**Journal of Hepatology**

**LONG-TERM ALBUMIN TREATMENT IN PATIENTS WITH CIRRHOSIS AND ASCITES**

---Manuscript Draft---

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<td>First Author:</td>
<td>Paolo Caraceni, MD</td>
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**Abstract:** Although proposed for the first time several decades ago, the possibility that long-term human albumin can be effective for treating patients with cirrhosis and ascites has become a topic of scientific and clinical discussion in the last decade. This review will critically analyze the data available highlighting the differences existing between studies, the controversial issues and the future perspectives related to such a treatment. Long-term albumin administration to patients with cirrhosis and ascites represents a completely different treatment perspective as compared to acute or short-term uses of albumin. Results from the ANSWER and the MACHT studies indicate that long-term albumin treatment can be effective, safe and able to modify the course of the disease provided that albumin is given in a sufficient amount and for a sufficient time to restore physiological levels and presumably functions of the circulating molecule, which are compromised, at least partially, in patients with decompensated cirrhosis. However, the discordant findings with other studies and several additional issues call for the critical need of further clinical studies and randomized trials to confirm the benefits of long-term albumin therapy on clinical outcomes. Other important areas for further research include the definition of the target population stratified according to the expected outcomes, biomarkers of response, the optimal dose and frequency of albumin infusions, stopping rules, and the cost-effectiveness of treatment in the different health-care systems worldwide, particularly in those where the logistic issues and costs related to periodic intravenous infusions can represent an important limitation to the implementation of this innovative approach in the clinical practice.

**Response to Reviewers:**

Ref: JHEPAT-D-21-02529  
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Dear Editors,

we thank the reviewers and editors for their comments. We substantially agree with their criticisms and the manuscript has been changed accordingly. In particular, the length of the manuscript has been cut more than 500 words and the parts on mechanisms of action and acute uses of albumin greatly shortened.

We hope that the revised manuscript will now meet your approval.

Kind regards,
On behalf of all co-authors,

Paolo Caraceni

Point by point response to the reviewers:

Reviewer #1.

This is a review about long-term albumin use in patients with cirrhosis and ascites. The Authors comprehensively reviewed and interpreted the current evidence. They also highlighted areas of uncertainties and provided suggestions for future research in this field. I have some minor comments:

1) Paragraph "Why albumin functions are useful in patients with cirrhosis and ascites". It would be better rephrasing "Why albumin infusion is useful in patients with cirrhosis and ascites".

The title of the paragraph has been rephrased. Hopefully the change will meet the reviewer's approval.

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A sentence describing the main finding of the study has been added.

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4) Comments about albumin threshold to be reached to achieve clinical benefits (page 17). Here the Authors seem to suggest that a 4 g/dl threshold has to be reached to maximize the benefits of albumin. However, a note of caution is mandatory here. Physicians could be tempted to give high doses of albumin in a short time to reach the 4 g/dl threshold, but the ATTIRE trial showed that this practice could be harmful leading to circulatory overload/pulmonary edema. Therefore any albumin threshold should be reached in the mid-term (weeks) instead of short term (days). Moreover, further prospective studies are necessary to prove that this threshold can be safely achieved and can maximize albumin benefit.

We fully agree with the reviewer. The following paragraph has been added in the text: "It also emerges that the target level should be reached in weeks and not days to avoid the risk of volume overload particularly in patients who appear more prone to develop this complication due to predisposing conditions".

5) "Challenges and open issues" paragraph. Albumin proved to be effective in patients with ascites and a study comparing long term albumin administration versus other strategies (e.g., transjugular intrahepatic portosystemic shunt) would be very interesting.

We agree with the reviewer and therefore a new paragraph dealing with this issue has been added in Chapter 6: "It would be also important to perform studies comparing
long-term HA treatment with transjugular intrahepatic portosystemic shunt (TIPS). Research questions to be answered are: which are the patients in which one treatment is superior to the other? can long-term HA and TIPS be not mutually exclusive and instead part of a sequential approach for optimizing the global management of patients with ascites?"

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The reviewer is right. We have changed the number of the reference.

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Caraceni et al. provided a detailed review on the potential benefit of long-term benefit of human albumin (HA) in patients with cirrhosis and ascites. The review is well-written, detailed and interesting for a broad spectrum of readers in the hepatology field. They discussed adequately to two main RCT in the topic (ANSWER and MACHT) and controversies. They opened new research perspectives. Comments:

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We agree with both comments of the reviewer. We have therefore summarized both parts on the acute/short-term use and the mechanisms of action of albumin by cutting more than 500 words. However, we have decided to leave a separate paragraph on acute/short-term treatments, mostly dedicated to the ATTIRE study, since we believe that one of the main message of this review is to highlight the different perspective of long-term albumin treatment from the current short-term uses.
February 26, 2022

Prof. Paolo Angeli  
*Editor in Chief, Journal of Hepatology*

Prof. Vincent Wong  
Prof. Jean-Francois Dufour  
Prof. Robert Schwabe  
Prof. Norah Terrault  
*Guest Editors, Supplement on “Therapeutic Breakthrough in Hepatology”*

Dear Editor and Guest Editors,

on behalf of my co-authors, I am submitting the revised version of the invited review entitled “Long-term use of albumin in patients with cirrhosis and ascites” by P. Caraceni, A. O’Brien and P. Gines.

We thank the reviewers and the editor for their comments.

We have shortened the manuscript by more than 500 words and reviewed the text according to their criticisms and suggestions.

A detailed point-by-point response to the comments of the editor and reviewers has been provided.

All the listed authors have contributed actively and approved the submitted manuscript.

We hope that the manuscript will meet yours and reviewer’s expectations.

Yours sincerely,

Paolo

Paolo Caraceni, M.D.  
*Associate Professor of Internal Medicine*  
Alma Mater Studiorum University of Bologna, Italy  
paolo.caraceni@unibo.it
Journal of Hepatology
Revised Submission Checklist

This form must be completed and submitted for all revised manuscripts. Without this form the manuscript will be returned to the corresponding author for completion.

Corresponding Author: Paolo Caraceni
Manuscript Number: 21-02529-1

Below, provide the page number(s) or figure legend(s) where the information can be located. Please make sure that all the information requested below is present in the manuscript.

