Profiling of lactate dehydrogenase isoenzymes in COVID-19 disease

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ARTICLE INFO

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ABSTRACT

Introduction

Serum total lactate dehydrogenase (LDH) activity was elevated and showed a positive correlation with disease severity and outcome in severe COVID-19 disease. However, it is still unknown whether the relative abundance or calculated activity of any LDH isoenzyme is predominately increased in COVID-19 subjects.

Methods

Twenty-two consecutive patients suffered from moderate or severe COVID-19 pneumonia were recruited into this study who showed enhanced total LDH activity. The ratio of LDH isoenzyme activities was further investigated using gel electrophoresis (Hydragel®, Sebia) with densitometric evaluation. Calculated activity values of these isoenzymes were correlated with routine laboratory parameters, the degree of lung

parenchymal affection based on chest CT and clinical outcome.

Results

Total LDH activity was raised in the range of 272-2141 U/L and significantly correlated with calculated LDH-3 and LDH-4 activities (r=0.765, P=0.0001; and r=0.783, P=0.0001, respectively). In contrast, the relative abundance of neither LDH isoenzyme was exclusively abnormal in COVID-19 patients. Calculated activity of LDH-3 and LDH-4 demonstrated a modest but statistically significant association with serum ferritin (r=0.437, P=0.042; r=0.505, P=0.016, respectively). When the relationship between the severity of pulmonary affection by SARS-CoV-2 infection and relative abundance of LDH isoenzymes was studied, a larger ratio of mid-zone fractions was observed in the presence of ≥ 50% lung parenchymal involvement. Finally, regardless of LDH isoenzyme pattern, abnormal relative ratio of LDH-4 and higher calculated LDH-3 and LDH-4 activity values were detected in subjects with unfavorable outcome.

Conclusion

No characteristic profile of LDH isoenzymes can be detected in COVID-19 pneumonia, however, elevated activities of LDH-3 and LDH-4 are associated with worse clinical outcomes.

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INTRODUCTION

Since the outbreak of the Coronavirus disease 2019 (COVID-19) pandemic in December 2019, the importance of clinical laboratory tests has emerged to manage the hospitalization of patients with different severity of COVID-19 related disorders, to distinguish severe and non-severe clinical conditions and to predict the outcome of the disease. For these purposes, an enormous amount of clinical data has recently accumulated to evaluate and validate the potential role of routinely available as well as novel laboratory biomarkers (1). There are several parameters which have been identified as independent risk factors to assess disease severity, such as C-reactive protein (CRP) (2,3), interleukin-6 (2,3), circulating ACE2 activity (4,5), D-dimer (3,6), total lactate dehydrogenase (LDH) (7-10) and cardiac markers, i.e. high-sensitive cardiac troponin I (cTnI) with myoglobin, CK-MB activity and NT-pro-BNP (11). In parallel, CRP (12) and total LDH (10,12) were useful to recognize early lung injury and failure, whilst total LDH (7,8,11), CRP (13), D-dimer (6,14) and soluble ACE2 activity (5) were able to predict unfavorable outcome of COVID-19. Furthermore, the combination of increased total LDH with other blood-based biomarkers or clinical parameters could aid the clinical estimation of COVID-19 severity and mortality (15,16).

Regular analysis of total LDH activity has got in focus in this disease, however, only limited amount of data is available on the profile of LDH isoenzymes that was analyzed in plasma samples of some COVID-19 subjects (17). Hence, our aim was here to further investigate the relative abundance and calculated activity of LDH isoenzymes in serum by gel electrophoresis in hospitalized COVID-19 subjects in connection with the disease severity and worse clinical outcome.

METHODS

Patients

In this study, 22 consecutive patients (13 males and 9 females) at the age of between (min-max) 27-81 years of age were recruited from March 1 to 14, 2021 at the Clinical Center and Gyula Kenézy Campus, University of Debrecen, Debrecen, Hungary (Table 1). These subjects suffered from severe (n=14) or moderate (n=8)

pneumonia at sampling time point and were confirmed to be positive for COVID-19 disease by reverse transcription polymerase chain reaction (RT-qPCR) test of a nasopharyngeal swab. All these patients underwent chest CT scan to evaluate the extent of pulmonary lesions, such as ground-glass opacities and consolidation using a visual scoring system. Also, enrolled subjects suffered from various diseases, such as hypertension, cardiomyopathy, diabetes mellitus, renal disorders, cataract or angina based on their pre-COVID-19 history (Table 1). Severely ill patients were transferred to the Intensive Care Unit (ICU), while those with moderate symptoms were treated at the Department of Infectious Diseases, Gyula Kenézy Campus, University of Debrecen, Debrecen, Hungary. Despite ICU treatment all severe subjects died of COVID-19 within 28 days of the initiation of the disease, while patients in moderate clinical status were effectively treated and survived (Table 1).

