Automated Quantification of Creatine Kinase MB Isoenzyme in Serum by Radial Partition Immunoassay, with Use of the Stratus Analyzer

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We evaluated the analytical and clinical performances of a new radial partition immunoassay for measuring the mass concentration of creatine kinase (CK)-MB in serum. All pipetting, washes, incubations and data reduction were performed in 8 min by the Stratus® (Dade) fluorometric analyzer. Within-assay and between-assay CVs were respectively 5.5% and 8.4% at 21 μg/L, and 4.2% and 3.4% at 48 μg/L. Assaying serial dilutions of serum samples with high CK-MB concentrations demonstrated excellent linearity. Results of the Stratus technique correlated well (n = 115, r = 0.98) with those of the Tandem-E CKMB II assay. There was no interference from hemolysis, bilirubin, rheumatoid factor, or added CK-MM (up to 3500 U/L); consequently, CK-MB can be determined in undiluted serum, even in the presence of high total CK activity. The mean CK-MB concentration in 105 blood donors was 1.9 (SD 1.3) μg/L. For seven myocardial infarction patients who received prompt fibrinolytic therapy, the mean CK-MB concentration was 4.5 (SD 1.8) μg/L at admission, and maximum concentrations, 119 (SD 94) μg/L, were recorded 16 h later. CK-MB returned to concentrations <10 μg/L within 72 h.

The major clinical application of the MB isoenzyme of creatine kinase (CK, EC 2.7.3.2) is the assessment of acute myocardial infarction (AMI) (1, 2).[1] To take full advantage of the increase of the cardiac isoenzyme in the first hours after onset of symptoms, a rapid, practical, and sensitive method for measuring CK-MB in blood is needed.

We report here an analytical and clinical evaluation of the Stratus® system in determining CK-MB in serum specimens. The Stratus assay procedure is based on radial immunopartition (3–7). In the CK-MB method, the solid phase consists of tabs of glass-fiber filter paper containing a pre-immobilized monoclonal anti-CK-MB antibody. The patient's sample is first applied directly to the central "reaction zone" of the tab, allowing CK-MB to bind to the solid-phase-bound antibody. Then, a buffered solution containing enzyme-labeled Fab' fragments of an anti-CK-BB monoclonal antibody is applied, initiating "sandwich" formation. Finally, a wash buffer containing a fluorogenic substrate is applied, eluting unbound conjugate to the tab periphery. The bound enzyme conjugate is quantified by measuring the rate of increase in fluorescence. The Stratus system, which presents the advantage of full automation, yields quantitative results in 8 min.

Materials and Methods

Instrumentation

Details of the design and operation of the Stratus radial partition fluorometric immunoassay analyzer (Dade Divi-

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[1] Nonstandard abbreviations: CK-MM, CK-MB, creatine kinase isoenzymes MM and MB; and AMI, acute myocardial infarction.

sion, Baxter Inc., Miami, FL 33152) appear elsewhere (8). No special modification of the instrument was required for the CK-MB assay.

Procedures

CK-MB determination. We determined CK-MB by radial immunopartition, using the Stratus analyzer. The CK-MB fluorometric enzyme immunoassay kit (Dade) contains the following:

CK-MB antibody tabs. A solution of mouse monoclonal antibody specific for CK-MB is complexed onto the surface of glass-fiber filter paper by the addition of goat antibody specific for the Fc portion of mouse IgG.

Enzyme-labeled anti-CK. Calf intestinal alkaline phosphatase (EC 3.1.3.1) is covalently linked to mouse monoclonal anti-CK-BB Fab'. The conjugate is solubilized in 2-[(carbamoylmethyl)amino]ethane sulfonic acid buffer (pH 7.0).

Substrate wash solution. The fluorogenic substrate consists of 4-methylumbelliferyl phosphate (1 mmol/L) in a diethanolamine buffer (pH 9.0). The cleavage of the phosphate group from 4-methylumbelliferyl phosphate, catalyzed by alkaline phosphatase, results in the production of 4-methylumbelliferone.

CK-MB calibrators. The lyophilized calibrators, when reconstituted, contain human-heart CK-MB concentrations of approximately 0, 4, 10, 25, 60, and 125 μg/L in human serum.

