# CHAPTER 9

## The release of CKMB, troponin I and troponin T after coronary artery bypass surgery with or without cardiopulmonary bypass, and after aortic- and mitral valve surgery.

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**Summary**
We prospectively investigated the release patterns of the biochemical markers CK-total, CKMB-activity, CKMB-mass, troponin I (two different methodologies troponin $I^{AxSYM}$ and troponin $I^{Access}$) and troponin T after various forms of cardiac surgery in patients without complications during the first 48 h after surgery. We studied patients undergoing coronary artery bypass grafting (CABG) with the use of cardio-pulmonary bypass (CPB) (group A, n= 36) or without CPB (group B, n=23). In addition, we analyzed cardiac markers in a group of patients undergoing aortic valve replacement (group C, n=14) or mitral valve replacement (group D, n=9). Preoperatively, all patients had normal renal, hepatic and cerebral function. Exclusion criteria included recent myocardial infarction, unstable angina and emergency procedures. Blood samples were collected before induction of anaesthesia (baseline), at the start of surgery, after release of aortic cross-clamping (CPB procedures), respectively opening of the graft(s) (procedures without CPB), admission to the intensive care unit, and at fixed daily moments (2am, 7am, 2pm, 9pm). The median areas under the curves of the different release patterns indicate that the values of all studied markers except CK-total, are the lowest in the CABG without CPB group. In the CABG with CPB sub-group of one or two anastomoses (n=10) CKMB-activity, CKMB-mass, and troponin T are lower compared to the three or more anastomoses sub-group (n=26). All markers except CK-total are lower in both CABG groups than in the aortic and mitral valve replacement groups. Comparison between the aortic and mitral valve replacement groups showed no difference in the examined markers. For most parameters, the highest 97.5 percentile values were observed at 6-8 hours after release of aortic cross clamp respectively opening of the graft(s). These values are for troponin $I^{AxSYM}$ 23, 3.5, 44, 50 µg/l respectively for the A, B, C, D patient groups, for troponin $I^{Access}$ 0.8, 0.15, 2.2, 2.2 µg/l respectively, for troponin T 0.6, 0.15, 1.0, 1.8 µg/l respectively, and for CKMB mass 34, 8, 80, and 80 µg/l respectively.
We conclude that the release patterns of cardiac markers after cardiac surgery depend on the type of and circumstances during surgery. Patients undergoing CABG without CPB show the lowest levels, whereas patients undergoing CABG with CPB show higher levels, and patients undergoing valve replacements show the highest levels.

## Introduction

Perioperative myocardial infarction is a serious complication of open heart surgery resulting in increased morbidity and mortality. Myocardial infarction after coronary artery bypass graft (CABG) surgery occurs in at least 5% of the patients (1). It is important to detect the acute myocardial infarction (AMI) early, to initiate immediately an appropriate therapy. Currently, the diagnosis AMI is assessed by changes of the ECG and increase in the release of biochemical markers. However, changes of the ECG are not very sensitive and specific in the peri-operative period for detection of AMI. Also, the conventional biochemical marker, CKMB, which is still used as the gold standard for the detection of myocardial tissue damage, is not cardiac specific (2)
With the introduction of the new markers troponin I and troponin T, it has become possible to discriminate between myocardial and skeletal muscle damage. Troponin I and troponin

