

Serum Myoglobin Determination: Laboratory and Clinical Evaluation

Robert E. Sonnemaker, Douglas L. Daniels, William E. Craig, John L. Floyd, and Robert F. Bode

Nuclear Medicine and Cardiology Services, William Beaumont Army Medical Center, El Paso, Texas

Quality assurance examination of a commercially available radioimmunoassay kit for determination of serum myoglobin level confirmed the measurement to be accurate, precise, and reproducible under all assay performance variables. Initial clinical evaluation in patients admitted to the Coronary Care Unit revealed comparable diagnostic parameters and earlier detection when compared with the present standard indicator of myocardial necrosis, creatine phosphokinase MB isoenzyme. Interpretation that an elevated myoglobin reflects acute myocardial infarction should be made only in the appropriate clinical context.

J Nucl Med 20: 120–124, 1979

The diagnosis of acute myocardial infarction is usually established on the basis of clinical presentation, electrocardiographic abnormalities, and serum enzyme determinations. An important limitation of these conventional criteria is the time necessary for a diagnostic pattern to unfold. The electrocardiographic pattern and complete serial enzyme documentation require days to evolve fully. The introduction of Tc-99m stannous pyrophosphate imaging to detect acute myocardial infarction has added additional information in patient evaluation but falls short in diagnostic sensitivity and specificity (1-3) and may require several days to indicate an abnormality.

Recent attention has been focused on the myoglobin molecule known to be liberated from cardiac muscle during acute myocardial infarction. The commercial development of a radioimmunoassay for the quantification of serum myoglobin* permits the widespread utilization of a new parameter for the diagnosis of acute myocardial infarction.

The purpose of our investigation was twofold: a) to assess the laboratory characteristics of the assay; and b) to evaluate the clinical efficacy and utility in the patient population suspected of having suffered an acute myocardial infarction.

METHODS

The radioimmunoassay is a sequential saturation analysis using I-125-labeled human myoglobin as the tracer. Rabbit antimyoglobin serum is the binding antibody and sheep antirabbit serum serves as the precipitating antibody. The entire procedure is performed at room temperature with 75 min required for incubation. A complete run requires approximately two technician hours.

Quality assurance examination of the radioimmunoassay included documentation of reproducibility, precision, specificity, accuracy, and stability of myoglobin activity in patient samples.

The population of patients under investigation included all patients admitted to the Coronary Care Unit over a 6-mo period. Serum myoglobin concentration was determined at 0, 4, and 12 hr postadmission and tabulated for retrospective correlation. Final clinical impression was determined by traditional means, including clinical presentation, electrocardiographic findings, and serial enzyme deter-

Presented in part at 25th Annual Meeting of the Society of Nuclear Medicine, Anaheim, California, June 1978.

Received Apr. 6, 1978; revision accepted Oct. 25, 1978.

For reprints contact: Robert E. Sonnemaker, Nuclear Medicine Service, 6720 Bertner, Houston, TX 77030.

minations independent of serum myoglobin levels. Diagnoses were classified as acute myocardial infarction (23 patients) or no acute myocardial infarction (113 patients), the latter including angina documented by ECG (20 patients) and typical or atypical chest pain without ECG abnormalities (93 patients). Acute myocardial infarctions were further subclassified as transmural or subendocardial. The normal distribution, mean value, and estimated standard deviation of serum myoglobin were derived from the patient group with no acute myocardial infarction. Myoglobin results were compared with those of the MB isoenzyme of creatine phosphokinase (CPK-MB) as determined by the selective activation technique.† Serum CPK-MB concentrations were determined at admission and every 8 hr thereafter for 3 consecutive days. Comparison included time of appearance of the initial abnormal values and time to peak abnormal values of both myoglobin and CPK-MB in patients with acute myocardial infarction. Additionally, diagnostic parameters were derived.

RESULTS

The standard curve plotted on two-cycle semi-logarithmic paper is sigmoidal in nature with shoulders at 30 ng/ml and 125 ng/ml (Fig. 1). Dilution to one tenth is required for quantification of samples containing >250 ng/ml of myoglobin.

