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Radioimmunoassay of serum myoglobin in screening for acute myocardial infarction

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Over an 8-month period 289 patients consecutively admitted to a coronary care unit were studied to assess the value of serum myoglobin determinations by radioimmunoassay in screening for acute myocardial infarction. Of the 289 patients 127 (44%) had an infarction. It was found that when blood sampling was done within 5 to 15 hours after the onset of chest pain this assay had a sensitivity, specificity, and positive and negative predictive values of more than 97%. In this study healthy young adults had a mean serum myoglobin level of 37 ± 11 (standard deviation) ng/ml, and values above 80 ng/ml were considered positive for acute myocardial infarction. False-positive results can be due to shock, vigorous exercise, skeletal muscle damage and severe renal failure, but, except for the last one, these conditions also cause an increase in the serum level of the creatine kinase isoenzyme CK-MB.

Au cours d'une période de 8 mois on a étudié 289 patients consécutifs admis dans une unité coronarienne afin d'établir l'intérêt du radioimmunodosage de la myoglobine sérique dans le dépistage de l'infarctus aigu du myocarde. Des 289 patients 127 (44%) avaient un infarctus. On a découvert que quand le prélèvement de sang était fait dans les 5 à 15 heures qui suivent l'apparition de la douleur thoracique, cette méthode de dosage possédait une sensibilité, une spécificité et une valeur prévisionnelle positive ou négative de plus de 97%. Dans cette étude, les jeunes adultes en bonne santé ont montré une concentration moyenne de myoglobine sérique de 37 ± 11 (écart type) ng/ml, et les valeurs supérieures à 80 ng/ml ont été considérées indicatives d'un infarctus du myocarde. Les faux résultats positifs peuvent être dûs à un état de choc, un exercice physique vigoureux, une atteinte des muscles squelettiques ou une insuffisance rénale grave; toutefois, sauf pour l'insuffisance rénale, ces affections entraînent également une augmentation du taux sérique de l'isoenzyme créatine kinase CK-MB.

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Acute myocardial infarction is diagnosed on the basis of a characteristic clinical history and typical changes in serial electrocardiograms (ECGs), serum enzyme findings and myocardial scintiscans.

Recent studies have shown that acute myocardial infarction results in the release of myoglobin and a transient increase in its serum level.[1] This can be demonstrated by a sensitive, specific radioimmunoassay,[2] now commercially available from several sources, that can be performed in 2 to 3 hours.

The levels of myoglobin in the serum of patients with acute myocardial infarction peak within 4 hours after admission to hospital, according to Stone and associates.[3] Only 50% of patients have high levels by 12 hours, and in most the level is back to normal by 24 to 36 hours.[3] Dogs with experimentally produced acute myocardial infarction have increased serum levels of myoglobin at 2 hours and maximum levels at 6 hours.[4] Stone and colleagues[5] simultaneously assayed myoglobin and creatine kinase in blood samples obtained at the time of admission from 42 patients with acute myocardial infarction and found that all 42 had an increased serum myoglobin level but only 19 had a high serum creatine kinase level. They also noted, as have others,[6] an increase in the serum myoglobin level in certain other conditions (Table I).

We undertook a study to assess the potential value of serum myoglobin determinations by radioimmunoassay as a method of screening for acute myocardial infarction in our coronary care unit.

Methods

Study design

Over an 8-month period 289 patients consecutively admitted to the coronary care unit of St. Joseph's Hospital, London, Ont. were studied. Initially blood

samples were taken at the time of admission and the following morning. Because the first sample could therefore have been taken as early as an hour and the second more than 24 hours after a myocardial infarction, in many patients the first sample was too early and the second too late to document the transient rise in the serum myoglobin level. To alleviate this problem, the samples from the last 164 patients were taken at the time of admission and 8 hours later. This approach ensured that in most patients one of the samples was taken within the critical 5 to 15 hours after the onset of chest pain suspected of being due to acute myocardial infarction.

In all cases ECGs were obtained daily for 3 days, and blood samples were obtained at the time of admission and on two succeeding mornings for determination of the serum levels of creatine kinase, the isoenzyme CK-MB and lactate dehydrogenase. One third of the patients underwent myocardial scintiscanning with technetium 99m stannous pyrophosphate.