T are peptides and they are part of the actin-myosin complex of the thin filament of striated muscle. The amino acid composition of myocardial troponin I and troponin T differ from those of the skeletal isoforms. Antibodies directed against these specific sequences of cardiac amino acids have been developed to measure the cardiac troponins (3,4)
Cardiac surgery may be indicated for patients with coronary or valvular heart disease. Different surgical strategies have been developed to treat these patients including bypass surgery or valve replacement. Coronary artery bypass grafting (CABG) surgery can be performed with or without (minimally invasive CABG (MICAB)) the use of cardiopulmonary bypass (CPB or heart lung machine). In patients undergoing the CPB procedure (e.g. CABG and valve surgery) the heart is arrested and protected by cardioplegia. During this period the heart is ischemic. At the end of the CPB the heart is reperfused and the cardiac action is resumed. This reperfusion after the ischemic period results in myocardial damage and eventually in necrosis (5). In contrast, during MICABG the heart keeps beating, and thus reperfusion injury is avoided (6). Consequently, these types of cardiac surgical procedures may result in different release patterns of the biochemical markers for myocardial damage. Moreover, release of the markers after surgery may not only be caused by the surgery itself, but also by myocardial infarction. The cutoff values of the cardiac markers for AMI patients presenting with acute chest pain complaints have already been reported (7,8,9). In contrast, these values are not well established for patients after cardiac surgery.
The aim of this study is to investigate the release patterns of the biochemical markers CK-total, CKMB-activity, CKMB-mass, troponin I and troponin T after various forms of cardiac surgery in patients without complications during the first 48 h after surgery.

**Patients and methods**
This study was approved by the medical ethical committee of the University Hospital Groningen. After informed consent, we prospectively studied patients scheduled for different types of elective open heart surgery e.g. coronary artery bypass surgery (CABG) with (group A, n= 42) or without the use of cardio-pulmonary bypass (CPB) (group B, n=25), aortic valve replacement (group C, n=14) and mitral valve replacement (group D, n=9). Preoperatively all patients had normal renal, hepatic and cerebral function. Exclusion criteria included recent myocardial infarction, unstable angina and emergency procedures.
*Anesthesia and CPB management*
Anesthesia was performed according to a fixed protocol (10). Preoperative data include a clinical examination, routine blood screening and ECG. Premedication consisted of diazepam (10-15 mg orally) 2 hours preoperatively. All routine medication was continued except diuretics and digoxin preoperatively. After insertion of a peripheral venous line and radial arterial cannulation under local analgesia (lidocaine 1%), anesthesia was induced with sufentanil (1-3 µg/kg) and midazolam (0.05-0.1 mg/kg). Tracheal intubation was facilitated with pancuronium (0.1 mg/kg). A flow directed pulmonary artery catheter was inserted via the right internal jugular vein. Anesthesia was maintained with a sufentanil (0.1 µg/kg/hour) and a midazolam (0.1 mg/kg/hour) continuous infusion and pancuronium. Volatile anesthetics were not used. After induction hydroxyethyl starch 6% solution and ringer lactate solution were used to maintain mean arterial blood pressure (MAP) $60 mmHg) and

