Title: Albumin infusion improves renal blood flow autoregulation in patients with acute decompensation of cirrhosis and acute kidney injury

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Conflict of interest:

Professor Rajiv Jalan has on-going research collaboration with Grifols, the manufacturers of albumin but this study was conducted at a time when no such collaboration existed.

Author contributions:

R.G.M. executed data analysis and interpretation and wrote the manuscript. L.N. performed statistical analysis and revised the manuscript. S.S. contributed to the acquisition of data and performed the laboratory determinations. R. M. contributed to the acquisition and interpretation of data, gave intellectual input and edited the manuscript. R. J. conceived and designed the study, contributed to the acquisition and interpretation of data, supervised the overall project, and edited the manuscript.

List of abbreviations:

AD	Acute decompensation of cirrhosis
AKI	Acute Kidney Injury
EA	Endothelial activation
ED	Endothelial dysfunction
ELISA	Enzyme linked immune-sorbent assay
HR	Heart rate
HVPG	Hepatic vein portal gradient
MAP	Mean arterial pressure
MDA	Malondialdehyde
IMA	Ischemia-modified albumin
RPF	Renal Plasma Flow
RBF	Renal Blood Flow
RPP	Renal Perfusion Pressure
SVR	Systemic Vascular Resistance
vWF	von Willebrand Factor

Abstract

Background and aims: In cirrhotic patients with renal failure, renal blood flow autoregulation curve is shifted to the right, which is consequent upon sympathetic nervous system activation and endothelial dysfunction. Albumin infusion improves renal function in cirrhosis by mechanisms that are incompletely understood. We aimed to determine the effect of albumin infusion on systemic hemodynamics, renal blood flow, renal function and endothelial function in patients with acute decompensation of cirrhosis and acute kidney injury.

Methods: Twelve patients with refractory ascites and 10 patients with acute decompensation of cirrhosis and acute kidney injury were studied. Both groups were treated with intravenous albumin infusion, 40-60 g/d over 3-4 days. Cardiac and renal hemodynamics were measured. Endothelial activation/dysfunction was assessed using von Willebrand factor and serum nitrite levels. F2α Isoprostanes, resting neutrophil burst and noradrenaline levels were quantified as markers of oxidative stress, endotoxemia and sympathetic activation respectively.

Results: Albumin infusion lead to a shift in the renal blood flow autoregulation curve towards normalization, which resulted in a significant increase in renal blood flow. Accordingly, improvement of renal function was observed. In parallel, a significant decrease in sympathetic activation, inflammation/oxidative stress and endothelial activation/dysfunction was documented. Improvement of renal blood flow correlated with improvement in endothelial activation ($r=0.741$, $p<0.001$).

Conclusions: The data suggest that albumin infusion improves renal function in acutely decompensated cirrhotic patients with acute kidney injury by impacting on renal blood flow autoregulation. This is possibly achieved through endothelial

stabilisation, and a reduction in the sympathetic tone, endotoxemia and oxidative stress.

Key words: liver cirrhosis, albumin, endothelial dysfunction, renal blood flow, acute kidney injury, refractory ascites, human

Introduction

Advanced liver failure is characterized by hemodynamic alterations initiated by portal hypertension and splanchnic vasodilation. In advanced cirrhosis, systemic vascular resistance (SVR) is markedly reduced and mean arterial pressure (MAP) is maintained through the activation of compensatory mechanisms (renin-angiotensin system, sympathetic nervous system and arginine-vasopressin hormone) [(1)]. Bacterial translocation [(2)] and bacterial infections [(3)] are factors that may further deteriorate this impaired circulatory state.

