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Serum LD-1 Activity in Suspected Acute Myocardial Infarction

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We have studied the diagnostic value of measuring lactate dehydrogenase (LD) isoenzyme 1 in serum of 331 cases of suspected acute myocardial infarction (AMI). At a discriminatory level of 200 U/L (Scandinavian Committee on Enzymes, recommended method for the determination of LD) LD 1 verified the diagnosis in 96% of the AMI cases and excluded it in 96% of the not-AMI cases when samples were drawn 24–72 h after onset of pain. The correlation between 24-h S-LD-1 and 16-h S-CK B activities was 0.94 in the AMI cases. We found that quantitation of serum LD-1 is diagnostically more reliable than the serum LD-1/LD ratio.

Additional Keyphrases: *heart disease · diagnosis of infarction · isoenzymes · relative value of discriminatory tests*

All five isoenzymes of lactate dehydrogenase (LD) are represented in normal human blood, serum (S), and myocardial extracts—in myocardium with a predominance of LD-1, in serum with a predominance of LD-2.[7]

The average proportion of LD-1 of the total LD in human myocardium is about 0.55 when determined by scanning of tetrazolium-stained lactate-to-pyruvate isoenzyme electrophoretograms and about 0.65 by specific LD-1 determination by the pyruvate-to-lactate reaction after immunoprecipitation of all four M-containing LD isoenzymes (see below).

Adding myocardial LD isoenzymes to normal serum will increase both S-LD-1 and S-LD-2 activities, finally to a point at which S-LD-1 equals S-LD-2. By still further addition of myocardial LD, S-LD-1 will exceed S-LD-2 activity: the traditional "flip" criterion (*1*). Obviously, the increase of S-LD-1 may be detected before the "flip" pattern occurs. Consequently, it may be expected that quantitative determination of S-LD-1 activity will detect an acute myocardial infarction (AMI) earlier after an acute episode than the flip pattern, thus providing an increase in diagnostic sensitivity. S-LD-1 determination may also be expected to show a high diagnostic specificity in complicated cases of suspected AMI such as S-CK BB after cardiac arrest with hypoxia (*2*), S-macro CK (*3*), or extracardial S-CK MB (*4*). S-LD-1 may thus provide a late verification of the diagnosis in the routine diagnostic strategy of the working group of the Scandinavian Committee on Enzymes (*5*). The Committee's strategy requires that the time of onset of symptoms be known and that the proposed time schedule can be followed. The relatively slow disappearance rate of LD-1 activity from serum makes it feasible to measure it in only one blood sample per day.

Consequently, we studied S-LD-1 in suspected-AMI cases in two Scandinavian hospitals, Nya Lasarettet Helsingborg (NLH) and Centralsjukhuset Kristianstad (CSK).

Materials

Subjects

NLH: The subjects here were 115 suspected-AMI patients consecutively admitted to the coronary-care unit. The Committee's diagnostic strategy (*5*) was followed strictly: S-CK and S-CK B were determined in blood samples drawn about 10 and 16 h after onset of acute symptoms. From 85 of these patients a 24-h blood sample was drawn for determination of S-LD-1. For classification, S-CK isoenzyme electrophoresis was carried out in all doubtful cases. S-Aspartate aminotransferase (ASAT, EC 2.6.1.1) and S-alanine aminotransferase (ALAT, EC 2.6.1.2) were determined every morning for up to three days after admission. Samples for S-lactate dehydrogenase (LD, EC 1.1.1.27), S-LD-1 determinations, and S-LD isoenzyme electrophoresis were drawn about 16 and 24 h and one month after the suspected AMI.

CSK: The subjects here were 216 suspected-AMI patients consecutively admitted to the coronary-care unit. Samples for S-ASAT, S-ALAT, S-LD, S-LD-1 assay were drawn every morning for at least three days after admission. All enzyme determinations were carried out according to the Committee's recommendations (*6*).

Classification into AMI and not-AMI. AMI was defined when chest pain was associated with a positive ASAT/ALAT pattern and (or) typical evolutionary electrocardiographic signs, supplemented by S-CK and S-CK B determinations (*7*). S-LD and S-LD isoenzyme assays were not used for classification.

Liquid Quality Controls in Glycerol

Human myocardium was homogenized in Tris HCl buffer, pH 7.2 (25 °C), at 4 °C in a Potter–Elvehjem homogenizer and centrifuged (4 °C, 5000 × *g*, 20 min). The clear supernate was adjusted to suitable activity with buffer and stabilized by adding an equal volume of glycerol (*8, 9*).

Pure human LD 5 (M_4) was a gift from Roche-Produkter AB, Sweden.

