

## Effective albumin concentration and cirrhosis mortality From concept to reality

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Albumin is the most abundant protein in human plasma that is produced exclusively in the liver. Traditionally, albumin has been thought of being important in providing plasma with its oncotic property and therefore its use in clinical practice has been mainly aimed at promoting plasma volume expansion. The past 10 years or so has seen an explosion in the knowledge of albumin biology such that it is now clear that it has multifunctional properties ranging from provision of oncotic pressure, immune regulation and endothelial stabilization to being a molecule that works in the intracellular compartment modifying several key pathophysiological mechanisms (reviewed in Garcia-Martinez *et al.*) [1]. It has been hypothesized that the pleiotropic effects of albumin in cirrhosis and its proven effectiveness in spontaneous bacterial peritonitis, prevention of post paracentesis circulatory dysfunction, hepatorenal syndrome and hepatic encephalopathy relates to these functional characteristics rather than simple volume expansion [1]. Indeed, albumin administration, but not hydroxyethyl starch, improves systemic hemodynamics in patients with spontaneous bacterial peritonitis by decreasing endothelial activation [2]. Moreover, in experimental cirrhosis in rats, albumin exerts a positive cardiac inotropic effect counteracting oxidative stress- and TNF- $\alpha$ -induced impairment of cardiac contractility [3]. Given the pathophysiological importance of cirrhotic cardiomyopathy in hepatorenal syndrome, it is likely that the beneficial effects of albumin in this setting are mediated, at least in part, by these mechanisms. The paper in the current issue of the *Journal of Hepatology* by Oettl *et al.* adds to that growing literature by suggesting that albumin may be irreversibly oxidized in advanced cirrhosis, and this reduction in albumin function predicts mortality [4].

Albumin is 67 kDa protein and because of its amino acid composition it has a negative net charge at pH 7, which makes it soluble and imparts its oncotic properties. From the functional

perspective, albumin can be considered to have three essential classes of functional domains. It has a metal binding domain, which gives it the property to bind to and remove highly toxic reactive metal species. Assays, which measure binding of cobalt to albumin have been developed and the molecule defective in this function is known as 'ischemia-modified albumin (IMA)'. The second is a group of domains that bind a variety of substances such as long-chain fatty acids, bilirubin, anions, bile acids, endotoxin, hormones, and eicosanoids. The function of these binding domains can be studied using electron paramagnetic resonance spectroscopy (EPR), which essentially measures the binding of and release characteristics of albumin using labeled stearic acid [1,5]. Studies of the functional capacity of albumin in cirrhotic patients using these techniques were described recently. Albumin functional impairment closely correlated with the degree of liver insufficiency. The IMA/total albumin ratio was significantly higher in non-surviving patients with acute-on-chronic liver failure (ACLF) leading to the concept of 'effective albumin concentration', which is dependent upon the functional characteristics of albumin rather than its quantity. Importantly, the investigators showed that albumin dialysis did not restore the functional ability of the native molecule suggesting that albumin in liver failure may be irreversibly destroyed [5]. The third major function of albumin is its thiol function, which is imparted by a free cysteine molecule. It is this property that was the main focus of the article by Oettl *et al.* [4].

The free cysteine (Cys-34) residue provides a single free redox-active thiol (-SH). This is capable of reducing oxidative stress through thiolation, nitrosylation, and oxidation. In healthy individuals, albumin exists mostly in a reduced form (with a free thiol). This is referred to as mercaptoalbumin (HMA). A small proportion of albumin exists as mixed disulfide compounds known as non-mercaptalbumin 1 (HNA1), representing a molecule that has lost its anti-oxidant property but can be returned to the HMA form using a reducing agent. In health, a tiny fraction is found in a highly oxidized form with cysteine 34 (as sulfenic or sulfonic acid) known as non-mercaptalbumin 2 (HMA2). It is the sulfonic form that is thought to constitute an irreversibly damaged albumin [1,6]. These three different fractions of albumin can be isolated and quantified by high performance liquid

