

CHAPTER 5

Analytical aspects of the automated CKMB_{1,2} and CKMM_{1,2,3} isoform determination and its relation to other biochemical markers.

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Summary

The automated (CK)MB_{1,2}/MM_{1,2,3}-isoform measurement, based on electrophoresis, has been simplified to the point that it has become possible to perform this analysis on a 24 h routine basis. We studied analytical aspects of this analysis and its clinical relevance in relation to other biochemical markers (CK total, CKMB activity, CKMB mass, myoglobin, troponin I and troponin T) in patients with acute myocardial infarction (AMI), patients with unstable angina pectoris (UAP), and healthy donors. Furthermore, the additional significance of the analysis was evaluated in patients with clinically unexpected, raised CKMB/CK total activities. The storage of serum at 4°C does not influence the MB₂/MB₁-ratios, whereas storage at 20°C changes them significantly. MM₃/MM₁ and normal MB₂/MB₁ ratios show lower coefficients of variation (3%) than increased MB₂/MB₁ ratios (9%). Between 2 and 30 h after myocardial tissue damage, AMI patients showed a characteristic change in CK isoform patterns. At a mean time of 3.6 h after the onset of symptoms we found raised MB₂/MB₁ ratios in 94% of these patients. With the information of the CK-isoform analysis, unexpected abnormal CK activities could be explained by CK-macro enzymes (Ig-bound and mitochondrial), insufficient CK-clearance capacity, enzyme activities 4 h after (re-)infarction, and raised CK-activity 15 h after skeletal muscle damage. We conclude that the CK-isoforms are relatively simple to assess; they are adequate tools with which to indicate the CK kinetics over a period lasting between 2 and 30 h after tissue damage with a single blood sample and a single analysis; the CK-isoform analysis has additional value in explaining inappropriate CKMB/CK total activities, and the MB₂/MB₁-ratios show to be one of the best early parameters for discriminating patients with AMI on admission to the hospital.

Introduction

The enzyme creatine kinase (CK) is composed of two subunits (M and B), and consists of 3 iso-enzymes (CK)-MM, (CK)-MB and (CK)-BB (1). The existence of CK-isoforms was initially reported in 1977 (2). The difference in structure between CK-isoforms is determined by the presence of the amino acid lysine at the C-terminal position of the CK-M-subunit. The iso-enzyme MM (3) consists of three isoforms (or 'subforms') MM₃ (tissue form), MM₂ (intermediate form) and MM₁ (plasma form). Two (CK)-MB-isoforms (4) are recognisable in the blood circulation: MB₂ (tissue form) and MB₁ (plasma form). Loss of the amino acid lysine from the CK-M subunits (in the blood circulation catalysed by the enzyme carboxypeptidase) is associated with a minor change in molecular weight and no change in catalytic activity. However, this loss does induce a unique isoelectric point characteristic of each CK-isoform. The CK-isoform assay methodologies based on chromatofocusing (3) isoelectric focusing (5), and immunoblot techniques (6) are not suitable for clinical use, because of the tedious and time-consuming procedure. With the introduction of the Cardio RepTM analyser, the CK isoform analysis has been automated in such a way that this analysis has become more accessible as a stat analysis for 24 h per day, 7 days a week, and could potentially be the diagnostic application of MM and MB isoforms on a routine basis (7).

The established enzymatic criteria for acute myocardial infarction (AMI) by measurements of plasma CK-MB activities every 4-6 h, has long been regarded as the most specific, sensitive, and cost-effective means (8,9) by which to diagnose myocardial tissue damage. Because the prognosis of the patient with AMI is related to the time elapsed between onset of symptoms and start of

therapy, much effort has been made to detect (new) early markers of myocardial tissue damage. Small protein molecules such as myoglobin (mol mass 17 kDa) (10,11), Troponin T (mol mass 33 kDa) (12,13), troponin I (mol mass 23.5 kDa) (14,15) and heart Fatty Acid Binding Protein (mol mass 15 kDa) (15) are such new markers. Glycogen isophosphorylase BB (17) and the CK isoforms (18,19) were also introduced as early markers for myocardial tissue damage.

