Lactate Dehydrogenase Isoenzyme-1/Total Ratio: Accurate for Determining the Existence of Myocardial Infarction

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We have gradually revised our medical protocols for measuring creatine kinase MB isoenzyme (CK-MB) and lactate dehydrogenase isoenzyme-1 (LD-1) because of identifiable problems in the use of an interpretation of CK-MB isoenzyme associated with slowly evolving or small myocardial infarcts. The use of thrombolytic therapy, or burn and trauma, each of which affects the rate of appearance and composition of isoenzymes present. Despite recent evidence of the efficacy of LD-1 isoenzyme measurement in the first 12 to 24 h of myocardial infarction, this test is not widely used because of overstated assumptions about the value of CK-MB. Here we studied the adequacy of the current isoenzyme assays by determining the value of CK-MB and LD-1 at optimum serum sampling times and establishing the contribution of individual and combined predictors to diagnostic efficiency. We conclude that the LD-1/total LD activity ratio in serum is superior to measurement of CK-MB or LD-1, or both, in the diagnosis of acute myocardial infarction. Moreover, this ratio is most valuable when interpretation of the result for CK-MB isoenzyme is equivocal in patients with small or evolving myocardial infarcts.

Additional Keyphrases: assay of CK-MB, LD-1, or both vs LD-1/total LD ratio • the small or evolving infarct • factors complicating interpretation

The concentration of the creatine kinase (EC 2.7.3.2) CK-MB isoenzyme in serum should be serially determined at 6-h intervals and the lactate dehydrogenase (EC 1.1.1.27) isoenzyme 1 (LD-1) can be assayed 6 h after the CK-MB concentration is greatest (1), with adjustment of the frequency of collection to special clinical requirements. This approach is a formalization of the conclusions of several investigations as to the best sampling times for assay (2-6) of CK-MB and LD-1. There is now substantial agreement that LD-1 increases within 12 to 24 h of the onset of infarction (3), becoming increased before the total LD activity reaches a peak at 48 h (7, 8), but there is no consensus as to the earliest time that LD-1 should be measured in serum. Moreover, there is even disagreement about whether this test is needed, based on recent teachings (9) and observed practice patterns.

Explanations for lack of agreement about the value of assay of LD-1 isoenzyme are not readily available. Jablonsky et al. (10) presented evidence that interest in such assay has declined because the accuracy of LD isoenzyme methods is not established. Further, they demonstrated that the LD-1/LD-2 ratio is increased in serum as early as CK-MB is in many patients with infarction.

In this study we have attempted to clarify the current confusion about the accuracy of isoenzyme tests used for assessing the existence and severity of myocardial infarction by addressing the issues most closely related to the medical use of the CK-MB and LD-1 isoenzymes in clinical practice.

The use of CK-MB has become popular, but interest in assay of LD-1 has declined for reasons unrelated to diagnostic accuracy, despite the objections of pathologists and clinical chemists, because of the large body of investigative research (11) attempting to define the relationship between serum CK-MB isoenzyme concentration and heart muscle damage, to determine the extent of myocardial damage associated with infarction, and to classify patients into groups with different risk of short-term and long-term mortality after infarction.

The demonstration of the diagnostic performance of CK-MB and LD-1 assay has misrepresented the actual performance of either method in a clinical setting, because of the numerous studies that are not suitably designed to measure their actual performance, owing to inappropriate sample size and (or) composition of the study populations and owing to grouping together of patients with increased values for serum enzymes but who are unrelated in the temporal relationship of changes in their serum enzyme concentration to the progression of infarction. Further, the electrophoretic cutoff values commonly establish a LD-1/LD-2 ratio of 1 at the 50th percentile of the disease population, but CK-MB amounting to 3% of total CK activity is at the 25th percentile. This practice continues and fuels confusion about the accuracy of these tests despite a recent article elucidating common errors in published method evaluations (12) because of inaccurate measurement of true error rates.

