Macro Creatine Kinase Type 2: Results of a Prospective Study in Hospitalized Patients

Wolfgang Stein,1 Jürgen Bohner,1 Walter Renn,2 and Rainer Maulbetsch2

We determined total CK activity with the N-acetylcysteine-activated method and residual activity after immunoinhibition of the CK-M subunits in the sera of 2018 patients consecutively admitted to our university hospital for internal diseases, and of 936 outpatients, regardless of the patients' diagnoses. We could detect not more than two types of macro CK: macro CK type 2, which we observed in the sera of 85 patients (prevalence, 3.7% for hospitalized patients), and macro CK type 1. Most patients showing macro CK type 2 were older than 50 years, but we additionally observed a second peak at 20-30 years of age. We saw no preponderance by sex. We detected macro CK type 2 predominantly in severely ill patients of all ages, mainly those with malignant tumors or cirrhosis of the liver. Our findings support the assumption that macro CK type 2 is the manifestation of mitochondrial CK in serum. Occasionally, macro CK type 2 disappeared from the circulation after amelioration of the associated disease. Its occurrence in serum nevertheless is a sign of a serious illness with high mortality but not inevitably a sign of impending death.

Additional Keyphrases: CK variants · mitochondrial CK · immunohibition · malignancies · cirrhosis of the liver · prevalence of macro CK · CK isoenzyme patterns · sex- and age-related effects · pediatric chemistry

In recent years, variant creatine kinases have been described, which occur in serum samples and which show higher molecular masses by size-exclusion chromatography (Mr > 200 000) than the normal-size, dimeric, cytoplasmic isoenzymes of CK (Mr = 80 000) (1-10).8 They therefore have been termed "macro creatine kinases." Subsequent investigations revealed the existence of two different types of macro CK, both showing increased molecular mass and true creatine phosphate and ADP-dependent catalytic enzyme activity in the so-called reverse reaction.

Macro CK type 1 is an autoantibody complex in which one of the cytoplasmic isoenzymes (almost exclusively CK-BB) is linked to an immunoglobulin (mainly IgG) (11, 12).

We named a second form "macro CK type 2" (13), because it differs from type 1 by its extraordinarily high energy of activation (14), its relative mobility during electrophoresis (15), its enzyme kinetics (16), and its apparent mitochondrial origin (13, 14).

Here we report our results concerning detection and prevalence of macro CK type 2 and the age and sex distribution of the respective patients as well as their principal diagnoses. Analytical aspects of macro CK type 2 and the results of macro CK type 1 will be or have been dealt with separately (17, 18).

Materials and Methods

Patients: We determined CK activity in the sera of patients who either were consecutively admitted to our university hospital for internal diseases and who had to stay for at least two days, or whose serum samples were consecutively sent from other departments or outpatient clinics of the university hospitals to our laboratory for determination of at least one enzyme activity. The study began November 1, 1980, and ended February 28, 1981. Patients' data (laboratory findings, diagnoses, etc.) were taken from the Diagnostic Information System (19) of the university hospital and processed electronically.

Screening for macro CK activity: In each serum we measured, the same day, total CK activity with the N-acetylcysteine-activated method (20) (reagents supplied by Boehringer Mannheim, Mannheim, F.R.G., and E. Merck, Darmstadt, F.R.G.) and the residual CK activity after immunoinhibition of the CK-M subunits with anti-CK-M antibodies (21) (E. Merck) at 25 °C, using an ACP 5040 analyzer (Eppendorf Gerätebau, Hamburg, F.R.G.) as described earlier (14). We corrected all results for blanks and residual adenylyl kinase (EC 2.7.4.3) activity. Because both types of macro CK are not affected by these anti-M antibodies (6, 17), all sera with residual CK activity after INH ≥ 10 U/L and sera of patients without myocardial infarction or skeletal muscle trauma but with residual CK activity after INH in the range between >5 U/L and <10 U/L were classified as "macro CK suspect." These sera were split into 200-μL aliquots and stored at -20 °C, with no additives.

Detection and differentiation of macro CK: An exact assay for macro CK has to be based on the proof of an increased Mr. We therefore confirmed or ruled out macro CK in each suspected case by exclusion chromatography. Macro CK was considered to be in fact present if we observed activity peaks of total CK and CK after INH in fractions 16-19 or 22-24 of the chromatograms (17) or if we observed at least three subsequently increasing CK activities of more than 5 U/L in these fractions. After each 15 runs nonspecifically bound material was removed from the column with 5 mL of a 1 mol/L NaCl solution.