S-LD isoenzyme electrophoresis was carried out in 10 g/L agarose gel in 75 mmol/L barbiturate buffer, pH 8.6, at 21 V/cm for 1 h, in cells with circulating cooled (8 °C) buffer. This was followed by the usual (*10*) tetrazolium staining, with incubation for 30 min at 37 °C.

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[7] Nonstandard abbreviations: LD, lactate dehydrogenase, EC 1.1.1.27; CK, creatine kinase, EC 2.7.3.2; AMI, acute myocardial infarction; S, serum; LD-1, LD-2, isoenzymes of lactate dehydrogenase ("H_4" and "H_3M" by another system of symbolism); NLH and CSK, two of the hospitals participating in this study; ASAT, ALAT, aspartate- and alanine aminotransferases, EC 2.6.1.1, 2.6.1.2; and B, M, subunits of CK isoenzymes; PV, predictive value; CPD, class prevalence of disease.

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Methods

Determination of S-LD-1 Activity

S-LD-1 was specifically determined by the recommended (*6*) method at 37 °C in sample supernates after immunoprecipitation of all M-subunit-containing LD isoenzymes with the "Isomune-LD" (Roche) reagent (*11*). Samples with S-LD activity exceeding 2000 U/L were diluted. The manufacturer's instructions were strictly followed: To 200 μL of serum we added 50 μL of anti-human LD M subunit antibody from goat, vortex-mixed for 10 s, and incubated for 5 min at room temperature. We then added 20 μL of second-antibody suspension (from a bottle with continuous stirring), vortex-mixed for 10 s, incubated for 5 min at room temperature, and centrifuged the suspension for 10 min at 1000 × *g*. S-LD-1 activity was determined in the supernates (*6*), centrifugal analyzers being used for the determinations.

NLH: The Rotochem centrifugal analyzer was used. Add 10 μL of sample to 500 μL of LD reagent A (*6*) in transfer discs. Start with 50 μL of reagent B (*6*, pyruvate). The reaction rate was monitored for 80 s at 10-s intervals, after a 5-s lag phase.

CSK: The Cobas Bio centrifugal analyzer was used. Five microliters of sample (for total LD) or 10 μL (for LD-1) was incubated for 150 s in reagent A (*6*) + diluent, 180 μL. Start with 20 μL of pyruvate; make 10 readings at 10-s intervals after a 20-s lag phase. The search for the linear part of the curve was performed according to the Cobas Bio program. Type of analysis: 3.

Graphic Evaluation of Clinical Test Data

All clinical test data are plotted in standard histograms (*5*) presenting the number of patients in the AMI and not-AMI groups as a function of the class of test results. The concepts are defined as follows. Diagnostic sensitivity: the fraction of true-positive results of all AMI patients. Diagnostic specificity: the fraction of true-negative results of all not-AMI patients. The traditional concept of predictive values (PV) (*1*) and posterior probabilities apply only to dichotomous tests with two classes of results—positive and negative. PV_{pos} = the fraction of AMI patients within the positive test class. This is the same as the prevalence of AMI patients within the positive test class. In analogy, we extended this concept to *n* test classes and calculated the fraction of AMI patients within each class of test results, i.e., the class prevalence of disease (CPD, *12*). CPD and $\overline{CPD}$ (class prevalence of not-disease) are complementary and may thus be plotted as a function of test class as a single CPD curve. The CPD curve shows the prevalence of AMI as a function of test class and, like diagnostic sensitivity, specificity, and predictive values, it varies between 0 and 1 (CPD scale on the ordinate). The fluctuations of the CPD curve represent the actual findings in the pilot studies and permit intuitive estimates of the discriminatory performance of the test.

Results

Titration of Isomune immunoprecipitation capacity with pure human LD-1 isoenzyme showed a residual LD activity of less than 0.1% at 2000 U/L, 2% at 3000 U/L, and 3% at 4000 U/L.

Day-to-day imprecision for total S-LD, S-LD-1, and fraction S-LD-1 is given in Table 1.

S-LD-1 in Patients with Suspected AMI

The distribution of S-LD-1 results is shown in the histograms (Figures 1–3). Details are given in the legends. At a constant discriminator of 200 U/L, the diagnostic sensitivity increased from 0.88 at 16 h after the onset of acute symptoms to 0.95 at 24 h, at a constant diagnostic specificity of 0.97 (16 h) or 0.96 (24 h). Similarly, at the same discriminator, 200 U/L, by the second or third day after AMI the sensitivity was 0.94 and the specificity 0.98.

In contrast, calculation of data as fraction S-LD-1 resulted in a poorer discriminatory performance, as illustrated by the results for days 2 and 3 (Figure 4).

