CASE REPORT

Macroenzyme Creatine Kinase in the Era of Modern Laboratory Medicine

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Among the conditions in which creatine kinase (CK)-MB activity is elevated in the absence of myocardial injury or infarction, macroenzyme (macro) CK merits special attention from clinicians. We present 2 cases, 1 with macro CK type 1 and the other with macro CK type 2, to stress the common clinical situations and diagnostic dilemma that clinicians encounter when evaluating patients with macro CK. Moreover, the rare conditions associated with macro CK, and the phenomenon of spuriously high CK-MB activity out of proportion to total CK, are discussed. The biochemical characteristics, clinical significance and potential implications of macro CK are reviewed within the scope of modern laboratory medicine. [*J Chin Med Assoc* 2010;73(1):35–39]

Key Words: creatine kinase, hepatitis C virus, isoenzymes, macro creatine kinase, myocardial infarction

Introduction

The introduction of rapid laboratory testing for creatine kinase (CK)-MB greatly revolutionized the diagnosis and management of acute myocardial infarction (AMI) in the 1970s and 1980s.[1,2] Conditions that are known to lower the sensitivity and specificity of CK-MB activity assays include false-positive results in renal failure patients, concurrent skeletal muscle and myocardial injuries, and many other conditions, such as non-cardiac surgery, chest trauma, asthma, malignancies and pulmonary embolism.[1–3] Among the conditions in which CK-MB activity is elevated in the absence of myocardial injury, the presence of macroenzyme (macro) CK merits special attention from clinicians. Macro CK is one of the most common macroenzymes, which are enzymes with a higher molecular mass than the corresponding enzymes that are normally found in serum.[4–7] Macro CK occurs in 2 major forms. Macro CK type 1 is an enzyme-antibody complex with a molecular weight greater than 200 kDa, and is formed by 1 of the CK isoenzymes (most often, CK-BB) and immunoglobulin (most often, IgG with a kappa light chain; occasionally IgA; and rarely, IgM).[8] In contrast to macro CK type 1, macro CK type 2 is a non-immunoglobulin-bound macroenzyme produced by a separate gene.[9] Macro CK type 2 is a polymer of mitochondrial CK with a molecular mass greater than 300 kDa.[6,10] Both macro CK types 1 and 2 are well known to cause falsely elevated CK-MB activity, which often leads to diagnostic confusion and unnecessary investigation for myocardial injury. The detection of macro CK requires careful laboratory interpretation, and sometimes additional biochemical tests are needed to establish the proper diagnosis.

In this report, we present 2 individual cases, 1 with macro CK type 1 and the other with macro CK type 2. In the case involving macro CK type 1, the importance of the differential diagnosis with a true AMI in a patient who presents symptoms mimicking acute coronary syndrome (ACS) is highlighted. In the case involving macro CK type 2, we stress both the common

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scenario and an unusually high CK-MB activity in association with the presence of macro CK type 2 in a cancer patient. The biochemical characteristics, clinical significance, and potential implications of macro CK are reviewed within the scope of modern laboratory medicine.

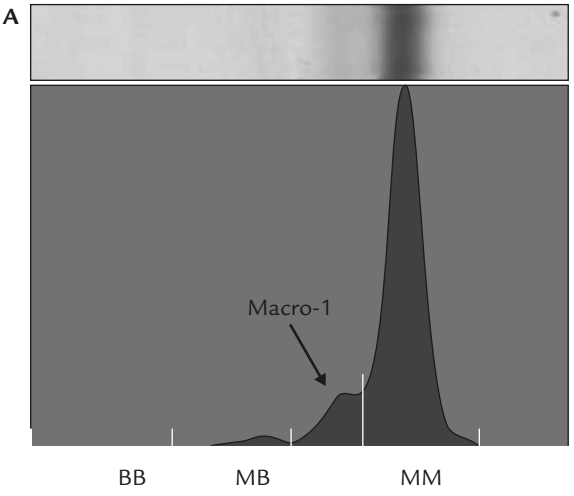
Case Reports

Case 1

A 72-year-old Taiwanese female suffered from intermittent chest tightness, and had undergone a serial work-up for AMI, including serial measurement of CK and CK-MB activities, troponin I levels and percutaneous coronary intervention, all of which revealed negative findings. The physical examination on admission revealed no remarkable findings. The patient had a history of asymptomatic chronic hepatitis C, without cryoglobulinemia. The blood biochemistry tests were normal, except for mildly elevated liver enzymes (alanine aminotransferase, 57 U/L; aspartate aminotransferase, 62 U/L) and increased total CK (311 U/L) and CK-MB (108 U/L). In addition, a serum CK isoenzyme electrophoresis was performed using an automated electrophoresis apparatus (Epalyzer-II; Helena Laboratories Inc., Saitama, Japan), which revealed the presence of macro CK type 1 with a percentage of 12.8% (35 U/L) (Figure 1). In the following 2 years of follow-up, the cardiac biochemistry profile showed persistent mildly elevated CK with the presence of macro CK type 1.

