Liver elastography in acute liver failure: a pilot study

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Supplementary Methods

Cytokine analysis

The V-PLEX Proinflammatory Panel 1 Human Kit (IFN-γ, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, TNF-α) from Meso Scale Diagnostics, LLC was measures as below.

Heparin plasma samples were 4-fold diluted. 50 µL of calibrators or sample were added to each well of a V-PLEX plate and incubated for 2 hours on a horizontal orbital microplate shaker at room temperature. Plates were then washed three times (20-fold wash buffer concentrate reconstituted). 25 µL of detection antibody solution were added to each well and incubated for 2 hours on a horizontal orbital microplate shaker at room temperature. 150 µL of a 2X Read Buffer T were added to each well and the plate was read with a MSD instrument. The average of the duplicate readings for each standard and sample were analysed and analyte concentrations established fitting a 4-parameter logistic calibration curve.

Enzyme-linked immunosorbent assay (ELISA)

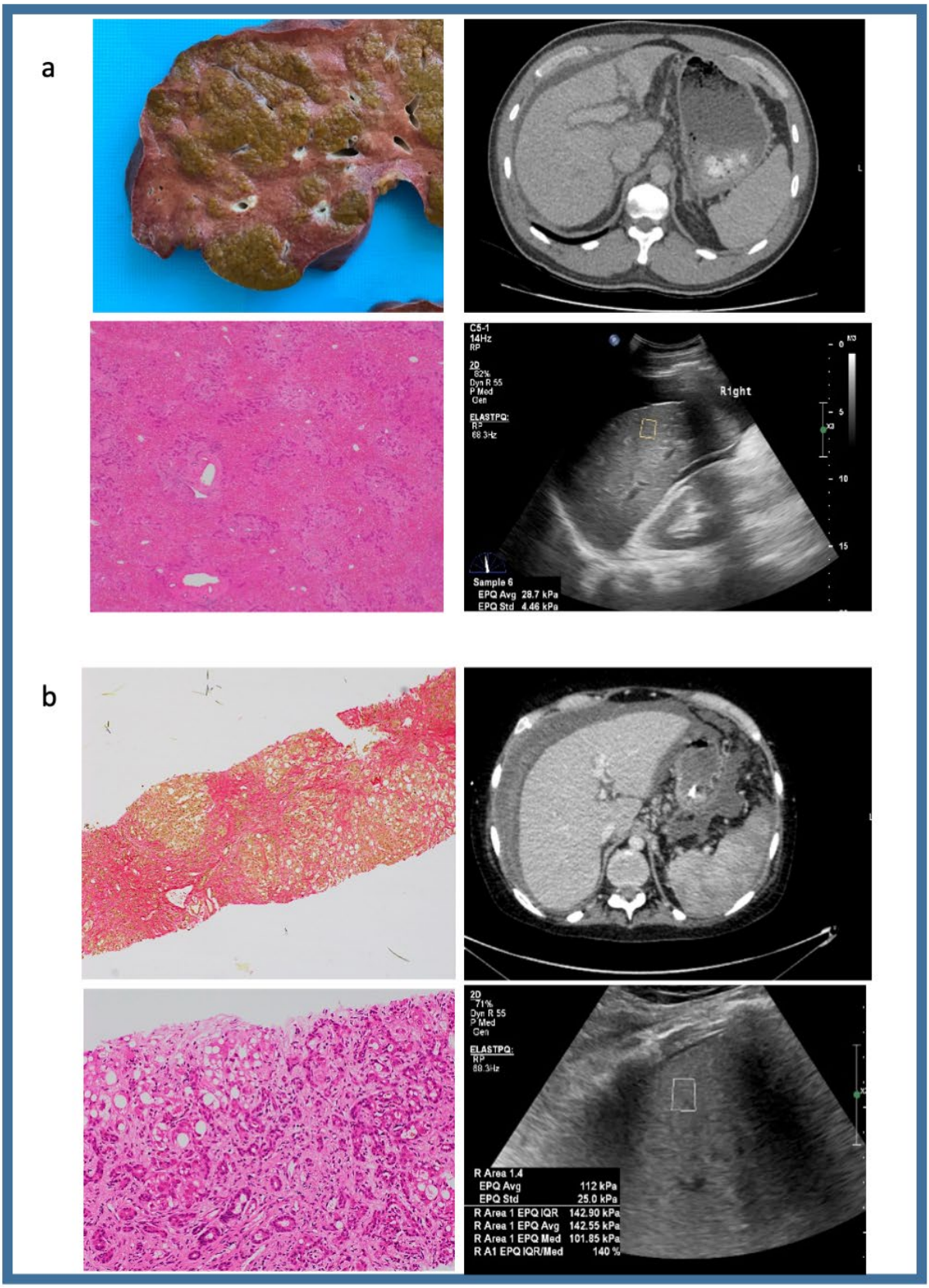
vonWillebrand factor (vWF) and soluble Platelet endothelial cell adhesion molecule-1 (sPECAM1) plasma concentrations were quantified according to the manufacturer's instructions briefly summarised below.