Reproducibility of the assay was good (Table 1). Coefficient of variation among 24 separate runs, three technologists and five separate kit lots was uniformly low for both low- and high-range control sera. When all assay performance variables over a 2-mo period were taken into account, coefficients of variation for low and high control sera were 5.4% and 3.0%, with all values falling within two standard deviations of the means.

Analysis of precision yielded similar results (Table 2). Ten within-run replicates of low- and high-value control sera resulted in coefficients of variation of 4.9% and 6.6%, respectively.

Linearity, as assessed by proportional dilution of two samples, is shown in Fig. 2. Correlation coefficients between data points and fit lines were 0.9995 and 0.9998. Linear regression analysis yielded y intercepts of +3.38 and -1.53, suggesting good assay specificity by their proximity to zero.

Further analysis of specificity reveals no cross reactivity with hemolysed blood. Samples containing varying amounts of hemolysate graded from 0-4+ (grossly bloody) yielded no significant change from the unhemolysed sample value of 43.0 ng/ml. The mean value for all samples was 43.5 ng/ml (estimated standard deviation = 0.8 ng/ml) with a coefficient of variation of 1.8%.

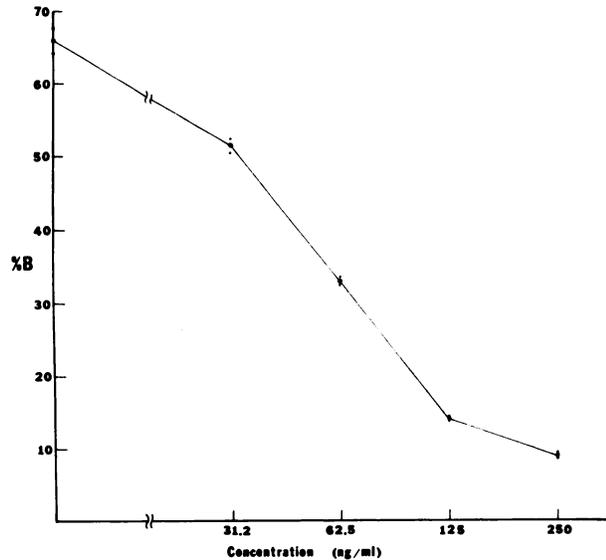


FIG. 1. Myoglobin standard curve constructed on two-cycle semilogarithmic paper: duplicate samples (small points) and mean values (large points) are plotted.

TABLE 1. SERUM MYOGLOBIN ASSAY REPRODUCIBILITY

	Coefficient of variation (%)		
	Low serum	High serum	All
Among runs (n = 24)	7.22	5.89	6.48
Among tech's (n = 3)	4.92	2.91	4.17
Among lots (n = 5)	2.86	4.18	3.42

TABLE 2. PRECISION OF SERUM MYOGLOBIN ASSAY: WITHIN-RUN REPLICATES

Serum sample	\bar{x} (ng/ml)	esd (ng/ml)	CV (%)
High value (n = 10)	188.1	12.4	6.6
Low value (n = 10)	32.9	1.6	4.9

Accuracy of the assay, evaluated by recovery of reference myoglobin*, prepared according to Stone et al. (17), was good for four values of added myoglobin ranging from 49.6-195.4 ng. The mean value recovered was 105.0% ± 4.1% (cv = 3.9%).

A definite trend for samples to lose myoglobin activity with multiple freeze-thaw cycles was established. The total myoglobin value of four samples decreased from 789 to 515 ng/ml over four freeze-thaw cycles, with an overall loss of 34.7% of initial myoglobin activity.

