Mass Concentration of Creatine Kinase MB Isoenzyme and Lactate Dehydrogenase Isoenzyme 1 in Diagnosis of Perioperative Myocardial Infarction after Coronary Bypass Surgery

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Recent advances in methodology allow the mass concentration of creatine kinase MB isoenzyme (CK-MB), and of lactate dehydrogenase isoenzyme 1 (LD1) to be determined quickly and easily as routine, emergency tests. We evaluated these tests as diagnostic criteria of perioperative myocardial infarction (PMI) after coronary bypass surgery. These tests were compared with the usual measurements of CK-MB activity by immunoinhibition and LD1 by electrophoresis and with other biological markers of myocardial infarction such as total CK, total LD, and aspartate aminotransferase. Sixty-one patients who underwent coronary bypass grafting were followed pre- and postoperatively by enzyme determinations and electrocardiography; a subgroup was monitored by myocardial scintigraphy. CK-MB mass appeared to be the best marker of PMI during the first 48 h, although LD1 was the marker of choice from days 2 to 4.

Additional Keyphrases: enzyme mass and activity compared · immunoinhibition · electrophoresis

Perioperative myocardial infarction (PMI) remains a major and frequent complication following coronary bypass surgery.6 Reports of its incidence vary from 8% to 35% (1, 2).

PMI is not a benign complication of coronary bypass surgery (3); its diagnosis has important prognostic implications (4). However, the diagnosis of PMI is difficult because there is no classic presentation; many perturbations of the various potential diagnostic criteria are related not only to PMI but also to the surgical procedure, hypothermia, hemolysis, and other factors. Consequently, the reliability of every criterion used for the diagnosis of PMI—electrocardiography (ECG), myocardial scintigraphy, and enzyme changes—has been criticized. Several authors (2, 5, 6) recommend the confirmation of at least two positive criteria (ECG, myocardial scintigraphy, or enzyme results) as evidence of PMI; patterns of enzyme release, obtained by multiple sampling, have been advocated by others (5–9). However, the use of myocardial scintigraphy and serial enzyme patterns in the early postoperative period are both difficult procedures and have been utilized mainly for research purposes; they cannot be considered routine tests.

Among the various biochemical approaches, determination of CK-MB activity is the most commonly used (1, 2, 5–7, 10), although there are conflicting reports regarding its clinical specificity (1, 2, 5–11). In particular, the high incidence of false-positive results has been well documented (5–7). More recently, the determination of CK-MB mass concentration by immunoenzymometric assays (7, 12, 13) represents an advance towards greater specificity. But this methodology is time consuming, as is determining lactate dehydrogenase (LD) isoenzymes by electrophoresis: neither can be applied as a routine procedure.

Recently, modified techniques of determining CK-MB mass concentration (immunoenzymometric assay) and LD1 isoenzyme (by immunoinhibition) have been developed whereby these enzymes can be determined more quickly, as emergency procedures, available 24 h/day. Our aim in this study was to evaluate these two determinations, to compare them with the other enzyme markers of PMI, and to determine whether either of these could be recommended as a reliable routine adjunct to ECG. We also evaluated their ease of performance and the best times for sampling. Cutoff values after coronary bypass surgery were established for all the enzymes tested. Finally, we assessed the interference of hemolysis in the LD assay.

Materials and Methods

Analytical Procedures

Enzyme assays. We measured creatine kinase (CK), LD, and aspartate aminotransferase (AST) in serum at 30 °C with commercially available kits (Boehringer Mannheim, Mannheim, F.R.G.) based on methods recommended by the International Federation of Clinical Chemistry. All these tests were performed with a Hitachi 705 multianalyzer (Tokyo, Japan). The CK-MB activity in serum was determined after immunoinhibition of the M subunit (CK-MB UV-test, no. 418234; Boehringer Mannheim). CK-MB mass concentration was measured with an immunoenzymometric assay (Tandem-E CK-MB; Hybritech Inc., San Diego, CA). LD1 was determined two ways: by immunoinhibition (Isomune; Roche Diagnostics, Nutley, NJ) (14) and after separation of the LD isoenzymes by electrophoresis on agarose gel (Beckman Instruments, Brea, CA). The electrophoretograms were scanned by densitometry at 600 nm (15).

Haptoglobin assay. We measured haptoglobin (Hp) by nephelometry (nephelometer analyzer; Behring, Marburg, F.R.G.).

Hemoglobin assay. We measured free hemoglobin (Hb) in plasma by spectrophotometry.