vonWillebrand Factor (Invitrogen, Thermo fisher)

Lithium heparin plasma samples were 8000-fold diluted. 100uL of diluted samples were added to each well, standards were diluted according to the protocol, incubated at room temperature for 2.5 hours on a microplate shaker. The following reagents were added sequentially with a washing passage in between: 100 µL of prepared biotin conjugate incubated at room temperature for 1 hour, 100 µL of prepared Streptavidin-HRP solution, incubated for 45 minutes,100 µL of TMB Substrate, incubated at room temperature for about 45 minutes, avoiding direct exposure to intense light. The enzyme reaction was stopped with 50 µL of Stop Solution. Absorbance was read with a spectro-photometer using 450 nm. Average of the duplicate readings for each standard and sample were analysed and analyte concentrations established fitting a 3rd order polynomial calibration curve.

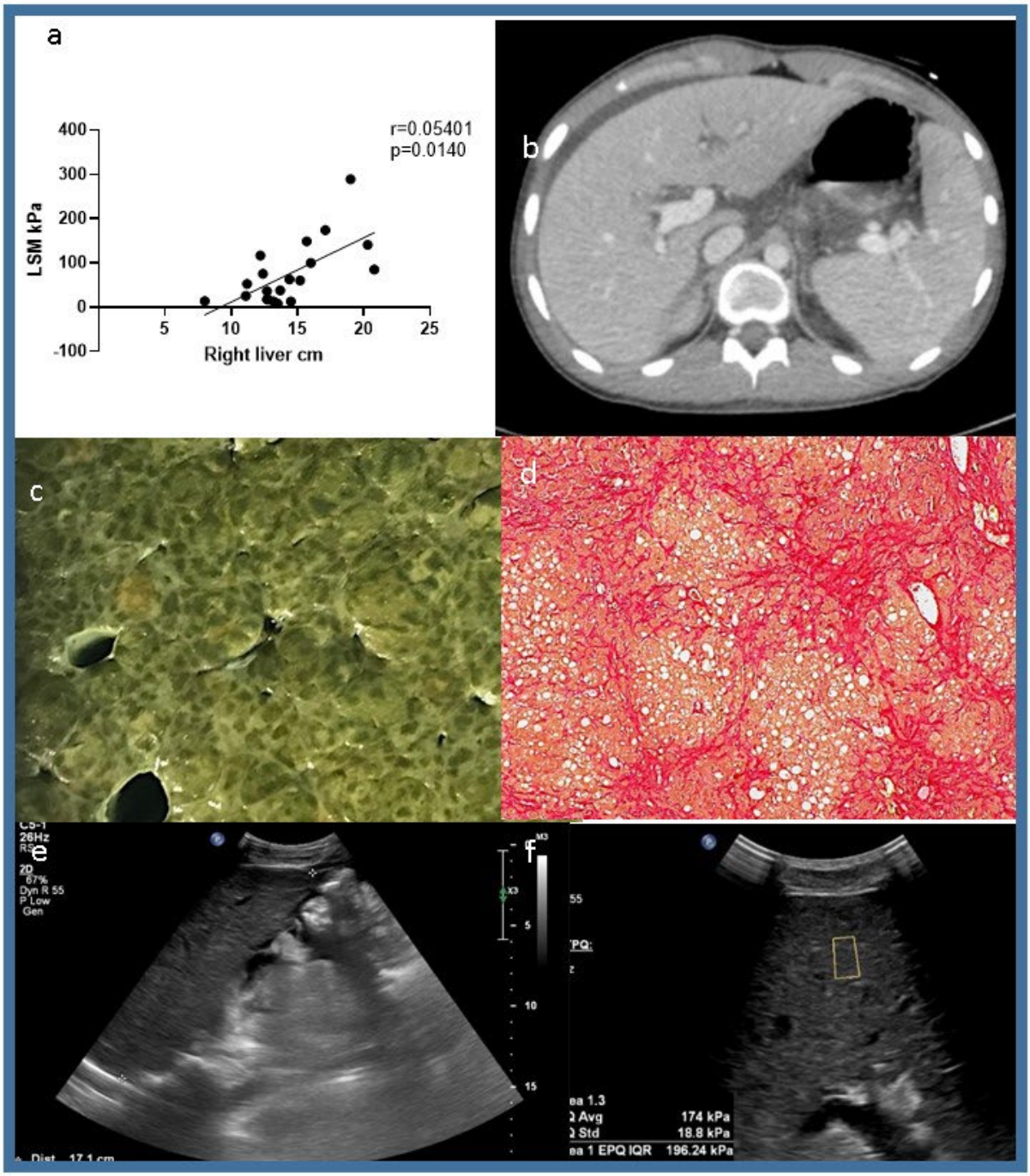
Soluble CD31/PECAM (Invitrogen, Thermo fisher)