Laboratory analyses

Total LDH activity and serum creatinine were determined by kinetic colorimetric assays on a Cobas® 8000 analyzer (Roche Diagnostics, Mannheim, Germany). In parallel, white blood cell (WBC) counts were determined by an Advia 2120 Hematology System analyzer (Bayer Diagnostics, Tarrytown, NJ, USA). The concentrations of C-reactive protein (CRP), ferritin, and cTnT were determined by electro-chemiluminescent immunoassay (Cobas® e 411 analyzer, Roche Diagnostics), while D-dimer was analyzed by immunoturbidimetry (BCS® XP, Siemens, Munich, Germany). The CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) equation was used to estimate the glomerular filtrate rate (GFR).

The five isoenzymes of LDH were separated by electrophoresis using LDH Hydragel® 7 kit (Sebia, Norcross, GA, USA) on alkaline buffered (pH 8.4) agarose gel. The separated isoenzymes were visualized using a specific chromogenic substrate, and the amount of formazan precipitate was proportional to the LDH enzymatic activity. A semi-automated HYDRASYS® electrophoresis instrument (Sebia) was applied to obtain gels ready for interpretation. The dried gels were processed for densitometry to achieve an accurate relative quantification of individual zones. Abnormal ratio of LDH isoenzymes was evaluated based on the manufacturer's instructions of LDH Hydragel® 7 kit.

Statistical analyses

Kolmogorov–Smirnov test was used for evaluation of the normality of data. To compare the data of two groups, we applied Mann–Whitney U test. Correlations between total LDH and LDH isoenzyme activities as well as the link between LDH-3 or LDH-4 activity and other laboratory parameters were determined using Spearman's test. Statistical significance was defined when P value was < 0.05. Statistical analyses were performed using GraphPad Prism software (version 6.01, La Jolla, CA, USA).

RESULTS

Based on routine laboratory tests, inflammatory clinical conditions were indicated by elevated WBC count, serum CRP and ferritin levels. Importantly, based on its upper reference limit (URL) *i.e.* 220 U/L, total LDH activity in sera was higher than normal in all recruited COVID-19 patients within the range of 272-2141 U/L (Table 1, Figure 1A). Moreover, total LDH activity was significantly higher in severe compared to non-severe COVID-19 patients (median [IQR] 947.5 [704.3-1307.0] vs 391.5 [331.8-895.8] U/L, P = 0.016) (Figure 1A). Although these subjects suffered from various comorbidities in the pre-COVID-19 era, these conditions did not substantially modulate LDH activities during COVID-19 disease (Table 1).

