A New Method for Reporting the Sources of Abnormal Activities of Lactate Dehydrogenase in Serum

Peter E. Ploitscher,1 Stephen H. Powell,2,3 and Jeffrey Fink2

We have developed and tested a new method to increase the diagnostic usefulness of measurements of lactate dehydrogenase (LDH; EC 1.1.1.27) isoenzymes. The method estimates the separate contributions from enzymatically distinct organ clusters (e.g., heart/kidney/erythrocyte, liver/muscle, lung) to the total activity of LDH in serum. To test this method, we monitored serum LDH isoenzymes over the entire hospital course of 73 patients admitted to the intensive-care unit with chest pain, myocardial infarction, or serious hemodynamic disturbances. The organ-specific estimates provided useful information beyond measurements of the original isoenzymes. The sensitivity and specificity of this new method in detecting acute myocardial infarction, as well as concomitant disorders involving the liver or lung, were significantly greater than those of other diagnostic indices or pathologists’ judgments. Serial plots of the organ-specific estimates may provide additional insight into evolving pathophysiological processes.

Additional Keyphrases: isoenzymes · myocardial infarction · data display · computer-aided diagnosis · economics of laboratory operation · medical decision-making · liver and lung disease

Recently, questions have been raised concerning the utility of measuring lactate dehydrogenase (LDH; EC 1.1.1.27) isoenzymes in the diagnosis of myocardial infarction (MI).4 Although such assays have modest sensitivity and specificity (1–6), concern remains that they fail to provide additional information beyond that derived from measurement of creatine kinase (CK; EC 2.7.3.2) isoenzyme MB or simpler, less-expensive assays of LDH 1 and total LDH (1, 7–12). Many sources of error, including complications and coexisting disorders, can simultaneously affect LDH isoenzymes, lessening their effectiveness for detecting individual diseases. In acute MI for example, abnormal amounts or distribution of LDH isoenzymes in the serum can be produced from passive congestion of the liver or lungs due to congestive heart failure, or from ischemic changes in several organs due to low cardiac output (6). In pulmonary embolism, which often must be distinguished from acute myocardial infarction, abnormal LDH results may be produced by concomitant hepatic injury due to venous hyper-

tension, myocardial injury due to ischemia, engorgement of the renal cortex, and hemolysis (6). Because these complications occur inconsistently and to various degrees, finding isoenzyme profiles characteristic of particular diseases has been difficult. Many standard indices have been proposed [e.g., LDH 1, 1:2, 2:4, 2:5 (vide infra)], but none unambiguously indicates the effects from any particular organ.

Here we propose a method to increase the diagnostic yield from measurements of LDH isoenzymes by directly estimating the separate contributions from various organs. We have compared this method with currently used LDH indices and with the pathologists’ judgments in the diagnosis of several disorders, including acute MI.

Materials and Methods

We selected cases for study from among admissions to the coronary-care/intensive-care units of the Cleveland VA Medical Center during 1984. We chose not to screen all consecutive admissions because of resource constraints and because we did not expect our method to be useful for all routine admissions. We expected it to be most helpful in detecting the presence or absence of acute myocardial infarction (MI) in complex cases involving the failure of multiple organ systems. Thus, we selected patients with clinically determined hemodynamic disturbances—those with documented MI, and a few patients who were admitted for chest pain but judged not to have an MI (by the criteria in Table 1, below). We excluded patients who died before we could collect samples on three days for isoenzyme determinations. Although we attempted no formal stratification of cases, we did try to select a clinically interesting spectrum of severities of acute MI and hemodynamic complications, believing that this would be the fairest initial challenge of our method and that its performance on such a sample would clarify its value to the population to which it most likely would later be applied.

Overall, 73 patients, approximately one-fourth of the total admissions during this time, were eventually chosen for study. For each of these, serial electrocardiograms were obtained, and CK and LDH isoenzymes were determined on blood samples collected daily.