cardiac index $2.2 l/m$^2$. All patients operated with CPB received dexamethason (1 mg/kg) after the induction of anesthesia.
Standard CPB technique with hypothermia was used in all patients of group A, C and D. Nonpulsatile flow was performed with a roller pump (Stockert, Munchen, Germany) and membrane oxygenator (Cobe Laboratories, Lakewood, CO) The circuit was primed with hydroxylethyl starch 6% solution (500 ml) and ringer solution (1000 ml). Heparin was injected into the central venous port of the pulmonary artery catheter (3 mg/kg) to obtain an activated clotting time greater than 400 sec before cannulation of the aortic root and the right atrium or the superior and inferior caval vein. Flow during CPB was maintained at 2.4 L.min/m$^2$ with mild hypothermia (32 $^o$C) as assessed by monitoring the nasopharyngeal temperature. Blood pH was regulated using "-stat management. For myocardial protection cardioplegic arrest during aortic cross clamping was obtained with cold (4 $^o$C) St Thomas solution infused into the aortic root or retrogradely into the coronary sinus. Administration of cardioplegia was repeated every 30 min. MAP was maintained between 60-90 mmHg during CPB and, if necessary, corrected with nitroglycerin (to decrease MAP) or phenylephrine (to increase MAP). Heparin was neutralized with protamine (3 mg/kg for CPB, and 0.5 mg/kg for non CPB procedures) within 10 min after weaning from CPB. Mannitol and ultrafiltration were not used during the entire procedure. Patients were weaned from bypass using dopamine.
Perioperative ECG-monitoring consisted of three-lead (I, II, V5) continuous automated ST-T segment analysis (Marquette, Milwaukee,WI. Postoperatively, full 12-lead ECG-registration was obtained at admission to the ICU, and at day 1, and day 2. The diagnosis AMI was established according to the WHO-criteria; ECG-changes (new Q-wave > 0.4 seconds, ST-elevation in two or more leads > 0.1 mV, and a typical rise and fall of CKMB. After completion of the study patients were excluded from further analysis if they experienced re-operation, AMI, or periods of sustained supra- or ventricular arrhythmia.
*Surgical procedure*
**Group A**: *conventional CABG*. Cardiopulmonary bypass was instituted by means of cannulation of the ascending aorta and right atrium (two-stage cannula). The mammary arteries and the right gastroepiploic artery were used as pedicled grafts. Occasionally, the saphenous vein graft and the radial artery were used as free-grafts. Cardiac arrest was obtained with infusion of cristalloid cardioplegic solution in the aortic root, and in the free-grafts whenever present.
**Group B**: *"off-pump" CABG (11).* "Off-pump" CABG was performed through a midline sternotomy. The mammary arteries and the right gastroepiploic artery were used as pedicled grafts. Coronary anastomoses were performed by means of mechanical stabilization of the anastomotic site and temporary segmental occlusion of the target coronary artery.
**Group C**: *aortic valve surgery*. Cardiopulmonary bypass was instituted by means of cannulation of the ascending aorta and right atrium (two-stage cannula). Cardiac arrest was obtained with infusion of cristalloid cardioplegic solution both antegrade in the coronary ostia selectively and retrograde through the coronary sinus.
**Group D**: *mitral valve surgery*. Cardiopulmonary bypass was instituted by means of cannulation of the ascending aorta and of both vene cavae selectively. Cardiac arrest was obtained with infusion of cristalloid cardioplegic solution in the aortic root. A standard

longitudinal left atriotomy was used to reach the mitral valve.

*Cardiac markers*

Blood samples were obtained before induction of anesthesia (base line), at the start of the surgery, during the surgical procedure after release of aortic cross clamping for CPB procedures and opening of the graft(s) for operations without CPB. Postoperatively, blood was collected directly at admission to the ICU, at fixed daily moments (2am, 7am, 2pm, 9pm) and at the second day postoperatively, together with routine blood sampling for patient care. All sampling times were recalculated to the time after the start of the procedure (baseline sample). Blood samples were immediately centrifuged at 1000 g and subsequently serum was separated from the cells. CK total and CKMB activity were measured immediately. For the determination of CKMB mass, cardiac troponin I and cardiac troponin T, the serum samples were stored at -20 °C until analysis.

CK-total and CKMB-activity measurements were performed with a Vitros analyzer (Ortho, Beerse, Belgium). The upper limits of reference range are for men 70 U/L, for women 50 U/L and for CKMB-activity 10 U/L.

Troponin I was measured using an Access (7) analyzer (Beckman, Mijdrecht, The Netherlands) and an AxSYM (8) analyzer (Abbott Diagnostics Division, Hoofddorp, The Netherlands). The upper reference limit of the Access analyzer is 0.1 µg/l, whereas the upper limit of reference range for the AxSYM analyzer is 2.0 µg/l.

CKMB-mass and troponin T were measured on an Elecsys 2010 (9) analyzer (Roche, Almere, The Netherlands). The upper limit of the reference range for CKMB-mass is 5.0 µg/l and for troponin T 0.1 µg/l.

*Statistical analysis*

Patient characteristics are expressed as mean ± SD. Results of the release patterns of the examined biochemical markers are smoothed and expressed as the 2.5-, 50- and 97.5-percentile of the concentrations from the individual patients at the various time points according to NACB recommendations (12). The area under the curve (AUC) of the release patterns of the various biochemical markers from the individual patients were calculated with the program AUCv1.0 (University Hospital Groningen, Dep. Path. and Lab.Med., Groningen, The Netherlands) using the trapezium method (13).

