Falsely Negative Laboratory Diagnosis for Myocardial Infarction Owing to the Concurrent Presence of Macro Creatine Kinase and Macro Lactate Dehydrogenase

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Macro creatine kinase (CK, EC 2.7.3.2) and macro lactate dehydrogenase (LD, EC 1.1.1.27) were both present in the serum of a 70-year-old woman with myocardial infarction. This interfered with the interpretation of the CK and LD isoenzyme analyses. Gel filtration and immunoprecipitation showed that the macro CK consisted of IgG and CK and the macro LD of IgG and LD. The IgG in this patient bound both MB and BB isoenzymes of CK, resulting in a macro CK complex that co-migrated with CK-MM on cellulose acetate electrophoresis. This situation led to a falsely negative laboratory diagnosis for myocardial infarction.

Lactate dehydrogenase (LD, EC 1.1.1.27) and creatine kinase (CK, EC 2.7.3.2) isoenzymes are useful in the diagnosis of acute myocardial infarction. Variants of these isoenzymes may interfere with their interpretation and quantification (1-8).

Macro CK, which in most cases is a complex between IgG and CK-BB, occurs in 0.5-1.5% of patients in whom CK isoenzymes are determined (2). This complex usually migrates between CK-MM and CK-MB on electrophoresis (2), but in some cases it co-migrates with CK-MM (8). The presence of macro CK can result in a false laboratory diagnosis of acute myocardial infarction with both ion-exchange (6) and immunoinhibition methods (1, 7).

Macromolecular LD is a complex between one or more isoenzymes and circulating IgA (3), IgG (4), or β-lipoproteins (5). The presence of macro LD may result any of the following on electrophoresis: abnormal number of LD isoenzyme bands, altered electrophoretic mobility of bands, and band broadening (4).

We describe here the findings in a patient with acute myocardial infarction who had both macro LD and macro CK in her serum, which interfered with the interpretation of the CK and LD isoenzyme patterns on electrophoresis.

Case History

A 73-year-old woman was admitted to the hospital for investigation of left-side weakness suspected to be due to ruptured intracranial aneurysm. She had had a myocardial infarction 10 years earlier. She was being treated with propranolol for angina, eltroxin for hypothyroidism, and sulindac for arthritis. Of significance at the time of admission were the above-normal LD and CK activities: 494 U/L (normal range 80–210 U/L) and 588 U/L (normal range 21–215 U/L), respectively.

While in the hospital, the patient developed sudden chest pain. The total activity of CK, LD, and aspartate aminotransferase (AST; EC 2.6.1.1) followed a pattern typical of myocardial infarction (9). Table 1 summarizes the data on enzyme changes. However, the isoenzyme patterns, as determined by electrophoresis, were unusual. No MB band was seen at any time after the infarction. The LD isoenzyme pattern was also difficult to interpret because of the abnormal electrophoretic mobility of the isoenzyme bands (see Figure 2).

The electrocardiogram showed ST-segment elevation and T-wave inversion, indicating an inferior infarction. A radionuclide scan with 99mTc-pyrophosphate confirmed the presence of a recent myocardial infarction. The patient's serum was examined for macro CK and LD.

Materials and Methods

Total CK, LD, and aspartate aminotransferase activities were determined with an aca (Du Pont, Wilmington, DE 19898) discrete analyzer.

LD and CK isoenzymes were separated by cellulose acetate electrophoresis (Helena Laboratories, Beaumont, TX 77704) according to the manufacturer's instructions. Serum immunoglobulins were measured by radial immunodiffusion (Behring Diagnostics, Hoechst Canada Inc., Montreal, Quebec H4R 1R6, Canada).

To estimate the molecular size of the CK and LD isoenzymes, we fractionated 1.0 mL of the patient's serum and control serum on a 2.5 × 33 cm Sephadex G-200 column (Pharmacia, Uppsala, Sweden), with 150 mmol/L NaCl in 50 mmol/L Tris HCl, pH 7.1, as buffer. A series of molecular markers was run to calibrate the column. The eluate was collected in 5.0-mL fractions, in which the protein was monitored by measuring the absorbance at 280 nm. CK and LD elution was monitored by determining enzyme activity with the aca in each fraction after concentration (with Minicon B15 concentrators; Amicon Corp., Lexington, MA 02173).

