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Immune dysfunction in cirrhosis: Distinct cytokines phenotypes according to cirrhosis severity

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ABSTRACT

Background/objectives: Cirrhosis associated immune dysfunction has been proposed to switch from a pro-inflammatory phenotype in stable cirrhosis to an immunodeficient one in patients with decompensated cirrhosis and acute-on-chronic liver failure. The aim of the present study was to compare serum cytokine levels between healthy patients, stable cirrhosis, and decompensated cirrhotic patients with and without development of acute-on-chronic liver failure (ACLF); and to explore whether any of the measured cytokines is associated with cirrhosis severity and prognosis in ACLF patients.

Methods: Patients were enrolled from October 2013 to May 2014 in two hospitals located in Buenos Aires. Cirrhotic patients with an acute decompensating event were enrolled accordingly to the development of ACLF defined by the CANONIC study group. There were two control groups: healthy subjects ($n = 14$) and stable cirrhotic patients ($n = 14$). Demographic, clinical and biochemical data were obtained. Seventeen cytokines were measured using Bio-Plex Pro Human Cytokine 17-plex Assay.

Results: Of the 49 decompensated cirrhotic patients enrolled, 18 (36.7%) developed ACLF. Leukocyte count, MELD score at admission, Clif-SOFA at admission and day 7 were significantly higher in the ACLF group ($p = 0.046$, $p < 0.001$, $p < 0.001$, $p < 0.001$ respectively) as well as short-term mortality ($p < 0.001$) compared to stable and decompensated cirrhotic patients. In comparison with healthy controls, stable cirrhotic and decompensated cirrhotic patients showed increased levels of pro-inflammatory and anti-inflammatory cytokines: IL-6, IL-7, IL-8, IL-10, IL 12, and TNF-α. Decompensated cirrhotic patients with the development of ACLF showed a significant decrease of IL-7, IL-10, IL-12, TNF-α, MCP-1 and IFN-γ, but a sustained response of IL-6 and IL-8. When evaluating cirrhosis severity, IL-6 and IL-8 correlated positively with MELD score, whereas only IL-6 correlated positively with Clif-SOFA score at day 7; IL-2 correlated negatively with Clif-SOFA at admission. In comparison with all scores, leukocyte count showed positive correlation and IFN-γ negative correlation with disease severity. When evaluating survival, only MELD and Clif-SOFA scores had a significant association with mortality.

Abbreviations: ACLF, acute-on-chronic liver failure; AD, acute decompensating event; G-CSF, granulocyte colony stimulating factor; GM-CSF, granulocyte macrophage colony-stimulating factor; HIV, human immunodeficiency virus; HLA-DR, human leukocyte antigen; IL-1β, interleukin 1 beta; IL-2, interleukin 2; IL-4, interleukin 4; IL-5, interleukin 5; IL-6, interleukin 6; IL-7, interleukin 7; IL-8, interleukin 8; IL-10, interleukin 10; IL-12, interleukin 12; IL-13, interleukin 13; IL-17, interleukin 17; IFN-γ, interferon gamma; MCP-1, monocyte chemo-attractant protein-1; MFI, mean fluorescence intensity; MIP-1β, macrophage inflammatory protein 1 beta; Gro, growth-regulated oncogene alpha; RANTES, regulated on activation, normal T cell expressed and secreted; SC, stable cirrhosis control group; TIPS, transjugular intrahepatic portosystemic shunt; TNF-α, tumoral necrosis factor alpha.

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Conclusions: Pro-inflammatory cytokines and chemo-attractant elements are increased in cirrhosis in comparison with healthy subjects, and display higher values concomitantly with cirrhosis progression. However, in acute-on-chronic liver failure an opposite cytokine pattern that can be resumed as a combination of immune paresis and excessive inflammatory response was observed. Several pro-inflammatory cytokines (IL-2, IL-6, IL-8 and IFN-γ) showed correlation with disease severity; their utility as prognostic biomarkers needs to be further studied.

1. Main content/introduction

Liver cirrhosis is the final stage of all progressive and chronic liver diseases. The natural history of cirrhosis was traditionally divided in two phases: a long-lasting initial phase denominated compensated cirrhosis, characterized by the absence of symptoms and excellent survival, followed by an advanced stage (i.e. decompensated cirrhosis), marked by the appearance of complications related to the presence of portal hypertension or liver dysfunction and associated with an elevated mortality rate [1]. Recently, a new clinical entity denominated acute-on-chronic liver failure (ACLF) has been identified as an alternative path in cirrhosis progression, characterized by its acute onset and poor prognosis [2]. Although several definitions were proposed, most of them had theoretical basis; currently, the only description based on observational data derives from a European prospective multicenter study named CANONIC (Clif Acute-On-Chronic Liver Failure in Cirrhosis). This consortium prospectively enrolled and studied 1343 hospitalized cirrhotic patients, defining ACLF as an acute deterioration of liver function in patients with cirrhosis, either secondary to superimposed liver injury or due to extra hepatic precipitating factors, which leads to end-organ dysfunction. The severity of this syndrome was objectively quantified by an *ad hoc* score and divided in three stages, according to the number or organ failures and increasing mortality observed [3].

