

Diagnosis of Myocardial Infarction by Serum Isoenzyme Analysis*

CHARLES R. ROE, M.D.

*Division of Pediatric Metabolism,
Duke University Medical Center,
Durham, NC 27710*

ABSTRACT

Serial monitoring of the serum isoenzyme patterns of creatine phosphokinase (CPK) and lactate dehydrogenase (LDH) in patients suspected of acute myocardial infarction has become a highly sensitive and specific diagnostic method. The predictable evolution of isoenzyme patterns following infarction permits diagnosis and recognition of early stages, recovery stages and extension of infarction in the individual. Usual therapeutic and resuscitative manipulations do not interfere with evaluation of patients with angina or following cardiopulmonary arrest without infarction. Despite significant elevations of serum enzyme levels following general and cardiac operative procedures, the occurrence of myocardial necrosis in the surgical population can be recognized by detection of the specific CPK-MB isoenzyme.

Introduction

The laboratory diagnosis of acute myocardial infarction (AMI) has been dependent upon measurement of several serum enzyme levels. In the late 1950's, serum glutamic oxalacetic transaminase and lactate dehydrogenase (LDH) were considered the most useful serum determinations for detection of myocardial damage.^{8,16} Since these two enzyme measurements reflect a variety of tissue sources, they had limited diagnostic specificity. Between 1964 and 1967, serum creatine phosphokinase (CPK) levels were reported to be a more specific

and reliable indicator of acute myocardial necrosis.^{2,3,13} However, even though CPK measurements were highly sensitive, they lacked specificity owing to serum elevations from skeletal muscle. In 1972, studies were published which presented methods for the routine detection of the highly specific CPK isoenzyme, CPK-MB.^{10,14} This isoenzyme is found in highest tissue concentration in myocardium. Many investigators have since chosen to combine serum analyses of LDH and CPK isoenzymes to establish the diagnosis of acute myocardial infarction.

This report will review the utility of combined isoenzyme analysis for the detection of cardiac injury in both the medical and surgical settings.

* Supported in part by grant HL-17670 of the National Institutes of Health.

Acute Myocardial Infarction in the Medical Patient

The clinical diagnosis of AMI depends upon the interpretation of historical, electrocardiographic and laboratory data. Each of these variables is known to have limitations. Prolonged substernal chest pain is often absent in patients with infarction⁶ and, if present, may be due to other causes. Similarly, although new "Q wave changes" on the electrocardiogram are highly specific for AMI, they are absent in approximately 30 percent of autopsy proven cases.⁵ Serum enzyme measurements lack specificity for diagnosis of myocardial infarction owing to their multiple organ origins.

Wagner et al¹⁵ evaluated the sensitivity and specificity of the electrocardiogram (ECG), total CPK, the LDH₁:LDH₂ ratio and CPK-MB in the diagnosis of AMI. In table I are presented the results of this study involving 328 patients admitted to the coronary care unit. The ECG was found to be highly specific but not sufficiently sensitive. Serum CPK measurements were sensitive but of lower specificity. The LDH₁:LDH₂ ratio was both sensitive and specific with a predictive value greater than the ECG and

equivalent to serum CPK measurement. The CPK-MB isoenzyme was observed to have the highest predictive value being 100 percent sensitive and 99 percent specific for the diagnosis of infarction. In this study, it was demonstrated that patients with CPK-MB but without diagnostic ECG were not at lower risk than those with CPK-MB and diagnostic ECG. The mortality was identical for both groups.

The highest degree of confidence in the laboratory diagnosis of AMI was obtained by analyzing serum samples for both LDH and CPK isoenzymes. In addition to diagnosis, the evolution of infarction in an individual patient could be recognized owing to the different time course of release of the cardiac isoenzymes. An example of the major characteristics of serial CPK and LDH isoenzyme analysis in patients with infarction is present in figure 1.

