

# Human serum albumin, systemic inflammation, and cirrhosis

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## Summary

Human serum albumin (HSA) is one of the most frequent treatments in patients with decompensated cirrhosis. Prevention of paracentesis-induced circulatory dysfunction, prevention of type-1 HRS associated with bacterial infections, and treatment of type-1 hepatorenal syndrome are the main indications. In these indications treatment with HSA is associated with improvement in survival. Albumin is a stable and very flexible molecule with a heart shape, 585 residues, and three domains of similar size, each one containing two sub-domains. Many of the physiological functions of HSA rely on its ability to bind an extremely wide range of endogenous and exogenous ligands, to increase their solubility in plasma, to transport them to specific tissues and organs, or to dispose of them when they are toxic. The chemical structure of albumin can be altered by some specific processes (oxidation, glycation) leading to rapid clearance and catabolism. An outstanding feature of HSA is its capacity to bind lipopolysaccharide and other bacterial products (lipoteichoic acid and peptidoglycan), reactive oxygen species, nitric oxide and other nitrogen reactive species, and prostaglandins. Binding to NO and prostaglandins are reversible, so they can be transferred to other molecules at different sites from their synthesis. Through these functions, HSA modulates the inflammatory reaction. Decompensated cirrhosis is a disease associated systemic inflammation, which plays an important role in the pathogenesis of organ or system dysfunction/failure. Although, the beneficial effects of HAS have been traditionally attributed to plasma

volume expansion, they could also relate to its effects modulating systemic and organ inflammation.

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## Human serum albumin (HSA) and the management of decompensated cirrhosis: Background and current indications

### History

Diuretics, antibiotics, and HSA are the most frequently used treatments for the management of patients with cirrhosis. According to the CANONIC study database [1], a prospective European investigation in 1348 patients with decompensated cirrhosis, HSA was indicated in 60% of the patients during hospital admission (Table 1).

The history of HSA started in 1940, when a long-term stable substitute of blood was required by the US military authorities to treat shock on the battlefield during the World War II [2]. It was used for first time in December 1941 in seven severely burned sailors after the attack on Pearl Harbor. At that period, the association of portal hypertension, hypoalbuminemia, and ascites was already known. Not surprisingly, ascites was one of the first indications of HSA. Three studies published between 1946 and 1949 assessing the effect of short- and long-term i.v. infusion of HSA in cirrhotic patients with ascites defined the first indications of HSA in cirrhosis [3–5]. Serum albumin concentration and urine volume increased in most patients. Peripheral edema also improved. However, only some patients showed improvement of ascites. First indication of HSA was, therefore, hypoalbuminemia in patients treated by frequent paracentesis. The introduction of spironolactone and furosemide in the early 1960's and the article by Hecker and Sherlock [6] first describing hepatorenal syndrome (HRS) lead to great changes in the management of ascites. The concept that paracentesis could be followed by rapid reformation of ascites and renal failure extended rapidly through the medical community and therapeutic paracentesis was formally proscribed. Only in 10% of patients not responding to diuretics (refractory ascites) was HSA prescribed to increase the plasma volume and diuretic effect [7]. In the 1970's LeVeen designed the first peritoneo-venous shunt [8]. It consisted in a multi-perforated intra-peritoneal tube connected to a unidirectional valve and to a second tube that subcutaneously reached the superior vena cava through the internal

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Abbreviations: HSA, human serum albumin; HRS, hepatorenal; PICD, paracentesis-induced circulatory dysfunction; SBP, spontaneous bacterial peritonitis; RCT, randomized controlled trial; LPS, lipopolysaccharide; TLR4, Toll-like receptor 4; PAMPs, pathogen-associated molecular patterns; DAMPs, damaged-associated molecular patterns; ROS, reactive oxygen species; RNS, reactive nitrogen species; HMA, human-mercapto-albumin; NMA, non-mercapto-albumin; NO, nitric oxide; CRP, c-reactive protein; RAS, renin-angiotensin system; SNS, sympathetic nervous system; ADH, antidiuretic hormone; PGs, prostaglandins; BDK, bradikinin; ACLF, acute-on-chronic liver failure; TNF $\alpha$ , tumor necrosis factor  $\alpha$ .



**Table 1. Use of albumin in patients with acute decompensation of cirrhosis in Europe.** Results from the CANONIC study database.

	Patients with hospitalization follow-up*	Patients receiving albumin during hospitalization
Patients (n)	699	425 (60.8%)
Bacterial infections (n)	398 (56.9%)	152 (38.2%)
SBP (n)	107 (15.3%)	53 (49.5%)
Non-SBP infections (n)	291 (41.6%)	65 (22.3%)
Paracentesis (n)	291 (41.6%)	225 (77.3%)
<5 L (n)	158 (22.6%)	107 (67.7%)
5-9 L (n)	137 (19.5%)	123 (89.8%)
>9 L (n)	55 (7.8%)	51 (92.7%)
HRS (n)	179 (25.6%)	75 (41.9%)

\*The CANONIC study was made in 1348 patients. The table only includes patients with hospitalization follow-up. There were patients with more than one complication requiring albumin administration.

**jugular vein.** The positive abdominal pressure and the negative intra-thoracic pressure facilitated the opening of the valve and the **continuous passage of ascites into the circulation.** LeVeen shunt was widely used in the management of refractory ascites for more than a decade. Following the introduction of LeVeen shunt, **HSA disappeared** from the therapeutic armamentarium of cirrhosis for more than a decade.

**Current indications of albumin**

**Management of ascites**

In 1988 a Spanish inter-hospital group [9] demonstrated that **paracentesis, if performed in association to HSA, was an effective and safe** therapy of ascites. They compared repeated large volume paracentesis (4 liters/day) associated with HSA (8 g per liter of ascitic fluid removed) vs. diuretics. The incidence of renal impairment, hyponatremia and encephalopathy was significantly **lower** in the paracentesis group. **No significant change** in plasma renin activity was observed indicating **no impairment in effective blood volume.** Survival probability was similar in both groups. In a second investigation, **total paracentesis (complete removal of ascites in only one tap)** associated with HSA was also found to be **safe** [10]. Treatment of ascites was therefore considerably simplified. Instead of requiring 2–4 weeks in hospital to compensate a tense ascites with diuretics, patients could be managed by **paracentesis in a single day** hospitalization regime [11]. Paracentesis associated with HSA was subsequently compared to peritoneo-venous shunting in patients with refractory ascites [12]. **Peritoneo-venous shunting was superior to paracentesis in the long-term control of ascites.** However, due to the high rate of complications associated with the prosthesis, the total time in hospital and the probability of survival was **similar** with both treatments. Based on these data, **paracentesis plus HSA was considered the treatment of choice for tense ascites.**