Our use here of a linear, stepwise discriminant analysis in which the candidate variables are introduced one at a time proved to be an elegant method for evaluating these tests after selection of an optimum sampling time that eliminates problems introduced by conjecture as to changes in the concentrations of isoenzymes in serum as related to the infarction process. By this method we were able to rank order individual tests and combinations of tests, to determine their relative diagnostic efficiencies as they would be used in a clinical setting. However, such a study does not resolve the current controversy about the need for subjective assessment of the patient by using the triad of chest pain, electrocardiographic changes, and CK and LD isoenzymes because of the lack of a reasonable "gold standard" such as multiple pooled gaited angiography that provides an objective measure with which all other test results can be compared.

The common clinical use of assay of CK-MB and the corresponding disregard for LD-1 assay are not based on the diagnostic efficiencies of these tests. Indeed, Roberts (13) and Wagner (9) have expressed dissatisfaction with the assay for quantifying the activity of individual LD isoenzymes in demonstrating that the LD-1 activity exceeds that

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1 Nonstandard abbreviations: CK-MB, isoenzyme of creatine kinase; LD, lactate dehydrogenase; AMI, acute myocardial infarction.

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variation just LD-1, because Together, thrombolytic increasingly methods clinical suboptimal. Consequently, this practice were changed, the alternative would have to be demonstrably more effective in the evaluation of cardiac damage. With respect to the second observation, LD-1 was known to be increased in all infarct patients within 24 h, even before the study of Bruns et al. (3), because it had been demonstrated that the LD-1 activity exceeds the LD-2 activity in patients with acute myocardial infarct by 3 to 5 h after CK-MB increase has reached a maximum (16), usually by the 12th to 16th hour after admission. But the studies of Henderson and associates (10, 15) have drawn attention to the fact that use of an LD-1/LD-2 ratio exceeding 1 as a reference value is inappropriate and that LD-1 increases just as early as MB does. On the other hand, Werner et al. (17) attempted to demonstrate that diagnostic efficiency is achieved by carrying out assays of CK-MB and hydroxybutyrate dehydrogenase (HBD) on each of two consecutive days, minimizing the importance of the method of LD-1 assay and the importance of the time of obtaining of serum samples (7, 5) in relation to the appearance and clearance rates for the increased CK-MB or LD-1 activity. Consequently, there has been a lack of consensus about the earliest time at which to sample serum for LD-1 determination, based upon agreement about an abnormal reference value that has little value. The unrealistic expectations as to the value of CK-MB assay, in contrast to the lack of interest in LD-1 assay, is increasingly evident when one considers deviations in CK-MB disappearance kinetics found in patients administered thrombolytic therapy (19), patients with clinically mild or slowly evolving infarct and borderline CK-MB elevation (20), or patients with massive burns and blunt trauma (21). Together, these studies tend to suggest that it is important to make serial determinations of the CK-MB and LD-1, because if the laboratory does not have control of the time of specimen collection the value of these determinations can be suboptimal. Even in some of the best studies of optimization of these assays, their specificity and efficiency in the clinical environment have not been established because of the small size of the study population (2, 3, 17) or because their skewed population distribution does not mirror a typical clinical infarct population with increased serum enzymes (20). Either of these flaws can result in inaccurate conclusions about the diagnostic performance of the individual methods (12).

The above studies constitute several significant attempts to define the efficacy of laboratory assays for CK-MB and LD-1, but there is a continuing lack of consensus and variation in practices because the true advantage of any given approach to these problems in cardiovascular management has not been demonstrated. This situation is also reflected in a survey that found electrophoresis being used for measuring CK-MB isoenzyme (22) in 74% of laboratories, even though it is known that serious error occurs in quantification by electrophoresis when there is a large difference in the concentrations of components (20), a more serious problem in measurement of the CK-MB isoenzyme than in measurement of LD isoenzymes (13). We recognize the problems encountered because of a standard cutoff of LD-1 activity exceeding LD-2 activity for reference to LD isoenzyme analysis (15) not acknowledged by Lott (23).

