Supplementary data to

DETERMINATION OF EFFECTIVE ALBUMIN IN PATIENTS WITH DECOMPENSATED CIRRHOSIS: CLINICAL AND PROGNOSTIC IMPLICATIONS

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Figure S1. The study population included 337 patients with liver cirrhosis. Eighteen patients with stable conditions were enrolled from outpatient clinic, while 319 patients were enrolled at the time of hospital admission due to acute decompensation. Seventy-eight patients met the criteria for the diagnosis of acute-on-chronic liver failure at admission, while 32 developed the syndrome within 30 days from admission.

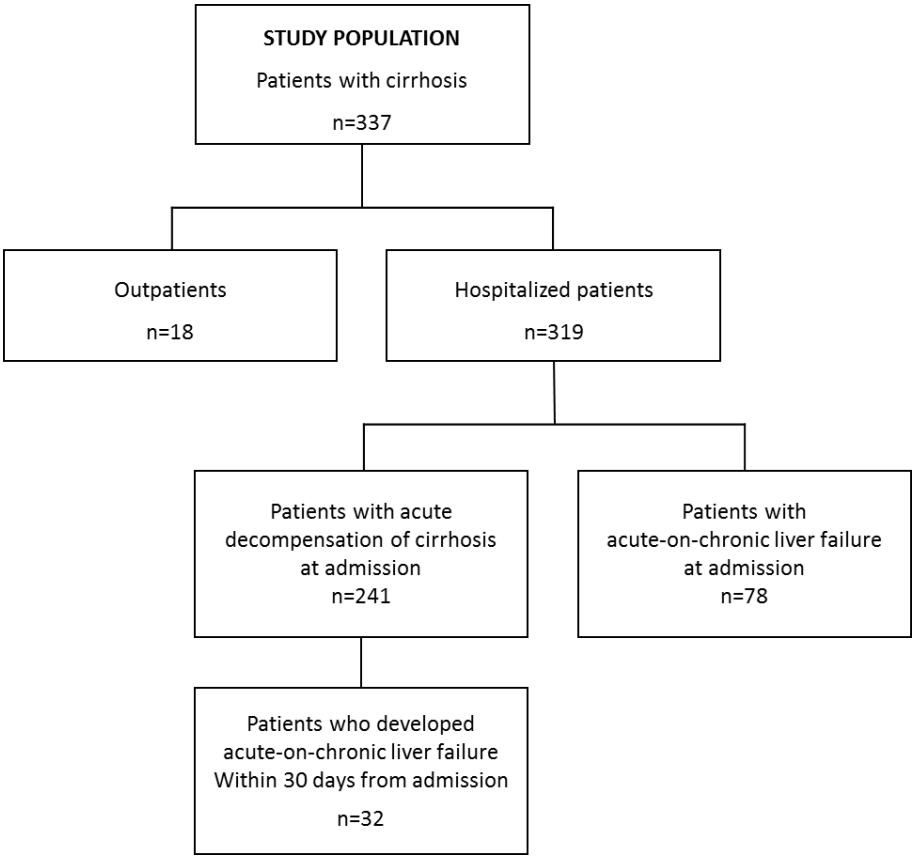

Figure S2. Principal component (PC) analysis loadings plot of functional parameters of albumin (Red arrows: Binding efficiency – BE, Detoxification efficiency – DTE, Binding coefficient A – KBA, binding coefficient B – KBB, and binding coefficient C – KBC) in patients with AD (Panel A) or ACLF (Panel B) at hospital admission. Disease severity scores, tAlb and eAlb were included as supplementary variables (Blue arrows). Numbers in brackets indicate the percent of the total variance explained by each principal component. Arrows directions indicate the strength of the contribution of each variable to the PC.

