levels were determined, and they underwent screening for bacterial infection through a standardized method according to specific suspected focal infections. Serum PCT levels were measured using a commercial immunofluorescent assay kit (Ref. #825.050, B.R.A.H.M.S PCT™ sensitive KRYPTOR™, B.R.A.H.M.S

GmbH, Hennigsdorf, Germany).

Data analysis

Descriptive data were displayed in tables and text to observe the characteristics of the patients and all variables that were measured. The difference in PCT levels among the groups was analyzed based on their average differences using a t-test on normal distributed data and Mann-Whitney analysis if the data were not normally distributed. The cutoff value of the serum PCT level was determined using receiver operating characteristic (ROC) curve analysis and area under the curve (AUC) measurement. Data were analyzed using SPSS 20.0 for Windows PC (SPSS Inc., Chicago, Illinois, USA).

Results

Patient characteristics

During the research period, 43 patients fulfilled the inclusion criteria. Five patients were then excluded for the following reasons: two patients had received treatment with antibiotics and three were found to have carcinoma. Finally, a total of 38 patients were registered and analyzed in this study. Pneumonia was the most common type of bacterial infection (50%), and two patients had two focal infections (**Table 1**). The characteristics of patients with decompensated liver cirrhosis are shown in **Table 2**.

Average difference of serum PCT levels between decompensated liver cirrhosis patients with and without bacterial infection

Table 3 shows the average PCT levels of decompensated liver cirrhosis patients with bacterial infection were significantly higher than those of patients without bacterial infection.

Determining the cutoff value of serum PCT level for diagnosing bacterial infection in decompensated liver cirrhosis patients

An ROC curve was used to assess the suitability of serum PCT levels for diagnosing bacterial infection in decompensated liver cirrhosis patients, based on sensitivity and specificity. An AUC value of 0.933 (CI 0.853-1.014) was established.

To determine the optimum cutoff value of the PCT level for diagnosing bacterial infection in decompensated liver cirrhosis patients, we used a curve of all the sensitivity and specificity values from the serum PCT level candidates. From this curve, we obtained the PCT value that had the highest sensitivity and specificity, namely 2.79 ng/ml.

Table 4 indicates that the cutoff value of the serum PCT level had a sensitivity of 87.5% and specificity, namely 2.79 ng/ml.

Table 4 indicates that the cutoff value of the serum PCT level had a sensitivity of 87.5% and specificity of 86.4%.

Discussion

Bacterial infection in liver cirrhosis patients is caused by several factors such as the gut microbiota, the permeability of the gut (gut barrier dysfunction), bacterial translocation, and immune system dysfunction, which can be acquired or due to genetic susceptibility. (1) The balance of gut bacteria is strictly controlled in order to prevent pathological bacterial translocation. This function is performed by the immune system by means of gut-associated lymphatic tissue (GALT). (11) In addition, portal hypertension causes mucosal edema that intervenes with the gut mucosal barrier and delays the gut transit time, leading to small intestine bacterial overgrowth. (12)

The tight junction between epithelial cells functions to prevent cellular leakage and inhibits the translocation of bacterial products. In liver cirrhosis patients, a change in the configuration of proteins forming the tight junction causes the loosening of the tight junction. (13,14) Bacterial invasion can then occur, particularly through transcytosis. One of the most important molecules controlling the tight junction and the transcytosis process is TNF-α, the level of which usually rises in the GALT of advanced liver cirrhosis patients. (15) The mediators that block the direct contact of gut bacteria with the epithelial surface and that appear to decrease in concentration in liver cirrhosis patients are immunoglobulin A, bile, and antimicrobial peptide. (16) Additionally, the decreased defense of Paneth cells is related to the decreased eradication of bacteria that invades the gut mucosal layer. (17) Gut macrophage activation releases IL-6 and nitric oxide, which further damage the gut barrier system and increase permeability to bacterial products. (1)