1) Submission

a) Title page: COI, Financial support, Authors’ contributions, keywords.  
Y

b) Structured abstract and lay summary  
Y

c) All tables and figures included, numbered correctly, with legends (p value and statistical test)  
Y

d) Supplementary data included in a single, separate word file  
N

e) A detailed point by point response to reviewers comments and changes highlighted in text  
Y

f) All authors to complete and upload an ICMJE conflict of interest form.  
Y

g) Graphical abstract

2) Materials and methods

a) Completed the CTAT form for all reagents and resource to be added to supplementary material  
NA

b) Identify the source and authentication of cell lines  
NA

c) Identify animal species, number of animals used, strain, sex and age.  
NA

d) For animal studies include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.  
NA

e) For qPCR data provide information according to the Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) guidelines  
NA

3) Human subjects

a) Identify the committee(s) approving the study protocol.  
NA

b) Include a statement confirming that informed consent was obtained from all subjects.

Completed, or reported on page(s) or figure legend(s):

Updated version November 2018
c) For randomized studies report the clinical trial registration number (at ClinicalTrials.gov or equivalent).

d) For phase II and III randomized controlled trials:
   I. Please refer to the CONSORT statement and submit the CONSORT checklist with your submission.
   II. Include all version of the study protocol and statistical plan (to be published as supplementary information)

e) Identify the inclusion/exclusion criteria in the selection process for the patients included in the study

4) Statistics
   a) State what statistical tests were completed and why
   b) Explain the sample size and how this size provides an adequate power to detect a pre-specified effect size.

5) Data deposition (Provide accession codes for deposited data)
   a) When using public databases:
      I. Identify the source and include a valid link
      II. When using databases that require permission, include a statement confirming that permission was obtained
   b) Data deposition in a public repository is mandatory for:
      I. Protein, DNA and RNA sequences
      II. Microarray data

Deposition is strongly recommended for many other datasets for which structured public repositories exist
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LONG-TERM ALBUMIN TREATMENT IN PATIENTS WITH CIRRHOSIS AND ASCITES

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*all authors contributed equally to the manuscript

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ABSTRACT

Although proposed for the first time several decades ago, the possibility that long-term human albumin can be effective for treating patients with cirrhosis and ascites has become a topic of scientific and clinical discussion in the last decade.

This review will critically analyze the data available highlighting the differences existing between studies, the controversial issues and the future perspectives related to such a treatment. Long-term albumin administration to patients with cirrhosis and ascites represents a completely different treatment perspective as compared to acute or short-term uses of albumin. Results from the ANSWER and the MACHT studies indicate that long-term albumin treatment can be effective, safe and able to modify the course of the disease provided that albumin is given in a sufficient amount and for a sufficient time to restore physiological levels and presumably functions of the circulating molecule, which are compromised, at least partially, in patients with decompensated cirrhosis. However, the discordant findings with other studies and several additional issues call for the critical need of further clinical studies and randomized trials to confirm the benefits of long-term albumin therapy on clinical outcomes. Other important areas for further research include the definition of the target population stratified according to the expected outcomes, biomarkers of response, the optimal dose and frequency of albumin infusions, stopping rules, and the cost-effectiveness of treatment in the different health-care systems worldwide, particularly in those where the logistic issues and costs related to periodic intravenous infusions can represent an important limitation to the implementation of this innovative approach in the clinical practice.

Abstract word count: 257
KEY-POINTS

- Albumin plays an essential role in human physiology.

- Both oncotic and non-oncotic properties of the albumin molecule are likely important for antagonizing key events in the pathophysiology of decompensated cirrhosis, such as circulatory dysfunction and systemic inflammation.

- In patients with decompensated cirrhosis, circulating albumin is low; moreover, albumin is damaged and dysfunctional.

- Long-term albumin administration to patients with cirrhosis and ascites represents a completely different treatment perspective as compared to acute or short-term uses of albumin.

- Long-term albumin administration appears to be an effective and safe treatment able to modify the course of the disease. It could be speculated that treatment benefits occur when albumin is given in a sufficient amount and for a sufficient length of time to restore the physiological levels and presumably functions of the molecule.

- Additional randomized clinical trials are needed to confirm the positive effects of long-term albumin administration on clinical outcomes

- Areas for future research include the definition of the target population stratified according to the expected outcomes, the biomarkers of response, the optimal dose and frequency of albumin infusions, the stopping rules, and the cost-effectiveness of treatment in the different health-care systems worldwide.
Human albumin (HA) administration is one of the most studied interventions in patients with decompensated cirrhosis (1). Randomized clinical trials (RCTs) have produced controversial data on the efficacy and safety of HA, likely as a consequence of the great variance in terms of indications, experimental design, type of patients enrolled, length of treatment, and dosage and frequency of infusions. Current HA indications are for acute or short-term (maximum 2 weeks) administration. Although proposed for the first time several decades ago, the possibility that HA can be administered for much longer to treat patients with ascites has become a topic of scientific and clinical discussion only in the last years.

This review will critically analyze the data available, the controversial issues and the future perspectives related to long-term HA treatment, highlighting the differences existing between studies and the other short-term uses of HA administration in patients with decompensated cirrhosis.

1. The albumin molecule in patients with decompensated cirrhosis

Albumin is synthesized by hepatocytes and continuously secreted into the circulation, without being stored in the liver (2). Albumin has a concentration of 3.5-5 g/dl and accounts for more than 50% of the total circulating protein content. It has a half-life of about 20-days in healthy adults and is continuously taken up and recycled by hepatocytes (3,4).

Albumin accounts for approximately 75% of plasma oncotic pressure, due to its high concentration and net negative charge and is therefore principally responsible for fluid distribution within the body's compartments (5). Albumin also has many other biological functions termed non-oncotic properties. It reversibly binds many molecules, including drugs, metallic ions, and multiple inflammatory mediators, potentially affecting systemic inflammation, immune response, antioxidant capacity and endothelial function (3,4) (Figure 1).
In patients with decompensated cirrhosis, the albumin molecule undergoes both quantitative and qualitative changes. Hypoalbuminemia has been considered a marker of advanced liver disease for decades. It correlates with the severity of cirrhosis and independently predicts the poor outcome of these patients (6,7). Hypoalbuminemia results mainly from the reduced synthesis in the diseased liver and the enhanced catabolism due to the structural alterations of the molecule; however, the hemodilution related to the expanded total plasma volume also contributes to its reduced plasma levels (4,8,9).