The onset of chest pain is generally considered to represent the beginning of infarction. Clearly, this is not always true. However, using onset of chest pain as the time of initiation of infarction, CPK-MB is observed within 2 to 12 hours. In experimental coronary occlusion, where the time of onset is clearly established, the interval to first appearance of CPK-MB and an altered LDH₁:LDH₂ ratio is 3 to 5 hours. In patients who are frequently sampled during the course of infarction, the CPK-MB isoenzyme appears in serum prior to the alteration of the LDH₁:LDH₂ ratio (figure 1, 12 hour sample). CPK-MB was found to persist in serum for 24 to 72 hours. Since the isoenzyme may disappear in 24 hours, sampling every 12 hours provides the greatest possibility of detecting CPK-MB in any patient suspected of AMI. The LDH₁:LDH₂ ratio is altered to the myocardial pattern between 12 and 24 hours following the event. This alteration is characterized by an increase in quantity of LDH₁ and LDH₂ with a greater

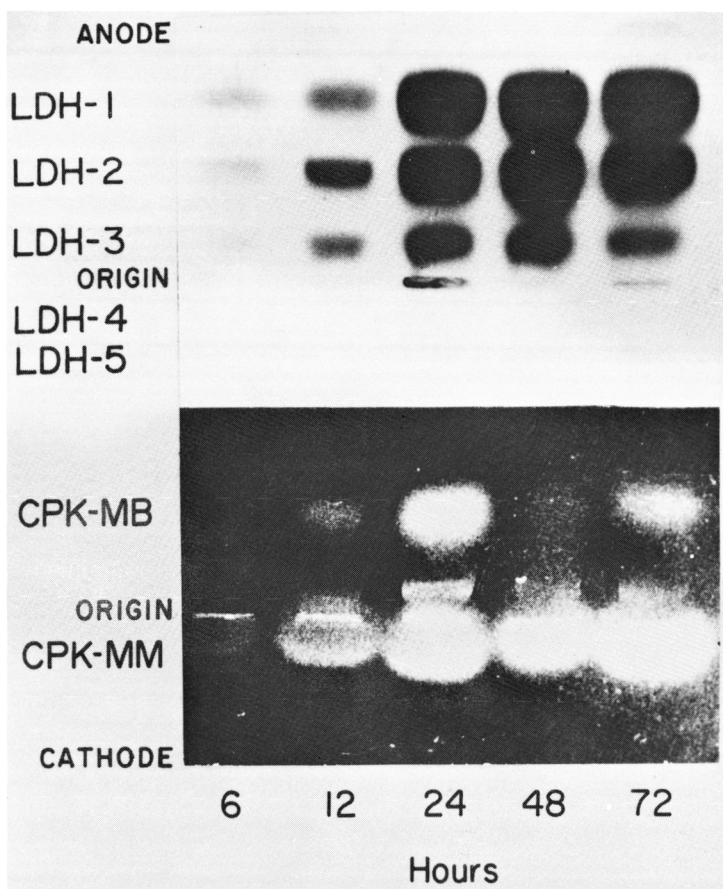
TABLE I

Parameter Sensitivity and Specificity*

Diagnostic Parameter	False		False	
	Neg. (%)	Sensitivity (%)	Pos. (%)	Specificity (%)
ECG	34	66	0	100
Total CPK	2	98	15	85
LDH ₁ :LDH ₂	10	90	5	95
CPK-MB	0	100	1	99

*The incidence of both falsely positive and falsely negative diagnostic parameters is shown. A parameter was considered falsely positive when all others (3) were negative. Likewise, a parameter was considered falsely negative when all others (3) were positive. Lack of false positives is indicative of a specific parameter and lack of false negatives indicates high sensitivity. (Reproduced by permission of *Circulation*.¹⁵)

FIGURE 1. Combined LDH (top) and CPK (bottom) electropherograms from sera of a patient with acute myocardial infarction. The samples were obtained 6, 12, 24, 48, and 72 hours following onset of chest pain. (From Roe, C. R., Limbird, L. E., Wagner, G. S., and Nerenberg, S. T.: Combined isoenzyme analysis in the diagnosis of myocardial injury: Application of electrophoretic methods for the detection and quantitation of the creatine phosphokinase MB isoenzyme. *J. Lab. Clin. Med.* 80:577-590, 1972.)



increase in LDH₁. This is the basic LDH pattern observed in myocardial extracts.

