Cretrans Kinase MB Isoenzyme in Serum of Uremic Patients: Electrophoresis and Quantification with the Corning Models 720 and 760 Fluorometers/Densitometers

To the Editor:

In previous studies with electrophoresis (1, 2), investigators have found increased creatine kinase (EC 2.7.3.2; CK) MB isoenzyme activity in serum of uremic patients who are undergoing maintenance dialysis and who had no evidence of acute myocardial infarction at the time their serum was studied. These authors imply that increased proportions of CK-MB isoenzyme may not indicate cardiac disease, thereby decreasing the reliability of this laboratory test in this group of patients.

At our hospital, most uremic patients maintained by dialysis have diabetic nephropathy; many have co-existent coronary atherosclerosis, and not uncommonly they complain of atypical chest pain. Moreover, they frequently have markedly abnormal, baseline electrocardiographic patterns, which makes new ischemic changes difficult to detect. In such patients, CK-MB results assume increased importance, and the decreased reliability of electrophoresis results suggested by previous studies (1, 2) would, if true, cause problems.

In an effort to clarify this issue, we quantified CK-MB isoenzyme activity by electrophoresis of serum from uremic patients without evidence of acute myocardial infarction. Our goals were to (a) determine the prevalence of CK-MB in serum of patients with renal failure and (b) compare the amount of CK-MB as quantified by two fluorometer/densitometers—the Model 720 (Corning Medical, Medfield, MA), which we have used in this laboratory for the past five years, and the Corning Model 760, which we have recently acquired.

We studied 81 uremic patients on maintenance dialysis treatment, 35 of them being treated by hemodialysis, and 46 by peritoneal dialysis. The median duration of dialysis was 17.5 months (range, two to 228 months). The mean age of these patients (51 men and 30 women) was 53.1 years (range, 24–94 years). The most common cause of renal insufficiency in 58 patients (76%) was diabetes mellitus. The median value for serum urea nitrogen was 78.5 μg/L (range, 36–202 μg/L); for serum creatinine, 10.6 μg/L (range 3.1–22.2 μg/L). No patient had clinical or electrocardiographic evidence of acute myocardial infarction at the time we sampled their serum.

For controls we studied 20 outpatients (nine men, 11 women) without renal failure or known cardiac diseases. The mean age of the control patients was 50.3 years (range, 29–79 years). All had normal concentrations of urea nitrogen (reference interval, 5–24 μg/L) and serum creatinine (reference interval, up to 1.1 μg/L) at the time of study.

We determined total serum CK activity and CK-MB activity by the modified Rosalki method (3). To determine total CK, we used a Multistat III microcentrifugal analyzer (Instrumentation Laboratory, Lexington, MA). As we described elsewhere (4), we quantified CK-MB isoenzyme activity by electrophoresis on agarose gel, using a system and reagents supplied by Corning, and using both models of fluorometer/densitometers for the same serum samples. The Model 720 instrument is completely automatic. We used the Model 760 instrument in its manual mode, adjusting the baseline according to the background fluorescence of each individual patient’s serum. To avoid quantifying non-CK fluorescent artifacts, discovered by viewing the gels before incubation with the CK-isoenzyme substrate set (Corning), we used the “suppressor” option of the Model 760 instrument.

Although the median total CK activity of each group was virtually identical, the prevalence of CK-MB isoenzyme activity in serum was increased in the uremic patients as compared with the control group (Table 1). Moreover, the quantity of CK-MB measured in serum depended upon which fluorometer/densitometer we used. If <5 U of CK-MB per liter of serum is considered to be within normal limits (5), then, according to results with the Model 720 instrument, half of the uremic patients had abnormally increased CK-MB activity in serum, triple the number indicated by the Model 760. There was no significant difference between the fluorometer/densitometers in values for the control group.

The difference in the amount of CK-MB activity quantified can be explained by the modes of operation of each instrument. The Model 720 instrument has both an automatic gain and an automatic zero: the maximum signal is amplified to produce full excursion of the pen and the minimum signal is considered zero. In serum with an intense signal (i.e., high total CK), all other fluorescence (including background fluorescence) is relatively minimized; when the signal is weak, both the signal and the background fluorescence are amplified. Because the sera from most of the uremic patients in this study had a low total CK (median, 64 U/L; reference interval; up to 155 U/L), the amplification signal led to a baseline. The Model 720 instrument, by including the baseline elevation in its automatic integration of the CK-MB peak, falsely quantified an increase in CK-MB. The great advantage of the Model 760 instrument is that the baseline can be adjusted manually: the tracing and its automatic integration can be modified by the user according to the background fluorescence of an individual patient’s serum, which is prominent in uremic patients. Results for CK-MB obtained with the Model 760 instrument can be approximated from the Model 720 results by manually drawing the correct baseline on the tracing, cutting out (with scissors) the CK isoenzyme peaks, and weighing the corresponding pieces of paper.

