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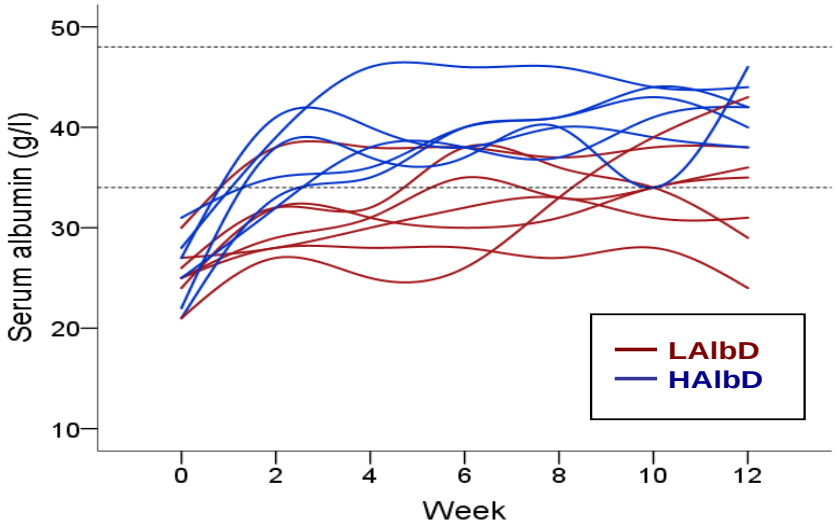
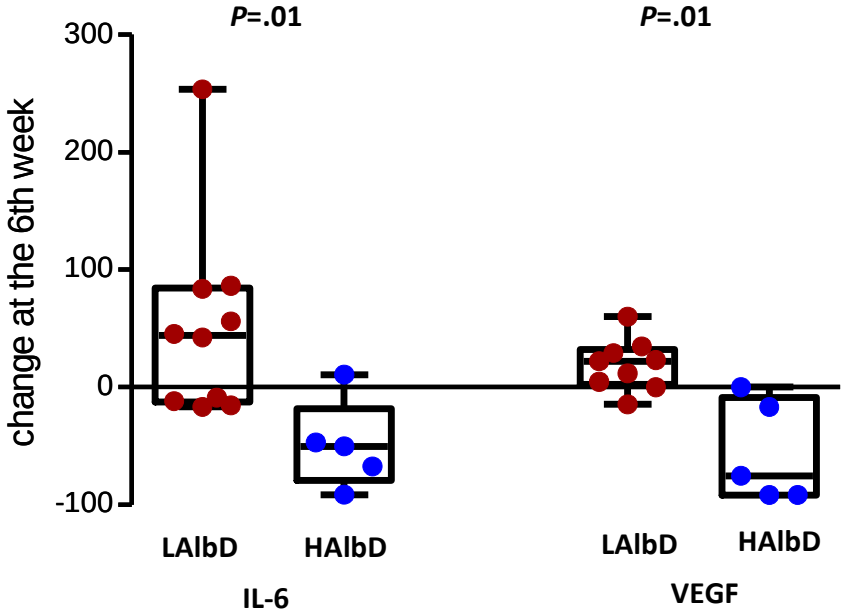
Effects of long-term albumin treatment on serum albumin levels and inflammatory cytokines
High albumin dose (HAlbD: 1.5 g/kg every week, blue figures) but not low albumin dose (LAlbD: 1 g/kg every 2 weeks: red figures) normalized serum albumin levels and decreased inflammatory cytokines





Effects of Albumin Treatment on Systemic and Portal Hemodynamics and Systemic Inflammation in Patients With Decompensated Cirrhosis

Short title: Pleiotropic Effects of Albumin Therapy

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List of abbreviations:

ACLF: acute-on-chronic liver failure; ANP: Atrial Natriuretic Peptide; BNP: Brain Natriuretic Peptide; BUN: Blood urea nitrogen; EASL-CLIF Consortium: European Association for the Study of the Liver-Chronic Liver Failure Consortium; HAlbD: high albumin dose; HCV: hepatitis C virus;

HCC: hepatocellular carcinoma; HRS: hepatorenal syndrome; HVPG: Hepatic venous pressure gradient; IL: interleukin; INR: international normalized ratio; IQR: interquartile range; LAlbD: low albumin dose; LV: Left ventricle; MAP: Mean arterial pressure; MELD: model for end stage liver disease; PAMPs: pathogen associated molecular patterns; PRA: plasma renin activity; PRC: plasma renin concentration; RCT: randomized controlled trial; SBP: spontaneous bacterial peritonitis; SVRI: Systemic vascular resistive index; TIPS: transjugular intrahepatic portosystemic shunt; TNFα: tumor necrosis factor alpha; VEGF: vascular endothelial growth factor.

ABSTRACT

Background & Aims: We investigated the effect of albumin treatment (20% solution) on hypoalbuminemia, cardiocirculatory dysfunction, portal hypertension, and systemic inflammation in patients with decompensated cirrhosis with and without bacterial infections.

Methods: We performed a prospective study to assess the effects of long-term (12 weeks) treatment with low doses of albumin (1 g/kg body weight every 2 weeks), and high doses (1.5 g/kg every week), on serum albumin, plasma renin, cardiocirculatory function, portal pressure, and plasma levels of cytokines, collecting data from 18 patients without bacterial infections (the Pilot-PRECIOSA study). We also assessed the effect of short-term (1 week) treatment with antibiotics alone vs. the combination of albumin plus antibiotics (1.5 g/kg on day 1 and 1 g/kg at day 3) on plasma levels of cytokines in biobanked samples from 78 patients with bacterial infections included in a randomized controlled trial (INFECIR-2 study).

Results: Circulatory dysfunction and systemic inflammation were extremely unstable in many patients included in the pilot-PRECIOSA study; these patients had intense and reversible peaks in plasma levels of renin and interleukin 6 (IL6). Long-term high-dose albumin but not low-dose albumin was associated with normalization of serum level of albumin, improved stability of the circulation and left ventricular function, and reduced plasma levels of cytokines (IL6, GCSF, IL1RN, and VEGF) without significant changes in portal pressure. The immune-modulatory effects of albumin observed in the Pilot-PRECIOSA study were confirmed in the INFECIR-2 study. In this study, patients given albumin had significant reductions in plasma levels of cytokines.

Conclusions: In an analysis of data from 2 trials (pilot-PRECIOSA study and INFECIR-2 study) we found that albumin treatment reduces systemic inflammation and cardiocirculatory dysfunction in patients with decompensated cirrhosis. These effects might be responsible for the beneficial effects of albumin therapy on outcomes of patients with decompensated cirrhosis. ClinicalTrials.gov no: NCT00968695 and NCT03451292

KEY WORDS: Liver-related complications; immune response; splanchnic hemodynamics; interventional trials

The first studies supporting the use of albumin treatment in cirrhosis were performed in the 1980´s and consisted of several randomized clinical trials (RCTs) demonstrating that paracentesis was a rapid, effective and safe therapy of ascites if performed with intravenous (IV) albumin administration (8 g per liter of ascitic fluid removed).[1] Sort et al. subsequently showed that treatment of spontaneous bacterial peritonitis (SBP) with antibiotics plus albumin (1.5 g/kg body weight at infection diagnosis and 1 g/kg on day 3) was associated with 60% reduction in the prevalence of type-1 hepatorenal syndrome (HRS), a special form of acute-on-chronic liver failure (ACLF), and in hospital mortality.[2] Ortega et al.[3] later on showed that the simultaneous administration of terlipressin and albumin (20-40 g/day for 7-14 days) normalized serum creatinine concentration in approximately 50% of patients with hepatorenal syndrome (HRS). Finally, the ANSWER study has recently shown that long-term (18 months) prophylactic administration of albumin (40 g every week) to patients with prior history of ascites is highly effective in preventing follow-up development of new episodes of ascites, refractory ascites, HRS, hepatic encephalopathy and bacterial infections, reducing hospital admissions and improving survival.[4] This successful research activity on the therapeutic use of albumin in cirrhosis contrasts sharply with the low number of investigations performed on its mechanisms of action.[1]

This article reports the results of the Pilot-PRECIOSA study, which was aimed to identify an albumin dosage that normalizes serum albumin concentration and to investigate the effects of the administration of this albumin dosage during 12 weeks on hypoalbuminemia, cardiocirculatory hemodynamics, effective blood volume, portal pressure and systemic inflammation (as estimated by the plasma levels of IL-6) in 18 patients with decompensated cirrhosis.

Recent investigations suggest that systemic inflammation plays a major role in the pathogenesis of acute decompensation and ACLF in cirrhosis.[5] The observation of a marked suppression of the plasma levels of IL-6 during albumin treatment in the Pilot-PRECIOSA STUDY, which suggests an immunomodulatory effect of albumin treatment, prompted us to

perform additional investigations to confirm this feature. These investigations consisted of the measurement of a large panel of inflammatory mediators in biobanking material from the Pilot-PRECIOSA study and from the INFECIR-2 study, a RCT aimed to compare the efficacy of antibiotics alone versus albumin-plus-antibiotics in patients with decompensated cirrhosis and bacterial infection unrelated to SBP[6].

Methods

The Pilot-PRECIOSA study and the INFECIR-2 study were approved by the corresponding Ethic Committees of each hospital involved. The informed consent forms of the two studies included the potential use of biobanking material for measuring serum albumin levels and plasma renin and cytokine concentrations.

The Pilot-PRECIOSA study

The Pilot-PRECIOSA study (IG0802, registered at ClinicalTrials.gov as: NCT00968695) is a proof of concept, open-label, multicenter, nonrandomized (single-group), prospective, phase 4, safety and dosage-exploratory investigation sponsored by Grifols with the aim to get preliminary information to design a currently ongoing multicenter randomized controlled therapeutic trial assessing the efficacy of long-term (1 year) albumin treatment in the prevention of ACLF and mortality in decompensated cirrhosis (PRECIOSA study, ClinicalTrials.gov: NCT03451292).

Investigators of the EASL-CLIF Consortium from three hospitals (Hospital Clinic and Hospital de Sant Pau from Barcelona and Hospital Ramón y Cajal from Madrid) participated in the design and implementation of the study, which started in July 2009 and was completed in April 2014. These hospitals use the same methodology for cardiocirculatory and hepatic hemodynamic studies and have large experience in cooperative hemodynamic, pathophysiological and therapeutic studies. Non-standard laboratory measurements (hormones and biomarkers estimating systemic inflammation) were centralized at the Hospital Clinic. The results of the Pilot-PRECIOSA study were submitted to embargo until the onset of the PRECIOSA study.

