Role of liver steatosis and transaminases elevations on hard clinical outcomes in patients with COVID-19

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Methods

Transient elastography

For this specific purpose, LSM were carried out only in consecutive admitted patients during the first 48 h after admission who were in stable condition without oxygen support or with mild-moderate O2 needs (Sa:FiO2 >300). The number of LSM performed was not preplanned and we tried to complete as many as possible after working ours depending on the availability of the members of the team with prior experience in performing liver elastography. The TE device utilized (Fibroscan 502 Touch) had availability of M and XL probes, that were used based on device requirements. The usual quality criteria (at least 10 valid measurements and an IQR/M ≤30%) were applied.

Statistics

In order to adjust the weight of AST by other well-known risk factors on COVID-19 outcomes, two approaches were taken. First, an exploratory adjustment using logistic regression analysis was performed. Selection of variables used to enter the logistic regression models was based on currently available literature, as well as on the results of univariate analyses. For this specific purpose, the composite death and/or ICU was preferred over death, due to the larger number of events and because it was regarded as a more realistic and complete representation of the whole impact of COVID-19, both for the individual patient and more globally for healthcare systems. Age and O2 saturation at admission were maintained as main covariates for adjustment based on their observed weight on outcomes, as well as on available literature and, most specially, on

their potential utility for rapid and objective triage, even in community settings. In order to further adjust the weight of AST with other well-documented blood prognostic markers available at admission (lymphocyte count, ferritin, LDH, IL-6 and DD), several combinations of these biomarkers were introduced in blocks of 3 (while keeping age and O2 saturation always in the model). In order to avoid overfitting, and given the total number of events of the composite endpoint, the 2 most stable variables where used to further adjust OR estimates for AST on the composite endpoint.

Second, a classification and regression tree (CART) analysis was conducted in order to study and illustrate the interaction between the same variables studied in the logistic regression analyses, as well as to identify best cut-offs for continuous variables. The method has been previously used by our group and others and has been described extensively elsewhere (1-3). In brief, the method allows for the construction of inductive decision trees through strictly binary splitting. This method is especially adept at detecting relevant interactions between variables, and allows identification of subgroups of patients that share a specific combination of clinical characteristics and a similar prognosis. In our study, the number of patients in terminal nodes was set to a minimum of 25 in order to maximize stability of estimates. Age was forced (as continuous variable) as first node variable for the reasons mentioned above. Cut-off points for continuous and ordinal variables were generated automatically by the model based on statistical cost assumptions. Finally, correlation between continuous variables was studied by linear regression, and logarithmic transformations were applied when required by differences in scale of variables in order to allow for a better visual interpretation of results.

Supplementary Table 1. Association of liver steatosis with outcomes.

	Death	p	Death/ICU	p
HSI		0.56		0.78
HSI<30	0% (0/29)		13.8 %(4/29)	
HSI 30-36	5.9% (6/102)		13.7% (14/102)	
HSI>36	4.1% (7/169)		13.6% (23/169)	
missing	5.7% (4/70)		18.6% (13/70)	
CAP		NA		1.0
≤250 dB/m	0% (0/51)		5.9% (3/51)	
>250 dB/m	0% (0/47)		4.3% (2/47)	
≤300 dB/m	0% (0/74)	NA	5.4% (4/74)	1.0
>300 dB/m	0% (0/24)		4.2% (1/24)	

HSI: Hepatic Steatosis Index (HSI = 8 × (ALT at admission/AST at admission) + BMI at admission (+ 2 if type 2 diabetes yes, + 2 if female)]; CAP: Controlled Attenuation Parameter

Supplementary Table 2. Association of baseline AST with outcomes (raw and stratified by age)