When **paracentesis is performed without HSA** or if HSA is substituted by **synthetic plasma expanders,** a high proportion of patients develop marked activation of the renin-angiotensin system, a feature known as **paracentesis-induced circulatory dysfunction (PICD)** [13–15]. The prevalence of PICD in patients not receiving volume expansion is 70%. In patients receiving dextran or polygeline it is also high (37.8%). **PICD** is due to an **accentuation of the arterial vasodilation** already present in cirrhosis and a lack of appropriated cardiac response [16–18] (Fig. 1). PICD, although asymptomatic, is a serious complication. It is not spontaneously reversible and is associated with shorter time to hospital readmission, **higher** incidence of **renal failure,** and shorter probability of **survival** [15]. A recent meta-analysis (17 trials,

1,225 patients) comparing HSA vs. alternative treatments (no volume expansion, synthetic plasma expanders or vasoconstrictors) has shown that **HSA significantly reduces the incidence of PICD and mortality** [19].

**Prevention of type-1 HRS associated with spontaneous bacterial peritonitis (SBP)**

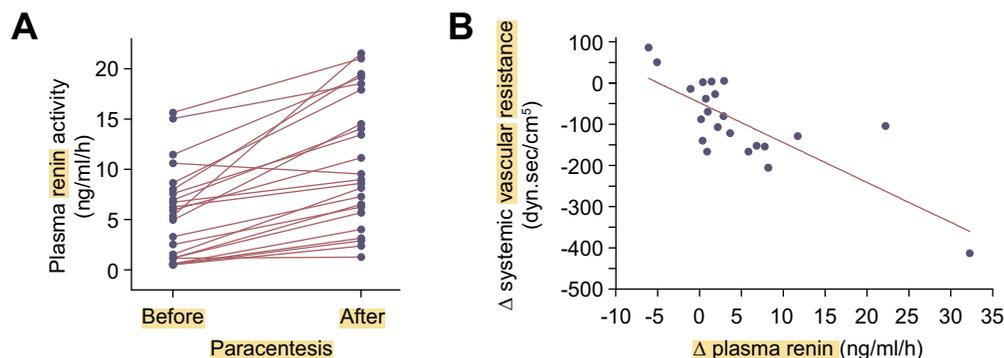
**Despite infection resolution, 20–40%** of patients with SBP develop type-1 **HRS** in relation to **arterial vasodilation,** acute impairment in cardiac function, and compensatory **activation** of the **renin-angiotensin and sympathetic** nervous systems [20–23] (Table 2). Type 1 **HRS** also **develops** in **cirrhotic** patients with **other** type of bacterial **infections** although the prevalence is **lower** [24–26]. The reason why SBP is such a frequent precipitating event of type-1 HRS is multifactorial. First, an **exaggerated inflammatory** response to **sepsis** occurs in patients with cirrhosis and ascites with an increase in plasma levels of **cytokines 20-fold greater** than in individuals **without cirrhosis** [26]. This feature has also been observed in experimental animals in which doses of bacterial endotoxin that do not produce any change in systemic hemodynamics in healthy **rats,** induce arterial hypotension and increase the plasma levels of **cytokines by 100-fold** in rats with cirrhosis and ascites [27]. Second, the **inflammatory** response to bacterial infection persists for a **longer duration** in cirrhosis. Finally, patients with cirrhosis and ascites **already have severe impairment** in cardio-circulatory and renal function, which predispose these patients to further deterioration in organ function [28]. In support to this contention, patients with SBP who have increased serum **creatinine** concentration or dilutional **hyponatremia** prior to infection, and those with intense inflammatory response (high concentration of polymorphonuclear leukocytes, tumor-necrosis-factor alpha and interleukin-6 in plasma, and ascitic fluid) are at **high risk** of developing type-1 **HRS** [23,29,30]. If **untreated,** type-1 HRS in patients with SBP is associated with a **mortality rate approaching 100%** [31].

In 1999 we reported the use of **HSA (1.5 g/kg b.wt. at infection diagnosis and 1 g/kg b.wt. at the third day)** in SBP [31]. HSA prevented cardiovascular dysfunction and this was associated with a **dramatic decrease** in the prevalence of **type 1 HRS** and hospital **mortality** (10% in the HSA group and 30% in the **control** group). A recent meta-analysis has confirmed these findings [32].

**Treatment of type-1 HR**

The use of **vasopressin analogs** and **HSA** for the treatment of type-1 HRS was based on **two features.** First, **arterial vasodilation** in cirrhosis occurs in the **splanchnic circulation** and **vasopressin analogs** act **preferentially** in this area [28]. Second, studies using

## Review



**Fig. 1. Paracentesis-induced circulatory dysfunction in patients not receiving HSA. Relationship to changes in systemic vascular resistance.** (A) Plasma renin activity before and after therapeutic paracentesis in cirrhotic patients with ascites not receiving HSA. Plasma renin activity increased in most patients indicating the development of paracentesis-induced circulatory dysfunction [13]. (B) Relationship between changes in systemic vascular resistance and of plasma renin activity. An inverse relationship was observed indicating that circulatory dysfunction is related to arterial vasodilation [17].

**Table 2. Effect of treatment with vasoconstrictors (ornipressin and terlipressin) and i.v. albumin in type-1 HRS (n = 15).\***

	Baseline (n = 15)	Day 7 (n = 9)	Day 14 (n = 7)
MAP (mmHg)	70 ± 8	77 ± 9	79 ± 12
PRA (ng/ml.h)	15 ± 15	2 ± 3	1 ± 1
NE (pg/ml)	1257 ± 938	550 ± 410	316 ± 161
Serum creatinine (mg/dl)	3 ± 1	2 ± 1	1 ± 1
GFR (ml/min)	9 ± 1	25 ± 2.5	41 ± 1.5

\*Data obtained from [34,35].

MAP, mean arterial pressure; PRA, plasma renin activity; NE, plasma norepinephrine concentration.

head-out water immersion, a procedure that expands the central blood volume, and vasoconstrictors showed that improvement in renal function in cirrhosis only occurs when both procedures are applied simultaneously [33]. In our first study we used ornipressin and HSA [1 g/kg b.wt. on the first day followed by 20 to 40 g/day] [34]. A remarkably improvement in circulatory function was observed with rapid suppression of the renin-angiotensin and sympathetic nervous systems, progressive normalization in serum creatinine and increase in renal perfusion and GFR (Table 3). Because treatment was associated with ischemic events in some patients, the study was repeated using i.v. terlipressin (1–2 mg/4–6 h) with identical results but no ischemic complications [35].