The simultaneous detection of this altered LDH pattern and CPK-MB in the same sample (figure 1, 24 hour sample) is diagnostic of acute cardiac necrosis. The CPK-MB isoenzyme has a shorter duration in serum than the myocardial LDH isoenzyme pattern (figure 1, 48 hour sample). When this combination is detected, the positive LDH pattern indicates infarction has occurred within 48 hours but the patient is in the recovery phase. Such a patient can then be monitored until either the LDH pattern returns to a normal relationship or extension of infarction occurs as evidenced by the reappearance of CPK-MB (figure 1, 72 hour sample).

The relatively brief duration of CPK-MB in serum allows detection of extension which might be clinically unrecognized. Therefore, serial monitoring of CPK and LDH isoenzymes provides qualitative insight into the course of an individual patient. If frequent quantitative measurements are obtained, the course of individual patients is seen to be dynamic and additional data become available to assess the clinical progress of patients (figure 2).

The presence of CPK-MB in the serum of a patient with coronary insufficiency does not necessarily mean "Acute Myocardial Infarction" in the traditional sense of transmural tissue necrosis producing new Q wave changes on the ECG. Resuscitative techniques have occasion-

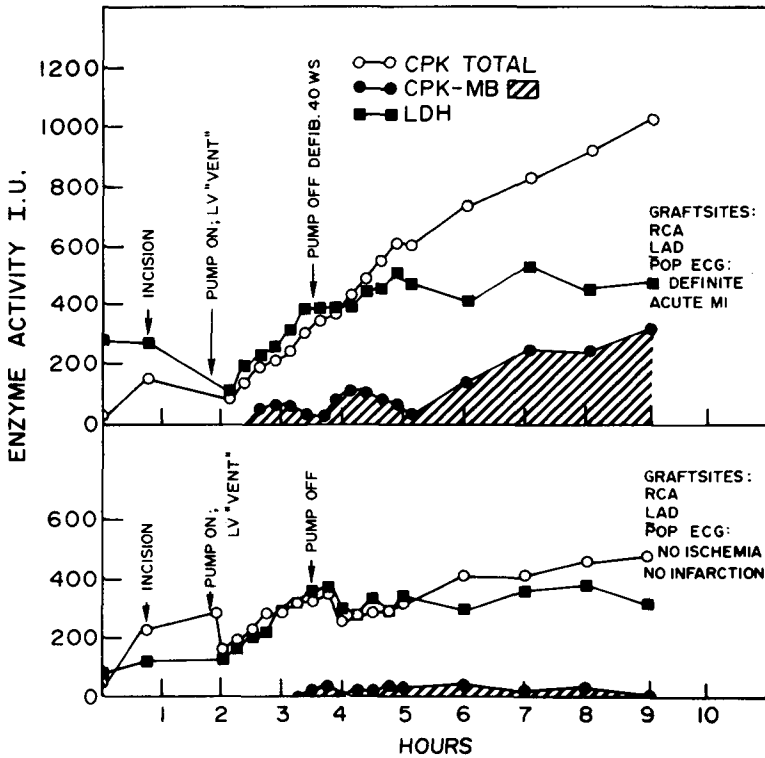


FIGURE 2. Serial measurements of CPK, LDH and the CPK-MB isoenzyme obtained every four hours in two patients with evolving acute myocardial infarction.

ally been associated with cardiac necrosis verified postmortem in patients who had CPK-MB detected in serum samples. The majority of cases requiring resuscitation following cardiopulmonary arrest without other evidence of AMI have not had detectable serum CPK-MB. Patients with angina, stable or unstable, have only CPK-MM present in the serum. The serum CPK elevations resulting from convulsions, injections, cardiac catheterizations, or direct countershock contain only CPK-MM in the serum. The specificity of CPK-MB for myocardial necrosis in the coronary care setting is, therefore, extremely good.

There are a number of other disorders in which CPK-MB is detected in the serum when the cardiac origin may not be proven. These conditions occur in general pediatric and medical patients and represent an area where laboratory diagnosis may be limited. The group as a

whole can be described as myopathic. The following conditions are included: Duchenne's muscular dystrophy, polymyositis-dermatomyositis, idiopathic myoglobinuria, severe peripheral vascular ischemia (gangrenous) and occasionally in Rocky Mountain spotted fever and Reye's syndrome.^{3,10,11,12} The source of CPK-MB is obscure since each of these clinical conditions also may have associated cardiac pathology. These disorders may also involve type II skeletal muscle fibers which contain CPK-MB. However, the LDH isoenzyme pattern and the magnitude of the serum CPK level frequently distinguish these disorders from acute myocardial infarction.¹⁰

In the coronary care setting, serial monitoring with CPK and LDH isoenzymes has specific diagnostic value greater than either the ECG or individual serum enzyme measurements. The diagnosis of infarction may be excluded with

certainty after 48 hours of monitoring resulting in shortened hospital stay, decreased cost and increased bed utilization in the coronary care unit.