Case 2

A 41-year-old Taiwanese female sought evaluation at our hospital for the acute onset of chest tightness and dyspnea. The physical examination revealed a mildly engorged jugular vein with clear breath sounds and no evidence of a cardiac murmur; however, a palpable mass measuring 4 × 1 cm was present over the left breast. The electrocardiograms and chest roentgenograms were normal. Biochemical testing of the blood showed an elevated total CK (359 U/L), with an abnormal CK-MB (1,367 U/L) by a CK-MB monoclonal antibody immunoinhibitory assay (Kanto Chemical Co., Inc., Tokyo, Japan), and an undetectable troponin I value. Chest computed tomography (CT) with contrast enhancement confirmed the palpable mass over the medial upper quadrant of the left breast. Chest CT also showed another metastatic nodule (1.5 cm in diameter) at the medial upper quadrant of the right breast, multiple osteolytic metastatic bony lesions involving the thoracic and lumbar spine, and multiple tiny pulmonary metastatic nodules within the lung fields bilaterally. After admission, an echocardiogram confirmed the presence of severe pulmonary hypertension with a measured pulmonary artery systolic pressure of 98 mmHg. Repeat serum biochemical



Fractions	%	Total unit (U/L)	Ref %
BB	0	0	< 4.5
MB	3.1	8	< 6.3
Macro-1	12.8	35	0
MM	84.1	230	93.5–100

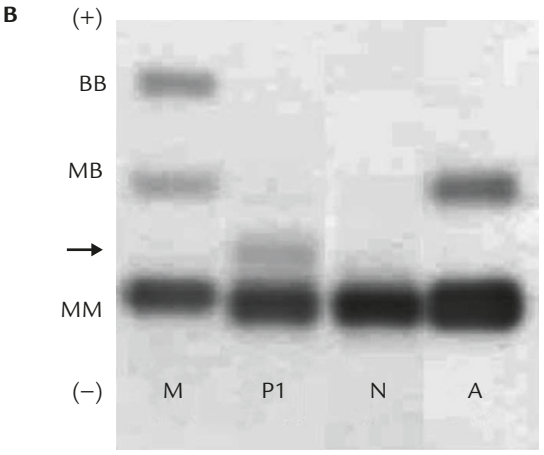


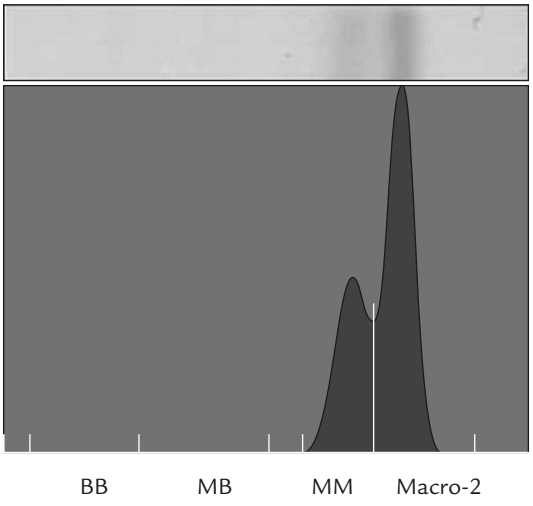
Figure 1. Electrophoresis results of serum creatine kinase (CK) isoenzymes of Case 1. (A) The peak patterns of electrophoresis by densitometry and the fractions of CK isoenzymes were analyzed automatically. (B) Agarose gel electrophoresis shows a positive band (arrow) migrating anodal to the CK-MM band, between the CK-MB and CK-MM bands, indicative of macro CK type 1. Macro-1 = macro CK type 1; BB = CK isoenzyme CK-BB; MB = CK isoenzyme CK-MB; MM = CK isoenzyme CK-MM; M = markers; P1 = patient (Case 1); N = normal control; A = acute myocardial infarction patient with elevated CK-MB.

tests showed persistently elevated total CK levels and abnormally high CK-MB activity levels. A CK isoenzyme electrophoresis (Epalyzer-II; Helena Laboratories Inc.) confirmed the presence of macro CK type 2 with a percentage of 63.3% (342 U/L), with a total CK of 540 U/L, and a CK-MM of 198 U/L (36.7%) (Figure 2). The patient also had positive signs of disseminated intravascular coagulopathy associated with an underlying malignancy. A pathologic diagnosis of stage IV invasive ductal carcinoma of the left breast was made based on the tissue biopsy.