We investigated analytical and clinical aspects of the automated CK isoform analysis on a routine basis by measuring CK isoform patterns from patients with AMI, patients with unstable angina pectoris (UAP), healthy donors, and patients with inappropriate CKMB/CK total activities (CKMB/CK total ratios >20% or clinically unexplainable raised CK total activities). The parameters CK total, CK-MB activity, CKMB mass, myoglobin, troponin I and troponin T were also analysed from the patients with AMI, those with UAP, and the healthy donors in order to determine the relevance of the CK isoforms in relation to these other markers.

Patients and methods

Patients

During a 3-month period, blood samples were collected at regular intervals from 39 consecutive patients (27 male [mean age 61, range 42-78 years], 12 female [mean age 63, range 48-83 years]) who suffered from an AMI in accordance with the WHO criteria (abnormal ECG, clinical signs, increased cardiac markers) [20]. The levels at which the cardiac markers were considered to be increased are given in Table 1. A total of 192 CK isoform analyses were performed from these patients in order to measure the MB2/MB1 and MM3/MM1 ratios in time after AMI. During a successive period of 2 months, CK isoforms were analysed as well as CK total, CKMB activity, CKMB mass, myoglobin, troponin I and troponin T from another 25 AMI-patients on admission to the hospital (19 male [age 57, range 35-85 years], 6 female [age 59, range 23-80 years]) from 54 UAP patients at admission to the hospital (39 male [age 62, range 35-85 years], 15 female [age 66, range 53-82 years]), and from 69 healthy donors (45 male [age 38, range 30-45 years], 24 female [age 37, range 31-44 years]). The interval between the onset of symptoms and admission to hospital was calculated from information given by the patient. This information was retrospectively validated by the time-related enzyme activity changes in blood after AMI. Furthermore, the CK isoforms were measured in sera from patients (age 55, range 36-71 years) with clinically unexplainable, raised CK-MB/CK total activities.

Methods

Blood was centrifuged for 10 minutes at 1000 g and 20°C in order to separate the blood cells from the serum. The stability of CK isoforms was tested by analysing 15 patient samples immediately after blood sampling and 24 h after storage of the sera at 4°C and at 20°C.

The CK-MM1,2,3/CK-MB1,2 isoform patterns were measured by electrophoresis on agarose gel using the Cardio RepTM (Helena Laboratories, Beaumont, USA). After electrophoresis at 25°C, 900 V, and 40 mA, the “reagent application” procedure, and determination of the optimum PMT voltage for scanning, the MB1 and MB2 bands are scanned. Modifying the standard procedure the operator must additionally edit, inspect and save the MM1,2,3 bands.

The MM3/MM1 and MB2/MB1 ratios are calculated by the analyser from the area under the curves of the densitometric scans. Five patient samples can be analysed simultaneously with one

standard sample on one gel in the same run. The whole procedure takes about 40 min [21]. The activities of CK total and CK-MB (based on immune inhibition) were measured on a Vitros 750C analyser (Johnson and Johnson, Beerse, Belgium). Myoglobin and troponin I were measured on an Access analyser (Sanofi Diagnostics Pasteur BV, Vlaardingen, The Netherlands). After incubation of patient serum with assay specific reagents, and separation of the bound and unbound fractions, a dioxetane chemiluminescent substrate provides a long-lasting luminescent signal which is proportional to the analyte concentration [22]. CKMB mass was measured on a MAGIA 7000 analyser (Merck, Amsterdam, The Netherlands). The principle of separation after the one step competition reaction of the enzyme-immunoassay is based on magnetisation of “paramagnetic micro particles”. After this procedure substrate is added for the chemical reaction catalysed by alkaline phosphatase [23]. Troponin T was measured with an Elecsys 2010 analyser (Boehringer Mannheim, Almere, The Netherlands). After incubation of serum with a biotinylated monoclonal troponin T- antibody and the addition of streptavidin coated micro particles, the complex becomes magnetically bound to the surface of the electrode. A photomultiplier measures a chemiluminescent emission after the application of a voltage to this electrode [24].

