Detection of Myocardial Infarct Extension by CK-B Radioimmunoassay

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SUMMARY Myocardial infarct extension after the acute event was defined as a second rise in the myocardial isoenzyme of serum creatine kinase (CK-B) after the initial return of CK-B to normal values. In 43 patients with acute myocardial infarcts, CK-B was measured by radioimmunooassay every 12 hours for 14 days. Nineteen patients had anterior transmural myocardial infarcts (AMI), 14 had inferior transmural myocardial infarcts (IMI) and 10 had subendocardial myocardial infarcts (SEMI). Infarct extension as detected by a second rise in serum CK-B occurred in six patients (32%) with AMI, two (14%) with IMI and two (20%) with SEMI; these differences are not statistically significant. Infarct extension for all patients combined was 23%. Four patients with AMI also had infarct extension as determined by recurrent chest pain, ECG alterations and other enzyme changes. In the other six, the infarct extension was undetected clinically. Four patients with AMI and infarct extension died within 3 weeks after hospitalization. We did not note any additional morbidity or mortality in patients with infarct extension who had AMI or SEMI. There was no significant difference in the frequency of previous myocardial infarction, history of hypertension, diabetes mellitus or smoking history in patients with and without infarct extension shown by serum CK-B isoenzyme elevations.

The measurement of serum CK-B values with a quantitative and sensitive assay suggests that myocardial infarct extension occurs more commonly than clinically recognized, but the frequency of extension may be less than that reported in patients in whom precordial mapping and total serum CK values were measured to identify this phenomenon.

MYOCARDIAL INFARCT EXTENSION several days after the acute event is generally thought to be the result of a second wave of necrosis after the initial ischemic event. It can be defined in a variety of ways, but clinically is characterized by recurrent chest pain associated with further electrocardiographic changes and re-elevation of myocardial enzymes. Only a few studies to detect the incidence of infarct extension have been performed, so the frequency with which infarct extension occurs is unclear. Rosati estimated a rate of 11–17% on clinical grounds, sometime during hospitalization, in 797 episodes of acute myocardial infarction. Using precordial ST mapping and total serum creatine kinase (CK) determinations for 14 days postinfarction, Reid et al. found an extension rate of greater than 50% — significantly higher than the incidence of infarct extension that was clinically suspected. Thus, myocardial necrosis may continue during apparently uneventful recovery in many patients with acute infarction. To assess more accurately the natural course of myocardial necrosis, we have used a quantitative and sensitive radioimmunooassay to measure serum values of the CK-B isoenzyme for 14 days after the onset of acute infarction.

Materials and Methods

Forty-three patients, ages 36–80 years, with a diagnosis of acute myocardial infarction were prospectively studied. Myocardial infarction was recognized by conventional means, with all of the following criteria: 1) chest pain consistent with the presence of myocardial infarction; 2) characteristic ECG changes for acute transmural myocardial infarcts or ST-T-wave alterations consistent with the presence of acute subendocardial infarcts; 3) elevation of serum CK, SGOT and LDH in a typical evolutionary pattern, with CK at least twice normal; and 4) the development of an abnormal technetium-99m stannous pyrophosphate (99mTc-PYP) myocardial scintigram, when performed.