Routine upper reference values for non-PMI patients. We determined the following upper reference limits for normal values: CK, 120 U/L; CK-MB activity, 15 U/L; CK-MB mass, 9 µg/L; LD, 350 U/L; LD1, 50 U/L; AST, 35 U/L; Hb, 0.8 g/L; and Hb, 0.775 µmol/L.

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6 Nonstandard abbreviations: PMI, perioperative myocardial infarction; CK, creatine kinase (EC 2.7.3.2); LD, lactate dehydrogenase (EC 1.1.1.27); AST, aspartate aminotransferase (EC 2.6.1.1); Hp, haptoglobin; Hb, hemoglobin; and ECG, electrocardiography.

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Patients

Coronary artery bypass. Sixty-one patients, 47 men and 14 women, ages 37–78 years (mean 60), underwent coronary bypass grafting as an isolated surgical procedure. Preoperatively, 28 patients had had a myocardial infarction, and 33 presented with unstable angina. There was no significant difference between the two groups for the mean age, sex, history, or ECG and enzyme results. Postoperatively, analysis of variance showed no significant difference in the number of grafts per patient (P = 0.086), but there was a significant difference between the two groups in the duration of extracorporeal circulation (P = 0.021) and the duration of aortic cross-clamping (P = 0.031).

Surgical procedure. All patients were treated with high-dose fentanyl and benzodiazepine anesthesia, extracorporeal circulation, hemodilution, and moderate (28 °C) hypothermia.

Follow-up. All patients were administered an ECG preoperatively, postoperatively in the recovery room, and on days 1, 2, 5, and 8 afterwards. CK, CK-MB activity, LD, LD isoenzymes, and AST were determined preoperatively in the recovery room (day 0), daily for four days and on day 8; day 0 = within 12 h after the start of the operation; day 1 = within 24 h after the start of the operation. CK-MB mass was determined in 32 patients preoperatively, in the recovery room, daily for three days, and on day 8. Hemolysis was monitored in 36 patients by measuring Hp and Hb in serum.

Of the 61 patients, 31 underwent a myocardial scintigraphy preoperatively and on day 2 after surgery. Results were graded according to accepted criteria (16). Scintigrams and ECGs were interpreted by an experienced observer who was unaware of the clinical evolution or the enzyme results, first in the immediate postoperative period and again three months later.

Criteria for PMI. We evaluated two different approaches to establishing the diagnosis of PMI. In the first approach, involving all 61 patients, we used two criteria (ECG and enzyme results). The ECG criterion was considered as the diagnostic one, which separated the PMI-positive group from the PMI-negative group. The aim of this approach was essentially to evaluate which of the biological markers was the most reliable adjunct to ECG. In the second approach, we evaluated 22 patients by using three criteria: ECG, enzymes, and myocardial scintigraphy. The diagnosis of PMI was confirmed when two of these criteria were positive. In this limited part of the study, we evaluated the reliability of each of these three criteria.

Determination of Cutoff Values

The 61 patients were separated into two groups: those with a negative ECG, the "NO PMI" group (n = 42); and those with a positive ECG, the PMI+ group (n = 19).

Samples from the NO PMI patients were used to establish the reference intervals for the various analytes in these conditions for coronary bypass surgery patients. We used the 95th percentile of the maximum value obtained during the peak period for every enzyme in the NO PMI group. Values greater than these cutoff limits were considered to be indicative of PMI+. The peak period considered for CK and CK-MB was day 0 to day 2. For LD and LD1, it was day 2 to day 4.

In the subgroup of 22 patients evaluated with three criteria, 15 were NO PMI patients (ECG and scintigraphy negative). We used their results to determine cutoff values similarly.

Because the main purpose of the study was to evaluate the reliability of different biological markers for PMI, patients at risk were preferentially included (by the wards). This explains the high percentage of PMI+ in both groups, which has no statistical significance.

Statistical analysis. Mean, median, standard error of the mean, and analysis of variance were calculated by standard methods.

Results

Cutoff Values

Cutoff values determined for the 42 patients with a negative ECG (first approach) were 800 U/L for CK, 50 U/L for CK-MB activity, 75 µg/L for CK-MB mass concentration, 700 U/L for LD, 200 U/L for LD1 by immunoprecipitation, ≤1.0 for LD1/LD2 ratio by electrophoresis, ≤46% for LD1/LD, and 50 U/L for AST. For the 55 patients with negative ECG and negative scintigraphy (second approach), the cutoff values were quite similar: CK 700 U/L, CK-MB activity 40 U/L, LD 500 U/L, and LD1 150 U/L.