Statistics

A paired t-test was used to compare the results of the stability study. The within run reproducibility was tested with sera from 2 patients. The samples with various levels for the MB2/MB1- and MM3/MM1-ratios were analysed five times each. One level was within the reference limits (mean MB2/MB1 0.85; mean MM3/MM1 0.33); the other level was beyond the reference limits (mean MB2/MB1 3.63; mean MM3/MM1 2.82). The run to run reproducibility was tested with a commercial control sample (Helena product nr 3320) at one level (MB2/MB1 ratio 1.13) for 41 runs in 23 days. This control serum contains no detectable MM3 fractions. Therefore, it was not possible to determine the run to run reproducibility of the MM3/MM1 ratio. The area under the curve, the sensitivity, the specificity with the corresponding 95% confidence intervals, and the cut-off values based on the most optimal point of the ROC-curves were calculated from the ROC curves (ROC 2.0, University Hospital Groningen).

Results

Analytical aspects. The storage of sample material during 24 hours at 4 EC did not influence the MB2/MB1 ratio ($p=0.768$). Whereas, storage at 20 EC showed a significant change of the MB2/MB1 ratio ($p=0.003$).

The reproducibility of the normal MB2/MB1 ratio showed a coefficient of variation (c.v.) of 3.5%, the c.v. of the MB2/MB1 level beyond the reference limits was 9.9%. The c.v. of the normal MM3/MM1 ratio was 3.0%, and the c.v. of the raised MM3/MM1 ratio was 2.8%. The run to run reproducibility of the MB2/MB1-ratios showed a c.v. of 8.8%.

Clinical aspects. Figure 1 shows the time related changes in CK-isoform patterns after myocardial infarction. The basic tissue turnover characteristic of the CK-isoform pattern is depicted in fig 1a. The top of the MB2-peak equals the MB1 peak; the MM3 peak is lower than the MM2- and MM1-peak. The first changes are observed approximately 2 hours after the myocardial tissue damage (fig 1b). The MB2 peak exceeds the MB1 peak and the MM3 peak exceeds the MM2-

and MM1-peak. By conversion of MB2 into MB1 as well as of MM3 into MM2 and of MM2 into MM1, the isoform-peaks are shifting in time from MB2 to MB1 and from MM3 through MM2 to MM1 (fig 1b - fig 1h). Until 18 hours after tissue damage, the MB2 peak exceeds the MB1 peak. Between 18 and 30 hours after myocardial damage MB2 is lower than MB1 and after 30 hours MB2 is comparable to MB1. From the MM-isoforms, MM3 is the most intense up to 9 hours after tissue damage. Between 9 and 20 hours MM2 is the most intense and after 20 hours MM1. After 30 hours the CK-isoform pattern has returned to that of basic tissue turnover (compare fig 1a with fig 1h).

In figures 2a-2b the patterns of the median MB2/MB1- and MM3/MM1-ratios with the 95% confidence intervals are shown up to 75 hours after AMI. About 2 hours after AMI the MB2/MB1 ratio starts to increase, the maximum is reached after approximately 8 hours and normalises after about 30 hours. The MM3/MM1 ratio also starts to raise about 2 hours after tissue damage, the maximum is reached after approximately 14 hours and normalises after about 30 hours.