Table 1						Main demographical, clinical and laboratory parameters of 22 consecutive COVID-19							
ID	Age (y)	Sex (F/M)	WBC (G/L)	CRP (mg/L)	Ferritin (ug/L)	Total LDH (U/L)	Abnormal relative abundance of LDH isoenzymes (%)	cTnT (ng/L)	GFR-EPI (mL/min/ 1.73 m2)	D-dimer (mg FEU/L)	COVID-19 severity	History, pre-COVID-19 comorbidities	28-day outcome
1	77	M	17.6	39.9	1401	272	-	17.1	24	1.0	moderate	HT, kidney stones	survivor
2	81	F	8.5	11.1	670	372	42.8 (LDH-2)	41.7	10	3.7	moderate	HT, drug induced nephropathy	survivor
3	63	F	24.2	67.3	949	737	31.1 (LDH-3)/ 14.4 (LDH-4)	13.1	81	1.5	severe	HT, stable angina	non-survivor
4	29	M	14.2	423.3	1391	955	42.1 (LDH-2)/ 28.0 (LDH-3)	10	71	0.7	severe	iron deficiency, epilepsy	non-survivor
5	32	M	3.1	16.8	245	331	42.6 (LDH-2)	16.8	90	0.5	moderate	inherited cardiomyopathy	survivor
6	64	M	7.2	37.5	1115	334	34.1 (LDH-1)/ 43.4 (LDH-2)	33.7	90	1.4	moderate	HT, cholecystectomy, cataract	survivor
7	73	M	13.7	21.1	549	411	-	16.5	90	0.5	moderate	HT, kidney stones, arthritis	survivor
8	71	F	9.4	92.2	907	426	26.5 (LDH-3)/ 13.8 (LDH-4)	13.1	80	0.9	severe	HT, polyarthritis	non-survivor
9	52	F	14.5	59.9	1715	1161	32.4 (LDH-3)/ 21.1 (LDH-4)	n.m.	90	0.9	severe	HT, instable angina	non-survivor
10	73	F	33.8	50.9	2000	749	43.7 (LDH-2)	12.9	76	3.7	severe	HT, cataract	non-survivor
11	27	F	8.2	94.3	985.3	979	31.9 (LDH-3)/ 21.0 (LDH-4)	10	81	1.3	moderate	cholangitis	survivor
12	68	M	7.6	112.5	2000	882	51.6 (LDH-5)	70.4	14	1.5	severe	HT, poststreptococcal GN	non-survivor
13	60	F	11.5	20.1	965	952	28.7 (LDH-3)/ 13.2 (LDH-4)	17.2	90	91.8	severe	HT, spinal osteoarthritis	non-survivor
14	65	M	17.3	12.8	1585	2141	18.5 (LDH-4)/ 18.3 (LDH-5)	n.m.	10	6.2	severe	HT, renal dysfunction	non-survivor
15	53	M	23.6	10.7	1418	1148	14.1 (LDH-4)/ 22.0 (LDH-5)	n.m.	90	2.1	severe	HT, cardiomyopathy	non-survivor
16	71	F	13.2	132.1	2000	943	31.2 (LDH-5)	93.7	17	2.2	severe	HT, nephrosis syndrome	non-survivor
17	49	M	17.2	40.6	2000	976	26.1 (LDH-3)/ 12.6 (LDH-4)	n.m.	60	2.3	severe	chronic alcohol consumption	non-survivor
18	65	M	11.8	9.9	516	1904	67.4 (LDH-1)	36.2	74	0.5	moderate	HT, iron deficiency	survivor
19	80	F	17.5	292	2000	606	17.6 (LDH-4)/ 29.9 (LDH-5)	n.m.	77	3.8	severe	HT, stroke, diabetes mellitus	non-survivor
20	46	M	43.8	156	1141	582	14.2 (LDH-4)/ 29.5 (LDH-5)	20	73	3.1	severe	aortic insufficiency	non-survivor
21	36	M	5.1	60.6	1160	655	32.8 (LDH-3)	10	90	0.8	moderate	cholecystectomy	survivor
22	51	M	12.5	13.3	2000	1746	28.3 (LDH-3)/ 16.8 (LDH-4)	53.1	90	22.9	severe	HT, diabetes mellitus	non-survivor

