Usefulness of Various Lactate Dehydrogenase Isoenzyme 1 Profiles after Myocardial Infarction*

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ABSTRACT

Isoenzyme profiles of lactate dehydrogenase (percent LD-1 of total LD, LD-1/LD-2 ratio, and absolute LD-1) have all been studied as late markers for myocardial infarction. It is known, however, that elevations of LD-5 frequently occur in this period as a result of liver congestion. Elevations of LD-5 may also occur as a result of complicating conditions. Such elevations could result in a reduced percent LD-1 of total LD, giving rise to false negatives. Receiver operating characteristic (ROC) curves were constructed for LD-1, LD-1/LD-2 and percent LD-1 of total LD from 285 specimens (124 patients) with suspected myocardial infarction. There was little difference in overall diagnostic power among the three assays. Using cutoffs determined from the ROC curves, 6 patients (18 specimens) were evaluated who appeared to be in the late period or who exhibited complicating conditions which could increase LD-5. In 14/18 specimens, increases in LD-5 resulted in false negatives by percent LD-1 of total LD. Only 5/18 specimens were false negatives by LD-1 or LD-1/LD-2. It is concluded that the percent LD-1 of total LD was affected by an increase in LD-5, and caution is recommended when using it.

Introduction

Because of differences in the time that various markers become elevated and remain elevated after a myocardial infarction (MI), two markers have generally been used to identify its occurrence; an early marker (prior to 24 to 36 hours) and a late marker (after 36 hours).1,2 Creatine kinase (CK; EC 2.7.3.2)—MB, and isoenzymes of lactate dehydrogenase (LD; EC 1.1.1.27) have been widely used as early and late markers, respectively.1,2 The development of new markers, such as troponins T and I,3,4 may eventually change this approach, but assay methods for these new markers are not generally available. Moreover, it may be some time before they are sufficiently validated in enough studies to prove greater diag-
nostic value. Thus, the traditional markers may continue to be widely used for years.

Conventionally, the LD-1/LD-2 ratio, obtained by electrophoresis, has been used as a late marker. In 1979, an ingenious immunoprecipitation method for measuring LD-1 was developed and later introduced as a kit. This approach was advantageous in that it could be semi-automated and potentially eliminate the need for the more tedious and time-consuming electrophoresis. Using this method, diagnostic sensitivity and specificity data for MI suggested that the absolute level of LD-1 was almost as accurate an early marker as CK-MB. More recently, several chemical inhibition methods have been devised for measuring LD-1. These can be completely automated; some are available in kit form, and appear to have become popular. Indeed, methods by 4 different manufacturers were listed on the 1993, College of American Pathologists Survey, EC-B.

Elevations of LD including LD-1 may be produced by non-cardiac sources. In these cases, although the absolute amount of LD-1 may increase, the relative level as compared to other isoenzymes may remain constant or even decrease. Presumably, under these conditions, the absolute level of LD-1 may not be as specific as the LD-1/LD-2 ratio. Thus, investigators have studied the use of percent LD-1 relative to the total LD (percent LD-1 of total LD) to normalize increases in LD-1 from non-cardiac sources. Several studies suggest that percent LD-1 of total LD is as accurate as the LD-1/LD-2 ratio, if not more so. Other studies question this finding, suggesting that while percent LD-1 of total LD may be accurate during the earlier period following MI, it loses sensitivity after 5 days when compared to LD-1 and LD-1/LD-2 ratio.

An automated method for LD-1 has been examined for use in our laboratory. Specimens with very elevated LD-5 consistently showed low levels of percent LD-1 of total even when the ratio of LD-1/LD-2 was elevated. Following an MI, there is often hepatic congestion owing to right sided heart dysfunction which causes an elevation in LD-5 during the late period. It was hypothesized that, although the percent LD-1 of total LD might be accurate for identifying MI during the early period, an elevation in LD-5 would cause the percent LD-1 of total LD to decline resulting in false negative values during the late period. A similar affect could occur as a result of patients experiencing an MI concomitantly with syndromes which cause an elevation in LD-5, such as systemic shock, liver disease, or cancer. This could create misdiagnosis in these patients. In order to test this possibility, LD-1, LD-2, and total LD were measured in 285 specimens from 124 patients, with suspected MI, from serum collected within 24 hours after entering the hospital, and 18 specimens from 6 patients who appeared to be in the late period or who showed complications from syndromes which elevate LD-5. The data indicate that the percent LD-1 to total LD should be used with caution in these types of patients.