Inclusion and exclusion criteria and patients evaluated

The study enrolled non-infected patients with decompensated cirrhosis and severe circulatory dysfunction as defined by the presence of ascites, renal dysfunction [serum creatinine≥ 1.2 mg/dl

or blood urea nitrogen (BUN) ≥ 25 mg/dl or dilutional hyponatremia (serum sodium ≤ 130 mEq/l)], high levels of plasma renin activity (PRA ≥ 2 ng/mlL.h) and need for diuretic treatment to prevent ascites recurrence (at least 200 mg of spironolactone or 100 mg of spironolactone and 40 mg of furosemide). PRA was used for assessing sequential changes in effective arterial blood volume. The exclusion criteria are detailed in the Supplementary Appendix.

One-hundred-thirty-five patients were evaluated, 72 were eligible, and among them 39 showed exclusion criteria. Of the 33 remaining patients, 12 were excluded for data analysis due to: 1. Lack of abnormal plasma renin activity (PRA < 2 ng/ml.h) at enrolment (2 patients); 2. Development of complications requiring treatment that interfere with the interpretations of the results (intensive care, liver transplantation and insertion of a TIPS, 3 patients); 3. Discontinuation of albumin treatment (7 patients). Three out of the remaining 21 patients died within the study period and 3 did not give informed consent for cardiocirculatory and hepatic hemodynamic assessment. The clinical characteristics at enrolment, and the main complications and causes of death during the study period are indicated in Supplementary Table 1.

Chronogram

Day 0

Samples were obtained for standard laboratory tests, serum albumin concentration, PRA (as marker of effective blood volume), plasma concentrations of atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) (markers of central blood volume expansion) and IL-6, followed by the hepatic and cardiocirculatory hemodynamic study. The methods for these studies have been previously described.[7] Immediately afterwards patients received the first albumin dose and they were followed-up for 20 weeks.

Weeks 1 to 12

The first 10 patients received an albumin dose of 1g/kg body weight every 2 weeks for 12 weeks (a total of 7 albumin treatments). PRA was measured every 2 weeks prior to each albumin dose in the first 5 patients and ad hoc weekly in the remaining 5. Plasma IL-6 and serum albumin concentration were measured every two weeks. An interim analysis in these first 10 patients showed that this dose of albumin was insufficient to normalize serum albumin concentration throughout the last 10 weeks of the study period in most patients (normal serum albumin concentration: 34-47 g/L). Accordingly, albumin dosage was increased to 1.5 g/kg body weight every week in the remaining patients. Therefore, this second group of patients received a higher albumin dosage per treatment and more albumin treatments (13) within the same time-period (day 0 and then every week for 12 weeks). Samples for PRA were taken ad hoc weekly during treatment. Samples for serum concentration of albumin and plasma levels of IL-6 were obtained every two weeks. For the description of the results, the group of patients who received albumin at a dose of 1g/kg every two weeks was defined as "Low Albumin Dosage" (LAlbD) group, and that receiving albumin at a dose of 1.5 g/kg every week as "High Albumin Dosage" (HAlbD) group.

14th Week

Two weeks after the last albumin dosage, the cardiopulmonary and hepatic hemodynamic study was repeated.

Post hoc measurements of cytokines, chemokines and other inflammatory markers

The post hoc assessment on the effects of albumin treatment on systemic inflammation was performed assessing a large panel of inflammatory mediators and biomarkers, including 24 cytokines, 10 chemokines, 4 growth factors and 6 markers of endothelial dysfunction (2), coagulation/platelet dysfunction (2) and monocyte activation (2), in biobanking material (September 2018).

The INFECIR-2 Study

The INFECIR-2 study is an EASL-CLIF Consortium investigator-promoted, phase 4, randomized, open-label, parallel, multicenter trial promoted by the Fundació Clínic (Hospital Clínic, University of Barcelona, Spain). It started in September 2014 and was finished in December 2016 (ClinicalTrials.gov: NCT02034279). The inclusion and exclusion criteria are detailed in the Supplementary Appendix. The study was aimed to assess the efficacy of short-term albumin treatment in the prevention of ACLF and hospital mortality in 136 patients with decompensated cirrhosis and acute bacterial infections unrelated to SBP. Eighteen patients were considered inclusion errors. Therefore, 118 patients were randomized to receive either antibiotics alone (antibiotics-alone group; n=57), or antibiotics plus two albumin doses, i.e., 1.5 g/kg at inclusion (day 1) and 1g/kg on day 3 (albumin-plus-antibiotics group; n=61). Plasma samples for biobanking were obtained at day 1, prior to the administration of the first albumin dose, at day 3, prior to the second albumin dose, and/or at day 7 in 48 and 47 patients, from the albumin-plus-antibiotics and antibiotics-alone groups, respectively. "On treatment" values of plasma cytokine levels given in the article represent the average of those obtained at day 3 and 7 (in patients with two measurements) or those obtained at day 3 or 7 in patients with only a single measurement. Both groups were similar regarding patient characteristics (except for the combined prevalence of ACLF and kidney dysfunction at baseline, higher in the albumin arm), type of infections and antibiotic therapy. The results of the INFECIR-2 study have recently been reported [6].

The current study used biobanking aliquots from the INFECIR-2 study for measurement of the serum concentration of albumin, the plasma concentration of renin (PRC) and the plasma concentration of the same panel of cytokines, chemokines, growth factors, and other inflammatory markers studied in the Pilot-PRECIOSA study. Measurements were performed at baseline and during treatment among 40 patients from the antibiotics-alone group and 38 patients from the albumin-plus-antibiotics group. The pre-specified criteria to select these 78 patients

were: 1. Availability of biobanking samples; 2. Infection receiving appropriate empirical antibiotic treatment; 3. Absence of severe complications within the first week of treatment that could affect the interpretation of the results; 4. Completion of one-week follow-up.

Laboratory methods,

Hormones and IL-6 were measured by radioimmunoassay (PRA), chemiluminescent immunoassay (PRC), immunoassay (ANP and BNP) and ELISA (IL-6). Measurement of the panel of cytokines, chemokines and other inflammatory mediators in patients from the Pilot-PRECIOSA and INFECIR-2 studies were performed using two multiplex immunoassays based on Luminex multi-analyte profiling technology. The plasma levels of sCD163 and sMR/sCD206 were determined by enzyme-linked-immunosorbent-assay. Methods are detailed in the Supplementary Appendix.

Statistical Methods

In the Pilot-PRECIOSA study, for a given patient on albumin treatment, there were several available results for serum albumin, PRA, and plasma IL-6. We averaged all the available values within the last 10 weeks of treatment to obtain a single "on-treatment" value for comparison with the corresponding baseline value.

Results are presented as median and interquartile range (IQR). For univariate analysis Mann-Whitney test and Wilcoxon signed rank test were used for not normally distributed variables. In all statistical analyses, significance was set at $P < 0.05$. Analysis were performed with SAS (version 9.4; SAS Institute Inc.; Cary, NC) statistical packages. Graphs were performed with GraphPad Prism (version 5.00, GraphPad Software, San Diego, CA).

Results

Baseline Clinical Characteristics Of The Patients Included In The Pilot-PRECIOSA Study

All the 18 patients included were admitted to hospital for the treatment of ascites; 3 had diabetes mellitus, 1 hepatocellular carcinoma and 2 minimal hepatic encephalopathy. Other characteristics at enrollment are illustrated in Supplementary Table 1.

Effect Of Long-term Albumin Treatment And Its Dosage On Serum Albumin Concentration (Pilot-PRECIOSA Study)

Thirteen out of the 18 patients included completing the sequential measurement of plasma albumin concentration had baseline hypoalbuminemia (serum albumin concentration <34 g/L). The effect of albumin treatment on serum albumin concentration was related to two factors. The first factor was albumin dosage. Although patients of the LAlbD group with baseline hypoalbuminemia (n=7) exhibited increases in serum levels of albumin during treatment, only one normalized the serum albumin concentration, i.e. had an increase in albumin level to a value ≥34 g/L in all measurements. In contrast, all patients of the HAlbD group with baseline hypoalbuminemia (n=6) normalized serum albumin concentration “on treatment” (P<0.001; Figure 1, Panel A). The median increase in serum albumin among patients receiving HAlbD or LAlbD, are detailed in Table 1, for all patients, and in Figure 1, panel B, specifically for patients with baseline hypoalbuminemia. Four out of the 5 patients with normal baseline serum albumin concentration (3 from the LAlbG and 2 from the HAlbG) showed relatively stable serum albumin concentration (always within the normal limits: 34-47 g/L) throughout the study (Figure 1, Panel C). The fifth patient exhibited an “on-treatment” increase in serum albumin, but this was also within normal limits.

The second factor influencing the effect of albumin treatment was the grade of hypoalbuminemia at baseline. There was a significant inverse correlation between the baseline

serum albumin concentration and the median change in serum albumin during treatment in both the HAlbD and the LAlbD groups (Figure 1, Panel D), the lower the baseline albumin concentration the higher the median increase in the serum albumin levels achieved during treatment. The response to albumin treatment at each level of serum albumin concentration was higher in patients receiving HAlbD.

Effect Of Long-term Albumin Treatment And Its Dosage On PRA (Pilot-PRECIOSA Study)

Long-term albumin treatment was surprisingly not associated with significant suppression in PRA in patients receiving both HAlbD and LAlbD, suggesting a minor effect on the effective blood volume (Table 1). Figure 1, panels E and F, show the individual time-course changes of PRA in patients receiving LAlbD and HAlbD, respectively. An intriguing observation was the extreme instability of effective blood volume, as indicated by the development of acute, high and transient positive peaks of PRA (increase in PRA >100% to levels over 10 ng/ml.h) in a significant number of patients. Peaks were observed more frequently in the LAlbD group (6 patients, 60%) than in the HAlbD group (1 patient, 12.5%) (P=0.04), suggesting that although albumin treatment was not effective in improving mean effective blood volume it was capable to stabilize circulatory function.

Effect Of Long-term Albumin Treatment And Its Dosage On Plasma IL-6 Levels (Pilot-PRECIOSA Study)

To explore the possibility that albumin treatment can affect systemic inflammation, we sequentially measured the plasma levels of IL-6 at day 0 and every two weeks after day 0 in the Pilot-PRECIOSA study. IL-6 is a paradigmatic proinflammatory cytokine whose plasma levels are increased in most patients with cirrhosis and systemic inflammation[6]. Nine patients from the LAlbD group and 7 from the HAlbD group had measurable levels of IL-6 at baseline and during treatment. The effect of albumin treatment on systemic inflammation in each patient could then be

estimated as the absolute or percent change of IL-6 between baseline value and "on treatment value" (Table 1). The median baseline value for plasma IL-6 levels in the 16 patients was well above the normal range, consistent with the existence of systemic inflammation in this group of patients. We arbitrarily defined that a patient developed significant immunomodulatory response to albumin treatment when the "on treatment" IL-6 level decreased by more than 20% below the baseline level. An outstanding finding of the current study was that the majority of patients receiving HAlbD (6 out of 7 patients, 85.7%) but only 1 out of 9 patients receiving LAlbD (11%; P=0.003 for between-group comparison) had a reduction of plasma IL-6 > 20%, suggesting that long-term treatment with HAlbD but not with LAlbD induces significantly immunomodulatory effect in patients with decompensated cirrhosis. Consistent with these findings, we found that the median reduction from baseline for IL-6 was significantly greater among patients receiving HAlbD than among those receiving LAlbD, whichever the way of expressing reduction, percentage or absolute values (Table 1).

