INTRODUCTION

Spontaneous bacterial peritonitis (SBP) is a serious complication in cirrhotic patients with ascites. It is characterized by infection of the ascitic fluid (AF) in the absence of any other intraabdominal infection. SBP occurs in 10 to 30% of hospitalized cirrhotic patients with ascites ***(Rimola et al., 2000)***. Without early antibiotic treatment, this complication is associated with a 30-50% mortality rate ***(Garcia-Tsao, 2001).***

The prognosis for SBP has improved dramatically since Conn's first description. This is due to earlier recognition and antibiotic treatment ***(Garcia-Tsao, 2001).*** Rapid detection and identification of bacteria in AF is the key to improving the survival of patients with SBP ***(Sugihara et al., 2009)***.

The severity of the liver disease is probably the most important predisposing factor for AF infection. Almost 70% of patients who develop SBP are Child-Pugh class C, with the remainder being class B. A serum total bilirubin level of 2.5 mg/dL is an independent predictive factor of SBP ***(Such and Runyon, 1998)***. Deficient AF bactericidal activity is the main intraperitoneal predisposing factor for the development of AF infection. A direct correlation between total protein level, complement components and opsonic activity explains why an AF total protein level of 1 g/dL is a risk factor for the development of AF infection ***(Such and Runyon, 1998)***

Currently, the diagnosis of SBP is made by confirming an increased amount of neutrocytes and identifying bacteria in the AF. However, the blood culture bottle method requires at least 5 days to identify bacteria. Thus, empirical antibiotic therapy is the standard approach to SBP while awaiting test results ***(Sugihara et al., 2009).***

Bacterascites (monomicrobial non-neutrocytic bacterascites) is the term used to describe the colonization of ascitic fluid by bacteria, in the absence of an inflammatory reaction in the bacterial fluid. By definition, the PMN count is < 250/mm^3 and bacterial culture is positive, while the patient may present with symptoms and signs of infection. The natural course of bacterascites, if untreated, is variable. Diagnosis of bacterascites can only be made 2-3 d after initial paracentesis (the time necessary for culture growth), and a repeat ascitic tap is recommended on day 3 ***(Runyon, 2006)***.

Culture-negative neutrocytic ascites is the term used to describe the clinical situation in which the ascetic PMN count is > 250/mm3 but fluid cultures fail to grow any bacteria. It is considered to represent the expected 20% failure rate of culture to isolate microorganisms ***(Koulaouzidis et al., 2009).***

In recent years, molecular diagnosis has played an increasingly important role in the rapid detection and identification of pathogenic organisms in clinical samples. The genetic variation of ribosomal genes in bacteria offers an alternative to culturing to detect and identify these organisms ***(Petti et al., 2005).***

These genes, such as 16S rRNA, demonstrate conserved sequence regions ideal for primer targeting, as well as regions of variability useful for species identification ***(Mignard and Flandrois, 2006).***

It appears that 16S rRNA gene sequencing is a more accurate and objective method than conventional phenotypic identification ***(Petti et al., 2005).***

Aim Of The Work

To compare the usefulness of the 16S rRNA-based DNA microarray with the conventional culture method in terms of detection of bacteria.

Secondary aims:

1- To identify the current causative bacteria for ascitic fluid infection and their antibiogram.

2- To identify the possible risk factors (both patient and ascitic fluid factors) for spontaneous ascitic fluid infection.

