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# Cardiac troponin I (cTn I) and the postmortem diagnosis of myocardial infarction

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**Abstract** In clinical practice several biochemical markers are used for the diagnosis of myocardial infarction. Because of its extremely high specificity for myocardial damage, cardiac troponin I (cTn I) is frequently used. The aim of this study was to evaluate the diagnostic efficacy of postmortem cTn I determinations in pericardial fluid and serum and to compare these results with other biochemical markers and with structural findings used to diagnose acute myocardial ischaemia. We studied 89 cadavers with a mean age of 51.38 ± 2.04 (SD 19.27 years). Cases were allocated to 1 of 4 diagnostic groups depending on the probable intensity of myocardial damage and cause of death. In pericardial fluid we obtained statistically significant differences for the four biochemical parameters, while in serum myosin heavy chains and myoglobin showed statistically significant differences. The highest levels of biochemical markers in pericardial fluid were observed in subjects who had died from definite myocardial infarction.

**Key words** Myocardial ischaemia · Biochemistry · Troponin · Postmortem · Pericardial fluid

## Introduction

Measurements of biochemical markers are recognised as important tests in the postmortem diagnosis of myocardial necrosis when such a lesion is suspected but cannot be established by routine histological methods (Luna et al. 1982; Stewart et al. 1984; Lachica et al. 1988; Burns et al. 1992; Pérez Cárceles et al. 1995 a, b). In such a situation pericardial fluid is the sample material of choice to use for the biochemical tests.

Recently studies about the postmortem applications of other markers (thyroid hormones, biogenic amines and antioxidant related enzymes) involved in sudden death have been published (Edston 1996; Hirvonen and Huttunen 1996; Ramos et al. 1997).

In clinical practice several biochemical markers are used for the diagnosis of myocardial infarction (MI) particularly the MB isoenzyme of creatine kinase (MBCK) and myoglobin. However, the specificity of both markers is questionable, since increased values of MBCK and myoglobin may occur in cases of skeletal muscle damage in the absence of detectable cardiac injury (Adams et al. 1994; Mair et al. 1995, 1996).

Because of its extremely high specificity for myocardial damage, cardiac troponin I (cTn I) is frequently used (Adams et al. 1993 a, b; Mair 1997) which is one of the thin filament-associated regulatory proteins of muscle (Bodor 1994; Etievent et al. 1995). The troponin complex consists of three subunits and is involved in the calcium-sensitive switch that regulates the interaction of actin and myosin in striated muscles. The third subunit cardiac troponin I (cTn I) (molecular weight 22,500 Da) is the inhibitory subunit of the troponin complex and was found exclusively in cardiac muscle (Adams et al. 1993 b, 1994; Larue et al. 1993; Mair et al. 1993, 1996; Bodor 1994; Etievent et al. 1995).

The aim of this study was to evaluate the diagnostic efficacy of postmortem cTn I determinations in pericardial fluid and serum using an immunoenzymometric assay (sandwich technique) and to compare these results with other biochemical markers and with structural findings used to diagnose acute myocardial ischaemia.

## Material and methods

From routine necropsies performed in the Institute of Legal Medicine, Cartagena (Murcia, Spain) 89 cadavers (65 males and 24 females) were selected and divided into 4 groups according to patient records, scene of death, autopsy and complementary toxicological and histological studies, depending on the probable inten-

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sity of myocardial damage and cause of death. The groups were as follows: (1) myocardial infarction (n = 25), (2) asphyxia (n = 30) (15 hanging and 15 drowning), (3) craniocerebral trauma and/or multiple trauma excluding chest trauma (n = 17) (all motor vehicle collisions), (4) other natural deaths (n = 17) (7 cases of cerebrovascular disease, 4 of pneumonia and pulmonary embolism, 2 of acute renal failure and 4 of acute haemorrhage). The mean age of the subjects was 51.38 ± 2.04 (SD 19.27 years, range 15–86 years). Mean survival time was 7.70 ± 3.48 h (range 0–242 h) and mean postmortem interval was 9.56 ± 0.74 h (SD 7.06 h, range 1–29 h). In group 2 those cases were included in which the cause of death was presumed to have involved myocardial suffering. The subjects in group 3 were used to analyse the behaviour of the biochemical markers of muscular origin being studied in the case of injury to skeletal muscle. Cardiopulmonary resuscitation was carried out in 14 subjects.

Pericardial fluid and serum from femoral vein blood were obtained using standard techniques and samples for biochemical analysis were stored at –40° C. Pericardial fluid and serum were tested in duplicate for cTn I, myosin, myoglobin and MBCK. cTn I was analysed by immunoenzymometric assay using commercial kits from Sanofi Diagnostic Pasteur (Marnes la Coquette, France); myosin by radioimmunoassay (RIA) using monoclonal antibodies (sandwich method) purchased from Sanofi Diagnostic Pasteur (Marnes la Coquette, France); myoglobin was tested by RIA using kits from Biomerica (Newport Beach, Calif.); MBCK was assayed with an immunoinhibition technique using a commercial kit (Boehringer Mannheim). When initial determinations showed concentrations higher than the clinical range, the samples were diluted with saline and stabilized with 3% albumin to adjust the concentrations to the clinical range in serum.