The mean and range of time of admission to the hospital of the AMI patients are 3.6 hours and 2 - 6 hours, respectively. The medians and ranges of the CK-total, CKMB-activity, CKMB-mass, the MB2/MB1- and the MM3/MM1-ratios, myoglobin, Troponin I, and Troponin T from the AMI- and the UAP-patients at admission to the hospital and the donors are shown in table 1. Table 1 shows that the median results of

Table 1. The medians and ranges of the test results of the indicated parameters from healthy donors, and at time of admission to the hospital from patients with AMI and patients with UAP.

parameter	unit	upper reference limit	d o n o r s		A M I patients		U A P patients	
			median	range	median	range	median	range
CK-total	U/L	70	37	12-159	150	37-1256	37	10-116
CKMB-act	U/L	10	2	1-4	13	2-58	3	1-9
CKMB-mass	µg/L	5.00	1.15	0.26-5.10	29.9	2.68-176.5	2.23	0.80-15.0
MB2/MB1		1.50	n.d.	n.d.-1.38	2.78	1.35-4.76	1.16	n.d.-2.63
MM3/MM1		0.60	0.18	0.04-1.80	2.35	0.72-5.03	0.70	0.38-2.44
myoglobin	µg/L	70	25	6-115	614	71-9840	32	20-323
Troponin I	µg/L	0.1	0.00	0.00-0.21	0.11	0.00-8.66	0.00	0.00-0.15
Troponin T	µg/L	0.1	0.00	0.00-0.03	0.14	0.00-5.95	0.01	0.00-0.42

n.d.: not detectable

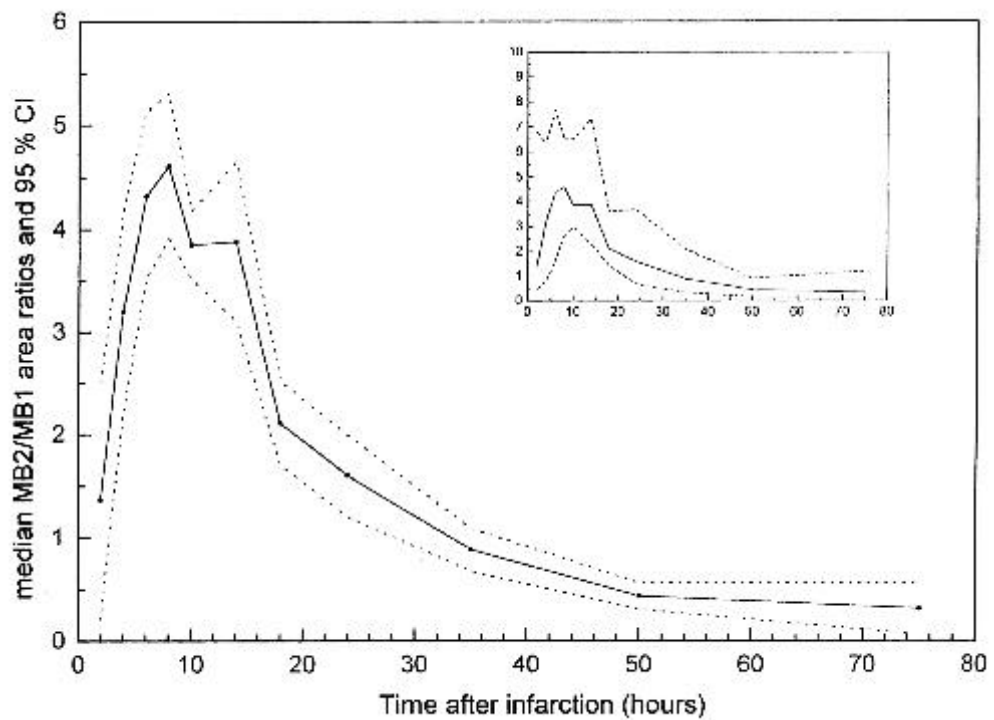
AMI: acute myocardial infarction

UAP: unstable angina pectoris.