Interpretation of the False-Negative Results

Table 1 gives the false-negative results observed with the different enzymes in the PMI+ group of 18 patients and in the subgroup of nine patients for whom CK-MB mass concentration was also determined. In the group of 22 patients who were scanned pre- and postoperatively, the three criteria (ECG, scintigraphy, and enzyme results) were in agreement in 20 patients: all negative for 15 patients, all positive for five. For one patient the scintigraphy was negative, but the other two criteria were positive. In another, a positive scintigraphy was supported by a CK-MB mass concentration exceeding the cutoff value, which contrasted with the negative test results for the other enzymes; an intermediate ECG result could not settle the diagnosis, so we considered the diagnosis of PMI as probable but not certain in this patient.

Evaluation of Routine Tests for Diagnosing PMI

Figure 1 shows the peak values for every enzyme in each patient of the NO PMI and PMI+ groups. From the results depicted in Figure 1 and Table 1, we conclude that AST,

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* Results not exceeding the cutoff values for each enzyme.

(4) All 61 patients (18 = PMI+) and (5) 32 patients whose CK-MB mass was determined (9 = PMI+).
CK-MB activity/CK, and CK-MB mass/CK have the lowest sensitivity.

The two different methods used to determine LD1—immunoinhibition (x) and electrophoresis (y)—give quite similar results, as demonstrated by the correlation \( y = 0.82x + 6.01 \) \( r^2 = 0.86 \).

As shown in Table 1 and Figure 1, between CK-MB activity (group E) and CK-MB mass (group F) determination, the latter has fewer negative results.

To evaluate the period of best discrimination of the CK, CK-MB activity, LD, and LD1, we determined the time course of release of these enzymes (Figure 2). The median and 95th percentile are indicated for both groups (NO PMI and PMI+). CK-MB activity discriminates both groups during days 1 and 2, and LD1 during days 2 to 4.

Effect of the Postoperative Hemolysis on Measurements

The marked decrease of Hp and increase of Hb during the first 24 h postsurgery confirm the in vivo perioperative hemolysis. Surprisingly, there was no correlation between the concentration of Hb and LD or LD1 in the first 24 h after surgery (Hb = 0.041 LD1 + 1.52; \( r^2 = 0.22 \)). This discrepancy was explained by the fact that the activity of LD measured by electrophoresis appeared to decrease; but the mass concentration, revealed by an immunoblotting procedure, was fully retained (unpublished observation).

Discussion

The main purpose of this study was to evaluate which of the enzyme markers are the best routine complementary criteria to an ECG. In the literature the criteria for diagnosing PMI after coronary bypass surgery vary. The three factors commonly used as good indicators of infarction are serial ECGs, serial myocardial scintigraphy with technetium 99m-labeled pyrophosphate, and serial enzyme analysis. Difficulties in interpreting these data in the patient after coronary bypass surgery have been reported with all these techniques and the reliability of each criterion has been criticized. In two studies the measurement of three criteria (ECG, myocardial scintigraphy, and CK-MB mass) have been performed concurrently (5, 6). Diagnosis of PMI was considered established when two criteria were positive. From these data, it is possible to evaluate the reliability of each criterion.

The ECG is considered by most authors as the basic criterion and is easy to perform. The first appearance of a new Q wave on the ECG is only indicative of a possible PMI. To be established, the diagnosis requires the persistence of this marker. Moreover, false-positive ECGs have been reported in several articles (5, 17). An early biological marker could help to confirm the diagnosis.

Myocardial scintigraphy is certainly a helpful criterion for PMI, but it is a difficult procedure that is not well accepted by the patients or the surgeons. Thus, at least one additional criterion is obviously needed, a routine procedure that is easy to perform and reliable, to increase the sensitivity.

Different enzyme markers have been used with variable success. Some authors (5-9) recommend establishing a
profile of enzyme release. Such a profile appears in their work to be sensitive and specific, but requires multiple sampling, which precludes its use as a routine procedure, although it must be considered as a valuable investigational tool. Wu (16) has evaluated the determination of the X-KM and CK-MB isozymes; however, he emphasizes that these are not routine procedures available as emergency tests to diagnose myocardial infarction.