Figure 1 **Analysis of the associations between serum LDH activities and different clinical parameters**
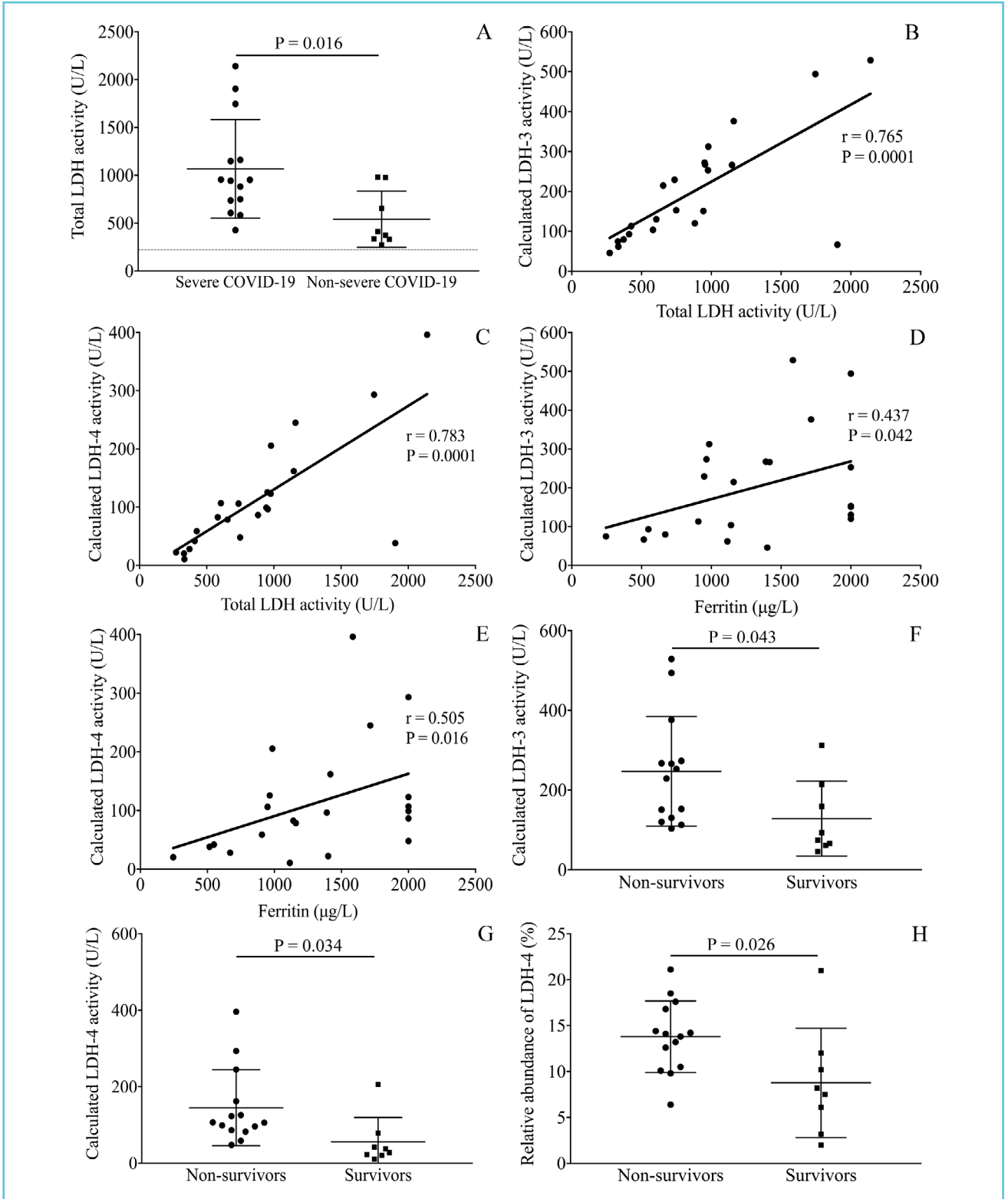
To further investigate the background of high total LDH activity, gel electrophoresis was performed to determine the relative abundance and to quantify the calculated activity of LDH isoenzymes. According to the subsequent densitometry analysis, LDH isoenzymes with increased activity had no universal pattern in COVID-19. Out of 22 subjects, nine patients showed a larger ratio of mid-zone fractions, *i.e.* increased LDH3 with or without LDH-4 or LDH-2, while single elevated LDH-2 activity was seen in case of three patients. Also, there were six individuals with increased LDH-5 activity with or without LDH-4 and two patients had higher LDH-1 level. In contrast, two persons did not show an altered ratio of LDH isoenzymes despite high total LDH activity. Overall, there was no typical profile of LDH isoenzymes in COVID-19 pneumonia (Figure 2).

In contrast, when we statistically correlated the measured activity of total LDH with the calculated activity of each individual isoenzyme, a significant relationship was observed between total LDH with LDH-3 activity (r=0.765, P=0.0001) (Figure 1B) and LDH-4 activity (r=0.783, P=0.0001) (Figure 1C), while no association was found with other isoenzymes (data not shown). The direct link between calculated LDH-3 and LDH-4 activity and other laboratory parameters typically altered in COVID-19 disease was also studied, and a modest but statistically significant association was demonstrated in case of both isoenzymes only with serum ferritin (r=0.437, P=0.042; r=0.505, P=0.016, respectively) (Figures 1D and E).

