Clinical Role of Recurrently Elevated Macro Creatine Kinase Type 1

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The typical creatine kinase (CK) isoenzymes include CK-BB, CK-MB, and CK-MM. Macro CK type 1, one of the atypical CK enzymes, has been identified in human serum, but the clinical significance still remains uncertain. In our laboratory, 105 patients who expressed serum macro CK isoenzyme type 1 were identified from March 2004 to March 2007. We found that macro CK type 1 recurred after at least one month in 16 patients. Clinical diagnoses were myopathy in 14 patients, sepsis in one, and acute coronary syndrome in one. The averages of serum total CK and macro

CK type 1 were 9,132 and 1,925 (U/L), respectively. The linear regression analysis between recurrent macro CK type 1 and total CK revealed a good correlation ($y = 3.5054x + 2381.3$, $R^2 = 0.7822$, $P < 0.001$). Three patients had critical illness, including one respiratory failure and two mortalities. Good linear correlation is documented between total CK and recurrent macro CK type 1. In conclusion, the macro CK type I isoenzyme recurred primarily in patients with myopathy. *J. Clin. Lab. Anal.* 22:186–191, 2008. © 2008 Wiley-Liss, Inc.

Key words: macro CK type 1; atypical creatine kinase; creatine kinase isoenzyme; electrophoresis; myopathy

INTRODUCTION

The typical creatine kinase (CK) isoenzymes include CK-BB, CK-MB, and CK-MM. CK-BB is mainly found in the brain, uterus, bladder, lung, prostate, and gastrointestinal tract. CK-MM and CK-MB are present in both skeletal muscle and myocardium, with CK-MM being the predominant isoenzyme. CK-MB is responsible for only 20–46% of the total CK in myocardium and very small amounts in skeletal muscle (1–4). Macro CK type 1, an atypical CK enzyme with higher than normal molecular mass, has been identified in human serum. It is a compound composed of CK-BB and immunoglobulin G(Ig)G that is created via an antigen-antibody reaction; in rare cases, CK-BB combines with IgA (5–8). During electrophoresis of CK isoenzymes, macro CK type I migrates between CK-MM and CK-MB, denoting a molecular weight that is slightly different from these two.

Although several studies have attempted to establish the correlation between macro CK type 1 and specific diseases, its possible pathogenetics or clinical significance still remains uncertain. Macro CK type 1 has been studied because it possibly affects the level of CK-MB

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and interferes with the diagnosis of acute myocardial infarction (8–16). The low prevalence of macro CK type 1 ranges from 0.54% to 2.3% (16–19). In our laboratory from March 2004 to March 2007, 105 patients who expressed the serum macro CK isoenzyme type 1 were identified. Moreover, there were 16 patients with recurrent macro CK type 1, and we tried to analyze the clinical and laboratory role of these cases of persistently elevated macro CK type 1.

MATERIALS AND METHODS

Subjects

The study population consisted of 105 patients with serum macro CK type 1 for whom CK isoenzyme analysis was ordered in our laboratory from March 2004 to March 2007. Among these 105 patients, we found the repeated presence of macro CK type 1 in 16 patients. Delayed clearance of immune-complex had been reported (3,8,9), so the inclusion criterion of this study was the recurrent presence of macro CK type 1 in a separate period of at least 1 month. We analyzed the clinical and laboratory data and utilized information from prior studies (20–23). This study received approval from our institutional review board.

Laboratory Analysis

CK isoenzymes were analyzed in an agarose-containing REP CK-16 isoenzyme gel with the Helena REP Analyzer system (Helena Labs, Beaumont, TX). The isoenzymes were separated on the gel according to electrophoretic mobility at 2 to 8°C. As expected, macro CK type I migrated between CK-MM and CK-MB. The gel was scanned in the REP system using the fluorescence mode to perform the quantitative evaluations. The normal quantitation range of CK-MM is 97–100%, CK-MB is 0–3%, and CK-BB is 0% according the study data from Helena Laboratories. The normal reference values for total CK are 15–130 U/L.

Clinical Approach

Patients received a physical examination and underwent a biochemical study that included liver and renal function, and electrolyte measurement. Magnetic resonance imaging (MRI), a nerve conductive study and electromyography or muscle biopsies were performed to establish the diagnosis of myopathy. Electrocardiography (EKG) and/or transthoracic echocardiography were performed if cardiovascular disease was suspected according to clinical symptoms.