Besides hypoalbuminemia, it has become evident during the last decade that the persisting inflammatory state of advanced cirrhosis induces molecular, structural and conformational changes of albumin that adversely affect its binding, transport and detoxification capacities (4,9). Albumin circulates predominantly in a reduced state with the free thiol group at the cysteine-34 (Cys-34) residue acting as a free radical scavenger for reactive oxygen and nitrogen species (3). Oxidative damage of the Cys-34, which can occur alone or in combination with other molecular changes, represents the most frequent post-translational alteration (10-12). Other structural changes include the truncation of N-terminal and C-terminal portions and the glycosylation of the molecule (11,12). As a result of these damages, albumin in decompensated cirrhosis becomes dysfunctional, showing an impairment of binding, detoxification and antioxidant activities, which parallels the severity of the disease (12,13). Damaged albumin isoforms may even be harmful since oxidized molecules have been shown to activate immune cells and promote inflammation (14,15) (Figure 1).

These findings have led researchers to propose the concept of an “effective albumin concentration”, which implies that the global function of albumin, resulting from both oncotic and non-oncotic properties, is related not only to its quantitative circulating level, but also to the preservation of its structure (12,16). As damage accumulates, the proportion of the albumin
molecules maintaining a fully preserved structure declines according to the severity of the disease (11,12). Interestingly, effective albumin concentration appears to discriminate the different stages of cirrhosis and predict outcomes significantly better than the total serum albumin concentration routinely measured by standard laboratory methods in daily clinical practice (12).

The combination of low circulating concentration of albumin and its dysfunctional quality observed in patients with decompensated cirrhosis provides the rationale for exogenous HA infusions aiming to restore the major physiological functions of the molecule.

2. Pathophysiological rationale for the use of albumin in patients with decompensated cirrhosis

Studies on the use of HA infusions were first reported more than 70 years ago (17), and all international guidelines currently recommend HA as the fluid of choice for volume expansion in patients with cirrhosis and ascites (18-19). Indeed, HA has been consistently shown to improve effective hypovolemia, reduce the activity of vasoconstrictor systems and increase mean arterial pressure in patients undergoing large-volume paracentesis (LVP) or suffering spontaneous bacterial peritonitis (SBP) or hepatorenal syndrome (HRS) (20-23).

Clinical and experimental data have raised the possibility of HA infusions having other beneficial properties beyond volume expansion (4,16,24). HA administration in patients with SBP improves systemic hemodynamics through mechanisms not directly related to volume expansion, but consistent with improvement of endothelial function (25,26). HA also appears to improve cardiac contractility in an experimental model of isolated perfused rat heart by reducing the activation of inflammatory mediators in the cardiac tissue (27). Furthermore, cirrhosis associated prostaglandin E2-mediated immune dysfunction was improved following HA infusion (28,29) and analyses of samples from two trials in cirrhosis patients demonstrated that HA is able to reduce systemic
inflammation (25,30). Finally, recent experimental evidence indicates that HA internalizes in immune cells and modulates their responses through interaction with endosomal toll-like receptor signalling (31).

Therefore, the potential benefits resulting from both oncotic and non-oncotic properties of the molecule provide the rationale for exogeneous HA infusions aiming to counteract the two major pathogenic drivers of poor outcome in patients with decompensated cirrhosis, namely effective hypovolemia and systemic inflammation/immune dysfunction (32) (Figure 2).

3. Long-term albumin treatment in patients with ascites

Five to ten percent of patients with compensated cirrhosis develop ascites every year, which represents the most frequent decompensating event of cirrhosis (1,33-35). Moderate and massive (grade 2 and 3) ascites usually requires long-term treatment, leads to recurrent hospitalizations, also caused by related complications (i.e., SBP, HRS, abdominal hernias, and restrictive ventilatory dysfunction), and impairs patient quality of life (36). Therefore, the contribution of ascites to the heavy health economic burden of decompensated cirrhosis is highly relevant (37). Finally, the development of ascites has a dramatic negative impact on patient prognosis, as the 1, 2 and 5-year mortality is approximately 30%, 50%, and 70%, respectively (34).

Based on its oncotic activity, chronic use of HA to treat ascites was proposed many decades ago, but the studies, uncontrolled and/or very small sized, failed to show a clear benefit (38,39). After almost 40 years, two RCTs, enrolling a relatively small number of patients, showed a better response of ascites to HA in addition to diuretics during hospitalization (40) and a significantly lower recurrence of grade 2-3 ascites associated with higher transplant-free survival in the group receiving long-term HA for a median follow-up of 84 months (41). More than 10 years after these pivotal studies, three clinical trials have been published in 2018 (42-44).
3.1 The ANSWER trial

The ANSWER study (42), a non-profit, Italian multicenter, open-label, pragmatic RCT, enrolled 431 patients with persisting non complicated grade 2 and 3 ascites requiring the combination of an anti-mineralocorticoid drug (at least 200 mg/day) and furosemide (at least 25 mg/day) to receive either standard medical treatment (SMT) or SMT plus 40 g of HA twice a week for the initial 2 weeks and then 40 g once a week.

HA administration improved the management of ascites, as the need of large-volume paracentesis and the incidence of refractory ascites decreased by about 50%. The incidence rate of other complications (i.e., SBP, non-SBP bacterial infections, hepatic encephalopathy (HE) grade III or IV, HRS type 1, renal dysfunction [serum creatinine >1.5 mg/dl], moderate hyponatremia or hyperkalemia) decreased also by 30-67%, leading to lower liver-related hospitalizations and days spent in hospital per year as compared to the control group, which were reduced by 35 and 45% respectively. A significantly better 18-month overall survival, which was the primary end-point of the study, was observed in patients receiving albumin, with a 38% reduction of the hazard ratio for mortality. The multivariable risks analysis for 18-month all-cause mortality considering TIPS placement or liver transplantation as competing events showed that HA treatment was the sole variable associated with increased survival. Furthermore, patients receiving HA had a better quality of life and HA treatment proved to be also cost-effective based on the reimbursement rates from the Italian National Health Service as compared to standard therapy. Finally, side-effects were similar between the two groups and no episodes of volume overload related to HA occurred, and only very few mild allergic reactions to HA infusion were reported.

