α-HBDH Is a Probably Higher Sensitive and Specific the Biomarkers of Poor Prognosis in Patients With COVID-19

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Research

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Abstract

**Background:** The coronavirus disease 2019 (COVID-19) that is caused by the severe acute respiratory syndrome-coronavirus2 (SARS-CoV2) has spread rapidly worldwide during the past nearly a year. SARS-CoV-2 particles spread through the respiratory mucosa and infect other cells, causing a storm of cytokines in the body, producing a series of immune responses, and causing multiple organ dysfunction, including the heart. Some patients present with cardiovascular system damage, such as palpitations and shortness of breath as the first or secondary symptoms. Previous studies suggested that LDH, α-HBDH, CK and CK-MB reflect myocardial function. Here, we aim to investigate whether these markers can predict poor prognosis of patients with COVID-19.

**Methods:** We collected data from 2338 patients with laboratory-confirmed COVID-19. Patients were then screened, and we focused on 49 moderate cases, 98 severe cases and 53 critical cases (27 recovered cases, 26 deaths). We divided these patients into non-critical group (n = 49) and critical group (n = 151). Then, we also divided the length of hospitalization into five time points, namely admission, 25%, 50%, 75% and discharge or death, according to the principle of interquartile distance. Blood was collected from patients on the above five time points. Patients with five blood tests were 49 moderate cases, 98 severe cases and 53 critical cases (27 recovered cases, 26 deaths). LDH, α-HBDH, CK and CK-MB of each group were collected for analysis.

**Results:** Our research found that α-HBDH and LDH of the critical groups significantly increased, diagnostic efficiency of LDH and α-HBDH have more advantages than that of CK and CK-MB compared with the non-critical group, and patients with α-HBDH greater than 182IU/L and LDH greater than 250IU/L at admission had lower survival rates. Then, CK, LDH, α-HBDH and CK-MB were observed dynamically in the 49 moderate cases, 98 severe cases and 53 critical cases (27 recovered cases, 26 deaths). It turns out that they increased progressively in the dead patients, while they decreased regularly in the severe case and the critical cases (recovered) (P < 0.001).

**Conclusions:** The prognosis of patients with abnormal α-HBDH was poor on admission. α-HBDH is a probably higher sensitive and specific the biomarkers of poor prognosis in patients with COVID-19. This predictor may help clinicians identify patients with a poor prognosis and may be useful for guiding clinical decision-making at an early stage.

**Background**

The coronavirus disease 2019 (COVID-19) that is caused by the severe acute respiratory syndrome-coronavirus2 (SARS-CoV2) has spread rapidly worldwide during the past nearly a year. As a novel infectious disease, COVID-19 has the ability of human-to-human transmission and can lead to acute respiratory distress syndrome (ARDS), multiple organ dysfunction and even death. It has become a global crisis with a tremendous socio-economic impact. On March 11, 2020, COVID-19 was declared by the World Health Organization (WHO) as a global public health emergency due to its pandemicity.

However, efficient indicators for predicting the poor prognosis of COVID-19 patients, therapeutic responses and disease outcome have not been fully investigated. Currently, these indicators include obesity, liver injury, chest CT, alteration of taste or smell, combined IL-6 and CD8+ T cell counts, serum amyloid A, cardiac troponin-I, homocysteine, elevated N-terminal pro-brain natriuretic peptide, angiotensin-219, lymphocyte-to-C-reactive protein ratio, albumin, peripheral lymphocyte count, neutrophil to lymphocyte ratio. However, most of these indexes are limited to specific populations, with small sample sizes, and are difficult to detect or to reproduce on a large scale. We found that many patients with COVID-19 had abnormal level of CK, LDH, α-HBDH and CK-MB. These indicators were the most available, efficient and economic examination, as this low-cost, easily acquired biomarker is readily available even in remote areas. Their significance for the mortality and severity of COVID-19 are essential in this pandemic situation.

SARS-CoV-2 particles spread through the respiratory mucosa and infect other cells, causing a storm of cytokines in the body, producing a series of immune responses, and causing multiple organ dysfunction, including the heart. The clinical manifestations of COVID-19 are mainly respiratory symptoms, but some patients present with cardiovascular system damage, such as palpitations and shortness of breath as the first or secondary symptoms. In addition, some people with basic cardiovascular disease (CVD) may have an increased risk of death. Therefore, it is important to understand the potential mechanisms of SARS-CoV-2 damage to the cardiovascular system. Previous studies suggested that LDH, α-HBDH, CK and CK-MB reflect myocardial function. The increased number of these cardiac injury markers is related to in-hospital mortality in patients with COVID-19. This study aims to
retrospectively analyze the time courses of LDH, α-HBDH, CK and CK-MB of cured and dead patients with COVID-19, in order to provide timely and effective treatment and reduce mortality in these patients.

