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Patterns of Lactate Dehydrogenase Isoenzymes 1 and 2 in Serum of Patients Performing an Exercise Test
Zvi Rotenberg, Itzhak Weinberger, Alex Sagie, Jacob Fuchs, Ehud Davidson, Oded Sperling,1 and Jacob Agnon2

Values for total lactate dehydrogenase (LD, EC 1.1.1.27) activity and LD isoenzymes were determined in serum from 56 patients and 40 healthy subjects before and 24, 48, and 72 h after they performed an exercise test. The mean (for all four times) total LD activity concentration and proportion of LD-2 were within the normal range for all 96 subjects. Mean LD-1 values for serum, although within the normal range in all subjects, were significantly higher in patients with positive exercise test results than in subjects with negative results: 75 (SD 12) U/L in 35 patients with ST depression >2 mm; 63 (SD 14) U/L in 16 patients with ST depression of 1–2 mm; 43 (SD 11) U/L in subjects with negative test results, by 48 h after the test. The LD 1:2 ratio was also markedly higher in the group of patients with positive test exercise results, especially in those with ST depression >2 mm (1.02, SD 0.06), compared with those subjects with negative results (0.60, SD 0.04). A similar trend was also found 24 and 72 h after the exercise test. We conclude that exercise-myocardial ischemia may lead to an increased LD 1:2 ratio in serum, and demonstrate a correlation between the degree of ischemia and the LD 1:2 ratio. Determination of the LD 1:2 ratio, even in the presence of normal total LD activity, may assist in the clinical evaluation of patients performing an exercise test.

Additional Keyphrases: myocardial ischemia · variation, source of · LD 1:2 ratio

Lactate dehydrogenase (LD, EC 1.1.1.27) isoenzymes in serum are sensitive and specific indicators of acute myocardial infarction (I–3). In about 80% of patients with acute myocardial infarction the LD 1:2 ratio is >1.0 (I–5). In myocardial ischemia, however, the activities of cardiac enzymes are seldom increased, there being only a few reports (6, 7) of an increased creatine kinase (CK, EC 2.7.3.2) MB fraction (CK-MB) in some patients with acute myocardial ischemia as long as 24 h after onset of chest pain. A possible pathological basis for this finding may be myocardial damage such as myocyteolysis and coagulation, as have been described in postmortem examination of patients with acute myocardial ischemia (8–12).

The aim of our study was to determine the patterns of LD isoenzymes 1 and 2 in sera from patients performing an exercise test and to discover whether there is a correlation between the LD 1:2 ratio and the degree of exercise-induced myocardial ischemia.

Materials and Methods

Subjects. The study population consisted of two groups:
Group A. Fifty-six male patients with proven coronary artery disease (history of acute myocardial infarction, or significant angiographically proven coronary artery obstruction, or both). Patients with congestive heart failure were excluded from the study. None of the 56 patients had clinical or laboratory findings suggesting hemolysis or renal injury. Their mean age was 60 (range 45–63) y.
Group B. Forty healthy men, not suffering from ischemic heart disease, mean age 55 (range 40–60) y.

Exercise test. The two groups of subjects performed a submaximal exercise test according to the modified Bruce protocol (13). Results of the test were classified as positive, when there was horizontal or downsloping depression or elevation of the ST segment by at least 1 mm and a duration of 0.08 s in any of the leads. This positive test result was subdivided into two groups: (a) ST depression 1–2 mm, (b) ST depression >2 mm. Test results were negative when the ST segment was depressed by <1 mm in the presence of a submaximal heart rate. Results were considered indeterminate when there was an absence of ischemic changes in patients who were unable to reach a submaximal heart rate.