Clinical Evaluation. Included in the clinical data analysis were 136 patients: 113 negative, 17 with

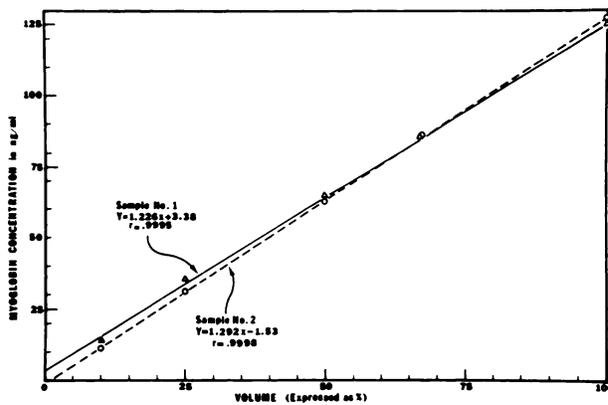


FIG. 2. Proportional dilution of two myoglobin samples confirms excellent test linearity. Ordinate shows measured myoglobin concentration; abscissa gives reciprocal of dilution factor, as percentage of original.

transmural infarction, and six with subendocardial infarction. Patients with trauma, intramuscular injection, or defibrillation were excluded. There were 113 patients identified as having no evidence for an acute myocardial infarction. The 339 serum myoglobin values from these patients ranged from 6.2 to 79 ng/ml. The normal value distribution approximated a standard normal curve with the mean at 33.0 ng/ml (kurtosis = 3.88, skewness = 0.72). Estimated standard deviation was 13.8 ng/ml, which placed mean + 3 s.d. at 74.4 ng/ml, very near the 79 ng/ml of the highest value obtained. Four of these patients demonstrated myoglobin values greater than the mean + 3 s.d. level (76.5, 78.0, 79.0, 79.0). Peak myoglobin values from 17 patients with transmural infarction ranged from 176 to 1100 ng/ml. Peak values from six patients with subendocardial infarction ranged from 120 to 250 ng/ml.

Myoglobin diagnostic parameters were all 100%. The high specificity reflects the exclusion of known causes for false-positive elevation. Chi-square analysis with Yate's correction demonstrates a highly significant association of myoglobin results with the final clinical impression (Table 3).

CPK-MB values from the 113 negative patients ranged from 0 to 24 IU/l. The mean value was 4.6 IU/l (s.d. = 3.4 IU/l, kurtosis = 5.84, skewness = 1.46). Eleven of the negative patients demonstrated CPK-MB values greater than the mean + 3 s.d. level of 14.8 (range: 15–24 IU/l) with the highest value (24 IU/l) 2.7 s.d. above the mean + 3 s.d. value. Peak CPK-MB values from patients with transmural infarction ranged from 27 to 490 IU/l, whereas peak values from patients with subendocardial infarction ranged from 16 to 70 IU/l. In contrast to the clear separation of abnormal from normal myoglobin values, there was considerable overlap between normal and abnormal CPK-MB values.

CPK-MB diagnostic parameters are as expected considering the overlap of values from normal and abnormal patient groups. Sensitivity = 100%, specificity = 90%, positive predictive value = 68%, negative predictive value = 100%, and overall diagnostic accuracy = 92% (Table 3). Chi-square analysis with Yate's correction yields a high degree of association with final clinical diagnosis.

Serial determinations of both myoglobin and CPK-MB permitted examination of both the time of first appearance and the time of appearance of peak abnormal values. The cumulative frequency distribution of the appearance of the first abnormal values for myoglobin and CPK-MB demonstrates that myoglobin abnormalities precede CPK-MB abnormalities by 12–24 hr in the great majority of cases (Fig. 3). Likewise, peak abnormal myoglobin values preceded peak abnormal CPK-MB values by at least 24 hrs in the majority of cases (Fig. 4).

DISCUSSION

Myoglobin, an oxygen-binding hememolecule of low molecular weight, was first reported in 1956 to be present in the serum and urine of patients with acute myocardial infarctions (4). Myoglobinuria accompanying infarction was substantiated by Strausser et al. (5) in 1966 and later by others (6–10); however, the relative insensitivity of measurement using precipitin and hemagglutination-inhibition procedures limited detection of myoglobin to urinary appearance. Although myoglobinuria had been suggested as the earliest parameter detectable in acute myocardial infarction (8–11), Kagel et al. (12), using a complement-fixation technique with increased sensitivity of measurement, showed that myoglobinuria was not a good indicator of the degree of myoglobinemia. Difficulties with radioiodination of human myoglobin by the traditional chlor-