Myocardial Infarction in the Surgical Patient

The surgical patient presents a unique challenge to enzymatic diagnosis of AMI. Serum enzyme levels are normally elevated following major general and cardiac operative procedures. The magnitude of elevation is variable and dependent upon the degree of trauma, need for transfusion and the extent of post-operative complications. CPK measurements which are both sensitive and specific in the medical patient setting are limited in the analysis of the post-operative course of the surgical patient.

In high risk general surgical patients having coronary disease but undergoing non-cardiac operative procedures, the frequency of post-operative myocardial infarction has been reported as 8 percent with an associated mortality of 53 percent.⁷ This high risk general surgical population presents a problem in diagnosis since the post-operative ECG as well as post-operative serum enzyme measurement are frequently non-specific. Serial monitoring of serum CPK and LDH isoenzymes in this population has detected cardiac damage as readily as in the non-surgical coronary care patient.

Two studies of high risk patients have shown that CPK-MB is detected post-operatively in 5 to 6 percent of these patients (table II). In a group of 20 patients, one was diagnosed as post-operative infarction by LDH₁:LDH₂ ratio and CPK-MB. Despite a non-specific post-operative ECG, cardiac necrosis was confirmed at autopsy.¹

A second series of 103 patients confirmed a 5.8 percent frequency of CPK-MB post-operatively in a similar

TABLE II
General Surgical Procedures: Incidence of CPK-MB in High Risk Patients

	Patients	CPK-MB	Incidence (Percent)
Series A	20	1*	5
Series B	103	6†	5.8

*Autopsy confirmation of myocardial necrosis.

†Five with EKG evidence of ischemia; one with gangrenous extremity.

population of patients with prior clinical history of myocardial infarction, hypertension or diabetes. Five of the six patients with CPK-MB had ECG evidence of ischemia post-operatively. The remaining patient had no clinical evidence of ischemia but had severe peripheral vascular insufficiency. This individual underwent right lumbar sympathectomy for relief of peripheral vascular insufficiency which proved unsuccessful. The extremity became gangrenous involving both type I and type II muscle fibers and required amputation. Following amputation, all serum enzyme and isoenzyme abnormalities returned to normal.

In this setting, it is not possible to distinguish between a cardiac or type II skeletal muscle source for CPK-MB. Except for severe peripheral vascular disease, the usual isoenzyme elevations observed in the general surgical patient are CPM-MM and LDH-5. Since these are the usual post-operative isoenzyme abnormalities, the presence of CPK-MB and an altered LDH₁:LDH₂ ratio indicates acute cardiac necrosis.

The most difficult setting for the laboratory diagnosis of myocardial damage in the surgical setting is the cardiac operative procedure. These operations, of necessity, involve some trauma to myocardium. Therefore, one would anticipate a higher frequency of detection of CPK-MB during the perioperative period.

Dixon and co-workers¹ studied 100 patients undergoing aortocoronary bypass for coronary artery disease. Serial sampling was limited to twice daily during the first 48 hours following operation and daily for three additional days. Forty-nine percent of these patients had no detectable CPK-MB post-operatively. Fifty-one percent had variable amounts of this isoenzyme noted in serum samples. Three general serial enzyme and isoenzyme profiles typified this population (figure 3, A,B,C). None of the 49 patients represented by profile A had post-operative infarction by ECG. Twenty-six of the remaining 51 patients had CPK-MB detected only in the first post-operative sample (figure 3,B). Four of these 26 (15.4 percent) had new Q wave changes on the post-operative ECG. In 25 of the 51 patients with detectable CPK-MB, this isoenzyme persisted for more than 18 hours post-operatively (figure 3,C). Seventeen of these 25 patients (68.0 percent) had diagnostic post-operative electrocardiograms. Therefore, 66 percent of patients with CPK-MB persisting for greater than 18 hours after aor-

tocoronary bypass are likely to have an ECG diagnosis of infarction.