	CK-isoform pattern		Time (h)	Characteristics
	MM ₃ , MM ₂ , MM ₁	MB ₂ , MB ₁		
a			0	MB ₂ ~MB ₁ MM ₃ <MM ₁ MM ₃ <MM ₂ MM ₂ <MM ₁
b			2	MB ₂ >MB ₁ MM ₃ >MM ₁ MM ₃ >MM ₂ MM ₂ <MM ₁
c			5	MB ₂ >MB ₁ MM ₃ >MM ₁ MM ₃ >MM ₂ MM ₂ >MM ₁
d			9	MB ₂ >MB ₁ MM ₃ >MM ₁ MM ₃ ~MM ₂ MM ₂ >MM ₁
e			12	MB ₂ >MB ₁ MM ₃ >MM ₁ MM ₃ <MM ₂ MM ₂ >MM ₁
f			18	MB ₂ ~MB ₁ MM ₃ <MM ₁ MM ₃ <MM ₂ MM ₂ >MM ₁
g			24	MB ₂ <MB ₁ MM ₃ <MM ₁ MM ₃ <MM ₂ MM ₂ <MM ₁
h			30	MB ₂ ~MB ₁ MM ₃ <MM ₁ MM ₃ <MM ₂ MM ₂ <MM ₁

Figure 1. Characteristic patterns of time-related changes in CKMM1,2,3/CKMB1,2 isoforms after myocardial tissue damage. From left to right the densitometric scans after electrophoretic separation of the (CK)MM3-, MM2-, MM1-, and the (CK)MB2-, and MB1 bands are shown. Time indicates the interval in hours after tissue damage. Time 0 is characteristic for basic tissue turnover.

A



B

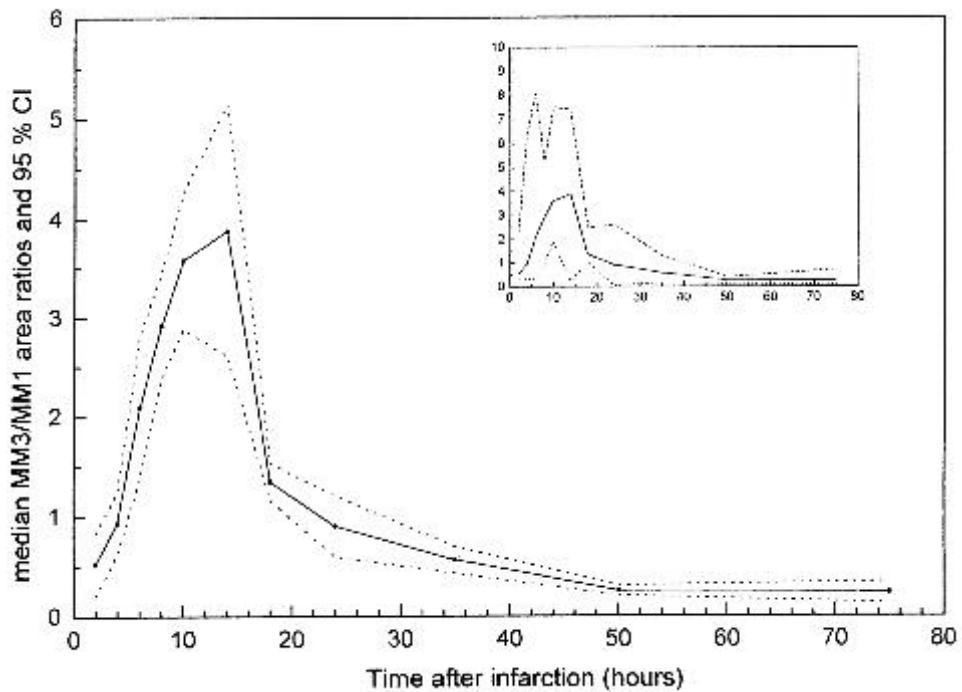


Figure 2A, B. The patterns of the median MB2/MB1 and MM3/MM1 ratios with the 95% confidence intervals (CI) up to 75 h after myocardial tissue damage. In the right upper corner the same median MB2/MB1 and MM3/MM1 ratios are shown with the 95% CI of all observed values.

