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REVIEW ARTICLE

Serum hydroxybutyrate dehydrogenase and COVID-19 severity and mortality: a systematic review and meta-analysis with meta-regression

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Abstract

Alterations in cardiac and renal biomarkers have been reported in coronavirus disease 19 (COVID-19). We conducted a systematic review and meta-analysis to investigate serum concentrations of hydroxybutyrate dehydrogenase (HBDH), a combined marker of myocardial and renal injury, in hospitalized COVID-19 patients with different disease severity and survival status. We searched PubMed, Web of Science and Scopus, between December 2019 and April 2021, for studies reporting HBDH in COVID-19. Risk of bias was assessed using the Newcastle–Ottawa scale, publication bias was assessed with the Begg's and Egger's tests, and certainty of evidence was assessed using GRADE. In 22 studies in 15,019 COVID-19 patients, serum HBDH concentrations on admission were significantly higher in patients with high disease severity or non-survivor status when compared to patients with low severity or survivor status (standardized mean difference, SMD = 0.90, 95% CI 0.74 to 1.07, $p < 0.001$; moderate certainty of evidence). Extreme between-study heterogeneity was observed ($I^2 = 93.5\%$, $p < 0.001$). Sensitivity analysis, performed by sequentially removing each study and re-assessing the pooled estimates, showed that the magnitude and the direction of the effect size were not substantially modified. A significant publication bias was observed. In meta-regression, the SMD of HBDH concentrations was significantly associated with markers of inflammation, sepsis, liver damage, non-specific tissue damage, myocardial injury, and renal function. Higher HBDH concentrations were significantly associated with higher COVID-19 severity and mortality. This biomarker of cardiac and renal injury might be useful for risk stratification in COVID-19. (PROSPERO registration number: CRD42021258123).

Keywords Hydroxybutyrate dehydrogenase · COVID-19 severity · Mortality

Introduction

While mass vaccination programs against coronavirus-19 (COVID-19) are being rolled out across the world, a significant number of infected people still require hospitalization for treatment and monitoring. An excessive systemic pro-inflammatory state is often observed in COVID-19 patients with overt respiratory compromise and acute respiratory distress syndrome (ARDS) [1, 2]. Significant extra-pulmonary clinical manifestations, particularly those affecting the cardiovascular system and the kidney, are also common in this group and independently predict excess mortality [3–7]. The exact mechanisms responsible for the cardio-renal involvement, albeit not fully established, are likely to involve a direct interaction with the causative agent, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and an excessive local inflammatory response with activation of oxidative stress-related pathways and cell apoptosis [8–11].

Organ-specific biomarkers of myocardial damage and dysfunction, e.g., creatine kinase, troponin, and B-type natriuretic peptide (BNP), and acute kidney injury, e.g., serum creatinine, are increasingly being studied to better predict clinical deterioration and adverse outcomes in

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hospitalized COVID-19 patients [5, 12, 13]. Pending further clinical validation of these biomarkers in prospective studies, the identification of additional biomarkers that reflect both myocardial and renal injury, singly or in combination, might be particularly attractive from a health economics and predictive standpoint. In this context, the availability of robust techniques, including machine learning algorithms, for the rapid development and validation of predictive tools combining different parameters in a rapidly developing pandemic might prove critical to enhance early risk stratification and facilitate the rational allocation of resources within healthcare systems under substantial pressure [14].

The enzyme hydroxybutyrate dehydrogenase (HBDH) is an established marker of cell death, particularly following cardiac and/or kidney damage, and can be measured in serum. HBDH primarily represents the activity of the lactate dehydrogenase isoenzymes 1 (LDH-1) and 2 (LDH-2) [15–21]. LDH-1 and LDH-2 are particularly expressed in myocardial tissues, red blood cells, and the kidney, and are primarily responsible for maintaining the equilibrium between acetoacetate and β-hydroxybutyrate in the biochemical pathways involved in the formation of ketone bodies [22].

Given the potential utility of HBDH as a combined marker of cardiac and renal damage, we sought to determine its pathophysiological role in COVID-19 by conducting a systematic review and meta-analysis of studies reporting serum HBDH concentrations in patients with different disease severity and survival status during follow-up. We hypothesized those patients with severe disease and non-survivor status had higher serum HBDH concentrations when compared with patients with mild disease and survivor status, indicating the presence of a more clinically overt cardiac and/or renal compromise in the former. A meta-regression analysis was further performed to investigate the presence of significant associations between the effect size of the between-group differences in HBDH concentrations and several plausible patient characteristics. The latter included age, sex, specific comorbidities, study design and endpoint, and markers of inflammation, sepsis, specific and non-specific tissue damage, and pro-thrombotic tendency.