Methods

Patients’ involvement and data collection

All hospitalized patients (n=2338) (admission date from February 10 to March 20, 2020) in Huoshenshan Hospital of Wuhan, diagnosed with COVID-19 based on their clinical symptoms (fever or respiratory symptoms) with typical changes in chest radiology and positive nucleic acid detection results, were involved in this study. Pharyngeal swab specimens of these patients were collected and used for COVID-19 viral nucleic acid detection using a real-time reverse transcriptase-polymerase chain reaction (RT-PCR) assay in the designated hospitals. Patients with positive test results were admitted to hospital and included in this study. All patients involved in this study were living in Wuhan during the outbreak period of COVID-19.

COVID-19 severity was defined according to the diagnostic and treatment guidelines for COVID-19 issued by the Chinese National Health Committee (version 7). Severe COVID-19 was designated when the patients had one of the following criteria: (a) respiratory distress with respiratory frequency \( \geq 30/\text{min} \); (b) pulse oximeter oxygen saturation \( \leq 93\% \) at rest; and (c) oxygenation index (artery partial pressure of oxygen/inspired oxygen fraction, \( \text{PaO}_2/\text{FiO}_2 \)) \( \leq 300 \) mm Hg. Critical COVID-19 was designated when the patients had one of the following criteria: (a) respiratory failure with mechanical ventilation; (b) shock; and (c) combination with other organ failure; requirement of ICU for monitoring and treatment.

A total of 2338 patients diagnosed with COVID-19 were included in this study, with 2126 non-critical patients (20 mild cases and 2106 moderate cases), 212 severe and critical patients (152 severe cases and 60 critical cases) (Figure 1). In order to facilitate the study, we divided the length of hospitalization into five time points, namely admission, 25%, 50%, 75% and discharge or death, according to the principle of interquartile distance. Blood was collected from patients on the above five time points. Patients with five blood tests were 49 moderate cases, 98 severe cases and 53 critical cases (27 recovered cases, 26 deaths) (Figure 1,2). The mean age for 200 patients was 66.4 years, ranging from 24 to 91 years old, and 125 (62.5%) patients were 60-80 years old. More than half (57.5%) of cases were male (Table1). The date of disease onset, hospital admission date and time of discharge or death, as well as the severity of COVID-19, were also recorded. The onset date was defined as the day when any symptoms were observed by the patients.

Laboratory testing

Blood testing for all patients was performed by the clinical laboratory of Huoshenshan Hospital of Wuhan. Medical laboratory results in this study, including the plasma levels of CK, LDH, α-HBDH and CK-MB, were collected for each patient. All medical laboratory data were generated by the clinical laboratory of Huoshenshan Hospital of Wuhan. As the disease progressed, the updated secondary results for laboratory findings (including levels of LDH CK, LDH, α-HBDH and CK-MB) during the hospital stay were also collected.

Statistical analysis

Categorical variables were expressed as a number (%), and continuous variables were summarized using interquartile range (IQR) values. To compare continuous variables for the data of different patient groups, variance analysis and the Kruskal-Wallis H(K) test were used appropriately. The frequencies of categorical variables were compared using the \( \chi^2 \) test, as appropriate. Statistical analyses were processed by the statistic package deal SPSS 23.0. ROC curve, line chart and survival analysis were processed by GraphPad Prism version 5.00 software (GraphPad Software Inc). P values of less than 0.05 were considered statistically significant.

Results

Demographics and clinical characteristics

Demographic information, clinical characteristics (including medical history, exposure history, comorbidities and symptoms), chest computed tomographic (CT) scan results, and laboratory findings of each patient were obtained from the electronic medical record system of Huoshenshan Hospital of Wuhan. Access was permitted by the hospital director.
Although COVID-19 infections clustered within 30 (15.0%) patients whose family members or friends were also infected with COVID-19 in this study, 170 patients (85.0%) did not have a clear history of exposure. About 74.5% of patients had one or more underlying comorbidity, the most common of which were chronic diseases, such as hypertension, diabetes and coronary heart disease. In particular, many patients concomitantly suffered from a variety of underlying comorbidities (Table 1).

Clinical symptoms of all patients at the onset of illness are shown in Table 2. The most common symptoms were cough, fever, chest tightness/dyspnea, fatigue and muscle aches. Less common symptoms were gastrointestinal symptoms, dizziness/headache, expectoration, sore throat etc. Frequently, many patients also had multiple symptoms at the same time.