Serum CK-B isoenzyme was measured by radioimmunooassay every 12 hours for 14 days after the onset of symptoms. The details related to the development of this radioimmunooassay and its use in myocardial infarct detection in patients have been previously reported. Briefly, antibodies were raised in rabbits to purified CK-BB isoenzyme. CK-BB isoenzyme was iodinated by conjugation labeling as described by Bolton and Hunter. The addition of a constant amount of 125I-CK-BB isoenzyme to serial dilutions of antisem from bleeding a single rabbit disclosed maximum binding of isoenzyme to antibody at an antisem dilution of 1:40,000; this dilution was used in the subsequent assays. Generally, a 20-μl aliquot of each serum sample was routinely assayed. For samples with CK-B values on the flat portion of the standard curve, the determination was repeated with 10 μl of serum. All determinations were performed in triplicate, and a standard curve was performed each day of testing. Separation of unreacted enzyme from
antibody-bound CK-BB isoenzyme was accomplished by the double-antibody method of using goat anti-
serum against rabbit IgG (Miles Laboratories, Elkhart, IN). Within a single assay, values with triplicate determinations agreed within 5%. The addition of known amounts (1, 2.5, 5, 10 and 20 ng) of unlabeled CK-BB isoenzyme to normal serum in four experiments resulted in an average recovery of 94 ± 3.2% (SEM). Cross-reactivity to other myocardial constituents revealed that only 0.01% of human heart phosphorylase b, 0.032% of human heart citrate synthase, 0.4% of human heart myoglobin and 0.009% of human skeletal muscle CK-MM isoenzyme could be detected with this assay. The lack of significant reactivity of CK-MM isoenzyme from human skeletal muscle points to the specific recognition of CK-MB or BB isoenzymes in this radioimmunoassay. Four separate experiments, performed to determine the cross-reactivity of the antibody to CK-BB isoenzyme for human heart CK-MB isoenzyme, revealed that an average of 44% of the serum level of this CK isoenzyme was detected. The lack of significant cross-reactivity with any of the other myocardial cellular constituents tested, and the recognition of approximately half the added amount of CK-MB isoenzyme from human heart, confirms that the antibody used in this assay is specific for the B portion of the CK-MB isoenzyme.

In 36 of 43 patients serum samples for CK-B isoenzyme determinations were obtained within 24 hours of the onset of chest pain. In the remaining seven patients, isoenzyme determinations were not begun until 36–48 hours after pain, but were subsequently obtained every 12 hours for 14 days. Each of these patients had sustained a classical acute myocardial infarction, and each one had normal serum CK-B values when tested 36–48 hours after the onset of symptoms. All samples were frozen within 1 hour of phlebotomy and stored at −20°C before analysis. Determinations were made with a specific antisera against radio-
labeled CK-BB isoenzyme as previously described. By this method serum CK-B values are elevated within 2–6 hours of acute myocardial infarcts, peaking at 10–12 hours and often returning toward normal levels within 24–36 hours after the clinical event. The radioimmunoassay is sensitive and specific for CK-B, identifying as little as 0.2 ng per assay tube; the anti-
tibody to CK-B does not bind specifically to the CK-MM isoenzyme or to other myocardial constituents against which it has been tested. Since this radio-
immunoassay detects the CK-B isoenzyme, it may demonstrate elevated CK-B values in patients with either acute myocardial infarcts or acute cerebral necrosis. It has also been suggested that occasion-
ally, patients with acute gastrointestinal injury and severe renal insufficiency may also have elevated serum CK-BB values. Therefore, patients with acute cerebral disease and those with acute gastrointestinal disease and/or severe renal insufficiency were not selected for study.

In 29 patients 99mTc-PYP myocardial scintigraphy was performed within 1–3 days of the acute myocar-
dial infarct, and in 15 of these repeated 5–10 days after the initial study. Each patient was imaged in the anterior, lateral and left anterior oblique projections 2 hours after the intravenous injection of 15 mCi 99mTc tagged to 5 mg of stannous pyrophosphate. A portable Searle 37 tube scintillation camera was used. Scintigrams were graded on a scale of 0 to 4+ by investigators unaware of the clinical status of the patient; those scintigrams graded as 2+ to 4+ were considered abnormal and indicative of the presence of myocardial necrosis. This method effectively identifies both acute transmural and subendocardial infarcts at our institution.

Infarct extension was defined in this study as either elevation or re-elevation of serum CK-B values above the normal range as determined by radioimmuno-
assay after initial return to normal levels. In one patient infarct extension was also considered to be present because of persistent elevation of serum CK-B levels over 4 days.