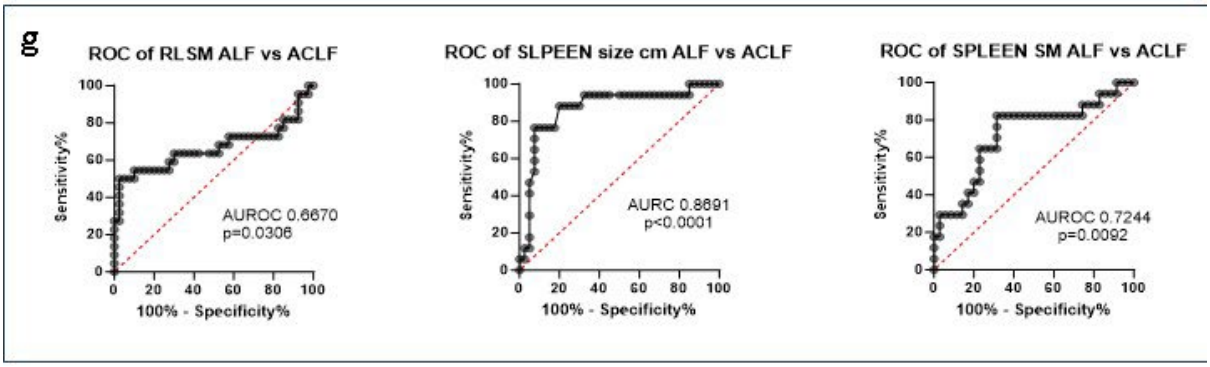
Lithium heparin plasma samples were 10-fold diluted. 100uLof diluted samples were added to each well, standards were diluted according to the protocol, after adding 50 µL of HRP-Conjugate to all wells, plate was incubated at room temperature for 3 hours on a microplate shaker. After washing, 100 µL of TMB Substrate Solution were added to all wells and plate was incubated at room temperature for about 10 minutes, avoiding direct exposure to intense light. The enzyme reaction was stopped by quickly pipetting 100 µL of Stop Solution. Absorbance was read with a spectro-photometer using 450 nm. Average of the duplicate readings for each standard and sample were analysed and analyte concentrations established fitting a 3rd order polynomial calibration curve.



Supplementary Figure 1. Two cases withs similar features at CT scan.

a) Submassive necrosis with map-like nodular areas intermixed with collapse areas. Liver weight 492g. Complete hepatocellular loss with no underlying fibrosis. LSM =28.7kPa±4.46; b) Micronodular cirrhosis and pericellular fibrosis (Sirius red). Steatosis, marked Mallory-Denk hyaline, and inflammation (top left). Marked ductular reaction with cholestasis. LSM112kPa±25.



