Unreliability of Immunochemical Determination of Lactate Dehydrogenase Isoenzyme-1 in Heparinized Plasma
Gifford Lum

Paired serum and heparinized plasma samples were assayed simultaneously for lactate dehydrogenase (EC 1.1.1.27) isoenzyme 1 (LD1) activity by a commercially available immunochemical procedure. For all sera specimens tested, only LD1 activity was detected. For heparinized plasma, random discrepancies in LD activity were noted at normal (Group I), borderline (Group II), and increased (Group III) total LD activity. Incomplete precipitation of LD-M subunits was confirmed by electrophoresis and occurred in eight of 15, four of 13, and eight of 22 instances (total: 20/50, or 40%) with a mean difference of 13, 10.6, and 10.2 U/L (8.3, 4.0, and 4.4%) in Groups I, II, and III, respectively. We conclude that heparinized plasma is an unsuitable sample for the immunochemical determination of LD1.

Additional Keyphrases: serum vs plasma samples • lactate dehydrogenase M-subunits • analytical error

Measurements of lactate dehydrogenase (EC 1.1.1.27; L-lactate:NAD+ oxidoreductase, LD) isoenzymes provide valuable clinical information, especially for the diagnosis of myocardial infarction. For the past six years, this laboratory has performed electrophoretic separation of the LD isoenzymes for patients with suspected acute myocardial infarction and for post-cardiac surgery patients. Because of the relatively large numbers of LD isoenzyme requests (approximately 35–40 per week), and in an effort to provide better laboratory service for the medical and surgical staffs at nights and on weekends, I decided to investigate the feasibility of substituting an immunochemical procedure for LD1 ("Isoimmune-LD"; Roche Diagnostic Systems, Nutley, NJ) for the present electrophoretic method.

Although the manufacturer of this kit specifically recommends the use of serum only for this assay, I decided to evaluate the suitability of using heparinized plasma: physicians often submit heparinized plasma samples; furthermore, because timing is extremely crucial in assessing the evolution of the LD isoenzyme pattern after myocardial infarction, and because clinical judgment is based on time-dependent specimens that cannot be retrieved or repeated, it is crucial to know whether there are any significant differences between heparinized plasma and serum for this procedure. I also subjected electrophoresis the total LD sample and the supernate obtained after immunochemical treatment (representing LD1 isoenzyme), to confirm the specificity of the immunochemical procedure for LD1.

Materials and Methods

Serum and heparinized plasma samples were collected simultaneously from unselected ambulatory and hospital-
chemically different between serum and plasma for LD₃ was detectable by electrophoresis.

Figure 1 illustrates the complete vs incomplete precipitation of LD-M subunits for paired serum and heparinized plasma samples (total LD activity = 280 U/L). Panel B shows complete precipitation of LD-M subunits in the serum specimen. In contrast, residual amounts of incompletely precipitated LD₂, LD₃, and LD₄ (16, 14, and 11%, respectively) were found in the matched plasma sample (panel D). These Figures demonstrate the effectiveness of immunoprecipitation with serum and the occasional ineffectiveness with heparinized plasma; they are representative of the discrepancies noted in all three groups of total LD activity.

Overall, for the patients' samples exhibiting discrepancies, the LD isoenzyme patterns for the heparinized plasma samples were (percent of total LD, mean ± SD) LD₁, 67.9 ± 10.5% (range 44.9–83.6%), LD₂ 12.3 ± 3.3% (range 8.2–20.9%), LD₃ 12.2 ± 5.2% (range 5.1–20.3%), and LD₄ 8.0 ± 3.5% (range 1.6–13.5%). LD₅ was usually not detected, but in five instances LD₅ was present in negligible amounts, ranging from 0.7 to 2.7%.

Thirty patients' samples showed no discrepancies between the LD₁ in serum and that in heparinized plasma samples (mean differences for LD₁, 0–3 U/L). Completeness of precipitation of LD-M subunits was confirmed by electrophoresis, which showed the presence of only LD₁ in all supernates tested. Hence, the incomplete precipitation of LD₂–LD₅ in samples of heparinized plasma is a random phenomenon because in many heparinized samples, only LD₁ was detected.

I attempted to elucidate the reason for incomplete precipitation of LD₂–LD₅ in several in vitro experiments. Heparin alone (50 to 5000 USP units/mL), added to a serum pool, did not inhibit in vitro binding of the anti LD-M antibody to LD-M subunits; only LD₁ was detected in the supernate of the heparin-treated specimen. Also, human fibrinogen, whether added to a serum pool alone (200 and 400 mg/mL to simulate normal serum concentrations) or with heparin (in above-normal concentrations of 1000 and 5000 USP units/mL), did not interfere with the immunochemical determination of LD₁ in the treated pooled serum specimen.

Discussion

Previous reports have focused primarily on the use of immunochemical LD₁ activity in clinical settings and in the use of this assay as an aid in the diagnosis of acute myocardial infarction (1–4). There have been no reports of comparison of results for LD₁ isoenzymes in serum and in heparinized plasma, and there are no instructions regarding the specificity of the anti-LD-M antibody for LD-M subunits in heparinized plasma in the manufacturer's insert. No claims for the reliability of LD₁ activity in heparinized plasma have been made by the manufacturer.

Previous investigators (5, 6), evaluating the capability of the anti-LD-M antibody in various situations, found that incomplete precipitation of the LD-M subunits occurred only in sera with high LD activities (>400 U/L), but that such discrepancies disappeared when the samples were appropriately diluted (5).

The reason for the discrepant results reported here for the immunochemical determination of LD₁ in heparinized plasma remains undetermined. Clearly, however, these discrepancies, occurring randomly in 40% of cases, make heparinized plasma an unsuitable specimen for the immunochemical assay.

I acknowledge the technical assistance of Teresa Sanford and Suzanne Carroll, who performed the electrophoretic separation of LD isoenzymes.

References


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Table 1. Activity of Immunochemically Determined LD₁ Isoenzyme in Serum and Heparinized Plasma in Discrepant Cases (Mean ± SD, and Range)

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum Activity</th>
<th>Plasma Activity</th>
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<tbody>
<tr>
<td>Group I (n = 8): normal total LD activity</td>
<td>143.3 ± 9.9</td>
<td>29.6 ± 7.5</td>
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<td>(127–158)</td>
<td>(14–39)</td>
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<tr>
<td>Group II (n = 4): borderline increased total LD activity</td>
<td>290.3 ± 23.6</td>
<td>48.7 ± 20.2</td>
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<td></td>
<td>(244–291)</td>
<td>(36–72)</td>
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<tr>
<td>Group III (n = 8): increased total LD activity</td>
<td>483.1 ± 132.3</td>
<td>146.4 ± 70</td>
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<td></td>
<td>(324–668)</td>
<td>(86–249)</td>
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Fig. 1. LD isoenzyme pattern in serum before (A) and after (B) immunochemical treatment, and in a paired sample of heparinized plasma before (C) and after (D) immunochemical treatment.

Note presence of residual amounts of LD₂–LD₅ in panel D. Total LD = 280 U/L.