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Sources and clinical significance of aspartate aminotransferase increases in COVID-19

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ABSTRACT

Background: Aspartate aminotransferase (AST) is often increased in COVID-19 and, in some studies, AST abnormalities were associated with mortality risk.
Methods: 2054 hospitalized COVID-19 patients were studied. To identify sources of AST release, correlations between AST peak values and other biomarkers of tissue damage, i.e., alanine aminotransferase (ALT) for hepatocellular damage, creatine kinase (CK) for muscle damage, lactate dehydrogenase for multiorgan involvement, alkaline phosphatase and γ-glutamyltransferase for cholestatic injury, and C-reactive protein (CRP) for systemic inflammation, were performed and coefficients of determination estimated. The role of AST to predict death and intensive care unit admission during hospitalization was also evaluated. All measurements were performed using standardized assays.
Results: AST was increased in 69% of patients. Increases could be fully explained by summing the effects of hepatocellular injury [AST dependence from ALT, 66.8% [95% confidence interval (CI): 64.5–69.1)] and muscle damage [AST dependence from CK, 42.6% (CI: 39.3–45.8)]. We were unable to demonstrate an independent association of AST increases with worse outcomes.
Conclusion: The mechanisms for abnormal AST in COVID-19 are likely multifactorial and a status related to tissue suffering could play a significant role. The clinical significance of AST elevations remains unclear.

1. Introduction

In December 2019, a novel viral respiratory illness caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged from Wuhan, China, and then rapidly spread worldwide. The 2019 coronavirus disease (COVID-19) is associated with pneumonia, acute respiratory distress syndrome, and multiorgan failure, leading to a high rate of morbidities and deaths [1]. Cases with a variety of extra-pulmonary manifestations, such as cardiac, neurological, gastrointestinal, ocular, cutaneous, and thromboembolic conditions, have been described [2]. SARS-CoV-2 has indeed a systemic distribution, with possible involvement of heart, liver, pancreas, and kidneys. This could be due to the ubiquitous expression of angiotensin-converting enzyme 2 receptors, the main viral target for cellular entry.

Since the initial studies carried out in China, up to 50% of patients hospitalized with SARS-CoV-2 infection were reported as having increased concentrations of aspartate aminotransferase (AST) in serum [3]. This evidence induced many authors to relate enzyme elevations with the presence of viral liver injury as part of disseminated systemic disease [4–6]. Immune-mediated inflammation typical of COVID-19 and hepatotoxicity of drugs employed in its treatment were also proposed as triggering mechanisms [7]. It is however well known that AST is found not only in liver, but also, in extremely elevated concentrations, in both myocardial and skeletal muscles, and, additionally, in kidney, pancreas, and lung [8]. Therefore, dysfunctions involving these tissues (even in COVID-19) may be responsible of AST elevations in blood. Quite importantly, in some studies but not in all, AST abnormalities were associated with the highest in-hospital mortality risk, with the

Abbreviations: SARS-CoV-2, severe acute respiratory syndrome coronavirus 2 COVID-19. 2019 coronavirus disease; AST, aspartate aminotransferase; URL, upper reference limit; ALT, alanine aminotransferase; CK, creatine kinase; LDH, lactate dehydrogenase; ALP, alkaline phosphatase; GGT, γ-glutamyltransferase; CRP, C-reactive protein; ICU, intensive care unit; IFCC, International Federation of Clinical Chemistry and Laboratory Medicine; HI, hemolysis index; R^2, coefficient of determination; IQR, interquartile range; OR, odds ratio; CI, confidence interval; P-5′-P, pyridoxal-5-phosphate; R, correlation coefficient; IL-6, interleukin-6.

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Table 1
Demographics and laboratory findings of COVID-19 patients included in the study.

	Total	URL	>URL	Nonsurvivors	Survivors	P	ICU	Non-ICU	P
Age, years	64 (51–77)	–	–	79 (71–85)	61 (49–75)	<0.0001	64 (55–71)	64 (50–78)	0.27
Male, no. (%)	1217 (59)	–	–	196 (69)	1021 (58)	<0.0001	150 (75)	1067 (57)	<0.0001
AST, U/L	47 (31–77)	34	69%	64 (43–104)	45 (30–74)	<0.0001	79 (50–145)	45 (31–73)	<0.0001
ALT, U/L	61 (30–112)	49 (men) 33 (women)	65%	55 (26–100)	62 (31–115)	0.033	112 (74–203)	57 (28–104)	<0.0001
CK, U/L	135 (69–306)	322 (men) 201 (women)	28%	294 (136–641)	119 (64–263)	<0.0001	369 (167–793)	120 (66–267)	<0.0001
LDH, U/L	362 (284–488)	220	91%	581 (445–755)	344 (273–449)	<0.0001	606 (496–736)	346 (275–456)	<0.0001
GGT, U/L	58 (33–114)	68 (men) 40 (women)	51%	76 (39–166)	55 (32–109)	<0.0001	164 (83–344)	52 (30–97)	<0.0001
ALP, U/L	79 (62–110)	115 (men) 98 (premenopausal women) 141 (post-menopausal women)	21%	101 (76–156)	77 (61–102)	<0.0001	126 (85–194)	76 (61–100)	<0.0001
CRP, mg/L	106.0 (44.4–181.2)	10	91%	217.1 (137.4–317.8)	89.7 (37.4–157.7)	<0.0001	289.5 (193.8–354.5)	94.0 (39.6–157.3)	<0.0001

