Upsala J Med Sci 92: 293–300, 1987

Release Characteristics of Enzymes Used in the Diagnosis of Myocardial Infarction

J. Christer Sylvén[1], Anders Kallner[2] and Flemming F. Larsen[1]

[1]*Department of Internal Medicine and* [2]*Department of Clinical Chemistry and Blood Coagulation, Karolinska Hospital, Stockholm, Sweden*

ABSTRACT

Release characteristics of S-LD, S-LD_1, S-ASAT, S-CK and S-CK-MB were studied in 47 consecutive AMI patients. In addition, previously obtained data for serum myoglobin (S-MYO) were compared. Serum was sampled at regular intervals after admission to the Coronary Care Unit (CCU). The release rate and half lives of the enzymes were calculated according to a one-compartment kinetic model. The time to peak values, the time of total release and the half lives were interrelated in the following order: MYO<CK-MB<CK<ASAT<LD_1<LD which coincides with the wellknown appearance and disappearance rates in serum. The ratio between mean peak values and upper reference limits followed the reverse order.

The finding that the release rate of enzymes and half-lives co-vary is hypothetically suggested to be attributed to differences in rate of membrane diffusion. There is indirect evidence that a slow indicator such as LD_1 reflects infarct size better than fast indicators with rapid release and removal such as MYO or CK-MB. However, these fast markers have a better signal to noise ratio, whereby they probably reflect changes in the infarction process better.

INTRODUCTION

A number of biochemical markers of acute myocardial infarction (AMI) are available such as myoglobin (MYO), creatine-kinase (CK) and its isoenzyme (MB), aspartate-aminotransferase (ASAT), lactate dehydrogenase (LD) and its isoenzyme LD_1. It is wellknown that the various markers have different "half-lives", i.e. the elimination from serum follows typical time courses where MYO and CK-MB have the shortest and LD the longest half-lives. In the present study, a model of elimination is chosen which allows an approximation of the release patterns of the markers from the myocardium into serum. It is assumed that the release begins simultaneously for all markers and at the time of beginning necrosis. The questions addressed are if the release rates of the different markers are similar and if the release therefore is completed at the same time and simultaneous to that of the fastests marker, MYO (8). The time course of changes in serum concentrations has been thoroughly described in many publications (e.g. 1, 5, 3, 7)

but answers to the questions posed in this study will also allow a discussion of the relation between peak values and serum entry, the influence of half life on peak values of serum enzyme concentrations and of the capacity of the marker to indicate infarct extension and size.

MATERIALS AND METHODS

Patients

47 consecutive patients (11 females) with AMI treated in a coronary care unit with New York Heart Association class I or II were included in the study. Their mean age was 62 ± 10 years. An AMI was subsequently diagnosed in all cases on the basis of 12-electrode ECG and daily enzyme determinations (ASAT, ALAT and LD) or autopsy findings. Venous blood was sampled on admission and after 6, 12, 18, 24, 48, 72 and 96 hours. None of the patients was known to have chronic renal failure, skeletal or muscular disorder, severe ethanol intoxication (or abuse) or any serious endocrine or neurological disease. Intramuscular injections were not given.

The study was approved by the Ethics Committee at the Karolinska Hospital, Stockholm, Sweden.

Methods

Total serum CK and LD and ASAT and ALAT were determined by routine methods recommended by the Scandinavian Enzyme Committee (6, 7). CK-MB was determined after chromatographic separation essentially according to Mercer (4). LD_1 was separated by an immunological procedure based on antiserum against the M subunit (Roche Products N.J., U.S.) (11).

The imprecision of these tests was expressed as the coefficient of variation and were for CK=4%, CK-MB=6%, ASAT=3%, LD_1=6% and LD=3%. The upper reference limits for the different enzymes were 0.2 (3% of CK), 0.7, 2.0 and 8.0 ukat/l, respectively.

Calculations

For each separate case and enzyme the apparent half-life and cumulative release or serum entry were calculated according to a one-compartment model of elimination from the serum pool as indicated by the equation (9).

$$CR = \sum_{i=1}^{n-1} \left(\frac{\Delta e_i}{\Delta t_i} + k_d * \frac{e_i + e_{(i+1)}}{2} \right) * \Delta t_i$$

Cumulative release, CR, is expressed in ukat/(l*h); Δe is the difference between two consecutive determinations of serum enzyme activity concentrations with a time difference of Δt. K_d is the apparent and individually calculated elimination constant. This was determined from the "final slope", or the "beta phase", occurring after the enzyme peak, defined as the maximal value observed on the curve representing the serum enzyme activity concentration vs time. The time to peak was the time for the peak value plus the time between onset of symptoms and admission. Completed cumulative release was considered to have been reached when the increment between two measurements was zero. The time to completed cumulative release was the time between onset of symptoms and the time for completed cumulative release.