In liver cirrhosis, an immune system dysfunction called cirrhosis-associated immune dysfunction occurs. (18) In addition to a decrease in monocytes, liver cirrhosis causes decreased numbers of numerous circulating immune cells, including neutrophils, naive T helper cells, cytotoxic T helper cells, and cluster of differentiation (CD) 27-positive memory B cells. (19) Together with the decreasing number of phagocytic mononuclear and neutrophil cells, phagocytosis ability and cell mobility also decline. T and B cells appear to become hypoproliferated in response to methogen and CD40/toll-like receptor (TLR) 9, while the natural

killer cells exhibit low cytotoxic activity. (20) Portal hypertension changes the direction of blood that had been flowing toward the liver, resulting in the detoxification process being reduced. The reticuloendothelial system also is impeded by a qualitative dysfunction that causes monocyte dysfunction, a decrease in complement factors level in serum, ascites, and problems with neutrophil phagocytosis. (1) Moreover, genetic variability affects susceptibility to bacterial infection. (2) Extracellular bacteria is recognized by TLR in cell membranes and intracellular nucleotide-binding oligomerization domain (NOD)-like receptors, causing the activation of nuclear factor kappa B (NF-κB) and triggering the release of antimicrobial peptides. TLR1 and TLR2 recognize tri-acylated lipoprotein from gram-positive bacteria; TLR4 recognizes LPS; and NOD2 recognizes muramyl dipeptide, which is one of the components in the wall of gram-negative bacteria.

The release of cytokines into the circulation from bacterial infections worsens the splanchnic and systemic vasodilations that have already occurred due to portal hypertension. The increase in vasodilation reduces effective arterial blood volume and activates the neurohormonal system (renin-angiotensin-aldosterone), leading to renal vasoconstriction and kidney failure. Bacterial infection can cause hepatorenal syndrome that can result in kidney failure. (1,3) Kidney failure then triggers proinflammatory overresponse through the release of inflammatory cytokines such as TNF-α and IL-6 and vasodilator hormones such as nitric oxide. Progressive changes that continue to occur cause multiple organ failure involving ACLF and septic shock. (1)

Procalcitonin is a calcitonin hormon precursor peptide that contains 116 amino acids. (21) The main source of PCT is the liver, but PCT is also produced in other organs and cells such as the lungs, kidneys, adrenal glands, monocytes, granulocytes, testis, prostate, and small bowel. (22) Procalcitonin expression is controlled by inflammation, which is induced by two main causes, namely direct activation of LPSs (and other toxins) by bacteria and indirect activation by proinflammatory cytokines such as IL-1β, IL-6, and TNF-α. How the mechanism functions is not understood in detail. However, PCT levels selectively increase the inflammation caused by bacterial infection. (23)

Bacterial infection induces the expression of the PCT gene, which leads to the release of PCT into serum from various tissues. This provides an accurate method for detecting bacterial infection, and an increase of PCT in serum confirms the presence of bacterial infection. (7) Increases in PCT levels usually occur in the systemic inflammatory process due to an infection etiology; hence, PCT levels can be used to differentiate the source of inflammation, for example, whether it is due to infection or other causes. (9) Research on serum PCT levels, CRP, IL-6, and lactate showed that the PCT level was the most effective marker for diagnosing sepsis. (24)

Systemic inflammation plays a key role in the pathogenesis of liver cirrhosis. The process starts with the damage of hepatocytes, which activates the immune system and leads to sterile systemic inflammation (compensated liver cirrhosis). Systemic inflammation continues through a stimulus from bacterial translocation and its products, such as LPS, which results in endotoxin tolerance. Endotoxin tolerance is defined as a condition involving the complete shutdown of the immune system, which is the basic mechanism for the worsening of decompensated liver cirrhosis or the occurrence of ACLF. (5) This is why PCT production increases in decompensated liver cirrhosis patients even without the presence of bacterial infection. Declining liver function does not affect the production of PCT. (25) In addition, the natural history of inflammation is connected to the risk of sepsis and death from sepsis in liver cirrhosis patients. (1,5)

Increases in serum PCT levels in decompensated liver cirrhosis patients without bacterial infection have been observed to be quite varied in several studies. Attar et al (10) reported that the average serum PCT level in patients with severe liver cirrhosis and massive ascites without bacterial infection was approximately 0.42±0.19 ng/ml. In cases without evidence of bacterial infection, PCT was reported to rise more than 0.5 ng/ml in liver cirrhosis patients with alcoholic and acute viral hepatitis. (10) Similar results were also observed in a study by Rahimkhani et al, (8) in which liver cirrhosis patients without bacterial infection exhibited average PCT levels of 0.59±0.16 ng/ml.