All data are expressed as median and interquartile range, except male no. and > URL percentage.
URL, upper reference limit; ICU, intensive care unit; AST, aspartate aminotransferase; ALT, alanine aminotransferase; CK, creatine kinase; LDH, lactate dehydrogenase; GGT, γ-glutamyltransferase; ALP, alkaline phosphatase; CRP, C-reactive protein.

recommendation to include this test in the timely monitoring strategy of COVID-19 patients [4,9–14].

Given the uncertainty of the information available about the source and clinical significance of AST increases in serum of COVID-19 patients, we thought it would be useful to examine a large cohort of these patients in order to accurately describe: a) the number of patients with AST above the upper reference limit (URL); b) correlations between AST values and other serum markers of organ or tissue damage to identify relationships and possible sources of AST release, i.e. alanine aminotransferase (ALT) for hepatocellular damage, creatine kinase (CK) for muscle damage, lactate dehydrogenase (LDH) for multiorgan involvement including lung, alkaline phosphatase (ALP) and γ-glutamyltransferase (GGT) for cholestatic injury (the latter being also susceptible to induction by drugs), and C-reactive protein (CRP) for systemic inflammation; and c) the role of AST, if any, in predicting transfer to the intensive care unit (ICU) and death during hospitalization for COVID-19.

2. Materials and methods

2.1. Study population

We performed a retrospective, observational study on a cohort of adult (age ≥ 18 years old) COVID-19 patients admitted between February 2020 and February 2021 to the "Luigi Sacco" academic hospital in Milan, one of the two national reference centers for infectious diseases in Italy. All patients had clinical and/or radiologic findings highly suggestive for COVID-19 at admission and SARS-CoV-2 infection was confirmed by detection of viral RNA on nasopharyngeal material, using a real-time reverse transcription polymerase chain reaction method. Electronic records of all patients were reviewed, and those having at least one AST result during the hospitalization period were included in the study. The Institutional Review Board approved the study (registration no. 2020/ST/159).

2.2. Methods

Patient data were extracted from the hospital information systems. For AST, only peak values during hospitalization were considered because enzyme abnormalities at admission might have been influenced by pre-existing liver disease or other basic conditions of patients. In addition to AST, serum ALT, CK, LDH, ALP, GGT, and CRP values were retrieved. For these tests, both results obtained on the same day of the AST peak value (used for correlation studies) and the highest value during the hospitalization period (used to capture the peak of these laboratory parameters and compare them against tested clinical outcomes) were collected. When the ICU admission was evaluated as outcome, in patients admitted to ICU the pre-admission peaks were retrieved.

All laboratory parameters were measured on the Alinity c platform using proprietary reagents and calibrators provided by Abbott Diagnostics [15,16]. Importantly, all enzymatic methods were previously validated by showing their optimal alignment to the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) reference measurement procedures [15]. The importance of using assays providing standardized results to allow the use of common URLs, enabling the universal application of results of clinical studies undertaken in different locations and permitting their unambiguous interpretation, was previously highlighted [17,18]. Adult reference intervals (all derived from previously performed ad hoc local studies) are: AST, up to 34 U/L; ALT, up to 49 U/L (men) and 33 U/L (women); CK, 47–322 U/L (men) and 29–201 U/L (women), LDH, 125–220 U/L; ALP, 43–115 U/L (men), 33–98 U/L (premenopausal women), and 53–141 U/L (postmenopausal women); GGT, up to 68 U/L (men) and 40 U/L (women); and CRP, up to 10.0 mg/L. Hemolysis index (HI) was automatically measured on all samples by the Alinity analyzer and the interfered test results were not reported to the wards and, therefore, not included in the study. The HI thresholds that blocked the reporting of numeric results were 25 (i.e., free hemoglobin concentration > 0.25 g/L) for LDH and 125 (i.e., free hemoglobin concentration > 1.25 g/L) for AST, the only two assays significantly interfered by hemolysis using the Alinity measuring system.

2.3. Data analysis

Linear regression analyses between all combinations of analyte results, obtained at the same time of AST peak values, were used to estimate coefficients of determination (R^2), which represent the proportion of the total variation in y (e.g., AST) that can be explained by its regression on $\times$ (other biomarkers). This permitted to identify relationships, if any, between AST increases and different organ or tissue damage specifically depicted by ALT (hepatocellular injury), CK (muscle damage), LDH (multiorgan involvement, including lung), ALP and GGT (cholestatic injury), and CRP (systemic inflammation).