Lung parenchymal involvement expressed in (%) caused by SARS-CoV-2 infection was variable among recruited patients with different disease severity based on chest CT examination (Figure 2). The extent of pulmonary lesions was significantly larger in non-survivors with severe symptoms compared to survivors having only moderate alterations (70 [50-76] vs 15 [50-76] %, P = 0.003) (data not shown). When the relationship between CT findings and abnormal relative percentage of LDH isoenzymes was studied, a larger ratio of mid-zone fractions was observed in patients suffered from ≥ 50% pulmonary parenchymal involvement. On the other hand, elevated relative abundance of LDH-2 alone was present at moderate (< 20%) parenchymal extension (Figure 2). Pneumonia and COVID-19 related severe liver failure together resulted in augmented LDH-5 ratio (n=6), while intravascular hemolysis in two critically ill patients showed high LDH-1 level (n=2). Based on these data, the severity of pulmonary affection was strongly related to abnormal relative abundance of LDH isoenzymes which belong to the mid-zone fractions, however, the manifestation of other comorbidities causing the release of other LDH isoenzyme(s) modified the overall results of LDH isoenzyme ratio.

Figure 2   **Evaluation of relative ratio of serum LDH isoenzymes by gel electrophoresis**
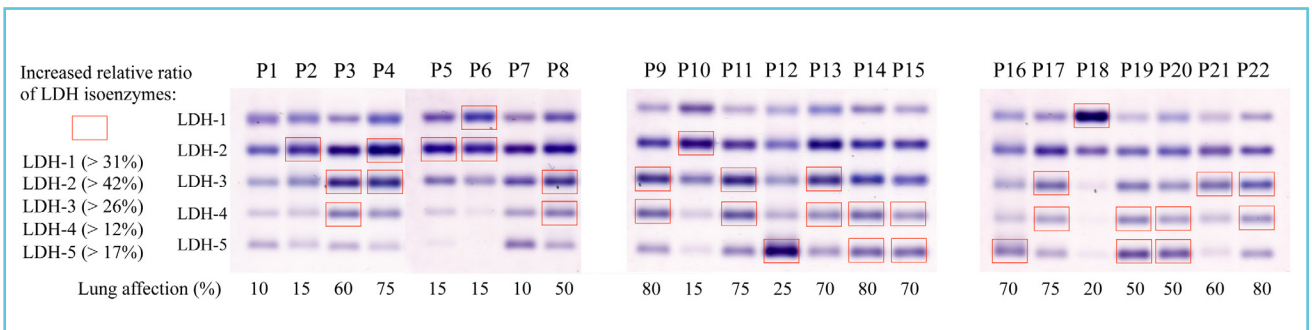
Finally, in regard to the clinical outcome, significantly larger activity values of LDH-3 (241.0 [127.7-299.0] vs 83.7 [63.0-200.9] U/L, P = 0.043) and LDH-4 (106.4 [85.5-182.7] vs 33.0 [20.9-69.4] U/L, P = 0.034) were seen in non-survivors vs survivors (Figures 1F and G). Furthermore, the relative abundance of LDH-4 (P = 0.026) but not LDH-3 (P = 0.368) was higher in patients with poor outcome than recovered subjects (Figure 1H).

DISCUSSION

In recent clinical studies, increased total LDH activity in sera has been investigated as a biomarker to estimate disease severity (7-10), to indicate early pulmonary damage (10,12) and to predict unfavorable outcome of COVID-19 (7,8,13). Since total LDH has been considered as a reliable biomarker for variable inflammatory conditions and related pulmonary damage for a long time, such as in sepsis, cardiovascular disorders or cancers (18), that is why the routine measurement of LDH needs to be verified in COVID-19 as well (8). However, it has not been revealed whether elevated total LDH level in COVID-19 was generally due to a release of one particular LDH isoenzyme. For this purpose, serum samples of 22 hospitalized patients with severe or non-severe COVID-19 disease showing abnormal total LDH activity were analyzed in this study to quantify the relative abundance and activity of LDH isoenzymes by gel electrophoresis. Apart from the analysis of LDH isoenzyme pattern, calculated activity values were correlated with other laboratory parameters and clinical data.