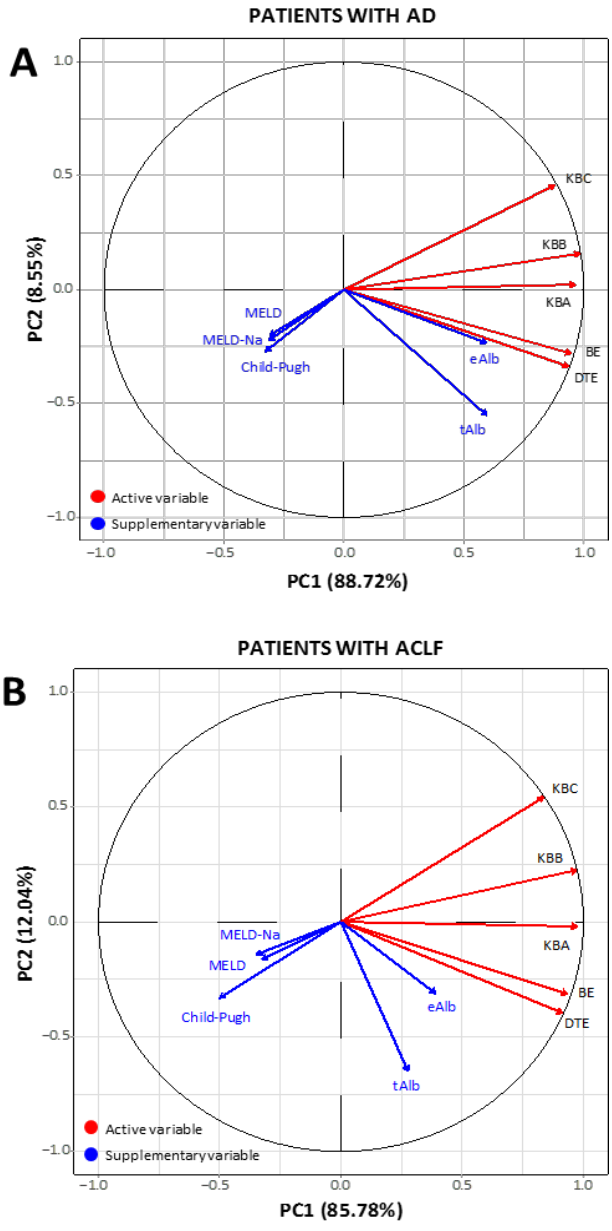

Figure S3. Impact of human albumin administration (20% commercial albumin solution) on total albumin concentration (tAlb, Panel A), native albumin relative abundance (nAlb, Panel B) and effective albumin concentration (eAlb, Panel C) in outpatients with cirrhosis and ascites presenting the indication to albumin administration (n=8).