A second important finding was that systemic inflammation was unstable in a significant number of patients (1 out of 7 receiving HAlbD and 4 out of 9 receiving LAlbD), with acute, high and reversible peaks of the plasma IL-6 (i.e., increases by at least 100% to levels over 100 pg/mL) during albumin treatment (Figure 2, panel A). The remaining 11 patients showed small changes (mainly patients receiving LAlbD) or marked reductions (mainly patients receiving HAlbD) of "on treatment" IL-6 (Figure 2, panel B).

Effect of Long-term Albumin Treatment And Its Dosage On A Large Panel Of Plasma Cytokines (Pilot-PRECIOSA Study)

The finding that elevated baseline plasma IL-6 levels as determined by ELISA can be reduced by albumin therapy in a dose-dependent manner, prompted us to investigate the effects of this treatment on the plasma levels of a large number of cytokines (24) in biobanking samples

obtained at baseline and at week 6 of albumin treatment in 10 patients from the LAlbD group and in 5 patients in the HAlbD group. In addition, we measured the plasma levels of the 24 cytokines in 25 healthy donors recruited at the Hospital Clínic Blood Bank.

Among the 24 cytokines measured, 11 were not detectable in any patient/healthy subject. Baseline values of all but two of the remaining 13 cytokines included in the panel were significantly higher among patients with decompensated cirrhosis than among healthy subjects, confirming the existence of full-blown systemic inflammation in decompensated cirrhosis (Table 2). In the next tables only changes in relevant cytokines are presented. Patients receiving LAlbD experienced only a small reduction or moderate increase during treatment in the plasma levels of these cytokines, a feature that contrasts sharply with the marked suppression of most cytokines in patients receiving HAlbD (Table 3, Figure 2, panels C and D). These results strongly suggest that long-term albumin treatment, if given at high dosage, has a significant immunomodulatory effect in decompensated cirrhosis reducing the degree of systemic inflammation.

Effects of Long-term Albumin Treatment On Systemic And Splanchnic Hemodynamics, Natriuretic Peptides, And Liver And Renal Function (Pilot-PRECIOSA study)

Treatment with HAlbD but not with LAlbD was associated with a significant increase in cardiac index, systolic volume and left ventricular stroke work index indicating an increase in left ventricular function (Table 4). There were no changes in most parameters estimating cardiac preload, including atrial pressure, pulmonary capillary wedged pressure, and plasma concentrations of ANP and BNP. There was, however a significant increase in mean pulmonary artery pressure in patients receiving HAlbD, although it might be related to improvement in right ventricular function. All patients had severe portal hypertension at enrollment. HAlbD and LAlbD treatment, however, was not associated with significant changes in hepatic venous pressure

gradient, a sensitive marker of portal pressure. There were also no major changes in other relevant standard laboratory parameters in both groups.

Effect Of Short-Term Albumin Treatment On Serum Albumin And Plasma Levels Of Renin And Of a Large Panel Of Inflammatory Cytokines in Patients With Infections (INFECIR-2 Study)

Next, we asked whether albumin therapy could have a reducing effect on plasma cytokine levels in patients with bacterial infections included in the INFECIR-2 study. Bacterial infections are known to result in an enhancement of the systemic inflammation already present in patients with decompensated cirrhosis.[7] This explains why baseline levels of TNF-α, IL-4, IL-6 and IL-10 were significantly higher among patients included in the INFECIR-2 study than among those included in the pilot PRECIOSA study (Table 2). As expected for a randomized trial, in the INFECIR-2 study, the baseline plasma cytokine levels were similar among patients assigned to receive antibiotics alone and among those assigned to albumin-plus-antibiotics (Table 5).

Treatment with antibiotics alone was not associated with significant changes in most cytokines. Only one showed a significant suppression (TNF-α) during treatment. In contrast, patients treated with albumin-plus-antibiotics had, during treatment, a significant decrease or a clear trend for a reduction in most cytokines (Table 5), suggesting that albumin associated with antibiotics was more effective than antibiotics alone in attenuating baseline systemic inflammation in patients with bacterial infections.

In the INFECIR-2 study, baseline values for serum albumin concentration were similar between patients of the antibiotics-alone group (26 [20 to 30] g/L) and those of the albumin-plus-antibiotics group (25 [19 to 30] g/L; P=0.91). The baseline activity of the renin-angiotensin system, estimated by PRC, was greater among patients of the albumin-plus-antibiotics group than among those of the antibiotics-alone group although difference was not statistically

significant (241.6 [46.3 to 903.0] µIU/mL and 125.0 [34.0 to 398.6] µIU/mL, respectively; P=0.25). Antibiotics alone were not associated with significant changes from baseline for serum albumin concentration (0 [-20 to 1.0] g/L) or PRC (-1.2 [-26.5 to 129.6] µIU/mL). In contrast, albumin-plus-antibiotics significantly increased serum albumin concentration (7.0 [4.0 to 10.0] g/L; P< 0.0001) and suppressed PRC [-40. 5 [-272.9 to -4.5], µIU/ml; P=0.002].

Effect Of Short-Term And Long-Term Albumin Treatment On Chemokines, Growth Factors, And Biomarkers Of Macrophage Activation, Endothelial Dysfunction And Coagulation/Platelet Function (Pilot-PRECIOSA and INFECIR-2 studies)

To have a comprehensive view of the effects of albumin treatment in both, the Pilot-PRECIOSA and the INFECIR-2 studies, we assessed a broad variety of soluble factors, including chemokines, growth factors and markers of macrophage activation and endothelial and coagulation/platelet dysfunction. As shown in Supplementary Tables 3 and 4, in both studies, albumin treatment was associated with minor or no changes in most of these factors, suggesting that it exerts its immunomodulatory effect mainly by influencing production and/or release of specific cytokines.

Discussion

Current albumin dosage in cirrhosis is based on empirical assumptions and on the concept that albumin mainly acts as a plasma volume expander.[8] Of note, albumin therapy, can have many other important biological effects since it is capable to bind and inactivate a wide range of endogenous and exogenous ligands.[1] The ability of albumin to bind pro-inflammatory molecules such as pathogen associated molecular patterns (PAMPs, e.g., the Gram-negative bacteria byproduct lipopolysaccharide),[9] prostaglandins, [10] nitric oxide[11] and reactive oxygen and nitrogen species[12] could be of greatest importance in the context of cirrhosis, because these molecules are involved in the pathogenesis of the systemic inflammation and circulatory and organ dysfunction/failure that characterizes decompensated cirrhosis and ACLF.[13] As the occurrence of these non-osmotic effects of albumin therapy in cirrhosis was elusive, there was an urgent need to address this question which gave rise to the present study.

The current article describes five important unreported observations on the pathophysiology and albumin treatment of decompensated cirrhosis. The first is that the long-term albumin dosage required to normalize serum albumin concentration is much higher than that used in all randomized controlled therapeutic trials so far performed.[4,14] The second is that circulatory dysfunction is not a steady state or a slowly progressive process, as it has been traditionally considered, but rather an extremely unstable condition. The third observation is that systemic inflammation in cirrhosis is also unstable with acute episodes of burst of circulating cytokines in the absence of any identifiable precipitating event. The fourth is that HAlbD but not LAlbD treatment is associated with significant improvement in left ventricular function in decompensated cirrhosis, which is currently considered as an important mechanism of systemic circulatory dysfunction.[15] Finally, and most importantly, the sequential assessment of the plasma levels of IL-6 during albumin treatment showed for the first time that long-term albumin treatment at high dosage has immunomodulatory effects in decompensated cirrhosis. The transcendence of

this later finding was the reason to complete the study with two additional investigations. The first was aimed to assess if the suppressive effect of albumin on IL-6 observed in the patients included in the Pilot-PRECIOSA study also extended to other cytokines and inflammatory molecules. The second was to investigate if the immunomodulatory effect observed during long-term treatment with HAlbD in patients without bacterial infection also occurs following short-term (one week) high albumin dosage treatment in patients with bacterial infections. For these objectives, we leveraged the availability of biobanking material from the Pilot-PRECIOSA and INFECIR-2 studies.

The initial albumin dose evaluated in the Pilot-PRECIOSA study (1 g/kg every two weeks during 12 weeks) was based on that used in the pioneer RCT by Gentilini et al[14] (25 g per week) exploring the effect of long-term of albumin treatment on the response to diuretics in patients with cirrhosis ascites, and in two RCTs exploring the long-term effect of albumin administration on the natural course of decompensated cirrhosis (the ANSWER study: 40 g of albumin every week[4], and the MATCH study: 40 g every 2 weeks[16]). The results of the current study indicate that a dose of 1g/kg, which is higher than the MATCH study dose and only slightly lower than the ANSWER study dose, was clearly insufficient to normalize serum albumin concentration in seven out of the eight patients with hypoalbuminemia included in the LALbD group. In contrast, our second albumin dosage (1.5 g/kg per week) rapidly normalized serum albumin concentration in all patients with hypoalbuminemia included in the HAlbD group.

The time-course changes of serum albumin concentration during albumin treatment suggest that the homeostatic feedback mechanism by which hepatic albumin synthesis is regulated by the serum albumin concentration[17] is fully operative in patients with advanced cirrhosis. Normalization of serum albumin concentration in patients with hypoalbuminemia receiving HAlbD occurred very rapidly (within 2 weeks) following the onset of albumin treatment but once normalized it remained within normal limits throughout the study despite the weekly

administration of albumin at a concentration of 20 g/dL (5 times higher than the normal serum albumin concentration). This rapid and intense initial increase in serum albumin concentration was probably the consequence of the combination of increased albumin synthesis by the liver secondary to hypoalbuminemia and the effect of the exogenous albumin administrations. In contrast, following normalization of serum albumin, the inhibitory effect of normo-albuminemia upon albumin synthesis precluded any further increase in serum albumin concentration despite continuous albumin treatment. The homeostatic feedback mechanism of serum albumin would also explain why albumin treatment did not increase serum albumin concentration in patients without hypoalbuminemia (for additional explanatory details see figure 1 legend).