Histological studies with H&E and acridine orange were carried out on heart tissue taken from ischaemic or necrotic zones (when present) and from five control samples (anterior and posterior walls of the right ventricle, anterior and posterior walls of the left ventricle and the interventricular septum) standardised for every individual. Pathologists who performed histological and histochemical sections were aware of the presumed diagnosis. When the H&E or acridine orange staining was positive we established the diagnosis of myocardial infarction (group 1). When the H&E method showed unspecific signs (congestion, interfibrillar edema, fibrosis, etc.) and/or was negative and the orange acridine was classified as unclear or negative, the cases were allocated to one of the three other groups according to the cause of death. Of the total number of cadavers submitted to autopsy, we selected 25 subjects with clear signs of having suffered myocardial infarction for contrast with the other groups diagnosed. In these cases there were also signs of previous coronary pathology (stenosis, atherosclerosis) in addition to the histological findings, and 15 showed clear pathomorphological findings of coronary thrombosis. Group 4 (other causes of natural death) included those cases in which both macro- and microscopic diagnosis, together with medical records and data from the scene of death, clearly excluded myocardial lesions.

For statistical analysis of the data, a multivariate analysis and a non-parametric test (Kruskal-Wallis Test) were used to compare groups. Specific contrasts for each variable grouped by diagnostic categories were also carried out using the Mann-Withney Test.

## Results

Haematoxylin-eosin staining showed 25 cases (28.08%) with specific signs of myocardial infarction corresponding to our group 1. With acridine orange staining we classified 20 cases as strongly positive (22.47%), 5 (5.6%) as positive and 6 (6.74%) as unclear. Table 1 shows the values (mean and SD) of the biochemical parameters in relation to the diagnostic groups. A non-parametric test (Kruskal-Wallis Test) was used to compare the mean values of the different biochemical markers in the diagnostic groups (Table 2). In pericardial fluid we obtained statistically significant differences for the four biochemical parameters, while in serum myosin heavy chains and myoglobin showed statisti-

**Table 1** Mean (x), standard deviation (SD) median and range values for the biochemical parameters in the diagnostic groups

| | Myocardial infarction (*n* = 25) | | | | Asphyxia (*n* = 30) | | | | Cranio/multiple trauma excluding chest trauma (*n* = 17) | | | | Others natural deaths (*n* = 17) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | x | SD | Median | Range | x | SD | Median | Range | x | SD | Median | Range | x | SD | Median | Range |
| *cTn I (ng/ml)* | | | | | | | | | | | | | | | | |
| Pericardial fluid | 2.4 | 1.4 | 1.8 | 6.5–0.3 | 1.7 | 0.9 | 1.6 | 3.7–0.03 | 1.1 | 0.7 | 1.16 | 2.3–0.01 | 0.4 | 0.5 | 0.05 | 1.8–0.0 |
| Serum | 1.0 | 1.2 | 0.8 | 4.1–0.0 | 0.5 | 1.0 | 0.1 | 4.0–0.0 | 0.6 | 0.6 | 0.65 | 2.0–0.0 | 0.2 | 0.4 | 0.07 | 1.5–0.0 |
| *Myosin (µU/l)* | | | | | | | | | | | | | | | | |
| Pericardial fluid | 1066 | 2935 | 131 | 11009–19 | 193 | 322 | 67.3 | 1277– 7.4 | 92 | 99 | 49.4 | 312– 7.4 | 90 | 112 | 40 | 462–10 |
| Serum | 781 | 2424 | 279 | 12 374– 7 | 1052 | 2701 | 228.6 | 12255–26 | 685 | 1041 | 231.3 | 3793–50 | 187 | 288 | 91.7 | 1219–28 |
| *Myoglobin (ng/ml)* | | | | | | | | | | | | | | | | |
| Pericardial fluid | 37 980 | 27660 | 40467 | 99583–1061 | 23309 | 30210 | 8415 | 104632–104 | 23914 | 26986 | 8484 | 75740– 84.5 | 1880 | 3779 | 259 | 15720– 67 |
| Serum | 1696 | 501 | 1706 | 3667– 764 | 1662 | 820 | 1759 | 3971–428 | 1948 | 891 | 1765 | 4314–410 | 980 | 1023 | 454 | 2925–123 |
| *MBCK (U/l)* | | | | | | | | | | | | | | | | |
| Pericardial fluid | 406 | 628 | 247 | 3126–14 | 190 | 288 | 76.7 | 1426–3.6 | 134 | 241 | 39.6 | 986–0.9 | 172 | 235 | 142 | 1020–2.4 |
| Serum | 216 | 235 | 131 | 994–14 | 283 | 285 | 164.5 | 922–7.5 | 190 | 183 | 136.3 | 866–0.3 | 190 | 183 | 140 | 516–4.3 |

