Influence of Autoantibodies to Creatine Kinase-BB on Assays for MB Isoenzyme

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We describe the influence of autoantibodies that bind creatine kinase BB (CK-BB) on the methods for MB isoenzyme. If these autoantibodies are present in patients' sera, they cause the formation of macro CK type 1 (immunoglobulin-linked CK-BB). In some of these cases they can bind not only endogenous CK-BB but also CK-MB without significantly affecting enzyme activity. Although these antibodies show distinctly less affinity for CK-MB than for CK-BB, they nevertheless bind CK-MB in these particular sera, because their concentration exceeds that of CK-BB isoenzyme. If a person with such autoantibodies has an acute myocardial infarction, the immunoinhibition method for CK-MB, which does not discriminate between CK-MB and CK-BB, will recognize the increase and peak of CK-MB with time, although persistent macro CK activity will be superimposed on the typical isoenzyme pattern. However, isoenzyme electrophoresis and recently introduced immunoenzymometric assays for CK-MB in these cases may be less sensitive for detecting myocardial infarctions, because the typical increase in CK-MB activity may be identified later in the progression of symptoms, or even be missed.

Additional Keyphrases: myocardial infarction · enzyme immunosassay · enzyme activity (specific) · immunoinhibition · isoenzymes · electrophoresis, agarose gel

In patients with suspected acute myocardial infarction (AMI),1 diagnosis has to be timely and exact: both false-positive and false-negative results can be very dangerous. Because of their high specificity and sensitivity, activities of total CK and the CK-MB isoenzyme are regarded as the best clinical chemical parameters to confirm or to rule out AMI (1). However, CK-MB cannot be an absolutely specific indicator for AMI, for it also is present in skeletal muscle (1–4). In principle, diagnostic specificity and sensitivity can be improved by applying an appropriate stepwise diagnostic strategy, as was shown for the immunoinhibition method for CK-MB (5). Additionally, much effort has recently been directed toward improving the technical specificity and sensitivity of the assays for CK-MB. Double-antibody techniques are now available, either as a combination of immunoinhibition by anti-CK-M antibodies and subsequent immunoprecipitation by a second antibody directed against the anti-M antibody (6, 7) or as solid-phase enzyme immunosassays (8–10) with subunit-specific anti-M and anti-B antibodies. In these latter assays CK-MB is detected by mass. The results of these mass determinations of CK-MB will be equivalent to results of a specific measurement of CK-MB activity if the antibodies react only with the intact, catalytically fully active enzyme. Additional or different clinical information may be obtained if the antibodies used in these solid-phase immunosassays also detect already inactivated enzyme protein.

In the recently developed enzyme immunosassays, CK-MB reacts successively (Behring) or simultaneously (Hybritech) with anti-B and anti-M antibodies. If the antibodies are specific and present in sufficient excess of the respective competing M or B subunits, then the determination of CK-MB should not be affected by the presence of CK-MM, CK-BB, or macro CK type 2 (11). Macro CK type 1 (11–14) in patients' sera results from CK-BB-binding autoantibodies. Although these autoantibodies generally show a high affinity for the intact CK-BB molecule (15), a certain cross reaction with CK-MB (16, 17) may nevertheless occur. The effect of this cross reaction is enlarged in serum samples because these autoantibodies are not saturated with CK-BB and usually exist in excess of CK-BB.

Our aim in this study was to point out a possible error during diagnosis of AMI and to confirm a previous case report (17) of falsely negative results with isoenzyme electrophoresis. At this time we do not yet know what proportion of sera with macro CK type 1 really interfere in various methods for CK-MB during diagnosis of AMI. Here we report the influence of CK-BB-binding autoantibodies on the sensitivity and specificity of electrophoretic and immunological determinations of CK-MB.

Materials and Methods

Samples: Serum samples were either analysed on the same day they were obtained, stored at 4 °C for no longer than 24 h, or were kept at −20 °C without any additives until assay. Macro creatine kinases were detected and differentiated as described previously (18). CK isoenzymes were prepared from human tissues as described elsewhere (16). Isoenzyme composition of the samples was checked by electrophoresis on agarose gel (18) and by isoelectric focusing in agarose gel (pH range: 5–8) according to the manufacturer's instructions (LKB Produkter, Bromma, Sweden). CK activity was located by overlaying the plates with filter paper soaked with reagent for total CK (see below), and viewed after 1 h at 37 °C.

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1 Nonstandard abbreviations: CK, creatine kinase (EC 2.7.3.2, ATP:Creatine N-phosphotransferase); CK-MM, CK-MB, and CK-BB: skeletal-muscle, heart, and brain types of cytoplasmic CK isoenzymes; AMI, acute myocardial infarction; and INH, immunoinhibition of CK-M subunits.

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Enzyme assays: We measured total CK activity with the "NAC reactivated" kit (nos. 14109-14111; E. Merck, Darmstadt, F.R.G.) (19) at 25 °C, using a photometer (Model M 1101; Eppendorf Gerätebau, Hamburg, F.R.G.) with a recorder. Residual activity after INH was also determined with "NAC reactivated" reagents (nos. 14109, 14110, 14112; E. Merck) (20) at 25 °C. Turnaround time for this determination of CK-MB was about 15 min.

We also performed enzyme immunoassays for CK-MB ("Enzygnost CK-MB", lot no. 966300, lot no. 460438; Behring Diagnostics, La Jolla, CA; "Tandem-E CK-MB", no. 14003, lot no. 4041262; and no. 149933, lot no. 4925602; Hybritech Inc., San Diego, CA) according to the manufacturers' instructions. One CK-MB determination by these methods took 2 to 3.5 h.