The above concerns led us to continue our search for the above-mentioned alternative approaches, using a combination of isoenzyme assays measured at optimum sampling times. We have attempted to confirm whether or not a patient has infarct by considering various observations, including the combined measurements of a test panel. We have re-examined whether measurement of LD-1 may be important in the evaluation of small or gradually evolving infarct (20) when concentration changes in measured serial CK-MB assays are not clear. We have also compared the value of measuring LD-1 activity with that of evaluating the ratio of LD-1 to total LD activity in serum (3, 5), particularly with respect to the effect of optimized sampling time on the quality of the patient's diagnosis. Using these test variables, we constructed functions to determine the efficiency of classifying patients, and we rank order the variables according to the contribution each makes as single or joint predictors in classifying patients.

Materials and Methods

Enzyme Assays

We measured CK-MB and LD-1 isoenzyme activities at 30 °C with kits for immunological inhibition of the CK B-unit activity (SmithKline Beckman Corp., Brea, CA) and immunological assay of LD-1 (Isomune; Roche Diagnostics, Nutley, NJ). CK-MB was serially assayed at 6-h intervals during the first 24 h after admission as previously described (4). We then assayed LD-1 in samples obtained from the same patients 6 h after the time of the CK-MB peak or 18 h after admission.

Subjects

The population studied includes 165 patients admitted to Bridgeport Hospital in 1984 and 1985 for infarct or its exclusion, who underwent treatment with thrombolytic agents, who underwent coronary artery bypass surgery, or who had massive trauma. A significant proportion had an increase in CK-MB without demonstrable infarct, as established by review of the medical record. The diagnoses were established by electrocardiography, clinical presentation, serial enzyme assays, and catheterization studies, and, for some, angiographic studies showing akineti myocardium according to standards established by the Section of Cardiology.

After initially grouping each patient into one of two categories, based on a detailed study of the medical records, we carried out statistical analyses. After careful examination of our patients and the data, a third category was identified, which consisted of patients with small myocardial infarct and borderline enzyme changes. In two instances the patient's physician provided a clinical history of presentation of symptoms between five and 10 days after the

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occurrence of infarct. Others in the group were indeterminate in that they had severe angina and coronary heart disease, and the possibility of slowly evolving infarct was considered over several days, but the diagnosis could not always be accurately established.

The patients in the study were then classified into three groups as follows: acute myocardial infarct (AMI) (n = 100), non-AMI (n = 41), and intermediate (n = 24). These patients' mean age was well over 60 years, but congestive heart failure was uncommon, the Killip class exceeding a functional status of 2, considered significant only when there are pulmonary infiltrates on roentgenographic examination (class 3) or by measuring reduced ejection fractions, which was not necessary in most cases. Therefore, we do not know the effect, if any, of mild congestive heart failure on results of our isoenzyme studies. However, four patients in Killip class 4 functional status developed cardiogenic shock, and for four the LD-1 activity exceeded 70 U/L, with LD-1 <24% of total LD activity. To this extent we could identify the effect of severe congestive heart failure on decreased LD-1 as a fraction of total LD activity.

We used basic statistical methods to measure central tendency, variation, and skewness and histogram evaluation by group for each variable. We then carried out one-way analyses of variance and discriminant function analyses to determine the best model for predicting group membership in either the AMI or non-AMI groups, and in the AMI, non-AMI, and intermediate groups.

**Results**

The population means and standard deviations for the peak CK-MB, for LD-1 isoenzyme activity, and for LD-1 as percentage of total LD activity show the overlap between the intermediate and the non-AMI groups (Table 1) for all variables and the broad range of percent LD-1 activity in the intermediate group. In this construction of reference ranges the activities of LD-1 and CK-MB were converted to log functions to reduce the skewness and differences in populations variances between AMI and non-AMI groups. The peak CK-MB and the LD-1 activity as percentage of total LD activity have the greatest and the least population standard deviations in AMI patients, respectively. When the CK-MB exceeds 11 U/L, a result for LD-1 isoenzyme activity that is <43 U/L probably excludes AMI, as demonstrated by the nonoverlapping ranges for LD-1 activity in AMI and non-AMI, when the intermediate group with a peak CK-MB activity up to 23 U/L is omitted. There is an overlap of AMI and non-AMI at LD-1 of 27 to 34% of total LD activity. Increase of LD-1 activity with CK-MB between 11 and 20 U/L may identify those patients in the intermediate group with AMI.