Endothelial cells regulate vascular tone and modulate blood flow towards different organs by synthesizing agonist and antagonist in response to different stimuli. Bacterial products [(4)], inflammatory mediators [(5)] and reactive oxygen species [(6)] may activate endothelial cells and induce endothelial activation (EA) and dysfunction (ED). These factors are frequently present in cirrhosis, and correlate with progression of the disease [(7)]. Renal blood flow (RBF) is tightly regulated to ensure a relatively stable renal perfusion regardless of daily fluctuations in MAP. Sympathetic activation is involved in this regulation [(8)]. Other factors such as renin-angiotensin system [(9)], inflammatory mediators and other as yet unidentified factors have been implicated in this RBF/RPP (renal perfusion pressure) relationship [(10)]. It has been shown that the RBF autoregulation curve (curve linking between RPP and RBF) shifts progressively to the right in cirrhosis, according to the severity of the liver failure and the degree of sympathetic activation [(11)]. Thus, RBF becomes progressively dependent on RPP.

Progressive hypoalbuminaemia is a common feature of cirrhosis. Recent studies have suggested that albumin is also functionally impaired in cirrhotic patients and associated with the higher risk mortality [(12)]. Infusion of human serum albumin

prevents and improves renal dysfunction in cirrhotic patients [13-17]. The exact mechanism through which these effects are seen is unclear. Understanding those mechanisms may help identify possible new pharmacological approaches in patients with renal failure and those at risk. Most studies suggest that this is consequent upon amelioration in the activated renin-angiotensin system. In patients with spontaneous bacterial peritonitis, albumin infusion resulted in a reduction in von Willebrand factor (vWF) and nitric oxide levels suggesting that it may improve ED [18].

Renal failure in cirrhosis, particularly hepatorenal syndrome is associated with extremely poor prognosis[19]. A significant number of patients with acute decompensation of cirrhosis (AD) does not fulfill criteria for the diagnosis of hepatorenal syndrome [20] and they are classified as having acute kidney injury (AKI) [21]. Although a lot is known about the pathophysiologic basis of effect of albumin infusion in patients with hepatorenal syndrome, its effects in patients with AD and associated AKI are not clear.

The aim of this study was to further understand the pathophysiological and clinical effects of albumin infusion in AD patients with AKI. Specifically, we sought to evaluate the effect of albumin infusion on systemic and renal hemodynamics, RBF autoregulation and endothelial function and, further investigate related mechanisms that might be implicated in the regulation of RBF using a group of patients with refractory ascites (RA) as controls.

Patients and Methods

Ethical considerations

The local ethics committee approved the study and the patients who took part gave their informed consent. The present study is a part of studies in patients with acute decompensation of cirrhosis recruited into observational and interventional studies following a prospective assessment [22-24]. A retrospective analysis of this data was performed in this study.

Patients

Twenty-two patients with clinical, biochemical, radiological and/or histological evidence of liver cirrhosis and controls in different studies over 5 years (2001-2006) were grouped into 2 different categories. The first group included patients (n=12) with chronic decompensation of cirrhosis due to refractory ascites (RA group). The second group of patients (n=10) recruited subjects with AKI in the setting of an acute decompensation of previously stable liver disease due to a precipitating event (AKI group). AD for this study was defined as a worsening in liver function over a period of 2 to 4 weeks manifested by increasing jaundice (> 100 µmol/L) and AKI. AKI was defined as a rise in serum creatinine of ≥50% from baseline or a rise of serum creatinine by ≥26.4 mmol/l in <48 h [21]. The precipitating factor associated to the AD and AKI was acute alcoholic hepatitis (n=7) or active infection (n=3). These patients received supportive therapy including treatment for the developed complications and organ failure according to the local protocols (corticosteroids if they had severe alcoholic hepatitis defined by Maddrey index above 32 and antibiotics if they had sepsis). None of these patients with AKI fulfilled the specific criteria for type I hepatorenal syndrome, therefore were not treated with vasoconstrictors. Patients were excluded if they were under

18 or over 80 years of age, in case of pregnancy, had hepatic/extrahepatic malignancy, active gastrointestinal bleeding, if they received vasoactive treatment (β-blockers, renin-angiotensin-aldosterone system inhibitors), immunomodulatory therapy or albumin infusion prior to entry in the study. Basal characteristics of the patients are summarized in table 1.