RESULTS

Patient Characteristics

From March 2004 to March 2007, 25,003 sets of CK isoenzymes were analyzed and 185 sets were positive for

macro CK type 1, with a prevalence of 0.73%. A total of 105 patients who expressed serum macro CK isoenzyme type 1 were identified. In 16 patients, macro CK isoenzyme type 1 recurred. The analytical data of CK isoenzymes and clinical characteristics of these patients are given in Table 1. The repeated presence of macro CK type 1 was found after 1 month and even up to 70 months. The decision to repeat the CK isoenzyme assays depended on the clinical situation when neurologic or cardiac problems were suspected, and the viewpoint of the physician in charge. These patients had been followed from 2 to 70 months (mean: 29 months). Totally, 84 sets of CK isoenzyme analysis were performed during follow-up and every patient received an average of 5.3 sets of CK isoenzyme assays. A total of 12 patients were male and four were female. Patient ages at the initial presentation ranged from 5 months to 70 years (mean: 20.3 years). There were 10 patients younger than age 20 years, five patients with ages from 20 to 50 years, and only one patient older than 50 years. All serum creatinine was within normal range.

Clinical Diagnosis

Clinical diagnoses were myopathy in 14 patients, one sepsis, and only one diagnosis of acute coronary syndrome (Table 1). Of the 14 diagnoses of myopathy, five were Duchenne muscular dystrophy, three were polymyositis, two were limb-girdle muscular dystrophy, one was Fragile X syndrome with muscular dystrophy, one was dermatomyositis, one was metal intoxication associated with polyneuropathy and myositis, and one was unascertained myopathy in a male patient whose brother had Duchenne muscular dystrophy. A muscle biopsy was performed on 10 patients and one patient participated in a MRI study. Among these 15 patients, cardiovascular diseases were excluded by clinical presentations, EKG, or echocardiography. A total of three patients had critical illness, including one respiratory failure (patient 7) and two mortalities (patients 8 and 11).

Activities of Total CK and CK Isoenzymes

The averages of the 84 sets of serum total CK, macro CK type 1, and CK-MB were $9,132 \pm 9,898$, $1,925 \pm 2,497$, and $787 \pm 1,103$ U/L, respectively. The enzyme fraction is expressed as the ratio of macro CK type 1 or CK-MB of total CK. The mean of macro CK type 1 and the CK-MB fraction was $14 \pm 11\%$ and $8 \pm 7\%$, respectively. Almost all CK-MB fractions were more than 4%, which is the upper limit for the definition of myocardial infarction, except for patients 2 and 12.

TABLE 1. Clinical characteristics and diagnosis in recurrent presence of macro creatine kinase type 1

No.	Age (years)	Sex	Mean Total CK (U/L)	Mean macro CK type 1 (U/L)	Mean CK-MB (U/L)	Diagnosis	Initial presentation	Others
1	6	M	16,355	4,345	1,741	DMD	Motor delay, lower extremity weakness	
2	35	M	2,370	175	86	LGMD	Distal to proximal progressive lower leg weakness	
3	10	F	6,063	1,092	250	LGMD	Bilateral lower limb weakness	
4	2	M	22,265	5,806	2,513	DMD	Muscular weakness, motor/speech delay	
5 ^a	1	M	26,202	6,452	3,400	Myopathy	Muscular weakness, speech/motor delay	
6	4	M	14,626	3,895	689	DMD	Tip-toe gait	
7	49	M	1,714	293	302	Metal intoxication, polynuropathy	Chest pain, general weakness,	Respiratory failure
8	0.4	M	1,126	261	336	Pneumonia, sepsis	Fever, shortness of breath	Sepsis, mortality
9	41	F	4,649	696	289	Polymyositis	4 limb weakness, upper limb worse	
10	5	M	23,441	4,881	1,903	Fragile X syndrome	Weakness of leg, speech delay	
11	70	M	1,697	68	91	ACS	Drowsiness, chest tightness	Sepsis, mortality
12	17	M	1,834	150	0	Polymyositis	4 limb weakness for 10 years	
13	49	F	1,407	127	141	Polymyositis	Muscle weakness	
14	28	F	3,715	1,117	191	Dermatomyocitis	General skin rash, 4 limb weakness	
15	3	M	2,2403	3,598	2,191	IgA GN	Muscle weakness, easily falls down	
16	7	M	2,3753	6,176	1,460	DMD	Lower leg weakness	

^aPatient 5 has family history of Duchenne disease.

