Time-Related Changes in the Diagnostic Utility of Total Lactate Dehydrogenase, Lactate Dehydrogenase Isoenzyme-1, and Two Lactate Dehydrogenase Isoenzyme-1 Ratios in Serum after Myocardial Infarction

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Using receiver-operating characteristic (ROC) curve and likelihood ratio analysis, we examined the diagnostic utility of total lactate dehydrogenase (LD; EC 1.1.1.27) activity (I), LD isoenzyme-1 activity (II), and the LD-1 percentage of total LD activity (III), LD-1/LD-2 (IV), and LD-1/LD-4 (V) in 347 persons admitted to the Cardiac Care Unit (of whom 173 were subsequently proven to have had myocardial infarction). Blood was sampled from these subjects at about 6-h intervals for up to 96 h from the onset of chest pain. Defining an “effective” test as one having an area under the ROC curve of ≥0.9, we determined the ranked utility (greatest to least) of these tests as V = IV > III > II > I. Tests III, IV, and V had, by this criterion, diagnostic effectiveness equivalent to measurements of creatine kinase-2 in serum but in samples obtained at later time intervals. The decision thresholds for both high (constant) test sensitivity and specificity varied with time, to differing extents, over the entire 96-h period, a finding with important diagnostic implications. We document positive and negative likelihood ratio values for each of these tests throughout the entire period of study.

Additional Keyphrases: receiver-operating characteristic curves · likelihood ratios · creatine kinase compared

Werner et al. (I), using serum enzyme data from a myocardial infarction (MI) population, showed that total lactate dehydrogenase (LD; EC 1.1.1.27) and α-hydroxybutyrate (or 2-hydroxybutyrate) dehydrogenase (no EC number assigned) provide decision (diagnostic) thresholds of constant sensitivity and specificity.4 By contrast, both total creatine kinase (CK; EC 2.7.3.2) and CK-MB (CK-2) showed a decrease in sensitivity for diagnosis of MI in the three days after the onset of pain. We have confirmed and extended this latter observation (2),6 first documented by Van Steirteghem et al. (3).

However, when we examined our findings based on measurements made at more frequent time intervals in a larger MI population than that used by Werner et al., we obtained a quite different set of patterns for the decision thresholds of total LD, LD-1 (both as U/L and percentage of total LD activity), LD-1/LD-2, and LD-1/LD-4. We include this latter LD ratio as a diagnostic marker for MI, because we have previously shown that it was superior to the more conventional LD-1/LD-2 ratio (4).

Therefore, in the present study, we document the changing temporal diagnostic utility of these various LD indices used for the diagnosis of MI. We have used receiver-operating characteristic (ROC) curve and likelihood ratio analyses (5, 6) on data obtained from blood collected over a 96-h period from the onset of chest pain in a cardiac-care-unit population. We compared the diagnostic efficacy of these indices on the basis of the area under the ROC curve and the positive and negative likelihood ratios for positive and negative test results, respectively. We show that both the diagnostic power and the decision thresholds of each of these tests change markedly with time after infarct. The implications of these findings have not been acknowledged sufficiently in the medical literature.

Materials and Methods

Samples and Patients: Our study group consisted of 347 patients, admitted to the Cardiac Care Unit of this hospital with chest pain (Table 1), in whom a diagnosis of MI was either later confirmed (n = 173) or excluded (n = 174). These diagnoses were established by one of us (G.J.), using our previously described criteria (7). Blood samples were collected for the latest 96-h period between the onset of chest pain and the visit of the diagnostic team. The study group consisted of 208 men (59.9%) and 139 women (40.1%), whose age range was 26 to 90 years (mean ± SD 61 ± 14.6 years). The MI diagnosis was based on clinical, electrocardiographic, and laboratory findings and was confirmed by myocardial enzyme analysis. Of the MI patients, 91 (53.3%) had a single-vessel disease, 34 (19.8%) had two-vessel disease, and 48 (27.7%) had three-vessel disease. In the non-MI group, 80 (46.1%) had a single-vessel disease, 61 (35.1%) had two-vessel disease, and 32 (18.9%) had three-vessel disease. Of the MI patients, 116 individuals (66.7%) had a non-ST elevation MI (NSTEMI), and 58 individuals (33.3%) had a ST elevation MI (STEMI). Of the non-MI group, 64 individuals (37.7%) had a non-ST elevation MI (NSTEMI), and 75 individuals (43.7%) had a ST elevation MI (STEMI).