Materials and Methods

Patients and Specimens

In our Medical Center, CK-MB and the LD-1/LD-2 ratio (all by electrophoresis) were used for biochemical evaluation of MI. Normally, three consecutive serum specimens are measured within 16 to 24 h, with the actual timing for each specimen collection varying between 4 to 8 hours. Occasionally, more than three specimens are measured over a longer
period of time. For this study, 285 specimens were measured from 124 patients over a period of about 3 months. Specimens selected for the study were the first three specimens from patients exhibiting at least one specimen with an elevated CK-MB, and an adjacently accessioned specimen set which was normal. These were used to develop receiver operating characteristic (ROC) curves. Besides, an additional 4 specimens were included from 3 patients who appeared to be in the late period or on whom more than 3 specimens were ordered. Specimens were denoted as positive or negative for MI according to the final diagnosis notes in the patient’s record which was based on evidence from ECG, enzymes, transesophageal echo cardiogram, and clinical impression.

**METHODS**

Total CK and LD were measured with a discrete analyzer.* Both CK-MB and the LD-1/LD-2 ratio were determined by electrophoresis.† The LD-1 was measured using an immunoassay kit (Isomune-LD®) with a discrete analyzer.‡ A program was used to calculate the receiver operating characteristic (ROC) curves.§ Areas under the ROC curves were compared using a two tailed area test computed by the ROC curve program. A p value ≤0.05 was considered significant. Other statistics were performed using software.¶

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‡ Cobas FARA II, Roche Diagnostic Systems Inc., One Sunset Avenue, Montclair, NJ 07042.
§ “Clabroc,” available from Dr. Charles E. Metz, Department of Radiology, University of Chicago Medical Center, 5841 South Maryland Avenue, Chicago, IL 60637-1470.
¶ “Statview IV,” Abacus Concepts, 1918 Bonita Avenue, Berkeley, CA 94704.

**Results**

In figure 1 are shown ROC curves depicting the diagnostic sensitivity and specificity for the LD-1 activity, the percent LD-1 of total LD, and the LD-1/LD-2 ratio for all of the specimens collected within the first 24 hours after admission to the hospital. The areas under the curves indicate the degree of diagnostic accuracy. Although the area under the percent LD-1 of total curve was less than the area under the other two, the difference was not significant (p = 0.30). The ROC curve for LD-1 activity obtained by electrophoresis is not shown, but the area under it was 0.77, which was not significantly different from the other curves.

It was determined that an appropriate cutoff level for the LD-1/LD-2 ratio should allow a false positive frequency (FPF) of no more than 15 percent. According to the ROC curve, this specificity would require a LD-1/LD-2 ratio of 0.8, which gave a true positive frequency (TPF) of 46 percent (figure 1). This cutoff level is very similar to the cutoff level of 0.76 identified by Leung and Henderson in a study of healthy subjects.16 Others have also identified optimal cutoff levels for LD-1/LD-2 in this range (0.7 to 1.0).1 At the same FPF (15 percent), the corresponding cutoff values for percent LD-1 of total LD and for LD-1 by the immunochemical method were 10.5 percent and 72 IU/L, respectively. The TPFs were 45 percent (LD-1 of total LD) and 48 percent (LD-1). Alternatively, when the LD-1/LD-2 ratio was compared with the percent LD-1 of total LD by linear regression line analysis, the best line was y = 14.065 (LD-1/LD-2 ratio)—0.756, with r = 0.75 and n = 283. By this approach, an LD-1/LD-2 ratio of 0.8 also resulted in a cutoff level of 10.5 percent for percent LD-1 of total LD. Thus, based on these statistical analyses, there appears to be little difference
among the markers as regards diagnostic accuracy. When a lower cutoff of 65 U/L for LD-1 (manufacturer’s recommendation) was applied to the ROC curve, the FPF was 18 percent and the TPF 53 percent, with a corresponding percent LD-1 of total LD cutoff of 9.8 percent.

In table 1 are depicted results for LD-1 and percent LD-1 of total LD analyzed by the immunochemical method and LD fractions assayed by electrophoresis for some patients who appeared to be in the late period after MI or who have concomitantly complicating conditions. In all cases, it can be seen that as the LD-5 fraction increases the percent LD-1 of total LD decreases. As a result, this percentage was below the cutoff level for MI in 14 of 18 measurements. On the other hand the LD-1/LD-2 ratio by electrophoresis and the absolute LD-1 level by immunochemical assay was below the cutoff for MI in only 5 of the 18 measurements. An analysis by Chi square using a two way contingency table indicating that this difference in sensitivity was significant (p = 0.002).

