Association of an Oxygen-Sensitive Lactate Dehydrogenase Isoenzyme, LDₖ, with LD-6 in Serum of Critically Ill Patients

Victoria A. Onorato,¹ Kenneth F. Manly,¹ and Adrian O. Vladutiu²,³

We measured a highly unusual, oxygen-sensitive lactate dehydrogenase, LDₖ, in the serum of six patients whose serum showed a band for LD-6 on routine agarose gel electrophoresis for LD isoenzymes. All these patients showed very high serum LDₖ activity, greatly exceeding the high values previously described in serum of patients with various malignant tumors. In two of the patients, LDₖ activity was low both before LD-6 was found in and after it disappeared from the serum, evidencing a correlation with LD-6. All of the six patients, five of whom died in the hospital, had severe hypotension. We suggest that hypoxia is responsible for the appearance of LD-6 in serum and that LD-6 is found in association with high LDₖ activity in serum of critically ill patients.

Recently, an additional lactate dehydrogenase (LD, EC 1.1.1.27) band has been described in electropherograms of serum from patients with various diseases, most of them critically ill. Indeed, most of these patients die soon after this band is found (1–9). This LD isoenzyme, designated LD₆, migrates the most cathodal, very close to LD-5. Furthermore, the total LD activity in the serum of patients who show LD-6 is very high (1–4, 9).

The tissue origin of LD-6 in serum is not known, but it has been found postmortem in liver and skeletal muscle tissue (3, 4). Several recently reported (4) properties of this isoenzyme (e.g., greater molecular mass, more basic than the other LD isoenzymes) suggest that LD-6 is not a usual LD isoenzyme bound to immunoglobulins (10, 11).

We wondered (7) whether LD-6, as seen in routine electrophoresis, was related (or even similar) to an unusual, oxygen-sensitive LD isoenzyme, designated LD₂ (15), found in cell infected with Kirsten sarcoma virus and in many malignant-tumor cells. Therefore, we measured LD₂ activity in the serum of six patients who exhibited LD-6 on routine electrophoretic separation of LD isoenzymes. Four of these patients died within 24 h after LD-6 was found; one patient died within a month after this finding; another patient survived and recovered.

Materials and Methods

Patients. Six patients (Table 1) admitted to the Buffalo General Hospital had blood drawn into evacuated glass tubes (Becton Dickinson, Orangeburg, NY 10962). The patients were all critically ill at the time of blood collection. Their high values for total LD activity prompted LD isoenzyme fractionation.

LD isoenzyme assay. An agarose gel electrophoresis system (Universal Film; Corning Medical, Palo Alto, CA 94302) was used. Sera with very high total LD activity were diluted to 900 U/L before electrophoresis, to avoid substrate depletion. Sera were assayed within 12 h of collection. The reference interval for LD-5 was 6 to 16% of the total LD activity. Samples of sera were also assayed by electrophoresis without the lactate substrate but with the color reagent added.

LDₖ assay. Sera were stored at –80°C for no longer than a month before being assayed. We had assured ourselves that LDₖ activity did not decrease during such storage. LDₖ was separated electrophoretically to measure its activity, as described by Anderson et al. (13). The gels were fixed in methanol/acetic acid/water (9/9/2 by volume) and scanned with a densitometer. The reference interval for LDₖ (i.e., the range of values we found for healthy individuals) was 0–10 U/0.1 mL (14). The units are not similar to the units used to express the total LD activity, but are arbitrary units as previously reported (12–15).

We also assayed LD₂ in the absence of the lactate substrate, substituting either 20 mL of glycerol or 17 mmol of ethanol per liter in incubating the plates. LD₂ purified from rat muscle by ultracentrifugation and column chromatography (G. R. Anderson and B. K. Farkas, submitted for publication to J. Biol. Chem.) was run as a control on each plate.

Other assays. Total LD, alanine aminotransferase, and aspartate aminotransferase activities were measured in serum with a continuous-flow analyzer (SMA-II; Technicon Instruments Corp., Tarrytown, NY 10591). Creatine kinase activity was assayed with commercial reagents (Biodynamics/BMC, Indianapolis, IN 46250). Arterial blood pH was measured with an IL 813 instrument (Instrumentation Laboratory, Inc., Lexington, MA 02173). The reference interval for LD was 100–250 U/L; for creatinine, 20–160 U/L; for alanine aminotransferase and aspartate aminotransferase, 5–50 U/L; and for arterial blood pH, 7.35 to 7.45.

