Diagnostic Efficiency of Four Lactate Dehydrogenase Isoenzyme-1 Ratios in Serum after Myocardial Infarction

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We compared the diagnostic efficacy of the ratios LD-1/LD-2, LD-1/LD-3, LD-1/LD-4, and LD-1/LD-5 in 69 documented cases of myocardial infarction. We used 149 patients with congestive heart failure and 67 patients with nonmyocardial infarct as controls. We used a computer program to produce receiver-operating characteristic curves, decision threshold plots, and likelihood ratios for these LD ratios at 6-h intervals up to 108 h after the onset of chest pain or hospital admission. All ratios in the myocardial infarction cases peaked around 36 h after the onset of chest pain, while those for the nonmyocardial and congestive cardiac failure cases did not change over the 108-h period. In all patients, LD-1/LD-2 increased by as much as 1.3 times, while LD-1/LD-4 and LD-1/LD-5 increased by 1.7 times (when LD-1 was <40%) and 3.4 times (when LD-1 was >40%), respectively, over control values. Optimum decision threshold values were obtained at 13-24 h (LD-1/LD-5), 31-36 h (LD-1/LD-4 and LD-1/LD-3), and 55-60 h (LD-1/LD-2) after onset of symptoms. The highest likelihood ratio was obtained with the LD-1/LD-4 ratio; therefore, we suggest that this is a better diagnostic test for myocardial infarction than LD-1/LD-2.

Additional Keyphrases: receiver-operating characteristic curves • decision threshold plots • likelihood ratios

Measurement of serum lactate dehydrogenase (lactate:NAD⁺ oxidoreductase; EC 1.1.1.27) isoenzymes—particularly changes in the anodic isoenzymes, LD-1 and LD-2—provides useful diagnostic information in myocardial infarction (MI) (1, 2). Moreover, these changes are often emphasized by also calculating the LD-1/LD-2 ratio (3), a ratio devised by Gambino and Galen (4) in the early 1970s to "capture the attention of potentially indifferent internists." The decision threshold for the LD-1/LD-2 ratio in myocardial infarction is conventionally accepted as 1.0 (1); this value was also adopted in a recent influential review (5). However, lower values, such as 0.76 or 0.74, have been suggested as the consequence of a careful series of determinations in healthy populations (6, 7); more sophisticated evaluation of such data by ROC curve analysis suggests a decision threshold of either 0.94 or 0.92 (8, 9).

Although the LD-1/LD-2 ratio appears to be the most obvious ratio to use in diagnosing myocardial infarction, the diagnostic usefulness of other ratios, such as LD-1/LD-3, LD-1/LD-4, and LD-1/LD-5, has not been investigated. Here, we assess the value of these ratios compared with the more usual LD-1/LD-2 ratio, in documented cases of myocardial infarction. We used a computer program to calculate ROC curves and LR values (9).

Materials and Methods

Samples and patients. We included patients (n = 69) admitted to the Cardiac Care Unit of this hospital with chest pain, in whom a diagnosis of acute myocardial infarction was later confirmed. Blood samples were drawn to determine concentrations of LD and LD isoenzymes [as well as other cardiac-related enzymes—e.g., total creatine kinase (EC 2.7.3.2) and CK-2 (CK-MB)—not included in this study] on admission, and at 4- to 6-h intervals thereafter, up to 108 h. These patients were subsequently divided into two groups based on serum LD-1 values (<40% (n = 29) vs >40% total LD (n = 40)). The anatomical sites of infarction in the group with <40% LD-1 were subendocardial (n = 14) and transmural (n = 15; five each being anterior, anteroseptal, or inferolateral); in the group with >40% LD-1, the sites were subendocardial (n = 8) and transmural (n = 32; eight anterior, one anteroseptal, three anterolateral, four anteroseptal, 14 inferior, one inferolateral, and one posterolateral). As we will show later, and as would be expected, the concentrations of serum enzymes involved suggest that the size of the infarction determines the magnitude of the LD-1.

Control subjects in Group I (non-MI) include patients admitted to this hospital with chest pain, but subsequently found not to have a myocardial infarction (n = 67), whereas those in Group II (congestive heart failure; CHF) include those patients who did not have a myocardial infarction, but who had a clinically established diagnosis of CHF, with LD-5 >16.1% of the total LD in serum during their hospital stay (n = 149).