In order to be able to compare the results of the AUC?s of the various markers, all test results were normalized by dividing the test result by the upper limit of the reference range of that particular marker (2). Differences between the median AUC of the four different heart surgery methodologies were analyzed using the Mann-Whitney U test and the Kruskal-Wallis test. $P < 0.05$ was considered to be statistically significant different.

**Table 1.** Patient characteristics.

| Type of surgery | men n | age years mean (SD) | women n | age years mean (SD) |
|---|---|---|---|---|
| CABG with CPB | 25 | 66 (9.8) | 11 | 68 (11.2) |
| CABG without CPB 19 | 61 (14.4) | | 4 | 63 (5.7) |
| AVR | 8 | 67 (9.2) | 6 | 65 (15.0) |
| MVR | 6 | 64 (13.9) | 3 | 73 (7.6) |

CABG: coronary artery bypass grafting; CPB: cardio-pulmonary bypass; AVR: aortic valve replacement; MVR: mitral valve replacement.

**Results**

Six patients from the CABG with CPB group were excluded because of AMI (n=2), atrial fibrillation (n=3) and re-operation (n=1). Data analysis is based on the remaining group consisting of patients without complications (total n=36). Two patients were excluded from the CABG group without CPB because of AMI (total n=23). The peri-operative period in the group of patients undergoing aortic valve (total n=14) and mitral valve replacement (total n=9) was uneventful. The patient characteristics are depicted in table 1.

In figure 1a and figure 1b the smoothed 2.5-, 50- and 97.5-percentile values of both troponin I methodologies, of troponin T, and of CKMB-mass are presented for the four groups of patients. For most parameters the highest measured values were at 6-8 hours after baseline. These values are for troponin $I^{AxSYM}$ 23, 3.5, 44, 50 ìg/l respectively for the A, B, C, D patient groups, for troponin $I^{Access}$ 0.8, 0.15, 2.2, 2.2 ìg/l respectively, for troponin T 0.6, 0.15, 1.0, 1.8 ìg/l respectively, and for CKMB mass 34, 8, 80, and 80 ìg/l respectively. For further analyses we subdivided the CABG with CPB population into two groups. The first group consisted of patients receiving one or two anastomoses (n=10), and the second group of patients received three or more anastomoses (n=26). The median areas under the curves with the corresponding ranges of the normalized release patterns from the examined biochemical markers after the various forms of heart surgery are summarised in table 2. From this table 2 it can be seen that the values for all measured markers, except CK-total, are statistically significantly lower ($p<0.05$) in the CABG without CPB group compared to the other types of surgery. In addition, in the CABG with CPB sub-group of one or two anastomoses CKMB-activity, CKMB-mass, and troponin T are statistically significantly lower compared to the three or more anastomoses sub-group. All markers except CK-total are lower in the two CABG groups than in the aortic and mitral valve replacement groups. Comparison between the aortic and mitral valve replacement groups showed no difference in the examined markers.

**Table 2.** Median area under the curve (range) of the normalised release patterns from the examined biochemical markers after various forms of heart surgery.

