A case of creatine kinase non-M activity in human plasma

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The enzyme creatine kinase (CK) [EC 2.7.3.2] is a dimeric molecule consisting of two separate subunits B and M, giving rise to three isoenzymes CK-BB, CK-MB and CK-MM. Most of the CK found in the plasma of normal individuals is the MM isoenzyme derived from skeletal muscle, while the CK-MB fraction originates predominately from cardiac muscle. Normally there is little, if any, CK-BB isoenzyme present in plasma. Other forms of CK can exist in the plasma in certain individuals and these include macro-forms, which include CK-BB bound to IgG (CK type 1) and also a mitochondrial isoenzyme (CK type 2).

In this article we describe a patient who presented with an abnormal plasma total CK activity and illustrate potential problems in the interpretation of plasma CK results, with particular emphasis upon possible causes of elevated plasma non CK-M activity.

CASE HISTORY

An 86-year-old woman presented to the casualty department in a state of collapse, dehydrated and with a 3 week history of vomiting. She was hypotensive, although a resting electrocardiogram (ECG) showed no evidence of a recent myocardial infarction. Her admission biochemistry blood results, obtained using a Kodak Ektachem analyser (Johnson & Johnson Clinical Diagnostics, Amersham, UK) showed (reference ranges in parentheses): plasma sodium 126 mmol/L (136-145), potassium 6.2 mmol/L (3.3-4.4), urea 34.0 mmol/L (2.7-7.8), creatinine 218 μmol/L (59-102), glucose 5.9 mmol/L (3.6-5.4), bilirubin 24 μmol/L (2-22), alkaline phosphatase 571 U/L (38-126), alanine transaminase 198 U/L (14-36), albumin 34 g/L (36-47) and creatine kinase 306 U/L (26-194). A CK-MB determination (assayed on the Kodak Ektachem analyser using immunoinhibition of the CK-M subunit) showed an apparent CK-MB activity of 600 U/L. A repeat assay of the plasma CK 24 h later showed a total activity of 1827 U/L and a very high CK-MB of >3000 U/L. In view of the discrepancy between the plasma total CK and CK-MB we performed plasma CK electrophoresis on the second sample, using agarose gels (Corning, Halstead, UK). The bands of CK activity were visualized using total CK and CK-MB reagents from Merck (Poole, UK), the latter containing antibodies to the CK-M subunit. Using this technique both CK-BB and CK-mitochondria were found to predominate (both being about 40% of total CK activity). The presence of melaena was noticed a few days later and ultrasound investigation of her abdomen revealed extensive hepatic metastases. Unfortunately, the patient died 4 days after admission. No post-mortem was performed and the source of her primary malignancy remains unknown.

DISCUSSION

The attending clinicians were presented with a patient with a possible acute myocardial infarction. A raised serum total CK activity led to the request for a CK-MB determination which was inappropriately elevated in comparison to the total CK activity. This patient illustrates the potential analytical problems of non-CK-M enzyme activity. An immunoinhibition method was used for the CK-MB determination, which involves addition of an antibody to the CK-M subunit to inhibit CK-M activity. Residual CK subunit activity is then multiplied by two to give the activity of the CK-MB, the assumption being that there is no CK-BB or other forms of CK present and that the enzymatic activity of the M and B subunits are the same. The spuriously high 'apparent' CK-MB activity alerted us to the possibility of the presence of non CK-M activity in the sample. This was confirmed by the plasma electrophoresis studies demonstrating the presence of CK-BB and mitochondrial-CK.
Causes of a raised creatine kinase non-M activity are summarized in Table 1. Elevated CK-BB has been reported in some patients with malignant disease, and it appears that changes in cell differentiation can result in increased production of CK-BB. Feld and Witte observed that CK-BB was present in the sera of over half their patients with prostatic carcinoma, and elevated serum CK-BB activity has been reported in other tumours including patients with metastatic small cell lung carcinoma without radiological evidence of their tumour. Anaplastic lung carcinoma and malignant tumours of the gastrointestinal tracts such as stomach and rectum also result in elevated serum CK-BB levels. Doran reported a case of a patient with acute necrosis of the large intestine in whom a transient elevation of serum CK-BB activity was found. Increased serum CK-BB levels have also been observed in breast carcinoma.