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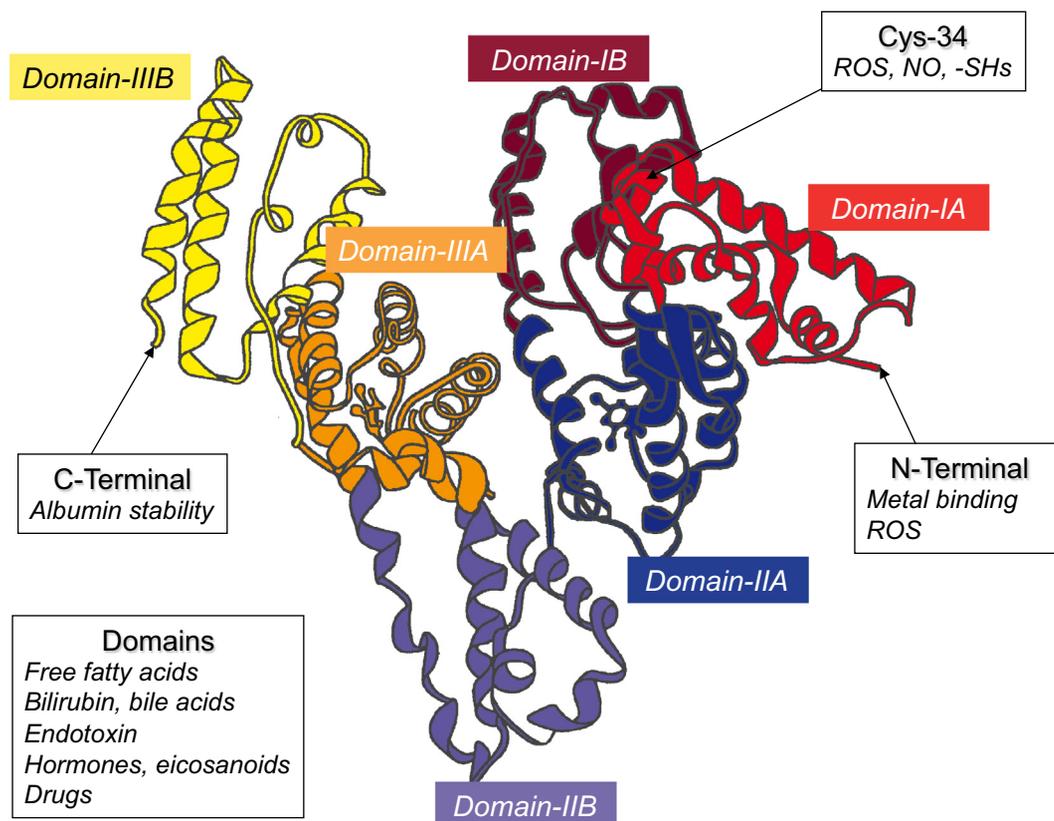
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**Fig. 1. Schematic representation of the albumin molecule illustrating the main binding domains.** Each site/domain is devoted to specific binding functions; the main ligands are also concisely reported. Cys-34: free cysteine in position 34; N-terminal: aminotermius; C-terminal: carbon terminal.

chromatography (HPLC). During aging [7] and different diseases, such as end-stage kidney disease [8] and liver failure [6], the fractions of the oxidized forms are increased. The paper by Oettl *et al.* extends this observation and shows for the first time that the proportion of HNA2 measured in the plasma of patients with advanced cirrhosis may predict mortality [4].