Traditionally, infarction in the peri-operative period has been termed "post-operative" infarction. Of the 100 patients studied by Dixon, less than 5 percent could be designated as truly late, post-operative infarcts. Oldham and co-workers⁹ studied a group of 39 patients undergoing the same operative procedure. Serial monitoring frequency was increased to study in detail the early operative interval beginning with anesthesia induction. In figure 4 are illustrated the type of data available from this analysis. In both patients, CPK-MB first appeared during the period of cardiopulmonary bypass. The higher levels of CPK-MB in the upper half of figure 4 were associated with an ECG infarct, evidence for which was detected 15 minutes after beginning cardiopulmonary bypass. The complete post-operative profile was identical to that observed in figure 3,C. The intra-operative profile in the lower half of figure 4, is similar to the complete profile seen in figure 3,B.

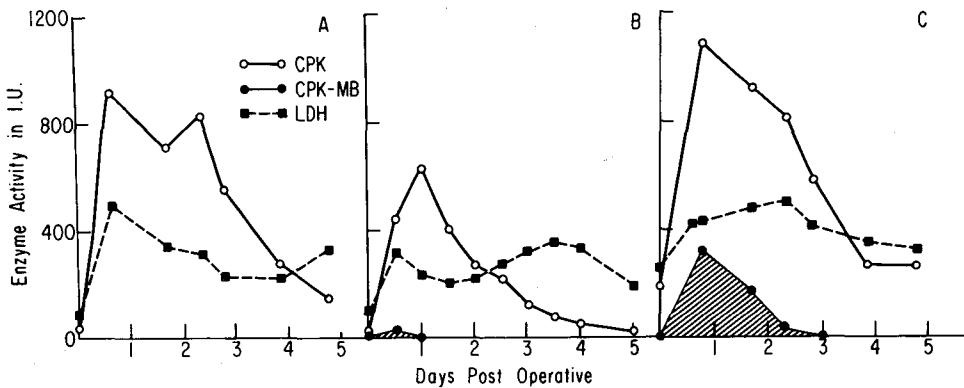
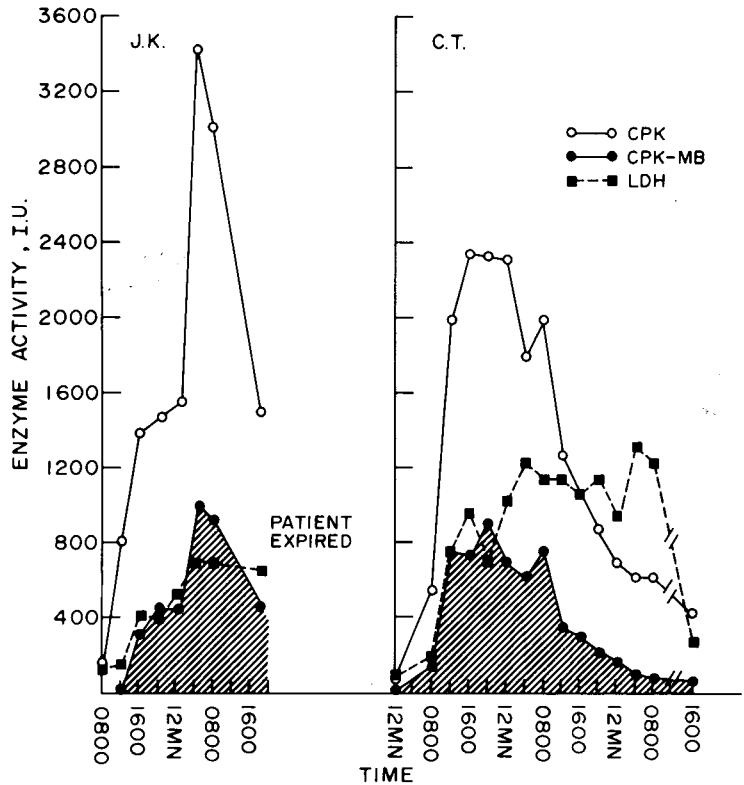


FIGURE 3. Serial measurement of CPK, LDH and the CPK-MB isoenzyme in patients undergoing aortocoronary bypass grafting. (A) Characteristic profile of the group of patients in whom CPK-MB was not observed. (B) Example of the group having transient appearance of CPK-MB without diagnostic ECG. (C) CPK-MB quantitation representative of the majority of patients with ECG confirmed acute myocardial infarction. Reproduced by permission of the American Heart Association, Inc. (From Dixon, S. H., Limbird, L. E., Roe, C. R., Wagner, G. S., Oldman, H. N., and Sabiston, D. C.: Recognition of post-operative acute myocardial infarction. *Circulation* 47 & 48, (Suppl. III):137-140, 1973.