all parameters from the donors are within the reference limits. The MB2/MB1-ratios could not be calculated from 64 out of 69 donors because the CK-MB activities were so low that the isoform bands on the electrophoresis gel could not be detected by the densitometer. The MB2/MB1-ratios were detectable, if the CKMB activities were more than 3 U/l. If the MB2 and MB1 bands could not be detected, the result was interpreted as negative. Therefore, for mathematical purposes (36), these ratios were assumed to be beyond the lowest measured patient ratio (0.50).

The medians of all parameters from the AMI-patients at time of admission to the hospital exceed the upper reference limits. From the UAP-patients the MM3/MM1 ratio is the only parameter, of which the median exceeds the upper limit of reference range.

In table 2 the characteristics for all 8 biochemical parameters of the ROC curves related to the three groups of individuals are presented. It concerns the areas under the curves, the sensitivities and the specificities (all 3 with the 95% confidence intervals), and the corresponding cut off values. Table 2 shows, that the areas under the curves of the various parameters except for Troponin I and Troponin T are hardly different. The MB2/MB1 ratios has the highest value for the donors vs. the AMI-patients and the second highest for the UAP vs. AMI-patients.

Figure 3 shows 5 CK-isoform patterns (patient 1-5) from patients with inappropriate CKMB/CK-total activities.

The CK-isoform pattern from patient 1 shows an extra peak, located between the MM1- and MB2- peaks; this extra peak approaches more the MM1- than the MB2-peak. For patient 2 the CK-isoform pattern shows an extra peak close to the MM3-peak in the direction of the cathode. The CK-isoform pattern of patient 3 with a permanently abnormal CK-total activity shows also consistently a very intense MM1-peak. The CK-isoform pattern from patient 4 shows that $MM3 > MM2 > MM1$ and $MB2 > MB1$. The CK-isoform patterns from patient 5 show firstly a basic tissue turnover pattern, secondly a pattern approximately 15 hours after tissue damage and thirdly a basic tissue turnover pattern with an intense MM1 peak.

Discussion

Analytical aspects. The in vitro stability of CK-isoforms at 4°C storage conditions during 24 hours show reliable results, which is in contrast to the results for storage at 20°C. These findings are confirmed by other investigators (37).

The within run reproducibility show acceptable results for the MB2/MB1-ratios within the reference limits and for the MM3/MM1-ratios within and outside the reference limits (c.v.'s less than 3.5%). Raised MB2/MB1-ratios show less reproducible results (c.v. up to 10%). This is caused, as has been reported earlier (32), by the inappropriate separation of the MB2 and MB1 bands by the densitometer, especially with high CKMB-activities.

Clinical aspects. From fig 2a-2b it may be concluded that the MB2/MB1-ratios maximises earlier in time than the MM3/MM1-ratios. This may be explained by the **Table 2**. ROC characteristics from the parameters CK-total, CKMB-activity, (CK)MB mass, (CK)MB2/MB1-ratio, (CK)MM3/MM1-ratio, myoglobin, Troponin I and Troponin T.

UAP-patients vs. AMI-patients 2-6 hours after the start of the complaints

Parameter	area under curve	c.o.v.	sensitivity	specificity
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CK-total	0.95	(0.89-1.00)	53	0.91	(0.76-0.99)	0.85	(0.74-0.94)
CKMB-act	0.92	(0.80-1.00)	6	0.86	(0.64-0.99)	0.87	(0.76-0.94)
CKMB-mass	0.95	(0.89-1.00)	5.77	0.93	(0.68-1.00)	0.84	(0.71-0.92)
MB2/MB1	0.96	(0.91-1.00)	1.96	0.95	(0.74-1.00)	0.88	(0.71-0.97)
MM3/MM1	0.92	(0.85-1.00)	1.09	0.89	(0.65-0.99)	0.91	(0.78-0.97)
myoglobin	0.98	(0.95-1.00)	255	0.93	(0.68-1.00)	0.94	(0.84-0.99)
Troponin I	0.90	(0.80-1.00)	0.11	0.53	(0.28-0.77)	0.98	(0.91-1.00)
Troponin T	0.78	(0.60-0.96)	0.02	0.80	(0.52-0.96)	0.69	(0.54-0.81)