LD assay. We determined total LD at 37 °C on the Cobas FARA analyzer (Roche Diagnostics, Etobicoke, Ontario, Canada, M9C 5S4), with the Scandinavian-recommended assay (10) with reagents supplied by Boehringer Mannheim Canada, Dorval, Quebec, Canada. Between-batch precision (as CV) was ≤2%.

LD isoenzymes. We separated the LD isoenzymes by electrophoresis on thin-layer agarose (Corning Universal Electrophoresis Film; Corning Medical and Scientific, Palo Alto, CA 94306) and quantified them by scanning with a Cliniscan fluorescence densitometer (Helena Laboratories, Beaumont, TX 77704). Between-batch precision (as CV) was <5% (LD-1 to LD-3) and <10% (LD-4 and LD-5). Upper reference limits for healthy subjects, established by this laboratory for routine application (11), were: total LD, 378 U/L; LD-1, 26%; LD-2, 39%; LD-3, 26%; LD-4, 16%; LD-5, 16%; and LD-1/LD-2 ratio, 0.75.

Test evaluation. We compared the diagnostic efficiency of the ratios LD-1/LD-2, LD-1/LD-3, LD-1/LD-4, and LD-1/LD-5 with a Mumps program (MSM-PC; Micronetics Design Department of Clinical Biochemistry and Medicine, University Hospital (University of Western Ontario), London, Ontario, Canada N6A 5A5. Address correspondence to this author, at: Department of Clinical Biochemistry, University Hospital, P.O. Box 5539, Station A, London, Ontario, Canada N6A 5A5.

*Nonstandard abbreviations: CK, creatine kinase; LD, lactate dehydrogenase; ROC, receiver-operating characteristic; LR, likelihood ratio; MI, myocardial infarction; CHF, congestive heart failure.

Received April 11, 1988; accepted June 6, 1988.

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Corporation, Rockville, MD 20850) installed in a Model 2000 personal computer (Tandy Corp., Fort Worth, TX 76102). This program prints operator-selected decision thresholds and plots ROC and decision level curves (9). The selection of the optimum decision threshold between the positive and negative test results is facilitated with the calculation of LRa (12) and their 95% confidence limits (9). We printed all graphs by using Lotus 1-2-3 (Lotus Development Corp., Cambridge, MA 02142-9984). We used Student's unpaired t-test to compare total CK and CK-2 values between the groups with LD-1 >40% and <40%.

Results

We initially compared the results for the entire MI population with the aggregated control groups. We determined the optimum decision thresholds for each LD-1 ratio by analyzing the data at 6-h intervals by ROC curve and decision level plots (9); these plots also permitted the selection of the optimum time interval for each ratio (Table 1). When we plotted the temporal development of the aggregated LD isoenzyme data for the MI population (as in Figure 1), the LD-1 values were clearly sequestered into two groups—those with LD-1 values above or below 40% of total LD. Accordingly, we conducted further analyses with two MI populations: those with an LD-1 <40% (n = 29) and those with LD-1 >40% (n = 40). Figure 1 illustrates the changes in LD isoenzyme proportions in these populations and in the non-MI and CHF controls over the 108-h period of this study. This subdivision was evidently based on infarct size; peak serum total CK and CK-2 values for these groups were significantly different (P <0.001).

A plot of the LD-1/LD-2, LD-1/LD-3, LD-1/LD-4, and LD-1/LD-5 ratios for the non-MI and CHF controls shows little variation over the 108 h (Figure 2, A and B). In both groups of the patients with MI, the LD-1/LD-2 ratio increased only slightly (1.3 times) compared with the controls. However, the other ratios were markedly increased in the MI groups (Figure 2, C and D). The largest increases were obtained with the LD-1/LD-4 and LD-1/LD-5 ratios; compared with values for the control groups, these ratios showed a mean increase of 1.7 times in the group with <40% LD-1 and 3.4 times in the group with >40% LD-1.