Table 1. Population Studied

<table>
<thead>
<tr>
<th>Non-myocardial infarction (n = 174)</th>
<th>Women, n (and %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y Range</td>
<td>Average</td>
</tr>
<tr>
<td>31–88</td>
<td>61</td>
</tr>
<tr>
<td>Myocardial infarction (n = 173)</td>
<td></td>
</tr>
<tr>
<td>Age, y Range</td>
<td>Average</td>
</tr>
<tr>
<td>25–90</td>
<td>60</td>
</tr>
<tr>
<td>Transmural infarction</td>
<td></td>
</tr>
<tr>
<td>Anterior</td>
<td>25 (20)b</td>
</tr>
<tr>
<td>Anterolateral</td>
<td>4</td>
</tr>
<tr>
<td>Anteroseptal</td>
<td>13 (10.4)b</td>
</tr>
<tr>
<td>Inferior</td>
<td>36 (28.8)b</td>
</tr>
<tr>
<td>Sub-endocardial infarction</td>
<td>36 (28.8)b</td>
</tr>
</tbody>
</table>

* Percentage of total.  b Percentage of male or female totals.

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drawn to determine concentrations of total LD and LD isoenzymes (as well as other analytes) at the time of admission and at 4- to 6-h intervals thereafter for as long as 96 h, although not all patients could be sampled at this frequency during their entire hospital stay. In all cases, sampling time is referred to the time of onset of symptoms.

**LD assay.** We determined total LD at 37 °C on the Cobas FARA analyzer (Roche Diagnostics, Etobicoke, Ontario, Canada M9C 5S4), using the Scandinavian-recommended assay (9) and reagents supplied by Boehringer Mannheim Canada, Dorval, Quebec, Canada. Between-run precision (as CV) was ≤2%.

**LD isoenzymes:** We separated the LD isoenzymes by electrophoresis on thin-layer agarose (Corning Universal Electrophoresis Film; Ciba Corning Diagnostics Corp., Palo Alto, CA 94306) and quantified them by scanning with a fluorescence densitometer (Cliniscan; Helena Laboratories, Beaumont, TX 77707). Between-run 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 (9), were as follows: total LD, 378 U/L; LD-1, 26% and 98 U/L; LD-2, 39%; LD-3, 26%; LD-4, 16%; LD-5, 16%; LD-1/LD-2 ratio, 0.75; and LD-1/LD-4 ratio, 2.0 (4).

**Test evaluation:** ROC curves, decision threshold curves, and likelihood ratios were produced as previously described (5, 6).

**Results**

**ROC curves:** Many of these curves are superimposed on each other at adjoining time intervals; we have therefore shown only data that differ between one time interval and another. These are shown for serum total LD activity (Figure 1, A and B), LD-1 expressed as U/L (Figure 1, C and D) or as % of total LD activity (Figure 1, E and F), LD-1/LD-2 (Figure 1, G and H), and LD-1/LD-4 (Figure 1, I and J).

**Decision thresholds:** The decision thresholds (cutoffs) for constant sensitivities (to "rule out" MI when the test result is less than the cutoff) and specificities (to "rule in" MI when the test result exceeds the cutoff) of 90%, 95%, and 99% are shown for each time interval for total LD (Figure 2, A and B), LD-1 expressed as U/L (Figure 2, C and D) or as % of total LD (Figure 2, E and F), LD-1/LD-2 (Figure 2, G and H), and LD-1/LD-4 (Figure 2, I and J).