Statistical Analysis

Chi square analysis was used to compare differences in serum CK-B values between the different groups of patients studied and to evaluate the presence of risk factors.

Results

Radioimmunoassay Measurements of Serum CK-B Isoenzyme

Patients were divided into three groups on the basis of whether they had acute anterior or inferior transmural myocardial infarcts or acute subendocar-
dial myocardial infaracts. Thirty-six of 43 patients had initial elevations of serum CK-B isoenzyme. The remaining seven did not have sera obtained initially for CK-B isoenzyme determination until 36–48 hours after the onset of infarction. In 30, the serum CK-B values returned to normal limits within 24–48 hours. Four patients had serum CK-B values return to nor-
mal within 60 hours after the onset of symptoms, and another within 72 hours. One patient had continued serum CK-B elevations for 4 days. There were no significant differences in the time from onset of chest pain to normalization of enzymes in the three groups.

Nineteen patients had anterior transmural infarcts. Fifteen had initial serum CK-B elevations. Four patients who were not part of this study until 36–48 hours after chest pain did not have initial serum CK-B elevations when the first sample was evaluated. However, each of these four had other clinical evidence of acute myocardial infarction and three had 4+ and one had a 3+ positive 99mTc-PYP scintigram, verifying the initial diagnosis of acute myocardial infarction. Six patients (32%) in this group developed in-hospital increases in serum CK-B levels suggestive of infarct extension several days after their CK-B values had returned to normal (fig. 1). Patients 1–3 had a single rise in serum CK-B limited to 24 hours on days 4, 10 and 14 after the onset of symp-
symptoms. All three had recurrent chest pain, ECG changes (new Q waves or R wave loss in two and ST-T-wave changes consistent with subendocardial infarction in two) and re-elevation of total serum CK. Two died, one 15 days and one 16 days after the onset of symptoms. Patient 1 had medically refractory left ventricular failure and patient 2 developed ventricular fibrillation. The third patient was discharged after a 3-week hospital stay with Killip class III heart failure, while on digoxin and diuretics. Patient 4 developed re-elevation of serum CK-B on days 8 and 10 after the onset of symptoms, with no new chest pain or ECG changes noted. On day 11 she had sudden cardiopulmonary arrest and could not be resuscitated. Patient 5 had continued elevations of serum CK-B from days 4-9. In spite of this he showed no clinical evidence of extension and was discharged Killip class I on no cardiac medications. Patient 6 was considered to have infarct extension because he had continuing elevations of serum CK-B isoenzyme for 4 days after admission, a pattern not seen initially in any other patients. He remained in Killip III congestive heart failure until his death on day 6, secondary to a low output state and pneumonia.

Fourteen patients with acute inferior transmural infarcts were also studied. All had initial serum CK-B elevations. Two patients had a second rise in serum CK-B values (fig. 2), one on day 7 and one on day 9, so the apparent myocardial infarction extension rate was 14%. Neither of these patients showed further myocardial necrosis, evident either by recurrent chest pain or definitive ECG changes.

Ten patients with acute subendocardial myocardial infarcts were also studied. Seven had initial elevations of serum CK-B isoenzymes, while in three serum CK-B levels were first measured 36-48 hours after the onset of chest pain. These three patients had positive 99mTc-PYP myocardial scintigrams and other clinical and enzymatic evidence of acute myocardial infarcts. Two patients in this group (20%) had re-elevations of serum CK-B isoenzymes later in their hospital courses (fig. 2). One, with an isolated peak in serum CK-B on day 3, had no clinical evidence for infarct extension. The other had increases in serum CK-B on days 4, 6, 7 and 9 after the onset of symptoms suggestive of myocardial infarction. This patient had chest pain judged to be angina on days 3, 4 and 6 after hospital admission.