Biomarkers were evaluated according to the following outcomes: a) hospitalization in ICU vs. hospitalization in non-intensive wards (non-ICU), and b) death during hospitalization (non-survivors) vs. hospital

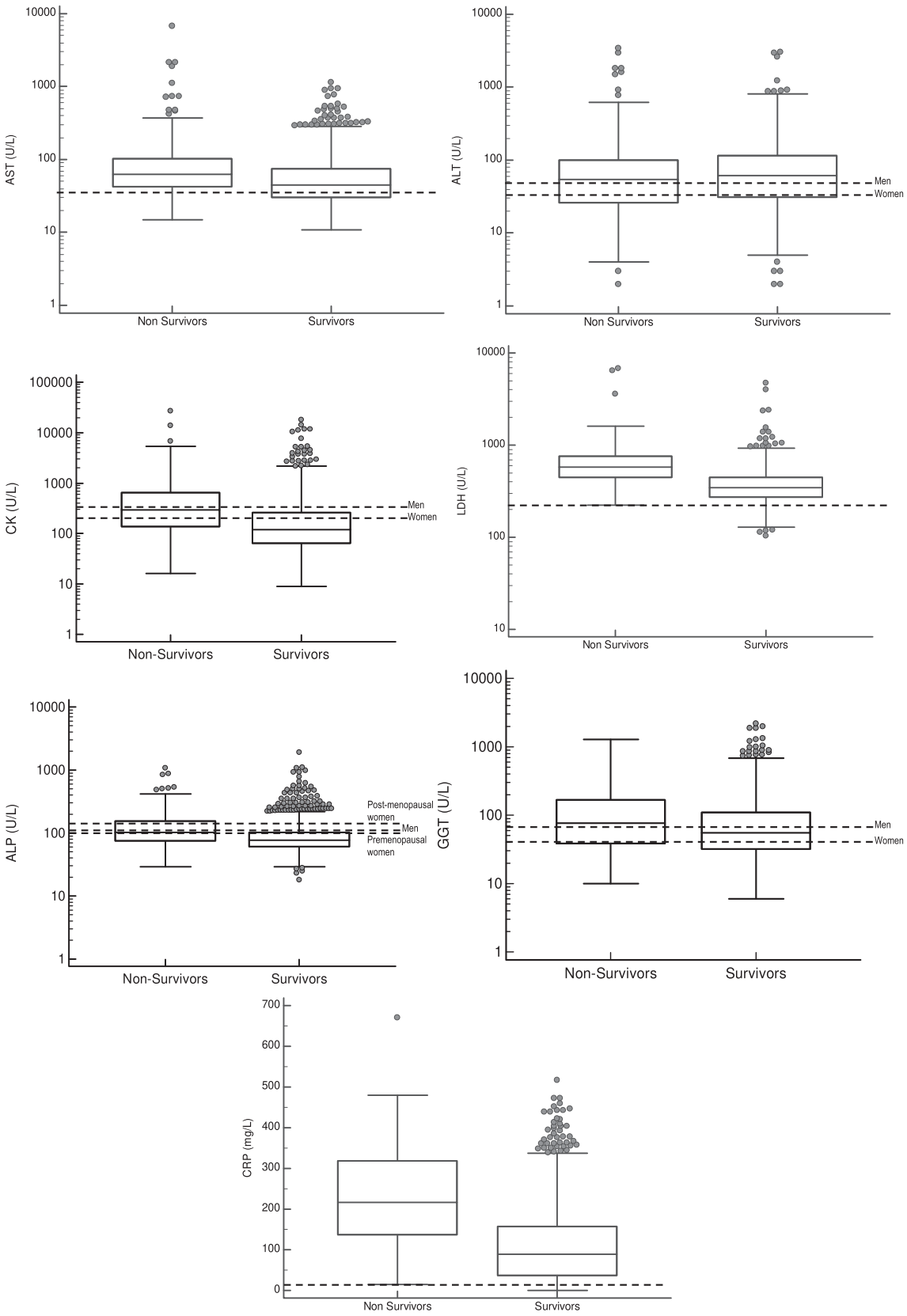

Fig. 1. Box and whisker plots showing the distribution of results of evaluated parameters in studied COVID-19 patients, according to the survival outcome. The dashed lines indicate the upper reference limits of each test. Note that, except for CRP, the scale of the *y*-axis is logarithmic. AST, aspartate aminotransferase; ALT, alanine aminotransferase; CK, creatine kinase; LDH, lactate dehydrogenase; GGT, γ-glutamyltransferase; ALP, alkaline phosphatase; CRP, C-reactive protein.