Thermal inactivation of CK-MB: Purified CK-MB from human heart was appropriately diluted with a normal serum (total CK activity 32 U/L; no detectable CK-MB), then stored at 4 °C overnight (pH at 37 °C: 7.6-7.7). We then warmed 500-μL aliquots at 37 °C, in the dark. Hourly, one of these aliquots was cooled in an ice-bath and then assayed for CK-MB by INH and both enzyme immunoassays.

Effect of macro CK sera on CK-MB assays: Electrophoresis: the IgG fraction of a serum containing macro CK-BB (patient Rud. M.) was chromatographically separated as described earlier (16). This enzyme-free IgG preparation then was incubated with purified human CK-MB for 30 min to form macro CK-MB. Subsequently, electrophoresis was performed (18). In a separate run, untreated serum from Rud. M. was supplemented with CK-MB to give a final CK-MB activity of 15 U/L, and assayed.

Immunoassays: To constant activities of CK-MB, appropriately diluted with bovine serum albumin (Enzyme Diluent; Du Pont, Wilmington, DE), we added increasing amounts of macro CK sera or, as control, normal serum. The endogenous concentration of CK-MB in both kinds of serum was less than 5 μg/L. After incubating these mixtures for 2 h at room temperature, we measured CK-MB by the enzyme immunoassays.

Results and Discussion

Effects on isoenzyme electrophoresis: Figure 1 shows why an AMI may be overlooked or detected later than usual in some cases of macro CK type 1. Serum of patient Rud. M. contains antibodies to CK-BB antibodies (IgG, subclass 1, kappa light chains) (16), which can bind not only CK-BB but also CK-MB without significantly influencing the catalytic activities of the isoenzymes (Figure 1, lane 2). In this serum, the antibodies are not all bound to endogenous CK-BB, so that some free binding sites are still available for complexation with additional CK-BB or—for, in the case of an AMI—with CK-MB. Binding CK-MB to IgG shifts the activity of the typical CK-MB band (lane 1) to the area of the macro CK type 1 bands (lane 2). Consequently, in such sera the typical CK-MB band in AMI will appear later, be weaker (compare CK-MB bands of lanes 3 and 5), or, as already shown in a previous case report (17), even will be missed. The extent of this binding and its influence on the diagnostic sensitivity of the electrophoretic methods depends on both the amount of CK-MB released during AMI and the affinity, specificity, and concentration of the individual autoantibodies.

Effects on enzyme immunoassays: The linear relationship between enzyme mass determination (x) and enzyme activity measurements (y) during thermal inactivation of CK-MB at 37 °C indicates that both enzyme immunoassays primarily react with catalytically active CK-MB and not with inactive enzyme protein: y(INH) = 0.19 + 0.288x(Behring)
CK-BB and CK-MB of these autoantibodies affected the results of the INH method unexpectedly. Typical curves for total and residual CK activities (after INH) for a patient with macro CK type 1 and for a patient with both AMI and macro CK type 1 are shown in Figure 3. In the first serum sample of the latter case, which by chance was taken the day before onset of acute symptoms, macro CK-BB activity is about 50 U/L as determined by INH and the ratio of CK activities as determined by the immunoinhibition method and the method for total CK exceeds the limit of 0.25, as is characteristic for macro CK (11). In the interval 10 to 48 h after onset of symptoms the typical increase and peak of total CK and CK-B activities are clearly seen, while the ratio shows an unexpected, but inverse course. Afterwards total CK activity returns to normal, whereas CK-B activity and the ratio again reach their former persistently high values. Even in these cases the enzyme activity patterns of total CK and CK-B activities allow a diagnosis of AMI. The "classical" and these "atypical" patterns differ mainly in their levels of CK-B activity. The dynamic changes in enzyme activity that are typical for an AMI, however, remain unaffected.

Although there is no urgent need to do so during diagnosis of an AMI, one can avoid measuring these elevated values caused by macro CK: technical specificity is improved if the immunoinhibition is followed by a confirmatory immunoprecipitation with second antibodies directed against the first anti-CK-M antibodies. Then "true" CK-MB is assayed (6, 7).

Patients with suspected myocardial infarction should be exactly diagnosed as early as possible after onset of symptoms. Especially early exclusion of an AMI has to be based, however, on results obtained for samples taken 10 to 16 after onset of symptoms. This delay results from the typical enzyme patterns after AMI and is inevitable, but it should not be increased further by waiting hours for the results of time-consuming and labor-intensive assays. We therefore need precise, easily performed, and cost-efficient CK-MB tests with high specificity and sensitivity, which can be performed 24 h a day, even during weekends and holidays. For reasons published previously (9, 26) and mentioned above, even the recently introduced enzyme immunoasays—like other methodologies—cannot totally fulfill all these requirements.

Three types of interferences by macro CK-BB in patients' sera are known to affect CK-MB determinations:

1. Direct interference of the whole Ig-CK-BB complex in methods not absolutely discriminating CK-BB from macro CK-BB: comigration or coelution of bands during electrophoresis (18) or chromatography, (13, 26), respectively;
2. interference by the CK-BB moiety of the complex in some immunological methods; and
3. interferences by the CK-BB-binding autoantibodies, which may "mask" CK-MB in electrophoretic assays, radioimmunoassays, and enzyme immunoasays.

Accordingly, we conclude that, regardless of the methods used for CK-MB, early exclusion and confirmation of AMI still requires a strictly defined diagnostic strategy (5, 7, 27–30), based on:

- determination of CK-MB only in samples from patients suspected of AMI with values for total CK activity in the upper normal range
- sequential sampling during the diagnostic "time window" within the first 20 h after onset of symptoms
- observation of the typical enzyme pattern over time, because performing only one CK/CK-MB determination will give insufficient diagnostic information.

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References