It is desirable to find a distribution function that can classify the patients as AMI and non-AMI based on the joint probabilities of some combinations of the predictors, as illustrated in the plots of log (LD-1) and log (CK-MB) against logit (%LD-1) shown in Figure 1. The transformed variables log (activity) and logit (percent) were used to form a joint distribution based on the previous findings of Bernstein et al. (24). Their use is based on the assumption that they are required to justify the estimation of a bivariate density by a classification procedure that minimizes the misclassification probabilities, especially when the function is applied to a new population. For the present studies, adequate results were obtained by using an assumption of normality for untransformed variables, even though we were aware of skewness in the AMI population. This was partly justified by examining histogram data and our attempt to decrease population variances, compared with earlier studies (24), introduced by measurements taken at nonoverlapping time intervals. Moreover, we were not interested here in applying this function to a larger population than that derived from this study. Linear regression analyses between the following candidate variables or transformations of these variables show significant correlation (r) in AMI patients: CK-MB, LD-1, 0.807; %LD-1, ln (LD-1), 0.726; logit (%LD-1), ln (LD-1), 0.724. Values for CK-MB and LD-1 are correlated within, but not outside of, the AMI group. The increase of the ratio of LD-1 to total LD activity also is correlated with LD-1 activity in the AMI group, as expected—a correlation that does not exist in the non-AMI or intermediate patients.

We carried out one-way analyses of variance between AMI and non-AMI and the intermediate group to determine whether there were significant differences between groups.

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**Table 1. Statistical Differences between Acute Myocardial Infarct (AMI) Patients and Other Groups**

<table>
<thead>
<tr>
<th>Reference range</th>
<th>Peak CK-MB, U/L</th>
<th>LD-1, U/L</th>
<th>LD-1/total</th>
<th>Peak CK-MB, LD-1/total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>%LD-1</td>
<td>%LD-1</td>
<td>%LD-1</td>
<td>%LD-1</td>
</tr>
<tr>
<td>AMI</td>
<td>55.1 (108.8)</td>
<td>128.8</td>
<td>48.8 (9.9)</td>
<td>20-300</td>
</tr>
<tr>
<td>24 (95)</td>
<td>(111.4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IM</td>
<td>16.2 (3.4)</td>
<td>53.4</td>
<td>36.3 (9.5)</td>
<td>10-23</td>
</tr>
<tr>
<td>Non-AMI</td>
<td>13.2 (8.3)</td>
<td>30.0</td>
<td>23.0 (8.1)</td>
<td>0-30</td>
</tr>
<tr>
<td>41 (15.9)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Standard deviations are in parentheses. *Peak CK-MB is taken 12 or 18 h after admission sample. *Peak CK-MB >18 U/L may be seen with macro BB-type CK; unstable angina is associated with values <22 U/L. * Approximately 22% of LD-1 representing nondisease patients have LD-1 >25%, and LD-1 <15% may reflect liver disease. **Intermediate group: patients with small acute myocardial infarct, late findings, and inconclusive findings for AMI.**

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**Fig. 1A. Distribution plot of log (LD-1) against logit (%LD-1) for AMI (open triangles) and non-AMI (closed triangles) patients.**

The use of transformed variables was considered necessary to estimate the bivariate mapping of patients by a procedure that minimizes misclassification errors.

**B. Plot of the distribution of log (CK-MB) against logit (%LD-1) between AMI (open circles) and non-AMI (closed circles) patients with a good separation between the groups.**
in the dependent variables (MB, LD-1, and percent LD-1). In Table 2 we compare F-values for the two- and three-group classification in the dependent variables, indicating the greater significance in the two-group than in the three-group classification, but particularly in the LD-1 and percent LD-1 distributions. The Scheffé post hoc tests showed that for MB and LD-1 the differences were significant between AMI and the other groups, but not for the intermediate and non-AMI groups. However, the percent LD-1 differed significantly in the intermediate group from both the AMI and the non-AMI groups. This is largely accounted for by the lower variances in the AMI and non-AMI groups for percent LD-1 activity.