Design

Patients with RA who agreed to take part in the study were admitted to the hospital and a radial artery and venous catheter were placed. A baseline blood sample was collected after 30 minutes of lying supine for further determinations. A hemodynamic study was performed at baseline, which included cardiac hemodynamics, hepatic venous pressure gradient (HVPG) measurement and RBF. All patients received 40-60 grams/day of albumin during 3-4 days and were again tested for hemodynamics and blood determination after the treatment (1 day after finalizing albumin therapy).

Hormonal Assays

Norepinephrine was measured using high-performance liquid chromatography and electrochemical detection (normal plasma concentration: 0-5 nmol/L).

Cytokines

IL-6 determination was performed using commercially available set (BioSource International, Belgium).

Respiratory Burst

The neutrophil functional study (an index of endotoxemia) was performed as previously described [25]. Whole blood from healthy volunteers was layered over 5 mL of Polymorphprep (Axis-Shield, Norway) and spun (30 min, 400g, room temperature). Neutrophils were harvested from the second interface and washed with phosphate buffered saline. Neutrophils were counted and re-suspended in

PBS at a density of $5x10^5$ in 50 µL; 50 µL of cell suspension and 50µL of plasma were used per assay. Viability (Trypan blue) was over 98%. The Phagoburst kit (Orpegen Pharma, Germany) was used to determine the percentage of neutrophils that produce reactive oxidants. Briefly, 100 µl of heparinized whole blood or 50µL of isolated neutrophils and 50 µL of plasma were incubated for 20 min with either 20 µl of opsonized Escherichia coli (2×10^7 bacteria) or without stimulus at 37 °C. The formation of reactive oxidants was monitored by the oxidation of dihydrorhod- amine 123 to rhodamine. To identify neutrophils, cells were stained with anti–CD16-PE antibody (Immuntools, Germany) and analyzed by fluorescence-activated cell sorting (Becton Dickinson FACS Canto II, FACS Diva 6.0 software, CA). Neutrophils were gated and the percentage of CD16-positive cells producing reactive oxygen metabolites calculated.

F2 Isoprostanes

F2 isoprostanes (free 8-isoprostane F2α) were assayed with an EIA kit (Cayman Chemical, Ann Arbor, MI) according to the manufacturer's instructions [(26)]. Briefly, 200 µl plasma was deproteinized with ethanol containing ^{3}H -prostaglandin E2 as an internal standard to account for losses. After centrifugation, the supernatant was dried, and 2 mL acetic acid was added and applied to a preconditioned C18 SPE cartridge (Waters, Milford, MA). The column was washed with water, dried, and eluted with high-performance liquid chromatography–grade hexane. The prostanoid fraction was eluted with 5 ml ethylacetate containing 1% methanol, eluant reduced to dryness and reconstituted in 450 µl of EIA buffer, 100 µl being used to determine recovery of ^{3}H-PGE2 and 50 µl added to the EIA plate with isoprostane tracer and antibody. Isoprostane levels were determined by reference to authentic standards and corrected for losses.

Malondialdehyde

Malondialdehyde (MDA) was measured using a modified thiobarbituric acid reactive substance assay described by Lapenna [12] wherein the major interfering component in the plasma is inhibited by addition of sodium sulphate.

Von Willebrand Factor assay

Plasma levels of vWF were assessed by in-house Enzime-Linked Immunoassay.

Nitrite-nitrate levels

Nitrite and nitrate were determined by a modified Greiss test and as previously described [27].