The most relevant finding of our study was the observation that both long-term and short-term albumin treatment, if given at high dosage, are associated with significant immunomodulatory effects in decompensated cirrhosis. Three lines of evidence supported this conclusion. The first derived from the sequential measurement of IL-6 during albumin treatment in patients included in the pilot-PRECIOSA study. The median reduction from baseline of plasma IL-6 levels was significantly greater among patients receiving HAlbD than among those receiving LAlbD. This finding is important considering that IL-6 has broad effect on immune and non-immune cells and often displays hormone-like characteristics that can affect homeostatic processes.[18] The second line of evidence derived from the analysis of the effect of albumin treatment on cytokines other than IL-6 in biobanking material from the Pilot-PRECIOSA study. This investigation, confirmed the observations of the first investigation. Treatment with HAlbD but not that using LAlbD was associated with significant decrease in plasma IL-6 during treatment. Moreover, it demonstrated that this effect also involved other keystone cytokines (e.g., G-CSF), confirming that long-term therapy with HAlbD but not that using LAlbD induces a significant and extensive immunomodulatory effect in decompensated cirrhosis. Finally, the third line of evidence was obtained from the analysis of biobanking plasma samples from the INFECIR-2 study.

Treatment with albumin-plus-antibiotics was associated with a rapid, significant and widespread suppression of the circulating levels of cytokines, an effect not observed with antibiotics alone. It was interesting to observe that the immunomodulatory effect of albumin in the Pilot-PRECIOSA and INFECIR-2 studies was related mainly to the inhibitory effect of albumin on cytokine production but not to an effect on other inflammatory molecules.

An intriguing finding of our study was the observation of one or two acute, intense and spontaneously reversible peaks of PRA and plasma IL-6 during albumin treatment in many patients included in the Pilot-PRECIOSA. There are reasons to suggest that the prevalence and frequency of these peaks in the current study are not representative of their actual prevalence and frequency in patients with decompensated cirrhosis. First, we monitored PRA and plasma IL-6 only once every week or two weeks during the study period. On the other hand, according to our data the duration of these peaks may range from less than one to two or more weeks. Therefore, we could have lost a significant number of peaks in our patients. Interestingly, the prevalence of PRA and IL-6 peaks was lower in patients receiving HAlbD than in those receiving LAlbD suggesting that treatment with HAlbD may prevent the occurrence of these acute episodes of aggravation of circulatory dysfunction and systemic inflammation in decompensated cirrhosis. Although the current study is the first demonstrating these abnormalities, the existence of such episodes of acute circulatory impairment and systemic inflammation had already been anticipated by the “Systemic Inflammation Hypothesis” as an explanation for the 40% prevalence of ACLF in patients without any identifiable exogenous precipitating event of the syndrome.[14,19] The proposed mechanism of such changes by the ”Systemic Inflammation Hypothesis” is the existence of transient bursts of translocation of viable bacteria or bacterial products from the intestinal lumen to the systemic circulation. Therefore, the potential futility of single measurements of renin and cytokines as surrogate markers of effective blood volume and

systemic inflammation in patients with decompensated cirrhosis has to be considered in the design of future studies.

Although circulatory dysfunction in cirrhosis has been traditionally attributed to splanchnic arterial vasodilation, there is now evidence that impairment in left ventricular function plays also a major role. In fact, cardiac index in cirrhosis falls progressively from compensated cirrhosis to decompensated cirrhosis and HRS.[15] Our data show that normalization of serum albumin concentration with long-term HAlbD treatment in non-infected patients with decompensated cirrhosis does not induce significant changes in central blood volume and portal pressure. However, it was associated with a significant improvement in left ventricular (LV) function. These observations are important for two reasons. The first is that they explain why treatment with HAlbD is generally not associated with variceal bleeding or pulmonary edema in decompensated cirrhosis without bacterial infections. Second, since systemic inflammation induces direct deleterious effect on heart function, our study supports the concept that the beneficial effect of albumin treatment in the management of organ dysfunction/failure in cirrhosis may be mediated, at least in part, by its immunomodulatory effect. In fact, this has also been observed in rats with carbon tetrachloride-induced cirrhosis, which develop evidences of systemic inflammation and inflammation in the cardiac tissue associated with severe impairment of LV contractibility, which reverses following albumin treatment.[20]

One of the strengths of our study is the use of multiple plasma samples whose prospective collection was pre-specified in the context of two well-designed multicenter controlled trials, of which one was randomized. A limitation of our study was the relative low number of patients included in the Pilot-PRECIOSA study. However, the most important finding of this investigation, the significant immunomodulatory effect of albumin treatment in patients with advanced cirrhosis, was confirmed by assessing the effect of albumin treatment on a large panel of inflammatory cytokines both in patients included in the Pilot-PRECIOSA study and in a

relatively large number of patients included in the INFECIR-2 study, thus offering solid additional arguments supporting our conclusions.

In summary, the current study allowed us to uncover important new findings related to the efficacy of albumin treatment in cirrhosis. The most outstanding were that high doses of albumin, but not low doses of albumin in patients with decompensated cirrhosis, have significant immunomodulatory effect, prevent a phenomenon revealed by the present study and which consists of “bursts” of circulatory dysfunction, improve LV function and correct serum albumin levels without inducing “albumin overdose”, probably because of the preservation of negative feed-back mechanisms controlling albumin synthesis, even in advanced liver disease. Since albumin is capable to bind and inactivate many inflammatory promoters such as PAMPs, bioactive lipid metabolites, reactive oxygen species and nitric oxide, the immunomodulatory effect of albumin could be related to this scavenging function. However, this explanation may be too simplistic, and further investigators are clearly needed to understand the anti-inflammatory effect of albumin treatment in cirrhosis.

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Figure legends

Figure 1

Changes in serum albumin concentration and plasma renin activity (PRA) induced by treatment with high albumin dosage (HAlbD, blue color in all panels) and low albumin dosage (LAlbD, red color) in the 18 patients included in the Pilot-PRECIOSA Study. **Panel A**: Individual changes in serum albumin concentration among the 13 patients with baseline hypoalbuminemia (serum albumin concentration <34 g/L). The horizontal lines indicate the upper and lower normal limits of serum albumin. All the six patients with hypoalbuminemia treated with HAlbD developed a rapid increase (within 2 weeks) in serum albumin concentration up to normal levels, remaining so during the remaining 10 study-weeks. In contrast, although all the 7 patients with baseline hypoalbuminemia treated with LAlbD increased the serum levels of albumin during treatment, only one normalized the serum albumin concentration. **Panels B to D**: Two factors influenced the response to albumin treatment. The first factor was the albumin dosage: Among the 13 patients with baseline hypoalbuminemia, the individual absolute median increase in serum albumin was almost double in patients receiving HAlbD than in those receiving LAlbD (**Panel B**); The second factor was the feedback mechanism by which baseline serum albumin concentration influences the hepatic synthesis of albumin. In most patients without hypoalbuminemia (**Panel C**), the inhibition of hepatic synthesis of albumin prevented the increase in the serum concentration of albumin to abnormal levels during albumin treatment. This feedback mechanism was also reflected by the close inverse correlation between the baseline serum albumin concentration and the mean increase in serum albumin during treatment (**Panel D**). The lower the baseline levels of serum albumin, the higher the absolute mean increase in the serum concentration of albumin in both the LAlbD and HAlbD groups. **Panel E**. Circulatory dysfunction was extremely instable during albumin treatment in patients receiving LAlbD, with high peaks of PRA in six patients.

Panel F. Circulatory instability was significantly improved in patients receiving HAlbD, with only one patients presenting one peak of PRA throughout treatment.

Figure 2

Changes in IL-6 and other cytokines induced by treatment with high albumin dosage (HAlbD, blue color in all panels) and low albumin dosage (LAlbD, red color) in the 15 patients included in the Pilot-PRECIOSA study with sequential cytokines measurements. **Panel A**. The degree of systemic inflammation, as estimated by repeated measurements of plasma IL-6 in baseline conditions and during treatment was extremely unstable in 4 patients receiving LAlbD and in one receiving HAlbD; **Panel B**. In the remaining patients there was a marked suppression of the circulating plasma levels of IL-6 (mainly in patients receiving HAlbD) or no-to-minor changes (mainly in patients receiving LAlbD); **Panels C and D**. Data derived from the assessment of a large panel of inflammatory cytokines at baseline and at week 6 showed that plasma levels of IL-6, VEGF, G-CSF and IL-1ra had a median reduction from baseline (IQR; %) which was significantly greater among patients treated with HAlbD than among those receiving LAlbD.

Table 1. Serum Albumin, Plasma Renin Activity And Plasma Levels of Interleukin (IL)-6 At Baseline and During Albumin Treatment In 18 Patients with Decompensated Cirrhosis Unrelated To Bacterial Plasma Levels For Infection Who Were Enrolled In The Pilot PRECIOSA Study And Divided Into Two Groups Depending Whether They Receive Low (LAlbD) Or High (HAlbD) dosage of Albumin.*

	Patients' Group		
	HAlbD group (n=8)	LAlbD group (n=10)	P value #
	Median (IQR)		
Serum Albumin Concentration			
Baseline (g/L)	27.6 (22.7 to 34.0)	26.5 (24.8 to 40.3)	0.83
"On treatment" Average Value (g/L)	39.2 (38.7 to 43.0)**	33.3 (31.8 to 37.9)***	0.004
Absolute Change (g/L)	12.7 (8.5 to 16.6)**	5.7 (-1.8 to 8.0)***	0.01
Percentage Change (%)	48.7 (26.7 to 71.3)**	20.2 (-4.1 to 32.5)***	0.04
Plasma Renin Activity			
Baseline (ng/mL.h)	5.5 (3.6 to 7.9)	7.9 (3.8 to 12.3)	0.41
"On treatment" Average Value (ng/mL.h)	4.9 (3.9 to 5.8)	6.9 (3.8 to 11.3)	0.17
Absolute Change (ng/mL)	0.2 (-4.2 to 1.3)	-0.4 (-5.5 to 5.8)	0.64
Percentage Change (%)	2.0 (-44.4 to 36.2)	-6.7 (-45.3 to 146.2)	0.69
Plasma IL-6 Concentration			
Baseline (pg/mL)	123.5 (51.5 to 151.5)	41.5 (25.8 to 75.0)	0.02
"On treatment" Average Value (pg/mL)	62.5 (24.5 to 93.6)**	57.5 (30.0 to 79.2)	0.76
Absolute Change (pgm/L)	-53.0 (-108.0 to -18.0)**	-3.2 (-11.1 to 30.0)	0.04
Percentage Change (%)	-56.0 (-68.8 to -24.2)**	-7.6 (-15.7 to 79.7)	0.04

* For each variable in each patient, the average value during treatment was obtained by using all the values of this variable, available "on-treatment". IQR denotes interquartile range, Cells colored in green show P values of less than 0.05. **P <0.05 for the within-group comparison with baseline values. ***P=0.05 for the within-group comparison with baseline values. # P value for the between-group comparison.