Because the difference in isoenzyme as quantified by the two instruments is medically important, knowledge of which fluorometer/densitometer is being used by the laboratory is essential to correct clinical interpretation of CK-MB isoenzyme results determined by electrophoresis. We do not mean to imply that the diagnosis of acute myo-

<table>
<thead>
<tr>
<th>Uremic patients</th>
<th>Median CK-MB U/L No. (and %) of patients with CK-MB &gt; 5 U/L</th>
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</thead>
<tbody>
<tr>
<td>720</td>
<td>5.0 (0–21.3) 41 (50.6)</td>
</tr>
<tr>
<td>760</td>
<td>1.8 (0–18.2) 13 (16.1)</td>
</tr>
</tbody>
</table>

Control patients
| 720 | 0 (0–3.0) 0 |
| 760 | 0 (0–1.6) 0 |
cardial infarction would be based on a single CK-MB result—aerial determinations are required for this diagnosis—but we suspect that falsely high CK-MB results obtained with the Model 720 instrument (and all other fluorometer/densitometers that are similarly designed) have led previous investigators (1, 2) to suggest that CK-MB isoenzyme activity can be increased in the serum of patients with renal failure as a result of renal failure per se, and to a degree that can mimic acute myocardial infarction. In this study, however, we have found that the electrophoresis results quantified by the Model 760 instrument agree with recent studies with ion-exchange column chromatography (6) and radioimmunoassay (7): most uremic patients without evidence of acute myocardial infarction have only slightly increased amounts of CK-MB in their serum, unlikely to be confused with the greater quantity of CK-MB in patients with acute myocardial infarction. Therefore, CK-MB as determined by electrophoresis in the serum of patients with renal failure is reliable.

During the course of this study, we also made two minor observations of interest. First, the albumin artifact found in 79 of 81 uremic patients was also present in 10 of 20 control patients. This suggests that the albumin artifact is not peculiar to renal failure or dialytic treatment. Second, like Jaffe et al. (7), who described a diffuse, non-CK-mediated fluorescent artifact in the CK-MB region of uremic patients' sera electrophoresed on cellulose acetate gels, we found a similar artifact, midway between the CK-MM and CK-MB regions on agarose gels, in 51 of 81 (62.9%) uremic patients but not in the control patients. This artifact, although easily seen before incubation of the gels with the CK-isoenzymes substrate, is difficult to detect after the incubation. However, its effect on the quantification of CK-MB is probably minimal except perhaps at very low total CK activity.

References


More on AIDS

To the Editor:

We would like to comment on the paper of Heriot et al. (1) describing the presence of monoclonal components (MC) in the serum of patients with acquired immunodeficiency syndrome (AIDS) or lymphadenopathy syndrome (LAS). They found MC in 14 of 24 patients with AIDS or LAS, all identified as IgG kappa.

Our experience includes four cases of AIDS and five cases of LAS, and only one showed MC. The patient in question is a four-year-old girl who was treated for a thoracic neuroblastoma at the Institute Gustav Roussy, Villejuif, France, in 1983, with heavy chemotherapy followed by two autologous bone-marrow grafts. After the grafts, she developed anemia, leukopenia, and thrombocytopenia, thus requiring many blood transfusions. For the last 14 months she has been suffering from many infectious diseases such as Pneumocystis carinii interstitial pneumonia, cytomegalovirus, virus B hepatitis, and gastrointestinal candidiasis. Laboratory investigations showed leukopenia (< 200/mm³) and a very low T₄/T₃ lymphocyte ratio. The anti-HTLV III test confirmed the suspicion of AIDS. The electrophoretic pattern showed two faint bands in the gamma region, both identified with immunofixation as IgG lambda immunoglobulin (Figure 1).

The observation of weak MC in hospitalized patients is very common. Such a pattern appears to be related more to the immunoglobulins concentration and the presence of an acute process than to the medical diagnosis (2). Moreover, we observed a lower frequency of MC in AIDS or LAS patients than did Heriot et al., and a different type of light chain. Therefore we believe that more observations are necessary to establish whether this electrophoretic pattern is related to AIDS/LAS syndrome or is only an immune response of restricted heterogeneity. In fact, as these authors themselves pointed out, it is not clear yet if the B-cell abnormalities in AIDS or LAS are primary or secondary to T-cell abnormalities and antigen challenge.

References


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To the Editor:

Heriot et al. recently reported the presence of paraproteins in the serum of patients with acquired immunodeficiency syndrome (AIDS) (1). Earlier, in another journal, we reported the presence of oligoclonal immunoglobulins in patients with AIDS (2). The overall incidence of paraproteins was similar in both studies. When we divided the AIDS patients into two subgroups—opportunistic infections (OI) and Kapo-"si’s sarcoma (KS)—we found oligoclonal banding in the overwhelming majority of KS without OI. Paraproteins and lymphocytic malignancies have been described in the past in patients with classical KS (3). Electrophoretic detection of paraproteins and oligoclonal immunoglobulins in the serum of AIDS patients aids in the differential diagnosis. The same technique is