Ischemia-modified albumin

Ischemia-modified albumin (IMA) was determined according to the cobalt-binding assay method[28]. Briefly, plasma was incubated with a cobalt chloride (1 g/L, 10 minutes) and dithiothreitol (1.5 g/L, 2 minutes) before dilution in saline prior to measurement at 470 nm (Agilent 8453 Diode Array, Agilent, UK). IMA was calculated from the difference between samples measured with and without dithiothreitol. Plasma albumin concentration was determined (COBAS Integra, Roche, UK) and IMA/albumin ratio was calculated (IMAR).

Haemodynamic assessment

Cardiac hemodynamics: Heart rate (HR), oxygen saturation, and electrocardiographic activity were recorded continuously. MAP was measured before and every 5 minutes after catheterization (model 86S; Hewlett Packard, CA). The pulmonary artery was catheterized using a Swan-Ganz catheter (Edward Life Sciences, CA) and cardiac output calculated using thermodilution method (Edwards Life Sciences). Each measurement was performed in triplicate, and the mean was calculated.

HVPG: the right hepatic vein was cannulated using a balloon-tipped catheter (Cordis, The Netherlands) under fluoroscopic guidance (SA-900U; Toshiba, Japan), and wedged measurements were made in triplicate (Omnipaque; Amersham Health, England). Careful attention ensured a wedged position. HVPG was calculated as the difference between the wedged and free hepatic venous pressures.

Measurement of RBF: Primed continuous infusion of *para*-aminohippuric acid (Sigma, England) was used to measure renal plasma flow using the Fick principle with simultaneous sampling of an artery and the renal vein 1 hour after starting the infusion. For *para*-aminohippuric acid determinations, 500 µl plasma was added to 50 µl trichloroacetic acid solution (50% wt/vol). Plasma *para*-aminohippuric acid concentration was determined spectrophotometrically. RBF was calculated as follow [(11)]:

Calculations:

1.Renal plasma flow(mL/min) was calculated as:

$$RPF = \frac{\text{PAH infusion rate x [PAH] infused}}{\text{[PAH] arterial - [PAH] renal vein}}$$

2.RBF (mL/min) was calculated as:

$$RBF = \frac{RPF}{\text{1-hematocrit}}$$

3.RPP (mmHg) was calculated as:

RPP = MAP – inferior vena cava pressure

4. Systemic vascular resistance (dynes/sec X cm^{-5}) was calculated as:

$$SVR = \frac{79.9 \text{ X (MAP-central venous pressure)}}{\text{cardiac output}}$$

Mathematical model of RBF autoregulation

A mathematical model was used to extrapolate the shape of the autoregulation curves from clinical measurements in each patient group (full description of the model is published[11]). Briefly, the model made simple physiological assumptions to link renal blood flow with renal perfusion pressure and a parameter *α* indicating the severity of autoregulation failure. The full model equation is:

$$Renal\ Blood\ Flow = k\ RPP^5\left(1 - \frac{a(RPP+a)^2}{b^2+(RPP+a)^2}\right)^4$$

where a = 0.95, b = 40.9 and k = 8.2×10^{-5}. The parameter *α* was estimated by employing a best fit approach which used measurements of RBF and RPPs. A value of *α*=0 represented a healthy state, whereas an increase in α indicated a worsening of the state.

Statistical analysis

Continuous variables are expressed as mean±SD or median (interquartile range). Normality of continuous variables was explored using the Shapiro-Wilk Test. Depending on the distribution of the variables, parametric or nonparametric tests were applied to study the within-subject (t test for paired samples or Wilcoxon test) or between-group (Student t test or Mann-Whitney rank-sum test) differences. The Pearson or Spearman correlation coefficient tests were used to assess correlations between variables. P value < 0.05 was considered statistically significant. Calculations were performed with Graphpad Prism 5.

Results

The patients included in this study received the dose of albumin in accordance with the study protocol and no significant major adverse effects were observed.