IMMUNITY IN CHRONIC LIVER DISEASE

Introduction

The immune system plays a dual role in the pathogenesis of cirrhosis such that, besides the role of immune-mediated inflammatory mechanisms, cirrhosis itself also leads to immune system dysfunction. The immune system mediates hepatocyte damage due to alcohol, virus infection or autoimmunity, leading to fibrogenesis through hepatic stellate cell activation. In addition, cirrhosis leads to impairment of the immune system with an inability to protect the host from bacterial infection and dysregulated immune cell activation ***(Albillos et al., 2014).***

Immune function of the liver:

The liver plays a role in immune system through two mechanisms. First, it plays a role in immune surveillance, defending against blood-borne pathogens via its double blood supply, thereby avoiding the systemic spread of microbial and dietary antigens arriving from the gut ***(Jenne and Kubes, 2013).*** This function is maintained by the delicate balance between immunity and the local immune tolerance to non-pathogenic exogenous material observed in the liver. The second mechanism is the synthesis of soluble molecules that are essential for an effective immune response ***(Racanelli and Rehermann, 2006).***

Immune surveillance of the liver:

The liver exerts its antimicrobial surveillance function through different types of resident antigen presenting cells and lymphocytes. The liver antigen presenting cells include Kupffer and sinusoidal endothelial cells, which comprise the reticuloendothelial system of the liver, and dendritic cells.

Kupffer cells present within the sinusoidal vascular space and represent the largest group of fixed macrophages in the body, and sinusoidal endothelial cells form a sieve-like, fenestrated endothelium. Kupffer cells are specialized at eliminating insoluble waste by phagocytosis through a variety of receptors can capture bacteria. Sinusoidal endothelial cells are responsible for the elimination of soluble macromolecules and colloidal waste by endocytosis. Kupffer and sinusoidal endothelial cells are also antigen presenting cells ***(Willekens et al., 2005).***

In addition, the liver contains populations of both resident and transiting T and B lymphocytes scattered throughout the parenchyma and the portal tracts that are important in the adaptive immune response. Further, the liver is rich in natural killer (NK) cells and unconventional lymphocytes (natural killer T and gamma, delta T cells), which have roles in innate immune responses of the liver ***(Schildberg et al., 2008).***

Role of the liver in the systemic immune response

Hepatocyte is the main source of proteins involved in innate and adaptive immune responses, including complement components and many secreted pattern-recognition receptors (PRRs), (e.g. C reactive protein, lipopolysaccharide[LPS]-binding protein [LBP], peptidoglycan-recognition protein, soluble CD14), which activate complement, induce opsonization and regulate immune cell function ***(Gao et al., 2008).*** The liver also produces other acute phase proteins, such as hepcidin, fibrinogen and proteinase inhibitors, which participate in the innate immune response and in controlling tissue damage and repair during inflammation ***(Liu et al., 2001).***

Additionally, liver cells express different membrane-bound or cytoplasmic PRRs, which recognize different bacterial and viral molecules. These include cell surface and endosomal toll-like receptors (TLRs), cytoplasmic nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs), and RNA helicases. Specifically, TLR4 is expressed on all types of liver cells and is involved in the uptake and clearance of endotoxins, and the production of cytokines ***(Crispe, 2009).***

Cirrhosis associated immune dysfunction (CAID):

This concept includes two main alterations: (a)-immunodeficiency, due to an impaired response to pathogens at

different levels of the immune system, and (b)-systemic inflammation, as a consequence of persistent and inadequate stimulation of cells of the immune system ***(Albillos et al., 2014).***

a. Cirrhosis-induced immunodeficiency:

Cirrhosis is associated with abnormalities in both innate and adaptive immune response leading to a state of acquired immunodeficiency through.

1- Damage to the liver's immune surveillance function:

Diminished function of the reticulo-endothelial system of the liver by sinusoidal fibrosis and capillarization, septal fibrosis with portal-systemic shunts, Kupffer cell loss and impairment of the synthesis of innate immunity proteins and of pattern recognition receptors (PRRs), reducing phagocytic activity. This reduces the clearance of endotoxin and bacteria from the blood, leading to bacteremia, metastatic organ infection, and persistent immune system stimulation. Therefore, cirrhosis is associated with a greater risk of bacterial infection and lower survival ***(Jenne and Kubes, 2013).***

2- Circulating immune cell damage:

Cirrhosis is associated with several abnormalities of the main circulating populations of immune cells (fig 1):