Comparison method. We used a solid-phase two-site immunoenzymometric assay (Tandem-E CKMB II; Hybritech Europe S.A., Sart Tilman, Liège, Belgium) (9), with the Hybritech "Photon" photometer. In this technique, samples containing CK-MB are reacted with a plastic-bead solid phase that is coated with a monoclonal antibody directed toward the B subunit of CK-MB, and with an enzyme-labeled monoclonal antibody directed toward the M subunit of the molecule. After formation of the solid-phase/CK-MB/labeled-antibody "sandwich," the bead is washed, then incubated with enzyme substrate (p-nitrophenyl phosphate). The amount of substrate turnover, determined colorimetrically, is directly proportional to the concentration of CK-MB in the test sample.

In some specimens, we also determined the CK-MB content after separation by discontinuous electrophoresis on polyacrylamide gel (Protean TM II Slab Cell; Bio-Rad Labs., Richmond, CA). We applied 20 μL of serum and performed the electrophoresis at 4 °C for 5 h at 400 V. The isoenzymes, made visible by specific CK staining, were measured densitometrically.

Total CK determination. We used an optimized spectrophotometric method (Enzyline® CK NAC; BioMérieux, Lyon, France) to measure total CK at 37 °C (reference limits 0–120 U/L) with an automated analyzer (Eris 6170; Olympus-Eppendorf, Hamburg, F.R.G.).

Evaluation of imprecision. Two pools of fresh patients' sera (low- and high-concentration pools) were aliquoted and frozen, then assayed to evaluate within-run and be-

tween-run precision. During this part of the study, we calibrated the Stratus system once a week.

Evaluation of linearity. Two serum samples with high CK-MB concentration were diluted (in successive twofold dilutions to a 64-fold dilution) with a serum pool containing no CK-MB (as confirmed by electrophoresis). The resulting samples, which covered the whole measuring range of CK-MB, were measured in duplicate.

Method comparisons. We collected 115 serum specimens from patients admitted to the University Hospital of Liège and for whom CK-MB had been requested on a routine basis. These specimens were assayed with the Stratus CK-MB method and with the comparison method.

Interference studies. Interference by hemoglobin was studied by adding increasing amounts of hemolysate to a serum specimen with low CK-MB content (3.3 $\mu g/L$) up to a maximum concentration of hemoglobin of 3.4 g/L, and assaying. We tested interference by bilirubin by mixing two fresh serum samples (one containing 4 mg of bilirubin and 4.5 μg of CK-MB per liter, the other 352 mg of bilirubin and 0.3 μg of CK-MB per liter) to prepare four specimens, and assaying. Interference by rheumatoid factor was tested with the same experimental model by mixing two fresh serum samples (one with no rheumatoid factor and 29 μg of CK-MB per liter, the other with 1230 int. units of rheumatoid factor and 0.9 μg of CK-MB per liter). In these assays, the maximum concentrations of bilirubin and rheumatoid factor were 176 mg/L and 615 int. units/L, respectively.

By combining in various proportions three serum samples with widely differing contents of total CK and CK-MB, we prepared six serum specimens with total CK activities increasing stepwise from 506 to 3545 U/L, but with a nearly constant CK-MB content (18.2 to 20.0 $\mu g/L$). The relative proportions of CK-MM and CK-MB in these six specimens, and the absence of CK-BB, were confirmed by electrophoresis. We then assayed each specimen by the Stratus technique and by the comparison method, without dilution.

Clinical Evaluation

Healthy subjects. We investigated 105 blood donors, 64 men (mean age ±SD: 43 ± 12 years) and 41 women (38 ± 9 years).

Acute myocardial infarction patients. We studied 18 patients, ages 30–69 years (mean 57, SD 9 years), who were admitted to the University Hospital, Liège, Belgium. AMI was diagnosed on the basis of a history of severe chest pain and electrocardiographic evidence of localized injury with typical ST-segment elevations. Total CK activity and CK-MB concentration were determined at admission, then every 4 h for 36 h and every 12 h thereafter until 72 h after admission. To determine the evolution of serum CK-MB after AMI in a homogeneous group of patients, we assayed samples from 11 subjects who had reached the coronary care unit within 4 h after the onset of the symptoms and whose total CK activities at admission were <150 U/L.

