Increased Lactate Dehydrogenase Isoenzyme 1 in Serum and Tumor Tissue of a Patient with Small-Cell Carcinoma

Robert H. Christenson,¹ ² Mark W. Scroggs,¹ ² and J. David Odom III¹

Histological examination of supraclavicular lymph node tissue obtained at biopsy from a 63-year-old man disclosed metastatic small-cell carcinoma. On admission and for four days subsequently, total lactate dehydrogenase (LD; EC 1.1.1.27) activity in serum was 6.5 times normal; studies of LD isoenzyme showed persistently increased LD-1, with LD-1 >LD-2. Isoenzyme electrophoresis of tissue homogenates prepared from the patient’s tumor also showed the LD-1 >LD-2 pattern. Isoenzyme studies for supraclavicular lymph node tissue from five control subjects showed contrasting isoenzyme patterns as compared with the patients in whom LD-2, LD-3, and LD-4 predominated. Because these abnormalities were persistent, they differ from the temporal sequence for LD usually seen in myocardial infarction. This emphasizes the importance of repetitive sampling for clinical interpretation of data on this enzyme.

Additional Keyphrases: cancer · lymph node tissue

Structurally, lactate dehydrogenase (LD; EC 1.1.1.27) is a tetramer, composed of four peptide-chain subunits. Each peptide-chain subunit is one of two electrostatically distinct types, either M (also designated A) and H (or B), accounting for the five isoenzyme forms of LD classified by electrophoresis. Ordinarily the ratio of LD-1 to LD-2 activity in serum is <0.76. In more than 80% of patients with myocardial infarction, however, this ratio is reversed, producing a pattern in which LD-1 exceeds LD-2. Monitoring total LD activity and fractionation of serum LD isoenzymes, particularly LD-1, LD-2, and the ratio of the two, helps in the differential diagnosis of myocardial infarction. Interpretation of LD isoenzyme patterns is complicated, because LD-1 is widely distributed and the LD-1 >LD-2 pattern may be seen in association with other conditions, including hemolysis and infarction of the renal cortex.

In this report, we describe a case of small-cell carcinoma that showed consistently increased total LD activity, an isoenzyme pattern with persistent increase in LD-1, and the LD-1 >LD-2 pattern in serum. A predominance of LD-1 and the LD-1 >LD-2 isoenzyme pattern were also found in surgically removed cancerous tissue from our patient but not in similar noncancerous tissue from controls.

Case History

This 63-year-old black man was hospitalized after recently developing night sweats, chills, and a palpable mass in the area of the left supraclavicular lymph nodes. Soon after hospital admission, histological examination disclosed metastatic small-cell carcinoma in a scalene lymph node biopsy. His past medical history included 60 pack-years of cigarette smoking and ethanol abuse, but the patient had no history or clinical signs of heart disease. At admission his total creatine kinase (EC 2.7.3.2) activity in serum was 22 U/L (normal reference interval 20–130 U/L), values for vitamin B₁₂ and folate were within normal limits, total LD activity was 917 U/L (normal reference interval 50–140 U/L), and the isoenzyme pattern was LD-1 >LD-2. Follow-up LD studies, done on each of the next four days, showed total LD activity and isoenzyme patterns consistent with those done on admission. Following a brief clinical course, the patient died of cardiopulmonary arrest.

Post-mortem examination showed a large necrotic tumor mass surrounding the bronchus and extending into the upper lobe of the patient's right lung. The mass fulfilled histological criteria for small-cell carcinoma; the bronchus was concluded to be the primary anatomical site of the patient’s disease. Although the patient’s left anterior descending coronary artery contained a focal atherosclerotic plaque, with about 20% occlusion, all coronary arteries were patent, and careful examination of the myocardium revealed no evidence of infarction.

Materials and Methods

Patient and control samples. Serum and cancerous left supraclavicular lymph node tissue were obtained from the patient and evaluated for LD isoenzymes. For use as controls, we obtained noncancerous left supraclavicular lymph node specimens from five subjects, three at autopsy and two at surgery.

The patient’s serum used for the LD isoenzyme studies was separated from cellular material within an hour of collection and stored no longer than 12 h at room temperature. Supraclavicular lymph node specimens, obtained from the patient and five controls, were immersed in a minimum volume of isotonic saline (NaCl, 150 mmol/L) and stored for not more than 24 h at 4 °C.

Tissue preparation. To prepare tissue extracts for the patient and controls, we made a cross-sectional incision approximately 5 mm from the tip of each supraclavicular lymph node. To each 5-mm lymph node tip, we added three drops of freshly prepared isotonic saline. The tissues were...
then vigorously ground to a fine slurry in a Dounce tissue homogenizer. Each tissue slurry was centrifuged for 5 min at 1000 x g; the resulting supernate was used for isoenzyme analysis.

Enzyme and isoenzyme analysis. We measured total LD activity kinetically at 30 °C by monitoring, at 340 nm, the reduction of NAD\(^+\) during the LD-catalyzed lactate-to-pyruvate reaction ( Worthington Statzyme\textsuperscript{a} LD (L-P) kit; Cooper Biomedical, Freehold, NJ 07728). We previously had determined a normal reference interval of 50–140 U/L for total LD activity in serum, using the reagents and conditions stated above.

LD isoenzymes, separated by electrophoresis on agarose gel, were detected by use of an LD staining kit containing NAD\(^+\), Nitro Blue tetrazolium chloride, methoxyphenazine methosulfate, and lithium 1-lactate (all from Corning Medical, Medfield, MA 02052). The relative activity of each isoenzyme on the gel film was determined by densitometry at 600 nm. Our laboratory’s normal reference intervals for serum LD isoenzymes are: 15–27% for LD-1; 28–38% for LD-2; 18–26% for LD-3; 7–15% for LD-4; and 7–17% for LD-5.