Renal function and biochemical changes

In this study we assessed renal function using different parameters (table 2). Patients with AKI had a higher plasma creatinine concentration (213±60.3 vs 94.8±13.3 µmol/L, $p<0.001$) and lower creatinine clearance (13±4.2 vs 29.3±7.1 mL/min, $p<0.001$) at baseline compared to patients with RA. Both groups showed a significant improvement of renal function following albumin treatment. Similarly, plasma sodium concentration and urinary sodium excretion increased in both groups. Patients with AKI had a marked sympathetic activation, which was significantly greater than in patients with RA (norepinephrine 8.2±2.1 vs 3.6±0.5 nmol/L, $p<0.0001$). A significant amelioration was observed in both groups.

Albumin concentration and function

Regarding to plasma albumin, albumin infusion increased plasma concentration and improve albumin function (IMAR) in both groups of patients (table 2).

Systemic, Cardiac, Renal and Hepatic Hemodynamics

The data on hemodynamics is summarized in table 3. Patients with RA had decreased SVR (normal range 1600-2400 dynes/s/cm^5) with the other parameters of cardiac hemodynamics within the normal values. They also exhibited lower RBF compared to the reference population (1000-1250 mL/min). After albumin infusion there was an increase in central venous pressure, MAP and RPP, together with an improvement in the RBF.

Patients with AKI showed worse hemodynamic state at baseline with lower SVR ($p=0.014$), MAP ($p<0.001$), RPP ($p<0.001$) and higher HR ($p=0.004$) compared to

patients with RA. Albumin infusion was associated with a significant improvement in the hyperdynamic circulation (increase in SVR and MAP and decrease in CO and HR). This was associated with a significant improvement in RBF.

In addition, no worsening in hepatic vein portal pressure gradient was observed following albumin administration. To the contrary, a significant decrease in the HVPG was observed in the subset of patients with AKI.

Impact of albumin infusion on RBF autoregulation

Renal autoregulation curves were estimated for both groups of patients before and after receiving albumin (Fig 1). AKI group exhibited a more impaired renal autoregulation with a more pronounced shift towards the right and a more marked loss in its sigmoid shape compared to patients with RA. In both RA and AKI groups, administration of albumin shifted the autoregulation curves towards the left, which denotes an improvement in the renal blood-flow autoregulation.

Endothelial function

Since endothelial cells modulate blood flow to different organs we conducted an endothelial assessment in these patients before and after albumin infusion. EA was assessed by measuring plasma levels of vWF. Plasma concentration of nitrate and nitrite were indicators of ED. Patients with AKI showed significantly higher plasma levels of vWF (463±73 vs 342±68, p=0.001), nitrate (82±26 vs 42±18, p<0.001) and nitrite (8±2 vs 4±1, p<0.001) before receiving albumin indicating more marked EA/ED (table 2). Both groups of patients exhibited a significant decrease in vWF, nitrate and nitrite following albumin treatment denoting an improvement in endothelial function (Fig 2). Interestingly, we observed that an increase in RBF following albumin infusion closely correlated with a decrease in the vWF a (r=0.741,

p<0.001), indicating that a rise in RBF is concomitant with improvement in endothelial activation (Fig 3).

Inflammation and oxidative stress markers

Since endotoxemia, inflammation and oxidative stress are factors able to induce EA/ED we investigated these conditions. Patients with AKI had higher endotoxemia level (% neutrophil burst 52±20 vs 37±13, p=0.04) compared to patients with RA. Albumin infusion was associated with a significant decrease in the neutrophil burst (ΔRA 12±10%, p<0.01; ΔAKI 17±12%, p<0.01). Accordingly, there was also a decrease in oxidative stress markers in both groups. Patients with RA exhibited a significant reduction in MDA and those with AKI showed a decrease in MDA and F2 isoprostanes levels (Fig 4). There were no significant changes in plasma levels of IL-6.

Discussion

This study shows for the first time that albumin infusion shifts the abnormal RBF autoregulation curve in the patients with RA and in those with AD and AKI to the left allowing greater renal blood flow for a given RPP. This shift was associated with an improvement of renal function. This improvement was demonstrable by an increase in albumin content and function, a reduction in inflammatory markers and oxidative stress, which together contributed to an improvement in endothelial function. We also make the novel observation that a measurable change in albumin function can be achieved with albumin infusion.