From each set of isoenzyme results, we attempted to determine the sources of abnormalities by estimating the separate contribution from five enzymatically distinct "types" or clusters of organs to the total LDH in serum. Cluster 1 included pancreas, lymph, adrenal, and thyroid; cluster 2 (hereinafter called the "liver/muscle-related cluster") included liver, muscle, tongue, and mucus; cluster 3 (hereinafter called the "heart-related cluster") included heart, kidney, and erythrocyte; cluster 4 included leukocyte and platelet; cluster 5 (hereinafter called the "lung-related cluster") included lung, colon, gallbladder, esophagus, duodenum, brain, thymus, prostate, and spleen. We defined these five "types" by a cluster analysis, using the published proportions of each isoenzyme in each organ. These proportions were averages from several studies in which extracts

1 Departments of Health Policy and Management, and Biostatistics, Harvard School of Public Health; and the Institute for Health Research, a joint program of the Harvard Community Health Plan and Harvard University, 677 Huntington Avenue, Boston, MA 02115.
2 Department of Medicine, Case Western Reserve University School of Medicine, Cleveland, OH.
3 Cleveland Veterans' Administration Medical Center, Cleveland, OH.

4 Nonstandard abbreviations: LDH, lactate dehydrogenase (EC 1.1.1.27); CK-MB, MB isoenzyme of creatine kinase (EC 2.7.3.2); MI, myocardial infarction.

Received December 30, 1985; accepted May 22, 1986.
from specific organs were added to sera and the resulting isoenzyme proportions measured (14). To measure isoenzymatic similarity between organs, we computed the correlations between the published proportions of isoenzymes in each pair of organs. Then we estimated the contributions from each isoenzymatically similar group of organs (i.e., each organ "type") to the total LDH activity. To estimate contributions for all five types, we used three different methods, described in the Appendix. One of these solved a set of linear equations involving the actual isoenzyme values for a particular patient at a given time, the published population means for each isoenzyme, and the average proportion of each isoenzyme in each organ type. Other methods enabled us to obtain confidence intervals for some of these estimates, using two different types of regression analysis.

To provide a basis for evaluating the diagnostic accuracy of these methods, we developed the diagnostic criteria for the disorders shown in Table 1. Using these criteria, we classified the patients as having or not having one or more of these disorders.

We first evaluated the ability of the heart-related estimate to discriminate acute MI by comparing it with the performance of standard diagnostic indices (LDH 1:2, LDH 1:total LDH, LDH 1, and total LDH) and with pathologists' diagnostic interpretations. We used the LDH values measured either 48 h after the episode of chest pain or 48 h after admission in the absence of chest pain. This excluded 14 cases in which chest pain occurred episodically over several weeks, or the onset was more than 48 h before admission, or non-cardiac sources in the heart/kidney/erythrocyte cluster were clinically apparent (two cases of acute hemolytic anemia, confirmed by decreased haptoglobin in serum, and one case of acute renal tubular necrosis, confirmed by finding renal tubular epithelial cells in the urinalysis). Optimum discrimination thresholds for the organ estimates and diagnostic indices were determined by using quadratic discriminant analysis. On the basis of these thresholds, we computed and compared the sensitivities and specificities of the indices. We also performed a "split-half cross-validation" by taking half the cases, determining the optimal threshold for them by discriminant analysis, and applying this threshold to classify the remaining cases in the other half. We then reversed the order of analysis and summed the results from both analyses to calculate overall sensitivity and specificity.

Similarly, we evaluated the ability of the lung-related and liver-related estimates to predict disorders of the respective organs according to the criteria in Table 2. For these analyses, we used the highest organ-specific estimate over the patient's course, and included all 73 cases. The organspecific estimates then were compared with pathologists' abilities to discriminate the causes of abnormal LDH results (i.e., the disorders listed in Table 1). Pathologists' diagnoses were denoted as "positive" for a particular organ-related estimate only if the organ was mentioned in the interpretive report as a possible source of LDH abnormalities.