Unraveling the pathophysiological pathway in advanced cirrhosis and ACLF is a major priority, since it would allow developing specific treatment strategies against factors related to poor prognosis and improve detection of sicker patients in order to modify liver allocation algorithms. In the CANONIC study, patients with ACLF had higher white cell count and plasma C-reactive protein levels than decompensated cirrhotic patients who did not developed ACLF; furthermore, both of these markers increased concomitantly with the number of organ failures, and white cell count was found to be an independent predictor of post-enrollment development of ACLF and ACLF-related mortality [3]. Based on these findings, an excessive systemic inflammatory response was proposed to be the causal factor for the morbidity and mortality observed in ACLF. Interestingly, systemic inflammation in response to bacterial infections was observed in only 30% of patients with ACLF, suggesting other "sterile" triggers may be involved [4]. An excessive inflammatory response has also been linked to the induction of ACLF by other authors. In a study by Mehta et al. that analyzed systemic hemodynamics and inflammation parameters in stable, decompensated and ACLF alcohol-related cirrhotic patients, the latter cohort portrayed significantly higher intrahepatic resistance and pro-inflammatory cytokines levels (TNF-α, IL-8 and IL-6) compared to the other groups [5]. In an ex-vivo study that evaluated cytokine production under baseline conditions and after stimulation by lipopolysaccharide in peripheral blood monocytes of advanced alcoholic cirrhotic patients and normal subjects, monocytes from cirrhotic patients were found to spontaneously produce six cytokines (TNF-alpha, IL-6, IL-8, MCP-1, RANTES and Gro) whereas normal monocytes only secreted small amounts of IL-8 and RANTES. These findings suggested an underlying pro-inflammatory phenotype in cirrhosis; importantly, none of the cirrhotic patients included had overt signs of infection [6].

However, systemic inflammation is not the only proposed pathophysiological pathway. An acquired alteration of the innate immune system in advanced cirrhosis and ACLF has been suggested to account for an inadequate response to pathogens, which ultimately derives into multiple organ failure and elevated mortality. This theory is based in alterations observed at different levels of the immune system; in a study that compared functional immune parameters between ACLF, compensated cirrhotic and septic patients, TNF-alpha production and monocyte HLA-DR levels were found to be severely decreased in sepsis and ACLF compared to stable cirrhosis, whereas IL-6 were highest in septic patients followed by ACLF subjects. Due to the similarities in the degree of cellular immune depression between ACLF and sepsis, the authors defined ACLF innate immune system alterations as a "sepsis like" immune paralysis [7]. In a cohort of alcoholic cirrhotic patients, a reversible neutrophil activation indicated by an increased resting burst and reduced phagocytic function was found to be associated with higher morbidity and mortality [8]. The migration capacity of this cellular subset was also proven to be altered in advanced cirrhosis when compared to stable cirrhosis [9]; and this was similarly portrayed by the detection of a functional exhaustion of monocytes leading to impaired pathogen clearance [10].

Systemic inflammation and an impaired immune response may not be mutually exclusive but operate simultaneously in decompensated cirrhosis and ACLF. Recently, a theory encompassing both features denominated cirrhosis-associated immune dysfunction (CAID) has been proposed. This syndrome describes excessive systemic inflammation as an initial phenotype in compensated cirrhosis, caused by persistent immune cell stimulation by bacterial and bacterial products translocation; under this constant stimulus, the immune response system eventually becomes exhausted and switches to a "immunodeficient" phenotype in late stages of decompensated cirrhosis, such as ACLF [11].

Despite the fact that there is plentiful evidence of the role of immune dysfunction in the pathogenesis in cirrhosis, the identification of objective, reproducible and readily-available surrogate biomarkers of this syndrome is still a work in progress. Several features have been analyzed, such as leukocyte count, procalcitonine and C-reactive protein; however, no clear cut-off points or extensive validation has been achieved so far [12]. Cytokines have also been proposed as prognostic tools in different stages of cirrhosis. In stable cirrhotic patients, they have been evaluated as tools in the detection of clinically-significant portal hypertension [13] and even as independent factors related to mortality in decompensated cirrhosis [14].

However, the recognition of prognostic biomarkers would probably be of utmost utility in ACLF, the most severe stage of decompensated cirrhosis, to improve current treatment strategies. Although several cytokines have been studied as part of the pathophysiology of ACLF and even suggested as prognostic factors, only a few were randomly selected, not allowing analyzing thoroughly its