RBF autoregulation in cirrhotic patients with refractory ascites and hepatorenal syndrome has been shown to be progressively shifted to the right with advancing liver disease and worsening of renal function [11] and correlates with the severity of sympathetic activation. This study extends these observations to patients with AD and AKI where more severe liver disturbance was observed. This observation has clinical implications since RBF and ultimately renal function become completely dependent on the RPP.

The current understanding of the pathophysiology of renal dysfunction in advanced liver failure is that the relative systemic hypovolemia due to portal hypertension and splanchnic vasodilation activates compensatory mechanisms such as sympathetic nervous system and renin-angiotensin-aldosterone system[1]. Additional factors may deteriorate this hemodynamic scenario such as bacterial traslocation [2], bacterial infections [3] and oxidative stress [29] leading to renal failure and increased mortality[30]. These abnormalities are common and progressive with the severity of the disease [31, 32].

Recent studies indicate that not only albumin concentration but also albumin function is progressively reduced in cirrhosis [12]. Albumin has demonstrated its ability to improve and prevent renal dysfunction in liver failure [13-17]. It has been generally accepted that its benefit relied on its oncotic properties. However, recent advances in the knowledge of albumin structure and function have disclosed non-oncotic potential benefits of albumin[18].

In agreement with these previous studies, we found that albumin infusion improved renal function in patients with refractory ascites and in those with AD and AKI. HRS type-1, a more severe form of renal failure was not investigated in this study since its optimum management requires additional vasoactive treatments. Interestingly, the hemodynamic assessment showed that albumin improves RBF even without significant changes in RPP, and increase SVR. The mathematical model reflects that albumin modifies the autoregulation curve towards normalization, suggesting that for a given RPP there is an improvement in RBF. Among the mechanisms modulating the RBF there are those that impact on renal arteriolar resistance. It is known that sympathetic activation alters renal vascular tone via α1-adrenergic mechanism in experimental models[33]. This is supported by the improvement in renal function following lumbar sympathectomy in cirrhotic patients [34]. Accordingly, increased plasma norepinephrine levels were associated with the shift of the RBF/RPP relationship in cirrhosis [11]. Here we observed that albumin infusion significantly decreased plasma norepinephrine levels, and therefore this could be a potential mechanism underlying the shift of the autoregulation curve. Amelioration in the compensatory mechanisms following albumin infusion reflected by the decrease in the norepinephrine levels could potentially explain the decrease in cardiac output documented in the AKI group.

Another factor contributing to this finding is that the second hemodynamic assessment took place 4-6 days after initiating albumin treatment suggesting that the increase in the CO post-albumin infusion could be maximum within the first hours decreasing thereafter [35, 36].

RBF autoregulation is complex and involves several mechanisms and mediators. Endothelial cells are responsible for regulating blood flow to different organs. They are able to respond to different stimuli by producing agonists and antagonists in order to maintain vascular tone, regulate inflammatory cell adhesion, migration and inflammation [37]. Endothelial cells can be activated by bacterial products (i.e. endotoxin)[4], inflammatory mediators such as tumour necrosis factor [5], reactive oxygen species [6] and activation of the renin-angiotensin aldosterone system [38] leading to ED [37]. It has been previously suggested that albumin might have positive effects on endothelial activation in patients with spontaneous bacterial peritonitis [14]. Importantly in this study we observed that the improvement in RBF closely correlated with amelioration in endothelial activation following albumin infusion. In addition, albumin infusion was associated with a decrease in endotoxemia and oxidative stress markers. In this regard, albumin has been shown to have the ability to bind and neutralize endotoxin in vitro and in vivo [39].