Many studies have been subsequently published on the use of terlipressin and albumin for HRS and their main conclusions are the following: (1) Terlipressin plus HSA reverses type-1 HRS (serum creatinine <1.5 mg/dl) in 40–65% of patients [36–38]. In less than 20% of responders type-1 HRS recurs after discontinuation of treatment but it may reverse again following retreatment. (2) The rate of response may increase if terlipressin is given by continuous infusion [39]. (3) Predictors of response include low baseline serum creatinine (<5 mg/dl) and bilirubin (<10 mg/dl), and increase in mean arterial pressure (>5 mm/Hg) within the first 3 days of treatment [40–42]. (4) Reversal of HRS is associated with improvement in survival [43,44]. (5) Noradrenaline is as effective as terlipressin [45–47]. (6) Reversal of type 1 HRS is significantly lower if terlipressin is given without HSA [48]. (7) Type-1 HRS may reverse with HSA alone [49–51]. (8) Patients with ongoing bacterial infections should be treated as soon as type-1 HRS is diagnosed [52]. (9) Treatment is not indicated in type-2 HRS because HRS recurrence is the rule [53].

**Table 3. Cardiovascular hemodynamics in 8 patients developing HRS after spontaneous bacterial peritonitis.\***

	At SBP diagnosis	Following SBP resolution	p value
Serum creatinine (mg/dl)	1.3 ± 0.6	2.5 ± 0.4	<0.02
BUN (mg/dl)	37.4 ± 9.5	81.2 ± 25.2	<0.02
MAP (mmHg)	83 ± 7	73 ± 8	<0.02
PRA (ng/ml.h)	18 ± 11	28 ± 12	<0.02
NE (pg/ml)	797 ± 226	1290 ± 415	<0.02
SVR (dyn.s.cm <sup>-5</sup> )	1137 ± 220	1268 ± 320	n.s.
CO (L/min)	5.7 ± 0.9	4.6 ± 0.7	<0.02
RAP (mmHg)	3.0 ± 3.0	4.6 ± 2.7	n.s.
PCWP (mmHg)	5.7 ± 4.0	7.4 ± 2.6	n.s.
HR (bpm)	93 ± 13	87 ± 9	n.s.

\*Obtained from [21].

Mean time elapsed between measurement was 6 days.

BUN, blood urea nitrogen; MAP, mean arterial pressure; PRA, plasma renin activity; NE, plasma norepinephrine concentration; SVR, systemic vascular resistance; CO, cardiac output; RAP, right atrial pressure; PCWP, pulmonary capillary wedged pressure; HR, heart rate.

#### Other potential indications for albumin in cirrhosis

There are four studies assessing long-term administration of HSA in cirrhosis with ascites. The rationale behind these investigations is that long-term improvement of circulatory function may prevent acute complications of cirrhosis, including recurrence of ascites, HRS, encephalopathy, and bacterial infections. The first investigation was a comparative study in 17 patients, 9 receiving between 184 to 558 g of HSA within 6 months [54]. All patients in the HSA group survived for more than 2 years. In the control group mortality rate was 30%. The second study was a randomized controlled trial (RCT) in 126 patients hospitalized for ascites [55]. Half of the patients received diuretics and half diuretics plus HSA [12.5 g/day during hospitalization, 25 g/week during the first year and 25 g/2 weeks during the second and third year]. Cumulative incidence of acute complications and hospital admission was significantly lower in patients receiving albumin. There were no significant differences in survival. Two other studies are still ongoing. The MUCH study (ClinicalTrials.gov, Identifier: NTC00839358) is a RCT comparing standard therapy vs. standard therapy, HSA (40 g every 2 weeks) and midodrine (an oral  $\alpha$ -adrenergic agonist) in 190 patients with ascites. The main end-points of the study are incidence of complications and survival. The ANSWER study

(ClinicalTrials.gov, Identifier: NTC01288794) is a RCT in 400 patients with ascites comparing standard therapy vs. standard therapy plus HSA (40 g twice weekly in the first two weeks and once weekly for one year). The main end points are mortality and incidence of refractory ascites.

The use of HSA for the management of non-SBP bacterial infections is an increasing indication in Europe (Table 1). There is data that these patients also develop type-1 HRS [25,22]. On the other hand a recent RCT in 110 patients (INFECIR-1 Study) showed that HSA [1.5 g/kg b.wt. at infection diagnosis and 1 g/kg b.wt. at the third day] has beneficial effects on renal and circulatory function [56]. A trend for better prognosis in patients receiving HAS was also observed. A second RCT (INFECIR-2 study, ClinicalTrials.gov, Identifier: NTC02034279) has been started by the EASL Chronic Liver Failure Consortium; 512 patients with non SBP bacterial infections and high risk of type-1 HRS and death will be included.

**Key Points 1**

- HSA is an extracellular heart-shaped three-domain protein of intermediate size ( $6.8 \times 10^4$  g/mol) that is synthesized in the liver and is the most abundant protein in plasma. It is a protein of remarkable solubility (50 mg/ml) owing to its high content (30%) in charged residues (39 Asp, 60 Glu, 58 Lys, 23 Arg) and performs a wide range of functions ranging from regulating oncotic pressure to scavenging reactive oxygen species. Both the amount and quality of HSA are important for health
- The structure of HSA, solved at high resolution in 1992, is stabilized by a remarkably large (17) number of intra-domain disulfide bonds that render the individual domains, that are of similar size and structure, very stable. Domains I and III, which are lobes in the structure, can fold autonomously but domain II, which is the core of the structure of HSA, cannot. This structural coupling provides a framework for the allosteric regulation of HSA, whereby the binding of ligands or chemical modifications in one domain generates a signal that can propagate to a different domain
- At the molecular level the most striking feature of HSA is its ability to bind to a wide range of ligands, which is intimately linked with one of its physiological roles, to transport relatively hydrophobic species that would otherwise not be soluble in plasma. This includes small molecules such as hormones, fatty acids, bilirubin and heme as well as metals such as Cu, Fe and Hg but also more complex substances such as lipopolysaccharide. In addition to endogenous ligands HSA can also bind to exogenous ligands such as drugs, greatly contributing to their bioavailability
- HSA can be modified irreversibly by non-enzymatic glycosylation of Lys side chains, mainly Lys<sup>525</sup>, as well as by oxidation, mainly of the only free Cys residue in the protein, Cys<sup>34</sup>. The former occurs in diabetic patients whereas the latter is frequent in acute on chronic liver failure. Although the mechanisms by which these chemical modifications alter the structural properties of HSA are not known there is substantial evidence that they alter the physiological functions of the protein, presumably by collective changes in its structure