ACS, acute coronary syndrome; DMD, Duchenne muscular dystrophy; GN, glomerulonephritis; LGMD, limb-girdle muscular dystrophy.

Fig. 1. The linear regression analysis between macro CK and total CK (A); between macro CK type 1 and CK-MB (B); and between macro CK type 1 and CK-MM (C).

Relationship Between Macro CK Type 1, Total CK, CK-MB, and CK-MM

The linear regression analysis between recurrent macro CK type 1 and total CK revealed a good

correlation ($y = 3.5054x + 2381.3$; $R^2 = 0.7822$, $P < 0.001$) (Fig. 1A). There is also a linear relationship between recurrent macro CK type 1, CK-MB ($y = 0.3753x + 64.722$; $R^2 = 0.7217$) (Fig. 1B) and CK-MM ($y = 2.1302x + 2316.6$; $R^2 = 0.5991$) (Fig. 1C).

DISCUSSION

This study is the first report about the recurrent presence of macro CK type 1. Our findings demonstrate that a good linear correlation exists between total CK and recurrent macro CK type 1 via linear regression analysis (Fig. 1). Of the 16 patients with recurrent macro CK type 1, clinical diagnoses were myopathy in 14 patients, one sepsis, and only one with acute coronary syndrome.

Correlation Between Recurrent CK Type 1 and Total CK

Our findings show that recurrent macro CK type 1 had good correlation with total CK. Total plasma CK, which is associated with the activities of myopathy, reflects the degree of muscle inflammation and cell damage (2). We propose that macro CK type 1 arises as a consequence of muscular damage and is possibly another biomarker of myopathy that is independent of CK-MM and CK-MB. It may also provide the degree of cell damage, but the availability of this value is limited due to its low prevalence.

Pathogenesis

Several studies revealed that macro CK type 1 consists of a binding complex of IgG and CK-BB due to an antigen-antibody reaction (5–8,17,18). One hypothesis is that CK might become antigenic after cellular destruction (8,19). It is consistent with the observation from our study that macro CK type 1 occurred predominantly in patients with myopathy. Delayed clearance of the immune complex had been reported (3,8,9). During our follow-up of five patients, macro CK type 1 was not detectable, which may demonstrate the recurrent nature of macro enzymes and their inability to persist. Moreover, the detection of macro CK may be due to the specific condition of the patients before the test, and therefore we may have underestimated the prevalence of recurrent macro CK. In the future, using newer methods such as proteomics to analyze the construction of macro CK type 1 production may provide us with more information.

Clinical Role and Prognosis

The clinical significance of macro CK type 1 remains unclear because of its low prevalence. Earlier studies disclosed that macro CK type 1 might be associated with myopathy, cardiovascular disease, gastrointestinal disease, or autoimmune disease (17–19). Our study discovered that macro CK type 1 was recurrently present in patients with myopathy, one with sepsis and only one patient with coronary artery disease. Except

patient 14, a case of dermatomyositis and IgA glomerulonephritis, there was neither clinical nor laboratory evidence of general autoimmune diseases in the other 15 patients. The prognostic value of macro CK type 1 is currently uncertain. Any correlation with the occurrence of critical illness, life-threatening complication, or death is not clear (17,19). In our follow-up, one respiratory failure and two deaths occurred. The titer of macro CK type 1 in these three patients was not higher than the others.

Limitations of the Study

This is a retrospective study with small case numbers. In our laboratory, the decision to repeat the CK isoenzymes assays depended on whether the physician in charge suspected any neurologic or cardiac problems, and this may have contributed to the selection bias. Recurrence of macro CK type 1 could be underestimated among our 105 patients, since it is possible that macro CK type 1 was absent initially but detectable in further follow-up or that macro CK type 1 presented in the moment when CK isoenzyme assays were not tested.

CONCLUSION

In conclusion, good linear correlation between total CK and recurrent macro CK type 1 was demonstrated by our study, and macro CK type 1 possibly reflects the degree of cell damage or disease activities. We also found that recurrent macro CK type I isoenzyme is primarily associated with myopathy.