The serum immunoglobulins IgG, IgA, and IgM were removed by immunoprecipitation. The appropriate ratio of specific antisera was incubated with serum or concentrated column fractions overnight to maximally precipitate these antibodies. CK and LD activities were determined in the supernates after centrifugation.

Results

Table 1 summarizes the total CK, LD, and AST activities before and after the patient's myocardial infarction. It is important to note that before the infarction the patient had a persistent increase in both LD and CK activity. The enzyme
Changes after the onset of chest pain were characteristic of myocardial infarction. The myocardial infarction was confirmed by electrocardiographic findings and $^{99m}$Tc-pyro-phosphate radionuclide scan. However, the CK and LD isoenzyme changes were not typical. The CK isoenzyme pattern was determined on several occasions after the onset of chest pain. No MB band was visible in any sample. Sample A (Figure 1) shows the CK isoenzyme pattern 6 h after onset of chest pain. The total CK activity peaked at about 19 h and the CK isoenzyme pattern (not shown) only showed a small shoulder on the anodal side of the CK-MM peak. The pattern was similar to that of sample E (Figure 1).

The patient's serum taken at 6 h after infarction was mixed with an equal volume of a commercial CK isoenzyme marker (Ortho Diagnostics Inc., Raritan, NJ 08869) containing all three human CK isoenzymes and with a serum specimen from a patient with a myocardial infarction. These samples along the unmixed specimen, diluted 1:1 with isotonic saline, were incubated overnight at 4 °C and electrophoresed on cellulose acetate. Figure 1 illustrates the CK isoenzyme patterns. The MB and BB bands were not apparent after mixing with the patient's serum. However, the intensity of enzyme activity in the CK-MM region increased. A small shoulder on the anodal side of the CK-MM band was also evident.

![Fig. 1. CK isoenzyme patterns photographed under fluorescent light](image)


The LD isoenzyme changes were also unusual (Figure 2). The LD isoenzyme pattern of the patient's serum 6 h after the infarction showed no distinct LD isoenzyme bands. Most of the LD activity was associated with a broad, asymmetric, slow-moving band with the peak near the normal position of LD 5. The normal LD bands were more apparent in the serum specimen taken 19 h after the infarction, when the LD activity was significantly increased. However, all of the bands were broadened relative to the normal isoenzymes. An LD 1:2 "flip" was apparent at this time, which is consistent with a myocardial infarction.

The LD and CK results were suggestive of the presence of both macro CK and macro LD isoenzymes in the serum of this patient, so we did Sephadex G-200 chromatography as described in Materials and Methods to estimate the size of the CK and LD isoenzymes. Figure 3 shows the elution pattern of the patient's CK activity in serum taken 19 h after the infarction. The larger CK activity peak has a relative molecular mass of approximately 100,000, which is the reported mass of

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Table 1. LD, CK, and AST Activities before and after Onset of Chest Pain in This Patient

<table>
<thead>
<tr>
<th>Hours after onset of LD chest pain</th>
<th>LD Activity, U/L</th>
<th>CK Activity, U/L</th>
<th>AST Activity, U/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>-72</td>
<td>434</td>
<td>588</td>
<td>33</td>
</tr>
<tr>
<td>0</td>
<td>362</td>
<td>524</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>370</td>
<td>756</td>
<td>60</td>
</tr>
<tr>
<td>19</td>
<td>524</td>
<td>1740</td>
<td>198</td>
</tr>
<tr>
<td>43</td>
<td>642</td>
<td>1304</td>
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</tr>
<tr>
<td>67</td>
<td>536</td>
<td>822</td>
<td>96</td>
</tr>
<tr>
<td>115</td>
<td>348</td>
<td>555</td>
<td>45</td>
</tr>
</tbody>
</table>

AST, aspartate aminotransferase.
normal dimeric human CK (10). However, 30% of the CK activity in this patient’s serum has an apparent relative molecular mass of 250 000. A control patient’s serum (not shown) showed a single peak of CK activity matching the elution position of this patient’s low-molecular-mass peak.