Total LDH activities were found abnormal in all cases ranging from slightly elevated level up to 9 times URL value and were significantly higher in severe compared to non-severe COVID-19 patients, which were in accordance with the latest clinical data (8,15). Although there was a positive correlation between calculated activity of LDH-3 or LDH-4 and measured activity of total LDH, neither of them was found to exclusively contribute to high total LDH activity in this cohort based on gel electrophoresis. Hence, there was no typical profile of LDH isoenzymes in COVID-19 pneumonia. Serrano-Lorenzo *et al.* have recently investigated the different LDH isoenzymes in plasma samples and showed no correlation between the activity of total LDH and its isoenzymes (17). In addition, these authors did not find any association of relative LDH activities with various routine hematological and chemical laboratory parameters in contrast to our results where LDH-3 and LDH-4 activities were related to serum ferritin levels. Due to the limited number of our patients, we could not determine the odds ratio of total LDH activity for the recognition of severe COVID-19 cases, but others have determined that LDH had a powerful predictive value for disease severity (9,10). Similarly, Poggiali *at el.* found that total LDH showed a substantial ROC-AUC value (0.76, P < 0.0001) at the cut-off value of 450 U/L to distinguish severe and moderate respiratory distress states in COVID-19 (12).

Based on chest CT examination, various degree of lung parenchymal involvement was detected among recruited patients with different disease severity. The level of pulmonary impairments was significantly larger in non-survivors with severe symptoms compared to survivors having only moderate alterations. These results are in line with clinical data of Canovi *et al.* suggesting a direct role of detectable lung lesions provoked by SARS-CoV-2 infection with induced inflammatory response and reduced oxygen saturation (19). In addition, there was a strong negative correlation between total LDH and PaO_2/FiO_2 values in a large group of COVID-19 subjects (12). In our study, when the relationship between CT findings and abnormal relative ratio of LDH isoenzymes was studied, a larger ratio

of mid-zone fractions was observed in patients who suffered from ≥ 50% pulmonary parenchymal involvement. On the other hand, elevated LDH-2 activity was present at moderate (< 20%) parenchymal extension. Accordingly, the severity of pulmonary affection was strongly related to abnormal activities of LDH isoenzymes which belong to the mid-zone fractions. However, pneumonia and COVID-19 related severe liver failure together resulted in augmented LDH-5 activity, while intravascular hemolysis caused high LDH-1 level. Although Serrano-Lorenzo and his colleagues have analyzed in-gel LDH isoenzymes in COVID-19 subjects, altered relative activities of these isoenzymes were not studied in detail in the aspect of other comorbidities and clinical outcome (17). In this study, based on their pre-COVID-19 history, subjects suffered from various diseases, such as hypertension, cardiomyopathy, diabetes mellitus, renal disorders, cataract or angina, but these conditions did not substantially modulate LDH activities in COVID-19 disease. Finally, considering the clinical outcome of these patients, significantly larger LDH-3 and LDH-4 activity values were seen in non-survivors vs survivors. Furthermore, the relative abundance of LDH-4 but not LDH-3 was higher in patients with poor outcome than recovered subjects. Recently, elevated total LDH activity has been described to provide a prognostic value for survival having a 16-fold increase in odds of mortality (7) and showing an increased risk for death at cut-off value of 395 U/L (13).

In conclusion, no characteristic profile of LDH isoenzymes can be detected in COVID-19 pneumonia, however, elevated calculated LDH-3 and LDH-4 activities are associated with unfavorable clinical outcomes. Based on these data, there must be a direct link between increased LDH activity and SARS-CoV-2 induced lung injury, but a more widespread tissue damage can simply overwhelm the relative activities of

LDH isoenzymes. Hence, further clinical studies are required with a larger number of patients to validate the usefulness of in-gel activities of LDH isoenzymes in COVID-19.

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Conflict of interest

There are no competing interests to declare among the authors of this work.

Ethical approval

The study was approved by the Scientific and Research Ethics Committee of the University of Debrecen and the Ministry of Human Capacities under the registration number of 32568-3/2020/EÜIG.