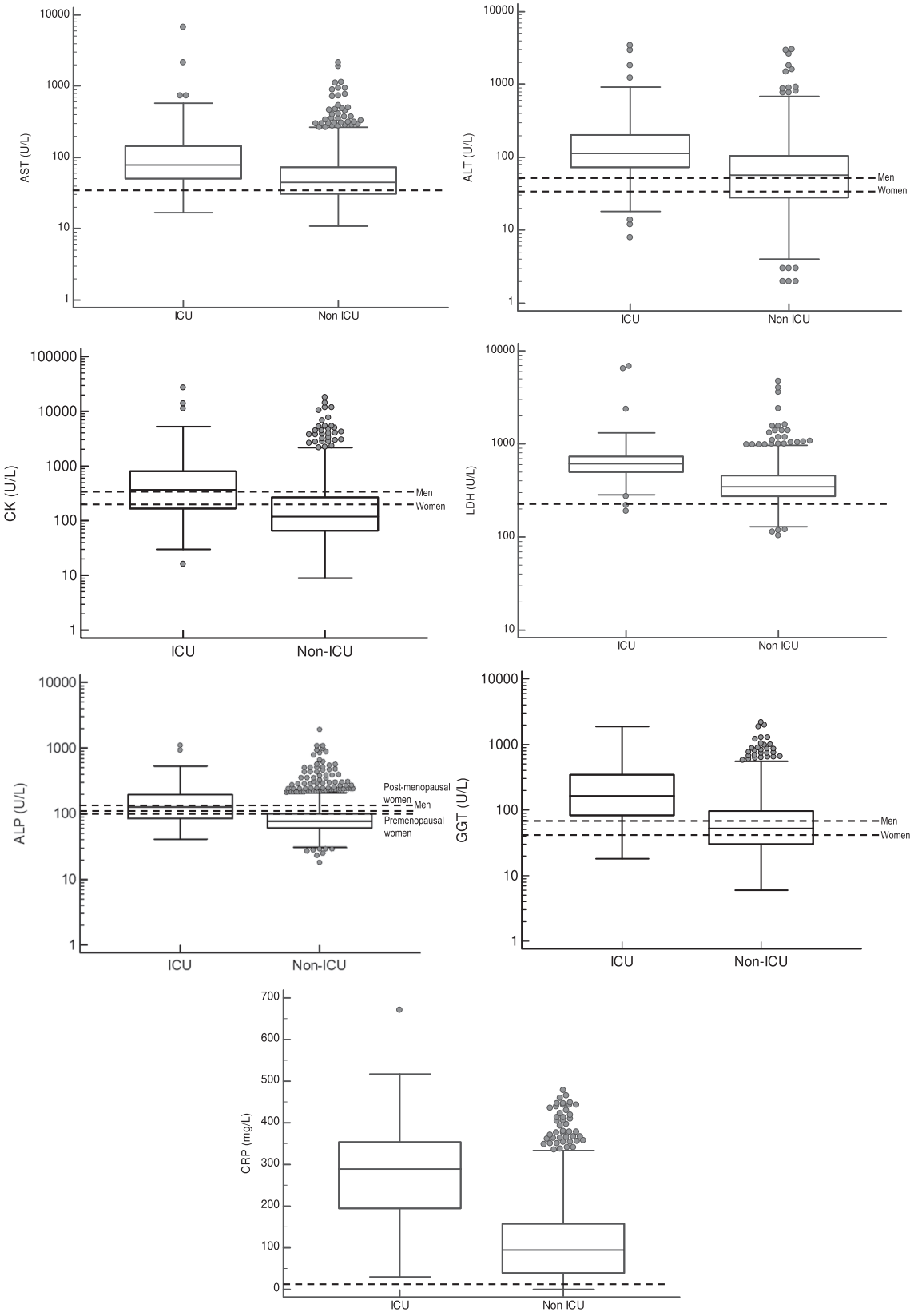

Fig. 2. Box and whisker plots showing the distribution of results of evaluated parameters in studied COVID-19 patients, according to the hospitalization in intensive care unit vs. hospitalization in nonintensive wards. The dashed lines indicate the upper reference limits of each test. Note that, except for CRP, the scale of the y-axis is logarithmic. ICU, intensive care unit; AST, aspartate aminotransferase; ALT, alanine aminotransferase; CK, creatine kinase; LDH, lactate dehydrogenase; GGT, γ-glutamyltransferase; ALP, alkaline phosphatase; CRP, C-reactive protein.

Table 2
Coefficients of determination (R^2) for the comparison data set.

	ALT	CRP	LDH	GGT	ALP	CK
AST	0.668	0.024	0.565	0.012	0.011	0.426
ALT		0.001	0.359	0.046	0.020	0.150
CRP			0.129	0.011	0.011	0.025
LDH				0.009	0.013	0.264
GGT					0.491	0.000
ALP						0.000

AST, aspartate aminotransferase; ALT, alanine aminotransferase; CRP, C-reactive protein; LDH, lactate dehydrogenase; GGT, γ-glutamyltransferase; ALP, alkaline phosphatase; CK, creatine kinase.

discharge after clinical recovery (survivors). Demographic and laboratory characteristics (peak values) were compared between patients divided in these categories. Data were reported as percentages for categoric variables and median with interquartile range (IQR) for quantitative variables. Differences between variables in different categories were assessed by applying a χ^2 test (categoric) and Mann-Whitney rank-sum test (quantitative). Univariate logistic regression was used to estimate variables' odds ratios (ORs) and their 95% confidence intervals (CIs) in relation to the two selected outcomes. A multivariate logistic regression model was then applied to variables significant at the univariate analysis. Final selection of variables included in the multivariate model was done by applying a stepwise approach. A P value < 0.05 denoted statistical significance. All statistical analyses were performed using MedCalc software version 13.0 (MedCalc Software bvba, Ostend, Belgium).