The overall rate of myocardial infarct extension for all three groups combined was 23% (10 of 43 patients studied). Eight patients had serum CK-B levels that demonstrated re-elevation of at least 100% above the baseline as determined by the mean serum CK-B isoenzyme values for 24-72 hours before extension. One patient had a 50% rise in serum CK-B isoenzyme followed by a 30% rise 48 hours later. The last patient's serum CK-B values did not return to normal until 4 days after myocardial infarction. There was no statistically significant difference in the frequency of extension rates among patients with anterior, inferior and subendocardial myocardial infarctions.

**Postmortem Examination**

Four patients in this series died in-hospital after their acute myocardial infarcts. All had acute anterior
myocardial infarcts and secondary elevation in serum CK-B isoenzyme values, suggesting extension of the previous myocardial infarcts. Only one of these patients had a postmortem examination, but this patient did have histological evidence of a large, healing, approximately 10-day-old transmural anteroseptal myocardial infarct, and a 1-2-day-old patchy subendocardial infarct and left ventricular and left atrial dilatation. This woman had developed re-elevation of serum CK-B isoenzyme values 8 and 10 days after the onset of initial symptoms, suffered cardiopulmonary arrest on day 11, and could not be resuscitated.

Myocardial Scintigraphy

Twenty-nine patients had an initial $^{99m}$Tc-PYP scan 1-3 days after their infarcts, all of which were judged to be abnormal. Fifteen patients had a second $^{99m}$Tc-PYP myocardial scintigram obtained 5-12 days later. Repeat scintigrams tended to fade, consistent with previous reports of $^{99m}$Tc-PYP scintigraphic changes obtained more than 6 days after myocardial infarction. Only two of these 15 patients had re-elevations of serum CK-B indicative of infarct extensions. One patient had no qualitative change in the second scintigram 3 days after the serum CK-B isoenzyme rise. The second patient had a decrease in $^{99m}$Tc-PYP uptake in the damaged region of the heart, in spite of isoenzyme elevations for 2 days before imaging. Six patients had a doughnut pattern on $^{99m}$Tc-PYP myocardial scintigraphy. One of these patients was noted to have infarct extension by serial serum CK-B determinations.

Risk Factors

Risk factors, including hypertension, diabetes mellitus, smoking and previous myocardial infarction, were examined. Smoking history (five of 10 vs 18 of 33) and the presence of diabetes mellitus (five of 10 vs 10 of 33) were similar in patients with and without infarct extensions. Only one of 10 patients with infarct extension had a history of hypertension, while nine of 33 without extensions had evidence of essential hypertension. Four of 10 patients with infarct extension had previous infarction as determined by a documented history of previous Q waves on ECG. A history of infarction was present in four of 10 patients with infarct extensions (40%) as opposed to four of 33 patients (12%) without extensions. These differences were not statistically significant, but larger numbers of patients must be evaluated to determine the biological significance of the presence of a previous myocardial infarct on this phenomenon.

Discussion

In previous studies we have used the radioimmunoassay for CK-B determination to detect the presence of acute myocardial infarcts in patients. The rationale for using this approach is that we wanted a sensitive and quantitative means to detect CK-B and consequently CK-MB isoenzyme elevations that would avoid detection of CK-M isoenzyme. One would expect serum CK-B elevations in patients with either acute myocardial infarcts or acute cerebral necrosis. Our preliminary results in 17 patients and
more recently in 40 additional patients (Willerson JT, Stone MJ: Unpublished data) with classical acute myocardial infarcts have suggested that this radioimmunoassay for CK-B measurement identifies the presence of classical myocardial infarcts with 100% sensitivity, and if patients with acute cerebral necrosis are excluded, 100% specificity (Willerson JT, Stone MJ: Unpublished data). Studies in occasional patients with acute gastrointestinal abnormalities or uremia suggested that some of these individuals have CK-MB or CK-BB elevations. Patients with acute cerebral necrosis also have elevated CK-B values. However, in our study we did not evaluate patients that simultaneously had acute myocardial infarcts and acute gastrointestinal, cerebral disease or uremia. In 10 patients that we studied with acute coronary insufficiency or unstable angina pectoris without classical acute myocardial infarcts, serial serum CK-B isoenzyme determinations at 12-hour intervals for 12 days have not demonstrated increases above normal; these individuals had a mean deviation from average serum levels of 4 ± 1.7 (SD) mg/ml (Willerson JT, Stone MJ: Unpublished data). Since serum CK-MB elevations by traditional testing and CK-B isoenzyme elevations by radioimmunoassay (Willerson JT, Stone MJ: Unpublished data) appear to identify the presence of acute myocardial infarcts in patients, we conclude that re-elevation of serum CK-B isoenzyme following return to normal values initially represents extension of the myocardial damage. In the one patient in whom a postmortem examination was performed, re-elevation of serum CK-B isoenzyme did identify the presence of subendocardial extension of an earlier myocardial infarct. Furthermore, all four patients who died in this study had re-elevation of serum CK-B isoenzyme in-hospital, suggesting extension of the initial myocardial infarcts.

