Macromolecular Creatine Kinase Type 1: a Serum Marker Associated with Disease

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The prevalence of circulating macromolecular creatine kinase type 1 (macro CK type 1 or CK-immunoglobulin complexes) is significantly higher in a patient population selected for CK isoenzyme assay than in age- and sex-matched blood donors (n = 1304). In >8000 patients studied, 49 individuals with macro CK type 1 were identified, yielding an overall prevalence of 0.61%. Macro CK type 1 complexes occurred more frequently in women and in patients older than 70 years, and were often associated with complications of cardiovascular disease, life-threatening conditions, and poor outcome. These latter clinical associations could arise, at least partly, from the selection of patients for whom CK isoenzyme analysis was ordered.

Additional Keyphrases: macroenzymes  autoimmunity  cardiovascular disease  autoimmune antibodies

Circulating enzyme forms with higher-than-normal molecular mass—so-called macromolecules—have been described for several clinically useful enzymes (1–4). Certain macroenzyme species have been clearly associated with disease states, e.g., oligomeric mitochondrial creatine kinase (CK; EC 2.7.3.2; macro CK type 2) with deep cellular necrosis (5, 6), and alkaline phosphatase (EC 3.1.3.1)-containing membrane fragments with cholestasis (7). Binding of circulating enzyme molecules to specific immunoglobulins (Igs) constitutes yet another common mechanism for macroenzyme formation. Thus far, the latter forms have not emerged as specific disease markers, although occasionally their appearance has paralleled autoimmune disease activity (1, 2, 8–10).

The presence of enzyme–Ig complexes can lead to misinterpretation of concomitantly increased (iso)enzyme activities (1, 2, 11) but, even more importantly, raises questions about their possible pathogenetic or diagnostic significance. The widespread implementation of methods for rapid indirect estimation or electrophoretic separation of isoenzymes in clinical chemistry has facilitated a more systematic detection of enzyme–Ig complexes in recent years (1, 4) and allowed rough estimates of their prevalence, ranging from 0.1% to 13.8%, depending on the type of enzyme, patient group, or methodology (1, 2, 12).

Circulating CK–Ig complexes (macro CK type 1) have been studied most, probably because their presence readily causes unexplained or disproportionate increases in apparent CK-MB activities as measured with widely used rapid diagnostic tests, based on immunoinhibition or ion-exchange chromatography (1, 2, 11). In electrophoresis of CK isoenzymes, the presence of an atypical enzyme fraction migrating between CK-MM and CK-MB is highly suggestive of macro CK type 1, whereas (high-performance) gel-permeation chromatography (HPGCP) can confirm the macromolecular nature of this enzyme activity and distinguish it from (fluorescent) artefacts or other enzyme activities (1–3).

Several studies have attempted to define the prevalence of macro CK type 1, but only a few involved a large number of patients (1, 2, 13–15). The largest studies so far have yielded estimates of 0.9%–1.2% for overall macro CK type 1 prevalence in patients, with the highest values in females and older subjects (2, 13–16). No clear-cut association of macro CK type 1 with a particular type of disease has as yet been established, although the macroenzyme has occasionally been detected in pediatric patients and even in apparently healthy individuals (2, 14, 17). However, because comparative data in healthy control populations are largely missing, it is impossible to decide whether enzyme–Ig complexes represent disease-associated markers or instead reflect physiological or preclinical events related to, e.g., aging or variations in natural autoimmunity or immune repertoire (18–21). We therefore compared the prevalence of the CK–Ig complex, macro CK type 1, in two populations: a patient population selected on a clinical basis for CK-isoenzyme analysis and an age- and sex-matched population of apparently healthy blood donors.

Materials and Methods

Subjects

The patient population studied consisted of 2140 individuals (1204 men and 936 women, including both in- and outpatients) for whom a CK isoenzyme analysis was ordered in our laboratory during 1987 for suspected cardiac, muscular, or neurological disease and (or) increased total CK activity. The age group between 20 and 70 years comprised 1304 patients (794 men, 510 women). An age- and sex-matched control group consisted of 794 men and 510 women blood donors (ages 20–69 years). For each 10-year age class, the number of men and women was identical to that of the corresponding category in the patient population. Belgian blood donors...
are selected according to a standardized protocol, including collection of anamnestic data (absence of medication, drug abuse, recent surgery, and various chronic or acute affections), physical examination (absence of injection marks on body, apyrexia, good general condition, weight >45 kg, normal blood pressure and pulse), and blood analysis (negative serology for several bloodborne infectious diseases, absence of anemia, normal liver tests, and protein electrophoresis). In addition, we reviewed the medical files of all patients (n = 49) in whom macro CK type 1 was detected between January 1987 and April 1990 among a population of 8080 patients selected for CK isoenzyme assay as above.