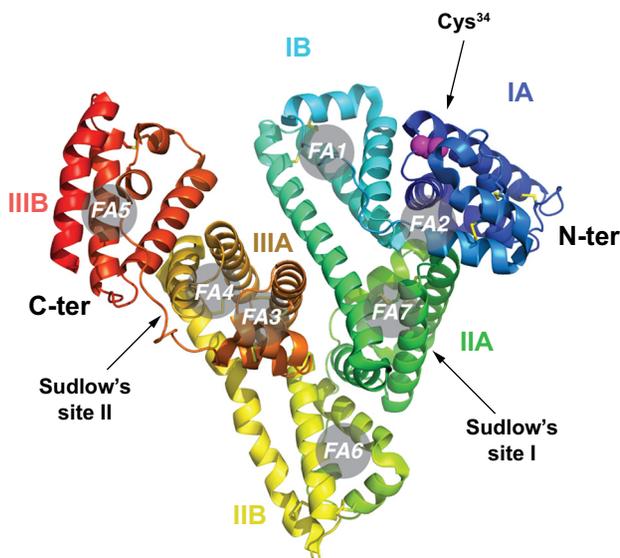
**Chemistry and physiology of HSA**

*Molecular structure and chemical properties*

HSA is a multi-domain protein stabilized by 17 disulfide bonds that confer a remarkable stability to its structure [57]. In addition to the 34 cysteine residues involved in disulfide bonds HSA also possesses a free cysteine (Cys-34) that is relatively solvent-exposed (Fig. 2) and plays an important role in its antioxidant activity [58,59]. The structure of HSA at low resolution (6 Å) was first solved in 1989 [60] and a high resolution atomic structure (2.8 Å) was reported in 1992 [57]. These revealed this protein to be composed of three domains of similar size (residues 1–197, 198–381, and 381–585) arranged in a heart shape and formed by eight α-helices. Owing to their structural similarity the domains can be divided in sub-domains (IA, IB, IIA, IIB, IIIA, and IIIB) that have functional relevance as they define the binding sites of many HSA ligands (Fig. 2) [61].

Due to the presence of a substantial number of intra-domain disulfide bonds the domains of HSA have significant stability. Domains I and III are particularly stable and a construct containing domains I and II [1–381] as well as a construct containing domains II and III [198–585] fold autonomously by adopting the same structure that they have in the full-length protein [62,63]. In spite of its large size and owing to its remarkable stability and its relatively simple structure, where residues that are connected by disulfides or in contact are not particularly distant in sequence, HSA can be refolded from the fully reduced state unassisted by molecular chaperones [64,65]. This greatly facilitates its synthesis in the laboratory and the study of its structural and biophysical properties [62].

Again owing to its relatively simple structure and modular nature HSA appears to be a very flexible protein. According to



**Fig. 2. Molecular structure of human serum albumin.** Crystal structure (pdb code 1e7i) of HSA with an indication of the its subdomains (IA, IB, IIA, IIB, IIIA and IIIB), of the N and C termini, of Sudlow's sites I and II and of the seven LCFA binding sites (FA1 to FA7). The heavy atoms of the side chain of residue Cys-34 are shown as purple spheres. LCFA binding sites also bind prostaglandins. Cys-34 binds ROS and RNS, including NO [61].

## Review

various experimental approaches the heart shape that the protein adopts in the crystal is likely to be only one in the range of inter-domain orientations that HSA samples show in solution. Experiments monitoring the rate of exchange between exchangeable hydrogens and water have shown these to be particularly labile, indicating that in addition to inter-domain motions HSA undergoes local unfolding events where the hydrogen bonds that stabilize its secondary structure are transiently disrupted [66].

Many of the physiological functions of HSA rely on its ability to reversibly bind to an extremely wide range of ligands to increase their solubility in plasma, to transport them to specific tissues or organs or to dispose of them when they are toxic. The structures of HSA bound to various of these substrates have been determined by X-ray crystallography, revealing that on many occasions the protein must experience substantial structural changes to accommodate the ligand and therefore strongly suggesting a functional role for its high flexibility. Although many binding sites for ligands have been reported in HSA the most important sites are commonly known as Sudlow's sites I and II (Fig. 2) which are found in subdomains IIA and IIIA, respectively.

Long chain fatty acids (LCFAs) are intermediates in lipid metabolism that circulate in plasma both in soluble form as well as associated with HSA. According to crystallographic studies they can bind up to seven sites in HSA (FA1 to FA7) including both Sudlow's sites, with site I corresponding to FA7 and site II corresponding to FA3 and FA4 [67]. It is however likely that many of these sites are secondary and therefore do not play an important role in LCFA binding in physiological conditions, where it is thought that up to two LCFA molecules, bound to Sudlow's site I, are bound to HSA at a given time.

Drugs such as warfarin, indomethacin, ibuprofen, diazepam are another heavily studied class of HSA ligands. Similarly to what is the case for LCFAs these molecules can bind to various sites in HSA and, although they tend to mainly interact with Sudlow's sites I and II, this can vary depending on the relative concentration of competing ligands and on the allosteric effects discussed below [68]. That these and other drugs can compete for HSA binding sites for their transport in plasma emphasizes the importance of understanding the physicochemical basis of their interaction with HSA [69].

Given that HSA is a flexible protein that can bind to various substrates in different sites there have been suggestions that it is an allosteric protein, i.e., that its affinity for specific ligands can depend on whether a second ligand is occupying a different binding site. Specific examples of such a behavior, among others, include an allosteric coupling between the binding sites of heme that binds to FA1 in sub-domain IB, and the drug warfarin, that binds to Sudlow's site I [70]. The structural, dynamical, and thermodynamic coupling between domains occurring in HSA is highly suggestive of this possibility as these are sufficient conditions to give rise to allosteric binding [71].