| Parameter | CABG + CPB[a] 1-2 anas[e] n=10 | CABG + CPB[a] $ 3 anas[e] n=26 | CABG + CPB[a] all patients n=36 | CABG - CPB[b] 1-2 anas[e] n=23 | Aorta VR[c] n=14 | Mitralis VR[d] n=9 |
|---|---|---|---|---|---|---|
| CK-total | 61 (29 , 153) | 97 (30 , 1126) | 90 (29 , 1126) | 96 (21 , 267) | 100 (17 , 354) | 132 (26 , 257) |
| CKMB-act | 12 (7 , 19) | 17 (10 , 68) | 16 (7 , 68)[$] | 8 (3 , 14)[#] | 25 (11 , 138) | 45 (16 , 126) |
| CKMB-mass | 83 (40 , 160) | 108 (72 , 339) | 104 (40 , 339)[$] | 24 (8 , 44)[#] | 196 (97 , 1280) | 268 (181 , 530) |
| Troponin T | 84 (18 , 166)[*] | 124 (54 , 241) | 106 (18 , 241)[$] | 5 (2 , 41)[#] | 198 (49 , 561) | 285 (193 , 662) |
| troponin I (AxSYM) | 111 (78 , 242) | 164 (67 , 234) | 151 (67 , 242)[$] | 6 (1 , 55)[#] | 246 (34 , 1022) | 428 (322 , 732) |
| troponin I (Access) | 116 (55 , 269) | 134 (19 , 532) | 122 (19 , 532)[$] | 14 (1 , 48)[#] | 288 (85 , 1621) | 252 (146 , 736) |

[a] Coronary Artery Bypass Grafting (CABG) with Cardio-Pulmonary Bypass (CPB);
[b] CABG without CPB;
[c] Aorta Valve Replacement;
[d] Mitralis Valve Replacement;
[e] number of anastomoses.

[*] statistically significant different from CABG + CPB $ 3 anastomoses;
[#] statistically significant different from all other methodologies;
[$] statistically significant different from Aorta VR and Mitralis VR.