Blood Samples

Blood was obtained by venipuncture of the antecubital vein, collected in commercial dry-塑料 tubes (Sarstedt, Haarsoede, Belgium, for patients, and Laborimpex, Brussels, Belgium, for blood donors), allowed to clot at room temperature, and centrifuged within 4 h after sampling at 1000 × g for 15 min. Sera were stored as previously described (3).

Detection of Macro CK Type 1

Macro CK type 1-containing samples were identified on basis of the following criteria: (a) apparently increased CK-B activity (>15 U/L) determined by immuno-inhibition assay (CK-MB, N-acetyl-L-cysteine-activated; Boehringer Mannheim, Mannheim, F.R.G.) performed at 30 °C with an RA-1000 analyzer (Technicon, Dublin, Ireland), which detects macro CK with 100% sensitivity but low specificity (3,11); (b) in CK-B positive sera, the demonstration of an abnormal CK band, typically migrating between CK-MM and CK-MB markers (I, 4), determined by Paragon® agarose electrophoresis (Beckman Instruments, Brea, CA) and quantified by densitometric scanning as previously described (3); (c) in sera with suggestive isoenzyme profiles, the presence of a peak of CK activity eluting at higher-than-normal Mₐ during HPGPC of whole serum (3). All samples with residual CK-B activity >20% of total CK activity and with macro CK type 1-like activity of at least 10 U/L, as judged by electrophoresis, proved positive for macro CK type 1 after HPGPC analysis. Failure to detect a high-Mₐ CK peak in a few samples appeared related to low macro CK type 1 activity by electrophoresis or pseudo-macro CK type 1 bands attributable to fluorescent artefacts in hyperlipemic sera (3). In samples with confirmed macro CK type 1, the macroenzyme activities estimated by HPGPC correlated well with electrophoretic data (3). No unstable complexes were noted; however, catalytically inactive complexes would go unnoticed if present (3).

Statistical Analysis

For differences in macro CK type 1 prevalence between various subject groups, we evaluated statistical significance by means of the Fisher exact test (Table 1) or the chi-square test with Yates correction (Table 2). The 95% confidence intervals for prevalence values were computed as described (22). The primary hypotheses tested in this investigation were (a) Is the prevalence of macro CK type 1 in a patient population (n = 1304; ages 20–69 years) significantly different from that in a group of blood donors, matched for age, sex, and extent (Table 1)? and (b) Do differences in macro CK type 1 prevalence between men and women, and between younger (20–69 years) and older (≥70 years) subjects occur within a (larger) patient population (n = 8080; Table 2)? According to Bonferroni adjustments, differences in prevalence were considered significant if P <0.017. Mean values of age and (macro) CK activity were compared in different subject groups by the Student's t-test for independent measurements (if n >30 for the samples compared) or by the Mann-Whitney test for smaller samples. All statistical tests were carried out two-sided at the 5% level of significance, unless indicated otherwise.

Results

Prevalence of Macro CK Type 1 in Patients and Blood Donors

Figure 1A represents the age and sex distribution of the 2140 patients studied, who were selected for routine CK-isoenzyme analysis during 1987. Among this population, 13 cases with circulating macro CK type 1 activity were identified according to the criteria defined in Materials and Methods; all patients but one were older than 50 years and nine were females (Figure 1B). Patients tested for CK isoenzyme composition were also, in general, older than 50 years (mean age ± SD, 61 ± 18 years) but 58% were men (Figure 1A). In contrast, of the total patient population attending our academic hospital during the same one-year period, only 38% were older than 50 years and only 44% were men (Figure 1C).

We next investigated whether the relatively low prevalence of macro CK type 1 in the studied patient population (13 of 2140, or 0.61%) was significantly higher than that in apparently healthy blood donors and, if so, whether age- or sex-related variations could be demonstrated. Because Belgian blood donors are recruited exclusively among 20- to 69-year-old subjects, the control group was matched with the patient group for age, sex, and numbers (Table 1). In the patient group, both total CK activity and macro CK type 1

| Table 1. Macro CK Type 1 Prevalence in Patients and Blood Donors |
|----------------------------------|-----------------|-----------------|
| **Ages, y** | **n** | **Mean (SD)** | **Prevalence of macro CK 1, %** |
| **Patients selected for CK electrophoresis** | | | |
| All | 2140 | 1204 | 936 | 61 (18) | 255 (709) | 0.61 (13) |
| ≥70 | 793 | 383 | 410 | 79 (6) | 279 (798) | 0.76 (6) |
| 20–69 | 1304 | 794 | 510 | 51 (13) | 237 (613)* | 0.54a (7) |
| **Blood donors** | | | |
| 20–69 | 1304 | 794 | 510 | 51 (12) | 86 (46) | 0 (0) |

* P <0.001 vs blood donors.

a P <0.016 vs blood donors. According to Bonferroni adjustments, differences in prevalence were significant for P <0.017.
prevalence were significantly higher than those in blood donors, for whom no case of macro CK type 1 was detected (Table 1). The macro CK type 1 prevalence was not significantly higher in patients ≥70 years than in the 20–69-year age group, nor was the macro CK type 1 prevalence significantly higher in women than in men.