Given its high stability HSA has a long half-life in plasma, of about 19 days [2]. During this relatively long time its chemical structure can be altered by oxidation as well as by non-enzymatic glycosylation, among other irreversible modifications. The accumulation of chemically altered HSA has been linked to specific pathologies such as cirrhosis [72] and diabetes [73]. The highly flexible nature of this protein and the growing body of evidence, suggesting that the various binding sites that contribute to HSA function are strongly coupled, suggests that such chemical alterations can have functional consequences. In this scenario

### Key Points 2

- The total extracellular pool of albumin of a 70 kg b.wt. healthy man is 360 g which represents  $3.2 \times 10^{21}$  molecules of albumin. The weight of the extracellular pool of albumin is similar to the total liver protein weight. 66% of the extracellular albumin is in the interstitial space and 33% in the intravascular space. The transfer of albumin from the intravascular to the interstitial space (transcapillary escape rate of albumin) is 4-5% per hour and approximately the same percentage is returned to the intravascular compartment throughout the lymphatic system; 80% of the extravascular pool is in the muscle and skin
- Albumin is synthesized only by the hepatocytes and it is not stored, rather the protein is rapidly secreted (synthesis occurs within 2-3 minutes and secretion within 25 minutes). Each hepatocyte synthesizes approximately  $15 \times 10^6$  albumin molecules per minute which represents a total hepatic synthesis of 13-14 g (3.5% of the total albumin pool) per day of newly synthesized albumin entering the intravascular space. An equivalent mass of albumin is catabolized. The half life of albumin is 19 days. An average molecule, therefore, leaves the circulation every 23 h, which means approximately 28 trips from and back to the circulation during its lifetime, and makes around 15,000 trips around the circulation
- The plasma concentration of albumin (35-45 g/L) and the total albumin pool are regulated by changes in albumin gene transcription and in albumin catabolism. The most important physiological factor regulating synthesis is the plasma concentration of albumin. Hypoalbuminemia stimulates albumin synthesis. Conversely, albumin infusion depresses albumin synthesis. The administration of other macromolecules (dextrans) has similar effects pointed to the colloid osmotic pressure as the function on which this regulation is based. Albumin catabolism is regulated by the total albumin pool. A decrease in albumin pool leads to a reduced albumin catabolism. Changes in albumin synthesis are less pronounced than changes in catabolism
- In patients with decompensated cirrhosis, albumin metabolism is much more complex than in healthy subjects, since there are additional mechanisms affecting synthesis and catabolism. For example, systemic inflammation is associated to a marked fall in albumin synthesis, a process that is mediated by cytokines (IL-6 and TNF $\alpha$ ). On the other hand, structural changes of the albumin molecule related to specific binding of ligands (i.e., glucose, reactive oxygen or nitrogen species) are associated to rapid degradation and catabolism of the modified molecules. Since these processes are present in patients with decompensated cirrhosis or ACLF, albumin replacement requires high doses in these patients. In fact, the amount of albumin given to a patient of 70 kg b.wt. with SBP (1.5 g/kg b.wt. at infection diagnosis and 1 g/kg b.wt. at the third day) represents 48.6% of the total extracellular pool of albumin

the oxidation or glycosylation of specific positions in the surface of HSA would cause structural changes that could propagate to different regions of structure either by concerted conformational changes or through local changes in the stability known to give rise to long range effects in simpler systems.

The best studied irreversible alteration of the structure of HSA is the **oxidation by reactive oxygen species** of Cys-34 from mercapto-albumin, which has the native thiol group, to non-mercapto-albumin, where the Cys side chain is involved in a disulfide bond with either glutathion or another HSA molecule [58,59]. The reaction can proceed further to the sulfinic and sulfonic acid derivatives of the side chain. An obvious consequence of this reaction is a **decrease in the anti-oxidant properties of HSA**, that rely on the thiol form of Cys-34, but there is also evidence that it can indirectly lead to decreases in other functions of HAS, such as its ability to bind drugs in Sudlow's sites [72,74].

### Physiology

#### Synthesis and metabolism

Albumin is synthesized by the liver and rapidly released to the intravascular compartment [2]. The total amount of albumin in humans is approximately 360 g, 120 being in the **intravascular** and 240 in the **extravascular** compartment. Intravascular albumin is constantly being exchanged (4–5%/h) through the endothelium with the extravascular pool. In organs having sinusoids or capillaries with **fenestrated endothelium** albumin can pass through the **large capillary gaps**. In the remaining capillaries with continuous endothelium, **albumin is transported by an active transcytotic mechanism** mediated by the gp60 receptor (albondin) [75–78]. There is a second group of receptors (gp18 and gp30) expressed in many tissues that governs degradation of albumin [78–81]. These surface cell receptors shows 1000-fold higher affinity for chemically modified albumin (i.e., oxidized albumin). Once internalized, this modified albumin is degraded. Finally, a third type of albumin receptor (FcRn) that rescues albumin from lysosomal degradation contributes to extend the albumin half life [78,82–84].

### Physiology

The effects of HSA rely in the following features: an **appropriated molecular mass** and **negative charge** generate **high oncotic pressure**; a **high solubility in water** and density of binding sites make the protein an ideal vehicle to **transport water-insoluble substances**; the effects of active exogenous and endogenous substances are modulated following binding to HSA; the high extracellular concentration of HSA magnified its biological actions.

Among the substances transported, **fatty acids** stand up. HSA is the **main fatty acid binding protein** in the **extracellular space**. It carries fatty acids from the **intestines** to the **liver** and from the liver to **muscle** and to and from **adipose tissues** [85,86]. Transportation of **unconjugated bilirubin** from the spleen or bone marrow to the liver is the best example of albumin transportation of a water insoluble endogenous metabolite to its elimination site [87]. Other endogenous substances that bind to albumin are the eicosanoids, bile acids, steroids, hematin, tiroxin, vitamin D, and folate [2,88]. Among the exogenous molecules transported by albumin there is a wide variety of drugs [2,69,89]. In addition to drug solubility, albumin binding decreases toxicity and **increases drug half-life** [69]. Drug binding to HSA can be affected

by other drugs that compete with specific sites in the molecule. As a consequence, an increase in the free fraction may lead to changes in pharmacokinetics and pharmacodynamics. Drug-albumin binding may be also altered in diseased states, including liver and renal diseases [58,90,91], due to the increase in endogenous substances that compete for binding sites, but pharmacokinetic consequences of this does rarely cause important effects [92,93].