Discussion

Elevated CK and CK-MB activity often leads to a suspicion of AMI or ACS, especially when patients present with chest symptoms mimicking angina pectoris, and the presence of macro CK can be troublesome. Although currently, the diagnostic confusion of AMI/ACS with macro CK can be resolved by careful identification of macro CK (by measuring the CK-MB mass assay which directly determines the antigenic amount of CK-MB, or by the determination of troponin levels[1,11,12]), in areas where CK-MB mass or troponin assays are not available, CK and CK-MB activity assays are still reliable, and the identification of macro CK avoids unnecessary and costly diagnostic procedures. Macro CK can usually be recognized on CK electrophoresis. Occasionally, other tests may be needed to unmask a macro CK co-migrating with CK-MM or CK-MB, such as the combined use of immunoprecipitation and electrophoresis,[13,14] the heat stability method,[15] or energy kinetics characterization.[16] Often, macro CK causes only a mild elevation (< 500 IU/L) in CK or a high CK-MB/CK ratio with a normal level of total CK.[17] Clinically, the absence of symptoms, the presence of symptoms atypical for the abnormal level of CK, or an isolated and persistently increased CK favor the presence of macro CK.[6,7,17]

CK-MB activity by immunoinhibition assay is based on the inhibition of all M subunits by anti-M antibody, allowing determination of residual B subunit enzyme activity by anti-B antibody. Both macro CK types 1 and 2 can cause a falsely elevated CK-MB activity. Increased amount of CK-BB-immunoglobulin complex (macro CK type 1) is resistant to inhibition by the anti-M antibody. On the other hand, macro CK type 2 is not structurally related to the M or B subunits, nor is it inhibited by monoclonal anti-M antibody. In our 2nd case, the measured CK-MB activity was nearly 3 times more than that of the estimated



Fractions	%	Total unit (U/L)	Ref %
BB	0	0	< 4.5
MB	0	0	< 6.3
MM	36.7	198	93.5–100
Macro-2	63.3	342	0

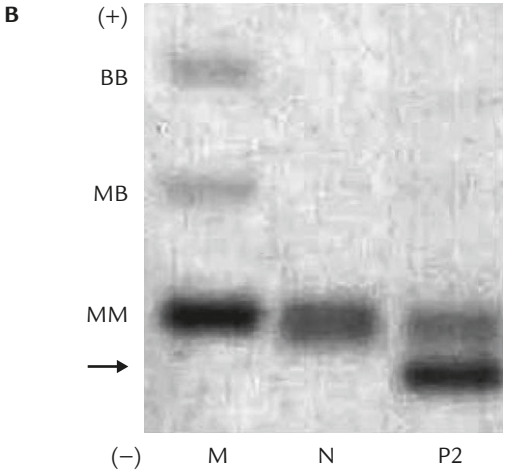


Figure 2. Electrophoresis results of serum creatine kinase (CK) isoenzymes of Case 2. (A) The peak patterns of electrophoresis by densitometry and the fractions of CK isoenzymes were analyzed automatically. (B) Agarose gel electrophoresis shows a positive band (arrow) migrating cathodal to the CK-MM band, indicative of macro CK type 2. Macro 2 = macro CK type 2; BB = CK isoenzyme CK-BB; MB = CK isoenzyme CK-MB; MM = CK isoenzyme CK-MM; M = markers; N = normal control; P2 = patient (Case 2).

macro CK type 2 by electrophoresis and vastly exceeded the total CK level, showing that macro CK type 2 abnormally reacted with the anti-B antibody during the immunoinhibition assay. Rarely, under certain pathological conditions, abnormally increased CK-BB may contribute to the falsely elevated CK-MB activity that may exceed total CK activity. Jap et al[18] reported a patient with rectal adenocarcinoma and an

extremely high CK-MB activity greater than the total CK level, in which the etiology was attributed to a marked elevated immunoglobulin-bound CK-BB complex (macro CK type 1) that was caused by the rectal cancer.

The prevalence of macro CK type 1 has been reported to range from 0.54% to 2.3%.[5] The clinical significance of macro CK type 1 remains of interest to clinicians, and there is no well-established association of a particular disease with macro CK type 1. Nevertheless, it has been reported to be associated with a variety of diseases, including hypothyroidism, malignancies, autoimmune diseases, myositis, and cardiovascular disease.[8,17,19,20] More rarely, macro CK 1 has been described in patients with irritable bowel syndrome, bronchopulmonary chronic illness,[8] or in association with other macroenzymes, such as macroamylasemia[21] and macro-lactate dehydrogenase.[22] Case 1 had no hepatitis C-associated cryoglobulinemia that might potentially explain the formation of immune complex. It remains unclear whether macro CK type 1 is associated with the hepatitis C carrier state.

Macro CK type 2 is detected in up to 3.7% of hospitalized patients.[23] It is frequently found in patients who are critically ill or who have widespread tissue damage, such as severe liver disease and disseminated malignancies.[6,8,10,23,24] In general, the presence of macro CK type 2 may be viewed as a warning signal of occult malignancies, a poor prognostic sign in patients with a malignancy, or a reflection of the severity of an underlying illness.[4–6,8,25]

In conclusion, macro CK can occur as an incidental finding in healthy individuals or as a marker of certain diseases (autoimmune diseases, cancer, severe liver disease, and serious illness). It is particularly important to recognize macro CK in patients with symptoms mimicking ACS, to avoid unnecessary specialist consultations and invasive procedures. Despite the usefulness of troponin assays, confirmation is required to completely replace CK and CK isoenzymes by troponins in AMI/ACS diagnosis. It is important for clinicians to understand the biochemistry and clinical significance of macro CK in the context of modern laboratory medicine.

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