In the present study, no significant difference was observed in the average age, sex, proportion, or proportions of specific types of hepatitis between decompensated liver cirrhosis patients with and without bacterial infection. A CTP score of class C was more prevalent in patients with bacterial infection. SIRS was found in 20 (52.6%) decompensated liver cirrhosis patients. Of those 20 patients, 7 (35%) did not present with bacterial infection. Statistically, this result indicated that the proportion of SIRS was significantly higher in decompensated liver cirrhosis patients with bacterial in-

infection than in those without infection, with a p value of 0.004 ($p<0.05$). A total of 8 (50%) of the decompensated liver cirrhosis patients with bacterial infection had leucocyte levels below 10,000/ul. Decompensated liver cirrhosis patients do not always show typical signs and symptoms of bacterial infection.

In this study, a significant difference was observed between the average PCT levels of decompensated liver cirrhosis patients with bacterial infection (3.607±0.643 ng/ml) and those without any bacterial infection (0.738±1.185 ng/ml), with a p value of 0.000 ($p<0.05$). The average serum PCT levels of decompensated liver cirrhosis patients with bacterial infection were 4.9 times higher than those of patients without bacterial infection.

Cai et al (25) reported that the average serum PCT level of 94 decompensated liver cirrhosis patients (3.02±3.30 ng/ml) was higher than that of compensated liver cirrhosis patients without bacterial infection (0.15±0.08 ng/ml), with a p value of <0.05. Lesinska et al (26) found that the serum PCT levels in 10 decompensated liver patients with spontaneous bacterial peritonitis (1.08±1.34 ng/ml) were significantly higher than those of 22 controls without bacterial infection (0.44±0.44 ng/ml).

By identifying the increase in the serum PCT level in decompensated liver cirrhosis patients without bacterial infection, we aimed to determine the cutoff value of the serum PCT level for decompensated liver cirrhosis patients with bacterial infection. A few studies have been aimed at establishing serum PCT cutoff values to diagnose bacterial infections in liver cirrhosis patients, with varied results. Li et al (27) studied 27 septic patients and 71 noninfection septic patients and identified a serum PCT level cutoff value of 0.49 ng/ml with 81.5% sensitivity and 87.3% specificity. Lazzarotto et al (6) reported a cutoff value of 1.10 ng/ml with 60% sensitivity and 80% specificity for 24 decompensated liver cirrhosis patients with bacterial infection out of a total of 81 liver cirrhosis patients. Connert et al (28) determined a cutoff value of 0.58 ng/ml with 92% sensitivity and 78% specificity for 36 decompensated liver cirrhosis patients out of 127 liver cirrhosis patients. Villareal et al (29) reported a cutoff value of 0.80 ng/ml with 83% sensitivity and 75% specificity for 69 liver cirrhosis patients with bacterial infection out of 255 liver cirrhosis patients treated in intensive care units. However, no studies have focused on the cutoff value of the serum PCT level for diagnosing bacterial infection in decompensated liver cirrhosis patients.

This study established that the serum PCT level was effective for diagnosing bacterial infection in decompensated liver cirrhosis populations. The ROC curve indicated that the optimum cutoff value was 2.79 ng/ml (87.5% sensitivity and 86.4% specificity). This serum PCT level cutoff value can be applied clinically in ruling out or confirming the diagnosis of bacterial infection in decompensated liver cirrhosis patients whose signs and symptoms of bacterial infection are not typical, particularly if sepsis has occurred. We can thereby prevent further worsening of their disease progression and subsequent death.

This study not only demonstrated that PCT levels could rise in decompensated liver cirrhosis patients without bacterial infection, but also determined a cutoff value of the serum PCT level for diagnosing bacterial infection, which is suitable for patients who are admitted to adult wards because of bacterial infection. This type of study has never been conducted in Indonesia, where the risk of bacterial infection remains one of the major health problems in the general population, and particularly among liver cirrhosis patients.

This study had some limitations, including lack of follow up of the patients to establish their prognosis. Only one point measurement of each patient's serum PCT level was performed because of the difficulty of determining when exactly their serum PCT levels started to rise, particularly if the patient was in a decompensated state and the signs and symptoms of decompensation and bacterial infection were overlapping. In addition, in this study, the change in the serum PCT level was not observed in treatment evaluation.