We differentiated macro CK type 1 from type 2 (17), using isoenzyme electrophoresis (15), radio-electrophoresis (15), and energies of activation (14).

Statistical methods: The chi-squared test was calculated for p > 0.95 according to Yates and Fisher (22). Prevalences and class prevalences of disease (CPD) were calculated according to Gerhardt et al. (23).

Results

Screening Procedure

During the four-month period, 2375 (1139 ♀, 1236 ♂) patients were consecutively admitted to our hospital; 2273
of them had to stay for at least two days, 311 (13%) of them were admitted to the intensive-care unit. Total CK was determined in these patients' sera, but due to holidays and weekends and other reasons in only 2018 (89%) of them (967 \( \delta \), 1051 \( \xi \)) was the immunoinhbitition test performed within 24 h of admission. During the same period immunoinhbitition was performed in an additional 936 samples from outpatients and patients hospitalized in other departments of the university hospitals. These numbers also include all patients who had to be readmitted (up to five times) during our study. Table 1 summarizes the results of the screening by immunoinhbitition. The average ages of the patients are given in Table 2. Two items are remarkable:

1. The total number of patients with macro CK type 2 (n = 85) exceeds the number of patients with acute myocardial infarction (n = 44).

2. The proportion of patients showing only CK-BB (in addition to CK-MM) and no macro CK at the same time was minute (0.2%).

**CK Activities in Sera of Patients with Macro CK Type 2**

Figure 1 shows the residual activity of CK after INH in the 86 cases with macro CK type 2 as a function of their total CK activity. Seventy-five of the 86 cases with macro CK type 2 are associated with normal, 10 cases with (mostly moderately) increased values for total CK activity. Residual activity after INH, however, often is increased and sometimes exceeds 20 U/L and even reaches 50 U/L. Therefore macro CK type 2 is characterized by a relative high percentage of residual CK after INH, and this ratio often exceeds 0.25. In the area typical for samples of patients with myocardial infarction (hatched area in Figure 1, those with total CK increased, residual CK after INH >10 U/L and 0.25 > ratio > 0.06) (14, 23, 24) we only detect three cases with macro CK type 2. In Figure 2 three typical enzyme-time curves of cases with macro CK type 2 are contrasted with patterns of uncomplicated myocardial infarction and skeletal muscle trauma, respectively.

**Prevalence of Macro CK Type 2**

**Dependence on enzyme activity.** At our most sensitive value (6 U/L) we determined a prevalence of macro CK type 2 of 3.7% for the inpatients (36 \( \delta \), 39 \( \xi \)) and 1.1% for the outpatients (six \( \delta \), four \( \xi \)). These prevalences differ significantly (chi-squared test), not only at the selected discriminator of 5 U/L but also at \( \approx 10 \) U/L and \( \approx 15 \) U/L. In the group of inpatients we detected six cases showing macro CK type 1 and type 2 simultaneously, with the activity of macro CK type 2 exceeding that of macro CK type 1 at the time of detection. Increasing the discrimination limit of the screening method decreases sensitivity and consequently the number of macro CK type 2 detected decreases drastically (Figure 3). If we assayed the samples using the same discrimination limits that we usually apply in the diagnosis of acute myocardial infarction ("S" in Figure 3: total CK increased and residual CK after INH >10 U/L), then the prevalence of macro CK type 2 of both groups of patients decreases to <0.1%.

**Dependence on age.** Table 2 demonstrates that, on the average, the inpatients with macro CK type 2 are about 55 years old. Their average age seems to exceed the average age of all inpatients by about five years. Further analysis (chi-squared test) verified this trend only for the group of female patients: 26 of the 36 women with macro CK type 2 exceeded the average age of all other hospitalized women without macro CK type 2. Furthermore, Table 2 shows the distribution of age in the whole group of patients with macro

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**Table 1. Screening for Macro Creatine Kinase by INH: Results for 2954 Samples (100%) from 2018 In- and 936 Outpatients**

<table>
<thead>
<tr>
<th>Residual CK after INH</th>
<th>CK isoenzyme type</th>
<th>No. patients</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥10 U/L (25 °C)</td>
<td>Macro CK type 2</td>
<td>37 (15 ( \delta ), 22 ( \xi ))</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>Macro CK type 1</td>
<td>34</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>CK-MB (AMI)</td>
<td>44*</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>CK-MM</td>
<td>132</td>
<td>4.5</td>
</tr>
<tr>
<td></td>
<td>CK-BB</td>
<td>8*</td>
<td>0.2</td>
</tr>
<tr>
<td>&gt;5 U/L (25 °C)</td>
<td>Macro CK type 2</td>
<td>85 (43 ( \delta ), 42 ( \xi ))</td>
<td>2.9*</td>
</tr>
<tr>
<td></td>
<td>Macro CK type 1</td>
<td>41</td>
<td>1.4</td>
</tr>
</tbody>
</table>

*Includes three patients with acute myocardial infarction (AMI) and macro CK. Normal size CK-BB: no CK-MB, no macro CK present. Includes six patients with macro CK type 1 and macro CK type 2 simultaneously.