<table>
<thead>
<tr>
<th>Table 1. Criteria for Diagnosis of Disorders Related to the Organ Clusters</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Heart/kidney/erythrocyte-related disorders</strong></td>
</tr>
<tr>
<td>MI</td>
</tr>
<tr>
<td>Acute renal tubular necrosis</td>
</tr>
<tr>
<td>Hemolytic anemia</td>
</tr>
<tr>
<td>No other acute necrotizing disorder</td>
</tr>
</tbody>
</table>

1518 CLINICAL CHEMISTRY, Vol. 32, No. 8, 1986
Table 2. Types of Cases Studied

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chest pain with ST change, no myocardial infarction (MI)</td>
<td>23</td>
</tr>
<tr>
<td>Myocardial infarction (MI)</td>
<td></td>
</tr>
<tr>
<td>Uncomplicated MI</td>
<td>33</td>
</tr>
<tr>
<td>MI with lung congestion</td>
<td>14</td>
</tr>
<tr>
<td>MI with liver congestion</td>
<td>11</td>
</tr>
<tr>
<td>MI with liver and lung congestion</td>
<td>4</td>
</tr>
<tr>
<td>MI with lung congestion and seizures</td>
<td>2</td>
</tr>
<tr>
<td>MI with liver and lung congestion and renal failure</td>
<td>1</td>
</tr>
<tr>
<td>Congestive heart failure without acute MI</td>
<td></td>
</tr>
<tr>
<td>Pulmonary congestion</td>
<td>11</td>
</tr>
<tr>
<td>Liver congestion</td>
<td>2</td>
</tr>
<tr>
<td>Liver and lung congestion</td>
<td>1</td>
</tr>
<tr>
<td>Liver congestion with seizures</td>
<td>1</td>
</tr>
<tr>
<td>Liver and lung congestion with seizures</td>
<td>1</td>
</tr>
<tr>
<td>Other</td>
<td></td>
</tr>
<tr>
<td>Seizures</td>
<td>6</td>
</tr>
<tr>
<td>Rhabdomyolysis</td>
<td>2</td>
</tr>
<tr>
<td>Pulmonary embolism</td>
<td>1</td>
</tr>
<tr>
<td>Lung cancer</td>
<td>1</td>
</tr>
<tr>
<td>Hemolytic anemia</td>
<td>1</td>
</tr>
</tbody>
</table>

Results

The organ-specific estimates of contributions to LDH abnormalities increased the sensitivity and specificity in detecting acute MI over pathologists' judgments and other diagnostic indices. Statistical discriminations were equivalent for both the linear equation and regression techniques. The organ-specific estimates for both methods yielded a 96% sensitivity and 100% specificity for the heart-related cluster. Traditional diagnostic indices did not perform as well—77% sensitivity and 90% specificity for LDH 1; 77% sensitivity and 93% specificity for LDH 2; and 80% sensitivity and 90% specificity for LDH total. Figure 1 illustrates that the heart-related cluster much more clearly distinguished MI from non-MI cases than did its closest competitor (LDH 1:total LDH). Furthermore, this gain was sustained when we performed the split-half cross-validation. The heart-related cluster still yielded 96% sensitivity and 100% specificity, better than LDH 1:total LDH, which had equivalent specificity but significantly lower sensitivity (71%; t = 3.8, p < .0001).

In comparing the organ-specific estimates to pathologists' LDH interpretations, we also found significant improvements. In the diagnosis of acute MI, for example, the model had equivalent specificity, but significantly higher sensitivity: 96% vs 85% (t = 2.3, p < .05). In the diagnosis of lung-related disorders (pulmonary vascular congestion, pulmonary embolism, or lung cancer), the new method had higher specificity, 93% vs 81% (t = 2.2, p < .05), and much higher sensitivity, 70% vs 30% (t = 4.8, p < .00001). For liver- or muscle-related sources, the new method still had moderately higher sensitivity (93% vs 89%) and much higher specificity, 83% vs 30% (t = 6.5, p < .00001).

One further test of the organ-specific estimates evaluated whether a summation of the estimated LDH due to each organ "type" approximated the total LDH (in excess of the normal mean) actually observed. The multiple correlation between the estimated and observed total LDH averaged .77, with most values above this (see Figure 2 for the results and see the Appendix for a technical discussion of the computation and meaning of these correlations). This degree of agreement was not expected because only three of the organ clusters (heart, liver, and lung) were used in this part of the analysis. Moreover, all of the cases wherein the summed organ estimates did not agree closely with the observed LDH values (r < .8) involved cases where they both were very low.