Results

No disease common to all six patients was identified. However, at the time LD-6 was observed in the serum all had multiple organ failure with hypotension, and five patients had documented episodes of hypoxemia and acidosis, which usually appeared during a respiratory arrest just preceding the LD isoenzyme assay. Of the six patients, five died (four shortly after LD-6 was observed) and one survived and recovered.

LD isoenzymes. LD-5 activity was high in all six patients; indeed by densimetry of the electrophoretic pattern this isoenzyme appeared to have the highest activity. In our electrophoresis system, LD-6 migrated very close to LD-5. In electropherograms for two patients additional bands were seen cathodal to LD-5 (Figure 1) or between LD-4 and LD-5 (Figure 2). LD-6 was defined as the first band cathodal to LD-5. The other additional LD isoenzymes were not quantitated. The percentages of LD-6 in all patients is given in Table 1.

When we assayed serum samples for LD isoenzymes without addition of the substrate reagent, no color was
Table 1. Clinical Findings in Six Patients with LD-6 in Serum

<table>
<thead>
<tr>
<th>Pl. no.</th>
<th>Age, sex</th>
<th>Total LD (U/L)</th>
<th>LD&lt;sub&gt;k&lt;/sub&gt; (U/0.1 mL)</th>
<th>LD-6&lt;sup&gt;a&lt;/sup&gt;</th>
<th>pH</th>
<th>CK&lt;sup&gt;b&lt;/sup&gt;</th>
<th>ALT&lt;sup&gt;b&lt;/sup&gt;</th>
<th>AST&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Diagnosis</th>
<th>Hospital course</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>76, F</td>
<td>2650</td>
<td>1050</td>
<td>11.4</td>
<td>7.14</td>
<td>329</td>
<td>1990</td>
<td>850</td>
<td>Coronary disease</td>
<td>Cardiogenic shock; died a month later</td>
</tr>
<tr>
<td>2</td>
<td>67, F</td>
<td>8700</td>
<td>4600</td>
<td>13.1</td>
<td>7.17</td>
<td>397</td>
<td>6850</td>
<td>11 300</td>
<td>Coronary disease</td>
<td>Coronary bypass surgery; renal failure; died 12 h later</td>
</tr>
<tr>
<td>3</td>
<td>69, M</td>
<td>&gt;10 380</td>
<td>7100</td>
<td>15.7</td>
<td>7.07</td>
<td>1286</td>
<td>2990</td>
<td>&gt;6000</td>
<td>Coronary disease</td>
<td>Cardiogenic shock; died 12 h later</td>
</tr>
<tr>
<td>4</td>
<td>84, F</td>
<td>ND&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4200</td>
<td>12.5</td>
<td>7.10</td>
<td>577</td>
<td>ND</td>
<td>ND</td>
<td>Cardiomyopathy; congestive heart failure</td>
<td>Respiratory arrest; died 24 h later</td>
</tr>
<tr>
<td>5</td>
<td>77, M</td>
<td>4326</td>
<td>723</td>
<td>10.7</td>
<td>7.10</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>Acute myocardial infarction; pulmonary edema</td>
<td>Respiratory arrest; died 12 h later</td>
</tr>
<tr>
<td>6</td>
<td>68, F</td>
<td>27 600</td>
<td>5200</td>
<td>13.8</td>
<td>6.99</td>
<td>2185</td>
<td>9750</td>
<td>&gt;30 000</td>
<td>Generalized atherosclerosis</td>
<td>Respiratory arrest; died 12 h later</td>
</tr>
</tbody>
</table>

<sup>a</sup>Expressed in percentage of total LD activity. <sup>b</sup>CK, creatine kinase; ALT, alanine aminotransferase; AST, aspartate aminotransferase. <sup>c</sup>ND, not determined after the finding of LD-6.

---

**Fig. 1. Electrophoretic pattern of LD isoenzymes, showing two additional bands cathodal to LD-5**
Note intense staining of LD-4 and LD-5. Anode is at the left.

**Fig. 2. Electrophoretic pattern of LD isoenzymes showing additional bands, two between LD-4 and LD-5, and two post-LD-5**
Note the very intense staining of LD-4. Anode is at the left.

---

As expected, when the LD<sub>k</sub> assay was performed, substituting glycerol for lactate as a substrate, the intense band that represented LD<sub>k</sub> disappeared but the faint band remained (Figure 3) and indeed was intensified when ethanol was substituted for lactate (Figure 4), suggesting that the second band is probably an alcohol dehydrogenase. Patients with carcinoma did not show the second, faint band in their serum but only the LD<sub>k</sub> band (Figure 3 and ref. 14).