Materials & methods

Search strategy, eligibility criteria & study selection

We conducted a systematic literature search, using the terms "hydroxybutyrate dehydrogenase" or "HBDH" and "coronavirus disease 19" or "COVID-19", in PubMed, Web of Science and Scopus, from December 2019 to April 2021, to identify peer-reviewed articles reporting serum HBDH concentrations in COVID-19 patients (PROSPERO registration number: CRD42021258123). The references of the retrieved articles were also reviewed to identify additional studies. Inclusion criteria were: (1) reporting continuous data on serum HBDH concentrations in COVID-19 patients, (2) investigating COVID-19 patients with different disease severity or survival status, (3) adult patients, (4) English language, (5) ≥ 10 participants, and (6) full-text available. Two investigators independently screened the abstracts and, if relevant, independently reviewed the full articles. A third investigator was involved in the case of disagreement. Data extracted from each article included the country where the study was conducted, clinical endpoint (disease severity or survival status), study design (prospective or retrospective), number of participants, age, sex, serum HBDH concentrations, and parameters included in meta-regression analysis (see details in the Statistical analysis paragraph). The Newcastle–Ottawa scale was used to assess the risk of bias, with a score ≥ 6 indicating low risk, 4–5 moderate risk, and < 4 high risk [23–25]. Certainty of evidence was assessed using the Grades of Recommendation, Assessment, Development, and Evaluation (GRADE) Working Group system, which considers the following criteria: study design (randomized vs. observational), risk of bias (Newcastle–Ottawa scale), unexplained heterogeneity, indirectness of evidence, imprecision of results (sample size, 95% confidence interval width, and threshold crossing), effect size (small, SMD < 0.5, medium, SMD 0.5–0.8, and large, SMD > 0.8) [26], and high probability of publication bias [27–29].

Statistical analysis

Standardized mean differences (SMDs) with 95% confidence intervals (CIs) were calculated to build forest plots and evaluate differences in HBDH concentrations between COVID-19 patients with mild vs. severe disease or survivor vs. non-survivor status (p-level of significance set at < 0.05). When studies reported HBDH concentrations as medians and interquartile ranges (IQR), the corresponding mean and standard deviation values were estimated according to established methods [30]. SMD heterogeneity across studies was assessed with the Q-statistic (p-level of significance set at < 0.10). Inconsistency across studies was evaluated using the I^2 statistic (I^2 < 25%, no heterogeneity; between 25 and 50%, moderate heterogeneity; between 50 and 75%, large heterogeneity; and > 75%, extreme heterogeneity) [23, 31, 32]. A random-effects model was used to calculate the pooled SMD and 95% CIs in the presence of significant heterogeneity. Sensitivity analyses were conducted to evaluate the influence of individual studies on the overall effect size using the leave-one-out method [23, 33]. The presence of publication bias was assessed with the Begg's adjusted rank correlation test and the Egger's regression asymmetry test (p-level of significance set at < 0.05) [34, 35]. We also

performed the Duval and Tweedie "trim-and-fill" procedure to further test and adjust for the possible presence of publication bias. This method recalculates a pooled SMD by incorporating the hypothetical missing studies as they actually existed, to augment the observed data so that the funnel plot is more symmetric [36]. Univariate meta-regression analysis was performed to identify possible contributors to the between-study variance. In particular, we investigated associations between the SMD of HBDH concentrations and the following biologically and/or clinically plausible variables: age, sex, clinical endpoint, diabetes, hypertension, and cardiovascular disease, biomarkers of inflammation (C-reactive protein, CRP, white blood cell count, WBC, neutrophils, lymphocytes), sepsis (procalcitonin), liver damage (aspartate aminotransferase, AST, alanine aminotransferase, ALT, albumin), renal damage (serum creatinine, urea), cardiac injury (creatine kinase-MB, CK-MB), musculoskeletal damage (creatine kinase), non-specific tissue damage (lactate dehydrogenase, LDH), and pro-thrombotic tendency (D-dimer, pro-thrombin time, PT, activated partial thromboplastin time, aPTT, fibrinogen). Statistical analyses were performed using Stata 14 (STATA Corp., College Station, TX, USA). The study was compliant with the PRISMA 2020 statement regarding the reporting of systematic reviews and meta-analyses [37].