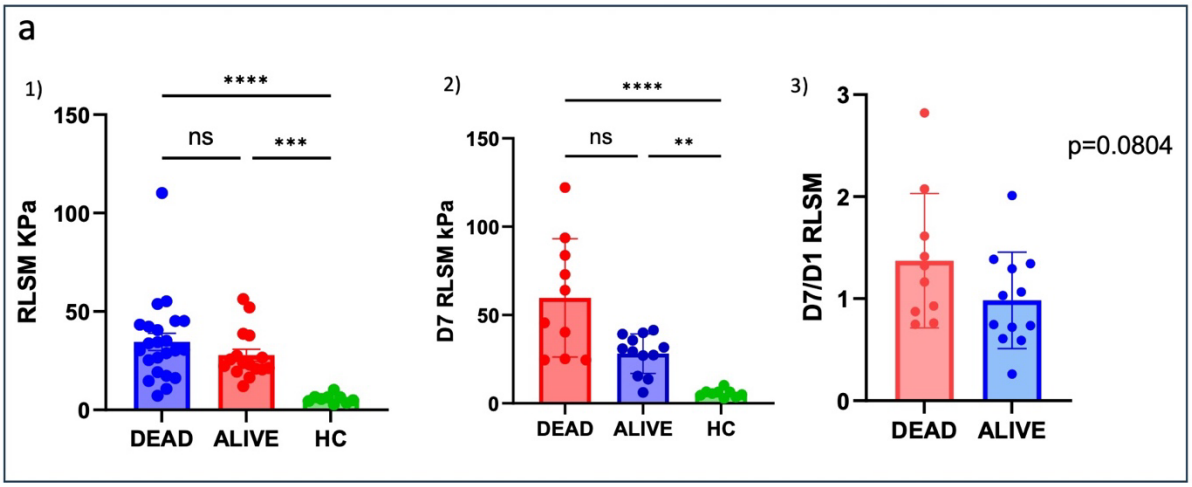


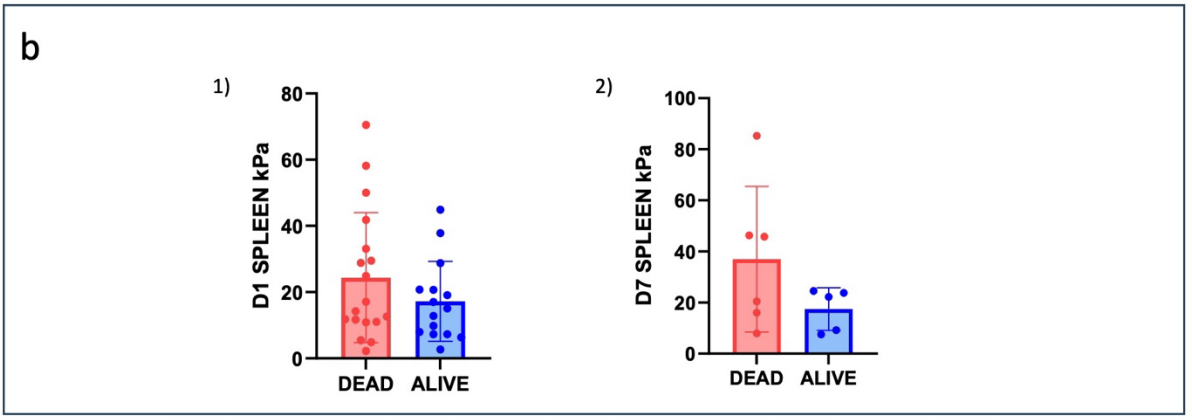
Supplementary Figure 2.

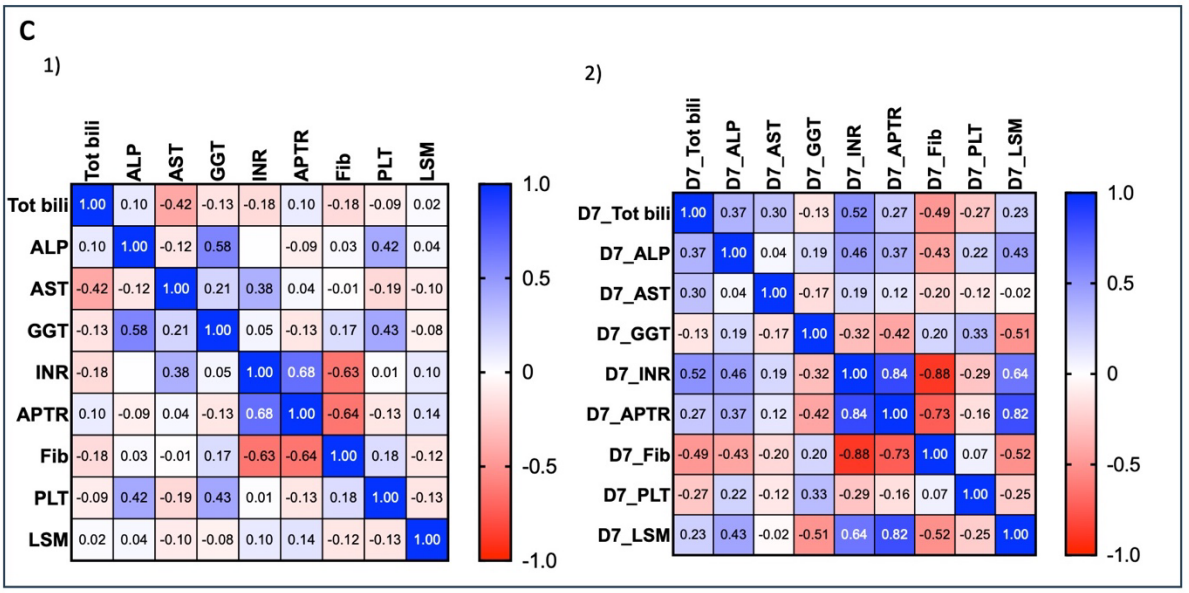
In ACLF, size and stiffness are linearly correlated, (a) Spearman r=0.05401, p=0.0140; Clinical Case showing CT imaging (b) and histology (c and d) in a patient with Wilson's disease; Liver size (e) measured as liver longitudinal diameter along an oblique line from the right hemi-diaphragm to the inferior tip of the right lobe in midaxillary line and ElastPQ (f) measurement showing average of 174kPa in the same patient.