**Likelihood ratios:** The positive and negative likelihood ratios* are illustrated for total LD activity (Figure 3, A and B), LD-1 expressed as U/L (Figure 3, C and D), or as % of total LD (Figure 3, E and F), LD-1/LD-2 (Figure 3, G and H), and LD-1/LD-4 (Figure 3, I and J).

**Discussion**

**Total LD activity**

The selected ROC curves (Figure 1, A and B) show that, as for total CK (2) and aspartate aminotransferase (EC 2.6.1.1.; AST) (6), there is no diagnostic value in the use of the total LD test result within the first 6 h after the onset of chest pain. Thereafter, until 48 h after the infarction, total LD performs less well than either total CK (2) or AST (6) if the area under the curve (AUC) is used to assess diagnostic utility. Total LD activity determinations during this period are thus of value only for adjusting the amount of sample to place on an LD isoenzyme electrophoresis plate, or, for calculating the activity concentration (U/L value) for LD-1. However, for the periods 19–24, 37–48, and 72–90 h after the onset of chest pain, total LD is diagnostically useful, with AUC values of about 0.9; these data

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7 The likelihood ratio for a positive or "abnormal" test result (positive LR) equals sensitivity/(1 – specificity), or true-positive rate/false-positive rate. A positive LR is the multiplier applied to the pre-test odds to calculate the post-test odds of disease; it should, therefore, be much greater than 1.

The likelihood ratio for a negative or "normal" test result (negative LR) equals (1 – sensitivity)/specificity, or false-negative rate/true-negative rate. A negative LR is the multiplier applied to the pre-test odds to calculate the post-test odds of nondisease; it should, therefore, be much less than 1.

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Fig. 1. ROC curves for total lactate dehydrogenase activity (A and B), LD-1 activity (C and D), LD-1 (%) (E and F), LD-1/LD-2 (G and H), and LD-1/LD-4 (I and J) in myocardial infarction, at selected intervals after infarction.

The meaning of the symbols is provided for each set of four intervals; note that the same symbol may represent different time intervals for each enzyme.
Fig. 2. Decision thresholds at 6-h intervals up to 95 h after the onset of a myocardial infarction for test sensitivities and specificities of 90% (○), 95% (△), and 99% (□).

Total lactate dehydrogenase activity (A and B), LD-1 activity (C and D), LD-1 (%) (E and F), LD-1/LD-2 (G and H), LD-1/LD-4 (I and J). The first box of each enzymes set shows test sensitivity; the second box, test specificity.
Fig. 3. Likelihood ratios calculated at 6-h time intervals up to 95 h after the onset of a myocardial infarction for test sensitivities and specificities of 90%, 95%, and 99%.

Total lactate dehydrogenase activity (A and B), LD-1 activity (C and D), LD-1 (%) (E and F), LD-1/LD-2 (G and H), and LD-1/LD-4 (I and J). The first box of each enzymes set shows the likelihood ratio for a positive test result; the second box, the likelihood ratio for a negative test result. Symbols as in Fig. 2.
merely confirm the well-known usefulness of LD during these time intervals (10, 11). For comparison, total CK, CK-2 (U/L), and total AST have maximal AUC values of 0.97, 0.99, and 0.96, respectively, at 19–24 h after an MI (2, 6). Note, however, that for the period 91–96 h (Figure 1B), the AUC for total LD drops to 0.75, which clearly limits its diagnostic value at that long of a time after infarction.

The decision thresholds increase in magnitude with time after infarction for all test sensitivities (Figure 2A), but tend to plateau during the second 24-h period after the onset of chest pain. Therefore, if a constant decision threshold is used—as is conventional—the test sensitivity will actually increase with time; i.e., in Figure 2A, the same decision threshold that gives a 90% sensitivity at the 0–6-h time interval gives a 99% sensitivity at the 13–18-h interval. Our more extensive observations, therefore, do not support the findings of Werner et al. (1), who described a constant test sensitivity for total LD activity. What are the implications of our analysis? The application of a high test sensitivity provides a rule-out threshold for MI when enzyme activity is below the threshold. Therefore, with a constant decision threshold, the rule-out criterion becomes more stringent with time after infarction.