Results

Study selection

The initial screening identified 240 articles. Of them, 215 were excluded because they were either duplicates or irrelevant. After a full-text review of the remaining 25 articles, three were further excluded due to missing data, leaving 22 studies for final analysis [38–59] (Fig. 1

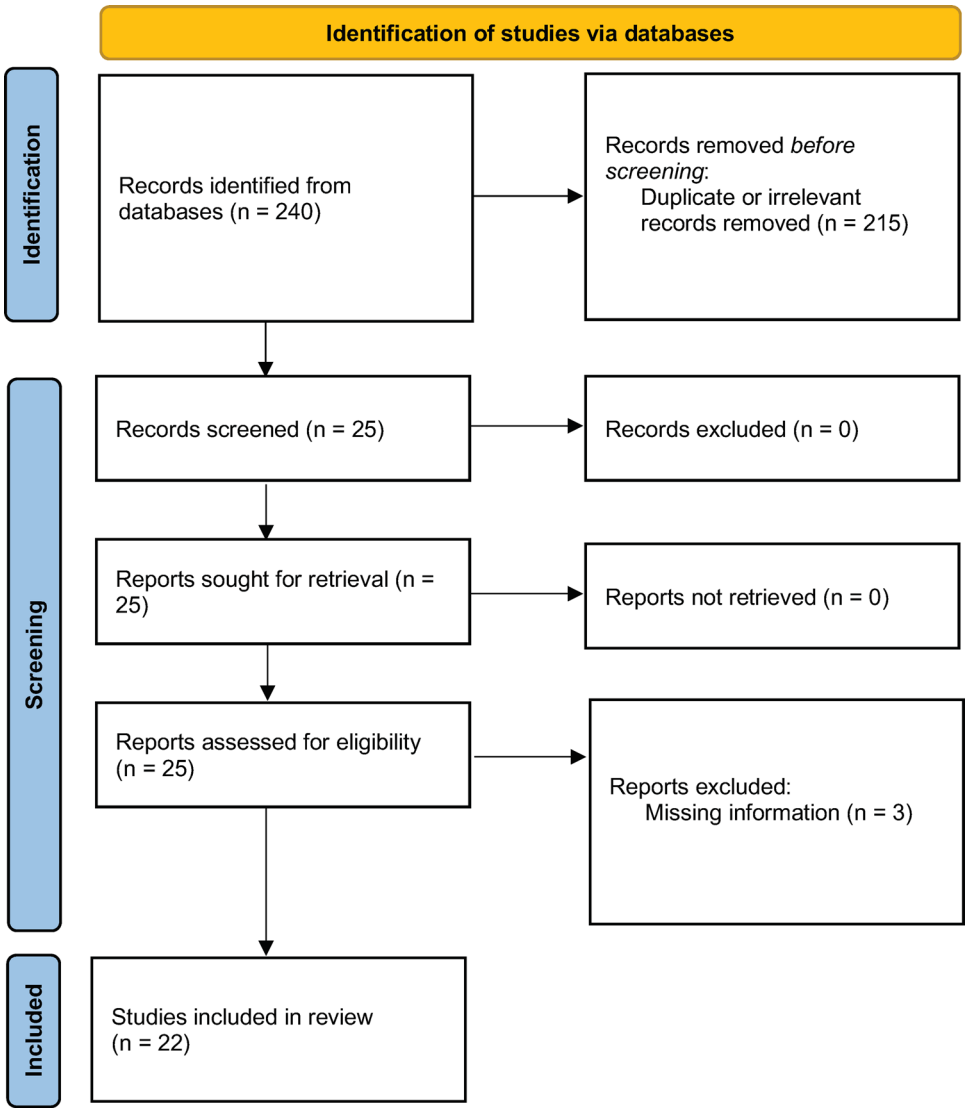

Fig. 1 PRISMA 2020 flow diagram

Table 1 Study characteristics

First author (Ref.)	Design	Endpoint	NOS (stars)	Mild disease or survivor status				Severe disease or non-survivor status			
				n	Age (Years)	Gender (M/F)	HBDH U/L (Mean ± SD)	*n*	Age (Years)	Gender (M/F)	HBDH U/L (Mean ± SD)
Ali A [38]	R	Survival	6	46	70	30/16	228 ± 58	17	71	9/8	413 ± 164
Bai Y [39]	R	Disease severity	8	2,694	59	1,331/1,363	144 ± 36	648	66	353/295	159 ± 32
Cen Y [40]	NR	Disease progress	8	409	69	181/228	301 ± 149	265	69	154/111	317/125
Cheng J [41]	R	Disease severity	5	91	NR	NR	170 ± 81	19	NR	NR	235 ± 114
Dong X [42]	R	Survival	8	65	54	30/35	206 ± 70	54	70	35/19	479 ± 188
Dong Y [43]	R	Disease severity	8	94	40	34/60	169 ± 59	53	60	29/24	208 ± 29
Guo J [44]	R	Survival	8	43	60	22/21	200 ± 75	31	68	21/10	368 ± 264
Huang J [45]	R	Disease severity	8	552	43	208/344	133 ± 42	635	59	329/306	163 ± 52
Kong M [46]	R	Disease severity	8	123	53	59/64	146 ± 74	87	68	45/42	218/194
Li P [47]	R	Disease severity	5	1,439	57	728/711	139 ± 31	1,515	63	765/750	169 ± 59
Liu W [48]	NR	Disease severity	8	120	35	36/84	152 ± 38	20	48	8/12	193 ± 21
Ma X [49]	R	Survival	8	134	63	68/66	273 ± 172	128	71	75/53	75 ± 53
Qin S [50]	R	Disease severity	8	1,467	56	748/719	139 ± 31	1,577	64	798/779	167 ± 56
Shi P [51]	R	Disease severity	5	88	41	40/48	145 ± 53	46	56	25/21	190 ± 65
Song CY [52]	R	Disease severity	8	31	48	16/15	182 ± 50	48	57	33/15	241 ± 72
Wang J [53]	R	Disease severity	8	509	46	265/244	164 ± 49	53	59	25/28	168 ± 69
Wu C [54]	R	ARDS	7	117	48	68/49	224 ± 73	84	59	60/24	354 ± 166
Xiao J [55]	R	Disease severity	8	203	45	82/121	142 ± 57	40	59	23/17	247 ± 352
Xie J [56]	NR	Disease progress	8	75	51	45/30	206 ± 65	29	66	18/11	281 ± 70
Yan X [57]	R	Survival	8	964	62	466/498	203 ± 82	40	68	27/13	449 ± 305
Yu Y [58]	R	Survival	8	204	55	100/104	201 ± 64	42	69	25/17	318 ± 80
Zheng X [59]	R	Disease severity	8	52	47	18/34	130 ± 29	28	56	16/12	153 ± 36