An outstanding feature of HSA is its **capacity to bind pro-inflammatory substances** and mediators of inflammation. **Lipopolysaccharide (LPS)**, lipoteichoic acid, and peptidoglycan are **surface components of gram-negative and gram-positive bacteria** that **activate** the innate immune system through **Toll-like receptor 4 (TLR4)** and induce **inflammation**. HSA **binds** these molecules by electrostatic and hydrofobic forces [94–97]. Cell activation by LPS requires an ordered interaction with several host proteins including LPS-binding protein, CD14 and co-receptor MD-2. Recent studies showed that albumin may also participate in LPS presentation to TLR4 facilitating disaggregation of LPS-Lipid A polymers and donation of monomers to CD14 [98–100]. This suggests that **albumin promotes the inflammatory response**. In contrast, LPS-albumin complexes are much less effective in immune activation than endotoxin-CD14 complexes [100]. Giving the abundance of albumin and the lower relative cell activation capacity of the LPS-albumin complex, albumin could play a role in **moderating the inflammatory response to bacterial infections**. Therefore, albumin could play a **dual role** either **stimulating** or **moderating** immune cells activation depending on pathophysiological conditions [98,100].

Systemic **inflammatory response** can be **triggered** by antigens derived from **bacteria** (Pathogens-Associated Molecular Patterns, **PAMPs**) or by **intrinsic factors** released into the circulation as a result of **trauma** or **cell injury** (Damaged Associated Molecular Patterns, **DAMPs**). Specialized **receptors** of the **innate immune system** recognized these factors and **release inflammatory mediators** among which **cytokines**, and reactive oxygen (**ROS**) and nitrogen species (**RNS**) are the most important [101,102]. Generation of **ROS** and **RNS** (superoxide, nitric oxide, hydrogen peroxide, hydroxyl radical, peroxynitrite, hypochlorous acid, nitrogen dioxide radical, hydroperoxide radical, and peroxy radical) by neutrophils, macrophages, and endothelial cells, which represent the **innate immune system**, is the **first line of defense** against sepsis [103–105]. The aim of the modification of the redox state in plasma and extracellular fluid is to **destroy the bacteria by energy depletion and oxidative damage of lipids, proteins, and DNA**. This extracellular oxidative “burst” however may be **transferred into mitochondria** leading to **cell dysfunction** and **organ failure** [103]. Extracellular fluid and the mitochondria dispose of antioxidant systems to prevent excessive oxidative damage [103–105]. **HSA is the main extracellular defense against oxidative stress**. It provides **80% of extracellular thiols**, which are **potent scavengers of ROS and RNS** [60,106,107]. The **glutathione system** is the most abundant **mitochondrial antioxidant**. Intracellular **albumin catabolism** represents a **source of sulfur-containing amino acids** for the **synthesis of glutathione**. Albumin therefore **modulates the intracellular levels of this antioxidant system** [108].

Two mechanisms account for the antioxidant effect of HSA. The first is related to its capacity to **bind** and inactivate free metals such as **cooper** and **iron**, which **catalyse** the formation of aggressive ROS [109,110]. HSA-bound bilirubin inhibits lipid peroxidation and represents an **indirect antioxidant effect** [111]. The

## Review

second and most important mechanism is related to the capacity of HSA to trap free radicals. Two-thirds of the HSA molecules exist in a reduced form with a free thiol group in the Cys-34 residue. Working as a free radical scavenger, the Cys-34 residue is able to trap multiple ROS and RNS [60,107]. As indicated, in physiological conditions the Cys-34 residue exists in two different forms, namely human-mercapto-albumin (HMA; reduced) in which the thiol group is in the free state and human non-mercapto albumin (NMA; oxidized) [60,107,112]. In this later form the thiol group may be present as a disulfide that is formed reversibly with Cys or glutathione or as sulfinic or sulfonic acid that is formed irreversibly. Irreversible albumin oxidation is associated with a loss of function and rapid degradation. Cys-34 is a good indicator for evaluating oxidative stress in the systemic circulation [60,107]. HSA contains six methionine residues which can also be oxidized [112,113].

Under nitrosative stress by nitric oxide (NO) or other nitrosylating agents, mercaptalbumin can be converted in nitroso-HSA. This is a reversible reaction, so that NO can be transferred to other molecules [114,115]. HSA also binds arachidonic acid, prostaglandins, thromboxanes, and leukotrienes [116–120]. HSA has two-sided effects on eicosanoids. In some cases the protein, which has intrinsic enzymatic activity, catalyzes their synthesis or degradation. In others (i.e., PGI<sub>2</sub>, thromboxanes, and leukotrienes) it stabilizes the molecule by delaying hydrolysis. HSA, therefore, functions as storage, carrier, and supplier of NO and eicosanoids to target sites to initiate physiological actions (vasodilation and inhibition of platelet aggregation) and as protector agents to diminish harmful biological effects if produced in too large amounts. By these mechanisms HSA modulates endothelial function and inflammation.

### Decompensated cirrhosis is associated with systemic inflammation

Three major features characterize decompensated cirrhosis. The first is multi-organ dysfunction. The second is a systemic inflammatory reaction with increased plasma and ascitic fluid concentration of cytokines and C-reactive protein (CRP) [121–129]. Finally, the third is an increased systemic oxidative stress with a high levels of oxidized HSA and of other markers of oxidative stress [58,72]. Systemic inflammation, oxidative stress, and organ dysfunction are moderate in patients with decompensated cirrhosis and severe in patients with acute-on-chronic liver failure (ACLF) [1,130] (Fig. 3). Translocation of bacterial products (i.e., lipopolysaccharide, bacterial DNA) or of viable organisms from the intestinal lumen to the circulation due to quantitative and qualitative changes in gut microbiota, impairment in intestinal mucosal barrier, increased epithelial permeability, and impaired intestinal immunity, are important mechanisms of systemic inflammation in cirrhosis [1,23,26,130,131]. However, systemic inflammation may also occur in response to acute liver injury (i.e., acute alcoholic hepatitis) or other mechanisms [1].

Closed interactions exist between bacterial translocation, local inflammation, and cardiovascular dysfunction in decompensated cirrhosis (Fig. 3). Activation of the intestinal immune system by bacterial translocation causes local release of NO and other vasodilators leading to the characteristic hyperdynamic circulation of cirrhosis and in more advanced stages, effective hypovolemia, activation of the renin-angiotensin system (RAS),

sympathetic nervous systems (SNS) and antidiuretic hormone (ADH), and ascites formation [28,130–134]. On the other hand, the activated sympathetic nervous system induces changes in the gut microbiota and impairs intestinal immunity, thus producing a vicious circle promoting the progression cardiovascular dysfunction [131]. A slow but progressive impairment of left ventricular function and cardiac output also develops in decompensated cirrhosis and contributes to circulatory dysfunction [135,136]. Recent data suggest that impairment in cardiac function in experimental cirrhosis is related to inflammation, tumor-necrosis factor  $\alpha$  (TNF $\alpha$ )-related activation of inducible NO-synthase and oxidative stress in the cardiac tissue [137].