We generated ROC curves for each of the LD-1 ratio values at 6-h intervals over the 108 h of study. From these 18 ROC curves, we selected six to illustrate the minimum and maximum areas, superimposed on the same axis (Figures 3 and 4). For example, in the 0–6-h period the ROC curve area for the LD-1/LD-2 ratio was 0.520 (SE 0.060), which represents a test with the least diagnostic usefulness (Figure 3A). The LD-1/LD-2 ratio in the 55–60-h period generated an ROC curve area of 0.905 (SE 0.053), the largest ROC curve area for this group. LD-1/LD-2 results

![Fig. 1. Mean LD isoenzyme values in serum, plotted at 6-h intervals after admission to hospital](image)

(A) For patients admitted with chest pain, later shown not to have had MI (n = 67), 0 h is the time of onset of chest pain. (B) Patients admitted with congestive heart failure (n = 148). 0 h is the time of hospital admission. (C) Patients with MI in whom the serum LD-1 value never exceeded 40% (n = 29), 0 h is the time of onset of chest pain. (D) Patients with MI in whom the serum LD-1 value exceeded 40% (n = 40), 0 h is the time of onset of chest pain. SD values did not exceed 2.5% for LD-1 in the control groups, or for LD-2 to LD-5 in the MI groups. For <40% and >40% LD-1, SD values were 4.4% and 10.8%, respectively. Individual LD isoenzymes are LD-1 (C), LD-2 (C), LD-3 (B), LD-4 (J), and LD-5 (E).
Fig. 2. Mean LD-1 ratios in serum plotted at 8-h intervals after admission to hospital
Panels A-D described in Fig. 1. Individual ratios are LD-1/LD-2 (C), LD-1/LD-3 (O), LD-1/LD-4 (□), and LD-1/LD-5 (△)

ROC CURVE LD-1/LD-2 (LD-1 < 40%)

ROC CURVE LD-1/LD-2 (LD-1 > 40%)

Fig. 3. ROC curves for the LD-1/LD-2 and LD-1/LD-3 ratios in serum from patients with MI at six time intervals after the onset of chest pain
generated in this latter time frame would, therefore, produce fewer false-positive values and be more diagnostically significant. The optimum time interval for the LD-1/LD-2 ratio was 55–60 h for both groups of MI patients (i.e., <40% or >40% LD-1), but the ROC curve area for >40% LD-1 was greater at 0.975 (SE 0.029), as illustrated in Figure 3, A and B. The ROC curve areas for the LD-1/LD-3 ratio gave a maximum of 0.934 (SE 0.038) and 0.985 (SE 0.017) for <40% and >40% LD-1, respectively, for the 31–36-h time intervals (Figure 3, C and D). For the LD-1/LD-4 ratio, the ROC curve areas were 0.924 (SE 0.045) and 0.994 (SE 0.005) for <40% and >40% LD-1, respectively, at 31–36 h after the onset of chest pain (Figure 4, A and B). The maximum ROC curve areas for the LD-1/LD-5 ratio were 0.919 (SE 0.032) at 13–18 h for <40% LD-1 (Figure 4C), and 0.936 (SE 0.029) at 19–24 h for >40% LD-1 (Figure 4D). In all cases, the ROC curve areas—the proportion of true-positive results—were greater for patients with >40% LD-1.

Based on these ROC curve evaluations, and the corroborating decision level plots (9) (not shown), the optimum time intervals for each LD-1 ratio for the diagnosis of myocardial infarction are listed in Table 2. We conclude that the LD-1/LD-5 ratio optimizes most rapidly, LD-1/LD-3 and LD-1/LD-4 ratios are intermediate, and the LD-1/LD-2 ratio optimizes at the longest time after the onset of chest pain.

The associated likelihood ratios (12) and 95% confidence ranges were also computed for these LD-1 ratios (Table 2). Values above 1.0 become significant positive-predictors of disease: the larger the value, the greater the predictive index.4 Depending on the type of control population used, the two best diagnostic indicators are the LD-1/LD-4 ratio, followed by the LD-1/LD-2 (or sometimes the LD-1/LD-3 ratio); the worst indices are the LD-1/LD-5 and, usually, the LD-1/LD-3 ratios.