The following cases illustrate graphically how the organ-specific estimates diminish the errors of standard indices and pathologists' judgments when plotted serially to monitor patients over time. They show how the new method can help recognize incipient hemodynamic complications and generate other diagnostic hypotheses.

Case 1. A 65-year-old white man was admitted with chest pain but no initial electrocardiographic (EKG) or isoenzyme changes. An MI was confirmed within 48 h based on the LDH 1:2 ratio and EKG. On the second day, he developed severe bradycardia and hypotension but responded to treatment. He was asymptomatic for three days but then developed recurrent severe chest pain and ventricular tachycardia. He was temporarily stabilized, but the following morning had a cardiac arrest and died. Autopsy revealed cardiac
hypertrophy and dilation with a recent massive organizing MI.

Although the raw isoenzymes showed a sustained LDH 1:2 flip (Figure 3a), the organ estimates (Figure 3b) show a much more pronounced escalation in LDH attributable to the heart, with no significant change in the other clusters. This suggests a continuously extending infarct, not nearly so apparent or economically illustrated in the raw isoenzyme profiles. Based on the raw data, the pathologist did acknowledge a possibility of extension but attributed most of the LDH increase to pulmonary and hepatic congestion. Possibly identifying the extent of this massive evolving infarction might have led to therapeutic intervention earlier, when the patient was asymptomatic.

Case 2. A 70-year-old white man was admitted, having experienced chest pain within the preceding 24 h. On the basis of the EKG ST segment depression, positive CK MB, and LDH flip, a seemingly uncomplicated nontransmural MI was diagnosed. Three days after admission he had recurrent chest pain with new ST depression. The pain resolved but EKG changes persisted, and he developed dyspnea, mitral insufficiency, and pulmonary edema. Subsequently, he underwent surgery to replace the mitral valve but developed refractory shock during surgery and died. Autopsy revealed a recent MI with papillary muscle involvement, severe pulmonary congestion, and micronodular cirrhosis of the liver.

The serial display of the organ estimates (Figure 4b) closely parallels the clinical course. Whereas the raw isoenzyme data (Figure 4a) were interpreted by the pathologist as resolving myocardial infarction with liver congestion, the organ estimates suggest recurrent myocardial necrosis causing mitral prolapse and subsequent pulmonary congestion. The pathologist overlooked evidence of the recurrent infarction, probably because there was no new LDH 1:2 flip between days 4 and 5. Interestingly, the clinical staff also believed recurrence unlikely because of an ambiguous history, EKG findings, and the absence of recurrent CK MB elevation. Total CK increased somewhat during this period but not enough to require isoenzyme fractionation.

Case 3. A 72-year-old black man was admitted with gastrointestinal bleeding, epigastric pain, nausea, dyspepsia, and EKG ST segment depression. He had diffuse rales but no hepatosplenomegaly or edema. His chest roentgenogram was consistent with pulmonary edema. CK MB was positive, and his hematocrit was 25. The clinical diagnosis was myocardial infarction with pulmonary edema probably precipitated by the stress of gastrointestinal bleeding. He made an uneventful recovery.

The isoenzyme pattern (Figure 5a) shows an LDH 1:2 flip and a strikingly increased CK 3. The pathologist interpreted the patterns as consistent with MI and hepatic congestion. The organ-specific estimates (Figure 5b), however, correlate much better with the clinical course and clearly parallel the hemodynamic aberrations present in the patient. The lung-related estimate is increased at the time of admission, peaking the next day. The liver/muscle estimate is also increased, but less prominently and rapidly drops off. Also, while evidence of acute MI (LDH 1:2 flip) exists for only half a day in the raw data, the heart-related estimate remains significantly increased during all eight days. This suggests why the new method may be much more sensitive for patients presenting at various times after the onset of acute MI.

Case 4. A 54-year-old white man with poorly controlled diabetes was transferred to the intensive-care unit for severe biventricular failure concomitant with dyspnea, orthopnea, bilateral pleural effusions, epigastric pain, and deteriorating renal function. He had been admitted to an outside
hospital three weeks previously with hyperglycemia, nausea, and vomiting. Upon transfer, a perfusion lung scan indicated a low probability of pulmonary embolism. EKGs did not show acute ischemic changes but CK MB was positive.