ARDS, acute respiratory distress syndrome; *NOS*, Newcastle–Ottawa quality assessment scale for case–control studies; *NR*, not reported; *R*, retrospective; *M*, male; *F*, female; *HBDH*, hydroxybutyrate dehydrogenase

and Table 1). A total of 15,019 COVID-19 patients were enrolled, 9,520 (48% males, mean age 55 years) with low severity or survivor status and 5,499 (53% males, mean age 63 years) with high severity or non-survivor status.

Study characteristics

All studies were conducted in China. Nineteen studies were retrospective [38, 39, 41–47, 49–55, 57–59], whilst the remaining three did not report the study design [40, 48, 56]. Clinical endpoints included disease severity based on current clinical guidelines (13 studies) [39, 41, 43, 45–48, 50–52, 55, 59], disease progress (two studies) [40, 56], occurrence of ARDS (one study) [54], and survival (six studies) [38, 42, 44, 49, 57, 58]. In all studies, serum HBDH concentrations were measured on admission.

Risk of bias

The risk of bias was considered to be low in 19 studies [38–40, 42–46, 48–50, 52–59], and moderate in the remaining three [41, 47, 51].

Results of individual studies and syntheses

The overall SMD in HBDH concentrations between COVID-19 patients with low vs. high severity or survivor vs. non-survivor status is shown in Fig. 2. In all studies, patients with high severity or non-survivor status had higher HBDH concentrations when compared to those with low severity or survivor status (mean difference range, 0.04 to 2.45), although the difference was not statistically significant in two studies [40, 53]. The pooled results confirmed that HBDH concentrations on admission were significantly higher in patients with high severity or non-survivor status (SMD = 0.90, 95% CI 0.74 to 1.07, $p < 0.001$) (Fig. 2). Extreme between-study heterogeneity was observed ($I^2 = 93.5\%$, $p < 0.001$). HBDH concentrations remained significantly higher (SMD = 1.00, 95% CI 0.72 to 1.27, $p = 0.001$; $I^2 = 93.8\%$, $p < 0.001$) in patients with high severity or non-survivor status after excluding the three largest studies (> 2,500 participants) [39, 47, 50], accounting for ~ 62% of the overall sample size.

Sensitivity analysis, performed by sequentially removing each study and re-assessing the pooled estimates, showed that the magnitude and the direction of the effect size were not substantially influenced (effect size range, between 0.81 and 0.95) (Fig. 3).

In univariate meta-regression, the HBDH SMD was significantly associated with WBC (t = 5.24, $p < 0.001$),