donors vs. AMI-patients 2-6 hours after the start of the complaints

Parameter	area under curve		c.o.v.	sensitivity		specificity	
CK-total	0.93	(0.87-0.99)	49	0.95	(0.76-0.99)	0.77	(0.65-0.86)
CKMB-act	0.95	(0.87-1.00)	5	0.91	(0.70-0.99)	1.00	(0.96-1.00)
CKMB-mass	0.97	(0.95-1.00)	5.77	0.93	(0.68-1.00)	0.99	(0.94-1.00)
MB2/MB1	1.00	(1.00-1.00)	1.35	1.00	(0.85-1.00)	1.00	(0.96-1.00)
MM3/MM1	0.98	(0.96-1.00)	0.72	1.00	(0.85-1.00)	0.94	(0.85-0.98)
myoglobin	0.98	(0.96-1.00)	71	1.00	(0.82-1.00)	0.95	(0.90-0.99)
Troponin I	0.76	(0.64-0.87)	0.02	0.71	(0.44-0.90)	0.93	(0.84-0.98)
Troponin T	0.89	(0.76-1.00)	0.01	0.87	(0.60-0.98)	0.99	(0.92-1.00)

UAP: Unstable angina pectoris; AMI: acute myocardial infarction; c.o.v.: cut-off value; units: CK-total and CKMB-activity U/l; CKMB-mass, myoglobin, Troponin I and Troponin T $\mu\text{g/l}$; values between parentheses are 95% confidence intervals.

kinetics of the MB and MM-isoforms. MB2 is directly converted into MB1, whereas MM3 is converted into MM1 via MM2. Therefore, there is more time for the MM3/MM1-ratio to raise. Approximately 30 h after myocardial tissue damage the MM- and MB-isoform patterns have returned to basic tissue turnover.

Regarding the test results of CK-total, CKMB-activity, CKMB-mass, MB2/MB1- and MM3/MM1-ratios, myoglobin, Troponin I and Troponin T, the MB2/MB1-ratio is at

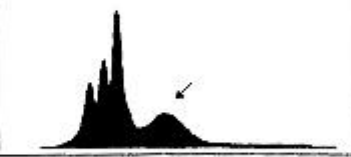
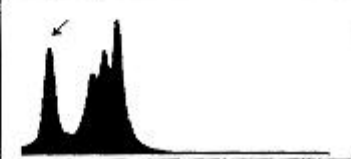





Patient	CK Isoform		Activity (U/l)		
	Pattern		Explanation	CK-Total	CKMB
	MM M M ₂ M ₁ M ₃	MM B ₂ B ₁			
1		macro enzyme Ig-bound	120	54	
2		macro enzyme mitochondrial	49	74	
3		prolonged CK-clearance	1007	4	
4		approximately 4 hours after (re-)infarction	310	20	
5		day 2 no remarks (normal)	411	6	
		day 3 approximately 15 hours after muscle damage	1505	10	
		day 4 raised MM1	2010	8	

Figure 3. Examples of CK isoform patterns from patients with different causes for unreliable CK-MB/CK total activities. The (CK)MM3, MM2, and MM1 peaks are shown from left to right. The (CK)MB2 and MB1 peaks are only detectable for patient 4. The arrows in the CK isoform patterns for patients 1-3 indicate the disturbances and relate to the explanation column.