LD assays. Total serum LD activity and proportions of LD isoenzymes were determined in each subject before and 24, 48, and 72 h after the exercise test. Total LD was measured by the method of Wacker et al. (14) at 37 °C, in a GEMSAEC centrifugal analyzer (Electro-Nucleonics, Fairfield, NJ). The normal reference interval in our laboratory is 100–225 U/L and the CVs for normal and abnormal concentrations of LD in serum are 3.2% and 4.2%, respectively. The proportions of the LD isoenzymes were determined by electrophoresis on cellulose acetate plates, with the Helena kit (cat. no. 5451) and instrumentation (Helena Laboratories, Beaumont, TX). The normal reference interval for LD-1 in our laboratory is 30–90 U/L; for LD-2, 35–100 U/L. The normal reference interval for the LD 1:2 ratio, based on data obtained in our laboratory for 150 healthy subjects and based on values in the literature (a), obtained by the same methods, for 200 subjects is 0.45–0.74.
Results

None of the 96 subjects developed acute myocardial infarction. No new Q-wave appeared in electrocardiograms, and there was no increase in cardiac enzymes (CK-MB and LD) in serum. Before the exercise test, total LD activity and LD isoenzyme activities in serum were within the normal reference interval in all subjects.

Table 1 shows the results of the exercise test for the two groups of subjects. Most of the patients with ischemic heart disease had a positive exercise test result, and most of them had ST depression >2 mm. None of the healthy group showed positive results for the exercise test; for most, the test result was negative.

LD data did not appear to differ by time of collection, between 24, 48, and 72 h. Table 2 shows the mean total serum LD activity, LD isoenzymes 1 and 2, and the LD 1:2 ratio 48 h after the exercise test in relation to results of the exercise test. Differences between the means were assessed by Student's t-test and were considered significant at P <0.05.

Total LD and LD-2 activities in serum were within the normal range in all 96 subjects and did not differ between groups. Mean (and SD) values for LD-1 in serum, although within the normal range in all subjects, were significantly higher after the exercise test in patients who had had a positive exercise test result than in subjects with negative results. The LD 1:2 ratio was also markedly higher after the exercise test in the positive results exercise group of patients (especially in those with ST depression >2 mm) than in those subjects with negative results. The LD data for the indeterminate results group did not differ from those for patients without ST depression.

Discussion

Sera of normal healthy individuals contain less LD-1 than LD-2 (4), the normal ratio of LD 1:2 being 0.45–0.75 (4). Because the myocardium has a preponderance of LD-1, with lesser amounts of LD-2, necrosis of the myocardium releases relatively more LD-1 than LD-2 into the serum, reversing the normal ratio. In about 80% of patients with acute myocardial infarction, an LD 1:2 ratio >1 (a "flipped" LD pattern) may be found (1–6). Vasudevan et al. (3) and Leung and Henderson (4) found a peak LD 1:2 >0.76 to be a more sensitive test for the diagnosis of acute myocardial infarction. In acute myocardial ischemia, on the other hand, the activity of cardiac enzymes is seldom increased (6, 7).

Our present study demonstrates that, although total LD activity in serum was normal in all subjects performing the exercise test, the LD 1:2 ratio was greater in sera of patients with positive exercise test results, like that usually found in patients with acute myocardial infarction. In those subjects with ST depression of 1–2 mm, the mean LD 1:2 ratio was 0.86 (SD 0.06), 48 h after the exercise test; in those with ST depression >2 mm, a flipped ratio was found: 1.02 (SD 0.04).

Subendocardial myocardyolysis and coagulation necrosis may be present in patients with myocardial ischemia who die after either a relatively brief (6–10) or a protracted (11) clinical course. Early myocytolysis is characterized by sarcoplasmic vacuoles that later enlarge and replace or displace the myofibrils. External to the subendocardial layer of myocytolysis are foci of coagulation necrosis. This necrosis differs from that typically seen in patients with acute myocardial infarction (11), in whom the pathognomonic histological finding is contraction-band necrosis, suggesting severely damaged myocardial cells.

The pathological findings described above may explain our findings that, even if total LD activity is within normal limits in patients with exercise-induced myocardial ischemia, the LD 1:2 pattern in the serum of these patients may be relatively high 24 to 72 h after the exercise test. These findings suggest that relatively more LD-1 than LD-2 is released from the foci of damaged myocardial cells undergoing myocytolysis or even coagulation necrosis, without development of the contraction-band necrosis or total tissue necrosis seen in patients with acute myocardial infarction.

