Opinion Paper

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Lactate dehydrogenase: an old enzyme reborn as a COVID-19 marker (and not only)

https://doi.org/10.1515/cclm-2020-1062
Received July 11, 2020; accepted August 11, 2020; published online August 24, 2020

Abstract

Background: Historically, the lactate dehydrogenase (LDH) measurement was introduced into Laboratory Medicine as component (together with creatine kinase (CK) and aspartate aminotransferase) of the classical enzyme triad employed for the diagnosis of myocardial infarction, which was subsequently replaced by CK-MB, and more recently by cardiac troponins. Afterwards, for many years, the clinical application of serum LDH measurement has been limited to the evaluation of anemias and to as a rough prognostic tool for certain tumors.
Content: In the last few years, significant changes have happened. First, the test has been confirmed as a robust predictor of poor outcomes in many neoplastic conditions. Furthermore, in the Revised International Staging System adopted in the 2015 by the International Myeloma Working Group, LDH acts as determinant of disease biology in differentiating myeloma stages. Finally, in the last few months, LDH is definitively reborn given its proven significant contribution in defining the COVID-19 severity.
Conclusions: This increased clinical role calls for an improvement of LDH assay standardization through the implementation of traceability of results of clinical samples to the available reference measurement system.

Keywords: clinical enzymology; lactate dehydrogenase; tumors; SARS-CoV-2 disease; standardization.

Lactate dehydrogenase (LDH) is a hydrogen transfer enzyme that catalyzes the oxidation of L-lactate to pyruvate with the mediation of NAD^+ as a hydrogen acceptor, with the reaction being reversible. LDH has a molecular weight of 134 kDa and is composed of four peptide chains of two types: M (or A) and H (or B), each under separate genetic control [1]. Its activity is present in the cytoplasm of many cells of the body and enzyme concentrations in various tissues are about 1,500 to 5,000 times greater than those physiologically found in serum. Therefore, leakage of the enzyme from even a small mass of damaged tissue increases the observed serum activity of LDH to a significant extent.

Because of its wide tissue distribution, serum LDH increases occur in a variety of diseases, but in the last years its clinical utility has been relegated to hematology and oncology [2]. Hemolytic anemias significantly increase LDH concentrations in serum and the enzyme values have been proposed as a diagnostic and prognostic marker of acute and chronic complications [3]. Marked increases of LDH activity—up to 50 times the upper reference limit—have been observed in the megaloblastic anemias. These anemias, usually resulting from the deficiency of folate or vitamin B12, cause the erythrocyte precursor cell to break down in the bone marrow (ineffective erythropoiesis), resulting in the release of large quantities of LDH. For monitoring purposes, LDH is relevant in predicting disease activity in leukemia, and the survival rate (probability of survival) and duration in Hodgkin disease and non-Hodgkin lymphoma [4]. Finally, in multiple myeloma a Revised International Staging System (RISS) has been developed, which combines markers of tumor burden (albumin, beta-2 microglobulin) with markers of aggressive disease biology (high risk cytogenetics and elevated serum LDH) [5].

In addition to its use in hematological diseases, serum LDH can also be employed when solid tumors are present. Patients with solid malignant disease often show increased LDH activity in serum; up to 70% of patients with liver metastases and 20 to 60% of patients with other non-hepatic metastases (e.g., lymph nodes) have increased LDH activity. Notably elevated LDH is observed in germ cell tumors (≈60% of cases) such as teratoma, seminoma of the testis, and dysgerminoma of the ovary, and, coupled with both α-fetoprotein and human chorionic gonadotropin, LDH is an important serologic marker for these tumors [6]. Elevated LDH levels are the product of enhanced glycolytic activity of the tumor and tumor necrosis due to hypoxia, the latter being associated with high tumor burden. Consequently,

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LDH elevation is harbinger of poor outcome in patients with several types of solid tumors. Recent meta-analyses have convincingly shown the value of LDH measurement in predicting both overall and progression-free survival in renal cell carcinoma, melanoma, lung cancer, osteosarcoma, and metastatic prostate cancer (Table 1).