Clinical and Biological Findings in 49 Patients with Macro CK Type 1

To eliminate the absence of sex- and age-linked differences in macro CK type 1 prevalence because of the limited number (n = 13) of patients with macro CK type 1, we analyzed clinical and biological data from 49 patients (ages 30–100 years) with detectable amounts of circulating macro CK type 1, identified in our laboratory between January 1987 and April 1990. For this period the overall prevalence of macro CK type 1 in patients was also 0.61% (95% confidence intervals: 0.45–0.80%) (Table 2). These subjects with macro CK type 1 were predominantly women and older than 70 years (Table 2). The men and women did not differ significantly in age and in macro CK activity, nor did older (≥70 years) and younger patients (20–69 years) differ with respect to enzyme activities (Table 2). Finally, the prevalence of macro CK type 1 in the selected patient population was significantly higher in females and in patients older than 70 years (Table 2).

Review of the patients' medical files revealed that the presence of macro CK type 1 could be associated with various pathologies, but most frequently with cardiac, vascular, and neurological complications of cardiovascular disease (51%) (Table 3). Although no clinical information was available for four patients, at least 19 of the remaining 45 subjects had very severe illnesses, as evidenced within a two-year follow-up, or biological markers of deep necrosis or cancer, such as CK-BB, mitochondrial CK, or monoclonal components (1, 2, 5, 6) (Table 3). Total (macro) CK activity was, however, not significantly higher in these patients, compared with the other macro CK type 1-positive patients. About 15% (seven of 49) of the macro CK type 1-positive patients had other clinical or biological evidence of autoimmune disease. Analysis of the referring clinical unit showed that 42% of all CK isoenzyme assays were ordered by the departments of cardiology, neurology, or intensive care, the latter unit alone accounting for 38% of the demands, primarily for suspected cardiac or neurological problems.

Discussion

First described in 1979 (23), circulating Ig–CK complexes (macro CK type 1) have been observed in pa-

Table 2. Macro CK Type 1 Prevalence According to Age and Sex

<table>
<thead>
<tr>
<th>Subjects</th>
<th>n</th>
<th>Age, years</th>
<th>Total CK actv, U/L</th>
<th>Macro CK type 1</th>
<th>Prevalence, no. (%)</th>
<th>95% confidence interval, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>49</td>
<td>71 (14)</td>
<td>145 (79)</td>
<td>56 (67)</td>
<td>49/8080 (0.61)</td>
<td>0.45–0.80</td>
</tr>
<tr>
<td>Women</td>
<td>33</td>
<td>74 (14)</td>
<td>151 (78)</td>
<td>58 (62)</td>
<td>33/5331 (0.93)</td>
<td>0.64–1.31</td>
</tr>
<tr>
<td>Men</td>
<td>16</td>
<td>67 (12)</td>
<td>133 (83)</td>
<td>52 (67)</td>
<td>16/4549 (0.35)</td>
<td>0.20–0.56</td>
</tr>
<tr>
<td>20–69</td>
<td>21</td>
<td>58 (10)</td>
<td>147 (86)</td>
<td>44 (59)</td>
<td>21/4921 (0.43)</td>
<td>0.26–0.65</td>
</tr>
<tr>
<td>&gt;70 y</td>
<td>26</td>
<td>81 (6)</td>
<td>143 (75)</td>
<td>65 (66)</td>
<td>28/2990 (0.94)</td>
<td>0.62–1.30</td>
</tr>
</tbody>
</table>

* P <0.002 vs women.

b P <0.01 vs age group 20–69 years. Based on Bonferroni adjustments, differences in prevalence are significant for P < 0.017.