RESULTS

Mean duration of symptoms at onset of the study was 6.2 hours (range 0.5-21.5 hours). Peak values of the different enzymes correlated significantly. The best correlation of those tested was found between LD_1 and ASAT (r=0.92) whereas those between ASAT and CK-MB (r=0.82) and LD_1 and CK-MB (r=0.82), were of the same order of magnitude.

Table 1. Time to peak concentrations and time to maximal rate of release after onset of pain and apparent half lives for LD, LD_1, ASAT, CK and CK-MB in 47 patients with AMI. Statistical interrelationships between successive markers are: xxx $p < 0.001$, xx $p < 0.01$, x $p < 0.05$, NS non-significant.

	LD		LD_1		ASAT		CK		CK-MB
Time to Peak conc (hours)	40±17	x	37±17	xxx	25±9	xxx	22±10	xxx	21±8
Time to Max rate (hours)	19±8	NS	19±7	xx	15±6	xx	14±6	NS	13±5
$t_{0.5}$ (hours)	87±37	xx	67±26	xxx	30±11	xxx	19±4	xxx	12±3

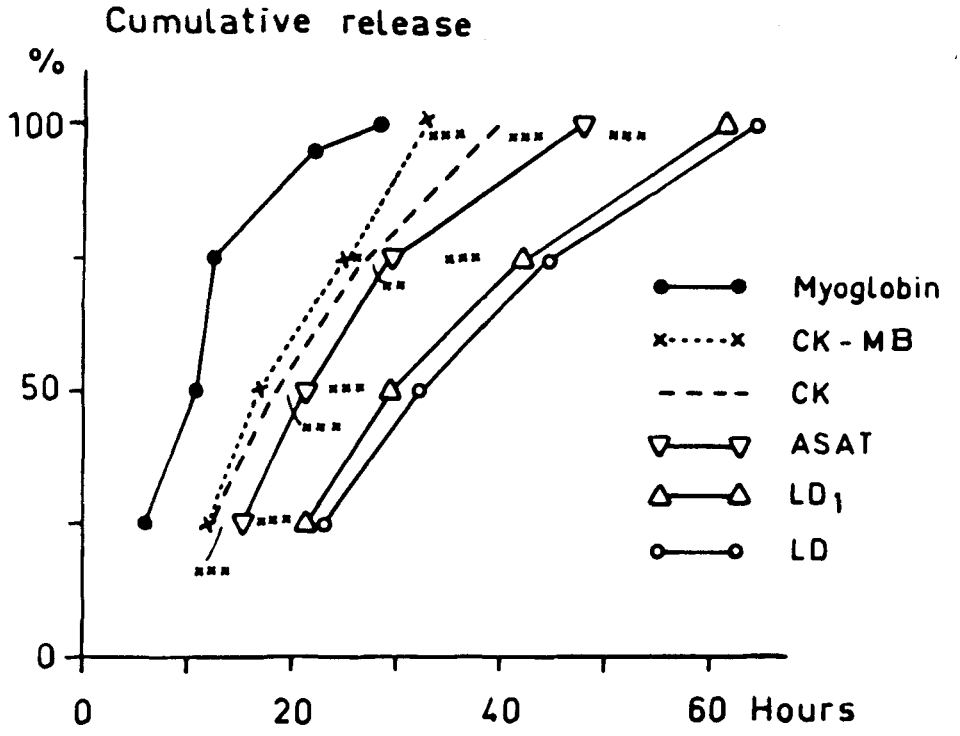

Figure 1. Cumulative release vs time for studied markers. Completed release is indicated as 100%. Values for MYO have been taken from a previous work (9) while values for the other enzymes are means from a series of 47 consecutive patients. xxx $p < 0.001$, xx $p < 0.01$, x $p < 0.05$, NS nonsignificant.

The main enzyme release characteristics are presented in Table 1. Fig. 1 shows the cumulative release for the different enzymes. Values for MYO are from a previous publication (9). The time to peak concentration of LD and LD_1 differ slightly and their time to completed cumulative release occurs simultaneously being ended about 60 hours after onset of anginal symptoms. The release of these markers is distinctly slower than the release of ASAT, CK and CK-MB. Even within this group there are differences, ASAT being the slower and CK-MB the faster enzyme. MYO has the fastest release being completed 24-30 hours post onset of symptoms.