The clinical and laboratory data were reviewed. The presence or absence of acute myocardial infarction was judged by the cardiologists by the usual criteria of clinical course, appearance of new Q-waves or ST-segment elevation and T-wave inversion on serial ECGs, and increases in levels of serum enzymes, especially CK-MB. Abnormal ^{99m}Tc stannous pyrophosphate scans of the myocardium helped in some cases. The serum myoglobin levels were not available to the cardiologists.

A retrospective analysis was then made to assess the correlation between the serum myoglobin levels and the discharge diagnosis.

Assays

The serum samples were assayed for myoglobin in duplicate by the only radioimmunoassay commercially available at that time (from Nuclear Medical Systems Inc., Newport Beach, California). Since then, several other kits have become available, and we now use one with the same protocol and comparable results (from Eiken Chemical Company Ltd., Tokyo).

The assays were carried out in accordance with the manufacturer's protocol, without modification, as follows: To each tube of 100 μl of standard, control or patient serum 100 μl of myoglobin antibody (rabbit) was added and the tube was incubated for 15 minutes. Then 100 μl of myoglobin labelled with iodine 125 was added and the tube was incubated for 30 minutes. To achieve separation 200 μl of sheep rabbit antiserum was added and the tube was incubated for a further 30 minutes, then centrifuged for 15 minutes at 750 $\times$ *g*. The supernatant was decanted, the pellet counted for 1 minute and the percentage of bound myoglobin read from the standard curve. The upper limit of normal for the serum myoglobin level established by the manufacturer, 80 ng/ml, was confirmed by analysis of our data.

The normal distribution, mean and estimated standard deviation of the serum myoglobin level were derived from data for 10 healthy adults aged 21 to 35 years and for patients without myocardial infarction. The effect of exercise and renal function on the serum myoglobin level was studied. Values above 250 ng/ml were simply reported as $>$ 250 ng/ml, and those below 31 ng/ml as $<$ 31 ng/ml.

The total serum creatine kinase activity was determined with a reaction rate analyser (LKB Instruments, Inc., Rockville, Maryland) and a CK NAC (N-acetyl-L-cysteine)-activated kit (Boehringer Mannheim Biochemicals, Indianapolis, Indiana) yielding an upper limit of normal of 135 U/l. In the serum samples with a creatine kinase level above 80 U/l the activity of CK isoenzymes was determined electrophoretically with a CK isoenzyme reagent kit (Beckman Instruments Inc., Fullerton, Calif.). A CK-MB value above 8 U/l in any of the three samples for each patient was considered to indicate acute myocardial infarction[7] and was designated "positive".

Results

The assay sensitivity at 90% B/B_0 (i.e., with 90% of the myoglobin bound) was found to be 25 ng/ml. The between-assay coefficients of variation for the sample with the lowest value, 34 ng/ml, and that with the highest value, 192 ng/ml, were 8.6% and 8.0% respectively (there were 86 assays, including four lots of antibody, performed by three technicians). The within-assay coefficients of variation for the same samples were 3.8% and 2.7% respectively.

The rates of recovery of myoglobin were 102%, 95% and 92% respectively when 31.2, 62.5 and 125 ng of myoglobin per millilitre was added to the patient's serum. Dilutional parallelism was demonstrated, with a linear regression of $Y = 199X - 7.7$ (where Y was the serum level of myoglobin and X the dilutional factor) and a correlation coefficient of 0.999 between expected and observed values.

The mean serum myoglobin levels in the four groups of subjects are shown in Table II.

Of the 289 patients in the coronary care unit 127 were classified as having an acute myocardial infarction and 162 patients as not. Fig. 1 shows the relation between the levels of myoglobin and CK-MB in the serum of these two groups of patients according to the time the blood samples were taken. It can be seen that

Table I—Conditions in which an increased or a normal serum myoglobin level has been found[5,6]

Increased level
After acute myocardial infarction
After open heart surgery
After vigorous exercise
With skeletal muscle damage
With shock
With severe renal failure
Normal level
In healthy adults
With chest pain but not acute myocardial infarction
With congestive heart failure but not acute myocardial infarction
After cardiac catheterization
After exercise on a bicycle ergometer
After intramuscular injections

a cut-off point of 80 ng/ml between normal and abnormal serum myoglobin levels gave the best separation of the patients into infarction and no infarction groups.