TABLE 3. DIAGNOSTIC PARAMETERS OF MYOGLOBIN AND CPK-MB ASSAYS

Result	Myoglobin*	CPK-MB†
True positive	23	23
True negative	113	102
False positive	0	11
False negative	0	0
Sensitivity	100%	100%
Specificity	100%	90%
Positive predictive value	100%	68%
Negative predictive value	100%	100%
Accuracy	100%	92%

* Chi square with Yate's correction = 128.6 (p < .0005)

† Chi square with Yate's correction = 78.6 (p < .0005)

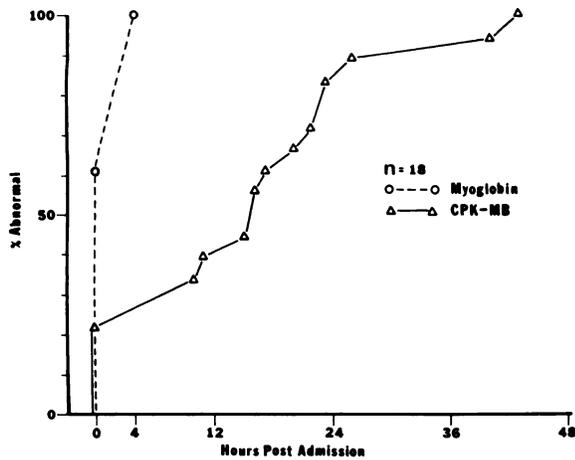


FIG. 3. Cumulative frequency distribution of time of appearance of initial abnormal values for myoglobin and CPK-MB in patients with infarct.

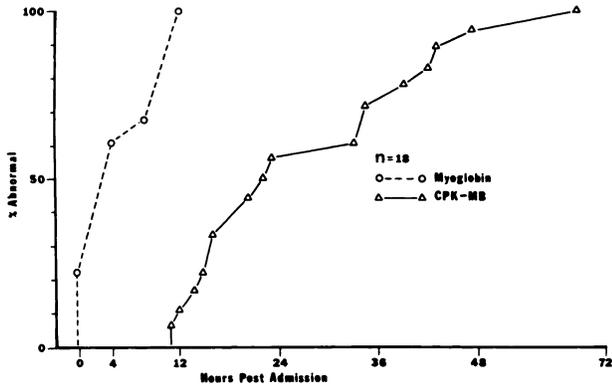


FIG. 4. Cumulative frequency distribution of time of appearance of the peak abnormal myoglobin level and CPK-MB value in patients with infarct.

amine-T reaction initially delayed the development of a radioimmunoassay, and efforts continue at improving the sensitivity of microimmunologic assays for myoglobin (13,14). The necessity to quantify the release of myoglobin into plasma motivated several groups to develop successful radioimmunoassays that were sensitive enough to detect serum myoglobin concentration in the ng/ml range (15-22).

The commercial radioimmunoassay evaluated in this investigation represents a technical improvement over the above assays in that an individual assay run may be completed and results made available in 2 hr as compared with more than 24 hr. In our evaluation of quality assurance, we have documented excellent reproducibility, precision, accuracy, and specificity. Our highest observed normal value of 79 ng/ml closely agrees with the 75-85 ng/ml upper limit of normal reported by others

(22,27). Overall, the procedure lends itself well to incorporation as a routine daily run in the radioassay laboratory.

Myoglobin is found in skeletal as well as cardiac muscle, and molecules of each origin are immunologically indistinguishable (23,24). Serum myoglobin concentration has been reported to be elevated in a variety of skeletal-muscle disorders, after extreme exertion, after intramuscular injection, and in chronic renal failure (25,26). Therefore, elevation of myoglobin levels should be interpreted only in the appropriate clinical context.