g) Receiver operating characteristic (ROC) curve for right liver stiffness measurement (RLSM), spleen size in cm and spleen stiffness measurement (SM) discriminating between ALF and ACLF.

*p<0.05, **p<0.01,***p<0.001,****p<0.0001


a
1)
****
ns
***
RLSM KPa
150
100
50
0
DEAD
ALIVE
HC
2)
****
ns
**
D7 RLSM kPa
150
100
50
0
DEAD
ALIVE
HC
3)
D7/D1 RLSM
3
2
1
0
p=0.0804
DEAD
ALIVE


b
1)
D1 SPLEEN kPa
80
60
40
20
0
DEAD
ALIVE
2)
D7 SPLEEN kPa
100
80
60
40
20
0
DEAD
ALIVE


c
1)
Tot bili ALP AST GGT INR APTR Fib PLT LSM
Tot bili 1.00 0.10 -0.42 -0.13 -0.18 0.10 -0.18 -0.09 0.02
ALP 0.10 1.00 -0.12 0.58 -0.09 0.03 0.42 0.04
AST -0.42 -0.12 1.00 0.21 0.38 0.04 -0.01 -0.19 -0.10
GGT -0.13 0.58 0.21 1.00 0.05 -0.13 0.17 0.43 -0.08
INR -0.18 0.38 0.05 1.00 0.68 -0.63 0.01 0.10
APTR 0.10 -0.09 0.04 -0.13 0.68 1.00 -0.64 -0.13 0.14
Fib -0.18 0.03 -0.01 0.17 -0.63 -0.64 1.00 0.18 -0.12
PLT -0.09 0.42 -0.19 0.43 0.01 -0.13 0.18 1.00 -0.13
LSM 0.02 0.04 -0.10 -0.08 0.10 0.14 -0.12 -0.13 1.00
1.0
0.5
0
-0.5
-1.0
2)
D7_Tot bili D7_ALP D7_AST D7_GGT D7_INR D7_APTR D7_Fib D7_PLT D7_LSM
D7_Tot bili 1.00 0.37 0.30 -0.13 0.52 0.27 -0.49 -0.27 0.23
D7_ALP 0.37 1.00 0.04 0.19 0.46 0.37 -0.43 0.22 0.43
D7_AST 0.30 0.04 1.00 -0.17 0.19 0.12 -0.20 -0.12 -0.02
D7_GGT -0.13 0.19 -0.17 1.00 -0.32 -0.42 0.20 0.33 -0.51
D7_INR 0.52 0.46 0.19 -0.32 1.00 0.84 -0.88 -0.29 0.64
D7_APTR 0.27 0.37 0.12 -0.42 0.84 1.00 -0.73 -0.16 0.82
D7_Fib -0.49 -0.43 -0.20 0.20 -0.88 -0.73 1.00 0.07 -0.52
D7_PLT -0.27 0.22 -0.12 0.33 -0.29 -0.16 0.07 1.00 -0.25
D7_LSM 0.23 0.43 -0.02 -0.51 0.64 0.82 -0.52 -0.25 1.00
1.0
0.5
0
-0.5
-1.0