The authors prospectively studied 67 patients who were admitted to the hospital with acute decompensation of cirrhosis of whom 18 patients were in the intensive care unit. In addition to the clinical parameters, they measured albumin function, specifically, HMA, HNA1, and HNA2 using HPLC, i.e., looking at the free cysteine status and they also measured the binding characteristics of albumin to danylsarcosine, which looks at the functional activity of binding site 2 using HPLC as well. They found that the concentration of both oxidized forms were significantly increased in the cirrhotic patients and the value of HNA2, in particular correlated closely with the MELD score, bilirubin, INR, and CRP, confirming that the values were reflected by the severity of liver disease and associated inflammation, both of which have been identified as predictors of ACLF [9,10]. Therefore, it was not surprising and important that they found a high predictive ability of HNA2 in determining 30- and 90-day mortality. In fact, the prediction of 30-day mortality was possibly higher than MELD score. A cut-off of 12% was considered optimal and identified a cohort with a very low survival rate. They observed a correlation between this and danylsarcosine binding which may suggest that the structural change due to oxidation of albumin

may have non-specific effects on other functional characteristics of albumin. They then validated these data in a second cohort of 40 similar patients from Innsbruck. Finally, in a small sample of 3 patients, they extended their findings using mass spectrometry and confirmed that the HNA2 fraction represented an irreversible damage to albumin as the modification of the Cys-34 was in the sulfonic acid form [4]. The data from this study significantly adds to the weight of the argument that albumin dysfunction is likely to be pathophysiologically important [1,4]. It would be useful to know in a larger cohort of patients whether HNA2 is a surrogate marker of ACLF or independent of it and whether changes in its concentration occur in the patients who deteriorate in the hospital (Fig. 1).

These data provide a possible explanation why the current albumin dialysis systems, such as the molecular adsorbents recirculating system (MARS) or Prometheus, have not been shown to improve the survival of patients with liver failure [11,12]. The concept these devices are based upon is the potential reversibility of albumin dysfunction in liver failure as the damaged albumin is not removed and remains in the circulation. In MARS, the patient's blood is dialysed against albumin across a membrane with a 55 kDa cut-off [11]. Therefore, if the patient's albumin is irreversibly destroyed, it cannot be regenerated. In Prometheus, the patient's is simply cleaned by filters in the secondary circuit [12]. An irreversibly destroyed albumin will therefore not be regenerated. By contrast, plasma exchange, which allows removal of the damaged molecule, has been recently shown to improve

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survival in acute liver failure patients but is limited by non-selectivity [13]. Based upon these data, newer devices that address albumin exchange are in development [14].

The data in this study generates numerous new questions. It is not clear whether the increased concentration of HNA2 detected in the patients who will die is only a marker or whether this may actually be harmful to cell function. The mechanism of formation of HNA2 remains to be defined. In the test tube, HNA2 can be generated by the addition of hydrogen peroxide. Therefore, it is likely that inflammation and the associated oxidative stress can produce HNA2 *in vivo*. An alternative question is whether the stressed hepatocyte is producing a functionally damaged albumin due to cellular stress leading to alterations in the folding of the albumin molecule. This will need to be investigated in future studies. The authors have clearly identified a potential biomarker. Larger numbers of patients will be required to confirm their data and it will be important to simultaneously study all the known albumin functions to tease out whether more than one function will further improve its prognostic ability. In order for this to happen, a simpler bedside test will need to be developed. Finally, it will be important to answer whether the concentration of HNA2 changes with either deterioration or improvement of liver function and whether instead of aiming at a target concentration of albumin, one should be aiming at a 'functional concentration' of albumin, i.e., a goal directed approach [1].

In conclusion, on-going research in the field of albumin biology in liver failure has opened up new translational possibilities which will impact on finding new indications for albumin use, functional characterization to define 'effective albumin concentration', and the use of these concepts to design new albumin dialysis systems.

### Conflict of interest

Rajiv Jalan has ongoing research collaboration with Grifols, a company that manufactures albumin and with GAMBRO a company that makes an albumin dialysis device, MARS. He has received lecture fees from Grifols. UCL have filed patents around the UCL-ARSENEL device.

Mauro Bernardi has received fees, as consultant and lecturer, by CLS Behring GmbH, a Company that manufactures albumin, as lecturer, by the Plasma Protein Therapeutics Association (PPTA) Europe, which represents the private sector manufacturers of

plasma-derived and recombinant analog therapies, and as lecturer, by Grifols Italia S.p.A.

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