Figure legends

Table 1. Main demographical, clinical and laboratory parameters of 22 consecutive COVID-19 patients. To investigate the background of increased total LDH activity in sera of subjects suffered from severe (n=14) or moderate (n=8) COVID-19 condition, gel electrophoresis was performed for the quantitation of relative abundance and activity of LDH isoenzymes. Enrolled patients showed elevated WBC count with high CRP and ferritin levels. cTnT was not determined

in all cases. Renal function and prothrombotic conditions were determined by GFR-EPI and D-dimer level, respectively. Based on the data collection by the clinicians, pre-COVID-19 comorbidities, COVID-19 disease severity and clinical outcome were also available. Abbreviations: WBC: white blood cells, CRP: C-reactive protein, cTnT: cardiac troponin T, GFR-EPI: glomerular filtrate rate calculated by CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) equation, HT: hypertension, poststreptococcal GN: poststreptococcal glomerulonephritis. n.m. means 'not measured'.

Figure 1. Analysis of the associations between serum LDH activities and different clinical parameters. (A) Comparison of total LDH activity in severe (n=14) and non-severe COVID-19 patients (n=8). (B, C) Correlation between total LDH and calculated LDH-3 and LDH-4 isoenzyme activities in the entire patient cohort. (D, E) Relationship between calculated LDH-3 and LDH-4 activity and serum ferritin concentration in recruited subjects. (F, G) Analysis of calculated activity of LDH-3 and LDH-4 between non-survivors (n=14) and survivors (n=8). (H) Association between the relative abundance of LDH-4 and 28-day outcome of COVID-19 patients. To compare the data of two groups, Mann–Whitney U test was used, while correlations were determined using Spearman's test. Dotted line in part A depicts the URL value of measured total LDH activity (i.e. 220 U/L).

Figure 2. Evaluation of relative ratio of serum LDH isoenzymes by gel electrophoresis. The five isoenzymes of LDH were separated by electrophoresis using LDH Hydragel® 7 kit on alkaline buffered (pH 8.4) agarose gel. The separated isoenzymes were visualized using a specific chromogenic substrate, and its amount was proportional to the LDH enzymatic activity according to densitometry. Abnormal relative ratio of LDH isoenzymes indicated by red quadrants was evaluated based on the manufacturer's instructions of the kit. P1-P22 indicate the ID number of enrolled patients. Below the gel images, the degree of lung parenchymal involvement (%) based on chest CT examination is depicted in the presence of various LDH isoenzyme patterns.

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REFERENCES

1. Tomo S, Karli S, Dharmalingam K, Yadav D, Sharma P. The Clinical Laboratory: A Key Player in Diagnosis and Management of COVID-19. EJIFCC. 2020;31(4):326-346.

2. Zhu Z, Cai T, Fan L, Lou K, Hua X, Huang Z, Gao G. Clinical value of immune-inflammatory parameters to assess the severity of coronavirus disease 2019. Int J Infect Dis. 2020;95:332-339.

3. Gao Y, Li T, Han M, Li X, Wu D, Xu Y, Zhu Y, Liu Y, Wang X, Wang L. Diagnostic utility of clinical laboratory data determinations for patients with the severe COVID-19. J Med Virol. 2020;92(7):791-796.

4. Nagy B Jr, Fejes Z, Szentkereszty Z, Sütő R, Várkonyi I, Ajzner É, Kappelmayer J, Papp Z, Tóth A, Fagyas M. A dramatic rise in serum ACE2 activity in a critically ill COVID-19 patient. Int J Infect Dis. 2021;103:412-414.

5. Fagyas M, Fejes Z, Sütő R, Nagy Z, Pócsi M, Bíró E, Bekő G, Nagy A, Szentkereszty Z, Papp Z, Tóth A, Kappelmayer J, Nagy B Jr. Circulating ACE2 activity predicts mortality and disease severity in hospitalized COVID-19 patients. Int J Infect Dis. 2021 Nov 25:S1201-9712(21)00876-6. doi: 10.1016/j.ijid.2021.11.028.

6. Ruetzler K, Szarpak Ł, Ładny JR, Gąsecka A, Gilis-Malinowska N, Pruc M, Smereka J, Nowak B, Filipiak KJ, Jaguszewski MJ. D-dimer levels predict COVID-19 severity and mortality. Kardiol Pol. 2021;79(2):217-218.