Table 3. Clinical Findings in 49 Macro CK Type 1-Positive Patients

<table>
<thead>
<tr>
<th>Clinical Findings</th>
<th>No. of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complications of cardiovascular disease</td>
<td>25</td>
</tr>
<tr>
<td>Suspected (pre)cancerous lesions</td>
<td>10</td>
</tr>
<tr>
<td>Gastrointestinal disease</td>
<td>10</td>
</tr>
<tr>
<td>Trauma or disease of muscles, bones, or joints</td>
<td>8</td>
</tr>
<tr>
<td>Markers of autoimmune disease</td>
<td>7</td>
</tr>
<tr>
<td>Lung disease</td>
<td>4</td>
</tr>
<tr>
<td>No relevant clinical information available</td>
<td>4</td>
</tr>
<tr>
<td>Severe illness; poor outcome*</td>
<td>19</td>
</tr>
</tbody>
</table>

* Within two years of follow-up: death, cachexia, precancerous lesions, local or disseminated cancers, or biological markers of deep necrosis or cancer, such as CK-BB, mitochondrial CK, or monoclonal components.
patients, including children, with various diseases, as well as in apparently healthy individuals (1, 2, 13–17). By comparing the prevalence of macro CK type 1 in a patient population with that of age- and sex-matched blood donors, we document here that such Ig–CK complexes are indeed preferentially associated with disease states.

As in previous reports (1, 2), macro CK type 1 was observed most often in women, despite the predominance of men in the selected patient group. Autoimmune diseases and circulating autoantibodies are generally more frequent in women, perhaps in part because of the immuno-enhancing effects of estrogens (24). Also in agreement with earlier data (1, 2, 12, 14, 16, 25), most macro CK-positive patients were older than 50 years. The prevalence of macro CK type 1 in patients >70 years exceeded that in the 20–69-year age group. However, the occurrence of macro CK type 1 cannot be envisaged as a mere consequence of immune senescence, because no such complexes were observed in older blood donors.

Why CK or other self-molecules might occasionally become antigenic is at present unclear. Several hypotheses have been proposed, including (1, 2, 18–20, 26) (a) release during cell destruction of self-antigens in an altered physicochemical form or from sequestered sites, (b) occurrence of molecular mimicry, (c) imbalance in the idiotype–anti-idiotype network as a consequence of dysregulation of tolerance or, conversely, as a defense mechanism against autoimmunity. Some patients with macro CK type 1 showed other clinical and biological signs of autoimmunity, which may point to a more global dysregulation of the immune system as an underlying cause for the occurrence of anti-CK antibodies. However, the association of the latter with various severe disease states indirectly favors the view that macro CK type 1 arises as a consequence of cellular damage in patients, mostly women and elderly people, with an increased tendency towards immune autoreactivity (24, 25). There is at present no indication that anti-CK antibodies actually cause cell destruction.

Although no clear correlation between severity of illness and titer of macro CK type 1 was observed, the occurrence of the latter often coincided with important clinical and biological findings, including life-threatening complications of cardiovascular disease, deterioration of general condition, death within less than two years after blood sampling, (suspicion of) generalized or local cancer, and biochemical markers of deep necrosis such as CK-BB and mitochondrial CK (6, 27). The associated increase in total CK activity may be due to decreased clearance of CK–Ig complexes and (or) increased CK release from damaged tissues (1, 2). Biological and clinical findings in macro CK type 1-positive patients could, at least in part if not completely, be explained by patient selection. In this respect, the frequent association of macro CK type 1 positivity with cardiovascular disease could largely result from the recruitment of patients for CK isoenzyme analysis in cardiology, neurology, and intensive care units, whereas poor outcome could also be linked to patient selection as well as to old age.

Although the possible clinical and prognostic value of macro CK type 1, if any, remains uncertain, its systematic detection by screening tests or electrophoresis is still warranted for the correct interpretation of increased CK (isoenzyme) activities and as a putative marker of associated (autoimmune) disease activity. Further investigations on the Ig-components involved in CK binding (e.g., IgA vs IgG) may yield new clues regarding the mechanism of immune-complex formation or possibly autoimmune disease in general. The design of class-specific radio-binding assays for the quantification of CK autoantibodies, e.g., through monitoring the binding of radiolabeled CK-BB tracer to serum after dissociation of immune complexes in acid medium, by analogy to insulin autoantibody assays (26), could provide more-sensitive markers of CK autoimmunity that would remain independent of CK activity measurements and immune-complex formation. This approach could give more reliable estimates of macro CK type 1 complexes or CK autoantibodies; favor the detection of high-affinity CK antibodies, which are more likely to be clinically relevant; and disclose associations between certain high-affinity CK-antibody subclasses and specific (autoimmune?) diseases (1, 2, 8–10, 12, 26).

In conclusion, macro CK type 1 is associated with disease and is likely to occur as a marker or a consequence of cellular damage in a minority of possibly predisposed individuals, predominantly women and elderly people.

The efficient secretarial help of L. Vermeir and the dedicated technical assistance of L. De Pree, M. De Winter, P. Goubert, F. Lebleu, and E. Verkest is gratefully acknowledged. Part of the blood donor samples were kindly provided by Dr. L. Muylle (Blood Transfusion Center, Edegem, Belgium) and information on patient populations by M. Duyck (Department of Informatics, Akademisch Ziekenhuis Vrije Universiteit Brussel).

References