Thin-Layer Agarose Electrophoresis of Lactate Dehydrogenase Isoenzymes in Serum: A Note on the Method of Reporting and on the Lactate Dehydrogenase Isoenzyme-1/Isoenzyme-2 Ratio in Acute Myocardial Infarction

Fred Y. Leung and Arthur R. Henderson

We assessed the clinical efficacy of a thin-layer agarose electrophoresis assay for lactate dehydrogenase isoenzyme estimation in the diagnosis of acute myocardial infarction. From a population of 228 patients admitted to the Coronary Care Unit with suspected acute myocardial infarction, all 101 positive cases (confirmed by clinical presentation and electrocardiographic changes) were correctly identified with lactate dehydrogenase isoenzyme-1 percentage of total activity above the reference range and with a lactate dehydrogenase isoenzyme-1/i isoenzyme-2 ratio of 0.76 or above. This ratio was between 0.45 and 0.74 for 250 healthy subjects. No falsely negative, but 12 falsely positive results were obtained from this Coronary Care Unit population, to give a sensitivity of 100% and a specificity of 90.5% for the ratio test.

Estimation of the serum isoenzymes of lactate dehydrogenase (EC 1.1.1.27; LD) in combination with creatine kinase (EC 2.7.3.2; CK) has become a well-established laboratory procedure for helping in the diagnosis of acute myocardial infarction (1). Because of methodological and (or) normal-value differences in many laboratories, the expression and interpretation of LD isoenzyme values have not been well defined (2, 3).

Possible misinterpretation of LD isoenzyme results when they are expressed in percentage of total activity rather than in international (IUB) enzyme units (U/L) has been suggested (4–6). An LD-1/LD-2 ratio greater than 1.0 (the classical "flipped" LD-1 pattern) is said to be absent in 20% of myocardial infarction cases, sometimes because of delay in obtaining a blood sample (7) or method differences which lower this critical ratio (8).

In this laboratory we use a thin-layer agarose electrophoresis assay (9) for LD isoenzyme estimation, which accurately measures the LD isoenzymes (10). This study was therefore directed to determining the clinical efficacy of this method, the reliability of the LD-1/LD-2 ratio, and the optimal method of expressing LD isoenzyme values in the diagnosis of myocardial infarction.

Materials and Methods

"Cardiac serum enzyme profiles," which include total and isoenzyme determinations for CK and LD, were prepared for all patients admitted to the Coronary Care Unit in University Hospital from February 1977 to June 1978. In this group of 228 patients, 101 had a diagnosis of acute myocardial infarction, confirmed by a history of acute onset of chest pain and by positive electrocardiographic changes.

Creatine kinase isoenzymes were determined electrophoretically with a CK isoenzyme reagent kit (Beckman Instruments Inc., Fullerton, CA 92634).

Lactate dehydrogenase isoenzyme analysis was performed by gel electrophoresis, with use of universal agarose film (Corning/ACI, Medfield, MA 02052) in a barbital buffer (50 mmol/L, pH 8.6). The fractions were localized by virtue of the conversion of NAD+ to NADH and the resulting fluorescence was scanned with a densitometer (Densicomp; Clifford Instruments Inc., Natick, MA 01760) at 405 nm (9, 10).

Total serum LD activity was determined by a modification of the Henry assay (11, 12).

Results and Discussion

The reliability of the electrophoresis methods was routinely assessed by daily analysis of lyophilized human-serum-based controls. The mean values (n = 25) plus or minus one standard deviation for CK isoenzymes in an Ortho control serum (lot no. 7R070) were 95.4 ± 11.4 U/L for CK-1, 194.4 ± 12 U/L for CK-2, and 310.2 ± 18.6 U/L for CK-3, with CV's of 11.9, 6.3, and 6.0%, respectively. LD isoenzyme electrophoresis on agarose gel was monitored routinely with Dade (lot no. EC-111) electrophoresis control. Mean values (n = 30) and standard deviation for LD isoenzymes 1, 2, and 5 were 21.7 ± 1.3% for LD-1, 35.1 ± 1.3% for LD-2, and 12.1 ± 1.3% for LD-5, with corresponding CV's of 6.1, 3.6, and 10.6%, respectively.

Normal reference ranges were established for serum CK and LD isoenzymes from data on 250 apparently healthy subjects. The upper reference limits for CK-1, CK-2, and CK-3 are 0, 4.3, and 11 U/L, respectively, with an upper limit of 135 U/L for total CK activity. These subjects' serum LD-1, LD-2, and LD-5 ranges (and percentages of total activity) were 35–75.6 U/L (14–26%), 55–113 U/L (29–39%), and 12–42.4 U/L (6–16%), respectively. In the group of 101 patients with a confirmed diagnosis of acute myocardial infarction, the CK-MB
values ranged between 8 and 954 U/L when assayed within the first 24 h after an episode of myocardial infarction.