These patients ranged between those suffering from major disease complications to those experiencing transmural and nontransmural (non Q wave) MI. The relationship between percent LD-1 of total, LD-1, LD-1/LD-2 ratio, and LD-5 expressed by spearman’s rank correlation coefficient are shown in table II.

Discussion

In recent years, direct LD-1 automated assays have become available in kit form and are now widely used. Several investigations have suggested that elevated LD-1 is a powerful marker for MI, and as good or better an early marker than CK-MB.\(^5,7,8\) Investigations have also suggested that the percent LD-1 of total LD is also comparable to CK-MB as an early marker,\(^6,7,8,9,17\) and better than the LD-1/
**TABLE I**

LD-1 Profiles from Patients Who Showed Elevated LD-5 After Myocardial Infarction

<table>
<thead>
<tr>
<th>Patient</th>
<th>Day After Entry</th>
<th>CK-MB</th>
<th>LD Isoenzymes (%)</th>
<th>LD1/LD-2</th>
<th>% LD-1 of Total</th>
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<tr>
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<td>Pos</td>
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<td>20</td>
<td>13</td>
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</table>

Day 1 designates the day of entry. Normal reference ranges in parenthesis. Values of lactate dehydrogenase were rounded off and may not equal 100 percent. Underlined values are those with elevated LD-5.

Pos = specimens with CK-MB greater than cutoff of 16 U/L and 5 percent, which is usually consistent with cardiac origin.

Neg = specimens less than this cutoff.

LD-2 ratio. The common cutoff for MI is an LD-1/LD-2 ratio of 1.0, but with any individual patient, the ratio may flip back and forth above and below this cutoff with consecutive measures. Some investigators recommend using a cutoff level as low as 0.76, which is very similar to the best cutoff level identified in the present study, because the sensitivity for identifying MI increases as the cutoff level is lowered.

**TABLE II**

Correlations Between LD-1, % LD-1 of Total, LD-1/LD-2 Ratio, and LD-5

<table>
<thead>
<tr>
<th>Parameters Compared</th>
<th>Rho</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>% LD-1 of total LD vs. % LD-5</td>
<td>-0.796</td>
<td>0.001</td>
</tr>
<tr>
<td>LD-1/LD-2 ratio vs. % LD-5</td>
<td>-0.345</td>
<td>0.155</td>
</tr>
<tr>
<td>LD-1 vs. % LD-5</td>
<td>-0.071</td>
<td>0.771</td>
</tr>
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The ROC curves shown here are very similar to that described by Onigbinde and associates for specimens collected within 24 h of chest pain.9 In the present study, little difference was found in overall diagnostic accuracy between the three types of LD markers (figure 1). Nor was a significant difference between the LD-1 activity by immunochemical assay and LD-1 by electrophoresis. It would be necessary to assay a much larger number of specimens to identify significant differences among the various indexes, which was not the intent of this study.

This study was designed to determine whether or not increases in the LD-5 fraction which may increase after an MI would cause a decrease in the diagnostic sensitivity of the percent LD-1 of total LD as compared to the other indexes. Rotenberg and associates showed that percent LD-1 of total LD, loses up to 38 percent of its sensitivity after 5 days, more than that of other LD-1 indexes.14 This deficiency is not apparent from the ROC curves developed in the present or most other studies,2,6,7,8,9,17 because the great majority of the patients are still in the early period with uncomplicated MI. In table I it is shown that in all 13 cases where LD-5 was elevated, the percent LD-1 of total LD test would have interpreted incorrectly. If the lower percent LD-1 of total LD cutoff of 9.8 percent, consistent with the manufacturer’s lower cutoff for LD-1, was used, 12 of the 13 specimens would have been misinterpreted. These sera were from patients who exhibited a wide variety of clinical conditions associated with MI. Evidence that elevations in LD-5 was directly responsible for this affects is shown in table II where percent LD-1 of total LD was strongly (inversely) related to the elevation of LD-5, while no significant relationships were observed between LD-5 and the other measures of LD-1.

Since most laboratories use CK-MB for identifying MI in the early period, and because the time after MI can only be determined in retrospect, for practical purposes LD-1 is used to identify patients in the late period who enter the hospital after CK-MB has returned to normal. Clearly, this accounts for only a small number of persons diagnosed with MI, too few to significantly affect ROC curves depicting the relationships between LD-1, LD-1/LD-2 ratio, and percent LD-1 of total LD in any single study over the first 48 hours. Still, as illustrated, misdiagnosis may occur in the small patient group that the test is designed to identified. Thus, the use of the LD-1/LD-2 ratio or LD-1 over the percent LD-1 of total LD test is recommended.

Acknowledgment

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References