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REFERENCES

1. Galen RS. The enzyme diagnosis of myocardial infarction. *Hum Pathol* 1975;6:141–155.
2. Bohlmeier TJ, Wu AH, Perryman MB. Evaluation of laboratory tests as a guide to diagnosis and therapy of myositis. *Rheum Dis Clin North Am* 1994;20:845–856.
3. Wolf PL. Common causes of false-positive CK-MB test for acute myocardial infarction. *Clin Lab Med* 1986;6:577–581.
4. Serdar MA, Tokgoz S, Metinyurt G, et al. Effect of macro-creatine kinase and increased creatine kinase BB on the rapid diagnosis of patients with suspected acute myocardial infarction in the emergency department. *Mil Med* 2005;170:648–652.

5. Mifflin TE, Bruns DE, Wrotnoski U, et al. University of Virginia Case Conference: macroamylase, macro creatine kinase, and other macroenzymes. *Clin Chem* 1985;31:1743–1748.
6. Urdal P, Landaas S. Macro creatine kinase BB in serum, and some data on its prevalence. *Clin Chem* 1979;25:461–465.
7. Bohner J, Stein W, Kuhlmann E, Eggstein M. Serum creatine kinase BB linked to immunoglobulin G. *Clin Chim Acta* 1979;97:83–88.
8. Remaley AT, Wilding P. Macroenzymes: biochemical characterization, clinical significance, and laboratory detection. *Clin Chem* 1989;35:2261–2270.
9. Stein W, Bohner J. Influence of autoantibodies to creatine kinase-BB on assays for MB isoenzyme. *Clin Chem* 1985;31:1189–1192.
10. Loshon CA, McComb RB, Bowers Jr GN. Immunoprecipitation and electrophoresis used to demonstrate and evaluate interference by CK-BB and atypical-CK's with CK-MB determinations by immunoinhibition. *Clin Chem* 1984;30:167–168.
11. Wu AH, Bowers Jr GN. Evaluation and comparison of immunoinhibition and immunoprecipitation methods for differentiating MB and BB from macro forms of creatine kinase isoenzymes in patients and healthy individuals. *Clin Chem* 1982;28:2017–2021.
12. Jacobs E, Anzalone T, Sarkozi L. Immunoprecipitation method for CK-MB analysis re-evaluated: influence of CK-BB and macro-CK on blank activities. *Clin Chem* 1988;34:585–588.
13. Sax SM, Moore JJ, Giegel JL, Welsh M. Atypical increase in serum creatine kinase activity in hospital patients. *Clin Chem* 1976;22:87–91.
14. Medeiros LJ, Greco FA, Walsh D, Gerson B. Macro creatine kinase type 1 with electrophoretic mobility identical to that of the MB isoenzyme. *Clin Chem* 1985;31:1393–1396.
15. Jay DW. Macro-creatine kinase co-migrating with CK-MB on electrophoresis. *Clin Chem* 1988;34:1920.
16. Sturk A, Sanders GT. Macro enzymes: prevalence, composition, detection and clinical relevance. *J Clin Chem Clin Biochem* 1990;28:65–81.
17. Lee KN, Csako G, Bernhardt P, Elin RJ. Relevance of macro creatine kinase type 1 and type 2 isoenzymes to laboratory and clinical data. *Clin Chem* 1994;40:1278–1283.
18. Tozawa T. Enzyme-linked immunoglobulins and their clinical significance. *Electrophoresis* 1989;10:640–644.
19. Laureys M, Sion JP, Slabbynck H, et al. Macromolecular creatine kinase type 1: a serum marker associated with disease. *Clin Chem* 1991;37:430–434.
20. Chu PH, Chiang CW, Hsu LA, Lin KH, Cheng NJ, Kuo CT. Low prevalence of coronary arterial diseases in Chinese Adults with mitral stenosis. *Chang Gung Med J* 2001;24:97–102.
21. Chu PH, Jung SM, Wu HH, et al. Apoptosis in primary cardiac tumors. *Int J Clin Pract* 2004;58:564–567.
22. Chu PH, Jung SM, Yeh TS, Wang CL. Mucin gene expression in cardiac myxoma. *Int J Clin Pract* 2004;58:306–309.
23. Chu PH, Jung SM, Lin HC, et al. Caspase-3-dependent apoptosis in cardiac myxoma: not associated with human papillomavirus or Epstein-Barr virus. *Modern Pathol* 2005;18:822–827.