The decision thresholds tend to be more stable with time for the test specificities of 90%, 95%, and 99% after the first 24 h (Figure 2B). Thus, a constant decision threshold will give a more stable rule-in cutoff where enzyme activities above that value are diagnostic of MI.

As would be expected from the foregoing, the likelihood ratios for a positive test result indicate that total LD activity has minimal diagnostic power. For example, the maximal likelihood ratios for a positive test result for total CK, CK-2, and total AST activities were 8, 90, and 12, respectively (2, 6), compared with a maximum of five for total LD activity. Note, however, that the higher the test sensitivity, the lower the magnitude of the likelihood ratio (Figure 3A). The maximal likelihood ratio for a negative test result depends, as expected, on the chosen specificity; the most useful period for such a result is during the second 24 h. However, a negative test result is of no value at a 95% specificity (i.e., the likelihood ratio remains constant at 1.0).

LD-1

**LD-1 activity:** The selected ROC curves (Figure 1, C and D) again show a pattern very similar to that for total LD activity except that the test performance of LD-1 (as judged by the AUC value) is clearly superior to total LD activity. Maximal diagnostic performance occurs during the 19–24-h period (AUC = 0.95); such a value is similar to that for total CK and AST (2, 6), but inferior to that obtained with CK-2 activity (2).

The decision thresholds for constant test sensitivity and specificity (Figure 2, C and D) show a similar pattern to those found for total LD activity; again, we were unable to confirm the claim of Werner et al. (1) that the use of a-hydroxybutyrase (approximately equivalent to the assay of LD-1) provides decision thresholds of constant sensitivity. The diagnostic value (Figure 3C) of a positive test result exceeds that of total LD activity, particularly at 60 h after the onset of chest pain. The pattern of likelihood ratios for negative test results (Figure 3D) is almost identical to that for total LD activity.

Bruns et al. (12) recommended the LD-1 (%) assay after comparing it with the LD-1 (U/L) assay, whereas Gerhardt et al. (13) and Rotenberg et al. (14) found the reverse. We believe such contradictory conclusions are due, in part, to a lack of appreciation of the changing decision thresholds with time, which we have demonstrated.

**LD-1 percentage:** The ROC curves (Figure 1, E and F) indicate a superior diagnostic performance to total LD and LD-1 (U/L), particularly after the first 24 h after the MI. Indeed, during the 67–72 h after infarction, this test has a diagnostic power (AUC = 0.99) equivalent to that of CK-2 (U/L) (2).

The decision thresholds are also slightly different from those for total LD and LD-1 activities (Figure 2E), the plots for LD-1 being flatter. This means, for example, that the rule-out criteria (using a test with high sensitivity) do not change so much with time. Notice also that the curves for the rule-in criteria (Figure 2F) are flatter, except for the line for 95% test specificity, which changes abruptly at the 45–48-h interval.

The LR values for a positive test result (Figure 3E) indicate that LD-1 (%) is, in general, a more useful test than is LD-1 (U/L). Likewise, a negative test result (Figure 3F) is consistently more useful (at 90% and 95% specificity during the first 48 h after infarction) than are similar negative values for total LD or LD-1 (U/L).

Isoenzyme Ratios

**LD-1/LD-2 ratio:** The ROC curves (Figure 1, G and H) indicate that this test is of diagnostic value from 13 h onwards after the onset of chest pain, as the AUC values are then >0.9. Indeed, this ratio has a diagnostic power (AUC = 0.99) at the 43–48-h period equivalent to that of CK-2 (U/L) (2). Also, this test retained an AUC in excess of 0.9 up to 96 h after the onset of chest pain.

The decision thresholds for constant sensitivity (Figure 2G) appear to plateau after 37 h from the onset of chest pain; this means that the criteria for ruling out an MI remain reasonably constant thereafter. However, up until this time, the criteria for ruling out an MI become more stringent with time, as with the other tests discussed above. The decision thresholds for ruling in MI (Figure 2H) are nearly constant for specificities of 90% and 99%, but the 95% line shows a sudden sharp increase at about 37 h after infarction.