3. Results

During the study period, we retrieved data from 2054 patients hospitalized for COVID-19. Of these, 284 (13.8%) died during the hospitalization period, whereas 1770 were discharged after clinical recovery. Furthermore, 199 (9.7%) of the 2054 patients were admitted to the ICU, whereas 1955 stayed in non-intensive care COVID units during the entire hospitalization period. Median patient age was 64 years (IQR: 51–77), 1217 (59.3%) being males. Demographic and laboratory data of the studied population are shown in Table 1. In non-survivors, age was significantly higher than in surviving patients, whereas the same median age was observed between ICU and non-ICU admitted patients. Furthermore, males suffered more frequently the two negative outcomes when compared with female patients. Values of all the examined laboratory tests were significantly different between groups for both the evaluated outcomes (Table 1; Figs. 1 and 2). AST was increased in 69% of cases, slightly more than ALT (65%), with an AST/ALT ratio value > 1 in 52% of patients. Interestingly, ALT median was slightly but significantly higher in survivors than in non-survivors (62 vs. 55 U/L). On the other hand, the great majority of patients (91%) showed an increase of CRP and LDH, confirming that these parameters are among the most susceptible COVID-19 biomarkers.

R^2 obtained by performing regressions between peak AST values and ALT, CK, LDH, ALP, GGT, and CRP values, measured at the same time, are reported in Table 2. These findings gave some important information. Particularly, in our COVID-19 population, AST increases could be fully explained by summing the effects of hepatocellular injury [AST dependence from ALT, 66.8% (95% CI: 64.5–69.1)] with muscle damage [AST dependence from CK, 42.6% (95% CI: 39.3–45.8)]. AST dependence from LDH (56.5%, 95% CI: 53.7–59.3) had the same explanation, as approximately 60% of the LDH increases could be in turn explained by summing the effect of hepatocellular damage [LDH dependence from ALT, 35.9% (95% CI: 32.6–39.2)] and of muscle damage [LDH dependence from CK, 26.4% (95% CI: 23.2–29.7)]. As expected, there was no dependence of AST increases on hepatocanalicular injury (approximately 99% of AST variation was not dependent from ALP and GGT), and on systemic inflammation [AST dependence from CRP, 2.4% (95% CI: 1.1–3.7)]. Fig. 3 shows the relationships of AST that were found significant.

At univariate analysis, ORs for death during hospitalization were significantly higher for older patients and for all evaluated tests (Table 3). On the other hand, only CK, LDH, and CRP were significant predictors of ICU admission (Table 4). It should be acknowledged however that ORs were very close to 1 in most cases. As a matter of fact, only high serum concentrations of CRP and LDH (and older age only for death) remained significantly associated with high ORs in the multivariate analysis.

4. Discussion

Since the beginning of SARS-CoV-2 pandemic, many authors have reported significant increases in AST activity concentrations in serum of COVID-19 patients, with results included in many *meta*-analyses [12,19–25]. Overall, the pooled incidence of AST elevation was 22.5% (95% CI: 18.1–27.6) among 47 studies and 11,914 adult patients [21]. However, authors did not pay enough attention to the analytical and post-analytical aspects of enzyme measurements that may significantly influence the obtained data. No studies discussed how assays used to determine AST (and other enzymes) comply with the available internationally agreed reference measurement system and, therefore, if they provided results standardized enough to allow the universal application of suggested URL thresholds. For measuring aminotransferases, almost all manufacturers still market assays with or without the addition of pyridoxal-5-phosphate (P-5′-P) coenzyme. However, it is impossible to compare results obtained by procedures for aminotransferases that do or do not incorporate P-5′-P, because the ratio of preformed holoenzyme to apoenzyme differs among individual clinical samples [8]. Unfortunately, in performing *meta*-analyses, no authors discussed how enzyme results were obtained and how much this may have influenced clinical results. Therefore, the practical implication and transferability of obtained results among different studies could have been markedly compromised. In our study, for the first time we paid attention to this issue by using methodologies that have been robustly validated in providing standardized results [15]. Only using these assays, it is possible to derive accurate URLs and use these thresholds to correctly estimate the frequency of test increases in COVID-19 [26]. Accordingly, we found AST more frequently increased in COVID-19 patients than in previous studies: approximately 70% of subjects displayed AST values $>$ 34 U/L, the URL we previously defined by using a robust approach [27]. ALT was also frequently increased (65% of cases), but curiously its behavior was inverted when compared to AST, with survivors showing slightly more elevated values than non-survivors. We can speculate that higher ALT values in survivors may denote a liver-related side effect of pharmacological treatments able however to improve the patient survival likeliness.