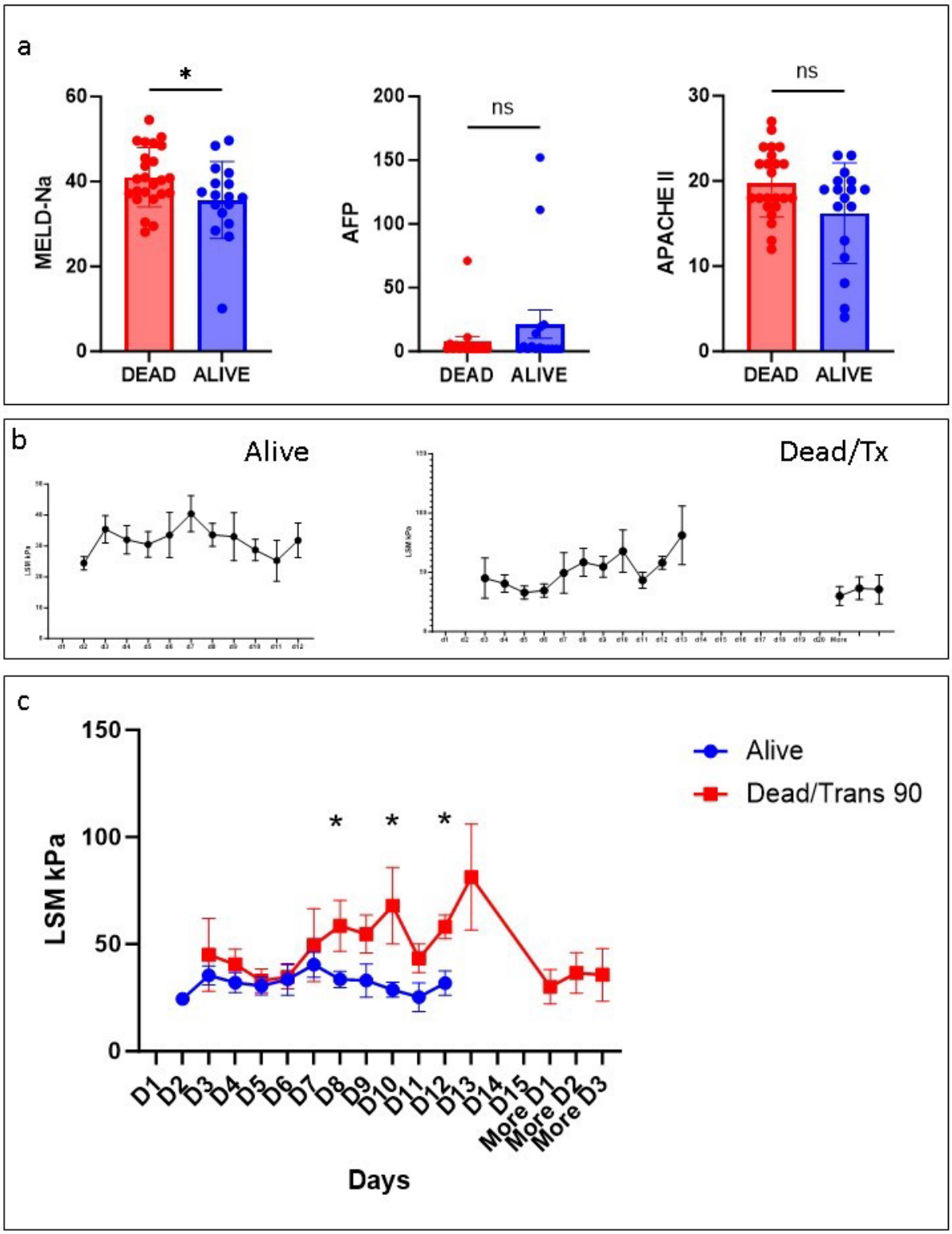
Supplementary Figure 3.

a) 1), At admission to liver intensive care LSM is not different between patients that spontaneously survive vs patients deceased or transplanted in the following 90 days. 2), At day 7 patients with increased LSM have poor prognosis (Kruskal-Walliss with Dunn's Multiple comparisons test). in both cases values are increased compared to healthy controls (HC). 3), the ratio between one week LSM and baseline is also increased in patients with poor prognosis despite not statistically significant (p=0.0804) .

b) Baseline spleen stiffness (1) is not different between patients alive at 90 days (17.22±12kPa) and those who die or were transplanted (24.37±19.6kPa), while at day 7 (2) patients with poor prognosis show increased values (17.45±8.36 vs 36.97±28.48kPa) .