**Radiological and laboratory findings at admission**

Abnormalities in chest CT images were detected in all patients. Of the 200 patients, 133 (66.5%) had multiple ground glass in both lungs; 51 (25.5%) patients had multiple patchy shadows in both lungs and 13 (6.5%) patients had multiple consolidation in both lungs (Table 2). There were significant differences between the moderate cases, severe cases, critical recovered cases and deaths (P < 0.001).

The blood tests of patients were collected at admission. The results showed that there were differences in CK, LDH, α-HBDH and CK-MB in the non-critical groups and critical groups (Table 3). Compared with the non-critical groups, the LDH and α-HBDH of critical groups significantly increased (P < 0.001), while their CK and CK-MB remained almost at the same level (P = 0.355, 0.051, respectively). Further analysis found that 67.3% of non-critical groups displayed normal level of LDH, while 62.9% of critical groups displayed abnormality levels (P = 0.001). The study also found 51.0% of non-critical groups displayed normal level of α-HBDH, while 71.5% of critical groups displayed abnormality levels (P = 0.021) (Table 4).

**Analysis of diagnostic efficiency of AUC of CK, LDH, α-HBDH and CK-MB in patients with COVID-19 at admission**

Receiver operating characteristic (ROC curve) analysis showed that AUC of CK and CK-MB were 0.544 (95% CI 0.458–0.630) and 0.596 (95% CI 0.503–0.683) (P < 0.05), while AUC of LDH and α-HBDH were 0.700 (95% CI 0.620–0.780) and 0.696 (95% CI 0.616–0.776) (P < 0.001) (Figure 3).

**Correlation analysis of CK-MB and LDH or α-HBDH in the non-critical groups and critical groups**

There was no correlation of CK-MB and LDH or α-HBDH in the non-critical groups (r = 0.2659 and P = 0.0648, r = 0.2066 and P = 0.1543, respectively). However, there was a positive correlation of CK-MB and LDH or α-HBDH in the critical groups (r = 0.2960 and P = 0.0002, r = 0.3182 and P < 0.0001, respectively). Furthermore, there was also a positive correlation of CK-MB and LDH or α-HBDH in the non-critical groups and critical groups (r = 0.3008 and P < 0.0001, r = 0.3119 and P < 0.0001, respectively) (Figure 4).

**Survival analysis of patients with COVID-19 on the admission**

A total of 200 patients with COVID-19 were divided into a 200IU group (180 cases) and a 200IU group (20 cases), a 250IU group (90 cases) and a 250IU group (110 cases), a 182IU group (68 cases) and a 182IU group (132 cases), a 24IU group (186 cases) and a 24IU group (14 cases), according to normal level of CK, LDH, α-HBDH and CK-MB, respectively. Analyzing survival, we found that there was no significant difference between the two groups according to level of CK and CK-MB (P = 0.5962 and 0.9676, respectively), while there was significant difference between the two groups according to level of LDH and α-HBDH (P < 0.0001 and P = 0.0075, respectively) (Figure 5).

**Dynamic changes of CK, LDH, α-HBDH and CK-MB in patients with COVID-19**

The blood tests of patients during their hospitalization were collected at admission, 25%, 50%, 75% and discharge or death. The results showed that there were differences in CK, LDH, α-HBDH and CK-MB at almost every time point in the four groups (Table 5). Compared with the time of admission, the CK, LDH, α-HBDH and CK-MB of dead patients increased gradually, while the CK, LDH, α-HBDH and CK-MB of other patients were decreasing gradually (Table 5 and Figure 6). Further analysis found that the CK, LDH, α-HBDH and CK-MB of death patients were significantly higher than that of other patients (P < 0.001). Through the longitudinal comparison, we also found that CK, LDH and α-HBDH of them were significantly changed in 200 patients, except CK-MB (P < 0.001). Further subgroup analysis revealed that LDH and α-HBDH of them gradually returned to normal in the severe case and the critical cases (recovered) (P < 0.001), while LDH and α-HBDH did not recover or even worsened in the dead patients (Table 5).


Discussion

We report here a retrospective analysis on 2338 pneumonia patients with laboratory-confirmed 2019-nCoV infection, and reviewed clinical records, nursing records, laboratory findings, and CT scans from the data of 200 patients with 5 blood tests. The important changes were presented in the CK, LDH, α-HBDH and CK-MB of patients with SARS-CoV-2 infection of different clinical types and at different follow-up time points in China. It was highly significant to study the level of α-HBDH of peripheral blood in patients with COVID-19 for accurate typing, illness evaluation, precise treatment, delaying the progression of the disease, and reducing mortality.