A post-hoc analysis has highlighted the importance of serum albumin concentration in the interpretation of the positive results of the study (45). With the schedule of HA administration followed in the ANSWER trial, serum albumin concentration increased from a median level of 3.1
g/dl to almost 4 g/dl after 1 month of treatment, and remained stable afterwards. In the control group, no rise above the baseline level was instead observed, so that the difference between the two groups (about 0.7-0.8 g/dl) was significant during the entire follow-up.

Furthermore, in patients undergoing long-term HA serum albumin concentration at 1 month of treatment, but not baseline serum albumin, directly correlated with the probability of 18-month overall survival, with the best discriminating cut-off to independently predict survival identified at 4 g/dl. The two baseline factors that independently predicted the achievement of this cut-off were serum albumin concentration and MELD score, so that the lower the baseline serum albumin or higher the MELD score, the lower the probability of reaching the threshold of serum albumin shown above (45).

The major limitation of the ANSWER study was of course related to its open label design. Although the absence of blinding reduces the internal validity of the study, the pragmatic design of the ANSWER trial could have produced an even higher external validity since other strengths, such as large sample-size, prolonged follow-up, and a hard primary endpoint, are satisfied. More important than blinding is the fact that weekly HA infusions led patients to be seen more frequently by health care professionals than those enrolled in the control group. Although patients in the ANSWER trial were usually not evaluated by physicians during the HA infusions and some of them received HA in residential services or even at home, it cannot be excluded a priori that the regular contacts with health care services and personnel may have produced a better general management, thus contributing to the improved outcomes. Again, the real-word assessment of the entire pathway of care related to the intervention under study is a core feature of pragmatic trials. It is also tempting to speculate that the need for regular intravenous infusions, if perceived by patients as beneficial to their health, could favor the adherence of such a challenging group of
patients to their overall pathway of care and incentivize them to overcome logistic limitations when present.

Finally, almost half of patients included in the ANSWER study had cirrhosis caused by hepatitis C, which remained untreated during the study, and patients with active alcoholism were not included. Thus, the effects of HA administration in those with alcohol-related cirrhosis and active drinking remains to be determined.

3.2 The “refractory ascites trial”

The core results of the ANSWER trial were confirmed by a prospective, non-randomized clinical trial performed in Padua, Italy, which enrolled 70 patients with cirrhosis and refractory ascites (43). Patients who received SMT + HA (20 g twice a week) had a significantly lower 24-month mortality than the 25 patients receiving the SMT. Treatment with HA was the sole independent protective factor of death and it was associated with a significantly lower cumulative incidence of re-hospitalizations due to HE, accumulation of ascites, and bacterial infections.

3.3 The MACHT trial

The midodrine and albumin for prevention of complications in patients with cirrhosis awaiting liver transplantation (MACHT) trial was a ground-breaking trial for two reasons (44). First, it is one of the first RCTs in patients with decompensated cirrhosis to evaluate the effect of combination therapy, midodrine (an alpha-adrenergic agonist) and HA, to improve outcomes of patients with cirrhosis; and second, it used a primary end-point that combines the most relevant complications of advanced cirrhosis (renal failure, hyponatremia, infections, HE and gastrointestinal bleeding).

Previous RCTs in cirrhosis had evaluated a single therapy, usually a drug, to treat or prevent a single complication of the disease (46). The rationale for the use of such combination of vasoconstrictor drug and HA was to normalize the impaired effective arterial blood volume thought to be responsible, at least in part, for some complications of cirrhosis (22,47,48). This
specific treatment combination was based on the positive results of midodrine together with HA in the treatment of HRS (49).

Unfortunately, the MACHT trial did not meet the primary endpoint and therapy with midodrine and HA was not associated with reduction in the incidence of complications or mortality in patients with decompensated cirrhosis awaiting liver transplantation. The only positive effect found was a moderate suppression of activity of renin-angiotensin and sympathetic nervous systems, suggesting a beneficial effect on circulatory function.

Speculation about possible causes of lack of efficacy of combined therapy in MACHT trial is challenging. One possibility is that midodrine, which has a relatively weak vasoconstrictor potency compared to that of terlipressin (22,50) did not produce a sufficient vasoconstriction in the splanchnic arteries and therefore was not capable of improving effective arterial blood volume sufficiently. Alternatively, but not mutually exclusive, the dose of HA used in the study (40 g every 2 weeks) could have been insufficient to produce the expected hemodynamic and non-hemodynamic effects of HA (25,32). In the treated group, serum albumin concentration increased from a mean of 30 g/L at baseline to a mean of 34 g/L at week 12. However, a similar increase was observed in patients from the placebo group, suggesting that the improvement was unrelated to therapy. Another possibility is that the duration of treatment was not long enough to cause a significant beneficial effect on patients. In fact, because many patients were transplanted quite rapidly during the study, the mean duration of treatment in the midodrine and albumin group was of less than 3 months (average 80 days). Finally, it is also theoretically possible that the hypothesis of the study was wrong. However, the hypothesis cannot be ruled out completely because the combined treatment fell short of achieving a normalization of effective arterial blood volume.

4. Acute and short-term treatment in patients with decompensated cirrhosis
HA treatment given from a single administration to a short-term course up to a maximum of 15 days, with the purpose of preventing or treating acute complications of decompensated cirrhosis, has been the main, if not the sole, use of HA for the last 40 years.

International guidelines consistently recommend HA infusion to prevent circulatory dysfunction after paracentesis and renal failure in patients with SBP or to diagnose and treat hepatorenal syndrome (HRS) (18,19). HA is also recommended by international guidelines in algorithms of diagnosis and management of acute kidney injury (AKI) and hypervolemic hyponatremia although solid evidence from clinical studies is still limited (18,19,51,52). In contrast, no benefits in survival were observed in RCTs assessing HA administration in patients with infections other than SBP (30,53,54). Noteworthy, HA infusion significantly increased the risk of pulmonary edema in one of these studies (54). Negative results were also observed in patients presenting with an acute episode of hepatic encephalopathy (55).