The correlation between S-LD-1, and fraction S-LD-1/S-LD in 38 cases of AMI (r = .71, not shown) was poorer than

Table 1. Day-to-Day Imprecision of Measurements in Two Laboratories (NLH and CSK)

		LD	LD-1	% LD
NLH	U/L	27.3 ± 0.85	17.8 ± 0.95	65 ± 2
(n = 48)	CV, %	3.2	5.3	3.1
CSK	U/L	29.0 ± 1.02	19.1 ± 0.73	66 ± 2
(n = 17)	CV, %	3.5	3.8	3.0

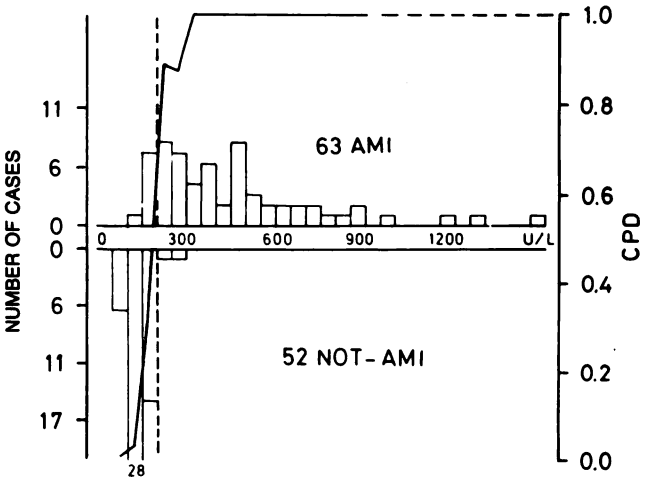

Fig. 1. Histogram showing the distribution of all test data for S-LD-1 samples 16 h after onset of acute symptoms

NLH Upper field: number of AMI patients (*ordinate*) as a function of S-LD-1 activity, class size 50 U/L (*abscissa*). ***Lower field:*** number of not-AMI (AMI-suspect patients classified as not-AMI by other means) as a function of S-LD-1 activity. ***Solid-line curve:*** class prevalence of disease (*CPD, ordinate*) = fraction of AMI within each class of test results plotted as a function of S-LD-1 activity. ***Broken vertical line:*** selected discriminator. Values of diagnostic sensitivity and specificity are given in the text

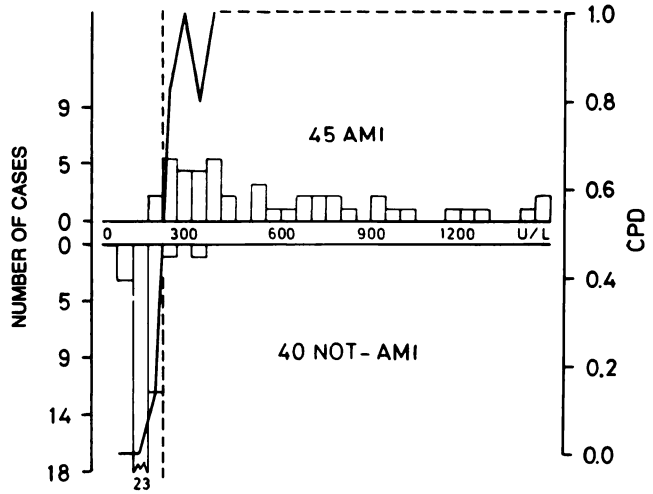

Fig. 2. Distribution of 24 h S-LD-1 results

Details in legend to Figure 1 and in the text

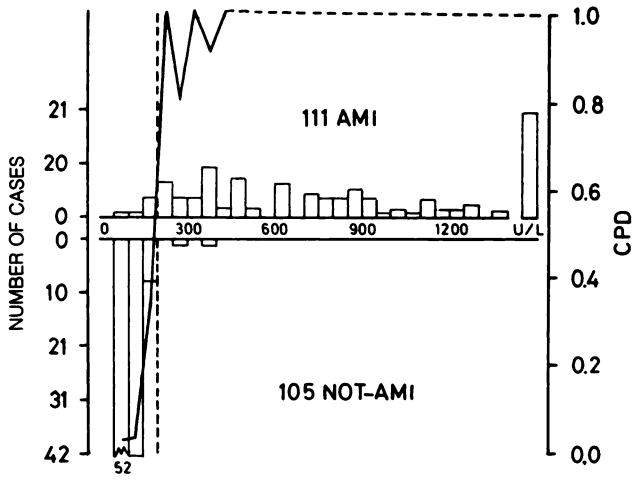

Fig. 3. Distribution of 2–3rd day S-LD-1 results (CSK)
Details in the legend to Figure 1 and in the text