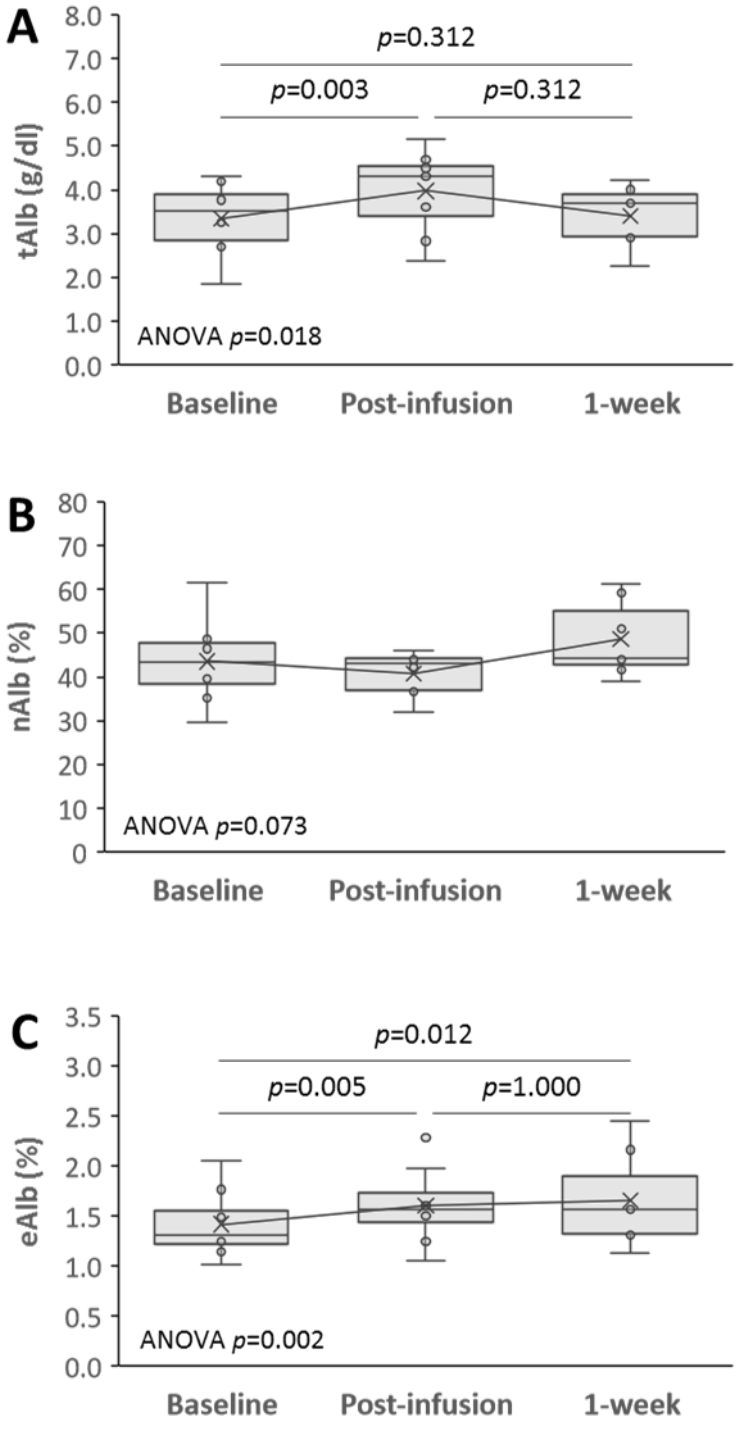

Figure S4. Impact of albumin administration (20% commercial albumin solution) on binding efficiency (BE, Panel A) and detoxification efficiency (DTE, Panel B) in outpatients with cirrhosis and ascites presenting the indication to albumin administration (n=8).

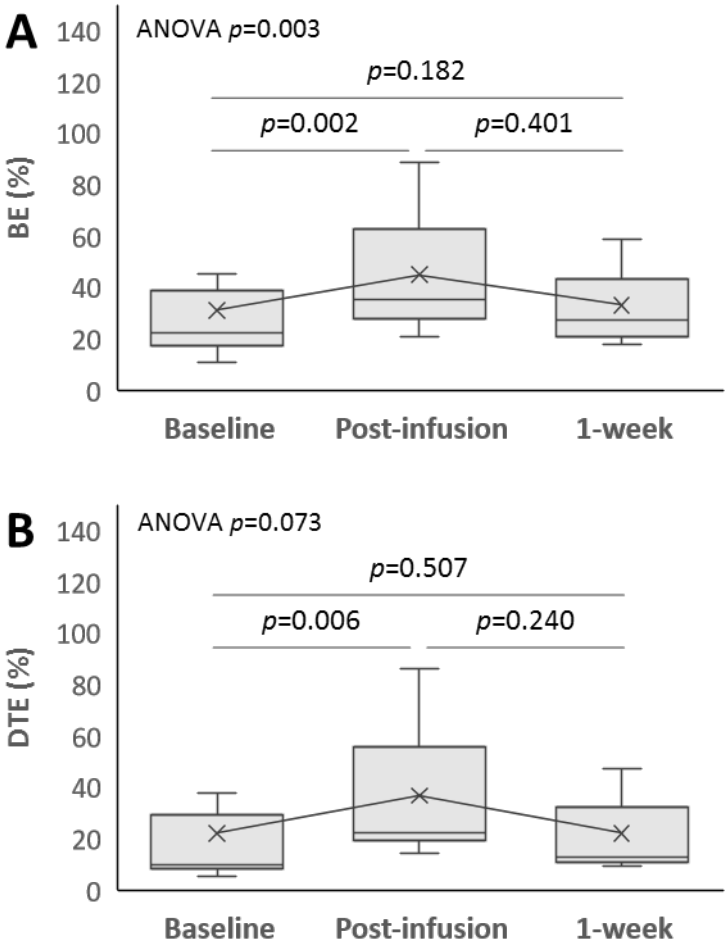

Table S1. LC-ESI-MS analysis of human albumin isoforms in plasma samples from healthy controls (n=21), outpatients with compensated cirrhosis (n=18) and hospitalized patients with acute decompensation (AD) (n=241) or acute-on-chronic liver failure (ACLF) (n=78) at admission.