Finally, the results of a large multicenter, open label, randomized, controlled trial assessing short-term HA administration to prevent complications in hospitalized patients have been recently published (56). In the ATTIRE (Albumin to Prevent Infection in Chronic Liver Failure) study, 777 patients with cirrhosis hospitalized with a decompensating even were included in the analysis. The treatment arm received daily intravenous infusions of HA to increase and maintain a serum albumin level of at least 3.0 g per liter throughout the trial treatment period of up to 14 days. The standard-care (control) group received HA only as recommended by guidelines (18,19), after large-volume paracentesis, during SBP, or for HRS. There were no significant differences in the composite primary end-point (infection, renal failure, or death) between treatment and control groups, despite the HA group patients receiving a significantly much higher HA dose compared to those in standard care. The HA group also had more severe or life-threatening serious adverse events, especially pulmonary oedema or fluid overload.
The trial’s greatest strength was the large number of patients recruited and multiple sites involved. Other strengths were that patients were recruited very soon after hospitalization (on average one day) and sub-group analyses including a time to event and threshold analysis that included missing data. Finally, crucial for an open-label trial ATTIRE achieved substantial differences in the amounts of 20% HA infused between the albumin treated group and control patients especially during the early part of the trial when an increased albumin level might have been expected to have benefit.

ATTIRE had several limitations, most obviously it was not blinded. 90% of recruited patients had alcohol-induced cirrhosis and results may differ for other causes of liver disease. The components of the composite end point (infection, renal dysfunction, and mortality) were not equivalent in severity; however, these do represent a common disease trajectory and move in line with each other and the 3 and 6-month mortality outcomes were also null. Inevitably in a trial of this size there was heterogeneity in patients recruited in terms of infection, antibiotic treatment and organ dysfunction and it is possible that a specific group of patients might have benefited that were not examined in the subgroup analyses. However, no biomarkers or clinical features that predict potential benefit from albumin infusions have been identified to enable such an approach to date. Thus, the results of this study do not support the use of HA in patients admitted for worsening or onset of a complication of cirrhosis with the aim of preventing the development of further complications of the disease during hospitalization.

5. Long-term albumin treatment: what we have learnt so far?

It appears evident that the data on efficacy and safety of HA are quite heterogeneous, so that some clinicians and researchers emphasize the benefits of HA in many conditions of
decompensated cirrhosis, whereas others are against any extension of its use beyond the few well-established indications.

A strong scientific debate is currently ongoing regards long-term HA administration in patients with ascites, which represents not only a novel indication, but also carries logistic and economic issues inherent to the need for chronic periodic intravenous infusions that render its applicability in clinical practice problematic.

It would be foolish to propose long-term HA administration for all patients with ascites who can be quite different in terms of clinical phenotypes and prognosis. As for any other intervention, the objective should be to define the target sub-group populations for whom the benefits are significant, the modalities of administration in terms of dosage, frequency and length of treatment, the assessment of response, and the absolute and relative contraindications.

At present, the clinical phenotype of cirrhosis who can benefit from long-term HA treatment appears to be represented mostly by patients with relatively stable conditions and at least grade 2 non complicated ascites despite a moderate dosage of diuretics. Patients who had recently resolved an acute complication of the disease yet still presenting with ascites are also amenable to treatment. For these types of patients, administration of long-term HA has been recently included among the medical treatment options for the management of ascites by the Italian Association for the Study of the Liver (AISF) (57). These recommendations suggest the use of long-term HA also in refractory ascites, because by adding HA some of the patients may become responsive to diuretics (43). It should be acknowledged, however, that Italy represents a sort of “unicum” with respect to other countries since long-term HA treatment is reimbursed by the National Health System and is currently standard of care in many hepatological centers.

A second important issue regards the modalities of treatment. At present, the data available indicate that two conditions, strictly interrelated, need to be achieved in order to optimize long-
term HA treatment: administering enough HA to have an impact on serum albumin concentration and for enough time to unveil the clinical benefits.

The first assumption is based on the comparison between the ANSWER and the MACHT trials (42,44) (Figure 3). While HA administration in the MACHT trial did not significantly influence serum albumin concentration, which remained almost identical to that of controls, this was not the case of the ANSWER patients who present a significant increase up to almost a median of 4 g/dl after 1 month of treatment to remain thereafter significantly higher than controls throughout all the 18 months of follow-up. These divergent findings were likely due to the lower amount of HA infused in the MACHT trial, which was less than half of the amount received by the ANSWER patients.

If increasing serum albumin concentration is needed for the therapy to be effective, the question arises on which is the target level to achieve during treatment. A post-hoc analysis of the ANSWER database provides interesting information to clarify this issue (45). First, the percentage of patients with normal serum albumin concentration (>3.5 g/dl) increased from the baseline 25% to almost 80% after 1 month of treatment. Second, serum albumin concentration reached after 1 month of treatment independently and directly correlated with the 18-month survival. Third, the best discriminating cut-off level of serum albumin concentration between patients receiving or not HA was 4 g/dl.

Thus, it appears that normalizing serum albumin concentration should be the target to obtain good clinical outcomes and a maximal benefit is reached with levels around 4 g/dl. Other observations support this assumption. The pilot PRECiosa trial, a pathophysiological study comparing the effects of high (1.5 g/kg every week) versus low (1 g/kg every 2 weeks) doses of HA given for 12 weeks in patients with decompensated cirrhosis and severe circulatory dysfunction, showed that the low dose protocol produced a significant increase of serum albumin level concentration without reaching the normal range, while the high dose protocol was able to
normalize serum albumin in all patients with a median level close to 4 g/dl (25). Interestingly, only patients receiving high doses of HA presented a significant improvement of cardiocirculatory dysfunction and systemic inflammation (25). Furthermore, even though the normal lower limit of serum albumin concentration has been set at 3.5 g/dl, more than 90% of healthy subjects of ages up to 80 years old present a serum albumin concentration higher, often quite higher, than 4 g/dl (58). Thus, the real physiological level of albumin in healthy individuals is at least around 4 g/dl.