7. Henry BM, Aggarwal G, Wong J, Benoit S, Vikse J, Plebani M, Lippi G. Lactate dehydrogenase levels predict coronavirus disease 2019 (COVID-19) severity and mortality: A pooled analysis. Am J Emerg Med. 2020;38(9):1722-1726.

8. Szarpak L, Ruetzler K, Safiejko K, Hampel M, Pruc M, Kanczuga-Koda L, Filipiak KJ, Jaguszewski MJ. Lactate dehydrogenase level as a COVID-19 severity marker. Am J Emerg Med. 2021;45:638-639.

9. Hu J, Zhou J, Dong F, Tan J, Wang S, Li Z, Zhang X, Zhang H, Ming J, Huang T. Combination of serum lactate dehydrogenase and sex is predictive of severe disease in patients with COVID-19. Medicine (Baltimore). 2020;99(42): e22774.

10. Han Y, Zhang H, Mu S, Wei W, Jin C, Tong C, Song Z, Zha Y, Xue Y, Gu G. Lactate dehydrogenase, an independent risk factor of severe COVID-19 patients: a retrospective and observational study. Aging (Albany NY). 2020;12(12): 11245-11258.

11. Han H, Xie L, Liu R, Yang J, Liu F, Wu K, Chen L, Hou W, Feng Y, Zhu C. Analysis of heart injury laboratory parameters in 273 COVID-19 patients in one hospital in Wuhan, China. J Med Virol. 2020;92(7):819-823.

12. Poggiali E, Zaino D, Immovilli P, Rovero L, Losi G, Dacrema A, Nuccetelli M, Vadacca GB, Guidetti D, Vercelli A, Magnacavallo A, Bernardini S, Terracciano C. Lactate dehydrogenase and C-reactive protein as predictors of respiratory failure in COVID-19 patients. Clin Chim Acta. 2020;509:135-138.

13. Turrini M, Gardellini A, Beretta L, Buzzi L, Ferrario S, Vasile S, Clerici R, Colzani A, Liparulo L, Scognamiglio G, Imperiali G, Corrado G, Strada A, Galletti M, Castiglione N, Zanon C. Clinical Course and Risk Factors for In-Hospital Mortality of 205 Patients with SARS-CoV-2 Pneumonia in Como, Lombardy Region, Italy. Vaccines (Basel). 2021;9(6): 640.

14. Chen Z, Xu W, Ma W, Shi X, Li S, Hao M, Fang Y, Zhang L. Clinical laboratory evaluation of COVID-19. Clin Chim Acta. 2021;519:172-182.

15. Gómez LC, Curto SV, Sebastian MBP, Jiménez BF, Duniol MD. Predictive Model of Severity in SARS CoV-2 Patients at Hospital Admission Using Blood-Related Parameters. EJIFCC. 2021;32(2):255-264.

16. Lagolio E, Demurtas J, Buzzetti R, Cortassa G, Bottone S, Spadafora L, Cocino C, Smith L, Benzing T, Polidori MC. A rapid and feasible tool for clinical decision making in community-dwelling patients with COVID-19 and those admitted to emergency departments: the Braden-LDH-HorowITZ Assessment-BLITZ. Intern Emerg Med. 2021 Jul 28:1-6. doi: 10.1007/s11739-021-02805-w.

17. Serrano-Lorenzo P, Coya ON, López-Jimenez A, Blázquez A, Delmiro A, Lucia A, Arenas J, Martín MA; COVID-19 '12 Octubre' Hospital Clinical Biochemistry Study Group. Plasma LDH: A specific biomarker for lung affectation in COVID-19? Pract Lab Med. 2021;25:e00226.

18. Drent M, Cobben NA, Henderson RF, Wouters EF, van Dieijen-Visser M. Usefulness of lactate dehydrogenase and its isoenzymes as indicators of lung damage or inflammation. Eur Respir J. 1996;9(8):1736-1742.

19. Canovi S, Besutti G, Bonelli E, Iotti V, Ottone M, Albertazzi L, Zerbini A, Pattacini P, Giorgi Rossi P, Colla R, Fasano T; Reggio Emilia COVID-19 Working Group. The association between clinical laboratory data and chest CT findings explains disease severity in a large Italian cohort of COVID-19 patients. BMC Infect Dis. 2021;21(1):157.