A very important characteristic is that the α-HBDH and LDH of the critical groups significantly increased, while their CK and CK-MB remained almost at the same level. Our research also found that 51.0% of non-critical groups displayed normal level of α-HBDH, while 71.5% of critical groups displayed abnormality levels; 67.3% of non-critical groups displayed normal level of LDH, while 62.9% of critical groups displayed abnormality levels. Therefore, compared with non-critical patients, LDH and α-HBDH in critically ill patients are significantly abnormal, which are more sensitive than CK and CK-MB. Previous studies suggested that LDH, α-HBDH, CK and CK-MB reflect myocardial function. In order to study the four of the biomarkers indicating myocardial injury, ROC curve was used to analyze, the results showed that diagnostic efficiency of LDH and α-HBDH have more advantages than that of CK and CK-MB (Figure 3).

Another very important feature is that there is a very slight correlation of CK-MB and LDH or α-HBDH in critical cases (Figure 4). This suggests that LDH and α-HBDH have similar clinical significance to CK-MB in critically ill patients. According to the study, LDH is a glycolytic enzyme which catalyzes the oxidation of lactic acid to pyruvate. It has five isozymes, LD1, LD2, LD3, LD4 and LD5. LD1 and LD2 mainly come from myocardium (the sum of LD1 and LD2 is α-HBDH); LD3 mainly comes from lung and spleen; LD4 and LD5 mainly come from liver and skeletal muscle (Figure 7). Therefore, it can be said that α-HBDH is a higher sensitive and specific the biomarkers of myocardial injury in patients with COVID-19. And in a previous study also showed that most of COVID-19 patients (75%) but COVID-19 patients (20%) had an abnormal α-HBDH. The results suggested that 2019-nCoV infected patient may result into cardiac injury.

The third feature is that α-HBDH and LDH can be used as a marker for poor prognosis in COVID-19, especially in critical patients. LDH and α-HBDH in dead cases with COVID-19 remained at a high level on admission. Through survival analysis, we found that patients with α-HBDH greater than 182IU/L and LDH greater than 250IU/L at admission had lower survival rates (Figure 5), which may indicate poor clinical prognosis. Therefore, CK, LDH, α-HBDH and CK-MB were observed dynamically (Figure 6). It turns out that they increased progressively in the dead patients, while they decreased regularly in the severe case and the critical cases(recovered). Previous studies have shown that LDH and α - HBDH are common indicators of cardiotoxicity. The evidence of myocardial damage during SARS-CoV-2 infection was evaluated by CK-MB, LDH and α - HBDH.

The cardiac injury related to SARS-CoV-2 infection can be explained by several mechanisms. The first mechanism is the systemic inflammatory response syndrome, such as the cytokine storm, which may contribute to the myocardial injury. Novel coronavirus pneumonia is a systemic disease. Its mortality was caused mainly by a respiratory failure due to severe acute pneumonia, but systemic inflammatory response syndrome, including can lead to myocardial damage, such as acute fulminant myocarditis with arrhythmic manifestations, even to a sudden cardiac death. In the heart, inflammatory changes with lymphohistiocytic infiltration were observed. The findings of Puntmann and colleagues, describing myocyte injury post-COVID-19 on MRI, suggest a clinical substrate for COVID-19-induced cardiac damage. Ruan Q et al. reported that 40% of deaths were associated with circulatory failure due to cardiac injury. The cardiac injury related to SARSCoV2 infection is considered as an important issue. They could relate to either virally induced inflammation, myocardial stress, ischaemia, drugs, micro vascular thrombotic occlusion, or combinations of these factors. The second one is that the serum myocardial enzyme spectrum increased abnormally after myocardial cell injury. Myocardial enzymes mainly exist in the heart tissues in good condition. When myocardial cells are damaged, it will cause the myocardial enzymes to overflow from the myocardial cells to the serum, which makes the content of serum central muscle enzymes increase abnormally. Therefore, the changes of myocardial enzymes can be used to reflect the degree of myocardial injury and the size of the lesion, especially CK-MB and α-HBDH, which is considered as the standard diagnostic parameter for various forms of cardiac injury. Accordingly, it is comprehensible that the patients with an increased number of cardiac injury markers have a higher incidence of mortality.
Our study has several limitations. Firstly, the lack of a randomized control group means that we cannot draw definitive conclusions. Second, this is a retrospective observational study with a limited patient population. Although the different time points in this analysis were used for the LDH and α-HBDH-related survival analysis, which have strengthened our findings; however, the sample size available was not of a sufficient size to conduct such an analysis. Further validation of α-HBDH in a larger patient population is necessary. Despite this, the present results confirm the prognostic ability of α-HBDH in COVID-19. Therefore, we believe that the predictors demonstrated here are meaningful. Greater effort should be made to tackle these issues in future studies.

