**Correction and prevention of hyponatremia in patients with cirrhosis and ascites – *Post hoc* analysis of the ANSWER study database.**

**Supplementary appendix**

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# **Study design, participants, and procedures**

The human Albumin for the treatmeNt of aScites in patients With hEpatic ciRrhosis (ANSWER) study was an investigator-initiated multicenter, randomized, parallel, open-label, pragmatic trial conducted in 33 academic and nonacademic Italian hospitals.+

The *inclusion criteria* were:

1. diagnosis of liver cirrhosis by liver biopsy or a combination of clinical, laboratory, radiological and endoscopic findings,
2. uncomplicated ascites with ongoing diuretic treatment with an anti-aldosteronic drug (at a dose ≥200 mg/day) and furosemide (≥25 mg/day), stable for at least 4 days before enrolment,
3. an esophagogastroduodenoscopy done in the past 12 months, abdominal ultrasonography done in the past 30 days, and laboratory tests required by the protocol in the past 7 days.

The *exclusion criteria* were:

1. Age lower than 18 years,
2. No written informed consent,
3. Inability to follow written consent,
4. Established diagnosis of refractory ascites,
5. Need for two or more paracenteses during the last month prior to enrolment,
6. Serum creatinine >1·5 mg/dl,
7. Organic nephropathy,
8. Hepatorenal syndrome (HRS) type 1 in the last 15 days prior to enrolment,
9. Gastrointestinal bleeding in the last 15 days prior to enrolment,
10. Ongoing endoscopic eradication of gastro-esophageal varices after recent gastro-esophageal variceal bleeding,
11. Bacterial or fungal infection, including spontaneous bacterial peritonitis (SBP), in the last seven days,
12. Hepatic encephalopathy grade III/IV,
13. Budd-Chiari Syndrome,
14. Patients with trans-jugular portosystemic shunt or surgical portocaval shunts (TIPS),
15. Known or suspected active hepatocellular carcinoma or other malignancies,
16. Previous liver transplantation,
17. Ongoing alcohol abuse (patients had to be abstinent for at least three months),
18. Antiviral therapy for hepatitis B or C started in the last six months,
19. Heart failure,
20. Respiratory failure defined as PaO2 < 60 mmHg,
21. Known or suspected hypersensitivity to albumin,
22. Previous albumin administration for the treatment of ascites in the last 30 days prior to enrolment,
23. Patients enrolled in other clinical studies for the treatment of ascites,
24. Use of experimental drugs for the last two months prior to inclusion in the present study,
25. Pregnancy and breast-feeding,
26. Females of child-bearing potential, unless they met one of the following criteria:

- post-menopausal for six months or more, and if post-menopausal for less than two years, a negative pregnancy test,

- surgical sterilization for more than one-month’s duration and a negative pregnancy test,

- intrauterine device in combination with a secondary barrier (e.g. diaphragm, condom or spermicide) and a negative pregnancy test.

Eligible patients were randomly assigned (1:1) to receive either standard medical treatment (SMT) or SMT plus human albumin (HA) with a computer-generated and blinded assignment sequence with randomly permuted blocks of four, stratified according to the need for therapeutic paracentesis in the month preceding enrolment (‘yes’ or ‘no’) and natremia (<135 mmol/L or ≥135 mmol/L).

SMT was aligned with the indications from the available clinical practice guidelines. Patients enrolled in the SMT plus HA group received an intravenous infusion of 20% HA in 50 mL vials in approximately 30–60 min at a dose of 40 g twice weekly for the initial 2 weeks, and 40 g weekly thereafter. The first dose was administered within 24 h after randomization. According to protocol, HA was infused by nursing personnel in outpatient settings, such as

hospital clinics, local health centers, or home-care services according to available facilities, whereas patients admitted to hospital received HA on the scheduled date during their hospital stay. No concomitant medications were forbidden during the study.

After enrolment, patients were assessed monthly for up to 18 months or study interruption or death. At each visit, clinical, laboratory, and instrumental data (if needed) were collected by the attending physicians. Data were recorded on an electronic case report form accessible via the internet. The study was interrupted when patients underwent liver transplantation or TIPS insertion, needed three or more therapeutic paracenteses per month, or refused to continue their participation in the study, or because of medical judgment.

# **Statistical analysis**

All continuous variables were tested for normality with the Kolmogorov–Smirnov test. Normally distributed variables were summarized by the mean and standard deviation, the median and 25th -75th percentiles were used in all other cases. Categorical variables were summarized by the absolute frequencies and percentage. Distribution of continuous variables among groups were analyzed by the unpaired Student’s t-test with Welch's correction to adjust for unequal variances or the one-way analysis of variance. The Mann-Whitney U test or the Kruskal-Wallis test were used to compare non-normally distributed variables. Contingency tables and Chi square or Fisher’s exact test were used to evaluate the relationship between categorical variables. The reverse Kaplan-Meier method was employed to estimate the median follow-up in patients with baseline hyponatremia randomized in the SMT or SMT+HA arm.

To compare the changes in serum sodium concentration during the 18-months follow-up in the SMT and SMT+HA groups, longitudinal modeling was performed using random coefficients linear regression (Rabe-Hesketh S. Multilevel and longitudinal modelling using Stata. Volume I: Continuous responses. College Station TX: Stata Press; 2021). The outcome variable of the regression model was plasma sodium and the predictor variables were treatment (discrete: 0 = standard; 1 = albumin), time (discrete: 0, 0.5, and thereafter 1 to 18 months by steps of 1 month), and a time X treatment (discrete per discrete) interaction. The random intercept was assigned to the patient and the random slope to month. Missing data were handled by maximum likelihood estimation (MLE) under the missing at random assumption (MAR).