FIGURE 4. Enzyme activity in two patients during the intra-operative and early post-operative period following coronary artery bypass surgery. The upper panel demonstrates a progressive rise in total CPK. CPK-MB first appeared after 30 minutes of cardiopulmonary bypass and persisted for 55 hours. This patient had post-operative ECG changes of acute infarction. In the lower panel, CPK-MB appearing after one hour and 10 minutes of cardiopulmonary bypass was transiently detectable for 6 hours. This patient had no changes of ischemia or infarction on post-operative ECG. (From Oldham, H. N., Jr., Roe, C. R., Young, W. G., Jr., and Dixon, S. H., Jr.: Intraoperative detection of myocardial damage during coronary artery surgery by plasma creatine phosphokinase isoenzyme analysis. *Surg.* 74:917-925, 1973.)



This high frequency monitoring protocol permitted identification of the operative interval in which patients first had detectable CPK-MB. In table III is shown this distribution in 30 patients within these intervals. The majority of patients had detectable CPK-MB while on cardiopulmonary bypass (47 percent) or following bypass (37 percent). No patients had CPK-MB appearing for the first time late following operation. Five patients (16 percent) had detectable serum CPK-MB prior to cardiopulmonary bypass. Additional studies indicate that pre-bypass appearance of CPK-MB in patients having aortocoronary bypass may be even more frequent.⁴ These studies emphasize the importance of this interval in the genesis of AMI in patients with severe coronary disease.

The amount of CPK-MB released during the perioperative period following aortocoronary bypass appears to have a relationship to the severity of the post-operative electrocardiographic diagnosis. The amount of CPK-MB is integrated over the duration of its presence in serum. This integral, referred to as a crude myocardial damage index (MDI) is expressed in units of IU/L-Hours. In

TABLE III
Initial Appearance of Detectable CPK-MB in 30 Patients*

Before Anesthesia	Before Pump	During Pump	After Pump	Post-Operative
1 (3%)	4 (13%)	14 (47%)	11 (37%)	0

*Reproduced by permission of *Surgery*.¹⁰

TABLE IV

Relationship of ECG Category to Myocardial Damage Index (MDI) Following Aortocoronary Bypass

ECG Category	Patients	%	Mean	Significance
			MDI	
Infarct	20	18	2145]P<0.01]]P<0.0001]
Ischemia or injury	50	46	221	
Non-specific ST or normal	39	36	63	

table IV is illustrated the relationship between these areas and the ECG category in 109 patients undergoing this operative procedure. There is a significant difference between the ECG categories of (1) infarction, (2) ischemia or injury patterns and (3) non-specific ST segment changes or no changes post-operatively.

Further study and evaluation of the clinical severity of coronary artery disease, anesthetic techniques, operative manipulations and myocardial preservation techniques will ultimately identify those factors related to intra-operative cardiac damage and should reduce both clinically significant and insignificant elevations of the CPK-MB isoenzyme during cardiac procedures. Although CPK-MB is expected more frequently during these operations, its elimination is desirable and may well reflect reduced individual risk.

Summary

Combined isoenzyme analysis (CPK and LDH) is both specific and sensitive for detection of acute myocardial necrosis in the medical, general surgical and cardiac surgical settings. The diagnosis of AMI can be excluded in patients whose ECG is non-diagnostic by serial monitoring. The usual resuscitative procedures or manipulations employed in the coronary care unit do not produce CPK-MB in serum. Patients with arrhythmias or angina without infarction have normal serum isoenzyme

patterns. Certain myopathic conditions involving type II skeletal muscle fibers have detectable serum CPK-MB which may be differentiated from cardiac origin by lack of confirmation by serum LDH isoenzyme patterns.

