MM Subisoenzymes of Creatine Kinase as an Index of Disease Activity in Polymyositis

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Creatine kinase (CK; EC 2.7.3.2), although the most commonly measured enzyme for assessing disease activity in polymyositis, is not always a reliable indicator of the extent and severity of myositis. Recently, the CK-MM isoenzyme has been found to undergo post-synthetic modifications upon release into the serum, such that electrophoretically identifiable sub-bands or subisoenzymes—MM₁, MM₂, and MM₃—are produced. To determine the diagnostic and discriminative value of these subisoenzymes in polymyositis, we analyzed CK and its MM subisoenzyme forms in serum samples from 22 patients with myositis and from 23 controls. In the presence of inflammatory myositis and increased total CK activity, MM patterns correlated with the clinical trend, often more accurately than did measurements of total CK. MM₁ proportions >30% of total CK-MM or ratios of MM₂ to MM₁ <1 were associated with an improving or stable condition, whereas MM₁ activity <30% or MM₂/MM₁ >1 reflected a deteriorating course of disease. Patients whose disease was assessed to be clinically deteriorating were clearly distinguished from patients with improving disease by their subisoenzyme patterns (p <0.01). Thus these patterns add significantly to the information obtainable by routine blood analysis.

Additional Keyphrases: electrophoresis, agarose gel · muscle disease

Polymyositis (PM) is a treatable myopathic disorder characterized by proximal muscle weakness, an abnormal electromyogram, and a muscle biopsy that demonstrates mononuclear cell infiltrates, degenerating and regenerating myocytes, and eventual muscle cell necrosis. Steroids and cytotoxic drugs are used to treat this disorder, but because of the morbidity associated with these agents investigators have sought serologic markers of the extent and severity of disease activity, which could be used to tailor treatment programs and thus reduce overall complications during therapy.

Previous indices of disease activity have included the Westergren sedimentation rate, and activities of aldolase, aspartate aminotransferase, and lactate dehydrogenase and its isoenzymes, but these often have proven insensitive, nonspecific, or occasionally misleading (1). Investigators exploring the incidence of antibodies unique to patients with PM have noted the antinuclear antibodies PM 1, Jo 1, and Mi (2–4) and have demonstrated antibodies to native muscle proteins such as myoglobin (5). Although the presence of these antibodies may be diagnostically useful, they provide little information regarding severity of the disease.

Measurement of creatine kinase (CK; EC 2.7.3.2) is now the most common enzyme assay used for assessing disease activity in PM (1), release of CK having been shown to result from damage to muscle tissue in various disorders (6). Although the activity of this enzyme in serum correlates generally with disease activity in many clinical conditions (e.g., myocardial infarction), it does not always accurately reflect disease activity in PM. Occasionally, serum activities of CK are normal or only minimally increased in patients with clinically active, biopsy-proven myositis, while serum CK may be high in patients whose disease is clinically assessed to be quiescent (1).

Recently, several variants of CK have been identified, e.g., macro-CK and post-synthetic variants of CK isoenzymes (7). In post-synthetic variants the M subunit of CK has been modified by a heat-labile, 190 000-Da factor in serum, the "CK conversion factor" (8–12). All classes of the CK-MM subisoenzymes—MM₁, MM₂, and MM₃—are normally present in human serum (11). Immediately after injury to muscle tissue the percentage of MM₁ increases, then MM₂ is sequentially converted to MM₂ and MM₃. The relative proportions of these subisoenzymes in serum may be under the control of a regulatory mechanism, as shown by the distinct differences in CK-MM patterns of controls and muscular dystrophy patients (7). This suggests that the patterns of CK-MM subisoenzymes may differ in various diseases. To extend the sensitivity and specificity of CK as a marker of tissue damage in PM, we have examined the post-synthetic variants of CK-MM in patients with PM and in appropriate controls. The results suggest that the post-synthetic variants of CK-MM (subisoenzymes) vary predictably with the clinical course of patients with PM, thus adding significantly to the information obtained by routine serologic study of these patients.

Materials and Methods

Patient Selection

Patients included in this study were selected without conscious bias from a retrospective chart review of all patients of the University of Michigan Arthritis Clinic during the past five years. All fulfilled the criteria of Bohan and Peter for PM (13), which include symmetrical weakness of limb-girdle muscles, biopsy evidence of necrosis of Type I and II fibers, increases of muscle enzymes in serum, an abnormal electromyogram, and characteristic dermatologic features. Included in this study were 15 patients with PM (group I and II, classification of Bohan and Peter), six patients with overlap syndromes (scleroderma-PM, five; rheumatoid arthritis-PM, one), and one with childhood-onset PM. In all, we determined subisoenzyme composition in 32 serum samples from patients with myositis and in samples from 23 controls. The control subjects included 15 healthy volunteers and eight patients with nonrheumatic disease who were receiving steroids.