Conclusions

In conclusion, α-HBDH is a probably higher sensitive and specific the biomarkers of poor prognosis in adults with COVID-19. This study revealed a feasible quantitative tool as a prognostic indicator for COVID-19. It may help clinicians identify patients with a poor prognosis and may be useful for guiding a physician for the strategy of treatment in SARS-CoV-2 infection.

Abbreviations

COVID-19: Coronavirus disease 2019
SARS-CoV2: Severe acute respiratory syndrome-coronavirus2
LDH: Lactate dehydrogenase
α-HBDH: α-hydroxybutyrate-dehydrogenase
CK: Creatine Kinase
CK-MB: Creatine kinase isoenzymes
RT-PCR: Reverse Transcription-Polymerase Chain Reaction
ARDS: Acute respiratory distress syndrome
WHO: World Health Organization
CVD: cardiovascular disease
ICU: Intensive Care Unit
CT: Computed Tomography
IQR: Interquartile range
SPSS: Statistical Product and Service Solutions
ROC: Receiver operating characteristic curve

Declarations

Availability of data and materials
All data generated or analysed during this study are included in this manuscript and its tables and figures.

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Compliance with ethical standards:
Clinical and laboratory information was collected approved by Huoshenshan Hospital of Wuhan. Moreover, when the information is obtained, the patient’s name, ID number, work unit, home address, contact person and telephone information are hidden, and there is no patient privacy exposure. Thirdly, Huoshenshan Hospital of Wuhan has stopped running before the article is completed. Fourth, this is a descriptive retrospective study. Therefore, this study has not been reviewed by the hospital ethics committee.

Transparency declarations:
None to declare.

Conflicts of Interest:
The authors declare no conflict of interest.

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Tables
| Clinical features | All patients | Diseases severity | | | | | F/x value | P value |
|-------------------|--------------|-------------------|---|---|---|---|---|---|
|                   | n=200        | (n=49)            | (n=98) | (n=27) | (n=26) |               |             |
| Age—mean (range)  | 66.4(24-91)  | 65.8(34-85)       | 66.0(24-91) | 65.7(37-87) | 69.1(25-89) | 0.577 | 0.631 |
| Age-groups-No (%) |              |                   |               |               |               |             |
| ≤60               | 50(25.0)     | 12(24.5)          | 25(25.5) | 8(29.6) | 5(19.2) | 5.141 | 0.526 |
| 60-80             | 125(62.5)    | 34(69.4)          | 60(61.2) | 16(59.6) | 15(57.7) |               |             |
| ≥80               | 25(12.5)     | 3(6.1)            | 13(13.3) | 3(11.1) | 6(23.1) |               |             |
| Sex-No (%)        |              |                   |               |               |               |             |
| Female            | 85(42.5)     | 22(44.9)          | 43(43.9) | 12(44.4) | 8(30.8) | 1.697 | 0.638 |
| Male              | 115(57.5)    | 27(55.1)          | 55(56.1) | 15(55.6) | 18(69.2) |               |             |
| Exposure history-No (%) |           |                   |               |               |               |             |
| Yes               | 30(15.0)     | 9(18.4)           | 17(17.3) | 2(7.4) | 2(7.7) | 3.169 | 0.366 |
| No                | 170(85.0)    | 40(81.6)          | 81(82.7) | 25(92.6) | 24(92.3) |               |             |
| Comorbidity-No,(%)|              |                   |               |               |               |             |
| 0                 | 51(25.5)     | 20(40.8)          | 22(22.4) | 5(18.5) | 4(15.4) | 13.412 | 0.145 |
| 1                 | 67(33.5)     | 12(24.5)          | 32(32.7) | 12(44.4) | 11(42.3) |               |             |
| Hypertension      | 33(16.5)     | 7(14.2)           | 16(16.3) | 5(18.5) | 5(19.2) |               |             |
| Diabetes mellitus | 6(3.0)       | 2(4.1)            | 3(3.1) | 1(3.7) | 0 |               |             |
| Coronary heart disease | 1(0.5)  | 1(2.0)           | 0 | 0 | 0 |               |             |
| Other             | 27(13.5)     | 2(4.1)            | 13(13.3) | 6(22.2) | 6(23.1) |               |             |
| 2                 | 34(17.0)     | 10(20.4)          | 15(15.3) | 5(18.5) | 4(15.4) |               |             |
| Hypertension+Coronary heart disease | 5(2.5)  | 4(8.2)           | 1(0.1) | 0 | 0 |               |             |
| Hypertension+Diabetes mellitus | 9(4.5)  | 2(4.1)           | 4(4.1) | 2(7.4) | 1(3.8) |               |             |
| Other             | 20(10.0)     | 4(8.2)            | 10(10.2) | 3(11.1) | 3(11.5) |               |             |
| ≥3                | 48(24.0)     | 7(14.3)           | 29(29.6) | 5(18.5) | 7(26.9) |               |             |
| Hypertension+Coronary heart disease+Diabetes mellitus | 3(1.5)  | 1(2.0)           | 2(2.0) | 0 | 0 |               |             |
| Hypertension+Coronary heart disease+X | 8(4.0) | 0 | 6(6.1) | 0 | 2(7.7) |               |             |
| Hypertension+Diabetes mellitus+X | 10(5.0) | 2(4.1) | 7(7.1) | 1(3.7) | 0 |               |             |
| Other             | 27(13.5)     | 4(8.2)            | 14(15.3) | 4(14.8) | 5(19.2) |               |             |