Serum CK-BB has also been found to be elevated in various brain disorders. Transient elevation of serum CK-BB was found within a few hours of an acute cerebral disorder (trauma, cerebrovascular or infective) by Kaste and co-workers. Using a radioimmunoassay, a number of groups have observed increased serum CK-BB levels in patients with cerebral pathology including seizures, cerebrovascular accidents, decreased consciousness and central nervous system infections. Neuromuscular disorders, such as Duchenne muscular dystrophy and inflammatory myopathies, also can show an increased serum CK-BB concentration possibly due to the degree of regeneration after damage.

Serum CK-BB concentration has been shown to be a sensitive index of brain damage following head injury. In this study, all patients with cerebral swelling or laceration had elevated serum CK-BB levels and fatally injured patients maintained high levels for several days after their injury, unlike the less seriously injured subjects whose serum CK-BB levels returned to normal within a few days. In another study on patients with head injury, those with subdural and intracerebral contusions showed the highest serum CK-BB levels but there was no correlation between recovery of the neurological lesion and normalization of the serum CK-BB concentration. Capocchi and colleagues observed that serum CK-BB activity was higher in patients with an acute stroke compared to controls and also that the serum activities correlated with the severity of the brain damage. Studies in preterm infants (below 34 weeks' gestation) found that serum CK-BB activity was a good predictor of periventricular haemorrhage.

Elevated serum CK-BB levels have been reported in patients undergoing aortocoronary bypass surgery. It is likely that some minimal brain damage could have occurred while the patient was undergoing bypass and thus a brain source for the isoenzyme cannot be excluded. After cardiac arrest, both serum and cerebrospinal fluid CK-BB were higher in those patients.
with residual neurological damage. There is also an increase in creatine kinase BB activity in the serum of patients who have just experienced a cardiac or respiratory arrest. Furthermore, Vaubourdolle and colleagues showed increased serum CK-BB activity after liver transplantation which they suggested could be useful as an index of sinusoidal injury.

Somewhat surprisingly, elevated serum CK-BB occurs in the rare autosomal dominant condition of osteopetrosis type II. Gram et al. suggested that serum CK-BB could be a marker of immature osteoclasts, although elevated serum CK-BB was not observed in patients with other sclerosing bone diseases. It has also recently been reported that elevated serum CK-BB activity can occur in myelodysplasia. Although elevated serum CK-B activity has been reported in chronic renal failure and patients undergoing haemodialysis, this may be a methodological artefact due to natural fluorescence of their serum. An as yet unexplained finding is the observation that serum CK-BB concentration can be transiently increased in some boys at the time of puberty.

Elevated serum mitochondrial-CK (CK-type 2) has been reported in various malignancies including leukaemia, lymphomas, breast, lung and gastrointestinal carcinomas. However, the use of this CK isoenzyme as a tumour marker was dismissed by Castaldo and co-workers who also found high serum mitochondrial-CK levels in patients with severe liver disorders such as cirrhosis. Furthermore, it was observed that the mitochondrial isoenzyme was elevated in the serum of patients with cardiogenic shock and was an ominous prognostic sign in these subjects. Chemnitz et al. present one of the early descriptions of serum non-CK-M activity and showed binding of CK-BB to immunoglobulin IgG (or sometimes IgA), also called macro creatine kinase type I. This combination has a molecular mass of greater than 200,000 Da and can be detected by electrophoretic or chromatographic techniques. There is evidence that macro-CK-BB results from autoantibodies that are produced against CK-BB, possibly associated with autoimmune disease (see Table 1).

CONCLUSION

Our patient illustrated the potential pitfalls of diagnosing myocardial infarction using a CK-MB immunoinhibition assay in the presence of non-CK-M activity. The patient's metastatic disease probably accounted for the observed CK-BB and mitochondrial-CK. We suggest that non-CK-M activity should be considered in cases of a spuriously elevated plasma apparent CK-MB activity, using an CK-M subunit immunoinhibition assay. Such non-M activity could be due to CK-BB, CK-mitochondria or CK-BB bound to IgG, and plasma electrophoresis can help determine the CK isoenzyme pattern. There is clearly a need for alternative methods for measuring serum CK-MB activity and, as the immunoinhibition methods can give falsely elevated results, immunoprecipitation or mass methods may help reduce diagnostic confusion. Laboratories using the immunoinhibition assay should be aware of this problem and of the danger of incorrect diagnosis of acute myocardial infarction in patients showing serum non-CK-M activity. In some cases, the apparent CK-MB activity measured in the serum of patients without myocardial damage may be residual adenylate kinase activity (liver) which has not been inactivated by inhibitors incorporated in the assay.

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