Disease Activity

Despite considerable controversy as to the best measure of disease activity, most practitioners base their assessment on...
(a) the recovery of normal muscle strength, evaluated clinically, and (b) assays of the activities of skeletal muscle enzymes, most commonly CK, a sensitive index of muscle tissue damage. However, several investigators have suggested that muscle strength alone may occasionally more nearly accurately reflect disease activity. There are also many case reports of patients with histological and electromyographic evidence of myositis but normal CK activity in serum (7). Because no single index has been accepted as adequate for monitoring disease activity in PM, the patients we investigated were classified as having either (a) inactive or stable PM, (b) deteriorating PM, or (c) improving PM on the basis of formal testing of muscle strength by the patients' individual primary physicians over at least three clinic visits. Formal muscle strength testing involves an evaluation of the mobility and strength of the flexor–extensor muscles. Because of the necessarily subjective and nonuniform nature of these determinations, such categorization represents the general impression of the individual primary physician. We also checked charts for endocrinological (thyroid, steroid, and diabetic myopathy), metabolic (hypokalemia, hypophosphatemia, and hypercalcemia), or neurological disorders that might affect motor strength, but none were found.

Serologic Studies

We measured total CK activity in serum, using an aco III discrete analyzer (DuPont Instruments, Wilmington, DE). CK isoenzyme activity was quantified by agarose gel electrophoresis with either the ACI (Corning Medical, Corning, NY) or the Paragon System (Beckman Instruments, Palo Alto, CA) and scanning densitometry, for which we used an ACD 18 densitometer (Gelman Sciences, Ann Arbor, MI) in the fluorescent mode. CK subisoenzymes were analyzed by a modification of the technique of Wevers et al. (8). We applied serum to sample wells of ACI agarose gels, diluting samples with CK activity >800 U/L with phosphate buffer (50 mmol/L, pH 7.4) in isotonie saline to a total activity of between 500 and 800 U/L. After electrophoresis of the gels for 100 min, we applied Corning substrate and incubated the gel plates at 37°C for 20 min. For sera with <100 U of total CK activity per liter we used the Paragon N-acetyl-1-cysteine-activated substrate system. Gels were dried for 30 min before which subisoenzyme activity was quantified as above with the scanning densitometer. We performed several determinations of serum subisoenzymes in duplicate to assure the reproducibility of these patterns; replicate determinations yielded similar data.

Freezing and thawing of serum samples stored for as long as six months had little or no effect on the subisoenzyme patterns, as Wevers et al. observed (8). Incubation of serum samples at room temperature for 12 h or longer altered subisoenzyme patterns quantitatively, although qualitatively the patterns were similar.

To avoid misinterpreting the results for CK in the presence of other forms of CK or its congeners, we screened 18 serum samples for macro-CK and for adenylate kinase. We found no interfering activity, in agreement with previous studies (7, 8, 11) in which MM subisoenzymes have been uniformly demonstrated to represent true CK activity.

Results

Using the nomenclature previously described by Chapelle and Heusghem (11), we designated the CK-MM subisoenzymes as MM₁, MM₂, and MM₃, based upon the modification state of the M subunits. Electrophoretically, MM₁ is the most cathodal and MM₃ the most anodal (Figure 1). All control subjects had normal CK activity and subisoenzyme patterns in which the percentage of MM₃ was greater than that of MM₁. Although mean activities of total CK were lower in the steroid-treated group, the subisoenzyme patterns were no different, implying that steroids do not affect subisoenzyme ratios (Table 1, Figure 2). The CK subisoenzyme patterns of our control subjects were nearly identical to those reported by Falter et al. (12).

As in previous studies of PM, total CK activity did not correlate well with clinically assessed disease activity. CK activity of several patients with PM was within the normal range: three patients in the inactive group and two in the deteriorating group (patients 8 and 9, Table 1). There was no consistent relationship between disease activity and quantitative CK activity among the groups, although most of the patients with either deteriorating or improving disease had greater activities of CK than did those with inactive or stable disease (Table 1).

Subisoenzyme patterns differed significantly among groups of PM patients. Among patients with stable disease, those with increased CK activity (patients 4–6) had more MM₁ than MM₃ activity. In those with normal CK activity, MM₁ was either approximately equal to (patient 3) or less than MM₃ (patients 1 and 2). Patients in the improving group, all of whom had increased CK activity, had patterns similar to those of patients in the stable group with increased CK activity, with %MM₁ > %MM₃. In contrast, but one patient in the deteriorating group had %MM₁ < %MM₃ (Figure 2). Statistical analysis by use of the unpaired t-test (14) revealed significant differences between patients in the deteriorating and improving groups when either the percent MM₁ (p < 0.01) or the ratio of MM₃ to MM₁ (p < 0.05) was evaluated. Differences between patients in the improving and control groups were also significant (p < 0.01, for percent MM₁ or MM₃/MM₁). Although most of our studies involved patients with idiopathic PM, we found similar relationships in patients with childhood-onset PM and "overlap" syndromes (patients 16–22).