Conclusion

The average serum PCT level of decompensated liver cirrhosis patients with bacterial infection is significantly higher than that of patients without bacterial infection. The cutoff value of the serum PCT level for diagnosing bacterial infection in decompensated liver cirrhosis patients is 2.79 ng/ml. Further study is necessary in order to validate the cutoff value of the serum PCT level obtained in this study. This can then be changed in serum PCT levels in decompensated liver cirrhosis patients with bacterial infection who have been treated. Serum PCT levels can then be applied to determine when to stop antibiotic treatment. Further research is necessary to determine the course of liver cirrhosis patients based on changes in serum PCT levels and the severity of liver cirrhosis (from a compensated to a decompensated state), due to its morbidity and mortality.

Compliance with ethical requirements

Rino Alvani Gani, Oska Mesanti, Marcellus Simadibrata, Suhendro, Irsan Hasan, Andri Sanityoso, and C. Rinaldi A. Lesmana declare that they have no conflict of interest. All procedures followed in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the .

Helsinki Declaration of 1975, as revised in 2008. Informed consent was obtained from all patients in the study.

Acknowledgement

We thank Wallace Academic Editing for editing the manuscript.

Table 1. Characteristics of patients with bacterial infection

Characteristic	N=16
Type of bacterial infection, n (%) - Pneumonia - Urinary tract infection - Spontaneous bacterial peritonitis	 8 (50%) 6 (37.5%) 4 (25%)
More than 1 focal infection	2 (12.5%)
Leucocyte (per µl), median (min-max)	10,700 (5900-18,600)
Procalcitonin (ng/ml), median (min-max)	3.71 (2.67-4.85)

Table 2. Characteristics of patients with decompensated liver cirrhosis

Characteristic	Without bacterial infection N=22	With bacterial infection N=16	p value
CTP score, n (%) - Class A - Class B - Class C	 10 (45.5%) 8 (36.4%) 4 (18.1%)	 0 (0%) 5 (31.2%) 11 (68.8%)	 0.001*
SIRS, n (%)	7 (31.8%)	13 (81.2%)	0.004*
Leucocyte (per µl, average±standard deviation)	4393±2136	11.231±4534	0.000**

Legend: CTP=Child-Turcotte-Pugh; SIRS=systemic inflammatory response syndrome; *=Chi square, $p<0.05$; **=independent t-test, $p<0.05$

Table 3. Average difference of serum PCT levels between decompensated liver cirrhosis patients with and without bacterial infection

Variable	With bacterial infection N=16	Without bacterial infection N=22	p value*
Procalcitonin (ng/ml, average±standard deviation	3.607±0.643	0.738±1.185	0.000

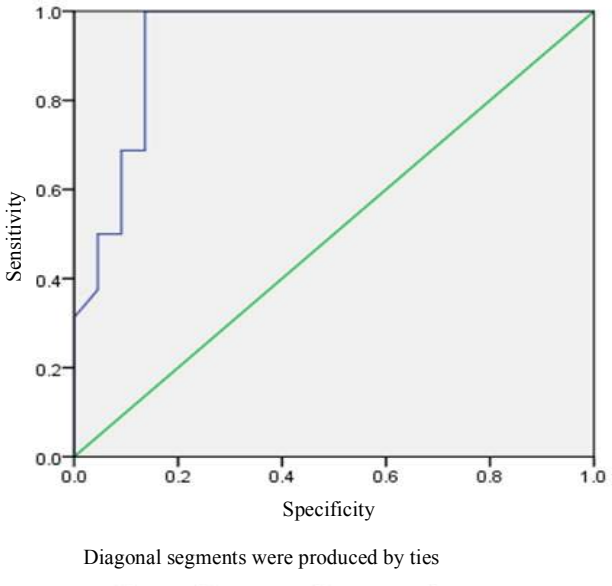
Legend: *=Mann Whitney test

Table 4. PCT level cutoff value of 2.79 ng/ml for diagnosing bacterial infection in decompensated liver cirrhosis patients

Cutoff value of PCT	Patients with bacterial infection	Patients without bacterial infection	N
≥2.79 ng/ml	14	3	17
<2.79 ng/ml	2	19	21
N	16	22	38
	Sensitivity: 87.5%	Specificity: 86.4%	

