Elevated plasma ICAM1 levels predict 28-day mortality in cirrhotic patients with COVID-19 or bacterial sepsis

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Supplementary materials and methods

High resolution CT chest for the severe covid-19 patients showed bilateral multifocal and diffuse ground glass opacities and interstitial thickening with peripheral predominance, typically CO-RADS 5 score. Samples of hospitalized moderate and severe covid-19 (with and without liver cirrhosis) patients were provided by National Disease Biobank of Institute of liver and biliary Sciences (ILBS), which has its own repository of covid-19 positive blood samples. Demographic and clinical data, including clinical symptoms or signs at presentation, laboratory and radiologic results during covid-19 management were collected from the biobank or the hospital records. From liver disease point of view, several parameters were noted for occurrence of any decompensating events such as ascites and encephalopathy. Also routine blood tests such as complete blood count, liver function tests, prothrombin time were recorded. In addition, for each cirrhotic patient; Child-Turcotte-Pugh (CTP) score and model for end-stage liver disease (MELD) scores were calculated.[1] The Charlson Comorbidity Index (CCI) based upon various comorbidities was calculated for all patients using an online calculator.[2] For the ICU patients, several other serum parameters including Ferritin levels, Procalcitonin, LDH, D-dimers were also recorded. Along with baseline SOFA, SOFA scores and CLIF-C OF scores of ICU patients were also calculated by an online calculator at day 3-5 post ICU admission as per the standard guidelines[3]. Organ Failure(s) were defined as follows: Liver (serum bilirubin ≥12.0 mg/dl), renal (creatinine ≥2 mg/dl or renal replacement), brain (HE III–IV grade), coagulation (INR ≥ 2.5), circulatory (vasopressor use for circulatory failure indication) and lung (SpO2/FiO2 ≤ 214 or mechanical Ventilation for lung failure)[3].

For ELISA assays, around 5 ml of venous blood was collected from hospitalised patients at day 1 of admission (ward or ICU) in 3·2% sodium citrate tubes and centrifuged at 4000 rpm for 20 min and plasma obtained were stored at –80°C. For the biobank samples, plasma was directly obtained and stored at –80°C. Commercial ELISAs were used to measure the

biomarker levels [(ICAM1 and vWF (R & D systems), Ang1, Ang2, vegfr1 (elabsciences)]. The dilutions used for each biomarker are given in Supp Table 1 and details of the ELISA kits are given in the Supplementary CTAT Table. The biomarker results were reported on a continuous scale, measured in picograms per millilitre (pg/ml) or log values. All measurements were performed according to the manufacturers' instructions. All standards, controls, and test samples were assayed in duplicate. Analysis of ELISA results were performed on logistic (4-PL) curve fit via an online logistic 4PL tool (www.myassays.com).

Fig. S1

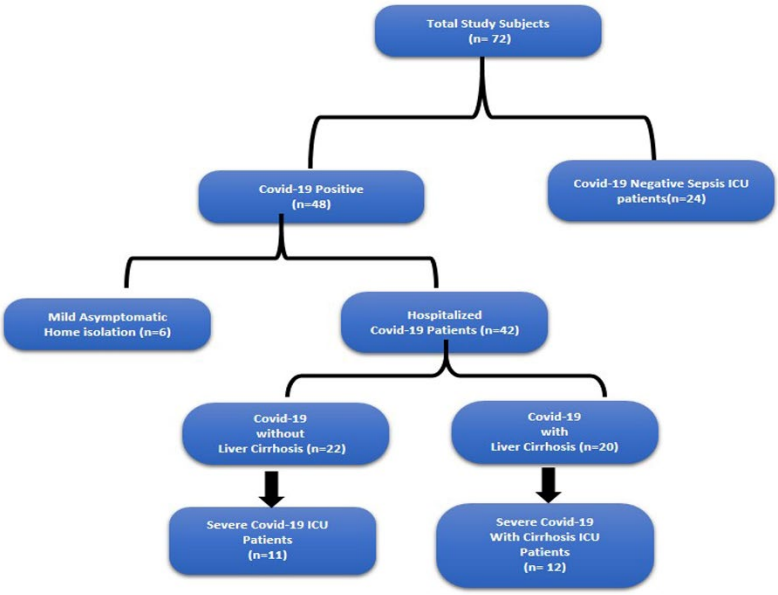

Fig. S1. Patients included in the Study. ICU: Intensive Care Unit

Table S1

Variables	Dilution factor of plasma samples
ICAM1	1:100
vWF	1:20
Vegfr1	1:2
Ang1	1:5
Ang2	1:5

Table S2
Levels of Endothelial biomarkers in mild and hospitalized Covid-19 patients alone