Afterload reduction was instituted and resulted in a modest increase in cardiac output and some clinical improvement in his passive pulmonary congestion. One day after the transfer to the unit, however, he became hypotensive, with labored respirations but no chest pain. He appeared to stabilize with mechanical ventilation, but on the third day developed acute bradycardia and refractory shock, and died. At autopsy, the heart was seen to be hypertrophied and dilated, with a recent MI. There was acute passive hyperemia of the lungs, liver, and spleen, and an organizing embolism in the right lobe of the lung.

Figure 6 shows that all the isoenzymes appear nonspecifically increased and relatively close together. No LDH flip occurred. A moderate predominance of LDH 5 gradually diminished over time. The pathologists noted this LDH 5 increase, attributing it to liver congestion. They also mentioned MI, because of a persistent and positive CK MB. However, the organ estimates (Figure 6b) provide a much clearer and more informative picture of abnormalities overlooked by the pathologists. The liver and lung estimates initially were above normal, but gradually tapered off. The lung estimate then increased again, at a time of recurrent dyspnea and hypotension, without a concomitant increase in the liver fraction, but with a parallel increase in the heart estimate. Thus, this lung-related increase may have been secondary to the pulmonary embolism noted at autopsy, perhaps exacerbated by left ventricular failure. The concomitant increase in the heart-related estimate may have resulted from either ongoing myocardial necrosis, acute renal tubular necrosis, or hemolysis associated with the pulmonary embolism.

**Discussion**

In this study, the new organ-specific estimates outperformed conventional LDH indices and pathologists' judgments in identifying acute MI. They also improved recognition of other disorders such as pulmonary edema, pulmonary infarct, and passive congestion of the liver.

The magnitude of these improvements were large, despite the estimates' vulnerabilities to several types of error. We recognized that the published isoenzyme proportions for each organ, utilized in calculating the organ-specific estimates, were based on necropsy data and thus might have differed from in vivo distributions in serum. Also, the isoenzyme proportions may vary among individuals or within an organ (e.g., necrosis of different regions of the heart). Most importantly, the average isoenzyme proportions for a cluster of related organs may differ from the true proportions from any individual organ. Thus, an extreme contribution of LDH from one organ may introduce error. When this leads to overestimation of one organ "type," negative values for another may result to compensate (with the linear equation or regression methods). The total contributions for all the organs add to the total LDH in excess of its normal mean. These negative values actually can be eliminated with an alternative regression type of method (see Appendix). The latter method is less clearly derived but had essentially equivalent performance. Alternatively, if these negative values exceed a significant range of analytical variability or if the correlation between observations and model predictions falls below a given threshold, the model can produce a "danger signal." The clinician may then choose to disregard the results. In actuality, however, the instances of poor model performance were rare (see Appendix). They mainly occurred when isoenzyme activities were low, probably due to imprecision in isoenzyme determinations at these levels. However, these were cases in which the organ estimates did not produce significant clinical information anyway.

Much more significant errors occurred with traditional diagnostic indices, especially in complicated cases. Unlike the organ-specific estimates, some of these indices may be derived from inexpensive assays not requiring electrophoresis. However, they result in a distressingly higher percentage of false negatives in the diagnosis of acute MI and lung-related disorders (e.g., pulmonary embolism). This error rate was higher than reported elsewhere (8-11), perhaps because other studies included fewer individuals with hemodynamic disorders. However, in these more complex cases, traditional indices seem clearly inferior to the organ-specific estimates.

Pathologists' judgments were also subject to error. To some extent, this might not have been surprising. One would expect pathologists to appear overly conservative, with an excess of false positives, since their evaluations were abstracted from the reports and merely indicated "possible" sources of LDH abnormalities. However, while they did over-report liver-related diagnoses, they unexpectedly under-reported lung- and heart-related disorders. We readily acknowledge that some experienced pathologists may have interpreted some of the isoenzyme profiles differently from the reports in our study—for example, not concluding there was a significant hepatic component in cases 1 and 3, previously illustrated. However, most of the important errors we found appear to be ascribable, not to equivocal interpretations, but to loss of distinctive patterns when multiple organ systems fail. Lung-related disorders...
often co-occur with others and lack distinguished isoenzyme profiles. Also, acute MI may lack its distinctive feature (the LDH 1:2 flip) when congestive heart failure co-occurs. Contributions from lung or liver can then increase the amount of LDH 2 relative to LDH 1. These explanations, of course, cannot entirely eliminate our surprise in finding that pathologists under-report true cases of acute MI relative to the organ-specific estimates. We had expected the pathologists to outperform the new method because they had access to CK MB results, usually the "gold standard" for acute MI.