Our investigation confirms the observations of others, that pronounced serum myoglobin elevations are an early accompaniment of acute myocardial infarction when subsequently followed by expected ECG and enzymatic evolutionary changes. However, final determination of whether serum myoglobin elevation alone represents frank necrosis remains to be done, although there is strong clinical evidence accumulating in support of this hypothesis. In our investigation of 20 patients diagnosed as having only angina, no elevation in myoglobin was observed. Stone et al. (27) reported similar results (42 of 44 patients) and did not observe elevations in patients with coronary-artery disease undergoing maximal treadmill exercise. Willerson et al. (28) found no elevation in serum myoglobin levels obtained in dogs following temporary 15-min occlusions of the LAD artery. Font et al. (29) reported constant myocardial creatine phosphokinase activity in the first 2 hr of ischemia during which there is a rapid decrease in creatine phosphate and adenine nucleotide levels. Thus, conventional cardiac-enzyme determinations may be an inadequate indicator of myocardial necrosis, although this may reflect relative insensitivity of measurement by conventional enzyme assays as demonstrated by Roberts et al. (30). Likewise, imaging with Tc-99m stannous pyrophosphate is a suboptimal test due to its relatively poor diagnostic sensitivity and specificity associated with nontransmural infarction (1-3). Because patients with acute nontransmural infarction share similar short- and long-term prognoses with those with transmural infarction (30), careful clinical management is indicated in patients suffering ischemic chest pain with subsequent significant elevation of serum myoglobin only.

This serum myoglobin assay—which is quickly performed and, following acute myocardial infarction, rapidly becomes abnormal and peaks early—has a potential to influence the overall management of patients. Early diagnosis may reduce the time required for observation in an intensive-care unit.

In addition, the early peaking of myoglobin release may provide a means to quantify infarct size as well as to assess infarct extension, which occurs in a significant number of patients (31).