1- Neutrophils are reduced in number due to sequestration by the spleen, with impairment of the phagocytic function of opsonized bacteria ***(Tritto et al., 2011).*** Neutrophils also show impaired chemotaxis to the infection focus, through reduced adhesion to microvascular endothelial cells and decreased transendothelial migration ***(Fiuza et al., 2002).***

2- Monocytes: cirrhosis is associated with monocytosis, but with impaired phagocytic function regardless of the etiology of cirrhosis ***(Seidler et al., 2012).***

3- B lymphocytes are profoundly affected in cirrhosis. Especially. In patients with alcoholic or HCV cirrhosis reduced peripheral blood absolute counts have been reported. The main observed defect in the B cell compartment is memory B cell dysfunction ***(Doi et al., 2012).***

4- T lymphocytes: cirrhosis is associated with depletion of T cells regardless of its etiology and is evident since the early stages of cirrhosis ***(Lario et al., 2013).***

5- Circulating NK cells are also defective in cirrhosis and show a poor response to cytokine stimulation ***(Tian et al., 2013).***

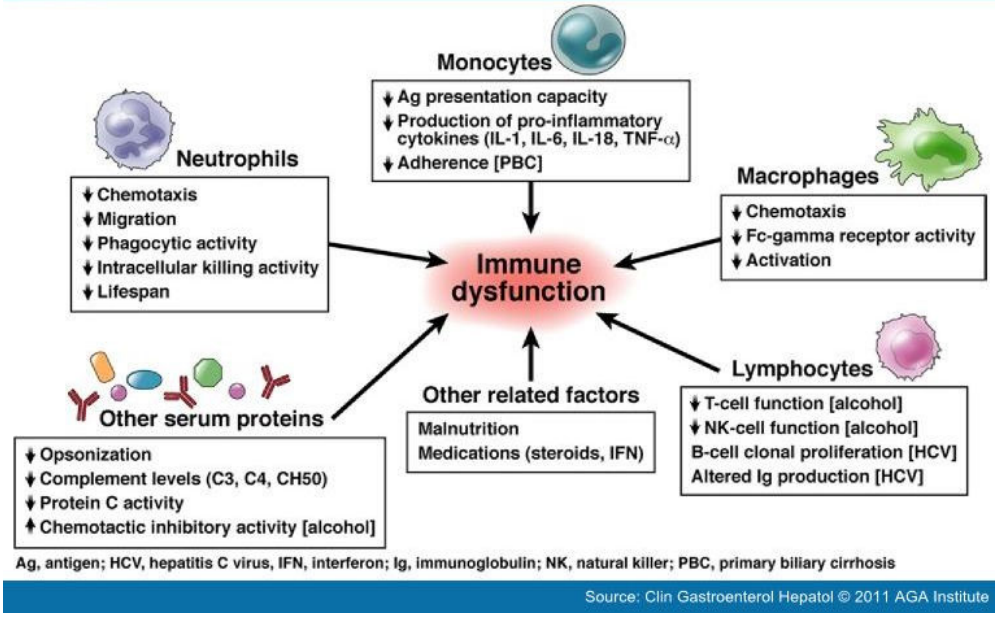

Figure (1): Cirrhosis induced immunodeficiency ***(Bonnel et al., 2011)***.

3- Gut-associated lymphoid tissue (GALT) damage:

GALT, which constitutes the first barrier of defense against antigens and pathogens entering from the intestine. The intestinal lymphoid tissue, distributed in Peyer's patches and mesenteric lymph nodes (MLN), and its effect or sites are present in the lamina propria and mucosal epithelium. In cirrhosis, GALT is under continuous stimulation of pathological bacterial translocation and the increased passage of bacterial products that results from a leaky gut and intestinal bacterial overgrowth. this results in an increased number of activated monocytes, dendritic cells and T lymphocytes at the intestine and MLN ***(Munoz et al., 2012).*** These activated cells cause the exaggerated expression of pro-/anti-

inflammatorycytokines at the lamina propria, mucosal epithelium and MLN, as well as increased phagocytosis by intestinal dendritic cells ***(Ubeda et al., 2010).***