The second assumption is that the benefits of long-term HA become evident after weeks of treatment, usually 1-2 months. Interestingly, in the ANSWER trial, the Kaplan-Meier curves of survival and refractory ascites (and also those of several other secondary end-points [Caraceni, personal communication]) started to diverge after 1-2 months of treatment once the increase in the albumin concentration had occurred and stabilized. The negative results of the ATTIRE trials provide an indirect confirmation to this hypothesis. The median length of HA treatment in the ATTIRE patients was only 8 days and, although the individualized protocol of administration significantly increased the very low baseline median serum albumin concentration (2.3 g/dl), it was not able to correct hypoalbuminemia, as the median serum albumin concentration reached a level little above 3 gr/dl throughout the entire 14-day follow-up (56). Along with these reasoning, it could be proposed the need of higher doses of HA in these very sick patients, which, however, would likely lead to an unacceptable risk of pulmonary edema if given in a short timeframe.

Thus, it can be concluded that long-term HA represents a completely different treatment paradigm compared to all other acute or short-term uses (Table 1). Acute or short-term treatment can last one or more days, but no longer than 2 weeks. They are usually applied in hospitalized patients (except in some of the patients subjected to after LVP or presenting AKI), either in regular wards or ICUs, but often very sick, with the goal of treating or preventing a specific acute complication. As treatment needs to become rapidly effective, high amounts of HA are often
infused in a relatively short time, thus raising safety issues related to volume overload at least in some complications, such as non-SBP bacterial infections (54) or HRS (59), and in patients admitted to hospital for an acute decompensation of the disease, as documented in the ATTIRE trial (56).

In contrast, long-term HA treatment lasts at least weeks, usually months or even years; it is usually initiated in relatively stable outpatients, but it can be started in hospitalized patients once complications are resolved, with the goal of controlling ascites and preventing other complications, thus modifying the course of the disease. HA doses are lower than those for acute indications and are distributed over a much longer time, thus making treatment safe. In this regard, no cases of volume overload and pulmonary edema have been described in the clinical trials assessing long-term administration (42-44), in contrast to the significantly higher incidence reported in some of the studies evaluating short-term HA treatment (54,56,59). The ATTIRE and ANSWER trials are examples of short- and long-term HA treatment, respectively (Table 2).

Based on all these considerations, it could be hypothesized that the goal of long-term HA administration should be to restore the physiological functions of albumin (both oncotic and non-oncotic properties), which are active against effective hypovolemia and systemic inflammation and are instead partially lost in patients with cirrhosis with ascites (4,9,32). In practical terms, the goal of long-term treatment should be filling the gap existing between the baseline patient serum albumin concentration and the on-treatment target serum albumin concentration corresponding to the physiological levels observed in healthy individuals (58) (Figure 4). As the extent of this gap is variable depending mostly on the starting level of serum albumin and on the severity of cirrhosis (45), the need emerges to go beyond a fixed dosage and schedule of HA administration - as used in the ANSWER study - to a more individualized treatment. It also emerges that the target level
should be reached in weeks and not days to avoid the risk of volume overload particularly in patients who appear more prone to develop this complication due to predisposing conditions.

In this perspective, the time-course changes of serum albumin concentration together with the clinical response in controlling ascites could be used as a guide to maximize the benefits of treatment, define stopping rules and optimize HA utilization. Further investigations are warranted to support this hypothesis.

6. Challenges and open issues of long-term albumin treatment

A number of important questions remain to be answered in clinical research on long-term HA administration in decompensated cirrhosis (Table 3). Investigation on HA is hampered by the lack of an objective biomarker of the effect of therapy. This is in turn responsible, at least in part, for the lack of dose-finding trials. In fact, all RCTs evaluating the efficacy of HA in different indications were performed using arbitrary doses, except for the ATTIRE trial (56), in which a preliminary study was performed to assess the dose required to increase serum albumin levels above 3 g/dL (60).

Which candidate biomarker should be used to assess response to therapy? Should this biomarker be serum albumin levels? In this regard, the post-hoc analysis of the ANSWER trial showed that normalization of serum albumin concentration at one month of therapy was associated with better outcomes and increasing serum albumin levels correlated with higher survival rates (45). However, the possible usefulness of effective albumin concentration reflecting the portion of the albumin molecule pool with normal structure and function merits also to be explored (12,61). Or should this biomarker be related to some desirable beneficial effects on systemic inflammation, circulatory or liver function, such as C-reactive protein, fatty-acid binding proteins, copeptin or cell death markers, to cite a few (10,62-65).
The availability of biomarkers in clinical practice would also help physicians to answer some other open questions related to long-term treatment: what is the minimum dose of HA and intervals between infusions that are effective? which are the stopping rules? when patients no longer need HA due to the improvement of their clinical conditions or treatment becomes instead futile?

Another clinical issue requiring further investigation regards the more precise definition of the target population that can benefit from long-term HA therapy. Comparison of patients from ANSWER and MACHT trials indicate that patients from the former trial had less advanced cirrhosis as indicated by lower median MELD scores (12-13 vs 17-16, respectively). Therefore, the possibility exists that HA is less efficacious in patients with more severe liver insufficiency. This possibility is intriguing and deserves investigation focused on the mechanism(s) that may be responsible for this, should this hypothesis be correct.

It would be also important to perform studies comparing long-term HA treatment with transjugular intrahepatic portosystemic shunt (TIPS). Research questions to be answered are: which are the patients in which one treatment is superior to the other? can long-term HA and TIPS be not mutually exclusive and instead part of a sequential approach for optimizing the global management of patients with ascites?

Other aspects of albumin therapy also deserve attention. Given the high cost and low availability of albumin, particularly in developing countries, the issue of cost-effectiveness is very important. Results from the ANSWER trial show that therapy is cost-effective because the extra-cost of HA administration is compensated for by the decrease in hospital readmissions related to prevention of cirrhosis complications (42). Specific analyses performed in other areas of the world including direct and indirect costs are needed to have the full picture of the cost-effectiveness of long-term HA treatment worldwide. Finally, weekly HA infusions lasting about 30-60 minutes may also cause significant logistic problems related to availability of space, journey of patients from home to
hospital, availability of nurses to perform intravenous infusions, and time required for treatment.

All these issues have to be taken into account should long-term HA treatment be implemented for patients with decompensated cirrhosis.