Table 2. Baseline Plasma Levels Of Cytokines Among Healthy Subjects (HS), Patients From The Pilot-PRECIOSA (P-PR) Study, And Patients From The INFECIR-2 (INF-2) Study.*

Cytokine	HS (N=25)	P-PR Study (N=15)	INF-2 Study (N=78)	P value		
				HS vs p-PR	HS vs INF-2	p-PR vs INF-2
TNFα						
Median level (IQR) — pg/mL	12.3 (11.5 to 16.9)	21.8 (16.0 to 30.6)	32.0 (21.9 to 49.8)	0.001	0.0001	0.04
Missing variable — no. (%)	0 (0)	0 (0)	0 (0)			
G-CSF						
Median level (IQR) — pg/mL	3.6 (2.4 to 5.7)	20.0 (8.8 to 156.9)	74.4 (32.0 to 155.5)	0.0008	0.0001	0.11
Missing variable — no. (%)	0 (0)	1 (7)	17 (22)			
IL-1ra						
Median level (IQR) — pg/mL	7.1 (3.8 to 13.2)	13.1 (9.1 to 32.8)	29.6 (8.3 to 76.5)	0.02	0.0001	0.26
Missing variable — no. (%)	0 (0)	0 (0)	0 (0)			
IL-6						
Median level (IQR) — pg/mL	0.9 (0.9 to 0.9)	10.5 (8.0 to 25.1)	37.1(22.6 to 107.6)	0.0001	0.0001	0.0001
Missing variable — no. (%)	0 (0)	0 (0)	0 (0)			
IL-10						
Median level (IQR) — pg/mL	1.1 (1.1 to 2.4)	2.7 (0.8 to 10.8)	10.9 (6.7 to 19.0)	0.20	0.0001	0.02
Missing variable — no. (%)	0 (0)	3 (20)	13 (17)			
IL-17A						
Median level (IQR) — pg/mL	0.7 (0.7 to 3.3)	17.7 (2.3 to 32.4)	3.2 (1.4 to 7.2)	0.0002	0.002	0.05
Missing variable — no. (%)	0 (0)	3 (20)	0 (0)			
IFNγ						
Median level (IQR) — pg/mL	3.0 (2.2 to 4.7)	6.7 (2.1 to 35.8)	6.8 (1.5 to 18.4)	0.11	0.04	0.49
Missing variable — no. (%)	0 (0)	0 (0)	0 (0)			
VEGF						
Median level (IQR) — pg/mL	24.4 (14.7 to 45.2)	59.0 (26.3 to 230.7)	61.0 (32.1 to 183.0)	0.02	0.002	0.77
Missing variable — no. (%)	0 (0)	1 (7)	32 (41)			

* Cells colored in green show P values of less than 0.05. The cell colored in yellow shows a P value of 0.05. IQR denotes interquartile range, TNF tumor necrosis factor, G-CSF granulocyte colony-stimulating factor, IL interleukin, IL-1ra interleukin-1 receptor antagonist, IFN interferon, and VEGF vascular endothelial growth factor.

Table 3. Plasma Levels Of Cytokines At Baseline And At The 6th Week Of Treatment In Patients Receiving Either Low Albumin Dosage (LAlbD) Or High Albumin Dosage (HAlbD) in The Pilot PRECIOSA Study.*

Cytokine	LAlbD Group (N=10)				HAlbD Group (N=5)				P Value for Between-Group Comparison	
	Undetectable Levels	Baseline Cytokine Level	Absolute Change from Baseline	Percentage Change from Baseline	Undetectable Levels	Baseline Cytokine Level	Absolute Change from Baseline	Percentage Change from Baseline	Absolute Change from Baseline	Percentage Change from Baseline
	no. (%)	*Median (IQR) — pg/mL*	*Median (IQR) — pg/mL*	*Median (IQR) — %*	*no. (%)*	*Median (IQR) — pg/mL*	*Median (IQR) — %*	*Median (IQR) — %*		
TNFα	0 (0)	20.3 (13.6 to 28.1)	1.8 (-0.7 to 3.5)	11.0 (-3.5 to 15.9)	0 (0)	30.9 (18.4 to 53.6)	-4.9 (-9.3 to 0.7)	-15.1 (-16.1 to 7.5)	0.12	0.19
G-CSF	0 (0)	20.0 (8.8 to 156.9)	4.9 (-1.4 to 13.8)	20.5 (-14.5 to 60.3)	1 (20)	47.3 (6.1 to 315.5)	-63.1 (-79.5 to -53.2)	-63.1 (-79.5 to -53.2)	0.05	0.03
IL-1ra	0 (0)	13.1 (10.2 to 35.3)	2.8 (-1.2 to 18.9)	28.8 (-15.2 to 53.6)	0 (0)	8.5 (6.7 to 29.3)	-4.0 (-7.0 to -2.8)	-70.3 (-82.9 to -13.8)	0.03	0.04
IL-6	0 (0)	8.9 (6.5 to 24.6)	0.8 (-2.5 to 7.4)	44.1 (-11.7 to 83.9)	0 (0)	10.7 (10.5 to 28.4)	-9.2 (-14.2 to -5.0)	-50.1 (-67.3 to -46.8)	0.01	0.01
IL-10	2 (20)	1.8 (0.6 to 10.8)	0.3 (-0.7 to 1.3)	-3.1 (-42.8 to 27.0)	1 (20)	5.6 (2.2 to 27.6)	-3.4 (-14.9 to 0.7)	-24.4 (-66.2 to 21.3)	0.35	0.43
IL-17A	3 (30)	24.7 (1.4 to 33.7)	0.65 (-0.9 to 9.8)	12.4 (-46.1 to 40.8)	0 (0)	15.4 (2.9 to 19.9)	-9.2 (-16.4 to -1.5)	-59.6 (-82.4 to -51.7)	0.07	0.14
IFNγ	0 (0)	5.6 (2.6 to 49.0)	0.2 (-0.6 to 2.2)	5.6 (-7.7 to 32.0)	0 (0)	8.7 (1.7 to 22.6)	-4.5 (-14.8 to -0.2)	-51.7 (-65.6 to -15.2)	0.12	0.12
VEGF	1 (10)	198.0 (56.1 to 230.7)	26.5 (0.0 to 50.8)	11.7 (0.0 to 29.2)	0 (0)	26.3 (23.5 to 50.1)	-8.4 (-17.6 to -4.0)	-75.2 (-91.4 to -16.8)	0.03	0.01

* Cells colored in green show P values of less than 0.05. Cells colored in yellow show a P value of 0.05. IQR denotes interquartile range, TNF tumor necrosis factor, G-CSF granulocyte colony-stimulating factor, IL interleukin, IL-1ra interleukin-1 receptor antagonist, IFN interferon, and VEGF vascular endothelial growth factor.

Table 4. Effects of LAlbD and HAlbD Administration On Cardiovascular And Splanchnic Hemodynamics, Cardiac peptides and Standard Liver And Renal Function Parameters

	Low Albumin Dose (n=10)		P value	High Albumin Dose (N=8)		P value
	Baseline	Week 14		Baseline	Week 14	
	Median (interquartile range)			*Median (interquartile range)*		
Systemic hemodynamics	**n=7**			**n=8**		
RAP (mm Hg)	6 (4-8)	4 (4-10)	0.87	8 (4-8)	9 (6-9)	0.26
MPAP (mm Hg)	16 (15-17)	15 (12-20)	0.55	15 (11-18)	18 (15-25)	0.02
PCWP (mm Hg)	10 (9-11)	8 (7-13)	0.74	11 (8-15)	12 (10-14)	0.11
Cardiac index (L/min/m^2)	3.9 (1.8-4.6)	3.8 (2.3-5.3)	0.09	4.2 (3.0-5.0)	5.3 (3.1-6.8)	0.04
Heart rate (bpm)	61 (59-82)	75(62-86)	0.21	69 (59-91)	68 (62-76)	0.21
Systolic volume (ml)	120 (40-127)	90 (52-135)	0.74	125 (85-145)	165 (110-190)	0.04
LV stroke work index (g.m/m^2)	48 (24-64)	44 (27-68)	0.74	54 (49-69)	82 (51-97)	0.04
SVRI (dyn.s/cm^5/m^2)	1158 (1042-3840)	1182 (944-2762)	0.18	1257 (952-1693)	1072 (728-1183)	0.09
MAP (mm Hg)	78 (63-88)	77 (75-85)	0.61	78 (74-85)	77 (66-84)	0.48
Cardiac peptides	**n=9**			**n=6**		
ANP (fmol/mL)	58 (23-84)	53 (37-64)	0.59	41 (13-87)	65 (29-155)	0.14
BNP (pg/mL)	82 (25-221)	37 (34-126)	0.45	41 (32-69)	49 (18-128)	0.46
Splanchnic hemodynamics	**n=7**			**n=6**		
FHVP (mmHg)	15 (9-17)	12 (5-17)	0.45	11 (8-14)	9 (5-10)	0.12
WHVP (mmHg)	35 (25-38)	30 (26-39)	0.67	32 (29-36)	28 (25-31)	0.21
HVPG (mmHg)	19 (15-20)	21 (14-22)	0.34	19 (17-27)	22 (18-25)	0.89
Liver and renal function	**n=10**			**n=8**		
AST (UI/L)	58 (27-78)	53 (26-69)	0.15	45 (40-112)	34 (31-55)	0.02
ALT (UI/L)	27 (19-46)	25 (16-34)	0.11	35 (28-56)	24 (21-39)	0.13
Serum creatinine (mg/dL)	1.3 (1.0-1.4)	1.0 (0.9-1.3)	0.06	0.9 (0.8-1.3)	0.9 (0.7-1.2)	0.24
BUN (m/dL)	26 (16-47)	22 (16-32)	0.16	20 (15-31)	24 (17-36)	0.25
Serum sodium (mEq/L)	132 (126-136)	133 (129-137)	0.67	130 (129-135)	132 (131-134)	0.87
Serum albumin (g/L)	27 (25-40)	35 (31-40)	0.06	27 (22-35)	40 (35-41)	0.03
Serum bilirubin (mg/dL)	1.8 (1.0-2.1)	1.8 (1.1-2.8)	0.41	3.7 (1.9-13.0)	1.9 (1.6-16.8)	0.40
INR	1.3 (1.1-1.6)	1.5 (1.1-1.7)	0.29	1.4 (1.3-2.3)	1.4 (1.3-2.5)	0.46
Child-Pugh score (points)	8 (6-10)	8 (7-9)	0.37	9 (8-11)	7 (6-8)	0.02
MELD score (points)	14 (11-17)	14 (9-17)	0.59	16 (13-26)	16 (13-27)	0.25

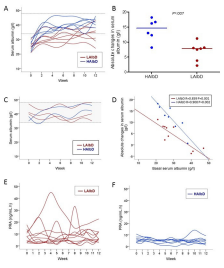
Cells colored in green show P values of less than 0.05. RAP denotes: right atrial pressure; MPAP: mean pulmonary artery pressure; PCWP: pulmonary capillary wedge pressure; LV: left ventricular; SVRI: systemic vascular resistance index; MAP: mean arterial pressure; ANP: atrial natriuretic peptide; BNP: brain natriuretic peptide; FHVP: free hepatic venous pressure; WHVP: wedge hepatic venous pressure; HVPG: hepatic venous pressure gradient; AST: aspartate aminotransferase; ALT: alanine aminotransferase; BUN: blood urea nitrogen; MELD: model for end stage liver disease.