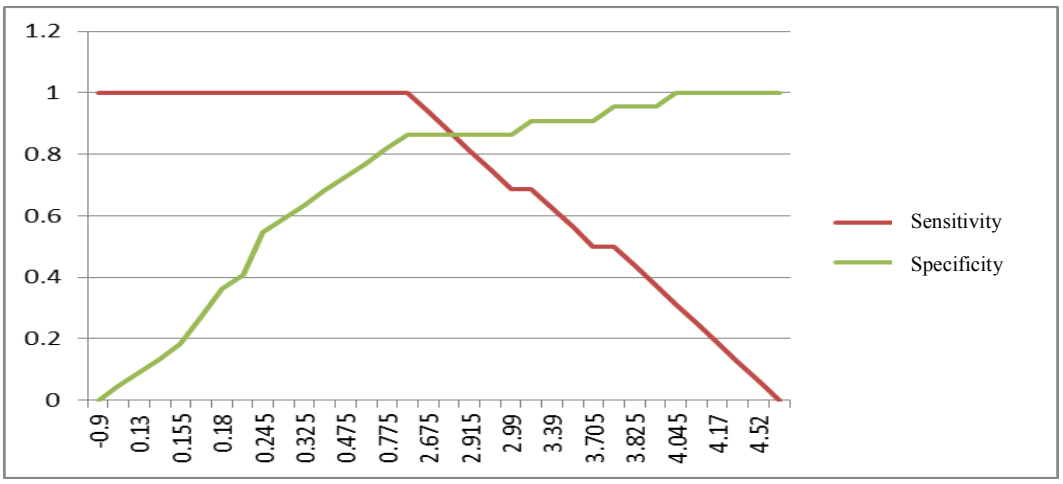
Legend: PCT=procalcitonin

Figure 1. ROC curve of PCT value for diagnosing bacterial infection in decompensated liver cirrhosis patients



Legend: PCT=procalcitonin

Figure 2. Intersecting curves of sensitivity and specificity for several PCT values