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**Table 2. Patients' Ages**

<table>
<thead>
<tr>
<th>Ages (yr) of patients</th>
<th>With macro CK type 2</th>
<th>Without macro CK type 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients</td>
<td>55.0</td>
<td>50.3</td>
</tr>
<tr>
<td>Women only</td>
<td>61.8</td>
<td>51.5</td>
</tr>
<tr>
<td>Men only</td>
<td>49.3</td>
<td>49.1</td>
</tr>
</tbody>
</table>

*Includes three patients with acute myocardial infarction (AMI) and macro CK. Normal size CK-BB: no CK-MB, no macro CK present. Includes six patients with macro CK type 1 and macro CK type 2 simultaneously.

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**Distribution of age of patients with macro CK type 2**

<table>
<thead>
<tr>
<th>Age class, yr</th>
<th>Fraction of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 30</td>
<td>0.147</td>
</tr>
<tr>
<td>30–49</td>
<td>0.227</td>
</tr>
<tr>
<td>50–69</td>
<td>0.389</td>
</tr>
<tr>
<td>≥ 70</td>
<td>0.240</td>
</tr>
</tbody>
</table>

*s.n.s.: difference not significant: chi square < limit of 3.84; s.: difference significant: chi square = 4.42.
Generally older people are more often admitted to hospitals for internal diseases. To eliminate this bias, we calculated class prevalences of macro CK type 2 for the various classes of age and checked whether the class prevalences are dependent on age. In the left part of Figure 4 we see a biphasic pattern of these class prevalences calculated for patients with macro CK type 2 and eight classes of age. Highest prevalences are seen in the class of the youngest patients and in the group of the 70- to 79-year-old patients, for which we obtained a prevalence of 5.2% for macro CK type 2. A minimum value of about 1% is found in patients between 30 and 39 years of age. Apparently there are differences for both sexes: in women we observe a significant increase of the class prevalence with age (chi squared = 8.28, limit = 7.82), documented in the right part of Figure 4. In the group of men there are the same two maxima we already observed for all patients with macro CK type 2. For men we therefore only observe a similar trend of increasing prevalences with age, but we cannot substantiate this by means of the chi-squared test.

Relation to Sex

Dependence on enzyme activity. The proportion of women in the group of inpatients with macro CK type 2 showing more than 5, 10, or 15 U/L of residual activity after INH slightly increased from 0.48 to 0.58 and 0.67. This tendency could not be confirmed by chi-squared test, which revealed no preponderance of women either within and between the different activity classes of macro CK type 2, respectively, or between patients with macro CK type 2 and all other hospitalized patients, of whom 48% were women. The same was true for the outpatients. With a high probability, sex is unrelated to the level of enzyme activity.

Dependence on age. Here we observe an increased proportion of women in the older age class, not only in the group of patients with macro CK type 2 but also in the reference group comprising all hospitalized patients. Only in the group of patients with macro CK type 2 who were older than 50 years could we ascertain a significant preponderance of women at a fraction of 0.60 (chi square = 5.25, limit = 3.84). For the younger patients with macro CK type 2 (<50 years) the chi-squared value was very close to the limit of 3.84, therefore a preponderance of one sex cannot be excluded totally. Furthermore, we could not detect sex-related differences between patients with and without macro CK type 2.

Diagnoses

Diagnoses of the patients with macro CK type 2 are collated in Table 3. In our hospital patients, the occurrence of macro CK type 2 apparently is associated with serious acute and chronic diseases. Most of these patients had...
malignancies and liver diseases. In contrast, the frequency of patients with diseases of the cardiovascular system in the group of patients with macro CK type 2 on the one hand and in the reference group on the other hand did not differ significantly. Furthermore, we were impressed to find macro CK type 2 in 33% of children's sera with residual CK activity after INH exceeding 10 U/L.

**Discussion**

The use of assays for the cytosolic isoenzyme CK-MB in the diagnosis of myocardial infarction has been well established; nevertheless, we still lack knowledge of the enzymology of CK. This lack is at least partly documented by the growing awareness of CK variants (25).