time of admission to the hospital one of the best of the examined parameters to discriminate AMI-patients from UAP-patients and healthy donors. This is supported by recently reported findings (38). The ROC-curves for the UAP-patients vs. the donors are not presented, since the areas under the ROC-curves are not significant. This implicates, that there are no reliable cut-off values to discriminate between these

populations. The parameter with the highest area under the curve (0.89) is the MM3/MM1-ratio, a parameter more representative for skeletal muscle than for myocardial tissue damage. Although Troponin I and Troponin T are more specific for myocardial tissue damage, they turn out to be less sensitive at time of admission to the hospital than the CK-isoform ratios, because these ratios are earlier raised in circulation after myocardial tissue damage.

Laurino et al. (37) reported comparable sensitivities and specificities of the mass measurements of CK-MB2, CKMB and myoglobin. In concordance with Roberts et al. (39), we prefer the measurement of MB2/MB1-ratios above CK-MB2 mass, because a release of minute amounts of MB2 into the plasma after myocyte necrosis will lead to a significant change in the MB2/MB1-ratio.

As the analyser reports only MM3/MM1- and MB2/MB1-ratios, the CK-isoform analysis cannot be used for the quantification of tissue damage. For this purpose other cardiac markers such as Troponin I or Troponin T should be used. However, only the CK-isoform analysis offers the possibility of indicating the interval between infarction and blood sampling in a single blood sample with a single analysis. So, we suggest to use the CK-isoform analysis as marker for early damage and as marker for indication of the interval between AMI and time of blood sampling.

Concerning the 5 CK-isoform patterns from patients with different causes for inappropriate CKMB/CK-total activities, the CK-MB/CK-total ratios >20% for patient 1 and for patient 2 were caused by CK macro-enzymes. The occurrence of these two types of CK macro-enzymes is a common phenomenon in a small part of the population (40,41). Type 1 is CK associated with immunoglobulin and type 2 appears to be oligomeric CK-mitochondrial. Most of the time an extraordinary raised CKMB/CK-total ratio (>20%) is caused by this macro-enzyme artifact (42). However, an accompanied raised CKMB-activity can never be excluded. With the investigated CK-isoforms technique it is possible to discriminate on a routine basis 24 hours a day between an increased MB-activity, a CK-macroenzyme, or a combination of both phenomena. Patients 3 - 5 are examples of the additional information of the CK-isoform analysis in comparison with the CK-isoenzyme analysis. With the information of the CK-isoform pattern the permanently abnormal CK-total activity (1004 U/l) from patient 3 can be explained by the also permanently raised MM1-isoform, indicating that the maximum CK-inactivation capacity of the lymphoid system is exceeded (43). Patient 4 and patient 5 are examples of the possibility to indicate the time elapsed after tissue damage with a single CK isoform analysis. With the result of the CK isoform analysis the correct time after (re-)infarction of 4 hours could be estimated for patient 4, from whom the clinician could not get reliable information about the time since onset of symptoms. Patient 5 had raised CK-total, CKMB activities (1505, 10 U/l, resp.) three days after Coronary Artery Bypass Grafting. On basis of the MM1,2,3/MB1,2 isoform pattern, it was concluded that there was no myocardial tissue damage, but only skeletal muscle damage caused by a phlebography on day 2, approximately 15 h before blood sampling.

From this study we conclude that the CK-isoform pattern is relatively simply to assess on a routine basis with the new analyser. However, the analyser should be improved for better reproducibility of raised MB2/MB1 ratios. The CK-isoform analysis has additional value in monitoring the CK-

kinetics over a period from 2 h until approximately 30 h after tissue damage. When the investigated parameters are compared, the MB2/MB1-ratio has shown to be one of the most reliable tests of the examined parameters to detect patients with AMI at time of admission to the hospital. Moreover, the CK-isoforms are additional tools in explaining inappropriate CKMB/CK-total activities.

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