7. Conclusion

Results from the ANSWER trial represent an important step forward in the investigation of albumin as therapeutic agent for patients with decompensated cirrhosis. Besides controlling ascites, long-term HA treatment appears to significantly prevent complications and hospitalizations and improve survival, thus representing one of the candidates for being the first disease-modifying pathophysiological intervention in patients with cirrhosis and ascites. However, the discordant findings with other studies and several open issues call for the critical need of further clinical studies and randomized trials. In this regard, a large multicenter open-label RCT assessing the “effects of long-term administration of human albumin on subjects with decompensated cirrhosis and ascites” (PRECIOSA study; NCT03451292) is underway and results are eagerly awaited, even if a double-blind design would have increased the strength of the findings.
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CONFLICTS OF INTEREST

*Dr. Caraceni* reports research grants from Grifols SA and Octapharma SA and personal fees from Grifols SA, Kedrion Biopharma SpA, CSL Behring SA and Biotest SA. *Dr. O’Brien* reports no conflicts of interest. *Dr. Ginès* reports grants from Grifols and personal fees from CSL Behring.

AUTHORS’ CONTRIBUTIONS

All authors contributed equally to the manuscript and approved the final draft for submission.
REFERENCES


**Table 1. Differences between acute/short-term and long-term albumin administration.**

<table>
<thead>
<tr>
<th>ACUTE/SHORT-TERM ALBUMIN TREATMENT</th>
<th>LONG-TERM ALBUMIN TREATMENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>It can last one or more days up to 2 weeks</td>
<td>It lasts at least weeks, usually months or sometimes years</td>
</tr>
<tr>
<td>Mostly hospitalized patients* (regular wards/intensive care units)</td>
<td>Outpatients (it can be started during hospitalization)</td>
</tr>
<tr>
<td>The goal is to treat or prevent acute complications</td>
<td>The goal is to treat ascites and influence the course of the disease by preventing complications</td>
</tr>
<tr>
<td>Effects should occur in hours or days</td>
<td>The effects become manifest usually after 1-2 months of treatment</td>
</tr>
<tr>
<td>High daily doses of albumin are infused within a short-time frame</td>
<td>Low doses of albumin are infused distributed over a long-time frame</td>
</tr>
<tr>
<td>Safety issues (pulmonary edema) in some cirrhosis complications§</td>
<td>Logistic issues (periodic intravenous infusions)</td>
</tr>
</tbody>
</table>

*patients subjected to large-volume paracentesis or presenting acute kidney injury can also receive albumin in outpatients settings.

§infections unrelated to bacterial spontaneous peritonitis (ref. #56), hepatorenal syndrome type I (ref. #65), and worsening or onset of an acute cirrhosis complication requiring hospitalization (ref. #62).
### Table 2. Main features of the ANSWER and ATTIRE trial. IQR: interquartile range; ITT: intention-to-treat; RCT: randomized clinical trial.

<table>
<thead>
<tr>
<th></th>
<th>ANSWER trial</th>
<th>ATTIRE trial</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Trial design</strong></td>
<td>Multicenter open-label RCT</td>
<td>Multicenter open-label RCT</td>
</tr>
<tr>
<td><strong>Study population</strong></td>
<td>Patients with stable cirrhosis and uncomplicated grade 2 and 3 ascites</td>
<td>Patients with cirrhosis hospitalized for worsening or onset of acute complications</td>
</tr>
<tr>
<td><strong>Intervention</strong></td>
<td>40 gr of albumin twice a week for 2 weeks, then 40 gr weekly up to a maximum of 18 months</td>
<td>Targeted to achieve and maintain a serum albumin level &gt;3.0 g/dl from day 3 up to a maximum of 14 days</td>
</tr>
<tr>
<td><strong>Primary endpoint</strong></td>
<td>18-month overall survival</td>
<td>Composite of incidence of all-cause infection, renal dysfunction and death between day 3 and 15</td>
</tr>
<tr>
<td><strong>ITT population</strong></td>
<td>431 (218/213)</td>
<td>777 (380/397)</td>
</tr>
<tr>
<td><strong>Baseline MELD score</strong></td>
<td>12 (10-15) / 13 (10-16)</td>
<td>19.5 (15.4-22.9) / 19.5 (15.4-23.4)</td>
</tr>
<tr>
<td><strong>Length of treatment in the intervention arm</strong></td>
<td>14.5 (5.0 - 18.0) months</td>
<td>8 (6 - 15) days</td>
</tr>
<tr>
<td><strong>Effect on serum albumin concentration</strong></td>
<td>Significant increase in the albumin arm (from 3.1 close to 4 gr/dl)</td>
<td>Significant increase in the albumin arm (from 2.3 to slightly above 3 gr/dl)</td>
</tr>
<tr>
<td><strong>Impact on survival</strong></td>
<td>Significantly Increased in the albumin arm</td>
<td>No difference between the two arms</td>
</tr>
<tr>
<td><strong>Impact on cirrhosis complications</strong></td>
<td>Significantly reduced incidence in the albumin arm</td>
<td>No difference between the two arms</td>
</tr>
<tr>
<td><strong>Risk of pulmonary edema</strong></td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>
Table 3. *Areas of research on long-term albumin treatment.*

<table>
<thead>
<tr>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Target population stratified according to the expected outcomes</td>
</tr>
<tr>
<td>Biomarkers of response to treatment</td>
</tr>
<tr>
<td>Optimization of doses and frequency of albumin administration</td>
</tr>
<tr>
<td>Stopping rules</td>
</tr>
<tr>
<td>Cost-effective analysis in health-care systems world-wide</td>
</tr>
</tbody>
</table>
FIGURE LEGENDS

**Figure 1.** Properties of the albumin molecule and major changes occurring to the albumin molecule in patients with decompensated cirrhosis. LPS: lipopolisaccharides. PGE$_2$: Prostaglandin E$_2$

**Figure 2.** Potential pathophysiological events antagonized by the oncotic and non-oncotic properties of the albumin molecule in patients with cirrhosis and ascites.

**Figure 3.** Comparison between the ANSWER and the MACHT trials. *Upper panel:* main features related to albumin treatment. *Medium panel:* changes in the median serum albumin concentration in the ANSWER trial. *Lower panel:* changes in the median serum albumin concentration in the MACHT trial. HA: human albumin; M: midodrine; SMT: standard medical treatment.