Seven of these patients had received a fibrinolytic therapy (with streptokinase); the other four were treated with intravenous heparin.

Results

Precision of the assay. We determined precision profiles from 20 determinations in the same run for the within-run tests, and from 20 consecutive determinations on different days for the between-run tests. Within-assay CVs were

4.2% and 5.5% and between-assay CVs were 3.4% and 8.4% for two pooled-serum specimens containing 48 and 21 μg of CK-MB per liter, respectively. The standard deviations ranged from 1.2 to 2.0 $\mu g/L$.

Assessment of linearity. The equations derived from the regression/correlation analysis of mean found values (*y*) vs calculated values (*x*) were $y = 0.997x + 1.58$ ($r = 0.999$) for the first sample investigated (from 1.5 through 94.0 $\mu g/L$, n = 6) and $y = 0.949x + 0.45$ ($r = 0.999$) for the other one (from 1.4 through 89.6 $\mu g/L$, n = 6). The minimum detectable concentration (mean + 2SD of zero standard, 20 replicates) was 0.2 $\mu g/L$.

Analytical recovery. Analytical recovery of CK-MB, added at concentrations of 17 and 34 $\mu g/L$ to two serum specimens containing 0.4 and 11.3 μg of CK-MB per liter, averaged 99.7% (range 91% to 104%).

Correlation between immunopartition (y) *and immunoenzymometric assay* (x). In the 115 serum specimens investigated, the CK-MB concentrations ranged from 0 to 108 $\mu g/L$ (Stratus technique). The results by both methods were related by the regression equation $y = 0.744x + 1.15$ ($r = 0.98$).

Interference. We saw no interference for hemoglobin concentrations up to 2.25 g/L. At 3.4 g/L, there was a very mild positive interference (CK-MB values 0.3 μg higher than expected). Assay of the hemolysate alone (6.75 g of hemoglobin per liter, absence of CK-MB confirmed by electrophoresis) gave a CK-MB value of 3.7 $\mu g/L$. Bilirubin and rheumatoid factor gave no detectable interference up to 178 mg/L and 615 int. units/L, respectively. Addition of CK-MM up to 3500 U/L altered none of the CK-MB results determined by the Stratus technique (92–106% of the expected CK-MB value). In contrast, the CK-MB values measured by the comparison technique were regularly decreased in the specimens containing CK-MM >1100 U/L; the specimen containing CK-MM at 3500 U/L gave only 39% of its expected CK-MB value.

Reference interval. In the 105 blood donors investigated, the mean serum CK-MB concentration was 1.9 $\mu g/L$ (SD 1.3 $\mu g/L$). Men demonstrated slightly, but not significantly, higher CK-MB concentrations than women (2.0 ± 1.3 vs 1.8 ± 1.2 $\mu g/L$). Using the nonparametric method of percentile estimation (10), we calculated the normal reference interval for serum CK-MB to be 0–6 $\mu g/L$.

Acute myocardial infarction patients. We observed a typical increase and decrease pattern for serum total CK activities in the 11 patients with short delays in admission. Total CK peak activities ranged from 841 to 4140 U/L in the streptokinase-treated group (n = 7) and from 1421 to 2400 U/L in the patients who received intravenous heparin (n = 4).