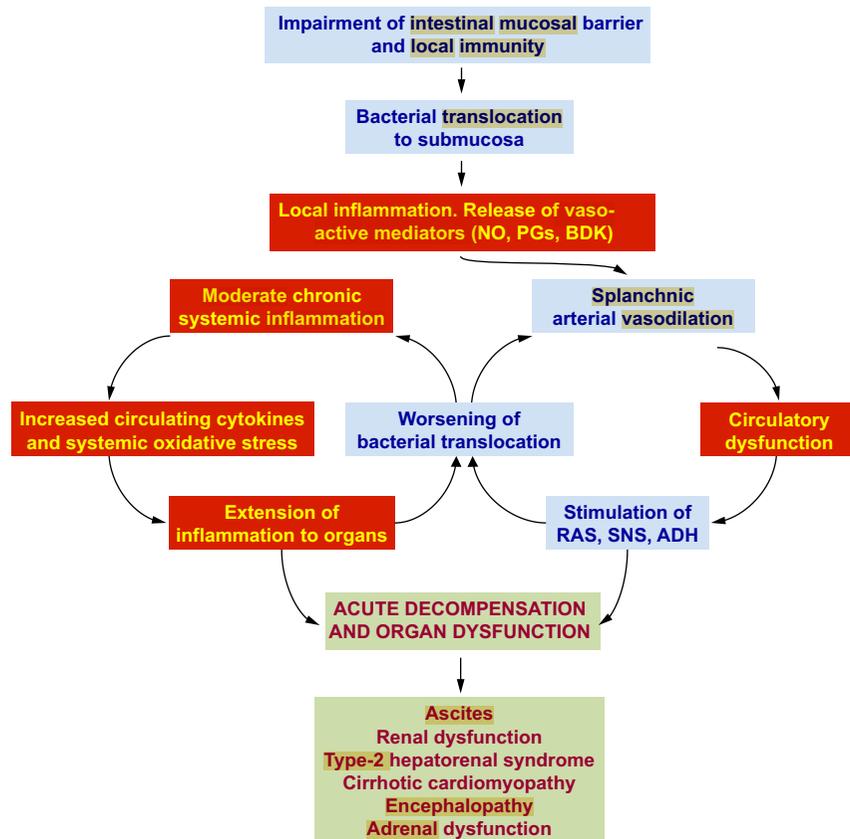
ACLF is characterized by acute development of organ failure(s) (liver, renal, brain, coagulation, circulation, respiration) in patients with compensated or decompensated cirrhosis [1]. ACLF develops in the setting of severe systemic inflammatory reaction due to bacterial infection, acute alcoholic hepatitis or other precipitating events [1,23]. The number of organ failures correlates directly with the degree of systemic inflammation (Fig. 4) [1]. Therefore, whereas systemic inflammation is chronic and moderate in decompensated cirrhosis, it is acute and severe in ACLF (Fig. 4). The mechanism of organ failure in ACLF is complex (Fig. 3). Acute impairment in cardiovascular function leading to intense organ hypoperfusion is a major feature [21]. However recent studies in sepsis suggest that extension of systemic inflammation to organs leading to abnormal distribution of blood flow within the microcirculation and cell dysfunction related to mitochondrial oxidative stress are also important mechanisms [138,139].

Bacterial infection is a frequent precipitating event of hepatic encephalopathy. Peripheral inflammation may affect cerebral function through afferent vagal nerves activated by cytokines at the site of inflammation, by lipopolysaccharide or cytokines that interact to brain in areas lacking the blood-brain-barrier or by diffusion to the brain of endothelial mediators [140–144]. Activation of microglia and synthesis of pro-inflammatory cytokines in the brain have also been demonstrated in experimental models of liver failure [141]. Circulatory dysfunction in patients with systemic inflammation reduces brain perfusion [145]. Finally, systemic inflammation increases the inhibitory effect of ammonia in brain function [146,147]. There are marked differences in hepatic encephalopathy between patients with decompensated cirrhosis and patients with ACLF [148,149]. In the first group hepatic encephalopathy is of low grade and diuretics the most frequent precipitating event. In contrast, hepatic encephalopathy in ACLF is severe and bacterial infections or acute liver injury the main precipitating factor. Organ dysfunction in cirrhosis therefore varies according to the mechanism and degree of systemic inflammation.

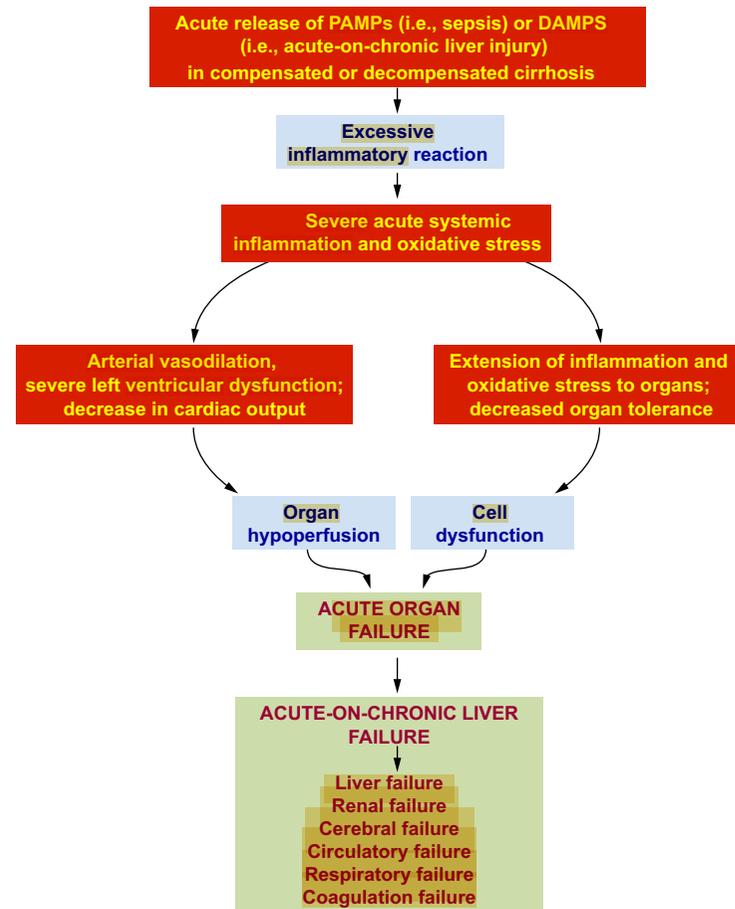
### HSA may have therapeutic effects unrelated to volume expansion

Considering the potential role of systemic inflammation in cirrhosis and the effect of HSA modulating the innate immune response and oxidative stress, it is rational to suggest that some effects of HSA (i.e., prevention and treatment of type-1 HRS) might be related to these features. Indeed, there are many steps in the process of acute decompensation of cirrhosis and ACLF that could be influenced by HSA (Fig. 3). There are three studies supporting this contention.