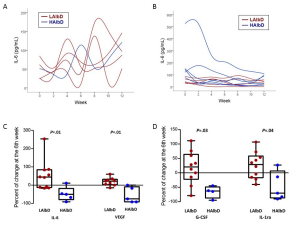
Normal values are: right atrial pressure: 2-10 mmHg; mean pulmonary arterial pressure: 10-25 mmHg; pulmonary capillary pressure: 6-14 mmHg; cardiac index: 2.5-4 L/min/m^2; SV: 60-100 ml; LV stroke work index: 45-75 g.m/m^2/beat; SVRI: 1970-2390 dyn.sec/cm^5/m^2; MAP: 80-95 mm Hg; Ejection fraction >50%; ANP: 9-24 fmol/mL; BNP: 4-37 pg/mL; HVPG: 1-5 mm Hg; hepatic blood flow: 1200-1500 ml/min

Table 5. Baseline Plasma Levels of Cytokines, And Their Changes During The First Week Of Treatment, In Patients From The INFECIR-2 Study Who Were Randomized To Receive Either Antibiotics Alone Or Albumin plus Antibiootics.*

Cytokine	Antibiotics Alone (N=40)						Albumin Plus Antibiotics (N=38)				P Value for Change From Baseline	
	Undetectable Levels	Baseline Level	Absolute change From Baseline	Percentage Change From Baseline	P Value for Change From Baseline		Undetectable Levels	Baseline Level	Absolute change from Baseline	Percentage Change from Baseline		
	no. (%)	*Median (IQR) — pg/mL*	*Median (IQR) — pg/mL*	*Median (IQR) — %*	*Absolute change*	*Percentage change*	*no. (%)*	*Median (IQR) — pg/mL*	*Median (IQR) — %*	*Median (IQR) — %*	*Absolute change*	*Percentage change*
TNFα	0 (0)	37.9 (23.3 to 50.0)	-2.8 (-12.6 to 3.2)	-8.2 (-28.9 to 11.9)	0.05	0.01	0 (0)	31.1 (21.2 to 45.3)	-3.4 (-14.7 to 3.1)	-16.2 (-40.5 to 12.8)	0.01	0.04
G-CSF	9 (23)	74.4 (19.2 to 185.5)	-3.4 (-55.3 to 11.5)	-21.2 (-91.4 to 21.5)	0.33	0.85	8 (21)	73.5 (33.6 to 115.0)	-41.5 (-65.1 to 8.4)	-58.6 (-88.8 to 30.2)	0.01	0.01
IL-1ra	0 (0)	29.6 (8.3 to 71.5)	-0.6 (-28.0 to 9.0)	-5.9 (-51.5 to 23.6)	0.37	0.31	0	29.9 (8.3 to 76.5)	-0.5 (-34 to 2.1)	-3.5 (-73.0 to 8.3)	0.05	0.28
IL-6	0 (0)	37.7 (18.3 to 94.7)	-7.0 (-19.9 to 20.7)	-14.8 (-43.5 to 66.4)	0.53	0.27	0 (0)	36.9 (23.9 to 158.9)	-7.7 (-33.1 to 0.3)	-23.0 (-55.0 to 3.8)	0.003	0.005
IL-10	8 (20)	10.7 (6.3 to 20.9)	-0.2 (-6.7 to 4.4)	-1.8 (-56.7 to 50.1)	0.74	1.00	5 (13)	11.0 (6.7 to 15.1)	-1.5 (-7.6 to 2.8)	-15.6 (-53.5 to 63.0)	0.03	0.03
IL-17A	0 (0)	3.7 (1.2 to 8.2)	0.1 (-1.7 to 1.3)	2.5 (-38.6 to 81.1)	0.92	1.00	0 (0)	2.7 (1.6 to 7.2)	-0.5 (-2.7 to 0.4)	-15.4 (-52.4 to 29.9)	0.05	0.09
IFNγ	0 (0)	4.8 (1.2 to 15.6)	-0.2 (-6.2 to 1.3)	-7.9 (-51.7 to 37.1)	0.17	0.52	0 (0)	8.4 (2.0 to 19.7)	-0.5 (-8.6 to 4.3)	-24.8 (-63.3 to 52.9)	0.48	0.24
VEGF	16 (40)	65.4 (45.6 to 204.0)	-18.5 (-41.4 to 18.2)	-16.4 (-45.3 to 29.4)	0.30	0.31	16 (42)	50.6 (24.1 to 183.0)	-13.9 (-49.0 to 11.9)	-24.7 (-36.9 to 32.5)	0.10	0.29

* Changes during the first week of albumin treatment were assessed between day 3 and day 7 after inclusion. There were no significant between-group differences in cytokine levels at baseline. Cells colored in green show P values of less than 0.05. Cells colored in yellow show a P value of 0.05. IQR denotes interquartile range, TNF tumor necrosis factor, G-CSF granulocyte colony-stimulating factor, IL interleukin, IL-1ra interleukin-1 receptor antagonist, IFN interferon, and VEGF vascular endothelial growth factor.





SUPPLEMENTARY APPENDIX

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Fernández J, Clària J, Amorós A, et al. Albumin Treatment in Decompensated Cirrhosis: Effects On Systemic And Portal Hemodynamics And On Systemic Inflammation

Supplementary Appendix

Table of contents

Supplementary Methods

Inclusion and Exclusion Criteria In The Pilot PRECIOSA Study

Inclusion criteria: age between 18 and 80 years, presence of ascites, renal dysfunction [serum creatinine≥ 1.2 mg/dl, blood urea nitrogen (BUN)≥ 25 mg/dl or dilutional hyponatremia (serum sodium ≤ 130 mEq/l)] and need for diuretic treatment to prevent ascites recurrence (at least 200 mg of spironolactone or 100 mg of spironolactone and 40 mg of furosemide).

Exclusion criteria: hepatorenal syndrome, refractory ascites (> 1 paracentesis/month), neoplastic disease including hepatocellular carcinoma over the Milan criteria, prior insertion of a transjugular intrahepatic portosystemic shunt, bacterial infections or GI bleeding within the previous 15 days, moderate to severe chronic heart (NYHA class II or IV) or pulmonary disease (GOLD III or IV), previous transplantation, active drug consumption, organic nephropathy, HIV infection, pregnancy and mental state that prevents the patient understand the nature, extent and consequences of the study, except for hepatic encephalopathy.

Inclusion and Exclusion Criteria In The INFECIR-2 Study

Inclusion criteria: age ≥18 years; diagnosis of liver cirrhosis established by histology or by the combination of clinical, analytical and ultrasonographic data; diagnosis of urinary infection, pneumonia, spontaneous or secondary bacteremia, skin and soft tissue infection, acute cholangitis or suspected bacterial infection; analytical data of renal and/or liver dysfunction [serum creatinine ≥ 1.2 mg/dl, serum sodium ≤ 130 mEq/l, serum bilirubin ≥4 mg/dl; patients with pneumonia or bacteremia required the presence of 1 or more of these criteria for inclusion, 2 or more were required in the rest]. Additionally, patients with urinary or suspected infections required the presence of at least 1 diagnostic criterion of systemic inflammatory response syndrome (SIRS) and serum CRP levels ≥1 mg/dl to be included.

Exclusion criteria: > 72h after infection diagnosis; acute or sub-acute liver failure; septic shock; endocarditis; fungal infection; severe acute respiratory distress syndrome ($PaO_2/Fi0_2$ ≤100), active or recent variceal bleeding (unless controlled for > 48h); type-1 HRS (IAC criteria); ACLF grade-3 (3 or more organ failures defined according to the Canonic Study criteria); renal replacement therapy; malignancy (except for hepatocellular carcinoma within Milan criteria or non-melanocytic skin cancer); moderate or severe chronic heart failure (NYHA class II, III or IV); severe chronic pulmonary disease (GOLD IV); previous liver transplantation; severe psychiatric disorders that prevent the patient from giving informed consent and from making autonomous decisions; HIV infection (except for patients under antiretroviral therapy with undetectable viral load, CD4 levels > 200/mm^3 and no previous history of opportunistic infections diagnostic of AIDS); contraindications to albumin (allergy, signs of pulmonary edema); albumin administration (≥ 80 g) in the last 2 days; spontaneous bacterial peritonitis co-infection; administration of any investigational drug within 90 days prior to randomization; pre-menopausal women not practicing an acceptable method of birth control; refusal to participate; patients who could not provide prior informed consent and when there was documented evidence that the patient had no legal surrogate decision maker and it appeared unlikely that the patient would regain consciousness or sufficient ability to provide delayed informed consent; physician and team not committed to intensive care if needed.

Laboratory methods

PRA was measured using radioimmunoassay (GammaCoat Plasma Renin Activity, DiaSorin, Saluggia, Italy, normal values: 0.3-2.5 ng/mL.h); PRC was measured using a chemiluminescent immunoassay Liaison Direct Renin on the LIAISON Analyzer (DiaSorin) (normal values 2.8-39.9 microUI/ml); ANP was measured by radioimmunoassay (Euro-Diagnostica, Arnhem, The Netherlands); BNP was determined by chemiluminiscence immunoassay run in an Advia Centaur XP (Siemens Health Care, Tarrytown, NY) (normal values: 9-24 f mol/l and 4-37 pmol/ml). The plasma concentration of IL-6 was measured by ELISA (Diasource, Louvain-la-Neuve, Belgium)

(normal values <5 pg/mL). Serum albumin measurement was performed using standard routine methods. Plasma levels of a panel of 24 inflammatory cytokines, 10 chemokines, 4 growth factors, 2 markers of endothelial dysfunction and 2 markers of coagulation/platelet function were performed using two multiplex immunoassays based on Luminex multi-analyte profiling technology (Human Cytokine/Chemokine Magnetic bead panel Premixed 38 Plex Kit and Human Sepsis Magnetic bead panel 1, Merk Millipore, Billerica, MA). Among the 24 cytokines measured, 11 were not detectable in any patient/healthy subject and were not included in this analysis. Signals were read in a Luminex 100 Bioanalyzer (Luminex Corp., Austin, TX). A five-parameter logistic regression model was used to create standard curves and to calculate the concentration of each sample with the standard version of the Milliplex Analyst software (Merck Millipore). The lower limit of detection of each analyte is indicated in supplementary table 2. Normal values given in table 2 were obtained from 24 healthy volunteers aged 18-65 years. The plasma levels of 2 soluble markers of macrophage activation (sCD163 and sMR/sCD206) were determined by enzyme-linked immunosorbent assays (Antibodies on line, Aachen, Germany).