When the characteristics of the different enzymes were compared, highly significant relations were obtained (Fig 2). Thus, the time to completed release correlated positively with the logarithm of the apparent half-life and negatively with the logarithm of the ratio between mean peak value and upper reference limit.

DISCUSSION

For the study of AMI development, release characteristics are of primary interest and in this study we have attempted to estimate the kinetics of the release of the enzymes. The release and disappearance of these serum markers are slow processes in relation to the distribution rate and therefore a kinetic one-compartment model may be a useful approximation for describing the time events. Using this concept, we have calculated the release by estimating rate constants for the disappearance of enzymes from serum after reaching a peak value (9). Dynamic parameters of two of the above-mentioned phases are thus presented.

The serum concentrations of the enzyme activities in the patients in this study follow the same pattern as that described in numerous reports during AMI (cf. 1). The cumulative release calculated according to the model reveals that the release of the five studied enzymes and MYO (data from a previous study) are subject to similar but gradually changing time courses. Thus, three main groups of release characteristics could be assumed, the fastest comprising MYO, an intermediary CK, CK-MB and ASAT and a slow group of LD and its isoenzyme LD_1.

As demonstrated above, the calculation of cumulative release includes the rate constant and this is estimated from the observed decline of the serum concentration of the enzymes. The means of the estimated in vivo half-lives show a wide variety (Table 2) but the plots of cumulative release reveal a high degree of parallelism. (Fig. 1) This is even more emphasized if the cumulative release is plotted against the logarithm of the time. The segments between 25% and 100% can then be described as straight lines with correlation coefficients between 0.97 (MYO) and 1.00 (LD and LD_1). Cumulative MYO release has been demonstrated to occur simultaneously with the cumulative change in the QRS vector of the electrocardiogram (13). Thus, MYO is released at the same time as the tissue loses

Table 2. Calculated half-lives for enzyme markers in AMI. (hours)

	LD	LD_1	ASAT	CK	CK-MB
Mean	86.5	67.0	29.9	8.6	12.2
SD	37.2	6.0	10.5	3.9	3.1
n	43	44	46	46	46

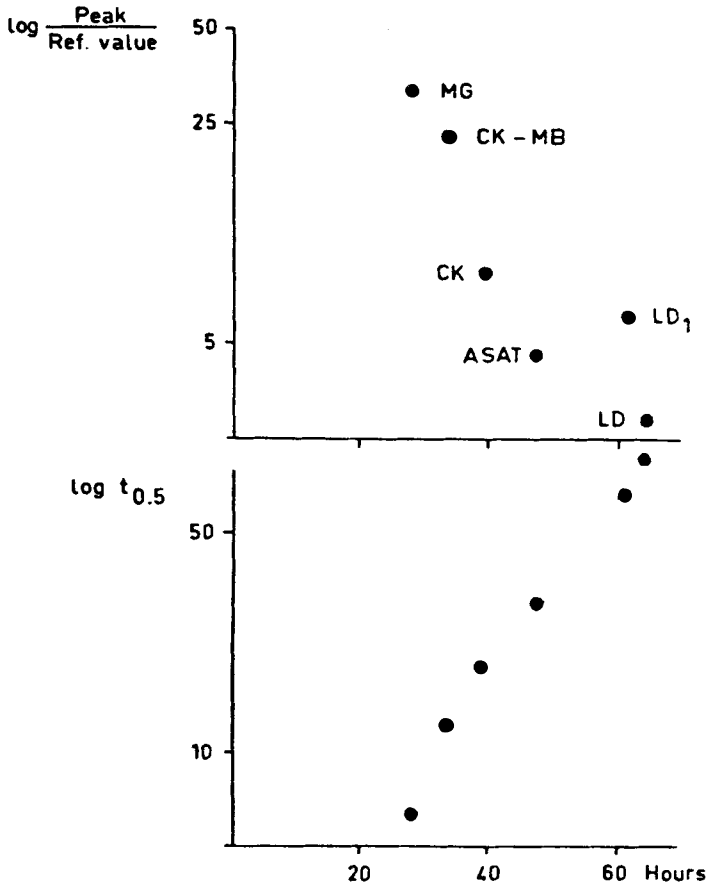

Figure 2. The logarithm of the ratio between peak value and upper reference limit (above) and the logarithm of the half lives (below) related to the times of completed release (from Fig. 1) for the studied markers. Values are means from a series of 47 patients except for MYO where values have been extracted from a previous work (9).

its electrical integrity and the release of MYO therefore appears to indicate the time course of cell necrosis of the myocardium. This process appears to be completed about 20 hours after the onset of symptoms. In the present study the markers studied had slower release rates and longer times of complete release than MYO. CK-MB had a release almost as fast as MYO while LD was slowest. Thus, these enzymes seem to be released from the necrotic areas at different rates and the release continues after the necrosis has been completed. This difference could be attributed to differences in the rate of membrane diffusion or

release from the subcellular structures which supposedly are damaged during the hypoxic state of the myocardium.