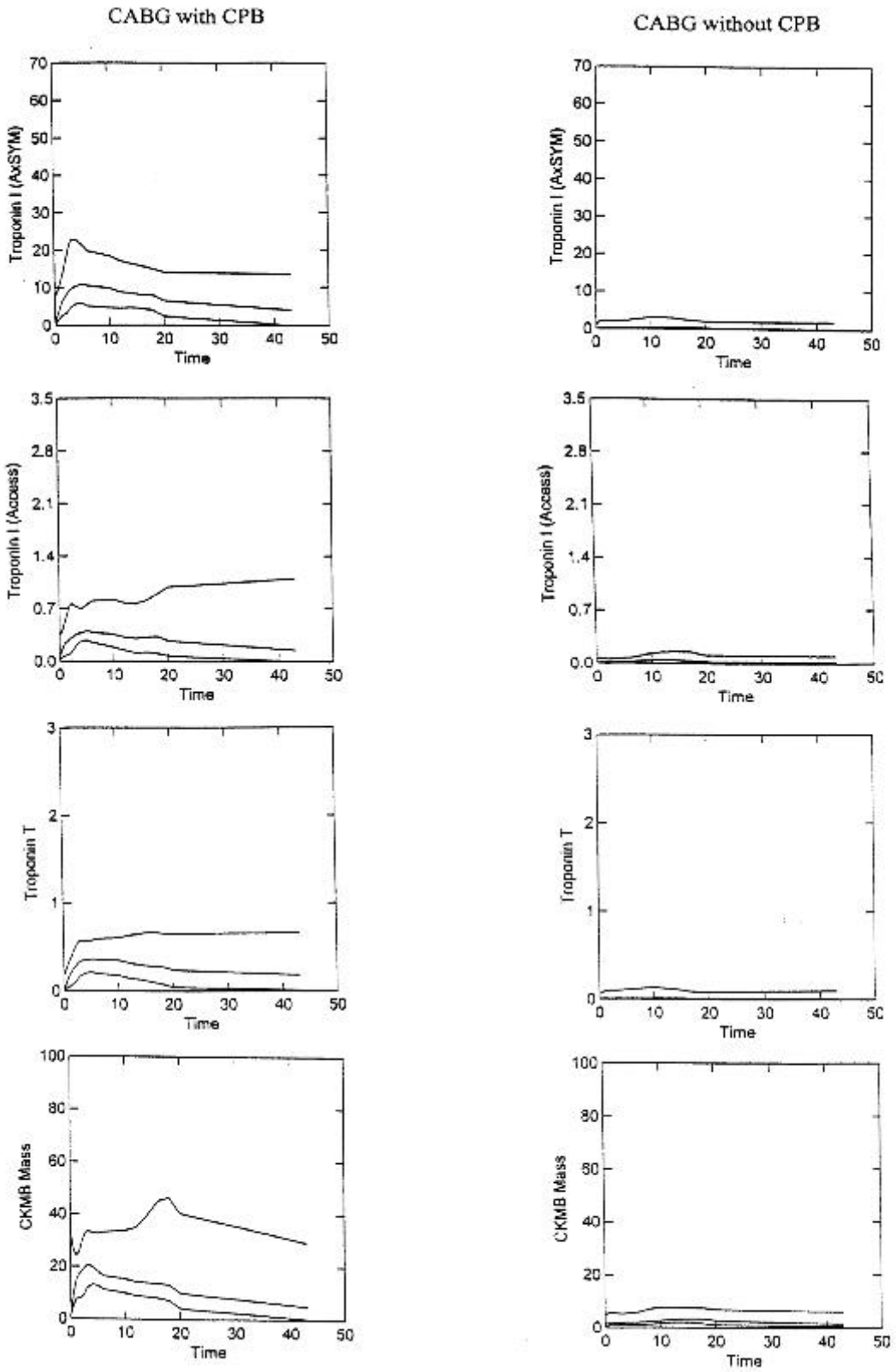


*Figure 1a.* The smoothed 2.5-, 50-, and 97.5-percentile values of the release patterns of two different troponin I methodologies, troponin T and CKMB mass (all concentrations in µg/l) are shown after coronary artery bypass grafting (CABG) with the use of cardiopulmonary bypass (CPB) and CABG without CPB. Time indicates hours after the start of the surgery.