Those patients classified here as having improving or stable disease but with persistently increased CK activity (patients 4–6 and 13–15, Table 1) often present clinicians with challenging management decisions. We studied these separately and compared them as a group with patients whose muscle strength was deteriorating. The %MM₁ activity clearly distinguished these patients at the p < 0.01 level, thus providing an index which could be used to facilitate management decisions. MM₁ > 30% of total CK-MM was associated with improving or stable patients; MM₁ < 30% was seen in patients with deteriorating disease activity.

In several patients (patients 6, 17, and 18) the subisoenzyme patterns changed during the course of their disease. In patient 6, this occurred in parallel with clinical improvement, despite almost no change in total CK activity (987 vs
Table 1. CK-MM Subisoenzymes in Serum in Polymyositis

<table>
<thead>
<tr>
<th>Patient, and disease statusa</th>
<th>Total CK, U/Lb</th>
<th>% of total CK-MM</th>
<th>Disease duration</th>
<th>Treatmentc</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,S</td>
<td>106</td>
<td>18</td>
<td>49</td>
<td>2.72</td>
</tr>
<tr>
<td>2, S</td>
<td>92</td>
<td>16</td>
<td>39</td>
<td>2.75</td>
</tr>
<tr>
<td>3, S</td>
<td>128</td>
<td>36</td>
<td>29</td>
<td>0.97</td>
</tr>
<tr>
<td>4, S</td>
<td>1480</td>
<td>46</td>
<td>28</td>
<td>0.56</td>
</tr>
<tr>
<td>5, S</td>
<td>497</td>
<td>41</td>
<td>28</td>
<td>0.75</td>
</tr>
<tr>
<td>6, S</td>
<td>943</td>
<td>37</td>
<td>32</td>
<td>0.86</td>
</tr>
<tr>
<td>7, D</td>
<td>987</td>
<td>28</td>
<td>34</td>
<td>1.32</td>
</tr>
<tr>
<td>8, D</td>
<td>401</td>
<td>15</td>
<td>35</td>
<td>3.27</td>
</tr>
<tr>
<td>9, D</td>
<td>80</td>
<td>21</td>
<td>26</td>
<td>2.52</td>
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<tr>
<td>10, D</td>
<td>63</td>
<td>14</td>
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<td>3.71</td>
</tr>
<tr>
<td>11, D</td>
<td>217</td>
<td>11</td>
<td>19</td>
<td>7.16</td>
</tr>
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<td>“Overlap” syndromes and childhood onset PMd</td>
<td>16, S</td>
<td>837</td>
<td>53</td>
<td>15</td>
</tr>
<tr>
<td>17, D</td>
<td>2660</td>
<td>21</td>
<td>38</td>
<td>41</td>
</tr>
<tr>
<td>18, D</td>
<td>9198</td>
<td>23</td>
<td>37</td>
<td>41</td>
</tr>
<tr>
<td>19, D</td>
<td>5240</td>
<td>23</td>
<td>33</td>
<td>44</td>
</tr>
<tr>
<td>20, D</td>
<td>240</td>
<td>21</td>
<td>33</td>
<td>46</td>
</tr>
<tr>
<td>21, D</td>
<td>6000</td>
<td>19</td>
<td>40</td>
<td>41</td>
</tr>
<tr>
<td>22, D</td>
<td>4580</td>
<td>19</td>
<td>32</td>
<td>49</td>
</tr>
<tr>
<td>Controls (mean ± SD)</td>
<td>80.3</td>
<td>±33.7</td>
<td>±6.3</td>
<td>±6.1</td>
</tr>
<tr>
<td></td>
<td>47</td>
<td>±20.6</td>
<td>±2.2</td>
<td>±3.5</td>
</tr>
</tbody>
</table>

*Normal <225 U/L.

*a Pred, prednisone; Aza, azathioprine; Ctx, cyclophosphamide.

*b Patients 16-20, scleroderma-myositis; 21, rheumatoid arthritis-myositis; 22, clinical-onset polymyositis.

c Results shown for two groups: 15 healthy subjects (top) and eight nonmyositis patients receiving steroids.

<table>
<thead>
<tr>
<th>% MM1</th>
<th>Stable Det. Imp. Control</th>
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Fig. 2. Patterns of MM1 subisoenzyme in PM patients whose clinical conditions are stable, deteriorating, or improving

Open circles and bars indicate mean ± 1 SD for each group. Statistical significance of differences is given in the text.