Note: X=COPD, Cerebrovascular disease, Malignant, Anemia, Hypoproteinemia, Arrhythmia, Liver cirrhosis, etc. 0,1,2, 3: the number of comorbidities.
| Clinical features                                      | All patients | Diseases severity | x value | P value |
|-------------------------------------------------------|--------------|------------------|---------|---------|
|                                                       | n=200        | moderate (n=49)   | severe (n=98) | critical (n=27) | recovered (n=26) | death (n=26) |
|                                                       |              |                  |          |         |
| Signs and symptoms—No./total No. (%)                  |              |                  |          |         |
| 1                                                     |              |                  |          |         |
| Fever                                                 | 6(3.0)       | 2(4.1)           | 1(1.0)  | 1(3.7)  | 2(7.7) | 5.777 | 0.449 |
| Cough                                                 | 2(1.0)       | 0                | 2(2.0)  | 0       | 0      |       |       |
| Chest tightness/dyspnea                               | 5(2.5)       | 2(4.1)           | 1(1.0)  | 0       | 0      |       |       |
| 2                                                     | 35(17.5)     | 5(10.2)          | 17(17.3)| 8(29.6) | 5(19.2)|       |       |
| Fever+Fatigue                                         | 6(3.0)       | 2(4.1)           | 2(2.0)  | 1(3.7)  | 1(3.8) |       |       |
| Fever+Cough                                           | 3(1.5)       | 1(2.0)           | 1(1.0)  | 0       | 1(3.8) |       |       |
| Fever+Chest tightness/dyspnea                         | 2(1.0)       | 0                | 1(1.0)  | 0       | 1(3.8) |       |       |
| Cough+Chest tightness/dyspnea                         | 11(5.5)      | 0                | 8(8.2)  | 3(11.1) | 0      |       |       |
| Chest tightness/dyspnea+Fatigue                       | 5(2.5)       | 2(4.1)           | 1(1.0)  | 2(7.4)  | 1(3.8) |       |       |
| Other                                                 | 5(2.5)       | 0                | 2(2.0)  | 2(7.4)  | 1(3.8) |       |       |
| ≥3                                                    | 154(77.0)    | 40(81.6)         | 77(78.6)| 18(66.7)| 19(73.1)|       |       |
| Fever+Cough+Fatigue                                   | 4(2.0)       | 1(2.0)           | 2(2.0)  | 1(3.7)  | 0      |       |       |
| Fever+Cough+Chest tightness/dyspnea                   | 22(11.0)     | 6(12.2)          | 9(9.2)  | 3(11.1) | 4(15.4)|       |       |
| Fever+Cough+Chest tightness/dyspnea+Fatigue           | 18(9.0)      | 7(14.3)          | 4(4.1)  | 4(14.8) | 3(11.5)|       |       |
| Fever+Chest tightness/dyspnea+Fatigue+Muscle aches    | 6(3.0)       | 1(2.0)           | 3(3.1)  | 1(3.7)  | 1(3.8) |       |       |
| Cough+Muscle aches                                    | 4(2.0)       | 0                | 3(3.1)  | 0       | 1(3.8) |       |       |
| Cough+Chest tightness/dyspnea+Fatigue+Muscle aches    | 7(3.5)       | 1(2.0)           | 4(4.1)  | 0       | 2(7.7) |       |       |
| Other                                                 | 50(25.0)     | 14(28.6)         | 27(27.6)| 5(18.5)| 4(15.4)|       |       |
| Chest CT images—No./total No. (%)                     |              |                  |          |         |
| Multiple patchy shadows of both lungs                  | 51(25.5)     | 0                | 23(23.5)| 14(51.9)| 14(53.8)| 44.147| 0.001 |
| Multiple ground glass of both lungs                    | 133(66.5)    | 45(91.8)         | 68(69.4)| 12(44.4)| 8(30.8)|       |       |
| Multiple Consolidation of both lungs                   | 13(6.5)      | 4(8.2)           | 5(5.1)  | 1(3.7)  | 3(11.5)|       |       |
| Other                                                  | 3(1.5)       | 0                | 2(2.0)  | 0       | 1(3.9) |       |       |