We conclude that exercise-induced myocardial ischemia may lead to an increased LD 1:2 ratio in serum. We found a correlation between the degree of ischemia and this ratio. Determination of the LD 1:2 ratio, even in the presence of normal total LD activity, may assist in the clinical evaluation of patients performing exercise tests.

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References


Automated Enhanced Latex Agglutination Assay for Rheumatoid Factors in Serum

J. W. Winkles, J. Lunec, and L. Gray

This improved assay of rheumatoid factors in serum, described here for use with the Baker "Encore" centrifugal analyzer, is efficient, with 250-sample throughput per hour; reproducible, with between-batch CV = 5% and within-batch CV = 2% (mid-assay range); and results correlate well (r = 0.9) with those by other methods. The method is fully quantitative and automated, involves no predilution steps, and can be adapted for use in a wide range of systems. It has a sensitivity of 96% and specificity of 80% in diagnosing rheumatoid arthritis.

Additional Keyphrases: reference interval • values for patients with various rheumatic diseases • diagnostic value, clinical sensitivity • centrifugal analyzer • latex slide, Waaler–Rose assay compared

Detection of rheumatoid factors (RF), autoantibodies directed against antigenic sites located in the Fc region of IgG (1), is important in the diagnosis of rheumatoid arthritis (RA) (2), and also in the prognosis of the disease, high concentrations being an indication of the future development of complications. Some investigations have suggested a correlation between the clinical activity of the disease and RF concentrations (3–5), but one of the reasons this has been difficult to show clearly is the poor precision of conventional assays.

Most laboratories measure RF by the Waaler–Rose assay (6, 7) and the latex slide test (2). At best these can only be considered semiquantitative, given the wide variation in titers obtained by different laboratories for analysis of the same sample. We have previously described the adaptation of enhanced latex immunoassay to automated analysis (8). We present here an improved RF assay and evidence of its usefulness in the diagnosis of RA.

Materials and Methods

Apparatus. We used an "Encore" centrifugal analyzer with its associated P1000 sampler (Baker Instruments Ltd., Windsor, U.K.), a Heraeus Christ minufuge GL (VA Howe Ltd., London, U.K.), and a probe sonicator (Model A180G, Ultrasonics Ltd., Shipley, U.K.) in this study.

Reagents and standards. Latex "polybead" polystyrene microspheres (25 g/L suspension), 57 (SD 5) nm in diameter, were obtained from Polysciences Ltd., Northampton, U.K.

Human IgG was obtained from Sigma Ltd., Dorset, U.K., as was bovine serum albumin, 220 g/L, in isotonic saline. RF standard material with a value of 250 int. units/mL, as assigned with use of the Bureau of Laboratories Provisional Reference Preparation for Rheumatoid Arthritis, was from BCL Ltd., Lewes, U.K. All other reagents were "Analar" grade, from BDH Ltd., Atherstone, U.K.

Samples. Blood was sampled from patients in rheumatology clinics at Selly Oak Hospital and from presumably healthy blood donors. Serum samples were obtained without preservative, stored at 4°C, and either analyzed within seven days or stored at −20°C until assay.

Preparation of stock latex reagent. This is essentially the same as for our previously published method for C-reactive protein (6), except that we added 10 mg of human IgG instead of the antibody. This amount of stock latex suffices for about 500 analyses.

Measurement of RF. Working latex reagent was prepared by diluting the stock reagent 11-fold with glycine-buffered isotonic saline, pH 8.2. We added 10 μL of sample, standard, or control to 220 μL of working reagent, using 20 μL of distilled water as the diluent to minimize carryover by the sample probe. The Encore analyzer mixed the solutions for 1.6 s; 4 s later a blank reading was taken at 340 nm, then at 2-s intervals between 90 and 100 s. The mean absorbance of these last five readings was calculated and corrected by subtracting the 4-s blank absorbance. Instrument settings other than the above timings and volumes are the same as in our previously published method for C-reactive protein (8) except that we used a spline curve fit and a temperature of 25°C. For RF controls we used patients' serum, pooled to give suitable concentrations of RF, then aliquoted and stored at −20°C. A working standard series was prepared by diluting the stock RF standard in phosphate-buffered...