Supplementary Table 1. Baseline Characteristics And Outcomes Of Patients Included In The Pilot PRECIOSA Study.*

	Patients (N=18)
Baseline data	
Age (years)	56 (50-64)
Male sex, n (%)	13 (72)
Alcoholic cirrhosis, n (%)	12 (67)
Previous variceal bleeding, n (%)	5 (28)
ß-blockers, n (%)	7 (39)
Previous SBP, n (%)	5 (28)
Long-term norfloxacin prophylaxis, n (%)	8 (44)
Previous hepatic encephalopathy, n (%)	7 (39)
Diabetes mellitus, n (%)	3 (17)
HCC, n (%)	1 (6)
Mild hepatic encephalopathy, n (%)	2 (11)
Ascites, n (%)	18 (100)
Blood leukocyte count, $x10^9$/L	4.2 (3.3-7.3)
Hematocrit, %	30 (27-34)
Platelet count, $x10^9$/L	88 (60-169)
Serum bilirubin, mg/dL	2.0 (1.4-4.0)
Serum albumin, g/L	27 (25-36)
INR	1.4 (1.2-1.8)
Serum creatinine, mg/dL	1.2 (0.8-1.4)
BUN, mg/dL	23 (15-35)
Serum sodium, mEq/L	132 (128-135)
Serum C-reactive protein, mg/dL	0.7 (0.3-1.1)
Child-Pugh score, points	9 (7-10)
MELD score, points	16 (13-17)
Main complications during 3-month follow-up	
Variceal bleeding, n (%)	2 (11)
Other gastrointestinal bleeding, n (%)	2 (11)
Non-SBP infections, n (%)	4 (22)

*The pilot PRECIOSA study was an open-label, multicenter, nonrandomized (single-group), prospective, phase 4 investigation of albumin treatment in patients with decompensated cirrhosis without bacterial infection. Continuous variables are expressed as median (interquartile range). SBP denotes spontaneous bacterial peritonitis, HCC hepatocellular carcinoma, INR international normalized ratio, BUN blood urea nitrogen, and MELD Model for End-Stage Liver Disease.

Supplementary Table 2. Lower Limit Of Detection, Expressed As The Minimun Detectable Concentration, For Each Analyte Measured In The Study*

Analyte	Minimum Detectable Concentration
EGF (pg/ml)	2.8
FGF-2 (pg/ml)	7.6
Eotaxin (pg/ml)	4.0
TGF-α (pg/ml)	0.8
G-CSF (pg/ml)	1.8
Flt-3L (pg/ml)	5.4
GM-CSF (pg/ml)	7.5
Fractalkine (pg/ml)	22.7
IFNα2 (pg/ml)	2.9
IFNγ (pg/ml)	0.8
GROα (pg/ml)	9.9
IL-10 (pg/ml)	1.1
MCP-3 (pg/ml)	3.8
IL-12P40 (pg/ml)	7.4
MDC (pg/ml)	3.6
IL-12P70 (pg/ml)	0.6
IL-13 (pg/ml)	1.3
IL-15 (pg/ml)	1.2
sCD40L (pg/ml)	5.1
IL-17 (pg/ml)	0.7
IL-1RA (pg/ml)	8.3
IL-1α (pg/ml)	9.4
IL-9 (pg/ml)	1.2
IL-1β (pg/ml)	0.8
IL-2 (pg/ml)	1.0
IL-3 (pg/ml)	0.7
IL-4 (pg/ml)	4.5
IL-5 (pg/ml)	0.5
IL-6 (pg/ml)	0.9
IL-7 (pg/ml)	1.4
IL-8 (pg/ml)	0.4
IP-10 (pg/ml)	8.6
MCP-1 (pg/ml)	1.9
MIP-1α (pg/ml)	2.9
MIP-1β (pg/ml)	3.0
TNFα (pg/ml)	0.7
TNFβ (pg/ml)	1.5
VEGF (pg/ml)	26.3
sICAM-1 (pg/ml)	17.7
sVCAM-1 (pg/ml)	10.7
tPAI-1 (pg/ml)	3.2
MIF (pg/ml)	2.7

*Minimum Detectable Concentration was calculated using MILLIPLEX Analyst 5.1. It measures the true limits of detection for an assay by mathematically determining what the empirical Minimum Detectable Concentration would be if an infinite number of standard concentrations were run for the assay under the same conditions.

Supplementary Table 3. Plasma Levels of Chemokines, Growth Factors, Markers Of Macrophage Activation, And Markers of Endothelial Dysfunction And Of Coagulation/Platelet Function, At Baseline And The 6th Week Of Treatment in Patients Receiving Either Low Albumin Dosage (LAlbD) or High Albumin Dosage (HAlbD) in the Pilot PRECIOSA Study.*

Variable	LAlbD group (N=10)				HAlbD Group (N=5)				P Value for Between-Group Comparison	
	Undetectable Levels	Baseline Cytokine Level	Absolute Change From Baseline	Percentage Change from Baseline	Undetectable Levels	Baseline Cytokine Level	Absolute Change from Baseline	Percentage Change From Baseline	Absolute Change from Baseline	Percentage Change from Baseline
	no. (%)	*Median (IQR) — pg/mL*	*Median (IQR) — pg/mL*	*Median (IQR) — %*	*no. (%)*	*Median (IQR) — pg/mL*	*Median (IQR) — pg/mL*	*Median (IQR) — %*		
Chemokines										
GROα/CXCL1	0 (0)	675.0 (256.0 to 916.2)	76.2 (-100.0 to 120.5)	19.1 (-19.0 to 58.4)	1 (20)	509.9 (287.9 to 1,717.1)	-157.6 (-1,230.1 to -65.5)	-31.3 (-57.1 to -10.5)	0.10	0.08
MCP-1/CCL2	0 (0)	307.1 (244.1 to 375.0)	-2.9 (-33.4 to 141.3)	-1.6 (-9.0 to 57.6)	0 (0)	292.8 (263.1 to 341.6)	107.4 (53.0 to 107.9)	20.8 (18.1 to 31.4)	0.66	0.76
MIP-1α/CCL3	4 (40)	7.0 (6.8 to 10.8)	-4.3 (-7.0 to 3.6)	-57.4 (-100.0 to 12.7)	1 (20)	6.6 (4.0 to 23.5)	-2.1 (-7.4 to -1.1)	-29.4 (-37.7 to -13.1)	0.75	0.59
MIP-1β/CCL4	0 (0)	41.7 (15.2 to 65.2)	2.8 (-9.8 to 5.4)	13.6 (-9.7 to 34.6)	0 (0)	35.3 (30.5 to 41.4)	-11.8 (-20.4 to -9.6)	-45.6 (-48.8 to -33.3)	0.07	0.02
MCP-3/CCL7	2 (20)	7.2 (3.8 to 14.7)	0.6 (-1.9 to 4.2)	15.7 (-17.7 to 42.3)	1 (20)	14.1 (4.5 to 23.3)	-3.7 (-12.9 to 0.0)	-26.2 (-55.2 to 5.1)	0.13	0.23
IL-8/CXCL8	0 (0)	48.8 (34.0 to 78.6)	-7.4 (-11.1 to 4.3)	-15.0 (-26.1 to 15.9)	0 (0)	75.1 (50.8 to 106.2)	-32.1 (-47.9 to -13.8)	-37.3 (-42.8 to -27.2)	0.08	0.10
IP-10/CXCL10	0 (0)	352.1 (187.2 to 509.9)	29.3 (-24.9 to 283.6)	15.6 (-9.7 to 85.9)	0 (0)	623.9 (187.8 to 830.5)	52.7 (-218.2 to 66.8)	35.6 (-35.0 to 40.3)	0.76	0.43
Eotaxin/CXCL11	0 (0)	126.8 (75.3 to 151.6)	11.1 (-12.4 to 31.4)	7.4 (-9.2 to 24.9)	0 (0)	128.7 (111.5 to 134.0)	-5.9 (-29.4 to -0.7)	-2.9 (-26.3 to -0.5)	0.29	0.36
Fractalkine/CX3CL1	2 (20)	45.3 (21.1 to 430.2)	-11.7 (-228.1 to 1.7)	-27.0 (-64.0 to 5.8)	1 (20)	22.7 (14.4 to 31.5)	-6.1 (-6.8 to -2.8)	-23.3 (-59.3 to -8.6)	0.49	0.73
MDC/CCL22	0 (0)	733.9 (547.0 to 1,289.0)	72.8 (-119.7 to 105.9)	6.6 (-26.2 to 17.8)	0 (0)	573.2 (529.9 to 809.5)	164.8 (36.9 to 193.8)	22.5 (8.4 to 28.8)	0.24	0.16
Growth Factors										
EGF	2 (20)	37.3 (12.9 to 63.3)	-3.7 (-15.3 to 11.0)	-10.9 (-38.4 to 134.1)	2 (40)	5.2 (5.2 to 45.4)	4.8 (-2.4 to 6.9)	15.3 (-46.4 to 92.5)	0.76	1.00
MIF	1 (10)	59.8 (53.9 to 63.7)	-1.0 (-48.7 to 14.3)	-3.2 (-32.8 to 31.2)	1 (20)	91.2 (55.8 to 207.2)	74.4 (25.1 to 5,394.3)	103.21 (5.51 to 8,366.60)	0.11	0.19
FGF2	1 (10)	58.9 (36.5 to 85.9)	3.5 (-3.2 to 14.8)	2.8 (-5.5 to 33.5)	0 (0)	70.7 (22.0 to 82.4)	-31.8 (-44.4 to 0.0)	-27.57 (-62.86 to 0.00)	0.27	0.20
Markers of coagulation/platelet function										
PAI-1	1 (10)	18862 (16401 to 19473)	513.0 (-2,709.0 to 2,706.0)	2.72 (-10.7 to 16.5)	1 (20)	9357 (8422.5 to 12299.5)	-2887.0 (-3,247.0 to 210.5)	-25.18 (-32.65 to 6.10)	0.39	0.14
sCD40L	0 (0)	612.8 (285.3 to 1497.0)	-184.8 (-471.7 to 0.0)	-18.15 (-52.8 to 0.0)	0 (0)	211.69 (43.8 to 635.2)	64.2 (-477.7 to 196.9)	146.58 (-34.18 to 447.03)	0.67	0.24
Supplementary Table 3. (Continued.)										