Thus, a preliminary evaluation suggests that the new method improves the use and interpretation of LDH isoenzymes. As several cases illustrate, serially displayed organ estimates based on this method may further elucidate pathophysiological processes and multiple organ involvement in the critically ill. The serial displays appear especially valuable in the diagnosis of recurrent or extending acute myocardial infarction.

More definitive future evaluations should determine whether the new method, perhaps through serial estimates, can improve MI survival predictions (15–16). Further studies should also clarify the value of organ-specific estimates as a preliminary screening test for pulmonary embolism, since both prescriptive anti-coagulation and pulmonary angiography entail significant risks. Future evaluations must also determine whether the organ estimates are useful when derived from data obtained in other laboratories and patient populations. Finally, careful cost-effectiveness analysis should determine whether and when the increased information provided by the organ-specific estimates justifies the increased cost of isoenzyme fractionation as compared with simpler, less expensive assays.

Only by answering these questions can we adequately judge the value of LDH isoenzymes. While these "little ticket" items can add up to big costs when performed repeatedly on many patients (17), they may ultimately reduce costs with the improved information provided by organ-specific estimates. The latter might increase diagnostic and prognostic confidence, enabling earlier discharge from the intensive-care unit or management in less costly intermediate-care facilities (18–19). If so, the present $10 charges for isoenzyme fractionation, even if recurrent, would seem very small when compared to repeated savings of several hundred dollars per hospital day. Also, just as minor expenses often add up to large costs, apparently minor improvements in patient management might add up to large gains in the aggregate quality of patient care.

Appendix

The following section discusses three methods that can be used to derive organ-specific estimates of LDH isoenzyme abnormalities. Each method was based on a cluster analysis that defines isoenzymatically similar and distinct "types" of organs. Correlations between the published proportions of isoenzymes in each pair of organs provided a measure of similarity between them. Recall that five enzymatically similar organ types were defined:

Cluster 1. Pancreas, lymph, adrenal, thyroid
Cluster 2. Liver, muscle, tongue, mucus (liver/muscle-related type)
Cluster 3. Heart, kidney, erythrocyte (heart-related type)
Cluster 4. Leukocyte, platelet
Cluster 5. Lung, colon, gallbladder, esophagus, duodenum, brain, thymus, prostate, spleen (lung-related type)

In the first method to estimate the contribution of each organ type to the total LDH (which we call "organ-specific estimates") we simply solved a set of linear equations for the unknown contributions.

To illustrate this method heuristically, suppose for example that we measured only two isoenzymes (L1 and L2) and that only two organ types (clusters 2 and 3) were potential contributors (this assumption is made only to simplify the explanation and is not necessary, as we shall see later).

Then to estimate the contribution of each organ cluster to the total LDH, we could write the following equations:

\[
\begin{align*}
\text{total } L1 &= \left( \% L1 \text{ in cluster } 2 \right) \times \left( \text{total LDH due to cluster } 2 \right) \\
&\quad + \left( \% L1 \text{ in cluster } 3 \right) \times \left( \text{total LDH due to cluster } 3 \right) \\
\text{total } L2 &= \left( \% L2 \text{ in cluster } 2 \right) \times \left( \text{total LDH due to cluster } 2 \right) \\
&\quad + \left( \% L2 \text{ in cluster } 3 \right) \times \left( \text{total LDH due to cluster } 3 \right)
\end{align*}
\]

Note then that if we divide all terms in the first equation by the percentage of L1 in cluster 3, divide all terms in the second equation by the percentage of L2 in cluster 3, and then subtract the second equation from the first, we obtain:

\[
\begin{align*}
\left( \% L1 \text{ in cluster } 3 \right) - \left( \% L2 \text{ in cluster } 3 \right) &= \\
&= \left( \% L1 \text{ in cluster } 2 \right) \times \left( \text{total LDH due to cluster } 2 \right) \\
&\quad - \left( \% L2 \text{ in cluster } 3 \right) \times \left( \text{total LDH due to cluster } 3 \right)
\end{align*}
\]