Table III shows the value of the two assays in screening for acute myocardial infarction in the entire group of 289 patients in the coronary care unit. There were 6 false-positive results with the myoglobin assay and 10 with the CK-MB assay. Because the serum level of CK-MB rose later, there were more false-negative results with blood samples obtained in the first 8 hours after admission.

Discussion

The precision and dilutional parallelism of the serum myoglobin assay were excellent and similar to what others have found.[8,9]

Early in our study it became obvious that when blood samples were taken at the time of admission and the next morning the transient rise in the serum myoglobin level was often missed: among the first 125 patients there were 27 false-negative results for 55 patients with acute myocardial infarction. This problem was solved by obtaining samples at the time of admission and 8 hours later, which ensured that we obtained samples from most patients within 5 to 15 hours after the onset of chest pain. Obviously patients who presented for treatment more than 15 hours after the onset of chest pain may have had blood samples taken only after the myoglobin level had returned to normal; in fact, this was responsible for two false-negative results.

The CK-MB assays were done on blood samples taken at the time of admission and on two succeeding mornings. Thus, the third sample could have been taken as early as 28 hours after admission; this no doubt explains the 85% sensitivity. This problem was

Table II—Serum myoglobin levels

Subjects	Mean ± standard deviation (ng/ml)
Healthy young adults (n = 10)	34.4 ± 7.7†
Patients undergoing long-term renal dialysis (n = 10)	171 ± 62‡
Patients in coronary care unit	
Without acute myocardial infarction (n = 162)	37 ± 11
With acute myocardial infarction (n = 70*)	194 ± 63

*Only those whose blood samples were taken 5 to 15 hours after the onset of chest pain are included here.
†Six levels were less than 31 ng/ml, three were 31 to 37 ng/ml and one was 55 ng/ml. The morning after a late-evening game of floor hockey the levels were 195 and 200 ng/ml in two subjects and normal in three.
‡Minimum, 90 ng/ml; maximum, over 250 ng/ml.

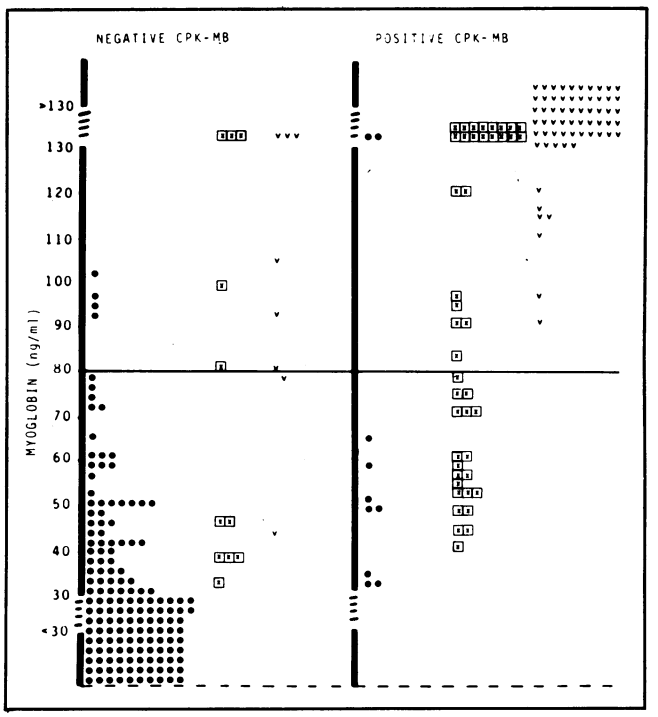

FIG. 1—Relation between levels of myoglobin and creatine kinase isoenzyme CK-MB in serum of patients without acute myocardial infarction (●), with infarction and blood samples drawn 5 to 15 hours after onset of chest pain (v) or with infarction and blood samples drawn outside this period (⊠).