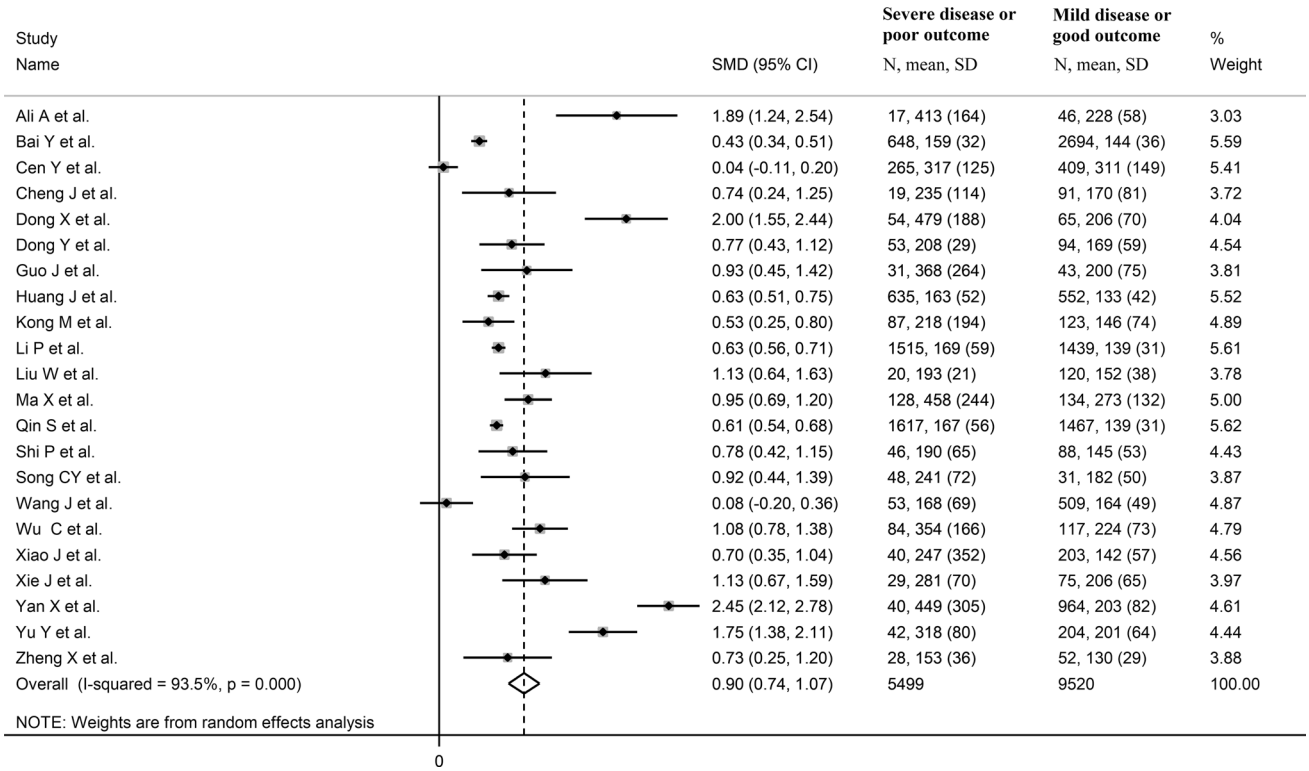

Fig. 2 Forest plot of studies reporting serum hydroxybutyrate dehydrogenase concentrations in patients with COVID-19

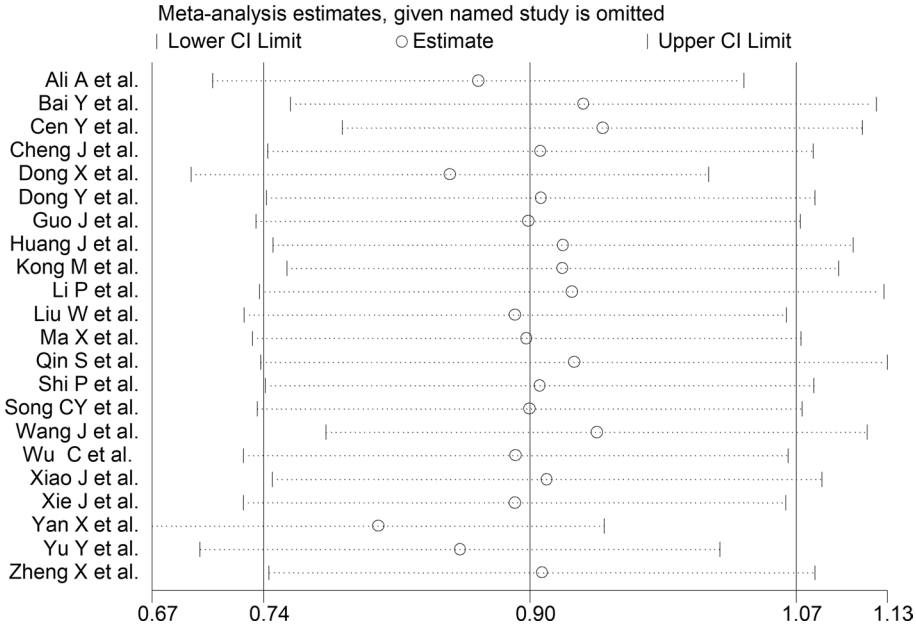

Fig. 3 Sensitivity analysis of the association between serum hydroxybutyrate dehydrogenase and COVID-19. The influence of individual studies on the overall standardized mean difference (SMD) is shown. The middle vertical axis indicates the overall SMD, and the two vertical axes indicate the 95% confidence intervals (CIs). The hollow circles represent the pooled SMD when the remaining study is omitted from the meta-analysis. The two ends of each broken line represent the 95% CI