Note: P values denote the comparison between moderate, severe and critical cases. Abbreviations: COVID-19, coronavirus disease 2019.[1,2, 3] the number of symptoms.
### TABLE 3  CK, LDH, $\alpha$-HBDH and CK-MB of non-critical groups and critical groups with COVID-19 on admissions

| Parameters | non-critical groups | critical groups | F/Z value | p value |
|------------|---------------------|-----------------|-----------|---------|
|            | (n=49)              | (n= 151)        |           |         |
| CK         | 50.2(33.5-79.4)     | 45.6(25.1-77.7) | 0.925     | 0.355   |
| (normal range 40-200IU/L) |                     |                 |           |         |
| LDH        | 217.1(176.2-272.3)  | 285.9(210.7-410.4) | 4.196     | $\leq 0.001$ |
| (normal range 120-250IU/L) |                     |                 |           |         |
| $\alpha$-HBDH | 179.7(145.4-223.1) | 237.8(178.8-346.0) | 4.127     | $\leq 0.001$ |
| (normal range 72-182IU/L) |                     |                 |           |         |
| CK-MB      | 9.5(7.0-12.6)       | 10.9(8.1-15.0)  | 1.947     | 0.051   |
| (normal range 0-24U/L) |                     |                 |           |         |

Note: P values denoted the comparison between non-critical groups and critical groups. Data are shown as median (IQR): IQR, interquartile range; CK, LDH, $\alpha$-HBDH and CK-MB collection time point of on admissions. Non-critical groups: moderate patients. Critical groups: severe and critical patients.

### TABLE 4  LDH and $\alpha$-HBDH of non-critical groups and critical groups with COVID-19

| Parameters | IU/L | non-critical groups | critical groups | x value | P value |
|------------|------|---------------------|-----------------|---------|---------|
|            | n (%)| n (%)               |                 |         |         |
| 120-250    | 33(67.3) | 56(37.1) | 16.68 | 0.001 |
| LDH        | 250-300 | 9(18.4)  | 29(19.2) |         |         |
| (normal range 120-250IU/L) |            |          |         |         |         |
| 300-350    | 3(6.1)  | 19(12.6) |         |         |         |
| ≥350       | 4(8.2)  | 47(31.1) |         |         |         |
| 72-182     | 25(51.0) | 43(28.5) | 9.712  | 0.021 |
| $\alpha$-HBDH | 182-232 | 14(28.6) | 49(32.5) |         |         |
| (normal range 72-182IU/L) |            |          |         |         |         |
| ≥282       | 4(8.2)  | 30(19.9) |         |         |         |