Taken together, the results of this study emphasize that the beneficial effects of albumin in improving renal function in cirrhosis is likely due to pleiotropic effects which considerably exceed its oncotic power. Specifically, this study points out that albumin may have an important role as an endothelial stabilizer in cirrhosis. The well-described anti-thrombotic effects of albumin in the microvasculature could also possibly explain this beneficial effect [40]. Moreover, albumin infusion

seems to be safe, since no significant side effects were observed. One of the potential side effects would be worsening in portal hypertension. In contrast to our expectation, in the AKI group a decrease in the HVPG was documented. The increase in SVR with a subsequent decrease in the splanchnic blood flow could contribute to this observation. Nevertheless, this results support the safety profile of albumin infusion regarding the risk of albumin precipitating an episode of variceal bleeding but this observation need to be confirmed in a larger cohort of patients. Another potential side effect would be clinical events related to overload specially in high-risk population. In our cohort the increased in CVP was not associated to major side effects, suggesting that a well selected population and close monitoring can minimize the risk.

This study has several limitations, which should be considered in interpreting the results of this study. On one hand we lack a control group with other plasma expander and therefore it is difficult to extrapolate these results. However, albumin infusion has been previously evaluated in controlled trials. Our observations here replicate the findings of these studies and explore the mechanisms underlying its clinical benefit. On the other hand, although this study support the role of albumin as an endothelial stabilizer, and suggest that it would be related through its capacity of neutralizing endotoxin and moderating oxidative stress, the specific mechanisms are still not fully clarified. In addition, the small sample size limits the strength of this work, but this is partly counteracted by the replication of results documented in larger cohorts.

In conclusion, this study enhances our understanding of the pathophysiological mechanisms by which albumin infusion improves renal dysfunction in cirrhosis and particularly in patients acutely decompensated who have superimposed

inflammatory insult. Our results point out an important role for albumin as an endothelial stabilizer among other functions resulting in improvement in RBF autoregulation, which contributes to an improvement in renal function justifying a suitable clinical trial.

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Tables

Table 1. Basal clinical and biochemical characteristics of patients

	Refractory Ascites (n=12)	Acute Kidney Injury (n=10)	p
Age (years)	59.8±10.9	56.5±13.9	0.54
Sex			0.65
Male	9 (75%)	6 (60%)	
Female	3 (25%)	4 (40%)	
Aetiology of cirrhosis			0.70
ALD	6 (50%)	7 (70%)	
HCV	3 (25%)	2 (20%)	
PBC	2 (17%)	1 (10%)	
NASH	1 (8%)	0	
BMI	24.3	23.4	0.65
Child's score	11(10-13)	14(13-14)	<0.0001
Precipitating event	none		<0.0001
Alc hep	-	7 (70%)	
Infection	-	3 (30%)	
Albumin (mg/mL)	26.5±4.1	22.3±2.9	0.01
Bilirubin (μmol/L)	67.5±27.5	293±188.5	0.0005
Protrombine time (sec)	16.9±2.6	21.8±3.7	0.002
MELD	14.7±2.3	30±5.4	<0.0001

Results are expressed as mean±SD, median (interquartile range) and N(%)

*p values were assessed by χ^2, Fisher´s exact test, t-test or Mann Whitney test.