A comparison of unit expression for LD-1 and LD-2 isoenzymes for these patients is shown in Figure 1. Total LD activities all exceeded the upper reference range of 320 U/L (331 to 3405 U/L). Corresponding results for LD-1 in the patients ranged from 109.7 to 1859 U/L, or 26.3 to 82.4% of the total activity. Both unit expressions gave 100% detection of the myocardial infarction cases with no falsely negative results.

The difference between the present findings and those of both Schneider’s and Konttinen’s groups (4, 5) is clearly due to the anodic bias of their techniques. The former group had an upper reference limit for serum LD-1 and LD-5 of 51% and 8%, respectively. Konttinen’s limits were 99 U/L (58%) and 6 U/L (4%) for LD-1 and LD-5, respectively. Our technique, which shows no anodic bias (10), gives values of 75.6 U/L (26%) and 42.4 U/L (16%) for LD-1 and LD-5, respectively.

The 101 true-positive cases in this study have LD-1/LD-2 ratios between 0.79 and 2.01 (Figure 1). The statistical mean of 1.22 and range of 0.76–1.88 for this ratio in the myocardial infarction patients are shown in Table 1. From data on 250 healthy subjects, the range is between 0.45 and 0.74. In these two groups the cut-off point for the diagnosis of infarction is 0.75. Values of 0.76 or above confirm the findings of previous workers (8, 13) for positive cases of myocardial infarction. Similar findings are given in Table 1, along with variation in the ranges between laboratories due to methodological and referent value differences. It should be noted that the critical ratio of 1.0 for LD-1/LD-2 did not always apply in this study and is similar to several reports (8, 14). In the group of 101 positive cases, 12 had a ratio below 1.0 and thus did not show the classical “flipped” LD pattern. All these cases with LD-1/LD-2 below 1.0 presented with an above-normal serum CK-2 and positive electrocardiographic changes. Therefore the absence of a “flipped” LD with CK-2 elevation does not exclude a diagnosis of myocardial infarction if the LD-1/LD-2 ratio is within the positive range established for the method being used.

It is important at this stage to stress other causes of an increase in serum LD-1 activity. Many are listed in standard texts, but in our experience by far the most important cause of false positives is hemolysis. The diagnostic hazards of angina from the anemia of megaloblastic anemia and hemolysis attributable to prosthetic heart valves are, by now, well known. However, the exercise of informed clinical judgment can considerably reduce this cause of false positives.

Since all our cases of myocardial infarction had a positive LD-1/LD-2 ratio, the test has a sensitivity of 100%. The specificity of 90.5% is similar to reported studies (8). In the Coronary Care Unit population studied (prevalence = 44%) the predictive value of a negative result is 100%, the predictive value of a positive result is 89.4%, and the efficiency of the test is 94.7%. In comparison, the CK-2 isoenzyme in this study has a similar sensitivity of 100%, but a slightly higher specificity and efficiency of 96.0 and 97.8%, respectively.

It is of interest that our results almost exactly duplicate those of Mercer’s group (8, 13), which were obtained by a rapid column method. We believe, however, that our procedure has the advantage of measuring the individual LD isoenzymes, although it must be accepted that for the investigation of anodic LD isoenzyme changes, the Mercer technique has the considerable benefit of rapidity coupled with analytical accuracy.

In conclusion, therefore, we have demonstrated the performance characteristics, for the diagnosis of myocardial infarction, of an accurate analytical method for LD isoenzyme

Table 1. LD-1/LD-2 Isoenzyme Ratio

<table>
<thead>
<tr>
<th>Ref.</th>
<th>No.</th>
<th>Normal subjects Range</th>
<th>Mean</th>
<th>Myocardial infarction patients No. Range</th>
<th>Mean</th>
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<tr>
<td>15</td>
<td>11</td>
<td>0.31–0.77</td>
<td>0.49</td>
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<tr>
<td>16</td>
<td>20</td>
<td>0.80–1.16</td>
<td>0.97</td>
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<td>17</td>
<td>51</td>
<td>0.49–0.98</td>
<td>0.70</td>
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<td>18</td>
<td>20</td>
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<td>100</td>
<td>0.30–0.62</td>
<td>0.46</td>
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<td>8</td>
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<td>0.51</td>
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<td>250</td>
<td>0.45–0.74</td>
<td>0.59</td>
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<tr>
<td>a</td>
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<td>b</td>
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</table>

* Not stated. b Present study.

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Fig. 1. Comparison of unit expressions for LD-1 and LD-2 isoenzymes of patients with myocardial infarction. Reference ranges are shown by shaded zones.
quantitation as regards the clinical efficacy of the method, the reliability of the LD-1/LD-2 ratio determination, and the method of expressing isoenzyme values.

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References