Earliest studies, focusing on the association between serum aminotransferase increases and outcome of COVID-19 patients, found that AST elevations were more frequent and prominent than ALT elevations in patients with severe clinical picture, better correlating with mortality risk [4,9]. According to Piano et al. [9], AST had "strong correlation" with ALT [correlation coefficient (R), 0.751] and "moderate correlation" with CK ($R = 0.319$), "reasonably suggesting that AST abnormalities reflected a hepatic injury". From these data, it is easy to calculate R^2 for estimating AST dependence from ALT, as a specific marker of hepatocellular injury, i.e., 0.564. Accordingly, approximately half of the variation of AST in COVID-19 should be explained with other extra-hepatic sources of enzyme release. While ALT is a specific marker of liver cell injury, with relatively lower concentrations in other organ tissues, AST is also produced by skeletal muscle cells, myocardiocytes, and in kidney, pancreas, and lung tissues. Accordingly, with this study we aimed to investigate in detail whether the frequent AST elevations observed in hospitalized COVID-19 patients can be explained by enzyme release from organs and tissues other than liver.

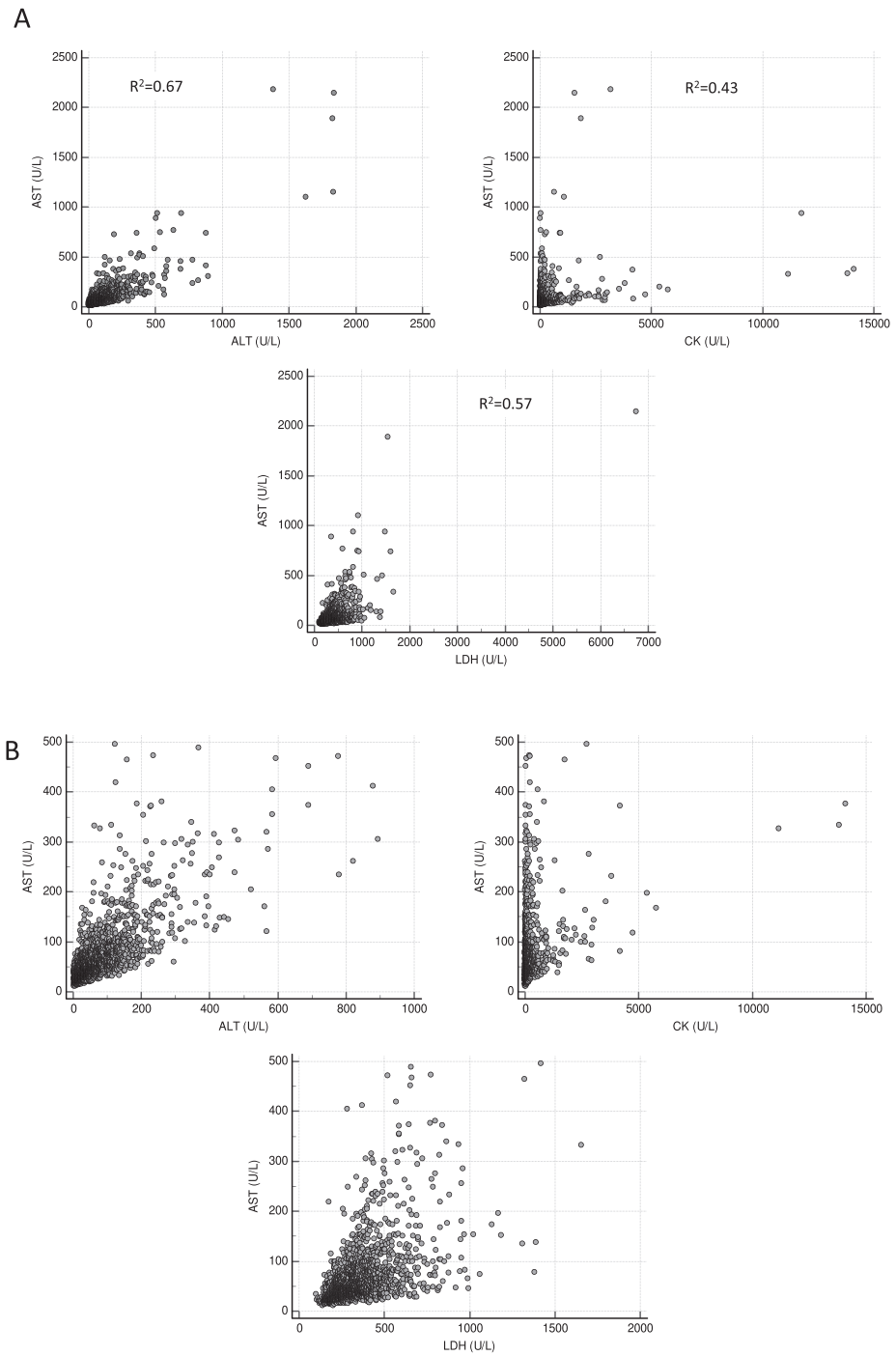

Fig. 3. Relationships of aspartate aminotransferase (AST) values at peak with alanine aminotransferase (ALT), creatine kinase (CK), and lactate dehydrogenase (LDH) obtained on the same day in studied COVID-19 patients. (A) All patients; (B) 20 patients with AST > 500 U/L removed. In part A), coefficients of determination (R^2) are reported.