	Healthy controls (n=21)	**Outpatients (n = 18)**	**AD (n = 241)**	**ACLF (n = 78)**	**P-value[a]**
HSA-DA (%)	3.82 (3.09 – 4.02)	3.01 (2.77 – 3.50)	2.67 (1.87 – 3.57) [§]	2.58 (2.03 – 3.84) [§]	0.006
HSA-L (%)	2.73 (2.25 – 3.71)	2.15 (1.84 – 2.40)	1.83 (1.02 – 2.89) [§]	1.79 (1.18 – 2.80) [§]	0.008
HSA+Cys-DA (%)	1.93 ± 0.61	1.93 ± 0.55 [@]	2.27 ± 1.18 [@]	2.81 ± 1.52 [#]	0.001
HSA(DHA) (%)	3.01 (2.79 – 3.26)	2.93 (2.67 – 3.15) [#]	1.44 (0.71 – 2.07) [#]	1.13 (0.00 – 1.65) [#]	<0.001
Native HSA (%)	49.01 ± 3.54	48.82 ± 3.78 [¥, @]	35.30 ± 8.84 [#]	29.62 ± 11.03 [#]	<0.001
HSA+SO_2H (%)	9.55 (8.68 – 10.82)	10.78 (10.60 – 11.04) [¥, @]	8.65 (7.29 – 9.85) [*, @]	7.91 (6.68 – 9.22) [#]	<0.001
HSA+SO_3H (%)	0.00 (0.00 – 0.00)	1.63 (1.51 – 1.73) [§]	1.39 (0.00 – 1.90) [§]	1.14 (0.00 – 2.01) [§]	<0.001
HSA(DHA)+Cys (%)	0.00 (0.00 – 0.58)	0.00 (0.00 – 0.00)	0.00 (0.00 – 0.00)	0.00 (0.00 – 0.78)	0.077
HSA+Cys (%)	17.91 ± 4.27	15.92 ± 3.35 [¥, @]	27.90 ± 8.06 [#]	32.08 ± 9.76 [#]	<0.001
HSA+Glyc (%)	8.25 (7.62 – 9.07)	8.27 (7.27 – 8.68) [¥, @]	9.45 (8.38 – 10.51) [#]	10.30 (8.76 – 11.82) [#]	<0.001
HSA+Cys+Glyc (%)	3.03 (2.40 – 3.56)	2.38 (2.08 – 2.64) [¥, @]	5.13 (3.76 – 6.67) [#]	6.32 (5.14 – 8.04) [#]	<0.001
HSA+2Glyc (%)	0.00 (0.00 – 0.00)	1.43 (1.28 – 1.67) [¥, @]	2.35 (1.81 – 3.29) [§, *]	2.84 (2.14 – 3.39) [§, *]	<0.001

Data are presented as relative amount (%); median and interquartile range or mean and standard deviation are reported according to data distribution. [a] Global ANOVA p value; Pairwise comparisons: [§]p<0.05 vs healthy controls; *p<0.05 vs outpatients; [¥]p<0.05 vs AD; [@]p<0.005 vs ACLF; [#]p<0.05 vs all.

AD: acute decompensation; ACLF: acute-on-chronic liver failure. HSA: human serum albumin; -DA: N-terminal truncated (-Asp-Ala); -L: C-terminal truncated (-Leu); +Cys-DA: Cysteinylated and N-terminal truncated; (DHA): conversion of a cysteine into dehydroalanine (DHA); Native HSA: Native Form of HSA; +SO_2H: Sulfinylated; + SO_3H: Sulfonylated; (DHA)+Cys: conversion of a cysteine into dehydroalanine (DHA) and Cysteinylated; +Cys: Cysteinylated; +Glyc: Glycated; +Cys+Glyc: Cysteinylated and glycated; +2Glyc: Glycated on two different sites.

Table S2. Glycated albumin isoforms in plasma samples from hospitalized patients with and without diabetes mellitus at admission.

	No diabetes (n=206)	**Diabetes (n=113)**	**P-value**
HSA+Glyc	9.42 (8.42-10.46	10.12 (8.77-11.38)	0.006
HSA+Cys+Glyc	5.21 (3.73-6.65)	6.08 (4.41-7.32)	0.009
HSA+2Glyc	2.35 (1.74-3.11)	2.68 (2.09-3.69)	0.008

Data are presented as relative amount (%); median and interquartile range are reported.
HSA: human serum albumin; +Glyc: Glycated; +Cys+Glyc: Cysteinylated and glycated; +2Glyc: Glycated on two different sites.

Table S3. Glycated albumin isoforms in plasma samples from non-diabetic outpatients with compensated cirrhosis and non-diabetic hospitalized patients with acute decompensation (AD) (n=241) or acute-on-chronic liver failure (ACLF) (n=78) at admission.