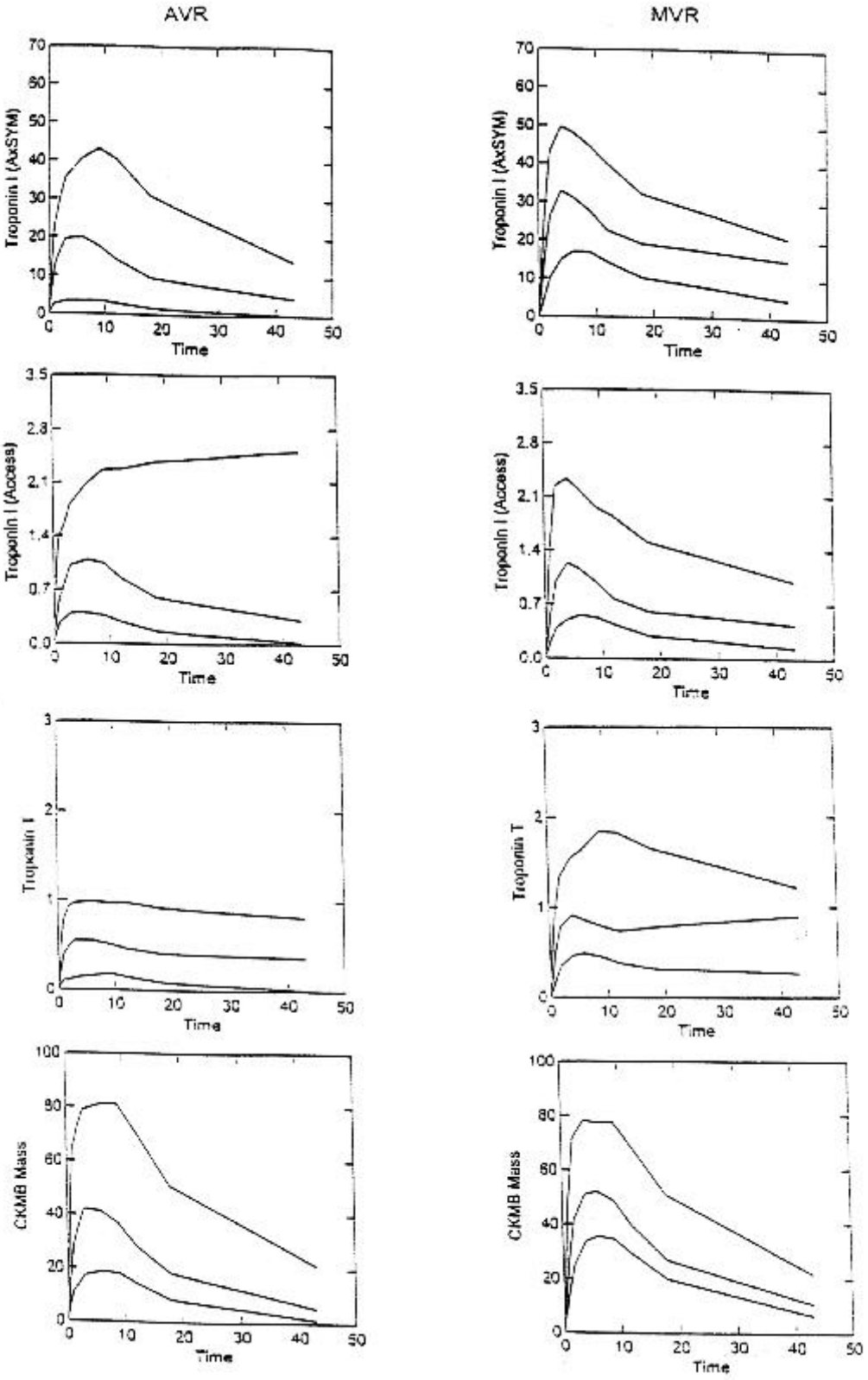


*Figure 1b*. The smoothed 2.5-, 50-, 97.5-percentile values of the release patterns of two different troponin I methodologies, troponin T, and CKMB mass (all concentrations in µg/l) are shown after Aortic Valve Replacement (AVR) and Mitral Valve Replacement. (MVR). Time indicates hours after the start of the surgery.

**Discussion**

This study demonstrates, that the release patterns of cardiac markers in patients after uncomplicated heart surgery are dependent on the type of surgery. Cardiac troponin I and cardiac troponin T are slightly increased from baseline in the group of patients after CABG without CPB. In contrast, all procedures using CPB resulted in significantly higher values of the release patterns of the examined cardiac markers. Release of troponins in CABG patients is lower than in valve surgery patients. Consequently, the measured 97.5 percentile values of the release patterns are different for the studied cardiac operations. These values increase after the start and reach the highest level after 6-8 hours. The lowest levels were obtained for the beating heart surgery group, the highest values for patients undergoing valve surgery whereas the values of the CABG with CPB patient group were in between.

For the detection of AMI in patients with chest pain, cut-off values are reported for all commercially available biochemical markers (7-9). In contrast, patients after heart surgery may experience a certain amount of myocardial injury. This injury is multifactorially determined including use of CPB, surgical technique, aortic occlusion and pre-existing coronary artery disease. This study clearly demonstrates that the release pattern of cardiac markers for each type of operation is different, Therefore, to detect AMI in the postoperative period dedicated cut off values related to the release time frame are necessary.