After coronary bypass surgery the significance of the usual reference limits of enzyme concentrations in serum are invalid as a consequence of the inevitable cardiac and extracardiac tissue damage occurring during the surgical procedure. We first had to delineate for each enzyme a cutoff value separating the group suggestive of NO PMI from the PMI+. These cutoff values were derived from the 95th percentile of all results obtained in the group of 42 patients with a negative ECG. To validate the discriminator values, we did the same in a subgroup of 15 patients NO PMI) assessed by both negative ECG and myocardial infarctigraphy. We obtained similar cutoff values.

The method of delineating these values naturally entails *ad factum* a high specificity (of 95%) for every enzyme. Under these conditions the reliability will be appreciated only through their sensitivity. The poor sensitivity of AST and total LD (Figure 1 and Table 1) eliminates these enzymes immediately, as could be expected from their nonspecific distribution in other tissues (i.e., muscle for AST and LD and leukocytes for LD).

Less expected, the ratio of CK-MB activity to total CK and of CK-MB mass to total CK were also quite insensitive. This resulted from a significant increase of total CK in the PMI+ group, which parallels that of CK-MB. We also noticed a tendency of higher LD5 values in this group. The reason for a total CK increase in the PMI+ group remains unknown, although it could be interpreted as a greater release of enzyme, perhaps from muscular anoxia. We are not aware of any reports or explanations of this in studies on coronary bypass surgery.

The CK-MB activity, despite its somewhat low sensitivity, appears helpful as an early marker (Figure 2), in agreement with other studies (2, 5–7). The most discriminant and reliable enzyme markers of PMI appear to be X-MB mass concentration and LD1; with the ECG as the basic criterion, their sensitivity is 75%.

CK-MB mass determined by an immunoenzymometric assay has been proved more specific than CK-MB activity and has been advocated as a first-choice marker (7), but because a fastidious technique is required, its routine use has been hindered. The recent availability of a quick emergency procedure to determine CK-MB mass concentration led us to evaluate this method in the last 32 patients. The results confirm our expectation: the CK-MB mass concentration appears to be the first-choice enzyme marker of PMI, with a higher discriminating power than X-MB activity (Figure 2, Table 1). To explain the higher sensitivity of CK-MB mass concentration vs CK-MB activity, some authors (7, 9) claim an in vivo inactivation of the enzyme, leading to a shorter half-life of the activity, than of the antigenic mass concentration. However, another work (12) reports similar half-lives of CK-MB activity and mass concentration. Whatever the explanation, CK-MB mass appears to be a better discriminator.

The other first choice, LD1, as shown in Figure 2 and Table 1, also appears to be a good discriminator of PMI, but a later period (days 2 to 4). Although our results corroborate a recently published study (8), this enzyme has been generally underevaluated in the field. LD1 was previously determined by electrophoresis, a time-consuming method that is not available on an emergency basis during the weekend or at night. This is probably one of the major reasons for the lack of interest in LD1 in the past. Another reason might be, in the special case of coronary bypass surgery, that some degree of hemolysis is always found after cardiopulmonary bypass, which leads to an increase of LD and LD1 in serum. This phenomenon was rarely mentioned by the other authors. In our study, hemolysis was indeed demonstrated; however, it was limited to the first 24 h and thus it did not interfere with the specificity of the increase of LD1 from PMI later (days 2 to 4). Moreover, we could demonstrate (by immunoblotting) a discrepancy between the activity of LD1 (low) and its mass presence (high), which leads us to consider possible inactivation of LD1 activity related to the extracorporeal circulation. We are not aware of other reports of this problem in the literature.

The recent availability of LD1 determination by a quick immunoinhibition method did stimulate a new interest in this marker; but the question remained whether this method is as reliable as electrophoresis (i.e., flipped LD1/LD2) (16, 19, 20). The very good correlation between both confirms the reliability of the immunoinhibition method, which appears to be a method of choice: quick and available at any moment. The ratio LD1/LD2 (with flipped LD1/LD2 values) is advocated as better than LD1 alone in many articles on myocardial infarction. Figure 1 shows that this ratio does not separate the two groups as clearly as do LD1 alone and the LD1/LD ratio.

In the special conditions of post-cardiac surgery, LD1 appears to be the most sensitive marker of the LD family. It becomes discriminant at a later period than CK-MB, after 48 h.

In conclusion, CK-MB mass concentration appears to be a promising marker, superior to the CK-MB activity assay, in evaluating PMI after coronary bypass surgery, but this needs to be confirmed in a larger series. Because recent advances in methodology allow this assay to be done at any moment as an emergency test, we expect it to become the biological marker of choice during the first 48 h, with LD1 being the marker of choice from days 2 to 4.

References