The diagnostic power of this test is shown in Figure 3, G and H. The likelihood ratio for a positive test result, at a sensitivity of 99%, is about 1 to 2. The decision threshold for such a test is between 0.5 and 0.7 (Figure 2G). If the sensitivity is reduced to 95%, the likelihood ratios vary between 2 and 22, depending on the time after infarction. Rather similar results are also obtained for a test sensitivity of 90%. Overall, the utility of this test is very similar to that of LD-1 (%) (Figure 3E). Likewise, the value of a negative test result may be useful if the chosen specificity is 95% (only until about 30 h after the infarct) or 90%, but it is clearly of no value at all if the test specificity is 99% (Figure 3F).

**LD-1/LD-4 ratio:** We previously examined the characteristics of this ratio by ROC curve analysis and suggested that it performed as well as the more conventional

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8 S. B. Rosalki has pointed out that we wrongly ascribed (in 4) the first use of this ratio to Gambino and Galen. In fact, the ratio was first reported by Cohen and others in 1964 (see 10), but Gambino and Galen were later instrumental in popularizing its use.
Table 2. Summary of Findings

<table>
<thead>
<tr>
<th>Test</th>
<th>Maximal area under the ROC curve</th>
<th>Periods (h after infarction) for which AUC is &gt;0.9, h</th>
<th>Maximal likelihood ratio for a positive test result</th>
<th>Minimal likelihood ratio for a negative test result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total LD</td>
<td></td>
<td>19–24, 31–36</td>
<td>19–24, 37–48</td>
<td>85–90, 49–54</td>
</tr>
<tr>
<td>LD-1(%)</td>
<td>67–72</td>
<td>13–96</td>
<td>19–24, 20.04</td>
<td>31–36, 0.02</td>
</tr>
<tr>
<td>LD-1/LD-2</td>
<td>67–72</td>
<td>13–96</td>
<td>31–36, 22.76</td>
<td>67–72, 0.01</td>
</tr>
<tr>
<td>LD-1/LD-4</td>
<td>55–60</td>
<td>13–96</td>
<td>31–36, 22.76</td>
<td>55–60, 0.01</td>
</tr>
</tbody>
</table>

* Test sensitivity of 90%.  Test sensitivity of 95%.  Test specificity of 90%.

LD-1/LD-2 ratio (4). In the present paper, we have carried this analysis a little further. In general, however, the comments regarding the performance of the LD-1/LD-2 ratio also apply here (Figures 1 and 2, and 3, I and J). Again, the very high performance of the ratio during the time 55–60 h after the infarction (AUC = 0.986) makes it equivalent, in diagnostic power, to the CK-2 assay (2), although at a later time after the infarction.

It is generally recognized that serum CK-2 (and, to a lesser extent, total CK) are the benchmark tests for the diagnosis of MI. Indeed, we have recently provided further detailed evidence for this assumption (2). However, we do not believe that a similarly detailed assessment has been made of the diagnostic value of the LD indices.

Our assessment of the value of the various LD indices in the diagnosis of MI is quite complex; a rapid overview of the main findings is summarized in Table 2. In the present study we document the diagnostic utility of these LD indices, and we show that:

- the diagnostic power of total LD, LD-1 (U/L), LD-1 (%), LD-1/LD-2, and LD-1/LD-4 (in terms of the relationship between true-positive and false-positive results) in serum yields areas under the ROC curve exceeding 0.9, particularly later than 24 h after MI.
- the diagnostic power of LD-1 (%), LD-1/LD-2, and LD-1/LD-4 (as defined above) is equivalent to that of serum CK-2 but at intervals of 67–72, 43–48, and 55–60 h, respectively, after infarction.
- the decision thresholds required to apply constant test performance (i.e., test sensitivity or specificity) change with time after MI.

References