**DECOMPENSATED CIRRHOSIS**

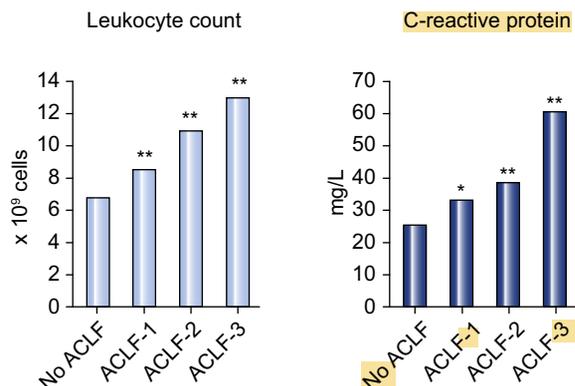


**ACUTE-ON-CHRONIC LIVER FAILURE**



**Fig. 3. Role of inflammation in the pathogenesis of decompensated cirrhosis and ACLF.** Hypothesis on the role of inflammation in decompensated cirrhosis (left panel). The initial event is inflammation of the intestinal submucosa and arterial vasodilation related to bacterial translocation. As the disease progresses effective arterial hypovolemia develops leading to stimulation of the RAS, SNS and antidiuretic hormone (ADH). The SNS reduces intestinal motility, increases bacterial overgrowth and inhibits the intestinal immune system, worsening bacterial translocation and closing the first vicious circle that perpetuates circulatory dysfunction. The second vicious circle includes the extension of local inflammation to the systemic circulation and the organs. The final effects are the complications associated with decompensated cirrhosis. The red boxes point out the steps that may be influenced by HSA. Hypothesis on the role of inflammation in ACLF (right panel). ACLF is the result of a severe acute systemic inflammation secondary to PAMPs or DAMPs. Two types of processes then develop. The first is a rapid and severe impairment in systemic vascular resistance and left ventricular function leading to organ hypoperfusion. The second is the extension of systemic inflammation and oxidative stress to the organs leading to abnormal distribution in blood flow within the microcirculation and cell dysfunction. Both features lead to organ failure(s) and ACLF. The red boxes point out the steps that may be influenced by HSA.

## Review



**Fig. 4. Relationship between systemic inflammation and ACLF grade.** Leukocyte count and C-reactive protein in patients with decompensated cirrhosis (no ACLF) and in patients with ACLF-1 (renal failure or single non renal organ failure associated with renal dysfunction or moderate hepatic encephalopathy; for further explanation see reference [1], ACLF-2 [2] organ failures) and ACLF-3 [3] or more organ failures. Normal values of C-reactive protein are below 5 mg/L. \* $p < 0.05$ ; \*\* $p < 0.001$  with respect to no ACLF. Results obtained from the CANONIC data-base [1].

### Key Points 3

- **HSA** is one of the most frequent **treatments** in patients with **decompensated** cirrhosis. Prevention of paracentesis-induced circulatory dysfunction, prevention of type-1 HRS associated to **SBP** and treatment of type-1 **hepatorenal syndrome** are the **main indications**. In patients with these indications HSA improves survival
- The effects of HSA rely on the following features: its relatively **large molecular mass** and the **negative charge** of the molecule generate **high oncotic pressure**; a high **solubility in water** and density of binding sites make the protein an **ideal** vehicle to **transport** water-insoluble substances; the effects of exogenous and endogenous substances are modulated following binding to HSA; the **high extracellular concentration of HSA** magnifies its biological actions
- **Decompensated cirrhosis** is associated to **moderate** and chronic systemic inflammation, which plays an important role in the pathogenesis of acute decompensation and organ/system dysfunction. Systemic **inflammation** is related to **translocation of bacterial products** from the **intestinal lumen** to the submucosal space and the systemic circulation
- In **contrast**, systemic **inflammation** in patients with **ACLF**, a syndrome characterized by **decompensated cirrhosis** and **organ failure(s)**, is **acute, severe** and frequently related to **spontaneous bacterial infections** (spontaneous **bacteremia** and **SBP**) due to **translocation of viable bacteria**. Systemic inflammation in ACLF may be also due to other types of infection and acute liver injury (i.e., acute alcoholic hepatitis)
- The **therapeutic** action of HSA in cirrhosis is probably related **not only** to **expansion** of the plasma **volume** but also to a **modulation** of systemic and organ **inflammation**

Two studies **compared** the systemic hemodynamic changes in uncomplicated patients with SBP treated with **albumin** or hydroxyethyl **starch** [150,151]. **Circulatory function only improved** in patients receiving albumin. An increase in left ventricular function, cardiac output, and peripheral vascular resistance was observed, indicating a simultaneous effect of HSA in the heart and in the peripheral circulation. An effect of HSA modulating the cardiac and endothelial effect NO was proposed to explain the different circulatory response on the basis of distinct changes in the plasma levels of NO metabolites and von Willebrand Factor between groups [152–154].

The third investigation was performed in cirrhotic rats with ascites [135]. Plasma volume expansion with HSA but not with hydroxyethyl starch improved left ventricular function *ex vivo*. This effect was related to a **normalization** of activated protein expression of **TNF $\alpha$**  and inducible **NO synthase**, MAD(P)H-oxidase activity and nuclear translocation of NF- $\kappa$ B in cardiac tissue, indicating a decrease of heart inflammation and oxidative stress.

### Conflict of interest

VA has a grant from Grifols. RG and XS declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript. The EASL Chronic Liver Failure Consortium has an unrestricted grant from Grifols.

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