neutrophils (t = 4.35, $p = 0.001$), lymphocytes (t = -2.92, $p = 0.01$), CRP (t = 2.73, $p = 0.01$), procalcitonin (t = 2.73, $p = 0.02$), AST (t = 6.94, $p < 0.001$), ALT (t = 3.14, $p = 0.006$), albumin (t = − 6.49, $p < 0.001$), LDH (t = 6.73, $p = < 0.001$), CK-MB (t = 4.94, $p = 0.001$), and urea (t = 4.58, p = 0.001). By contrast, no significant correlations were observed with age (t = − 0.23, $p = 0.82$), sex (t = 0.81, $p = 0.43$), creatinine (t = 0.99, $p = 0.34$), CK (t = 1.93, $p = 0.08$), D-dimer (t = 1.30, $p = 0.21$), fibrinogen (t = − 0.41, $p = 0.69$), PT (t = 0.77, $p = 0.46$), aPTT (t = − 0.68, $p = 0.51$), cardiovascular disease (t = 0.87, $p = 0.41$), diabetes (t = 0.59, $p = 0.56$), and hypertension (t = 1.61, $p = 0.13$).

Sub-group analysis showed that the SMD in studies reporting disease severity (SMD = 0.60, 95% CI 0.51 to 0.70, $p < 0.001$; $I^2 = 68.6\%$; $p < 0.001$) was significantly lower (t = 5.16, $p < 0.001$) than that in studies reporting survival (SMD = 1.66, 95% CI 1.10 to 2.21, $p < 0.001$; $I^2 = 91.8\%$, $p < 0.001$), with a relatively lower between-study variance in the former ($I^2 = 68.6\%$ vs $I^2 = 91.8\%$) (Fig. 4). Moreover, in a sub-group of studies (< 500 patients) there was no

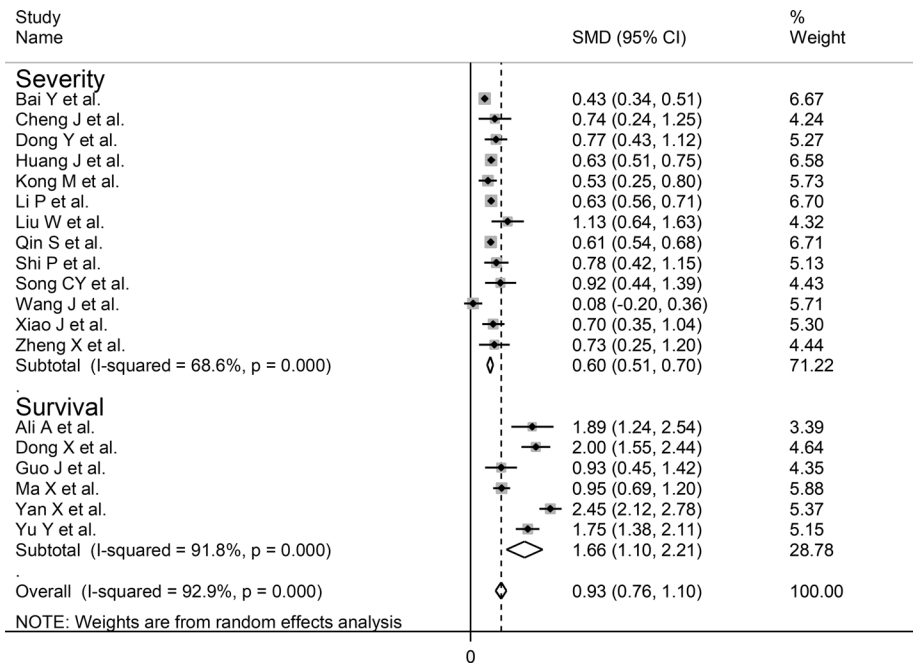

Fig. 4 Forest plot of studies reporting serum hydroxybutyrate dehydrogenase concentrations in patients with COVID-19 according to disease severity or survival status

heterogeneity, but still significantly higher HBDH concentrations, in patients with severe disease (SMD = 0.74, 95% CI 0.60 to 0.87, $p < 0.001$; $I^2 = 0.00\%$, $p = 0.61$) (Fig. 5).

Publication bias

There was significant publication bias (Begg's test, $p = 0.02$; Egger's test, $p = 0.02$). Accordingly, the "trim-and-fill" method identified nine potential missing studies to be added to the left side of the funnel plot to ensure symmetry (Fig. 6). The effect size, albeit reduced, remained significant (SMD = 0.56, 95% CI, 0.38 to 0.73, $p < 0.001$).