Then if we divide both sides by the term in brackets, we obtain:

\[
\begin{align*}
\left( \% L1 \text{ in cluster } 3 \right) - \left( \% L2 \text{ in cluster } 3 \right) &= \\
&\left( \% L1 \text{ in cluster } 2 \right) \times \left( \text{total LDH due to cluster } 2 \right) \\
&\quad - \left( \% L2 \text{ in cluster } 2 \right) \times \left( \text{total LDH due to cluster } 3 \right)
\end{align*}
\]

All of the quantities on the left side of this equation are measured or known from published data; thus we can estimate from these the total LDH ascribable to cluster 2. Likewise, by subtracting (percentage of L1 in cluster 2) \times (total LDH due to cluster 2) from both sides of equation 1 and then dividing by the percentage of L1 in cluster 3, we obtain:

\[
\text{total } L1 - \left( \% L1 \text{ in cluster } 2 \right) \times \left( \text{total LDH due to cluster } 2 \right) = \left( \% L1 \text{ in cluster } 3 \right)
\]

Since we have estimated the total LDH due to cluster 2 and have measures or estimates of the other quantities on the left side of the equation, we now can use this to compute the other previously unknown quantity, the total LDH due to cluster 3. We have solved two equations for two unknown quantities.

* In the method we have developed, we actually use corrected values for total L1 and L2—we first subtract the mean L1 values from total L1 and the mean L2 from total L2. The percent of L1 and L2 in each cluster are averages of the published isoenzyme proportions across each organ in the cluster.
We can analogously do this by writing five equations (for the total values of each isoenzyme, 1–5) to solve for five unknowns (the total LDH due each isoenzymatically similar cluster of organs); i.e., we assume:

\[ L = \beta X + \mu \]

where

- \( L = \{ L_i \} \) indicates the observed value of each isoenzyme, \( i = 1, \ldots, 5 \)
- \( X = \{ X_{ij} \} \) represents the proportion of isoenzyme \( i \) in organ cluster \( j \) (as defined in the Methods section), \( i = 1, \ldots, 5 \), and \( j = 1, \ldots, 5 \).
- \( \mu = \{ \mu_i \} \) is the population mean value for each isoenzyme.

Then, given the observed values of \( L \) and published or derived values of \( X \) and \( \mu \), we solved for \( \beta = (\beta_1, \beta_2, \beta_3, \beta_4, \beta_5) \), which is the amount of total LDH contributed by organ clusters 1 to 5. This method has the clinical advantage of allowing crude estimation of all five organ clusters but does not provide confidence intervals for them.

A second method to derive organ-specific estimates is through regression analysis. Because we have only five measurements (isoenzymes) at each time, we decided to try to estimate and provide confidence intervals for three clusters, at most. In our cases, the heart-, liver-, and lung-related types accounted for nearly all of the variance and thus seemed sufficient; however, the investigator may use any three of the five listed above.

To estimate the organ sources we then assume:

\[
L = \mu + \beta X + \epsilon \\
X = (X_1, X_2, X_3) \\
O = (O_1, O_2, O_3) \\
E(\epsilon) = O V(\epsilon) = \sigma^2 I \text{ and } \epsilon \sim N(O, \sigma^2 I)
\]

Although intuitively a weighted regression analysis (20) would seem potentially preferable to this method, to account more adequately for interdependencies between the residuals, we have found empirically that assuming independence provides much more adequate predictions in terms of the total variance accounted for by the regression. This may be due to differences in the correlations between errors for different types of cases (myocardial infarction, congestive heart failure, etc.). Better estimates of these could eventually provide further improvements, but such improvements are likely to be minor in view of the current performance levels.