The general surgical patient can be evaluated by these laboratory tests in the same manner as patients in coronary care. Patients undergoing cardiac operative procedures in which cardiac manipulation is inherent will have a higher frequency of CPK-MB detection than general surgical patients. The level of integrated CPK-MB released during the perioperative period following aortocoronary bypass procedures correlates with the severity of the electrocardiographic diagnostic category.

References

- DIXON, S. H., LIMBIRD, L. E., ROE, C. R., WAGNER, G. S., OLDHAM, H. N., and SABISTON, D. C.: Recognition of post-operative acute myocardial infarction. *Circulation* 47 & 48, (Suppl. III):137-140, 1973.
- DUMA, R. J. and SEIGEL, A. L.: Serum creatine phosphokinase in acute myocardial infarction. *Arch. Intern. Med.* 115:443-451, 1965.
- HESS, J. W., MACDONALD, R. P., FREDERICK, R. J., JONES, R. N., NEELEY, J., and GROSS, D.: Serum creatine phosphokinase activity in disorders of heart and skeletal muscle. *Ann. Int. Med.* 61:1015-1028, 1964.
- ISOM, O. W., SPENCER, F. C., FEIGENBAUM, H., CUNNINGHAM, J., and ROE, C. R.: Pre-bypass myocardial damage in patients undergoing coronary revascularization: an unrecognized vulnerable period. *Circulation* 52(Suppl. II):119, 1975.
- JOHNSON, W. J., ACHOR, R. W. P., BURCHELL, H. B., EDWARDS, J. E.: Unrecognized myocardial infarction. *Arch. Intern. Med.* 103:253-261, 1959.
- KANELL, W. B. and FEINLEIB, M.: Natural history of angina pectoris in the Framingham study. *Amer. J. Cardiol.* 29:154-163, 1972.
- MAUNEY, F. M., EBERT, P. A., SABISTON, D. C.: Post-operative myocardial infarction: a study of predisposing factors, diagnosis, and mortality in a risk group of surgical patients. *Ann. Surg.* 172:497-503, 1970.
- NYDICK, I., WROBLEWSKI, F., and LADUE, J. S.: Evidence of increased serum glutamic oxalacetic transaminase activity following graded myocardial infarcts in dogs. *Circulation* 12:161-168, 1975.

9. OLDHAM, H. N., ROE, C. R., YOUNG, W. G., and DIXON, S. H.: Intra-operative detection of myocardial damage during coronary artery surgery by plasma creatine phosphokinase isoenzyme analysis. *Surgery* 74:917-925, 1973.
10. ROE, C. R., LIMBIRD, L. E., WAGNER, G. S., and NERENBERG, S. T.: Combined isoenzyme analysis in the diagnosis of myocardial injury: application of electrophoretic methods for the detection and quantitation of creatine phosphokinase MB isoenzyme. *J. Lab. Clin. Med.* 80:577-590, 1972.
11. ROE, C. R., SCHONBERGER, L., GEHLBACH, S. H., WEIS, L. A., and SIDBURY, J. B.: Enzymatic alterations in Reye's syndrome: prognostic implications. *Pediatrics* 55:119-125, 1975.
12. SEXTON, D. J., BANKS, P. M., WEIG, S., and ROE, C. R.: Late appearance of skin rash and abnormal serum enzymes in Rocky Mountain spotted fever: a case report. *J. Pediat.* 87:580-582, 1975.
13. SMITH, A. F.: Diagnostic value of serum creatine kinase in a coronary care unit. *Lancet* 2:178-182, 1967.
14. SOMER, H. and KONTTINEN, A.: A method allowing the quantitation of serum creatine kinase isoenzymes. *Clin. Chim. Acta* 36:531-536, 1972.
15. WAGNER, G. S., ROE, C. R., LIMBIRD, L. E., ROSATI, R. A., and WALLACE, A. G.: The importance of identification of myocardial-specific isoenzyme of creatine phosphokinase (MB Form) in the diagnosis of acute myocardial infarction. *Circulation* 47:263-269, 1973.
16. WROBLEWSKI, F.: The clinical significance of serum enzyme alterations associated with myocardial infarction. *Amer. Heart J.* 54:219-224, 1957.