Variables	Mild Non-hospitalized patients (n=6) Median (min-max) pg/ml	Hospitalized patients (n= 22) Median (min-max) pg/ml	P value
ICAM1	35733.5 (5310-54930)	67475 (30930-261600)	<0.0001*
vWF	7156 (4351-12312)	24720 (5011-63560)	0.009*
Vegfr1	1034.5 (675.8-2667)	3600 (851.8-13744)	0.002*
Ang1	1282.4 (781-10643)	4708 (909.7-13744)	0.06
Ang2	932.5 (612-1823)	3572 (754-13059)	0.0005*

'' denotes significant p values (Mann-Whitney U test). Significance was taken as p< 0.05.*

Table S3
Biomarker levels in Survivors and Non-Survivors in Intensive care unit (n=47)

Variable ln(2) values	Survivors Median (min-max) pg/ml n=14	Non-survivors Median (min-max) pg/ml n=33	P value
ICAM1	82200 (30930-236200)	258000 (86970-353600)	<0.0001*
vWF	28220 (5011-63650)	28340 (5965-56650)	0.62
Vegfr1	2043 (688-5054)	1016 (762-7619)	0.04*
Ang1	2815 (587-11476)	1409 (466-9608)	0.02*
Ang2	2823 (628-11146)	3040 (1072-12695)	0.13

'' denotes significant p values (Mann-Whitney U test). Significance was taken as p< 0.05.*

Table S4
AST and ICAM1 as Independent Mortality Predictors in Patients admitted in Intensive Care Unit (n=47)

	Univariate Analysis		Multivariate Analysis	
Risk Factor	**Crude HR (95% CI)**	**P value**	**Adjusted HR (95% CI)**	**P value**
Age	0.88	0.99 (0.97-1.0)		
Sex	0.25	0.54 (0.18-1.57)		
CCI score	0.33	1.1 (0.89-1.4)		
Ddimers[#]	0.04*	4.3 (0.8-22.35)		
Ferritin	0.19	1.34 (0.86-2.11		
LDH[#]	0.068	1.737 (0.96-3.14)		
NLR	0.17	1.03 (0.98-1.08)		
PCT	0.97	1 (0.89-1.12)		
Albumin	0.89	0.95 (0.52-1.75)		
Bilirubin	0.25	1.04 (1-1.08)		
Sodium	0.98	1 (0.95-1.04)		
AST[#]	0.01*	1.96 (1.12-3.4)	1.87 (1.05-3.31)	0.03*
ALT[#]	0.36	0.78 (0.47-1.31)		
INR	0.05	1.42 (0.97-2.08)		
Creatinine	0.01*	1.28 (1.07-1.54)		
ARDS	0.76	1.12 (.51-2.44)		
ICAM1[#]	0.007*	3.002 (1.366-6.65)	2.94 (1.30-6.65)	0.01*
vWF[#]	0.96	0.98 (0.51-1.88)		
Vegfr1[#]	0.22	0.66 (0.34-1.28)		
Ang1[#]	0.75	0.93 (0.62-1.4)		
Ang2[#]	0.52	0.85 (0.53-1.38)		

'[#]' denotes that Log values of these parameters were taken. '' denotes significant p values (Cox regression). Significance was taken as p< 0.05. HR: Hazard Ratio; CI: Confidence Interval. CCI: Charlson comorbidity index; LDH: Lactate dehydrogenase; NLR: Neutophil to lymphocyte ratio; PCT: Procalcitonin; MELD: Model for end-stage liver disease; CTP: Child-Turcotte-Pugh; SOFA: Sequential organ failure assessment. CLIF-SOFA: Chronic liver failure-sequential organ failure assessment.*

Table S5
Predictive factors of 28-day mortality in Liver Cirrhosis Patients with Severe Covid-19 or Sepsis in Intensive Care Unit (n=36)

Predictive factors	AUC	Cut-off point	Youden Index	Sensitivity %	Specificity %
ICAM1	0.74 (0.56-0.93)	12.3	44.7	72	72.7
MELD	0.60 (0.38-0.83)	29.5	23.6	60	63.6
SOFA	0.82 (0.66-0.98)	11	44.7	72	72.7
CLIF-OF	0.83 (0.67-0.97)	12	52.7	80	72.7

AUC: Area Under the Curve; MELD: Model for end-stage liver disease; SOFA: Sequential organ failure assessment. CLIF-SOFA: Chronic liver failure-sequential organ failure assessment.

Supplementary references

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3. Pan HC, Jenq CC, Tsai MH, Fan PC, Chang CH, Chang MY, et al. Scoring systems for 6-month mortality in critically ill cirrhotic patients: a prospective analysis of chronic liver failure - sequential organ failure assessment score (CLIF-SOFA). Aliment Pharmacol Ther. 2014;40:1056-1065.