For the streptokinase-treated patients, the mean CK-MB concentration in serum was 4.5 (SD 1.8) $\mu g/L$ (mean ± SD) at admission, but increased rapidly during the following hours and averaged 97.9 $\mu g/L$ 8 h after admission (Figure 1). At peak (16 h after admission), CK-MB concentrations ranged from 38 to 295 $\mu g/L$ (mean 119, SD 94 $\mu g/L$). On day 3, the mean CK-MB concentration had decreased to 3.2 $\mu g/L$ (range 0–9.7 $\mu g/L$). In the patients who received heparin, CK-MB reached its peak 20 h after admission, 4 h later than in the streptokinase-treated group. After heparin treatment, however, the amplitude of the changes was similar to those observed after fibrinolytic therapy, maximum concentrations ranging from 45 to 308 $\mu g/L$ (mean 126, SD 103 $\mu g/L$). After 72 h, the heparin-treated patients
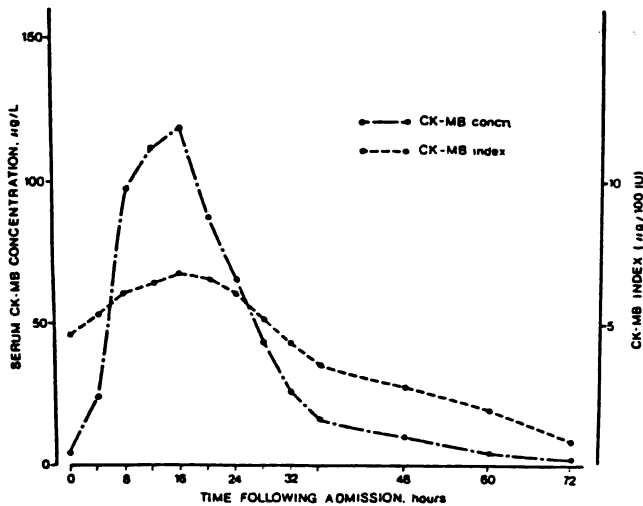
Fig. 1. Evolution of the mean serum CK-MB concentration, as measured with the Stratus assay, and of the mean CK-MB index during the first 72 h of hospitalization in AMI patients with short delays in admission (fibrinolytic therapy)

demonstrated a mean CK-MB concentration of 9.3 µg/L (range 0–20 µg/L).

At each measurement time, we also calculated the index [(CK-MB/total CK) × 100] corresponding to the mass concentration of CK-MB in micrograms per 100 units of total CK activity in the sample. For the streptokinase-treated group, the index values ranged from 2.6 to 8.1 (mean 4.7) at admission. A progressive increase was recorded in the following hours (Figure 1). The curve of the mean index values reached its maximum 16 h after admission (mean 6.8, range 4.3–9.0). Mean index values decreased to 4.3 and 0.9, respectively, 32 and 72 h after admission. In the patients who received heparin, we observed a similar evolution of the index, maximum values (range 4.1–19.5, mean 7.9) being recorded 20 h after admission.

Discussion

In this study, we investigated the analytical performance of the Stratus CK-MB method. Precision studies demonstrated within-run and between-run CVs <5.5% and 8.5%, respectively. The limit of detection for CK-MB was 0.2 µg/L; the linearity of the technique, evaluated from 1.4 through 95.5 µg/L, was excellent. Results by the Stratus technique correlated well with those by the Hybritech Tandem-E CK-MB II technique (r = 0.98). There was no detectable interference by hemoglobin, bilirubin, or rheumatoid factor up to 2.25 g/L, 178 mg/L, and 615 int. units/L, respectively. With the Stratus technique, CK-MB can be determined in the presence of large amounts of CK-MM (at least up to 3500 U/L). This allows CK-MB to be measured in specimens with high total CK content without dilution. The method has also the added advantage of quickness (8 min for a result if the calibration curve is stored) and full assay automation.

Reference values determined by the Stratus technique for serum CK-MB were 0–6 µg/L, which compares well with those obtained by others using an immunoenzymometric assay (9). Serial determinations performed in AMI patients showed the typical increase and decrease in CK-MB during the first 72 h after the attack. Within 24 h of admission, CK-MB increased to concentrations exceeding, on average, more than 20 times the upper limit of the reference interval. In most cases, the value had returned to normal by day 3. Confirming previous results (11), CK-MB peaked earlier in the patients treated by streptokinase than in those who received heparin. The amplitude of the changes was, however, similar in the two groups of patients. In the patients investigated, the mean value of the CK-MB index, which expressed the CK-MB concentration as a percent of total CK activity, exceeded 4 during the first 32 h after admission.

In conclusion, we have validated a rapid, convenient radial partition immunoassay for CK-MB in serum and have demonstrated its clinical applicability when used at a coronary-care unit for diagnostic evaluation of AMI patients.

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