The incidence of myocardial infarct extension has been variably estimated, depending on the method and definition. Mathey et al. used the term infarct extension to define a group of patients who showed continued total CK release for 24–72 hours after their infarcts, estimating a 62% immediate extension rate. Other studies have evaluated total serum CK and ECG changes for 2–3 weeks after infarction, attempting to define extension as a second discrete event, rather than as an initial variability in the pattern of CK release. Reid et al. demonstrated an 86% extension rate by precordial ECG mapping and a 57% rate by serial total CK analysis a mean of 5.8 days postinfarction in 14 patients with anterior transmural myocardial infarcts. Madias estimated a 48% extension rate using the precordial ECG mapping technique. Thus, previous studies have suggested a significant incidence of myocardial necrosis occurring several days to weeks after the initial infarct. However, these methods for detecting myocardial infarct extension are less specific than desired.

In determining the frequency of in-hospital infarct extension a recent select autopsy evaluation of patients dying after acute myocardial infarction is significant. In this study Hutchins and Bulkley identified infarct "expansion" ("acute dilation and thinning of the area of infarction not explained by additional myocardial necrosis") and "extension" ("histologically more recent foci of contraction band necrosis around an infarct") as complications of acute myocardial infarcts. Infarct "expansion" occurred in 59% of 76 consecutive myocardial infarctions 30 days old or less that were examined; infarct extension occurred in only 17%. Clinically diagnosed infarct extension (new pain, ST-segment elevation, rise in serum CK level and increased congestive heart failure) occurred in 18% of patients in this select series. However, this study evaluated a select patient population and does not allow one to precisely determine the frequency of these phenomena in patients who survive their infarcts.

Factors such as ischemia, myocardial potassium alterations, digitalis therapy, pericarditis and ventricular aneurysm may alter precordial ST segments. Many abnormalities other than infarct extension can result in increases in total serum CK values. As the optimal timing for interventions to protect ischemic myocardium appears within the first few hours after the infarct, or even prophylactically, certain pharmacological and/or physiological interventions continued beyond the first few days of acute myocardial infarction could be beneficial in limiting the frequency and extent of necrosis.

We sought to establish the incidence of myocardial infarct extension more definitively, using a quantitative and sensitive radioimmunoassay to measure serum CK-B values. We and others have recently developed a radioimmunoassay in order to make available a more sensitive and quantitative measurement of serum CK-MB or CK-B levels. It has a relatively short appearance time in the serum, allowing convenient study of recurrent enzyme rises, and serum CK-MB is more specific (and possibly more sensitive) than total CK in the detection of myocardial necrosis. Since enzyme release, and, specifically, CK-MB enzyme release has been suggested as a marker of irreversible cellular damage, it is an appropriate, and possibly the best measurement in documenting further ischemic injury. Others have shown that increases in serum CK or CK-MB isoenzyme correlate with the presence of acute myocardial infarcts in experimental animals and patients.