In our previous reports (14, 15) we described some basic findings for sera of a few selected cases; now we present more detailed data on the occurrence of macro CK type 2 in patients admitted to a hospital typical of university hospitals for internal medicine. This study is, to our knowledge, the first prospective study in which the diagnosis of macro CK type 2 is based on the following rigid criteria:

- Residual CK activity after INH >5 U/L (after correction for blanks and adenylate kinase activity) associated with an $M_r$ ranging between 250 000 and 350 000, and in some cases additionally exceeding 750 000.
- High apparent activation energy (>75 kJ/mol) and high thermal stability (fraction of activity remaining after heat inactivation =0.4) (17).
- Characteristic isoenzyme pattern after isoenzyme electrophoresis at two different pH values and isoelectric focusing (17).

One aim of our study was to find out how many different types of macromolecular CK variants may be detected in a clinic population. We therefore chose as a screening method the INH method, a sensitive, quantitative method easily allowing specific determination of non-CK-M activity. From own experiments (6, 15, 17) and from the literature we learned that mitochondrial CK (26, 27) and all macromolecular CK variants described so far are not significantly inhibited by the anti-M antibodies used, even if CK-MM is supposed to be part of the respective CK variant (1, 9, 10). Consequently we could expect not to overlook any of these CK variants that might be present in samples from the 2954 patients. At the end of the differentiation step we were astonished to see that, in our clinic, there is no such variety in CK patterns as might be deduced from the literature. Besides the ordinary cytosolic CK isoenzymes CK-MM and CK-MB, respectively, and macro CK type 1 we detected only the following patterns (and frequency of patterns):

- macro CK type 2: 63/2954, macro CK type 2 and CK-BB: 18/2954,
- macro CK type 2, CK-BB, and CK-BB' (= "pseudo-MB") (17, 28-31): 4/2954

Therefore each instance of increased CK activity after INH could be explained by either CK-B activity or macro CK type 2, or a combination of both.

The frequency with which macro CK type 2 is detected depends most critically on the sensitivity of the methods applied and the population studied. The different prevalences between in- and outpatients already reflect a relationship between macro CK type 2 and those special disorders that are more often seen in patients of our hospital for internal diseases with a large department of oncology than in outpatients. Depending on the discrimination limits used, its prevalence in our hospital ranges from <0.1% to 3.7% (Figure 3). The results of this study on the one hand agree very well with results of our former retrospective study comprising 14 201 patients, in which we applied the same decision criteria as generally used in the diagnosis of myocardial infarction (total CK increased and CK after INH >10 U/L) and determined a prevalence of less than 0.1% (13). On the other hand, our results nevertheless are comparable with results of previous studies, which sometimes lack information on the sensitivity of the methods or discrimination limits used during screening or which only refer to "cathodic bands" and unexpectedly high activities after INH, respectively, rather than to proved macro CK. Wu and

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*Table 3. Diagnoses of Patients with Macro CK Type 2*