An indication of the amount of cardiac damage is reflected in the area under the curve of the release pattern of a cardiac marker (14). In order to compare the quantity of myocardial tissue damage for the various forms of surgery, the areas under the curves from the numerous release patterns were calculated using the trapesium method. As not all examined biochemical markers are expressed in the same units, all results were normalised by dividing the testresults by the upper limit of the reference range. The use of areas under the curves in order to compare different types of heart surgery is more reliable than comparing 'peak' (e.g. highest measured) concentrations, since the peak concentration does not have to be the real maximal concentration. Moreover, working with one sample collected at the estimated peak moment demands fixed time intervals from intraoperative events, i.e. opening cross clamp respectively opening grafts. Fully individualized blood sampling is impractical in an intensive care unit. Most often routine blood collection in the ICU takes place at fixed protocollized time points. Therefore working with area under the curves is not only less complicated but also more accurate. However, for a reliable estimation of the AUC at least 3 to 4 sample points are necessary (for instance every 6 hrs during the first 24 hrs postoperatively). Table 2 shows that patients undergoing CABG without the use of CPB have the lowest areas under the curves values. Cardiac markers did hardly change from baseline and thus these patients did hardly experience myocardial damage. The other groups experienced a certain amount of damage. The areas under the curves from the patient category CABG with CPB are statistically significant lower than those for the mitral valve and aorta-valve replacement. A number of commercial methodologies is available for the determination of cardiac troponin I whereas there is only one manufacturer for the patented troponin T. This study shows that all types of surgery except the beating heart surgery result in elevation beyond the upper limit of the reference range of each cardiac marker concentration, and thus, in measurable cardiac damage as a result of the surgical procedure

itself. Comparison between the AUC's of the different surgery groups shows, that all examined cardiac markers except CK-total can differentiate between the types of surgery. These data show evidently, that CK-total is not applicable for discrimination between the various groups. In contrast to both troponin I methodologies, can troponin T discriminate between the CABG patients with 1 or 2, and the CABG patients with 3 or more distal grafts. This finding suggests that troponin T may be clinically more sensitive to monitor myocardial damage in the peri-operative period.

So far, most studies reported results from heterogeneous groups of patients undergoing bypass and/or valve replacement surgery. Furthermore, no discrimination has been made between patients with and without complications or only one new cardiac marker has been investigated.

Banning et al. (15) reported the release patterns of cardiac troponin T, CK and CKMB-isoenzyme after coronary bypass graft surgery. For cardiac troponin T peak values were reported of 3.5 µg/l. These values are higher than we found, however, they used the first generation cardiac troponin T reagent, which was known to be susceptible to interference by skeletal muscle tissue. Etievent et al. (16) investigated cardiac troponin I values after aortic valve replacement and after CABG. In contrast to our findings, they reported higher values for the CABG group than for the aortic valve replacement group at 6 and at 12 hrs. Harff et al. (17) reported the results of several biochemical assays after CABG. Most of these markers concern the conventional cardiac enzymes and from the new parameters only the (first generation) cardiac troponin T was measured. For this latter marker a 90th-percentile value of 0.79 µg/l was reported, which is in good agreement with our reported 97.5th-percentile value of 0.7 µg/l. Gensini et al. (18) investigated the release patterns of cardiac troponin I in forty-two patients undergoing CABG. Eight patients experienced AMI and these patients had higher cardiac troponin I values than the remaining uncomplicated group of patients. Moreover, the discriminative power of troponin I was higher than that of the CKMB-isoenzyme. The results of this study cannot be compared with our results, since they use an other cardiac troponin I methodology. Alyanakins et al. (19) investigated forty-one (CABG, n=17; valve replacement, n=24) patients undergoing heart-surgery. Post-operatively these patients were subdivided into three groups. Group 1 consisted of five patients with Q-wave myocardial infarction, group 2 contained twelve patients with nonspecific ECG changes and/or need of inotropic support and group 3 (n=24) showed no complications. For group 1 patients significant higher values of cardiac troponin I, which methodology was different from the methodology we used, were reported than for group 3 patients. Group 2 patients showed peak cardiac troponin I values between those of group 1 and group 3. The investigators concluded that cardiac troponin I might be useful for the diagnosis of perioperative myocardial infarction.

In conclusion we report that the release patterns of cardiac markers after heart surgery depend on the type of and the circumstances during surgery. Highest values are reached 6-8 hours after start of the procedure. Patients undergoing CABG without CPB show the lowest levels, whereas patients undergoing CABG with CPB show higher levels, and patients undergoing valve replacements show the highest levels.

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