An inverse relation between the logarithms of the ratio peak-value/reference value vs. time for complete elimination exists (Fig. 2a). On the average, a more than 30-fold increase of MYO is obtained whereas for LD, the slowest of the markers, the increase is only abut three-fold. A relationship between the means of half-lives and the time for complete elimination also exists, indicating a longer half-life for the slow markers (Fig. 2b).

Markers which combine a high serum entry rate with a short half life will be particularly suited to follow rapid changes in the AMI process (1). Usually markers combine a low serum entry rate with a long half-life or high rate with a short half-life. We therefore have access to suitable tools to follow the process of AMI (MYO and CKMB) as well as tools suitable for evaluating the size and progression of AMI (LD and ASAT). The greater variability in CK-MB is thus probably due to a biological rather than an analytical variation. Availability of LD_1 and CK-MB improves the diagnosis and supervision of AMI and eliminates the need for determination of ASAT. A combination of a fast and sensitive marker (e.g. MYO and CK-MB) and slow and specific marker (LD_1) would seem to be of optimal value in the monitoring and evaluation of AMI.

ACKNOWLEDGEMENTS

This study was supported by the Swedish National Association against Heart and Lung Diseases, and the Karolinska Institute "Fonder".

REFERENCES

1. Bruns, E.D., Emerson, J.C., Intemann, S., et al.: Lactate dehydrogenase isoenzyme-1: changes during the first day after acute myocardial infarction. Clin Chem 27:1821-1823. 1981.

2. Loogna, E., Sylvén, C., Groth, T., Mogensen, L.; Complexity of enzyme release during acute myocardial infarction in a controlled study with early Nifedipine treatment. Eur Heart J. 6:114-119. 1985.

3. Lott John A., and Stang John M. Serum Enzymes and Isoenzymes in the Diagnosis and Differential Diagnosis of Myocardial Ischemia and Necrosis. Clin Chem 26/9:1241-1250. 1980.

4. Nealon DA, Hendersson RR. Separation of creatine kinase and isoenzymes in serum by ion-exchange column chromatography (Mercer's method, modified to increase sensitivity). Clin Chem 21:392-397. 1975.

5. Rosano, T.G., Sanders, L.A., Kenny, M.A., Clayson, K.J., & Strandjord, P.E.: Myoglobin Concentrations and Muscle-Enzyme Activities in Serum after Myocardial Infarction and Cardiac Arrhythmia. Clin Chem 23/5:868-870. 1977.

6. Sederholm M, Sylvén C. Correlation between vectorcardiographic changes and myoglobin release during acute myocardial infarction. Cardiovasc Res. 17:589-594. 1983.

7. Smith A.F. Enzymes and routine diagnosis. In Enzymes in cardiology. (Eds Hearse DJ, de Leviis I pp. 190-246. Wiley and Sons. New York. 1979.

8. Sobel, B.E.: Biochemical estimation of infarction. In: The first 24 hours in myocardial infarction. (Eds Kaindl F, Pachinger O, Probst P) Gerhard Witstrock Publishing House, Baden-Baden-New York-Cologne. 1978.

9. Sylvén JCH. Myoglobin and creatine kinase rates of release during acute myocardial infarction. Clin Cardiol 3:324-328. 1980.

10. Swinscow TDV. Statistics at Square One. Brit Med Ass London. 1976.

11. Usategui-Gomez M, Wicks RW, Warshaw M. Immunochemical determination of the heart isoenzyme of lactatedehydrogenase (LD_1) in human serum. Clin Chem 25:729-734. 1979.

12. Recommended Methods for the Determination of Four Enzymes in Blood. Scand J Lab Clin Invest 33:291. 1974.

13. The Committee on Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology. Recommended method for determination of creatine kinase in blood. Scand J Lab Invest 36:711. 1976.

Correspondence
Christer J. Sylvén, M.D.
Dept. of Medicine
Danderyd Hospital
S-182 88 DANDERYD, Sweden