<table>
<thead>
<tr>
<th>Diagnoses</th>
<th>With resp. disease</th>
<th>Prevalence*</th>
<th>Pat. with resp. disease</th>
<th>Prevalence*</th>
<th>Chi-squared test* (limit = 3.84)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diseases of the cardiovascular system</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coronary heart disease</td>
<td>12</td>
<td>0.16</td>
<td>232</td>
<td>0.15</td>
<td>&lt;0.05, n.s.</td>
</tr>
<tr>
<td>Heart insufficiency</td>
<td>5</td>
<td>0.07</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>5</td>
<td>0.07</td>
<td>95</td>
<td>0.06</td>
<td>&lt;0.05, n.s.</td>
</tr>
<tr>
<td>Apoplexia</td>
<td>1</td>
<td>0.01</td>
<td>44$^d$</td>
<td>0.02</td>
<td>&lt;0.05, n.s.</td>
</tr>
<tr>
<td>Malignancies</td>
<td>30</td>
<td>0.41</td>
<td>367</td>
<td>0.24</td>
<td>9.76, s.</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>7</td>
<td>0.10</td>
<td>59</td>
<td>0.04</td>
<td>6.04, s.</td>
</tr>
<tr>
<td>Leukemia</td>
<td>1</td>
<td>0.01</td>
<td>34</td>
<td>0.02</td>
<td>&lt;0.05, n.s.</td>
</tr>
<tr>
<td>Bone-marrow transplant</td>
<td>3</td>
<td>0.04</td>
<td>4$^d$</td>
<td>0.002</td>
<td>24.9, s.</td>
</tr>
<tr>
<td>Liver diseases</td>
<td>18</td>
<td>0.25</td>
<td>63</td>
<td>0.04</td>
<td>58.4, s.</td>
</tr>
<tr>
<td>Cirrhosis</td>
<td>12</td>
<td>0.16</td>
<td>20</td>
<td>0.01</td>
<td>75.5, s.</td>
</tr>
<tr>
<td>Other acute diseases</td>
<td>Infecions: 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coma diabeticum</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Porphyria</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other chronic diseases</td>
<td>Inflammation: 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic renal failure</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Data from a study on lactacidosis, run in the same hospital, n = 1557 patients (39). $^a$: the respective disease is found more often in the group of patients with macro CK type 2 than in the reference group not showing macro CK type 2. The value for chi square exceeds the limit of 3.84. n.s.: no significant difference between both groups of patients. The value for chi square is <3.84. *Prevalence = no. of diseased patients/no. of all patients. *Data from this study (n = 2375 patients).
Bowers (32, 33) screened hospital patients by the immunoinhibition method and isoenzyme electrophoresis and determined a prevalence for cathodic bands of 0.52% (28/5000) on using a discrimination limit for CK-B of 10 U/L (20°C), which is approximately comparable to CK-MB = 10 U/L (25°C) [temperature conversion factor = 2.13 (17)]. Similar results were obtained by Yuzu et al. (7), who determined a prevalence of 0.8% for cathodally migrating, proved macro CK after electrophoretic screening of 356 unselected adult hospital patients, and Kanemitsu et al. (34), who reported 0.9% (5/550). Based on a ratio of CK after INH vs total CK >0.20 we calculated a prevalence of macro CK type 2 of 1.9%. Using the same discrimination limits for sera with cathodic bands of CK activity, Bayer et al. (35) found a prevalence of 1.34% (16/1191) in unselected hospitalized patients. We therefore conclude that each of the groups investigated the same phenomenon and that macro CK type 2 and their cathodic bands are identical. If the patients are selected, than a higher prevalence is to be expected for severely ill or older patients: up to 5% of hospitalized women (≥70 yr) and about 4% of the men (≥70 yr) showed macro CK type 2.

The enzyme patterns of patients with macro CK tend to remain fairly constant during several hours (Figure 2), and the ratio of residual CK after INH to total CK often exceeds 0.25, thereby allowing these samples to be differentiated from samples from patients with myocardial infarction.

If we observed macro CK type 2 not only in adults but also in children. There is no distinct preponderance for men or women, apart from the group of the oldest patients, in which there is an increased proportion of women. This may indirectly indicate the higher life expectancy for women. In our hospital, patients with macro CK type 2 and patients not showing macro CK differ significantly in the seriousness of their diseases. Among the 73 patients with macro CK type 2 whose medical records could be examined, 41% suffered from a malignant disease: 33% had a carcinoma, mainly with metastases; four patients suffered from leukemia, with three of them developing a graft-vs-host disease after bone marrow transplantation. In the patients with malignancies the CK variant was seen in all serum samples during the patients' stays in the hospital. In one patient who died from prostatic cancer we detected the same macromolecular mitochondrial CK in the mitochondrial fractions of the primary tumor and of metastases taken at necropsy as we had already found in his serum. This again points to the fact that the tumor and its metastases can directly release mitochondrial CK and sometimes CK-BB, and that the serum enzyme pattern in these cases roughly reflects "the tumor burden" (36, 37), the stage of the disease (13). It remains to be explained what kind or types of tumors release creatine kinase and to be elucidated what conditions cause the typical serum enzyme patterns of macro CK alone and macro CK type 2 and CK-BB simultaneously. We propose that when a specific, quantitative, and practicable assay for macro CK type 2 will become available, this test may be a better indicator of malignant and metastatic disease than the CK-BB RIA (38), which (e.g.) is influenced by CK-MB. In another patient's serum we detected macro CK type 2. Her colon carcinoma, however, could not be diagnosed before a second admission a few months later.

A second group of patients suffered from liver diseases. Of all patients with macro CK type 2, 16% were admitted to the hospital because their liver cirrhosis was in an acute phase. During convalescence, the activity of macro CK type 2 decreased, but usually it did not totally disappear. In these cases macro CK type 2 apparently is released from dying or regenerating liver cells, which contain measurable amounts of mitochondrial CK (36).

A patient in the third group—those with acute, severe, but non-malignant diseases—suffered from Lyell syndrome (toxic epidermal necrolysis). In this case the origin of macro CK type 2 remained obscure, its enzyme activity paralleled the course of disease (Figure 2), decreased with improvement of disease, and totally disappeared before the patient's discharge.

This study was supported by the Deutsche Forschungsgemeinschaft.

References


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