Note: P values denoted the comparison between non-critical groups and critical groups. Data are shown as median (IQR): IQR, interquartile range; LDH and $\alpha$-HBDH collection time point of on admissions. Non-critical groups: moderate patients. Critical groups: severe and critical patients.
| Parameters | time | All patients (n=200) | moderate (n=49) | severe (n=98) | critical recovered (n=27) | critical death (n=26) | x value | P value |
|------------|------|----------------------|----------------|--------------|--------------------------|------------------------|---------|---------|
| CK         | 1    | 46.7(27.9-77.4)      | 50.2(33.5-79.4)| 39.1(24.4-67.8)| 54.3(20.2-124.3)         | 73.5(34.7-120.5)       | 11.332  | 0.01    |
|            | 2    | 35.3(22.5-60.7)      | 39.7(23.9-55.0)| 31.9(20.8-47.7)| 39.9(23.1-65.8)         | 65.2(44.7-105.7)       | 20.376  | 0.001   |
| α-HBDH     | 3    | 35.6(21.9-54.1)      | 42.2(23.6-58.4)| 28.3(20.6-41.6)| 41.2(17.9-59.0)         | 111.8(39.6-206.3)      | 30.56   | 0.001   |
| LDH        | 1    | 146.7(98.8-351.9)    | 217.1(176.2-272.3)| 247.4(195.8-307.8)| 313.9(259.7-493.8)       | 465.3(357.9-557.7)     | 55.799  | 0.001   |
|            | 2    | 225.2(186.7-284.9)   | 201.9(165.3-253.3)| 216.6(178.7-255.0)| 263.8(211.1-371.7)       | 383.3(301.4-560.9)     | 45.648  | 0.001   |
| α-HBDH     | 3    | 209.0(181.9-265.0)   | 189.9(176.3-236.9)| 199.8(174.7-228.3)| 233.1(205.9-255.6)       | 394.4(323.6-527.6)     | 58.014  | 0.001   |
| LDH        | 1    | 203.4(177.0-248.4)   | 189.0(169.5-211.3)| 199.3(172.8-229.8)| 211.4(193.3-246.5)       | 409.7(285.7-495.5)     | 58.791  | 0.001   |
|            | 2    | 202.5(170.2-243.8)   | 182.0(155.9-227.6)| 194.0(177.023.0)| 211.3(182.6-236.7)       | 512.9(452.8-744.3)     | 64.755  | 0.001   |
| α-HBDH     | 3    | 172.9(146.1-217.1)   | 156.9(138.5-189.5)| 162.2(143.5-190.0)| 191.5(175.2-218.3)       | 335.7(258.9-417.3)     | 57.429  | 0.001   |
| LDH        | 1    | 166.7(144.4-204.7)   | 151.8(141.5-172.7)| 162.7(139.0-185.3)| 174.7(153.8-203.2)       | 338.2(247.5-410.8)     | 57.609  | 0.001   |
|            | 2    | 163.7(142.6-196.1)   | 151.2(127.5-182.1)| 158.1(141.2-184.7)| 171.9(156.5-192.0)       | 432.4(357.7-519.3)     | 64.473  | 0.001   |
| α-HBDH     | 3    | 53.151               | 10.54            | 42.757        | 30.679                   | 7.689                 |         |         |
| 1 | 10.3(8.0-14.5) | 9.5(6.95-12.6) | 9.4(7.40-13.3) | 14.3(10.7-18.0) | 14.5(12.0-18.1) | 29.169 | 0.001 |
|---|---|---|---|---|---|---|---|
| 2 | 9.2(7.2-13.2) | 7.7(6.4-11.5) | 8.8(6.5-11.6) | 10.3(8.9-15.8) | 17.9(12.6-27.3) | 41.271 | 0.001 |
| CK-MB | 3 | 9.1(7.0-13.5) | 9.0(6.3-12.2) | 8.4(6.8-11.2) | 9.2(7.4-13.0) | 19.55(12.1-22.9) | 31.175 | 0.001 |
| (normal range 0-24IU/L) | 4 | 9.5(7.3-12.4) | 9.1(7.6-12.0) | 8.7(6.7-11.3) | 10.3(6.9-14.2) | 16.3(12.0-26.9) | 34.788 | 0.001 |
| 5 | 9.3(7.2-13.6) | 8.5(6.6-10.4) | 8.8(7.1-11.2) | 8.8(6.7-13.6) | 37.9(21.9-73.8) | 61.728 | 0.001 |
| x value | 7.204 | 5.29 | 4.46 | 15.022 | 25.551 |
| P value | 0.126 | 0.259 | 0.347 | 0.005 | 0.001 |

Note: P values denoted the comparison between moderate, severe, critical and death cases. Data are shown as median (IQR); IQR, interquartile range; 1, 2, 3, 4, 5: CK, LDH, α-HBDH and CK-MB collection time point of admissions, 25%, 50%, 75% and discharge during their hospitalization.

Figures
Figure 1
Study flow chart

Figure 2
Time point of blood tests collection in during hospitalization

Figure 3
ROC curve of CK, LDH, α-HBDH and CK-MB in patients with COVID-19
Figure 4

Correlation analysis of CK-MB and LDH or α-HBDH in non-critical groups and critical groups
Figure 5

Prognosis of CK, LDH, α-HBDH and CK-MB in patients with COVID-19 on the admission
Figure 6

Dynamic changes of CK, LDH, α-HBDH and CK-MB in patients with COVID-19
Figure 7

Distribution of LDH isozymes LD1, LD2, LD3, LD4 and LD5 in human body