The effect of hemolysis (13) on these ratios is shown in Table 3, derived from our previous observations (14). We previously noted that there are two populations of erythrocytes—the majority, which have LD-1 > LD-2, and a group in which LD-1 < LD-2, in which no amount of hemolysis will cause the LD-1/LD-2 ratio to exceed 1.0. In the former group, the LD-1/LD-4 and particularly the LD-1/LD-5 ratios may increase to very high values.

Discussion

An ideal LD ratio is one that, in the presence of disease, shows an increase in the value of the numerator but a concomitant decrease in the value of the denominator, thus amplifying the difference between nondisease and disease. After a myocardial infarction, the relative proportions of

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4 Pre-test odds × likelihood ratio (LR) = post-test odds. When LR is >1.0, the post-test odds (of disease presence) are greater than the pre-test (prior) odds of disease presence.
Table 2. Optimum Values for Various LD-1 Ratios in Myocardial Infarction

<table>
<thead>
<tr>
<th>Test</th>
<th>Optimum time window after infarction, h</th>
<th>Decision threshold</th>
<th>Likelihood ratio</th>
<th>95% confidence interval</th>
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</thead>
<tbody>
<tr>
<td>LD-1 &lt; 40% (n = 29)</td>
<td></td>
<td>55-60</td>
<td>0.90</td>
<td>1.93</td>
</tr>
<tr>
<td>LD-1/LD-2</td>
<td></td>
<td>31-36</td>
<td>1.30</td>
<td>1.26</td>
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<tr>
<td>LD-1/LD-3</td>
<td></td>
<td>31-36</td>
<td>3.00</td>
<td>2.84</td>
</tr>
<tr>
<td>LD-1/LD-4</td>
<td></td>
<td>13-18</td>
<td>3.25</td>
<td>1.45</td>
</tr>
<tr>
<td>LD-1/LD-5</td>
<td></td>
<td>55-60</td>
<td>1.10</td>
<td>2.22</td>
</tr>
<tr>
<td>LD-1/LD-2</td>
<td></td>
<td>31-36</td>
<td>2.00</td>
<td>1.98</td>
</tr>
<tr>
<td>LD-1/LD-3</td>
<td></td>
<td>31-36</td>
<td>4.00</td>
<td>2.99</td>
</tr>
<tr>
<td>LD-1/LD-4</td>
<td></td>
<td>19-24</td>
<td>3.00</td>
<td>1.57</td>
</tr>
</tbody>
</table>

*Same “windows” are optimum for both comparisons.

Table 3. Effects of Hemolysis on Serum LD-1 Ratios

<table>
<thead>
<tr>
<th>Ratio</th>
<th>Control (non-hemolyzed) samples</th>
<th>LD-1/LD-2</th>
<th>LD-1/LD-3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>&lt;1.0</td>
<td>&gt;1.0</td>
</tr>
<tr>
<td>LD-1/LD-2</td>
<td>0.79 ± 0.06 a</td>
<td>0.93 ± 0.03</td>
<td>1.06 ± 0.01</td>
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<tr>
<td>LD-1/LD-3</td>
<td>1.36 ± 0.20 b</td>
<td>1.50 ± 0.23</td>
<td>2.07 ± 0.07</td>
</tr>
<tr>
<td>LD-1/LD-4</td>
<td>2.39 ± 0.55 b</td>
<td>5.23 ± 1.60</td>
<td>12.66 ± 1.79</td>
</tr>
<tr>
<td>LD-1/LD-5</td>
<td>2.20 ± 0.70 b</td>
<td>6.16 ± 2.10</td>
<td>30.78 ± 7.20</td>
</tr>
</tbody>
</table>

*Derived from some of the data in ref. 14.

a Mean ± SD; n = 5; hemoglobin was varied between 1 and 16 g/L.

serum LD-1 and LD-2 increase while those of the nonanodic LD isoenzymes (LD-3, LD-4, and LD-5) decrease (Figure 1, C and D). Therefore, on theoretical grounds alone, any ratio of LD-1 with the nonanodic LD isoenzymes is likely to be superior to the LD-1/LD-2 ratio. This conclusion appears to be borne out by the magnitude of change in these nonanodic ratios after a myocardial infarction (Figure 2, C and D). However, such a conclusion may be modified in clinical practice; e.g., LD-5 may be increased in serum after a myocardial infarction as a result of CHF, causing an increase in the denominator of the LD-1/LD-5 ratio and thus dampening the magnitude of change. Clearly, unusual destruction of platelets, which increases LD-3 in serum, may similarly dampen the change in the LD-1/LD-3 ratio. This emphasizes the crucial importance of exploring the effect of the clinical spectrum on test efficacy (15).