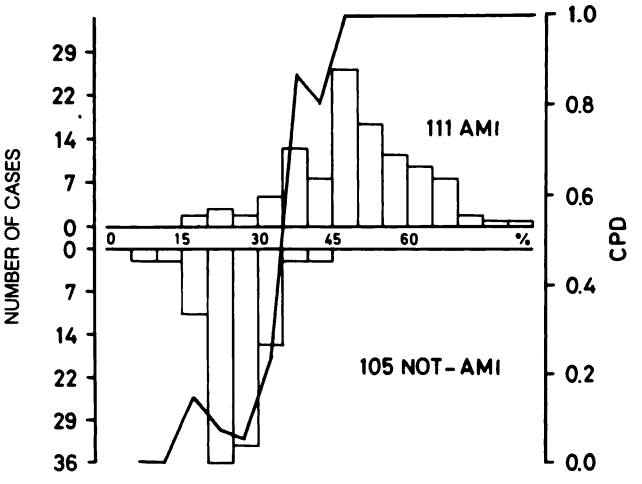

Fig. 4. Distribution of 2–3rd day fraction S-LD-1 (CSK)
The CPD curve shows more fluctuations than found for S-LD activities. A discriminator at 0.35 will give a diagnostic sensitivity of 0.90 and a specificity of 0.96. A discriminator at 40% S-LD-1 will give a sensitivity of 0.77 and a specificity of 0.98. Similar results were found for the NLH study

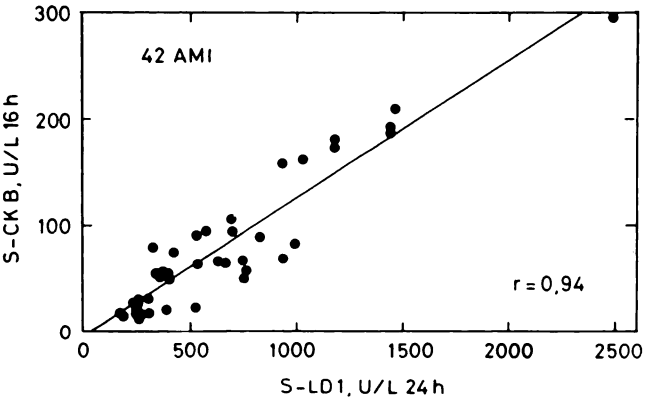

Fig. 5. Correlation between 16-h S-CK B and 24-h S-LD-1 activities (r = .94)

the correlation (r = .94) between 16-h S-CK B and 24-h S-LD-1 (Figure 5).

Discussion

In the time interval 24–72 h after onset of symptoms, an increased S-LD-1 above 200 U/L verified 96% of all the AMI, and an S-LD-1 value of less than 200 U/L excluded 96% of the not-AMI. The coefficient of correlation between 24-h S-LD-1 and 16-h S-CK B was .94. These data confirm that S-LD-1 determination can verify or exclude AMI. The addition of 24- to 50-h S-LD-1 to the Committee's (*6*) diagnostic strategy (*5*) seems to be the most powerful tool for the diagnosis of AMI:

1: sequential application of clinical signs,
2: 16–20 h S-CK,
3: S-CK B determinations of all S-CK positive samples.

A key question, currently being debated (*13–20*), is whether S-LD-1 activity or fraction S-LD-1/S-LD is the better discriminator between AMI and not-AMI. In our opinion, the main argument for the fraction LD-1 is that it might have a higher diagnostic sensitivity in minor infarctions in patients with low-normal S-LD activity. However, we found a diagnostic sensitivity for 24- to 50-h S-LD-1 of 0.94–0.95 at a diagnostic specificity of 0.97–0.98. Similar results were reported by Fogh-Andersen et al. (*19*). Furthermore, S-LD activity is increased in a variety of not-AMI conditions, including heart failure with liver stasis (*2, 19*), thus decreasing the fraction S-LD-1/S-LD and causing false negatives. The nonspecific variation in S-LD is indicated by the low correlation coefficient, r = .70, between S-LD-1 and fraction S-LD-1/S-LD in AMI. Similarly, a correlation coefficient of .71 can be calculated from the 20 AMI cases in Figure 1 of the paper published by Wang et al. (*15*). The overlap and number of false-negative results may be partly explained by increased S-LD-5 in cases of liver stasis.

The technique for S-LD-1 determination 24 h after admission that we used in this study with Isomune-LD in centrifugal analyzers is precise and rapid. Only one main problem must be noticed: hemolysis increases serum LD-1 activity. It has, however, recently been shown (*21*) that if all samples with visible discoloration are eliminated the interference from this source is practically precluded.

The results indicate that S-LD-1 is more reliable than the S-LD-1/S-LD ratio. In clinical practice, myocardial necrosis and the onset of symptoms are not always synchronized. Therefore LD-1, which has a long biological half-life in blood serum, seems an especially suitable assay for the diagnosis of AMI.

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