Certainty of evidence

The initial level of certainty for serum HBDH SMD was considered to be low because of the observational nature of the selected studies (rating 2, ⊕ ⊕ ⊖ ⊖). After taking into account the presence of a low risk of bias in 19 out of 22 studies (upgrade one level), a generally extreme

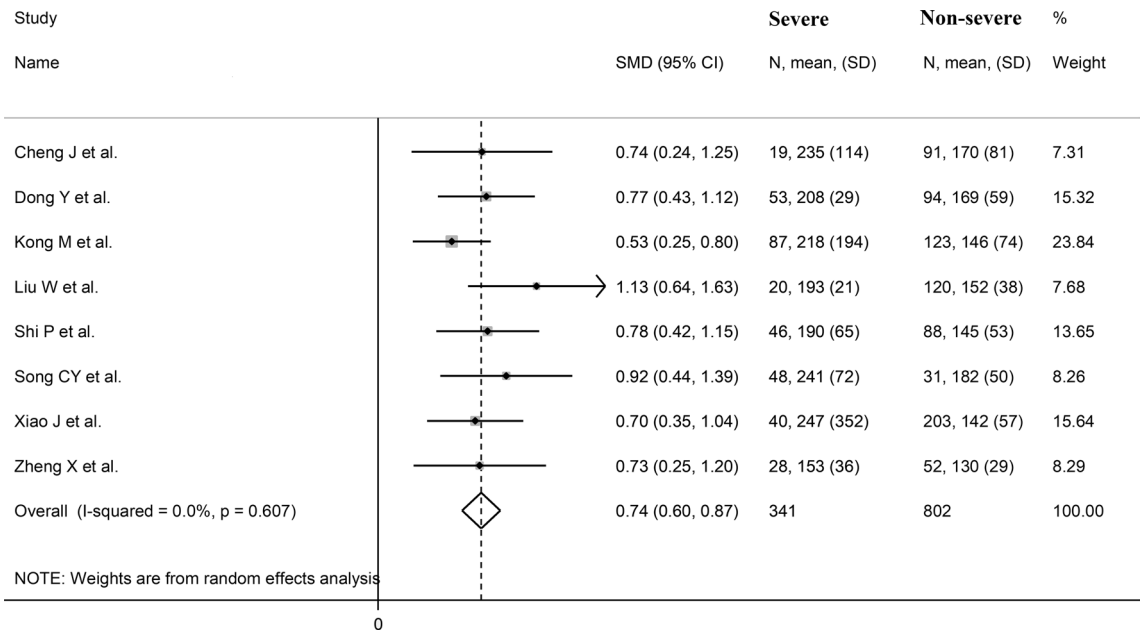

Fig. 5 Funnel plot of a sub-group of eight studies that were homogeneous for endpoint and number of recruited patients ($n < 500$)

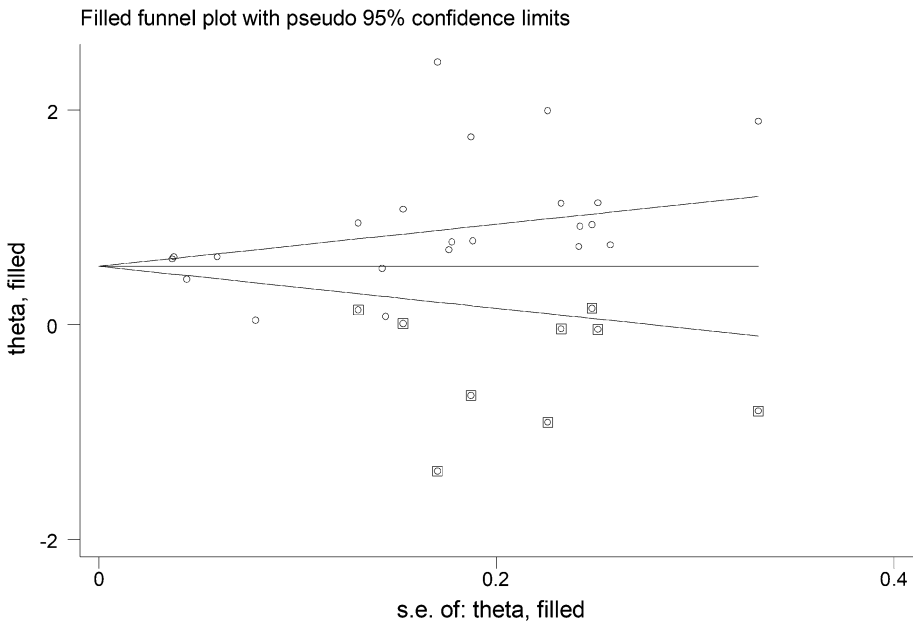

Fig. 6 Funnel plot of studies investigating low vs. high severity or survivor vs. non-survivor status after "trimming-and-filling". Dummy studies and genuine studies are represented by enclosed circles and free circles, respectively

and unexplained heterogeneity (serious limitation downgrade one level), the lack of indirectness (no rating change required), the relatively low imprecision (relatively narrow confidence intervals without threshold crossing, upgrade one level), the large effect size (SMD = 0.90, upgrade one level), and the presence of publication bias (downgrade one level), the overall level of certainty was considered moderate (rating 3, ⊕ ⊕ ⊕ ⊖).