	Outpatients (n = 12)	**AD (n = 153)**	**ACLF (n = 53)**	**P-value[a]**
HSA+Glyc	7.83 (6.96-8.5)	9.3 (8.39-10.29) *	10.05 (8.47-11.63) *	< 0.001
HSA+Cys+Glyc	2.34 (2-2.46)	4.77 (3.49-6.43) [#]	6.27 (5.04-7.62) [#]	< 0.001
HSA+2Glyc	1.32 (1.2-1.52)	2.25 (1.71-3.05) *	2.61 (2.06-3.27) *	< 0.001

Data are presented as relative amount (%); median and interquartile range are reported. [a] Global ANOVA p value; Pairwise comparisons: [#]P<0.05 vs all; *p<0.05 vs outpatients.
AD: acute decompensation; ACLF: acute-on-chronic liver failure. HSA: human serum albumin; +Glyc: Glycated; +Cys+Glyc: Cysteinylated and glycated; +2Glyc: Glycated on two different sites.

Table S4. Spearman rho correlation between total albumin concentration (tAlb), native albumin relative abundance (nAlb) and effective albumin concentration (eAlb) and plasma levels of Interleukin-6 (IL-6) and Tumor Necrosis Factor-α (TNF-α) in the whole series of outpatients and hospitalized patients with acute decompensation (AD) or acute-on-chronic liver failure (ACLF) at hospital admission (n=337).

	tAlb (g/dL)	**nAlb (%)**	**eAlb (g/dL)**
IL-6 (pg/mL)	-0.334**	-0.307**	-0.425**
TNF-α (pg/mL)	-0.164*	-0.234**	-0.257**

*p<0.05; **p<0.01

Table S5. Total (tAlb) and effective (eAlb) albumin concentration (g/dL) in patients with or without bacterial infection, renal dysfunction defined as serum creatinine ≥ 1.5 mg/dL and < 2.0 mg/dL, renal failure defined as serum creatine ≥ 2.0 mg/dL, ascites and grade III and IV encephalopathy at hospital admission. Data are reported as median and 25th – 75th percentiles.

		Yes	**No**	**P-value[a]**
Bacterial infection (n=79)	tAlb	2.71 (2.32 – 3.24)	3.09 (2.55 – 3.62)	0.027
	eAlb	0.91 (0.69 – 1.17)	1.02 (0.75 – 1.32)	0.006
Renal dysfunction (n=34)	tAlb	3.01 (2.62 – 3.32)	3.01 (2.45 – 3.50)	0.909
	eAlb	1.03 (0.65 – 1.23)	1.03 (0.78 – 1.33)	0.241
Renal failure (n=37)	tAlb	2.87 (2.37 – 3.58)	3.01 (2.46 – 3.49)	0.487
	eAlb	0.82 (0.57 – 1.01)	1.03 (0.75 – 1.32)	0.001
Ascites (n=202)	tAlb	2.98 (2.45 – 3.49)	3.02 (2.56 – 3.52)	0.683
	eAlb	0.96 (0.68 – 1.28)	1.05 (0.87 – 1.36)	0.001
Encephalopathy grade III/IV (n=61)	tAlb	2.90 (2.60 – 3.40)	3.02 (2.41 – 3.52)	0.852
	eAlb	0.96 (0.66 – 1.15)	1.01 (0.75 – 1.33)	0.049

Table S6. Discriminating performance of disease severity scores versus the development of acute-on-chronic-liver failure within 30 days from admission in hospitalized patients with only acute decompensation at admission. Data is presented as area under the Receiver Operating Characteristics curve (AUROC) and 95% confidence interval (CI).

	AUROC (95% CI)
MELD	0.750 (0.652 – 0.848)
MELD-Na	0.734 (0.636 – 0.883)
CLIF-c AD	0.729 (0.642 – 0.817)
Child-Pugh	0.698 (0.603 – 0.793)

Table S7. Discriminating performance of disease severity scores versus 90-day mortality in hospitalized patients with cirrhosis at admission. Data is presented as Area under the Receiver Operating Characteristics curve (AUROC) and 95% confidence interval (CI).

	AUROC (95% CI)
MELD	0.700 (0.627 – 0.773)
MELD-Na	0.727 (0.661 – 0.793)
CLIF-C AD	0.770 (0.708 – 0.831)
Child-Pugh	0.692 (0.626 – 0.758)