At the end of 2019, an outbreak of atypical pneumonia of unknown cause was detected in Wuhan, China. The etiological agent of this disease was identified to be a novel coronavirus, named "severe acute respiratory syndrome coronavirus 2" (SARS-CoV-2). The disease caused by SARS-CoV-2, named COVID-19, has since spread worldwide warranting the recognition as a pandemic by the WHO. About 5% of COVID-19 subjects ultimately develop a full-on acute respiratory distress syndrome, requiring admittance to an intensive care unit (ICU) to administer invasive mechanical ventilatory support. Given the elevated risk of death, it is vital to ascertain the patient's risk for poor outcome and many laboratory tests have been proposed to this scope [7]. Among others, there is now convincing evidence linking LDH levels to the severity of COVID-19 disease, reflecting both direct lung damage and more widespread tissue injury [8, 9]. In a recently published study on a large case-series of COVID-19 patients, with multivariate statistical analysis including the majority of debated biomarkers, high serum concentrations of LDH (together with low serum concentrations of albumin) remained significantly associated with higher odds of death [odds ratio (OR), 161.5 (95% confidence interval (CI): 2.28–11,422.8; p=0.019], while low LDH activities were associated with lower odds of ICU admission [OR, 0.06 (95% CI: 0.01–0.54); p=0.011] [10]. The best LDH cut-offs were >731 U/L, associated to a positive predictive value (PV) of 0.84 (95% CI: 0.70–0.93) and a positive likelihood ratio (LR) of 19.7 (95% CI: 9.1–42.7) for death prediction, and <425 U/L, associated with a negative PV of 0.99 (95% CI: 0.97–1.00) and a negative LR of 0.10 (95% CI: 0.03–0.30) for intensive treatment, respectively. One of the major strengths of the published results was represented by the use of an LDH measuring system (Alinity Abbott Diagnostics) which optimal standardization was previously verified and validated [11]. This allows the direct transfer of the selected LDH cut-offs in other settings, providing that the related institutions also use assays that produce standardized results. As two method principles using different substrates (lactate and pyruvate) are still commercially available, there is indeed a real possibility to obtain significantly different results in different laboratories [12]. One of the major concern about the relevance of the LDH results in COVID-19 patients, as reported in the recent literature, appears indeed the lack of the information about the measurement methods that, in the majority of the published papers, are not provided. On several occasions, I previously stressed how the issue of measurement standardization represents an

Table 1: Results of the recently published meta-analyses evaluating the prognostic value of serum lactate dehydrogenase in different solid tumors.

Authors	Reference	Tumor	No. of studies (patients)	Outcome	Results
Shen et al.	PLoS ONE 2016;11:e0166482	Renal cell carcinoma	25 (6,278)	Overall survival	HR = 2.13 (95% CI, 1.69–2.69)
			9 (2,905)	Progression-free survival	HR = 1.74 (95% CI, 1.48–2.04)
Zhang et al.	Int J Surg 2020;79:66-73	Metastatic renal cell carcinoma	27 (6,581)	Overall survival	HR = 2.15 (95% CI, 1.85–2.51)
			9 (2,057)	Progression-free survival	HR = 1.76 (95% CI, 1.49–2.10)
Gao and Ma	Panminerva Med 2017;59:332–7	Melanoma	13 (4,036)	Overall survival	HR = 1.97 (95% CI, 1.62–2.40)
Deng et al.	Medicine 2018;97:38(e12524)	Lung cancer	14 (4,084)	Overall survival	HR = 1.49 (95% CI, 1.38–1.59)
Fu et al.	Medicine 2018;97:19(e0741)	Osteosarcoma	15 (1,938)	Overall survival	HR = 1.87 (95% CI, 1.58–2.20)
			9 (2,585)	Event-free survival	HR = 1.78 (95% CI, 1.51–2.10)
Mori et al.	Clin Genitourin Cancer 2019;17:409-18	Metastatic prostate cancer	43 (11,590)	Overall survival	HR = 2.07 (95% CI, 1.75–2.44)
			12 (2,587)	Progression-free survival	HR = 1.08 (95% CI, 1.01–1.16)

CI, confidence interval; HR, hazard ratio.

absolute priority for optimizing health care [13]. Only the use of assays providing standardized results allows the use of common decision limits, enabling the universal application of results of clinical studies undertaken in different locations or times and permitting their unambiguous interpretation [14]. This is a central concept as comparability of LDH measurements among different measuring systems still has major drawbacks: This is mainly the result of using methods with different analytical selectivity for this enzyme, obtaining results that may not be traceable to the internationally accepted reference measurement system [15]. In this situation, the *in vitro* diagnostics manufacturers can play a central role in paving the road to standardization of LDH measurements by recalibrating biased assays with suitable calibration approaches [16].

Thirty years ago, Morton Schwartz asked if LDH, after years of use as component (together with creatine kinase and aspartate aminotransferase) of the classical enzyme profile employed for the diagnosis of acute myocardial infarction, could be reborn as a cancer marker [17]. The following experience has shown that, for many years, the clinical application of serum LDH measurement has been limited to the evaluation of anemias and to roughly prognostication of certain tumors [1]. In the last few years, significant changes have however happened. First, the test has been confirmed as a robust predictor of poor outcomes in many neoplastic conditions (Table 1). Furthermore, in the RISS adopted in the 2015 by the International Myeloma Working Group, LDH acts as determinant of disease biology in differentiating myeloma stages. Finally, in the last few months, LDH is definitively reborn given its proven significant contribution in defining the COVID-19 severity. This increased clinical role calls for an improvement of LDH assay standardization through the implementation of traceability of results of clinical samples to the internationally recommended reference measurement system [18]. This represents the indispensable prerequisite for the optimal clinical use of the important information provided by the LDH measurement.

Research funding: None declared.

Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Competing interests: Authors state no conflict of interest.

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