The first consequence of intestinal and MLN inflammation in cirrhosis is systemic inflammation ***(Munoz et al., 2005).*** In addition, this intestinal inflammation precipitate intestinal barrier failure. It is due to the increased pro-inflammatory cytokine production (e.g. TNFa, IFN□, IL-6) by intestinal immune cells disrupting epithelial tight-junctions leading to further increased translocation of bacteria and their products, creating a vicious cycle ***(Du Plessis et al., 2013).*** In addition, there is a reductionin the production of a-defensins and RegIII proteinsby the intestinal mucosal cells. These peptides are needed to maintain microbiota host homeostasis, and their deficiency could induce intestinal dysbiosis and bacterial translocation ***(Teltschik et al., 2012).***

b- Cirrhosis-induced systemic inflammation:

It is defined as the persistent stimulation of immune cells in cirrhosis due to the increased production of pro-inflammatory cytokines with elevated serum levels and the up regulated expression of the markers of cell activation. The severity of this state of systemic inflammation parallels that of cirrhosis itself, as assessed by the Child-Pugh score, and is particularly intense in cirrhosis with ascites ***(Trebicka et al., 2013).***

Pathogenesis of systemic inflammation:

In advanced cirrhosis, the immune response leading to systemic inflammation is initiated when bacteria from the intestinal lumen reach the extraintestinal sites (i.e. gut bacterial translocation). Pathogen-associated molecular patterns (PAMPs) from enteric bacterial organisms and/or damage-associated molecular patterns (DAMPs), originating from the host tissue upon injury, recognize host pattern recognition receptors (PRRs), expressed on innate immune cells. Activation of these receptors leads to expression of activation surface molecules (cytokine receptors, adhesion molecules) on immune cells and upregulation of cytokines (pro- and anti-inflammatory lymphokines and monokines), chemokines and growth factors, which are released to recruit and activate additional inflammatory cells. Notably, not only live bacteria, but also the episodic, persistent inflow of PAMPs (including LPS, lipopeptides, glycopolymers, flagellin and bacterial DNA) into the hepato-splanchnic circulation contributes to the systemic inflammatory response (Fig. 2) ***(Gonzalez-Navajas et al., 2008).***

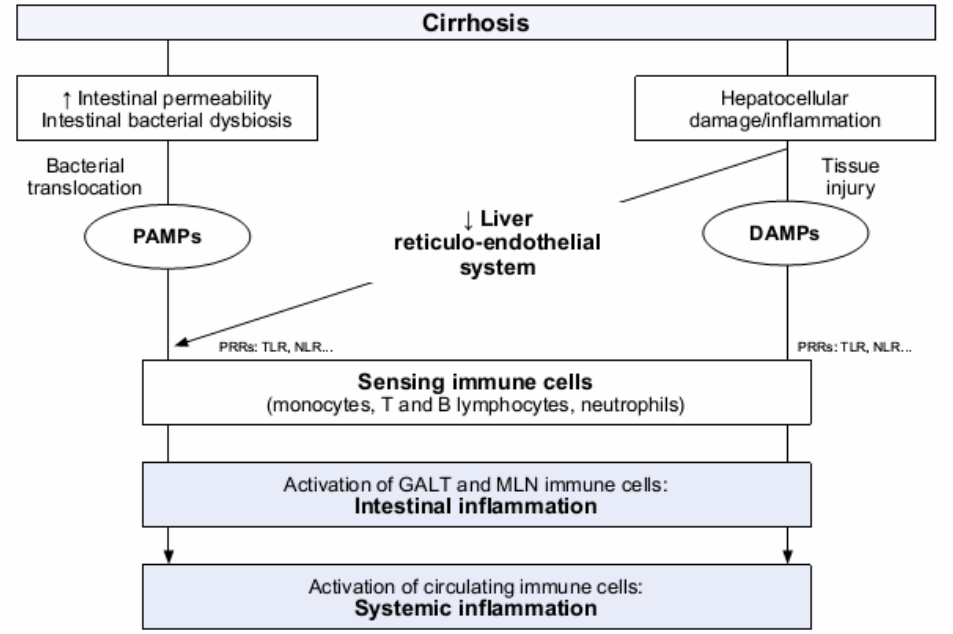

Figure (2): Pathogenesis of systemic inflammation in cirrhosis: ***(Gonzalez-Navajas et al., 2008).***