Table 2. Biochemical and humoral changes after albumin infusion

	Refractory Ascites		Acute Kidney Injury	
	Before	**After**	**Before**	**After**
Creatinine (µmol/L)	94.8±13.3	83.6±12.6**	213±60.3	154.4±42**
Clearance Creatinine (mL/min)	29.3±7.1	41.1±10.9***	13±4.2	28.3±10.3***
Urea (mmol/L)	4.8±2.6	4.1±1.9	13.2±3.4	10.7±3.4***
Albumin (g/L)	26.5±4.1	29.3±4.2**	22.3±2.9	26.7±3.5***
IMAR	0.019(0.006)	0.011(0.004)**	0.026(0.009)	0.021(0.008)**
Bilirubin (µmol/L)	67.5±27.5	75.2±27.5***	293±188.5	330.2±201.1*
Serum Sodium (mEq/L)	127.8±3.2	130.6±3***	133.4±4.2	134.6±3.8
Urinary Sodium 24h (mmol/L)	15.8±4.6	25.5±6.1***	8.3±4.6	15.3±7.2**
Norepinephrine (nmol/L)	3.6±0.5	2.6±0.6***	8.2±2.1	5.3±1.3***
IL-6 (pg/mL)	0.02(0.01)	0.01(0.01)	0.04(0.09)	0.02(0.07)
Neutrophil burst (%)	37±13	25±11**	52±20	36±12**
vWF (U/dL)	342.3±67.9	280.3±68.8***	463.0±72.8	372.9±52.5**
Nitrate (µmol/L)	41.6±18.2	28.9±10.2**	81.8±26.3	56.5±23.0**
Nitrite (µmol/L)	3.6±1.2	2.9±0.9*	8.4±2.1	6.5±1.2**
MDA (µmol/L)	6.7±3.4	4.2±1.4**	6.7±2.1	4.9±1.5**
F2a (pg/mL)	255.0±64	181.2±47.5	403.3±227	275.2±117.8*

Results are expressed in mean±SD or median(interquartile range)

*p<0.05, **p<0.01, ***p<0.001, paired samples t-test or Wilcoxon matched pairs test

Table 3. Changes in cardiac, hepatic and renal hemodynamics after albumin infusion

	Refractory Ascites		Acute Kidney Injury	
	Before	**After**	**Before**	**After**
Cardiac hemodynamics				
Heart rate (beats/min)	85±10	81±8**	99±9	88±9***
Mean arterial pressure (mmHg)	83±3	86±5*	69±3	72±4*
Central venous pressure (mmHg)	7.6±2.4	9.1±2.1*	10.6±2.4	14.2±2.3***
Cardiac output (L/min)	7.8(1.1)	7.8(0.9)	9.2(3.1)	7.4(2.5)**
Systemic vascular resistance (dynes/s/cm^5)	759(146)	780(62)	498(188)	627(292)**
Hepatic hemodynamics				
HVPG (mmHg)	19.5±2.0	19.3±2.3	22.2±3	19.6±2.9***
Renal hemodynamics				
Renal perfusion pressure (mmHg)	73.3±3.4	77±5.3**	63.5±5.6	66.4±5.1
Renal Blood Flow (mL/min)	560±40	695±57***	277±49	430±81***

Results are expressed in mean±SD or median(interquartile range)

*p<0.05, **p<0.01, ***p<0.001, paired samples t-test or Wilcoxon matched pairs test

Figure legends

Figure 1: Assessment of renal blood flow. Clinical measurements along with theoretical autoregulation curves obtained for each patient group. Best-fit values for α were: α = 8.9 and α = 5.6 in groups with refractory ascites pre and post albumin infusion, and α = 18.44, α = 12.05 in patients with AKI pre and post albumin. A decrease in the value of α post albumin treatment indicates an improved state. Open square (□): refractory ascites, cross (+): refractory ascites post albumin, open circle (o): AKI, star (*): AKI post albumin.

Figure 2:. Modification in markers of endothelial activation (vWF) and endothelial dysfunction (nitrate/nitrite). Albumin infusion was associated to a significant decrease in plasma level of von-Willebrand factor and nitrate/nitrite denoting an improvement in endothelial activation and dysfunction.

Figure 3: Correlation between improvement in endothelial activation and RBF following albumin infusion: The more pronounced the improvement in the endothelial activation, the greater the increase in the RBF.

Figure 4: Changes in markers of oxidative stress (MDA, F2 alpha -free 8-isoprostane F2α-) in both groups of patients following albumin infusion. Patients with refractory ascites (RA) showed a decreased in MDA whereas patient with AKI had a reduction in plasma levels of both markers.