Results

Table 1 shows the patient's data for total LD activity, percent LD-1, and LD-1/LD-2 ratio in serum as measured on each of the four days after hospital admission. For total LD in serum during this period, we observed activities that consistently were 6.5 times the upper limit of the normal range. Concurrent serum LD isoenzyme assay during this same period showed persistently increased LD-1, as compared with normal. For all serum specimens we obtained during this four-day period, the LD-1/LD-2 ratio was greater than unity.

On the fourth day of hospitalization a left supraclavicular lymph node was resected from the patient, and blood was obtained concurrently. The LD isoenzyme patterns, and densitometric scans we obtained after electrophoresis of the patient’s serum and tumor tissue homogenates, are shown in representations a and b of Figure 1. A comparison of the increases in percent LD-1, and the LD-1 >LD-2 patterns, indicate that the source of the patient’s abnormal serum LD-1 activity is tumor.

LD isoenzyme data for supraclavicular lymph node tissue from the patient and control subjects are shown in Table 2. For all five control-subject tissues, the isoenzyme proportions are strikingly similar, the composite activities for LD-2, LD-3, and LD-4 in these normal tissues accounting for 79% ± 2% (mean ± SD) of total LD activity. For our patient's tissue, however, LD fractionation revealed markedly different proportions for these isoenzymes. Their composite activities in the cancerous tissue accounted for only 51% of the total, nearly 30% (or 15 SD) less than in the controls.

Table 1. The Patient’s Total LD and LD\(_1\)/LD\(_2\)

<table>
<thead>
<tr>
<th>Time after admission, h</th>
<th>Total LD acy, U/L</th>
<th>LD(_1) acy, % of total LD</th>
<th>LD(_1)/LD(_2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>917</td>
<td>37</td>
<td>1.09</td>
</tr>
<tr>
<td>24</td>
<td>952</td>
<td>34</td>
<td>1.03</td>
</tr>
<tr>
<td>48</td>
<td>1090</td>
<td>34</td>
<td>1.03</td>
</tr>
<tr>
<td>72</td>
<td>1050</td>
<td>35</td>
<td>1.06</td>
</tr>
<tr>
<td>96</td>
<td>987</td>
<td>36</td>
<td>1.09</td>
</tr>
</tbody>
</table>

Table 2. LD Isoenzyme Patterns (%) for Tissue from the Patient and Noncancerous Controls

<table>
<thead>
<tr>
<th>Patient</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>LD-1</td>
<td>34</td>
<td>9</td>
<td>11</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>LD-2</td>
<td>24</td>
<td>18</td>
<td>17</td>
<td>13</td>
<td>16</td>
</tr>
<tr>
<td>LD-3</td>
<td>15</td>
<td>37</td>
<td>31</td>
<td>37</td>
<td>38</td>
</tr>
<tr>
<td>LD-4</td>
<td>12</td>
<td>26</td>
<td>28</td>
<td>28</td>
<td>25</td>
</tr>
<tr>
<td>LD-5</td>
<td>16</td>
<td>10</td>
<td>13</td>
<td>17</td>
<td>13</td>
</tr>
</tbody>
</table>

\* All control samples were from noncancerous suprachlavicular lymph nodes.

Data for LD-1 in tissue were also disparate between the controls and our patient. As shown in Table 2, LD-1 represented only 5–10% of total LD activity in control tissue specimens, but was the predominant isoenzyme in the patient’s tissue, accounting for 34% of total LD activity. Using data presented in Table 2, we calculated the LD-1/LD-2 ratio for the tissue samples. For the control group samples, this ratio averaged 0.47, substantially less than the ratio of 1.42 for the patient's tissue.

Discussion

We describe a patient with small-cell carcinoma and consistently increased serum LD activity that, upon fractionation, showed persistently increased LD-1 and LD-1 >LD-2 in the absence of heart disease, infarction of the renal cortex, or specimen hemolysis. Isoenzyme electrophoresis of tumor tissue homogenates from the patient also showed increased LD-1 and LD-1 >LD-2. Results of similar isoenzyme studies of noncancerous lymph node tissue from a group of control subjects, however, were markedly dissimilar, with LD-2, LD-3, and LD-4 predominating.

LD isoenzymes in tissue from human breast tumor (2), adenocarcinoma of the colon (3), and other tumor types (4, 5) reportedly show LD fractionation patterns in which LD-4 and LD-5 predominates. A prevalence of LD-4 and LD-5 in tumor tissue is not ubiquitous, however. In fact, some cancers typically show a predominance of LD-1 as well as the LD-1 >LD-2 pattern, as was seen in our patient. Such cancers include adenocarcinoma of the ovary (6), bronchial cancer (7), various stages of malignant hematological disease (8), seminoma (9), and selected germ cell tumors (10). According to a report by Tanaka et al. (4), LD fractionation studies with homogenates from small-cell carcinoma tissue typically show isoenzyme patterns in which LD-3 predominates. The patient we describe here, however, having histo-
logically verified small-cell carcinoma, showed increased LD-1 activity and the LD-1 \( \rightarrow \) LD-2 pattern in serum and tumor tissue, an observation we could not find previously documented.

In many patients having "uncomplicated" myocardial infarction, the activity of LD-1 exceeds that of LD-2 within 5 to 15 h after acute onset of symptoms, with peak total LD activity occurring 48 h after infarction (11). The patient presented here, having occult disease with no evidence of myocardial infarction, showed persistently increased total LD activity, increased LD-1, and the LD-1 \( \rightarrow \) LD-2 pattern throughout the four-day period studied. This difference from the temporal sequence characteristic of acute myocardial infarction emphasizes the importance of appropriate repetitive sampling for clinical interpretation of data for LD and its isoenzymes.

References