From our results, AST increases can be fully explained by summing the effects of hepatocellular injury with muscle damage. Unfortunately, it is quite difficult to establish if the nature of the liver injury observed in COVID-19 patients is directly mediated by the virus or caused by concurrent factors, such as an immune-mediated damage due to the hyperinflammatory response caused by the SARS-CoV-2 infection, the anoxia in a setting of respiratory failure, which is the hallmark of COVID-19, or a drug-induced liver injury by medical treatments, such as IL-6 receptor antagonists or antiviral drugs [3,7,10]. An early article by Wang et al. [5] described the finding of spiked particles, attributed to SARS-Cov-2, in the cytoplasm of hepatocytes of two COVID-19 patients with elevated aminotransferases. However, this work was later criticized because a confirmatory testing for viral nucleic acids was never performed and, therefore, the observed particles could have been a non-specific finding [28]. A more recent study by Chornenkyy et al. [29] did perform molecular analysis on autopsy tissue samples of nine COVID-19 patients and found evidence of viral RNA in all analyzed lung specimens, but only in less than half of liver specimens. Furthermore, this positivity did not correlate with laboratory and histologic findings and therefore the pathophysiological and clinical significance of the finding remains unclear.

As for muscle damage, it is possible that SARS-CoV-2 may induce a

Table 3
Univariate and multivariate logistic regression analyses for predictors of death during hospitalization of studied COVID-19 patients.

	Univariate Analysis		Multivariate Analysis	
	Odds Ratio (95% CI)	**P**	**Odds Ratio (95% CI)**	**P**
Age	1.075 (1.064–1.087)	<0.0001	1.087 (1.070–1.104)	<0.0001
AST	1.003 (1.002–1.004)	<0.0001		
ALT	1.001 (1.000–1.001)	0.045		
CK	1.0002 (1.0001–1.0004)	<0.0001		
LDH	1.004 (1.004–1.005)	<0.0001	1.003 (1.003–1.004)	<0.0001
GGT	1.001 (1.000–1.002)	0.002		
ALP	1.003 (1.002–1.004)	<0.0001		
CRP	1.010 (1.009–1.011)	<0.0001	1.009 (1.008–1.011)	<0.0001

CI, confidence interval; AST, aspartate aminotransferase; ALT, alanine aminotransferase; CK, creatine kinase; LDH, lactate dehydrogenase; GGT, γ-glutamyltransferase; ALP, alkaline phosphatase; CRP, C-reactive protein.

Table 4
Univariate and multivariate logistic regression analyses for predictors of admission in intensive care unit during hospitalization of studied COVID-19 patients.

	Univariate Analysis		Multivariate Analysis	
	Odds Ratio (95% CI)	**P**	**Odds Ratio (95% CI)**	**P**
Age	0.999 (0.990–1.007)	0.727		
AST	1.0009 (0.9996–1.0022)	0.245		
ALT	0.999 (0.997–1.001)	0.150		
CK	1.0001 (1.0000–1.0002)	0.021		
LDH	1.002 (1.002–1.003)	<0.0001	1.001 (1.001–1.002)	<0.0001
GGT	1.0004 (0.9993–1.0015)	0.525		
ALP	0.998 (0.995–1.002)	0.224		
CRP	1.007 (1.006–1.008)	<0.0001	1.006 (1.004–1.008)	<0.0001

CI, confidence interval; AST, aspartate aminotransferase; ALT, alanine aminotransferase; CK, creatine kinase; LDH, lactate dehydrogenase; GGT, γ-glutamyltransferase; ALP, alkaline phosphatase; CRP, C-reactive protein.

myositis similar to the one observed in severe influenza infections, contributing to elevate AST activities in COVID-19 [30,31]. Myalgia is indeed a common symptom, present in more than one third of COVID-19 patients [32]. Benedé-Ubieto et al. [33] recently suggested to use an AST/ALT ratio value > 1 to identify non-hepatic sources of tissue injury and enzyme release, such as muscle damage. These authors found that 75% of their COVID-19 population had an elevated AST/ALT ratio, suggesting a non-hepatic source of AST elevation in many subjects. Even in our population, most subjects (52%) had an AST/ALT ratio > 1. We can therefore consider unwise what Bloom et al. have proposed, namely joining COVID-19 to the list of other liver conditions associated with AST-predominant aminotransferase elevations, together with alcohol-associated liver disease and cirrhosis, "reflecting a unique virally mediated mechanism of hepatic injury, with mitochondrial injury as a possible underlying mechanism" [6].