**Figure 4.** The “filling the gap” hypothesis. The goal of long-term albumin administration should be to fill the gap existing between the pre-treatment serum albumin concentration and the physiological serum albumin concentration observed in healthy individuals (ref. #62). As the extent of the gap depends mostly by the pre-treatment serum albumin level and the severity of the liver disease, the amount of HA needed to fill the gap (grey arrows) may vary at the individual patient level. The dotted black lines correspond to the lower (3.5 g/dl) and upper (5.0 g/dl) limits of the lab references for defining the normal range of serum albumin concentration measured with standard methods in the daily clinical practice. The red line corresponds to the level of serum albumin concentration during treatment, which has been found associated to optimal outcomes (ref. #45). Modified from ref. #45
ALBUMIN IN HEALTHY INDIVIDUALS

ONCOTIC ——— PROPERTIES ——— NON-ONCOTIC

Regulation of fluid distribution
  Negative net charge
  High molecular weight
  High plasma concentration

Binding, transport, detoxification
  Many endogenous and exogenous compounds including drugs
Antioxidant activity
  Free radical and metal ion scavenging
Endothelial stabilization
Hemostatic effect
Immune/inflammatory responses modulation
  LPS binding, activation of pro-inflammatory genes, modulation of intracellular redox state, PGE₂ binding

Most abundant circulating protein
(50-60% of the total proteins)
Reference lab range: 3.5-5.0 g/dl

ALBUMIN IN PATIENTS WITH DECOMPENSATED CIRRHOSIS

Structural damages
  Reversible and irreversible oxidation
  Glycation
  N- and C-terminal truncation
  Dimerization

Impaired functions
  Reduced antioxidant activity
  Reduced binding/detoxification capacities
  Pro-inflammatory effect
  Others?

Reduced plasma concentration
  Reduced synthesis
  Increased catabolism
  Increased trans-capillary rate

Figure 1
Figure 2

CIRRHOSIS

- PORTAL HYPERTENSION
  - BACTERIAL TRANSLOCATION
    - PAMPs
  - LOCAL AND SYSTEMIC INFLAMMATION OXYDATIVE STRESS
    - IMMUNE DYSFUNCTION

- TISSUE DAMAGE
  - DAMPs

- IMMUNE DYSFUNCTION
  - CIRCULATORY DYSFUNCTION (effective hypovolemia)
    - ARTERIAL VASODILATION
      - Cardiac dysfunction
    - IMMUNOPATHOLOGY MITOCHONDRIAL DYSFUNCTION
    - KYDNEY (and other organs)
      - HYPOPERFUSION
    - SINGLE or MULTIPLE ORGAN DYSFUNCTION/FAILURE

ALBUMIN
Figure 3

<table>
<thead>
<tr>
<th></th>
<th>ANSWER</th>
<th>MACHT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Design</td>
<td>Randomized open-label</td>
<td>Randomized double blind placebo-controlled</td>
</tr>
<tr>
<td>Length of treatment (median)</td>
<td>14.5 months</td>
<td>63 days</td>
</tr>
<tr>
<td>Baseline MELD score (median) SMT/SMT+HA or SMT+M+HA</td>
<td>12/13</td>
<td>17/16</td>
</tr>
<tr>
<td>Dosage and timing of albumin administration</td>
<td>40 g twice a week for 2 weeks then 40 g once a week</td>
<td>40 g every 2 weeks (no loading dose)</td>
</tr>
<tr>
<td>Impact of treatment on serum albumin concentration</td>
<td>Significant increase (0.7-0.8 g/dl) in the albumin arm</td>
<td>No differences between the 2 groups</td>
</tr>
</tbody>
</table>

**ANSWER**

![Serum albumin (g/dL) vs. Months graph](image)

**MACHT**

![Serum albumin (g/dL) vs. Months graph](image)
Figure 4

Serum albumin (g/dl)

Pre-treatment albumin level

Severity of liver disease

Low

High
ALBUMIN IN HEALTHY INDIVIDUALS

ONCOTIC ——— PROPERTIES ——— NON-ONCOTIC

Regulation of fluid distribution
- Negative net charge
- High molecular weight
- High plasma concentration

Most abundant circulating protein (50-60% of the total proteins)
Reference lab range: 3.5-5.0 g/dl

Binding, transport, detoxification
- Many endogenous and exogenous compounds including drugs
- Antioxidant activity
  - Free radical and metal ion scavenging
- Endothelial stabilization
- Hemostatic effect
- Immune/inflammatory responses modulation
  - LPS binding, activation of pro-inflammatory genes, modulation of intracellular redox state, PGE₂ binding

ALBUMIN IN PATIENTS WITH DECOMPENSATED CIRRHOSIS

Structural damages
- Reversible and irreversible oxidation
- Glycation
- N- and C-terminal truncation
- Dimerization

Impaired functions
- Reduced antioxidant activity
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- Pro-inflammatory effect
- Others?

Reduced plasma concentration
- Reduced synthesis
- Increased catabolism
- Increased trans-capillary rate
PORTAL HYPERTENSION

BACTERIAL TRANSLOCATION
PAMPs

LOCAL AND SYSTEMIC INFLAMMATION
OXIDATIVE STRESS

ARTERIAL VASODILATION
Cardiac dysfunction

CIRCULATORY DYSFUNCTION
(effective hypovolemia)

ALBUMIN

IMMUNE DYSFUNCTION

KYDNEY (and other organs)
HYPOPERFUSION

SINGLE or MULTIPLE ORGAN DYSFUNCTION/FAILURE

IMMUNOPATHOLOGY
MITOCHONDRIAL DYSFUNCTION

CLICK HERE TO ACCESS/DOWNLOAD; Figure; Figure 2.pptx
### Design

<table>
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### Length of treatment (median)

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### Baseline MELD score (median)

<table>
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<tr>
<td>SMT/SMT+HA or SMT+M+HA</td>
<td>12/13</td>
<td>17/16</td>
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</table>

### Dosage and timing of albumin administration

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<td>40 g every 2 weeks (no loading dose)</td>
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### Impact of treatment on serum albumin concentration

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<tr>
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<td>No differences between the 2 groups</td>
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</tbody>
</table>

### Impact of treatment on serum albumin concentration

**ANSWER**

![ANSWER chart](Figure3.png)

**MACHT**

![MACHT chart](Figure3.png)
Figure 4

Severity of liver disease:
- Low
- High

Serum albumin (g/dl):
- Pre-treatment albumin level
- Albumin Gap

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