Four discriminant analyses, conducted with use of CK-MB, LD-1, and percent LD-1 in various combinations to form a vector of predictors, accounted for the differences among groups between the AMI and the other groups, because the members of the intermediate group blended in with both the AMI and non-AMI, so that this group could not be effectively separated. Nevertheless, percent LD-1 and LD-1 or MB activity accounted for some separation, with percent LD-1 contributing the most to the separation. Table 3 shows the results of four discriminant analyses executed for the two-group separation of AMI and non-AMI patients, with MB, LD-1, and percent LD-1 used in various combinations. Testing for differences between the two groups, the F ratio was 45.54 with 1.3 degrees of freedom. Therefore, the null hypothesis was rejected.

While four predictors were effective in significantly discriminating between the two groups, the best model was one that utilizes predictors in the following order: percent LD-1, LD-1, and MB. For this analysis the eigenvalue was 1.00. The canonical correlation was 0.71 with 50% variance explained. The Wilks Lambda statistic was calculated to be 0.50. The scaled coefficients of the discriminant function that was obtained (chi square = 34.9, df = 3, p <0.01) are: 0.84 (percent LD-1), 0.17 (LD-1), and 0.14 (MB), which gives group centroids, calculated on the study population, of 0.84 for AMI and 1.54 for non-AMI. Using this classification procedure, we correctly classified 93% of the AMI and 85% of the non-AMI population.

### Table 2. One-Way Analyses of Variance

<table>
<thead>
<tr>
<th>Variable</th>
<th>Groups</th>
<th>Degree of freedom</th>
<th>F*</th>
</tr>
</thead>
<tbody>
<tr>
<td>MB</td>
<td>2</td>
<td>1,139</td>
<td>28.1</td>
</tr>
<tr>
<td>LD-1</td>
<td>2</td>
<td>1,139</td>
<td>62.8</td>
</tr>
<tr>
<td>%LD-1</td>
<td>2</td>
<td>1,139</td>
<td>131.2</td>
</tr>
<tr>
<td>MB</td>
<td>3</td>
<td>1,161</td>
<td>20.99</td>
</tr>
<tr>
<td>LD-1</td>
<td>3</td>
<td>1,161</td>
<td>41.29</td>
</tr>
<tr>
<td>%LD-1</td>
<td>3</td>
<td>1,161</td>
<td>69.88</td>
</tr>
</tbody>
</table>

*All are significant at the p <0.01 level.

Discussion

Our studies re-establish the importance of measuring LD-1 isoenzyme for the accurate assessment of AMI—even though there is great reliance on serial CK and CK-MB measurements in many institutions—not only because of the reliability of measuring LD-1 activity within the first hospital day (3, 5, 10, 16). Jablonsky et al. (10) recently reported that the LD-1/LD-2 ratio (reflecting the increase in LD-1 isoenzyme fraction) appears to be increased even earlier than the CK-MB fraction or the LD-1/total LD ratio, and supported their previous work (15) and that of Bruns et al. (9) suggesting the superiority of measuring the percent LD-1 over measuring LD-1 isoenzyme activity despite an inadequately small study population (12). While almost all of the patients in the study of Bruns et al. (3) had LD-1 activity exceeding 40% of the total LD activity, a cutoff for LD-1 activity at 40% of LD activity corresponds with a flipped LD-1/LD-2 isoenzyme ratio, which is above the 50th percentile of patients with AMI, whereas a ratio of 0.76, reported to be significant by Leung and Henderson (15), is only at the 25th percentile of patients with AMI. It remains to be confirmed whether there is any difference between a cutoff established for LD-1/LD-2 ratio at 0.76 (10, 15) and LD-1/total LD activity of 27% in serum, which should refer to the same percentile of patients with AMI as a 3% CK-MB cutoff. While our studies have shown a significant population of AMI patients with increased LD-1 isoenzyme at the time of admission, more acceptable error rates are obtained 12 h later (5), possibly 6 h before the CK-MB activity is greatest (10).