*p<0.05, **p<0.01,***p<0.001,****p<0.0001, ns= Not Statistically Significant

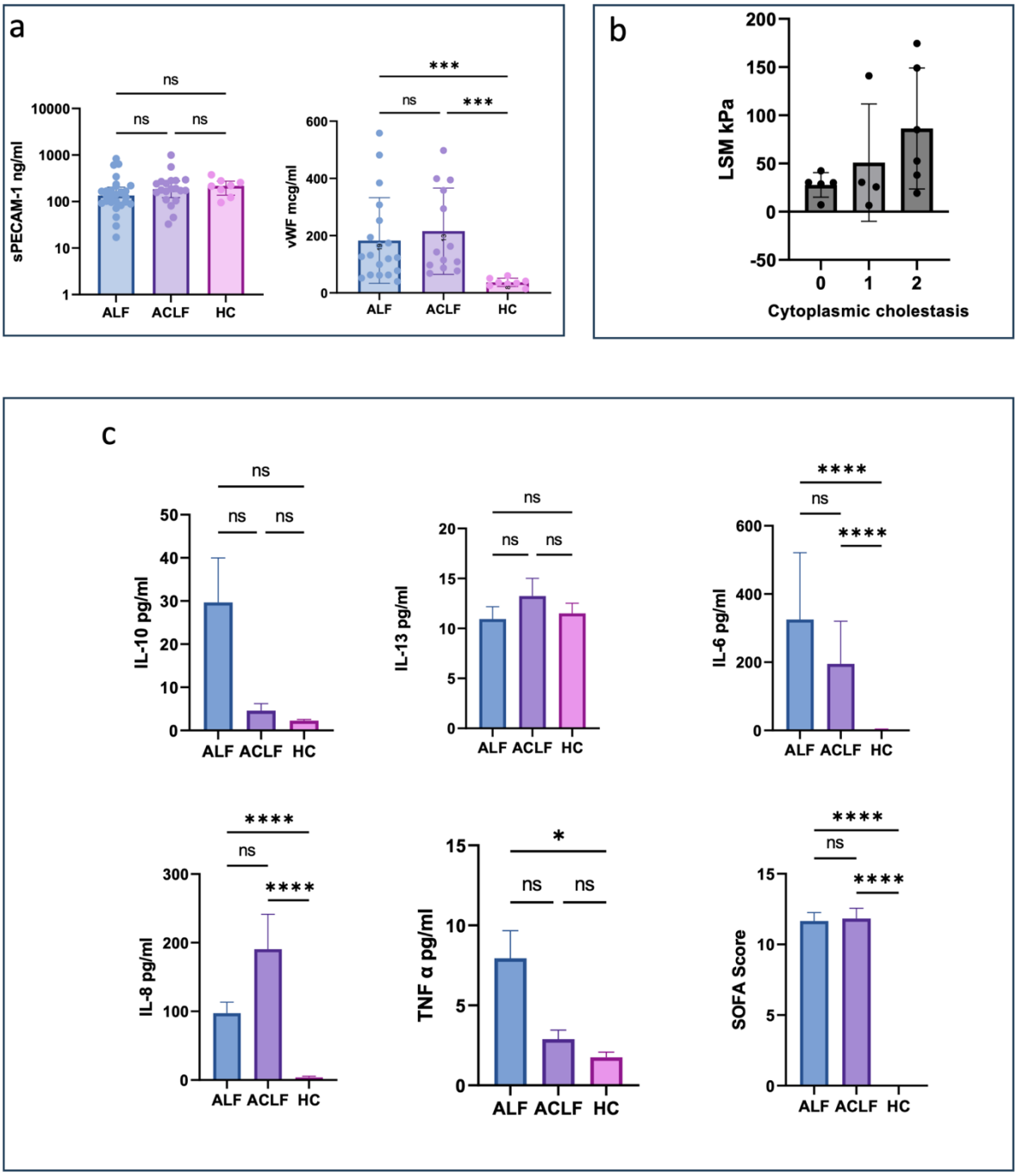
c)Correlation matrix showing lack of correlation on day 1 (1) and significant direct correlation of LSM with INR (Spearman r=0.6447, p=0.0029), APTR(r=0.8176,p<0.0001) and fibrinogen (r=-0.5215,p=0.022) on day 7(2) of admission.