In the 43 patients in the present series, the overall incidence of myocardial infarct extension, as determined by a second rise in serum CK-B isoenzyme, was 23% (10 patients). Myocardial infarct extension occurred between the 3rd and 14th day, mean 7 ± 3 days (SD) after the initial onset of symptoms suggestive of infarction. In only four of these patients (9%) was myocardial infarction clinically documented. This is consistent with Rosati's retrospective review of 797 patients with acute myocardial infarction, of whom 11% showed definite clinical evidence for infarct extension on the basis of recurrent pain, ECG and en-
zyme changes. Thus, measurement of serum CK-B levels by radioimmunoassay suggests that at least twice as many patients have infarct extension as are identified by traditional clinical parameters. The highest rate of myocardial infarct extension (32%) was in patients with acute anterior transmural infarcts. Using serum CK-B isoenzyme measurements, our data suggest this rate is considerably lower than previously estimated by total CK measurements and precordial ST mapping. In other patients with anterior transmural infarction. If we had used persistent elevation of serum CK-B isoenzyme values for 48 hours or longer as evidence of infarct extension two of 14 of the patients with initial (within 24 hours of onset of symptoms) and then serial determinations of serum CK-B isoenzyme values would have been considered to have had infarct extension by this assessment. Both of these patients had other evidence of infarct extension by serum CK-B measurements; thus, the overall incidence of infarct extension estimated by these techniques in patients with anterior infarcts would have remained 32%.

In our study the incidence of myocardial infarct extension in patients with acute inferior transmural myocardial infarcts was 14%, and in those with acute subendocardial infarcts, 20%. Using persistent elevation of serum CK-B isoenzyme values for 48 hours or longer as an indication of infarct extension in those with acute inferior infarcts, four of 14 patients, or including both these and later re-evaluations of CK-B isoenzyme 36% of patients, would have been considered to have had infarct extensions. None of the patients with acute subendocardial infarcts had persistent elevation of serum CK-B isoenzyme values for 48 hours. In both patients with acute inferior and subendocardial infarcts, the extensions were clinically unrecognized and they had no immediately associated morbidity or mortality. Reid et al., using precordial ECG mapping and total serum CK measurements, also suggested that patients with nontransmural infarcts had lower infarct extension rates (one out of five patients). However, precordial ECG mapping and ST-segment analysis have definite methodological limitations in this group, and are currently unsuitable for following patients with acute inferior infarcts to identify infarct extension.

We also evaluated the qualitative role of 99mTc-PYP scintigraphy in assessing infarct extension using serial myocardial scintigrams in the present study. 99mTc-PYP myocardial scintigrams were unchanged or tended to fade in two patients regardless of the presence of infarct extension, suggesting that the magnitude of infarct extension in the patients with new serum CK-B elevations was not sufficient to allow quantitative recognition of infarct extension. However, the precise ability of “hot spot” (99mTc-PYP) and “cold spot” (thallium-201) myocardial scintigraphy in recognition of relatively small extensions of myocardial infarcts will have to be studied in more patients in whom extension occurs and probably cannot be tested reliably without a three-dimensional quantitative measurement of infarct size, as might become available using computer-assisted reconstruction techniques and/or a tomographic camera with non-invasive emission radionuclides.

In summary, the incidence of myocardial infarct extension in patients after acute myocardial infarction appears to be less than previously suspected. There were no significant differences in infarct extension rates among patients with acute anterior, inferior and subendocardial myocardial infarcts. Significant morbidity and mortality were associated with extension of anterior infarcts in these series. These patients may represent a group in which it is possible to test certain physiologic and pharmacologic approaches to limiting infarct extension in the first 2 weeks after the acute event. Infarct extensions in patients with inferior and subendocardial infarcts were clinically unrecognized in this study and had no important associated morbidity or mortality, but it would be expected that large extensions in other similar patients might be recognized and have associated clinical problems.

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