LD activity in the serum specimen taken at 6 h after the infarct was eluted as a single asymmetrical peak on Sephadex G-200 (Figure 4). Its mean molecular mass was estimated at 200 000. This is higher than the mass of normal LD, which is approximately 140 000 (10). The serum specimen taken 19 h after the infarct was also eluted through this column (not shown). The LD activity peak eluted in a position between $M_r$ 160 000 and 200 000, with a skew towards the higher-molecular-mass side.

Immunoprecipitation was used to determine the nature of these macro enzymes. The column fractions containing high-molecular-mass CK activity eluted from the Sephadex G-200 column were pooled and concentrated. Removal of IgG by precipitation with specific antibody concomitantly removed 75% of the CK activity in this fraction. Removal of IgM and IgA did not influence the CK activity.

Immunoprecipitation of the LD activity was done, with use of unfraccionated serum. Antibody to IgG precipitated 80% of the total LD activity in the sample taken 19 h after infarction. Again, removal of IgG and IgM by immunoprecipitation had no effect on serum LD activity.

**Discussion**

Binding of CK and LD to immunoglobulins to form macro CK and macro LD, respectively, has already been described (1–8), but this, to our knowledge, is the first reported case of macro CK and macro LD appearing in the serum of the same patient.

The relative molecular mass of the macro CK activity (250 000) and its immunoprecipitation with antibody to IgG are consistent with a complex consisting of CK and IgG. Most previous reports have shown macro CK to be a complex of IgG and the isoenzyme CK-BB. In this patient the IgG forming the macro complex binds both MB and BB isoenzymes. In fact, this patient had a clearly demonstrated myocardial infarction on the basis of electrocardiographic results and $^{99m}$Tc-pyrophosphate scan, but did not have any evidence of MB on electrophoresis of her serum at any time after her infarction. We suggest that MB was released from the infarcted myocardial tissue and was bound by IgG to form a macro complex, which co-migrated with CK-MM.

On electrophoresis, macro CK usually migrates between MM and MB and can therefore be readily distinguished from the normal isoenzymes. In this patient the presence of macro CK-MB resulted in a falsely negative result by electrophoresis. This is in contrast to reports that macro CK results in falsely high values for apparent CK-MB if determined by immunoinhibition (1, 7) and ion-exchange methods (6).

The increased molecular mass and the precipitation of 80% of the total LD activity with antibody to IgG are consistent with the presence of an IgG:LD complex. However, the estimated molecular mass (approx. 200 000) of the macro LD was lower than the expected molecular mass of 250 000. The band broadening of the LD isoenzyme bands on cellulose acetate and the asymmetry of the LD activity peak on Sephadex G-200 suggest that the macro LD complex may have been partly dissociated during migration or elution. The sample taken 19 h after infarction shows even greater dissociability of the macro LD complex on both cellulose acetate, as indicated by the isoenzyme pattern, and Sephadex G-200, as evidenced by the even lower apparent relative molecular mass.

The pathogenesis and clinical significance of circulating antibodies capable of binding serum enzymes is not known. Macro CK is not associated with any common pathology. However, it is found more commonly in elderly women (8). Macro LD has been observed in apparently healthy individuals (5) and in patients with a wide variety of disorders, including autoimmune disease (11). The patient discussed here did have a polyclonal increase in IgG and also has arthritis of unknown etiology.

The long biological half-lives of macro CK and macro LD
complexes often result in an unexplained increase in the apparent serum CK and LD activity, respectively, and this may lead to further investigations (4). The presence of macro CK and macro LD is probably the explanation of the persistent elevation of CK and LD prior to the infarction in this patient.

References