When these clinical perturbations are taken into account—being reflected in the likelihood ratio—the magnitude of the LR values indicates the diagnostic power of each ratio (Table 2). For the four groups of patients investigated, the LD-1/LD-4 ratio was, in each, the most powerful diagnostic test for myocardial infarction, followed by the LD-1/LD-2 ratio or the LD-1/LD-3 ratio, depending on the nature of the control group and the magnitude of the LD-1 increase. The ratio with the poorest diagnostic utility was LD-1/LD-5 (usually) or LD-1/LD-3, again depending on the control group and the magnitude of the LD-1 change.

ROC curve analysis is probably the most satisfactory technique for comparing tests and establishing, for the particular use of the diagnostic test (discovery, exclusion, or confirmation), the optimum decision threshold (16), although there are acknowledged difficulties in preparing and interpreting these curves (17). Several alternatives have been suggested (17-19), but our approach—providing ROC curves, individual and cumulative frequency plots, decision level plots (18) and LR calculations (12) by an integrated microcomputer-based program (9)—appears to answer most of these objections.

Indeed, the use of such a program permits a much more detailed examination of the data. For example, the spectrum (15) of the control population alters, as expected, the optimum decision threshold for the diagnosis of myocardial infarction. This effect can be appreciated by comparing the decision thresholds for LD-1/LD-2 in Tables 1 and 2. Also, the magnitude of the change in disease can also affect these decision thresholds. For example, when there is >40% LD-1, the optimum decision thresholds for each ratio are higher than when there is <40% LD-1 (Table 2). We infer that the infarct sizes of these two groups differ, given the significant differences in their total CK and CK-2 activities in serum (see Results). Evidently, the extent of tissue damage also influences the optimum decision threshold.

We previously showed that the serum LD-1/LD-2 ratio becomes abnormal as rapidly as the serum CK-2 value in many patients with a myocardial infarction (20). However, we did not formally evaluate the time of optimum abnormality after the onset of chest pain. It is therefore surprising that, despite the rapidity of significant change, the LD-1/LD-2 ratio does not reach optimal diagnostic utility until 55 h after the onset of chest pain. We also found it interesting that this ratio test is not the most diagnostically effective ratio to use.

Our evidence unequivocally demonstrates that the best diagnostic LD ratio is LD-1/LD-4. This is because in practice LD-4 in serum decreases proportionally after a myocardial infarction and does not appear to be dampened by associated clinical events (unlike serum LD-3 and LD-5). Compared with the conventionally preferred LD-1/LD-2 ratio, the LD-1/LD-4 ratio optimizes earlier (31 h vs 55 h) and its LR value is ~50% greater. Our experience with patients receiving thrombolytic therapy (21) is so limited that we cannot comment, with any certainty, on the effect of successful reperfusion. However, we suggest that such an effect would further reduce the time required to reach the optimum diagnostic time windows (see Tables 1 and 2).

The LD-1/LD-2 ratio is a surprisingly robust test despite the theoretical objections (outlined earlier) against its use. Presumably this is because no common clinical events perturb this ratio in myocardial infarction except hemolysis (see Table 3) or renal infarction—which, in our experience, are uncommon events in the Cardiac Care Unit. Feldman (22) and Politser et al. (23) noted the advantage of separately calculating the contribution of each organ system to the total LD activity and LD isoenzyme patterns in serum;
however, until these approaches are more generally available, most laboratories will rely on interpretation of LD isoenzyme patterns and the ad hoc calculation of LD ratios that appear to have clinical utility.

We therefore conclude that the use of the serum LD-1/LD-2 ratio as a diagnostic test for myocardial infarction could, with advantage, be replaced or supplemented by the serum LD-1/LD-4 ratio.

We thank Tom Pellar for his help with the various Mumps programs used in this investigation.

References