Immune recognition of bacteria and PAMPs in cirrhosis occurs both locally in the gut associated lymphoid tissue (GALT) and mesenteric lymph nodes (MLN) and the peripheral blood ***(Tazi et al., 2005).*** In addition, immune cells already activated in the GALT and MLN may enter the peripheral blood and spread causing systemic inflammatory response ***(Ubeda et al., 2010).*** Upon interaction, PRRs' mediated immune response takes place leading to gene expression and to the synthesis of numerous pro- and anti-inflammatory cytokines, chemokines, cell adhesion molecules, and immunoreceptors that causing enhanced phagocytic activity ***(Tritto et al., 2011),*** vascular endothelial injury, synthesis of acute phase proteins by the liver, chemotaxis of leukocytes to

the sites of inflammation, mainly the liver, and activation of leukocytes at the systemic level ***(Wiese et al., 2014).*** The expression of PRRs, such as TLRs or NLRs, is distinctively upregulated on cells of the innate immune system in cirrhosis with ascites ***(Tritto et al., 2011).***

In cirrhosis, the increased intestinal permeability, due to compromised epithelial integrity, intestinal bacterial overgrowth and dysbiosis, caused by disruption of host microbiota homeostasis and intestinal and general immune defense impairment leads to increase the translocation rate of bacteria and /or their products which favors immune cell activation and builds up a systemic inflammatory state ***(Wiest et al., 2014)***.

Damage-associated molecular patterns (DAMPs) and sterile particulates, released from necrotic hepatocytes, activate circulating immune cells and elicit an inflammatory response in cirrhosis. Sterile inflammation, induced by DAMPs, is evident in the acute hepatic injury by acetaminophen, during ischemia/reperfusion, and in the low-chronic hepatic injury by alcoholic and non-alcoholic steatohepatitis ***(Zhu et al., 2011).***

Dynamic pattern of the CAID phenotypes:

The immune disturbance of cirrhosis varies according to disease stage (compensated, decompensated, acute-on-chronic liver failure [ACLF]), the extent of liver injury, and the presence of environmental stimulation, induced by signalling

from persistent episodic bacterial translocation. Cirrhosis-associated immune dysfunction is the result of two concurrent and interlinked processes, namely systemic inflammation and damage of the immune system response that come into play as cirrhosis progresses. In the compensated pre-ascitic stage, DAMPs from stressed and damaged tissue, mainly necrotic hepatocytes, activate circulating immune cells. Cirrhosis progression distorts hepatic architecture and cellular organization and impairs its functional capacity. These events compromise the immune surveillance function of the liver, both locally by damaging the reticulo-endothelial system and systemically by impairing the bactericidal role of phagocytic cells through the reduced synthesis of proteins involved in the innate immune response and of PRRs ***(Albillos et al., 2014).***

In the decompensated, ascitic stage of cirrhosis, gut bacterial translocation occurs at a high rate and PAMPs released from the leaky gut further activate the immune system and aggravate systemic inflammation. Immune response reprogramming occurs after constant PAMPs pressure and the predominantly ‘‘pro-inflammatory’’ CAID phenotype switches to the predominantly ‘‘immunodeficient’’ one of severely decompensated cirrhosis with extra-hepatic organ failure. So, bowel decontamination eliminates the bacterial stimulus and partially normalizes intestinal dendritic cell functions. This explains the fact that bowel decontamination in cirrhosis improves survival beyond mere infectious prophylaxis ***(Fernandez et al., 2006).***