Markers of macrophage and lymphocyte activation		*ng/mL*				*ng/mL*				
sCD163 (ng/ml) (9/4)	1 (10)	464.7 (86.1 to 631.0)	13.1 (-26.6 to 49.1)	6.8 (-46.6 to 24.6)	1 (20)	616.9 (488.0 to 692.6)	-31.0 (-131.6 to 156.6)	-5.25 (-20.98 to 24.00)	0.70	0.94
sMR/sCD206 (ng/ml) (9/4)	1 (10)	152.5 (120.7 to 287.5)	19.0 (-7.8 to 57.9)	9.7 (-2.7 to 48.0)	1 (20)	237.4 (183.9 to 309.6)	37.2 (-33.3 to 69.4)	14.04 (-8.63 to 31.72)	0.94	0.82
Markers of endothelial dysfunction		*µg/mL*				*µg/mL*				
sICAM-1	1 (10)	0.06 (0.04 to 0.08)	-0.0003 (-0.005 to 0.01)	-0.48 (-9.12 to 4.72)	1 (20)	0.13 (0.09 to 0.25)	-0.004 (-0.06 to -0.0003)	-3.29 (-17.44 to 0.14)	0.39	0.32
sVCAM-1	1 (10)	0.28 (0.20 to 0.31)	-0.005 (-0.06 to 0.05)	-1.54 (-14.01 to 27.08)	1 (20)	0.36 (0.25 to 0.43)	-0.05 (-0.11 to -0.03)	-20.66 (-30.10 to -12.44)	0.14	0.19

*The pilot PRECIOSA study enrolled patients with decompensated cirrhosis unrelated to bacterial infection. The LAlbD group received 1 g/kg b.w. of albumin every two weeks and the HAlbD group received 1.5 g/kg b.w. of albumin every week. Levels of molecules were measured using Luminex and enzyme immunoassays. The usual symbol of molecules was given together with its alias for most of them (usual symbol/alias). P values were calculated with the use of nonparametric tests. The cell colored in green shows P values of less than 0.05. IQR denotes interquartile range, GROα growth-regulated alpha protein, CXCL C-X-C motif chemokine ligand, MCP monocyte chemotactic protein, CCL C-C motif chemokine ligand, MIP macrophage inflammatory protein, IL interleukin, IP-10 10 kDa interferon gamma-induced protein, MDC macrophage-derived chemokine, EGF epidermal growth factor, MIF macrophage migration inhibitory factor, FGF fibroblast growth factor, sCD163 soluble CD163, sMR, soluble mannose receptor, sICAM-1 soluble intercellular adhesion molecule, sVCAM-1 soluble vascular cell adhesion molecule, PAI-1 plasminogen activator inhibitor 1, and sCD40L soluble CD40 ligand.

Supplementary Table 4. Baseline Plasma Levels of Chemokines, Growth Factors, Markers Of Macrophage Activation, And Markers of Endothelial Dysfunction And Of Coagulation/Platelet Function, and Their Changes During the First Week of Treatment, In Patients From The INFECIR-2 Study Who Were Randomized To Receive Either Antibiotics Alone Or Albumin-Plus-Antibiotics.*

Variable	Antibiotics Alone (N=40)						Albumin-Plus-Antibiotics (N=38)					
	Undetectable Levels	Baseline Level	Absolute Change From Baseline	Percentage Change from Baseline	P Value For Change From Baseline		Undetectable Levels	Baseline Level	Absolute change From Baseline	Percentage Change From Baseline	P Value For Change From Baseline	
	no. (%)	*Median (IQR) — pg/mL*	*Median (IQR) — pg/mL*	*Median (IQR) — %*	*Absolute change*	*% change*	*no. (%)*	*Median (IQR) — pg/mL*	*Median (IQR) — pg/mL*	*Median (IQR) — %*	*Absolute change*	*% change*
Chemokines												
GROα/CXCL1	0 (0)	241 (107 to 519)	21 (-47 to 354)	6.5 (-20.7 to 95.6)	0.13	0.75	0 (0)	247 (123 to 520)	-1.7 (-145 to 93)	-1.3 (-61.0 to 61.4)	0.60	0.87
MCP-1/CCL2	0 (0)	268 (175 to 504)	4.7 (-62.9 to 72.2)	1.0 (-27.1 to 35.9)	0.80	1.00	0 (0)	318 (208 to 549)	-35 (-96 to 33)	-9.8 (-23.2 to 22.7)	0.11	0.14
MIP-1α/CCL3	13 (33)	10.3 (2.7 to 21.7)	-0.5 (-2.2 to 0.9)	-4.4 (-15.4 ; 8.2)	0.32	0.33	10 (26)	9.4 (5.3 to 16.0)	-2.4 (-9.5 to 1.1)	-29.6 (-61.4 to 22.6)	0.01	0.09
MIP-1β/CCL4	3 (8)	24.2 (16.6 to 39.5)	-6.5 (-14.0 to 0.4)	-23.8 (-43.4 to 6.9)	0.01	0.03	6 (16)	30.4 (11.6 to 52.6)	-0.4 (-15.2 to 4.2)	-3.0 (-41.7 to 27.8)	0.24	0.58
IL-8/CXCL8	0 (0)	51.3 (20.6 to 96.2)	0.2 (-14.2 to 18.9)	1.0 (-36.3 to 47.8)	0.44	0.63	0 (0)	62.7 (27.3 to 100)	-6.6 (-28.1 to 5.2)	-13.5 (-35.1 to 25.2)	0.54	0.14
IP-10/CXCL10	0 (0)	1175 (870 to 2468)	-183 (-472 to 32)	-9.8 (-40.3 to 3.0)	0.004	0.04	0 (0)	1313 (803 to 2136)	-161 (-558 to 233)	-1.7 (-41.7 to 17.4)	0.30	1.00
Eotaxin/CXCL11	0 (0)	76.3 (56.5 to 141.4)	-0.6 (-26.3 to 9.5)	0.1 (-28.0 to 17.9)	0.52	1.00	0 (0)	101.7 (51.4 to 149.4)	16 (-16.9 to 21.2)	0.8 (-14.7 to 33.4)	0.66	1.00
Fractalkine/CX3CL1	13 (33)	89.9 (25.4 to 363.1)	-5.9 (-47.1 to 22.0)	-15.1 (-29.4 to 65.8)	0.25	0.70	0 (0)	67.2 (19.2 to 128.3)	0.0 (-18.7 to 36.6)	0.0 (-43.8 to 153.2)	0.56	1.00
MDC/CCL22	0 (0)	348 (257 to 523)	2.7 (-75.4 to 79.1)	0.8 (-19.4 to 27.8)	0.97	0.87	0 (0)	371 (246 to 573)	21.1 (-79 to 112)	5.7 (-27.7 to 28.5)	0.43	0.42
Growth factors												
EGF	0 (0)	6.3 (2.8 to 37.5)	0 (-7.0 to 11.2)	0.0 (-7.1 to 17.6)	0.73	0.85	0 (0)	2.8 (2.8 to 37.9)	0 (-2.0 to 0)	0.0 (-16.7 to 0.0)	0.60	0.81
MIF	0 (0)	198 (121 to 391)	4 (-182 to 108)	3.9 (-40.1 to 90.5)	0.91	0.87	2 (5)	226 (118 to 708)	8 (-198 to 168)	5.1 (-32.2 to 81.5)	0.90	0.62
FGF2	0 (0)	45.0 (13.3 to 79.0)	-2.0 (-21.8 to 5.7)	-9.6 (-34.6 to 17.9)	0.29	0.19	0 (0)	30.1 (15.1 to 74.6)	0 (-7.2 to 12.6)	0.0 (-19.4 to 53.1)	0.49	0.86
Markers of coagulation/platelet function												
PAI-1	0 (0)	49535 (35875 to 84200)	-67 (-15592 to 15755)	0.1 (-21.0 to 34.2)	0.87	1.00	2 (5)	62250 (36870 to 79825)	-610 (-28682 to 24980)	-2.7 (-33.4 to 64.0)	0.92	1.00
sCD40L	0 (0)	150.9 (72.3 to 386)	-4.8 (-58.9 to 110.1)	-3.9 (-38.6 to 103.1)	0.72	0.87	0 (0)	115.9 (46.7 to 412.5)	4.4 (-84.5 to 43.9)	5.4 (-34.7 to 107.8)	0.73	0.87

Supplementary Table 4. (Continued.)												
Markers of macrophage and lymphocyte activation		*ng/mL*						*ng/mL*				
sCD163	1 (3)	760 (539 to 1208)	84 (-342 to 578)	13.2 (-34.9 to 75.0)	0.45	0.52	1 (3)	704 (514 to 1152)	250 (-266 to 733)	27.2 (-28.1 to 137.3)	0.20	0.62
sMR/sCD206	1 (3)	190 (135 to 302)	21 (-85 to 144)	13.2 (-34.9 to 75.0)	0.52	0.52	2 (5)	181 (131 to 289)	34 (-77 to 175)	27.2 (-28.1 to 137.3)	0.27	0.62
		µg/mL						*µg/mL*				
Markers of endothelial dysfunction												
sICAM-1	0 (0)	0.5 (0.3 to 0.8)	-0.05 (-0.2 to 0.1)	-12.9 (-36.5 to 25.8)	0.22	0.27	1 (3)	0.5 (0.3 to 0.9)	-0.04 (-0.19 to 0.09)	-6.3 (-27.6 to 40.6)	0.34	0.32
sVCAM-1	0 (0)	1.9 (1.2 to 2.5)	-0.08 (-0.6 to 0.3)	-5.7 (-30.3 to 13.9)	0.14	0.27	3 (8)	1.8 (1.2 to 2.6)	0.16 (-0.6 to 0,7)	8.9 (-26.4 to 49.4)	0.64	0.74

* The INFECIR-2 study enrolled patients with decompensated cirrhosis and infection unrelated to spontaneous bacterial peritonitis. Levels of molecules were measured using Luminex and enzyme immunoassays. The usual symbol of molecules was given together with its alias for most of them (usual symbol/alias). Changes during the first week of albumin treatment were assessed between day 3 and day 7 after inclusion. There were no significant between‐group differences in cytokine levels at baseline. P values for within-group comparisons were calculated with the use of nonparametric tests. Cells colored in green show P values of less than 0.05. IQR denotes interquartile range, GRO-α growth-regulated alpha protein, CXCL C-X-C motif chemokine ligand, MCP monocyte chemotactic protein, CCL C-C motif chemokine ligand, MIP macrophage inflammatory protein, IL interleukin, IP-10 10 kDa interferon gamma-induced protein, MDC macrophage-derived chemokine, EGF epidermal growth factor, MIF macrophage migration inhibitory factor, FGF fibroblast growth factor, sCD163 soluble CD163, sMR, soluble mannose receptor, sICAM-1 soluble intercellular adhesion molecule, sVCAM-1 soluble vascular cell adhesion molecule, PAI-1 plasminogen activator inhibitor 1**,** and sCD40L soluble CD40 ligand.