In our study, a relevant relationship was also found between AST and LDH, which is a more ubiquitous tissue marker. However, the extent of this correlation corresponds to the sum of the influences on LDH by hepatocellular injury and muscle damage, confirming liver and muscle as the primary sources of AST release in COVID-19, in an approximate 60%-40% proportion. Regarding the positive relationship between AST and LDH, other studies have previously found an association between these two markers in COVID-19 patients with similar dependence (R^2 between 0.384 and 0.6) [34,35]. Conversely, in our population AST peak values did not show any relevant relationship with the inflammatory marker CRP. This goes against findings from the study by Effenberger et al. [35], where the enzyme was found to significantly correlate with inflammatory proteins such as CRP and interleukin-6 (IL-6), with R^2 of 0.38 for CRP and 0.48 for IL-6, measured at the time of patient admission. However, in our study, for testing correlation we used CRP values obtained on the same day of the AST peak value. Other studies gave relationships between AST and CRP closer to ours ($R^2 = 0.048$) [36].

Our data confirm those from previous studies in which COVID-19 patients who had been admitted to the ICU had higher concentrations of ALT and AST than non-ICU patients [10,20]. In our population, however, both AST and ALT did not remain significant at multivariate logistic regression for either of the tested outcomes. This may oppose the hypothesis that liver damage, if any and whichever the cause, can be considered an independent prognostic factor in COVID-19. On the contrary, it is probably just a feature linked to disease severity, which presents with several risk factors for developing liver injury such as anoxia, hyperinflammation, and administration of hepatotoxic drug regimens. More in general, in critical patients, organ and tissue injury may be caused by changes in hemodynamics and oxygen delivery and a hypoxic status can cause increases in AST in the setting of respiratory failure, shock, or cardiac failure [7]. On the other hand, our study confirmed that LDH and CRP are independent predictors of admission to the ICU and in-hospital death in COVID-19 [17,37]. The increase of LDH reflects tissue/cell destruction and the link between LDH levels and COVID-19 severity may reflect both a direct lung injury or a more widespread organ damage, including liver and muscle [38]. The hyperinflammatory status typical of COVID-19, measured by CRP, was also strongly associated with poor outcome [39,40].

Our study also showed increases in GGT (in 51% of patients) and ALP (in 21% of patients), and therefore a cholangiocellular injury, possibly secondary to some drug toxicity, could be suspected in about a fifth of COVID-19 patients [41]. In the group of patients with elevated GGT activity and normal ALP, a pattern that controverts the hypothesis of cholangiocyte injury, other mechanisms, including GGT induction by drugs, should be considered [8]. Some authors speculated a possible link between GGT and increased oxidative stress and chronic inflammation [24].

Our study has strengths and limitations. In addition to paying special attention to the preanalytical, analytical and post-analytical aspects of laboratory measurements, to our knowledge, it is the first study specifically designed to thoroughly assess the origin of AST elevations in COVID-19 patients using a specific statistic (R^2) giving this information. Additionally, we looked closely at the relationship between AST and outcomes, expanding on prior studies to determine whether AST is truly a useful biomarker in predicting adverse outcome in COVID-19. Study limitations should also be mentioned. Firstly, patients with preadmission liver-related comorbidities were not excluded. However, pre-existing advanced liver disease was reported in 2% of COVID-19 patients of the Milan area, precluding a significant role of these conditions in influencing the prevalence of AST abnormalities in our study [42]. Furthermore, in a pooled analysis, the possible presence of a preexisting chronic liver disease did not alter the outcome of COVID-19 [21]. Secondly, as a retrospective and observational study, some bias is inevitable, especially regarding data entry. In addition, it was not possible to characterize all the possible additional sources of liver injury, including the use of hepatotoxic COVID-19 experimental medications. Therefore, we cannot exclude that in our study the ability of abnormal AST in predicting transfer to ICU or death during hospitalization may have been dampened by these relevant confounders.

5. Conclusions

In conclusion, the pathogenetic mechanisms for abnormal AST frequently present in COVID-19 are likely multifactorial and, while direct SARS-CoV-2 infection in hepatocytes and/or cholangiocytes appears unlikely, a status related to hypoxia and tissue suffering could play a significant role. Our data failed to demonstrate an independent association of AST increases with worse outcomes. Therefore, the clinical significance of these AST elevations in COVID-19, which are probably of multifactorial origin, remains unclear and they appear to be a disease nonspecific epiphenomenon.

CRediT authorship contribution statement

Elena Aloisio: Data curation, Formal analysis, Investigation, Supervision, Writing – original draft. **Giulia Colombo:** Data curation, Formal analysis, Investigation, Writing – original draft. **Claudia Arrigo:** Data curation, Formal analysis, Investigation, Writing – original draft. **Alberto Dolci:** Supervision, Writing – review & editing. **Mauro Panteghini:** Conceptualization, Methodology, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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