Supplementary Figure 4.

a) Model for End-Stage Liver Disease(MELD)-Sodium (Na) was reduced in patients spontaneously survived, Alpha-fetoprotein (AFP0 and the Acute Physiology And Chronic

Health Evaluation (APACHE) II score was not different between the two groups. B) Analysis of liver stiffness measurement (LSM) according to day of illness onset rather than admission to ITU. Acute patients were generally transferred to King's College Hospital on day 2-3 of illness. In the dead/transplant (tx) graph you can see 4 patients were transferred after more than 20 days of illness (subacute liver failure), all of them were transplanted. c) Both curves show LSM in the alive and dead populations (Ordinary two-way ANOVA with Tukey's Multiple comparisons), the difference between the curves is significant at day 8 of disease onset and persists during the illness.



Supplementary Figure 5.

a) Soluble platelet/endothelial cell adhesion molecule (sPECAM-1) was not different between ACLF and HC, while and von Willebrand factor (vWF) was increased in acute liver failure syndromes compared to control HC=8, ALF=20, ACLF=12

b) LSM according to Cytoplasmic cholestasis (0,1,2)

d)When ACLF patients were divided according to the LSM, using 50 as cut off, the group with lower LSM showed increase IL-6 (p=0.0054), no difference in Soluble platelet/endothelial cell adhesion molecule (sPECAM-1), von Willebrand factor (vWF) white cell count (WCC), platelets(PLT), C-reactive protein (CRP), Aspartate transaminase (AST),

c) Cytokines are increased in both acute liver failure syndromes compared to controls. Same trend is seen in SOFA score

*p<0.05, **p<0.01,***p<0.001,****p<0.0001, ns= Not Statistically Significant

Supplementary Table

Supplementary Table 1								
Study Code	Death within 90 days	Transplant within 90 days	**KCC**	**PLEX**	**FFP**	**RBC**	**Cryo**	**PLT**
IMET A176	No	No	No	No	No	No	No	No
IMET A192	No	No	No	No	No	No	No	3
IMET A205	No	No	No	No	No	No	No	No
IMET A207	No	No	No	Yes	2	5	4	3
IMET A213	No	No	No	No	No	2	6	No
IMET A226	No	No	Yes	Yes	No	3	No	1
IMET A229	No	No	Yes	Yes	No	14	36	9
IMET A233	No	No	No	No	No	No	No	No
IMET A240	No	No	Yes	Yes	No	2	No	No
IMET A243	No	No	No	Yes	No	No	4	No
IMET A274	No	No	No	Yes	12	22	6	7
IMET A286	No	No	No	No	No	No	No	No
IMET A287	No	No	No	No	No	No	No	No
IMET A289	No	No	No	No	No	No	No	No

IMET A322	No	No	No	No	No	13	8	7
IMET A325	No	No	No	No	No	5	No	No
IMET A327	No	No	No	No	No	1	No	No
IMET A178	Yes	No	Yes	Yes	2	14	15	2
IMET A214	Yes	No	No	No	12	24	4	2
IMET A225	Yes	No	Yes	Yes	No	4	5	2
IMET A232	Yes	No	Yes	Yes	No	4	2	2
IMET A235	Yes	No	Yes	Yes	No	7	8	1
IMET A241	Yes	No	Yes	Yes	10	22	47	20
IMET A260	Yes	No	Yes	No	No	1	18	No
IMET A261	Yes	No	No	No	No	3	8	No
IMET A288	Yes	No	No	No	No	3	1No	No
IMET A304	Yes	No	No	No	No	1	No	No
IMET A184	Yes	No	Yes	Yes	15	21	47	6
IMET A276	No	Yes	Yes	No	6	2	15	6
IMET A177	No	Yes	Yes	No	No	No	2	No
IMET A193	No	Yes	Yes	No	No	1	4	No
IMET A195	No	Yes	Yes	No	2	4	6	1
IMET A219	No	Yes	Yes	No	No	No	2	No
IMET A227	No	Yes	Yes	No	No	No	No	No
IMET A237	No	Yes	Yes	No	No	No	No	No
IMET A251	No	Yes	Yes	No	2	8	8	1
IMET A305	No	Yes	No	No	No	2	8	No
IMET A315	No	Yes	Yes	No	No	No	8	No
IMET A316	No	Yes	Yes	No	No	1	6	No
IMET A183	Yes	Yes	Yes	Yes	No	10	15	8

Blood product received by each patient during the ITU stay including the referring hospital (until discharge, liver transplant or death)
KCC: King's College Criteria, PLEX: Plasmapheresis, FFP: fresh frozen plasma, RBC: red blood cells, Cryo: cryoprecipitate, PLT: platelets