The cumulative incidence of hyponatremia correction within 3 months was calculated according to the Kaplan-Meier method and compared by the log-rank test.

To assess episode of hyponatremia and deal with the different length of follow-up, we firstcomputed the incidence rate (IR) (number of events per person per year) and 95% CI using the exact method on the basis of the Poisson distribution. Subsequently, to compare the IRs in the two groups, we calculated the incidence rate ratio (IRR) and 95% CI using the SMT group as the reference category, as it has been done in the core-study manuscript.

All tests were two sided and values of p less than 0.05 were considered statistically significant. Analyses were performed using the Statistical Package for Social Sciences (SPSS version 25; IBM Corp., Armonk, NY) and R software (version 3.5.1; www.r-project.org).

# **Supplementary Table 1.** Baseline characteristics of patients with normonatremia at inclusion in the ANSWER study, comparing subjects in the SMT and SMT+HA arms.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  |  | **SMT****(n=139)** | **SMT+HA****(n=143)** | **P value** |
| **Demographic data** |  |  |  |
|  | Age (years) | 62 (53 - 70) | 63 (52 - 69) | 0.702 |
|  | Male sex  | 98 (71%) | 98 (69%) | 0.796 |
| **Aetiology of cirrhosis** |  |  |  |
|  | Viral | 45 (32) | 47 (33) | 0.264 |
|  | Alcohol  | 46 (33) | 45 (32) |
|  | NASH  | 10 (7) | 5 (4) |
|  | Viral and alcohol  | 12 (9) | 21 (15) |
|  | Viral and NASH  | 9 (7) | 4 (3) |
|  | Other  | 17 (12) | 21 (15) |
| **Clinical features**  |  |  |  |
|  | Body mass index (Kg/m2) | 26 (24 - 29) | 26 (23 - 28) | 0.358 |
|  | Systolic arterial pressure (mm Hg) | 110 (110 - 120) | 115 (110 - 122) | 0.499 |
|  | Diastolic arterial pressure (mm Hg) | 70 (65 - 80) | 70 (60 - 73) | 0.074 |
|  | Mean arterial pressure (mm Hg) | 83 (80 - 92) | 83 (79 - 90) | 0.414 |
|  | Heart rate (beats per minute) | 68 (62 - 76) | 70 (64 - 77) | 0.646 |
|  | Ascites  |  |  |  |
|  |  | Grade 2  | 113 (81) | 122 (85) | 0.425 |
|  |  | Grade 3  | 26 (19) | 21 (15) |
|  | Hepatic encephalopathy grade I/II  | 13 (9) | 12 (8) | 0.836 |
| **Hematologic and biochemical data** |  |  |  |
|  | White blood cells (103/mmc)  | 4.6 (3.52 - 5.73) | 4.99 (3.62 - 6.6) | 0.046 |
|  | Haemoglobin (g/dL)  | 11.5 (10.3 - 12.9) | 11.9 (10.7 - 13.1) | 0.220 |
|  | Platelets (103/mmc)  | 90 (61 - 126.5) | 86 (63 - 131.5) | 0.387 |
|  | Serum creatinine (mg/dL) | 0.99 (0.8 - 1.14) | 0.9 (0.77 - 1.11) | 0.410 |
|  | Serum sodium (mmol/L) | 138 (136 - 139) | 138 (136 - 139) | 0.573 |
|  | Serum potassium (mmol/L) | 4.3 (3.95 - 4.67) | 4.4 (3.97 - 4.68) | 0.456 |
|  | Serum bilirubin (mg/dL) | 1.8 (1.2 - 2.64) | 1.7 (1.04 - 2.67) | 0.330 |
|  | Serum albumin (g/dL) | 3.2 (2.86 - 3.5) | 3.1 (2.7 - 3.5) | 0.228 |
|  | International normalized ratio (INR) | 1.33 (1.19 - 1.5) | 1.3 (1.19 - 1.46) | 0.449 |
| **Prognostic scores** |  |  |  |
|  | Child-Pugh class |  |  |  |
|  |  | Class A  | 25 (18) | 24 (17) | 0.591 |
|  |  | Class B  | 89 (64) | 99 (69) |
|  |  | Class C  | 25 (18) | 20 (14) |
|  | Child-Pugh score | 8 (7 - 9) | 8 (7 - 9) | 0.851 |
|  | MELD score | 13 (10 - 16) | 12 (10 - 14) | 0.352 |

*Data are n (%), median (IQR) or mean (SD). SMT: standard medical treatment (SMT); HA: human albumin; NASH: non-alcoholic steatohepatitis; HCC: hepatocellular carcinoma; MELD: model for end-stage liver disease.*

# **Supplementary Table 2.** Individual doses of diuretics administered during the studyin patients with baseline hyponatremia (Na<135 mmol/L) randomized in the SMT or SMT+HA arms.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **SMT****(n=74)** | **SMT+HA****(n=75)** | **P value** |
| **Antialdosteronic drug** |  |  |  |  | 0.559 |
| Daily dose (mg) | 182 | (113 – 225) | 172 | (83 – 222) |  |
| Cumulative annual dose (mg) | 66,370 | (41,119 – 82,238) | 62,724 | (30,465 – 81,060) |
| **Furosemide** |  |  |  |  | 0.526 |
| Daily dose (mg) | 36 | (18 – 56) | 31 | (17 – 53) |  |
| Cumulative annual dose (mg) | 13,216 | (6,625 – 20,338) | 11,415 | (6,123 – 19,194) |

*SMT = standard medical treatment (SMT). SMT+HA = standard medical treatment plus human albumin.*