Discussion

In our systematic review and meta-analysis, serum HBDH concentrations on admission were significantly higher in hospitalized COVID-19 patients with severe clinical manifestations or who died during follow-up when compared to those with mild disease or who survived. The magnitude of the observed SMD value, 0.90, indicates that the between-group differences are likely to be biologically and clinically significant [26]. Although an extreme between-study heterogeneity was observed, the sequential omission of individual studies did not substantially affect the overall SMD. In meta-regression analysis, significant associations were observed between the SMD of HBDH concentrations and markers of inflammation (WBC, neutrophils, lymphocytes, and CRP), sepsis (procalcitonin), liver dysfunction (AST, ALT, albumin), non-specific tissue damage (LDH), myocardial injury (CK-MB), and renal damage (urea).

HBDH has been traditionally investigated as a marker of myocardial injury in animal and human studies [15–21, 60–63]. However, its use has been progressively replaced by other biomarkers, e.g., troponin, CK-MB, and BNP, in the routine diagnosis and monitoring of patients with ischaemic heart disease and/or heart failure. Further studies have suggested an additional role of HBDH as a marker of renal injury, although the evidence supporting this proposition is relatively limited compared to cardiac injury [20]. As previously described, HBDH represents LDH-1 and LDH-2 activity. LDH is composed of four peptide chains of two different types, the heart (H) subunit and the muscle (M) subunit. LDH-1 is composed of four H subunits (H_4) whereby LDH-2 is composed of three H subunits and 1 M subunit (H_3M) [64]. Both LDH-1 and LDH-2 isoenzymes predominate in the cardiac muscle, erythrocytes, and the kidney [64]. Therefore, it is plausible to speculate those elevations in serum HBDH concentrations reflect the presence of cardio-renal tissue damage, and that this alteration is more common in COVID-19 patients with severe clinical manifestations and/or adverse outcomes.

The observed associations, in meta-regression analysis, with established biomarkers of inflammation, sepsis, and liver dysfunction suggests those elevations in HBDH concentrations might provide additional information regarding the presence of a pro-inflammatory state and hepatic involvement, unlike currently available markers of cardiac and renal injury. While this suggests a substantial pathophysiological role of HBDH in COVID-19, further studies are required to determine whether the assessment of this enzyme on admission can be incorporated into specific predictive tools for early decision-making in this patient cohort. Furthermore, in contrast with the significant associations observed with CK-MB and urea, markers of cardiac and renal injury, respectively, no significant correlations were observed between the SMD of HBDH and creatinine, another marker of renal injury. One possible explanation is the reported fluctuation in serum creatinine concentrations in medical inpatients during the first week after admission. For example, in a study of 2,293 newly admitted patients, a > 20% variation in serum creatinine concentration was observed in 46% in the three to seven days post-admission [65]. Additional studies are required to further investigate the association between serum HBDH and creatinine concentrations over time.

The extreme between-study heterogeneity represents a significant limitation of our study, although this issue was no longer present when considering a sub-group of relatively small studies (< 500 participants) investigating disease severity. Furthermore, there was significant publication bias, according to the Begg's and Egger's tests and the "trim-and-fill" method. Another limitation is that all the identified studies were conducted in China, which affects the generalizability of the results. Finally, in no study was a serial measurement of HBDH conducted. The latter might provide additional information regarding the potential role of this biomarker in monitoring disease progress and response to specific therapies.

In conclusion, our systematic review and meta-analysis with meta-regression has shown that higher serum HBDH concentrations on admission, indicating cardio-renal compromise and, possibly, excessive systemic inflammation and hepatic involvement, are significantly associated with the presence of severe clinical manifestations and the risk of mortality in hospitalized COVID-19 patients. Further prospective studies are warranted to determine whether single or serial HBDH assessments, together with other clinical, demographic, or biochemical parameters, can improve our capacity to predict clinical outcomes in this group and optimize the rational allocation of resources, also in terms of designing specific care pathways that include the appropriate transfer to the intensive care unit or other aggressive management strategies.

Author contribution Study design: AZ, PP, CC, AAM; Data collection: AZ, PP; Data analysis: AZ, PP; Data interpretation: AZ, PP, CC, AAM; First draft: AAM; Subsequent drafts: AZ, PP, CC, AAM.

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Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

Ethics approval Not required as this was a systematic review of published studies.

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