	Death % (n)			Death/ICU % (n)		
	Yes	No	p	Yes	No	p
Overall cohort			0.03			0.002
AST normal (n=200)	2.5% (5)	97.5% (195)		9.5% (19)	90.5% (181)	
AST elevated (n=167)	7.2% (12)	92.8% (155)		21% (35)	79% (132)	
Age						
<65			0.46			0.22
AST normal (n=140)	0% (0)	100% (140)		8.6% (12)	91.4% (128)	
AST elevated (n=120)	0.8% (1)	99.2% (119)		13.3% (16)	86.7% (104)	
≥65			0.05			0.001
AST normal (n=60)	8.3% (5)	91.7% (55)		11.7% (7)	88.3% (53)	
AST elevated (n=47)	23.4% (11)	76.6% (36)		40.4% (19)	59.6% (28)	

Supplementary Table 3. Logistic regression adjustment of the weight of AST on the composite endpoint death and/or ICU

	Univariate		Multivariate adjustment	
	OR (95% CI)	P-value	OR (95% CI)	P-value
AST (U/L)	1.007 (1.001-1.013)	0.03	1.004 (0.998-1.011)	0.207
Age (yr)	1.039 (1.02-1.07)	<0.0001	1.015 (0.989-1.042)	0.256
O2 saturation (pulse-oxymeter) (%)	0.882 (0.832-0.936)	<0.0001	0.930 (0.869-0.996)	0.037
Lymphocyte count (10E9/L)	0.998 (0.998-0.999)	<0.0001	0.999 (0.998-0.999)	0.001
D-Dimer (µg/mL)	1.000 (1.000-1.000)	0.006	1.000 (1.000-1.000)	0.035

ICU: intensive care unit;

All variables are baseline

	Univariate		Multivariate adjustment	
	OR (95% CI)	P-value	OR (95% CI)	P-value
AST elevated (0=no, 1= yes)	2.526 (1.384-4.611)	0.003	2.316 (1.189-4.510)	0.014
Age (yr)	1.041 (1.02-1.07)	<0.0001	1.015 (0.998-1.042)	0.291
O2 saturation (pulse-oxymeter) (%)	0.882 (0.832-0.936)	<0.0001	0.933 (0.870-1.000)	0.049
Lymphocyte count (10E9/L)	0.998 (0.998-0.999)	<0.0001	0.999 (0.998-0.999)	0.001
D-Dimer (µg/mL)	1.000 (1.000-1.000)	0.006	1.000 (1.000-1.000)	0.041

ICU: intensive care unit;

All variables are baseline

AST elevated: men >50 IU/L, women >35 IU/L

	Univariate		Multivariate adjustment	
	OR (95% CI)	P-value	OR (95% CI)	P-value
AST elevated (0=no, 1= yes)	2.526 (1.384-4.611)	0.003	2.893 (1.553-5.388)	0.001
Age (yr)	1.041 (1.02-1.07)	<0.0001	1.026 (0.998-1.053)	0.065
O2 saturation (pulse-oxymeter) (%)	0.882 (0.832-0.936)	<0.0001	0.915 (0.859-0.975)	0.001
Hypertension (0=no, 1= yes)	2.351 (1.309-4.222)	0.004	1.738 (0.888-3.403)	0.11

ICU: intensive care unit;
All variables are baseline
AST elevated: men >50 IU/L, women >35 IU/L

	Univariate		Multivariate adjustment	
	OR (95% CI)	P-value	OR (95% CI)	P-value
AST elevated (0=no, 1= yes)	2.526 (1.384-4.611)	0.003	2.973 (1.589-5.561)	0.001
Age (yr)	1.041 (1.02-1.07)	<0.0001	1.029 (1.003-1.055)	0.065
O2 saturation (pulse-oxymeter) (%)	0.882 (0.832-0.936)	<0.0001	0.908 (0.851-0.968)	0.001
Type-2 diabetes (0=no, 1= yes)	1.902 (0.946-3.828)	0.071	1.939 (0.900-4.179)	0.091

ICU: intensive care unit;
All variables are baseline
AST elevated: men >50 IU/L, women >35 IU/L

Supplementary Fig. 1. Association between baseline AST and LDH and the composite endpoint death and/or ICU in the cohort.

Correlation between the variables and stratified occurrence of the composite endpoint per quadrants according to AST and LDH baseline values.
ULN: Upper limit of normal.
AST: aspartate transaminase (IU/L), LDH: Lactate dehydrogenase (IU/L)