FOOTNOTES

- * Nuclear Medical Systems, Inc.
† Calbiochem

REFERENCES

1. COWLEY MJ, MANTLE JA, ROGERS WJ, et al: Technetium-99m stannous pyrophosphate myocardial scintigraphy: Reliability and limitations in assessment of acute myocardial infarction. *Circulation* 56: 192-198, 1977
2. WERNER JA, BOTVINICK EH, SHAMES DM, et al: Acute myocardial infarction: Clinical application of technetium 99m stannous pyrophosphate infarct scintigraphy. *West J Med* 127: 464-478, 1977
3. BERMAN DS, AMSTERDAM EA, HINES HH, et al: New approach to interpretation of technetium-99m pyrophosphate scintigraphy in detection of acute myocardial infarction. Clinical assessment of diagnostic accuracy. *Am J Cardiol* 39: 341-346, 1977
4. KISS A, REINHART W: Ueber den nachweis des myoglobins im serum und in harn nach herzzinfarkt. *Wien Klin Wochenschr* 68: 154-155, 1956
5. STRAUSSER HR, ROTHFELD EL, BUCSI RA: Isolation and preservation of human myoglobin for use in immunologic detection of myoglobinuria. (31208) *Proc Soc Exp Biol Med* 122: 621-624, 1966
6. KAGEN LJ: Immunologic detection of myoglobinuria after cardiac surgery. *Ann Intern Med* 67: 1183-1189, 1967
7. ADAMS EC Jr, ELLIOT TA: Urinary myoglobin in myocardial infarction. *JAMA* 211: 1013-1014, 1970
8. LEVINE RS, ALTERMAN M, GUBNER RS, et al: Myoglobinuria in myocardial infarction. *Am J Med Sci* 262: 179-183, 1971
9. BERNSTEIN H, SARANCHAK H: Myoglobinuria: A diagnostic test for acute myocardial infarction. *Circulation* 48 (Suppl No. 4): 39, 1973 (abst)
10. KESSLER HA, LIEBSON PR, MATTENHEIMER H, et al: Acute myocardial infarction diagnosed by myoglobinuria. *Arch Intern Med* 135: 1181-1183, 1975
11. SARANCHAK HJ, BERNSTEIN SH: A new diagnostic test for acute myocardial infarction. The detection of myoglobinuria by radioimmunodiffusion assay. *JAMA* 228: 1251-1255, 1974
12. KAGEN L, SCHEIDT S, ROBERTS L, et al: Myoglobinemia following acute myocardial infarction. *Am J Med* 58: 177-182, 1975
13. KAGEN LJ, BUTT A: Myoglobin binding in human serum. *Clin Chem* 23: 1813-1818, 1977
14. BOESKEN WH, BOESKEN S, MAMIER A: Human myoglobin: Preparation, quantitation and standardization. *Res Exp Med* 171: 71-78, 1977
15. LWEBUGA-MUKASA JS, LIBBY P, BLOOR CM, et al: The evaluation of serum myoglobin following experimental coronary occlusion. *Circulation* 48 (Suppl No. 4): 129, 1973 (abst)
16. JUTZY RV, NEVATT GW, PALMER FJ, et al: Radioimmunoassay of serum myoglobin in myocardial infarction. *Am J Cardiol* 35: 147, 1975 (abst)
17. STONE MJ, WILLERSON JT, GOMEZ-SANCHEZ CE, et al: Radioimmunoassay of myoglobin in human serum. Results in patients with acute myocardial infarction. *J Clin Invest* 56: 1334-1339, 1975
18. STONE MJ, WATERMAN MR, WILLSON NR, et al: Radioimmunoassay of serum myoglobin in the diagnosis of acute myocardial infarction in patients. *Circulation* 54 (Suppl No 2): 29, 1976 (abst)
19. KLOCKE FJ, VISCO JP, REICHLIN M: Myoglobinemia as an index of myocardial infarction and/or ischemia. *Circulation* 54 (Suppl No. 2): 29, 1976 (abst)
20. ROSANO TG, KENNY MA: A radioimmunoassay for human serum myoglobin: method development and normal values. *Clin Chem* 23: 69-75, 1977
21. ROSANO TG, SANDERS LA, JOHNSON ES, et al: Myoglobin concentrations and muscle-enzyme activities in serum after myocardial infarction and cardiac arrhythmia. *Clin Chem* 23: 868-870, 1977
22. REICHLIN M, VISCO JP, KLOCKE FJ: Radioimmunoassay for human myoglobin: initial experience in patients with coronary heart disease. *Circulation* 57: 52-56, 1978
23. KAGEN LJ, CHRISTIAN CL: Immunologic measurements of myoglobin in human adult and fetal skeletal muscle. *Am J Physiol* 211: 656-660, 1966
24. KAGEN LJ, LINDER S: Synthesis of myoglobin by muscle polyosomes. *Biochem Biophys Acta* 195: 523-530, 1969
25. KAGEN LJ: *Myoglobin: Biochemical, Physiological and Clinical aspects*. New York and London, Columbia University Press, 1973, pp 79-116
26. JUTZY RV, NELSON JC, LEWIS JE, et al: Myoglobinemia in conditions other than myocardial infarction. *Circulation* 54 (Suppl No. 2): 29, 1976 (abst)
27. STONE MJ, WATERMAN MR, HARIMOTO D, et al: Serum myoglobin level as diagnostic test in patients with acute myocardial infarction. *Br Heart J* 39: 375-380, 1977
28. WILLERSON JT, BUJA LM, KULKARNI P, et al: Factors which influence myoglobin release from cardiac cells and the localization of a specific antibody to myoglobin in myocardial tissue. *Clin Res* 25: 458A, 1977 (abst)
29. FONT B, VIAL C, GOLDSCHMIDT D: Enzymes level and metabolites release in the ischemic myocardium. *J Molec Med* 2: 291-297, 1977
30. ROBERTS R, PARKER CW, SOBEL BE: Detection of acute myocardial infarction by radioimmunoassay for creatine kinase MB. *Lancet* 2: 319-322, 1977
31. MADIAS JE, GORLIN R: The myth of acute "mild" myocardial infarction. *Ann Int Med* 86: 347-352, 1977
32. REID PR, TAYLOR DR, KELLY DT, et al: Myocardial-infarct extension detected by precordial ST-segment mapping. *N Engl J Med* 290: 123-128, 1974