Legend: PCT=procalcitonin

References

1. Strauss E. The impact of bacterial infections on survival of patients with decompensated cirrhosis. Ann Hepatol 2014;13:7-19.
2. Lesmana LA, Lesmana CRA, Cahyadinata L, Angga B, Pakasi LS. Clinical presentations and outcome of bacterial infection in liver cirrhotic patients. Paper presented at: APASL Conference; 2014 Mar 12-15; Brisbane, Australia.
3. Prin M, Bakker J, Wagener G. Hepatosplanchnic circulation in cirrhosis and sepsis. World J Gastroenterol 2015;21:2582-92.
4. Pleguezuelo M, Benitez JM, Jurado J, Montero JL, De la Mata M. Diagnosis and management of bacterial infections in decompensated cirrhosis. World J Hepatol 2013;5:16-25.
5. Dirchwolf M, Ruf AE. Role of systemic inflammation in cirrhosis: from pathogenesis to prognosis. World J Hepatol 2015;7:1974-81.
6. Lazzarotto C, Ronsoni MF, Fayad L, Nogueira CL, Bazzo ML, Schiavon JLN, et al. Acute phase proteins for the diagnosis of bacterial infection and prediction of mortality in acute complications of cirrhosis. Ann Hepatol 2013; 12:431-9.
7. Schuetz P, Albrich W, Mueller B. Procalcitonin for diagnosis of infection and guide to antibiotic decisions: past, present and future. BMC Med 2011;9:107.
8. Rahimkhani M, Einollahi N, Khavari Daneshvar H, Dashti N. Survey of serum procalcitonin in cirrhotic patients. Acta Med Iran 2013;51: 153-6.
9. Acevedo J, Fernandez J. New determinants of prognosis in bacterial infections in cirrhosis. World J Gastroenterol 2014;20:7252-9.
10. Attar BM, Moore CM, George M, Ion-Nedelcu N, Turbay R, Zachariah A, et al. Procalcitonin, and cytokines document a dynamic inflammatory state in non-infected cirrhotic patients with ascites. World J Gastroenterol 2014;20: 2374-82.
11. Hooper LV, Littman DR, Macpherson AJ. Interactions between the microbiota and the immune system. Science 2012;336:1268-73.
12. Pande C, Kumar A, Sarin SK. Small-intestinal bacterial overgrowth in cirrhosis is related to the severity of liver disease. Aliment Pharmacol Ther 2009;29:1273-81.
13. Assimakopoulos SF, Tsamandas AC, Tsiaoussis GI, Karatza E, Triantos C, Vagianos CE, et al. Altered intestinal tight junctions' expression in patients with liver cirrhosis: a pathogenetic mechanism of intestinal hyperpermeability. Eur J Clin Invest 2012;42:439-46.
14. Assimakopoulos SF, Charonis AS. Uncovering the molecular events associated with increased intestinal permeability in liver cirrhosis: the pivotal role of enterocyte tight junctions and future perspectives. J Hepatol 2013;59:1144-6.
15. Munoz L, Albillos A, Nieto M, Reyes E, Lledo L, Monserrat J, et al. Mesenteric Th1 polarization and monocyte TNF-alpha production: first steps to systemic inflammation in rats with cirrhosis. Hepatology 2005;42:411-9.
16. Lorenzo-Zuniga V, Bartoli R, Planas R, Hofmann AF, Vinado B, Hagey LR, et al. Oral bile acids reduce bacterial overgrowth, bacterial translocation, and endotoxemia in cirrhotic rats. Hepatology 2003;37:551-7.
17. Teltschik Z, Wiest R, Beisner J, Nuding S, Hofmann C, Schoelmerich J, et al. Intestinal bacterial translocation in rats with cirrhosis is related to compromised Paneth cell antimicrobial host defense. Hepatology 2012;55:1154-63.
18. Malik R, Mookerjee RP, Jalan R. Infection and inflammation in liver failure: two sides of the same coin. J Hepatol 2009;51:426-9.
19. Tritto G, Bechlis Z, Stadlbauer V, Davies N, Frances R, Shah N, et al. Evidence of neutrophil functional defect despite inflammation in stable cirrhosis. J Hepatol 2011;55:574-81.
20. Tian Z, Chen Y, Gao B. Natural killer cells in liver disease. Hepatology 2013;57:1654-62.
21. Becker KL, Nylen ES, White JC, Muller B, Snider RH, Jr. Clinical review 167: Procalcitonin and the calcitonin gene family of peptides in inflammation, infection, and sepsis: a journey from calcitonin back to its precursors. J Clin Endocrinol Metab 2004;89:1512-25.
22. Morgenthaler NG, Struck J, Chancerelle Y, Weglohner W, Agay D, Bohuon C, et al. Production of procalcitonin (PCT) in non-thyroidal tissue after LPS injection. Horm Metab Res 2003;35:290-5.
23. Nijsten MW, Olinga P, The TH, de Vries EG, Koops HS, Groothuis GM, et al. Procalcitonin behaves as a fast responding acute phase protein in vivo and in vitro. Crit Care Med 2000;28:458-61.
24. Muller B, Becker KL, Schachinger H, Rickenbacher PR, Huber PR, Zimmerli W, et al. Calcitonin precursors are reliable markers of sepsis in a medical intensive care unit. Crit Care

Med 2000;28:977-83.
25. Cai ZH, Fan CL, Zheng JF, Zhang X, Zhao WM, Li B, et al. Measurement of serum procalcitonin levels for the early diagnosis of spontaneous bacterial peritonitis in patients with decompensated liver cirrhosis. BMC Infect Dis 2015;15:1-7.
26. Lesinska M, Hartleb M, Gutkowski K, Nowakowska-Dulawa E. Procalcitonin and macrophage inflammatory protein-1 beta (MIP-1beta) in serum and peritoneal fluid of patients with decompensated cirrhosis and spontaneous bacterial peritonitis. Adv Med Sci 2014;59:52-6.
27. Li CH, Yang RB, Pang JH, Chang SS, Lin CC, Chen CH, et al. Procalcitonin as a biomarker for bacterial infections in patients with liver cirrhosis in the emergency department. Acad Emerg Med 2011;18:121-6.
28. Connert S, Stremmel W, Elsing C. Procalcitonin is a valid marker of infection in decompensated cirrhosis. Z Gastroenterol 2003;41:165-70.
29. Villareal E, Vacacela K, Gordon M, Calabuig C, Alonso R, Ruiz J, et al. Usefulness of procalcitonin for diagnosing infection in critically ill patients with liver cirrhosis. Med Intensiva 2016;40:84-9.