943 U/L). Patient 17 illustrates the correlation between CK-MM subisoenzymes and disease activity over time (Table 2). That the subisoenzymes provided a more nearly accurate reflection of disease activity clearly demonstrates that even modest improvements in muscle strength over brief periods of time are reflected in the patterns of CK-MM subisoenzymes. The changes often preceded changes in total CK activity (e.g., 9198 vs 9300 U/L, Table 2).

Discussion

Bohan and Peter (13) demonstrated that although comparison of quantitative CK activity among groups of patients with PM is not helpful, trends in serum activities, but not single values, often reflect disease activity in individuals and are useful in the management of these patients. CK has therefore been used as an objective adjunct in assessing disease activity.

This study demonstrates that variations in the CK-MM subisoenzymes reflect the trend of PM at a single point in time. In almost all the patients thus far examined (Table 1, Figure 2) clear distinctions between patients with improving and deteriorating disease were apparent. Furthermore, patients whose disease was stable but who had increased CK activity were also distinguishable from those in the
deteriorating group. The proportion of MM₂ was qualitatively less important than that of MM₁ and MM₃, which varied according to the clinical status of the myositis. Although we have no immediate explanation for this, the relative constancy of the MM₂ contribution is not unique to our study; the contribution of MM₂ reported by Falter et al. (12) is similar to that in our polymyositis patients, and Yasmin et al. (7) demonstrated essentially no change in the MM₂ percentage of normals vs. muscular dystrophy patients, although the MM₁/MM₃ ratios differed in these two groups.

For correctly determining the subisoenzyme patterns, proper collection and processing of sera is important. To avoid the gradual conversion of MM₁ to MM₃ in serum stored for long periods of time, the storage should be at −20 °C, at which temperature the "CK conversion factor" is inactive. This ensures reproducible subisoenzyme measurements. Because the diagnostic use of electromyography in PM can damage muscle tissue, one should also obtain samples before any invasive procedures are performed.

The number of subisoenzymes detected depends on the separation media utilized, ranging from three on agarose to as many as 21 by isoelectric focusing (18). We chose a commercially available agarose system in routine use for the separation of CK isoenzymes in the clinical laboratory. Although the bands are not as highly resolved as with isoelectric focusing, the value of distinguishing three bands is apparent from the data presented here. Results obtained from all three electrophoretic techniques may demonstrate a superior classification of disease states.

The biochemical processes involved in the maintenance and regulation of CK-MM subisoenzymes in serum have not been completely elucidated. Wevers et al., using muscle tissue homogenates in vitro, convincingly demonstrated that CK-MM is sequentially converted from MM₁ to MM₂ to MM₃ by the action of a conversion factor in serum (8–10). Recently the calcium-dependent (16) conversion factor has been identified as a carboxypeptidase (17). Increases in MM₁ are found in vivo immediately after muscle trauma, surgery, or myocardial infarction (7, 10–12). In chronic muscular disorders such as Duchenne's muscular dystrophy or PM, an equilibrium is established among the MM subisoenzymes, the proportion of each subisoenzyme present being governed by the quantity of CK converting factor present. The increases in MM₂ in chronic muscular disorders may be caused by the leakage from tissue of CK converting factor (7), increased synthesis of converting factor as an acute-phase reactant, or some mode yet to be elucidated (15, 18). Perhaps direct measurement of converting factor activity (19, 20) in PM might more sensitively reflect disease activity than either CK or its subisoenzymes. We are currently addressing this question in studies in our laboratory. It is possible that CK-MM may also be converted before release or leakage of CK from muscle. Two studies have demonstrated multiple forms of CK-MM in muscle biopsies (15, 18). Guslit and Jacobs (15) have suggested that some unidentified inhibitors may also play a role in this biochemical process. These inhibitors could also be co-regulators of CK-MM subisoenzymes and the patterns seen in various diseases.

The biological half-lives of the subisoenzymes may contribute to the patterns observed in sera. Sobel et al. (21) indicate that the modified CK-MM forms MM₂ and MM₃ have longer half-lives than does purified MM₁ from muscle. Again, the exact nature of both the release of CK from muscle and the mechanisms involved in the clearance of CK are not completely understood.

An inherent problem in introducing a new assay purported to reflect disease activity is the initial dependence of the assay's utility on the previously described measures of the severity and extent of disease. The clinical severity of muscle disease reported for the patients in this study was assessed as objectively as possible. The extent to which the conclusions drawn thus far will prove valid can only be verified in future prospective studies.

We conclude that the determination of CK-MM subisoenzymes will provide a useful new tool in managing patients with PM. By permitting a direct assessment of muscle-tissue integrity, therapy with drugs that produce significant morbidity such as steroids, cytotoxic agents, or other new therapeutic modalities should be improved. Such guidelines have become increasingly important as survival in PM has continued to improve and traditional treatment programs are being questioned.

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References