A more important reason for caution in the interpretation of the results of the regression is that the confidence intervals are probably somewhat narrower than the true intervals, which would really include the possibility of errors in the published parameter estimates between and within individuals. This is not a serious flaw, because the potential error from false positives is generally less than that from false negatives, and because clinical judgment ultimately must be used in interpreting the clinical significance of the results anyway. In the future, however, as better estimates of the errors in our parameter estimates become available, we may be able to obtain more nearly accurate confidence intervals through errors-in-variables methods (21).

A final reason for caution arises from the possibility of negative results for the organ-specific estimates. We recognized that these might have been due to systematic parameter error—the use of average values for the proportions of each isoenzyme in each organ cluster rather than proportions for an individual organ (e.g., the exact published proportions of each isoenzyme in the heart when solely the heart is expected to contribute). However, perhaps because of other, nonsystematic individual variations in parameters, and because multiple organs in a given cluster may simultaneously contribute, we found empirically that models with parameters based on individual organs performed more poorly. Thus, we decided to recommend use of the present model, with two important safeguards. A "danger signal" from the computer program can be emitted (a) if negative results occur beyond a (conservatively) significant range of analytical variability or (b) if the correlation between actual and predicted total LDH falls below a predefined criterion. The clinician can then choose whether to ignore the results.

Fortunately, our model seldom performed poorly. To test its adequacy, we computed the correlation between the "predicted" and actual total LDH as the multiple correlation between the mean isoenzyme proportions in each organ cluster and the observed total LDH, in excess of the normal mean (with the regression intercept term set equal to zero). By this criterion, a poor fit of the original regression model (r < .8) occurred in less than 4% of all the (nearly 400) measurements we analyzed.

One might criticize our use of correlations as an index of the adequacy of our model, because they are not very sensitive to deviations in the "beta weights" (here the organ-specific estimates). Also, the way we have chosen to compute them (by setting the intercept equal to zero) does not enable us to interpret them as indicating the ability of the model to make predictions, beyond those of the mean total LDH values in our population (which would be possible if an intercept were used). While we did correct the total LDH to be predicted for the normal mean values before the analysis, we intentionally decided not to include an intercept (and thus correct also for the overall means). Our reason for not doing this was that the overall means would be influenced by different disease types and extents in different cohorts of patients and thus would be of little use in making predictions in future cases. Thus, computing a correlation (with an intercept) that could be interpreted as the model's ability to improve upon predictions from the overall means would have had little significance. Hence, we believe that our method of computing correlations was the fairest indicator of model performance. Also, we provided even more compelling evidence of the adequacy of our model. We showed that it outperformed any existing standard diagnostic indices in the actual diagnosis of acute myocardial infarction.

Recognizing that the mere possibility of negative values in organ-specific estimates, despite their lack of effect on model performance, still could disturb some clinicians, we developed yet a third method for computing them. The derivation of this method had a less clear interpretation, but it almost assured elimination of potentially negative values for the organ-specific estimates.

\[
\text{If we define } P_l = \frac{L_l - \mu_l}{L_T - \mu_T} \text{ where } L_T = \sum_{i=1}^{5} L_i
\]

CLINICAL CHEMISTRY, Vol. 32, No. 8, 1986 1523
and then suppose \( \log \frac{P_i}{1 - P_i} = \beta' X_i + e_i \quad i = 1, \ldots, 5 \)

and if we use logistic regression to estimate

\[ \beta = [D' D]^{-1} D' L_T \]

then \( \beta = \left[ \begin{array}{c} \beta_1 \\ \beta_j \end{array} \right] (L_T - \mu_T) \) is an estimate of the contribution of organ cluster \( j \) lying in the interval \([0, L_T - \mu_T]\).

This method produces results indistinguishable from the previous two in terms of overall accuracy. In the future, we will need to see whether it will produce better or worse discrimination than the alternative methods on a larger sample of cases.

In preparing this paper, we benefited from discussions with Peter Kampthorne, Donna McClish, and Robert Galen. We also wish to thank Timothy Clark and J. J. Loes for their assistance.

Dr. Politzer is recipient of Research Career Development Award LM0080 from the National Library of Medicine. Dr. Powell is a Kaiser Family Foundation faculty scholar in general internal medicine. This research was supported in part by grants LM04132 and LM04088 from the National Library of Medicine, by the Veterans’ Administration Health Services research program, and by the Keck Foundation.

References