Our studies confirm the findings of Elser and McKenna (20) and show the difficulty of distinguishing AMI from non-AMI patients with multiple problems. The patients with continuous angina and small or slowly evolving infarcts may show an increase in the CK-MB isoenzyme, no increase, or a borderline increase. The occurrence of AMI, however, is associated with an increase in LD-1 isoenzyme that is correlated with the CK-MB increase. The main advantage of the LD-1 assay is, first, the increase of the LD-1 activity as a percentage of the total LD activity, and then the prolongation of the LD-1 increase, which does not have the rapid decline seen with CK-MB. Further, there is unreliability of CK-MB in other situations associated with its rapid disappearance from serum (19, 21). This is of particular importance in a hospital that is a trauma center and that has a major cardiovascular surgery program.

The problem of variability in the increase of CK-MB isoenzyme associated with conditions other than myocardial infarction is best illustrated by a 61-year-old woman who was admitted with a previous history of coronary artery bypass surgery and a complaint of abdominal pain, who underwent small-bowel resection for superior mesenteric artery occlusion. This patient had an increased CK-MB activity concentration that reached 90 U/L in 48 h, and the activity was 8% of the total CK activity in serum. Analysis for LD-1 isoenzyme established an LD-1 activity of 136 U/L, 11% of the total LD activity. The LD-1/total LD activity, in this instance, helped to exclude the possibility of AMI in the face of above-normal serial CK-MB and LD-1 activities.

This is the first large study attempting to compare LD-1 and CK-MB taken at optimum or near-optimum sampling times and to assess a population of more than 150 patients taken from surgical, medical, and coronary intensive-care units. It is a continuation of a long-term study attempting to
accurately assess the performance of predictors of the existence and severity of AMI. We have provided evidence that the percent LD-1 isoenzyme is a more nearly accurate predictor than is the peak CK-MB activity, the LD-1 isoenzyme activity, or a combination of the two. More importantly, we demonstrate how we can test these methods and establish a combination of measures from these observations to classify patients. We do this by determining the contribution of each of several candidate predictors to the accurate classification of patients. The current study also reinforces the importance of optimizing sampling times by virtue of the fact that more accurate error rates were obtained than were achieved with a nonparametric approach in our earlier studies (24). However, we do not discount the possibility of obtaining better error rates in classifying patients by using a nonparametric classification and a substantially larger population to reduce the effects of unequal variances between groups. By choosing the best nonoverlapping time intervals for which isoenzyme increases occur we establish the most probable profile for determining the occurrence of AMI, and therefore, minimize some problems of incorrect assumptions about the distribution of data used for classifying patients as much as could be done by using more robust statistical approaches.

The ambiguous results of a three-group classification scheme illustrates the difficulty of developing an artificial-intelligence approach for improving the interpretation of laboratory information. The high significance of percent LD-1 as a candidate predictor is associated with the fact that several patients with known AMI of more than two days duration had increased LD-1 activity as a percentage of total LD activity. An attempt to accurately classify these patients by using a combination of predictors with CK-MB as a variable resulted in decreased confidence in classifying a patient as AMI because of the rapid disappearance of the CK-MB from serum. Thus, it was difficult to determine to which group these individuals belong by using the combined probabilities for LD-1/total LD and CK-MB activities to form a joint distribution. This can be a problem clinically in a patient with anginal chest pain and negative electrocardiographic changes if a small myocardial infarct is present, but it does not appear to be a problem when a physician is confronted with strong clinical evidence in addition to the laboratory findings. Depending on the weight of evidence, the ambiguous laboratory data are accepted or rejected.

Finally, we find no need for recommending the assay for LD-1 isoenzyme activity before the peak of the CK-MB increase, despite the recently presented findings of Jablonsky et al. (10), because we think that, given a reliance on the subjective assessment of patients data from CK-MB isoenzyme along with clinical and electrocardiographic findings, we are interested in a greater diagnostic efficiency of LD isoenzyme-1 assayed 12 to 18 h after admission than earlier sampling allows. A larger study should show that measurement of the LD-1/total LD ratio or the LD-1/LD-2 ratio taken at too early a time decreases the probability of the sensitivity estimation, as demonstrated by Linnet (12) and reduces the value of the information. We also find no justification for the current overreliance on CK-MB isoenzyme in the absence of an objective "gold standard" for clinical diagnosis.

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References