**Other assays.** Total LD activity was markedly increased in serum at the time LD-6 was observed; the values ranged from 945 to 27 600 U/L (Table 1). The activities of alanine aminotransferase and aspartate aminotransferase were very high (range, 1990 to 9750 U/L for the former and 850 to >30 000 U/L for the latter) and the creatine kinase activity was also increased (range, 329 to 2185 U/L).

In all these patients the arterial blood pH was subnormal (range, 7.17 to 6.99).

**Discussion**

The presence of LD-6 in serum signifies a very poor prognosis. Many patients were moribund at the time LD-6 was found and most died in the hospital. Of the 51 patients reported so far (1–6, 9), 43 (82%) died within a month from the time LD-6 was detected in their serum. We could not find obvious differences between the patients in our study who died and the patient who survived, except that their arterial blood pH was lower. In our hands, the serum LD-6
activity persisted after more than six months of storage in the freezer, even though there were several freezing–thawing cycles during this time.

All our patients and the great majority of those described by others (3, 4) had hypotension and acidosis. How long should hypoxia persist and how severe it must be to induce LD-6 in organs and its subsequent release in serum is not known. We have studied a 92-year-old male with chronic obstructive lung disease and pneumonia that accounted for a marked respiratory insufficiency, with arterial blood pH 7.20 and arterial pO₂ 40 mmHg. The LD activity was 2290 U/L, with LD-5 composing 56% of the total. However, we did not find LD-6 in his serum and LD₅ activity was only 83 U/0.1 mL. This shows that an increase in total LD and LD-5 activities in serum is not always accompanied by the appearance of LD-6. Probably hypoxia also explained the high activities of creatine kinase, alanine aminotransferase, and aspartate aminotransferase released from muscles and liver in the serum of our patients.

Very recently, it was reported that LD-6 from the liver is not lactate dependent and may in fact be an ethanol dehydrogenase (8). However, we saw LD-6 bands (albeit very weak) in the absence of lactate in only two patients. The LD₅ assay revealed both a true LD activity and an ethanol dehydrogenase activity, the former substantially exceeding the latter. Until it is clearly proven that the sixth band seen in LD electrophoresis of the serum of critically ill patients is not lactate dependent, we prefer to refer to this dehydrogenase by the established designation, LD-6 (1–6).

LD₅ has been found in cells infected with Kirsten murine sarcoma virus; anaerobic shock can induce transcription of the genes for this protein in uninfected rat cells (15). In previous studies it has been shown that liver and striated muscle contain LD₅ (13). Tumor tissues have 10- to 100-fold greater LD₅ activity than does adjoining non-tumor tissue (13).

Our findings in the patients just described suggest that LD₅ and LD-6 are related in some way. Indeed, both isoenzymes are cathodal, more basic than the other LD isoenzymes. The estimated pI of LD₅ is >8.6 (13) and the pI of LD-6 ranges between 9.0 and 9.6 (4). A purified LD₅ preparation with a total LD activity of 960 U/L, obtained from rat muscle, showed on aerobic LD electrophoresis one band only, exactly at the same location as LD-6. The presence of LD-6 in serum was always accompanied by a very high activity of LD₅, and in the serum from a patient LD₅ activity markedly decreased when LD-6 disappeared. Moreover, in another patient the LD₅ activity was very low before the finding of LD-6 and drastically increased when LD-6 was found in the serum.

It may be that LD-6 and LD₅ are distinct but related isoenzymes, induced in some organs (mainly liver) and released from them into the circulation during severe hypoxia. We do not know whether the most cathodal faint bands seen in the anaerobic electropherograms for serum LD₅, which seem to represent ethanol dehydrogenase, are part of LD-6 as it appears in routine LD isoenzyme (aerobic) electrophoresis or are related isoenzymes. It is likely that severe hypoxia accounts for the release from organs (e.g., liver and striated muscle) and the detection in serum (and perhaps for the induction in some organs) of several dehydrogenases, including ethanol dehydrogenase and a lactate dehydrogenase (LD₅).

The fact that LD₅ activity is increased in malignant tumors and also in serum from some patients with various carcinomas (14) might imply that LD-6 could also be found in tumors if LD-6 and LD₅ were related. However, the LD₅ activities in serum from patients with cancer have been much lower than those from patients with LD-6 (14).

Antibodies to LD₅ could help to detect smaller amounts of this enzyme in an immunoassay, and together with antibodies to LD-6 could be used to better define the relationship between LD-6 and LD₅.

This work was supported in part by a grant from the Sklarow Fund. We thank Dr. G. Anderson (Roswell Park Memorial Institute) for the gift of purified LD₅ and Mrs. Jacqueline Teitz for typing the manuscript.

References