Table III—Value of assays for myoglobin and the creatine kinase isoenzyme CK-MB in the serum in screening for acute myocardial infarction

Variable	Assay; time after admission that blood samples were taken: Myoglobin; 0 and 8 h (n = 164)	CK-MB; 0 and 8 h (n = 164)	Myoglobin; any time (n = 289)	CK-MB; three times up to 48 h (n = 289)
No. of patients				
With acute myocardial infarction	70	70	127	127
Without acute myocardial infarction	94	94	162	162
No. of results				
True positive	68	44	98	108
True negative	92	91	156	152
False positive	2	3	6	10
False negative	2	26	29	19
Sensitivity, %	97.1	62.9	77.2	85.0
Specificity, %	97.9	96.8	96.3	93.8
Positive predictive value, %	97.2	93.6	94.2	91.5
Negative predictive value, %	97.8	77.8	84.4	88.9
Accuracy, %	97.6	92.3	87.9	90.0

beyond our control, as the sampling routine was established by the coronary care unit, and the samples were obtained and tests performed by the biochemistry laboratory. Prolonging the sampling period no doubt would raise the sensitivity up to the 98% reported by Kubasik and associates.[10]

It was interesting that a game of floor hockey late in the evening resulted in high serum myoglobin levels in two of five healthy young men; strenuous activity should therefore be remembered as a cause of false-positive test results. In the patients in the coronary care unit there were 10 false-positive results with the CK-MB assay and 6 with the myoglobin assay, but in 5 of the 6 there were other reasons for the high myoglobin levels (e.g., cardioversion, shock, sepsis and poor renal function), as others have found[5,8] (Table IV). Our false-positive rate for the myoglobin assay, 3.7% (6/162), is less than the 20% reported by Witherspoon and Kubasik and their colleagues.[8,10] They reported levels above 50 ng/ml as positive, choosing the upper limit of normal on the basis of results in ambulatory volunteers (laboratory personnel), whereas we reported levels of 80 ng/ml and above as positive, choosing, like Sonnemaker and coworkers,[9] our upper limit of normal from data for patients in a coronary care unit with chest pain but not acute myocardial infarction. The cut-off point of 80 ng/ml eliminates the false-positive results due to mild or moderate renal failure (serum creatinine level above 1.0 mg/dl [88 μmol/l] but less than 2.5 mg/dl [220 μmol/l]). In addition, when the blood samples were obtained at the time of admission and 8 hours later, using the higher cut-off point for normal results yielded a false-negative rate of only 2.8% (2/70). Sonnemaker and coworkers[9] had no false-negative or false-positive results but admitted excluding patients with conditions known to cause false-positive results.

The sensitivity, specificity, and positive and negative predictive values of the serum myoglobin assay were all over 97% in our study (Table III). Thus, in our institution, with our mix of patients, this assay is superior to serial determination of the serum CK-MB level as currently carried out. In addition, the serum myoglobin assay provides a way to screen earlier for acute myocardial infarction.

Table IV—Details of the cases in which there were false-positive results of the serum myoglobin assay

Case no.	Clinical features	Serum myoglobin level (ng/ml)	Result of CK-MB test
1	Cardiac arrest, cardioversion and renal failure; patient died	250	-
2	Pneumonia, sepsis and pulmonary embolism; patient died	96	+
3	Ruptured aneurysm, shock and respiratory failure	250	+
4	Congestive heart failure; serum creatinine level 2.5 mg/dl (220 μmol/l)*	94	-
5	Chest pain, cause not determined; serum creatinine level 2.6 mg/dl (230 μmol/l)*	100	-
6	Angina	87	-

*Normally less than 1 mg/dl (88 μmol/l).

Conclusion

The serum myoglobin assay is an excellent early screening test for acute myocardial infarction, and it could influence the overall management of patients suspected of having this condition if performed within the appropriate time after the onset of chest pain, with awareness of the conditions that will cause nonspecific increases in the serum myoglobin level.

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