**Table 2** Kruskal-Wallis Test used for the biochemical values in the diagnostic groups

| Variable | df | Statistic | *P* |
|---|---|---|---|
| cTn I. Pericardial fluid | 3 | 32.6905 | 0.0000 |
| Myosin. Pericardial fluid | 3 | 10.5159 | 0.0147 |
| Myoglobin. Pericardial fluid | 3 | 29.3875 | 0.0000 |
| MBCK. Pericardial fluid | 3 | 11.6260 | 0.0088 |
| cTn I. Serum | 3 | 5.7513 | N.S. |
| Myosin. Serum | 3 | 8.6181 | 0.0348 |
| Myoglobin. Serum | 3 | 9.7532 | 0.0208 |
| MBCK. Serum | 3 | 1.7476 | N.S. |

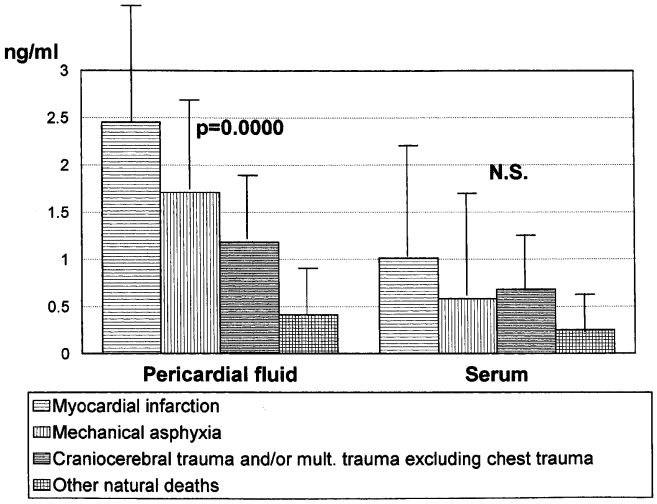


**Fig. 1** Mean concentrations of cTn I in pericardial fluid and serum

cally significant differences. The highest levels of biochemical markers in pericardial fluid were observed in subjects who had died from definite myocardial infarction. Figure 1 shows the mean concentrations of cTn I in pericardial fluid and serum.

The highest values for myosin in serum were obtained in group 2 and the lowest in the group of subjects who had died of natural causes, which also showed the lowest concentration of myoglobin. In pericardial fluid, the Mann-Withney Test pointed to statistically significant differences in cTn I, myosin, and MBCK levels between the group of subjects who died of myocardial infarction and the other groups. For myoglobin statistically significant differences were observed between the group of subjects who died of natural causes and the other groups (Table 3).

The correlation matrix showed statistically significant correlations between the diagnostic group and the levels of myosin heavy chains ($P = 0.045$), myoglobin ($P = 0.000$), and cTn I ($P = 0.000$) in pericardial fluid. Statistically significant correlations also existed between the diagnostic groups and the levels of myoglobin ($P = 0.042$) and cTn I ($P = 0.025$) in serum. We found no correlation between the levels of biochemical markers in serum or pericardial fluid and the use of cardiopulmonary resuscitation.

We analysed the values of cTn I observed in pericardial fluid in the group of subjects who had died of myocardial infarction (group 1) and in group 4 (other natural deaths). The mean concentration in group 1 was 2.45 ng/ml, while only one case (4%) of the 25 in this diagnostic group showed values of below 1 ng/ml. In group 4, (N = 17) in which the mean value of cTn I was 0.41 ng/ml, only two cases (11.76%) showed values higher than 1 ng/ml.

**Table 3** The Mann-Withney Test of the values of biochemical markers in pericardial fluid

| Variable | Groups | *Probability* |
|---|---|---|
| cTn I | 1–2 | 0.025 |
| | 1–3 | 0.002 |
| | 1–4 | 0.000 |
| Myosin | 1–2 | 0.026 |
| | 1–3 | 0.006 |
| | 1–4 | 0.010 |
| MBCK | 1–2 | 0.015 |
| | 1–3 | 0.001 |
| | 1–4 | 0.039 |
| Myoglobin | 4–1 | 0.000 |
| | 4–2 | 0.000 |
| | 4–3 | 0.001 |

Groups: 1 = Myocardial infarction; 2 = Asphyxia; 3 = Craniocerebral trauma and/or multiple trauma excluding chest trauma; 4 = Other natural deaths

On the other hand, groups 2 (asphyxia) and 3 (cranial/multiple trauma excluding chest trauma) make it possible to analyse the behaviour of the different markers in situations which may involve myocardial suffering. No difference was observed in the cTn I levels on the pericardial fluid of these two groups and those measured in group 4 (other natural death). This suggests that cell necrosis is a precondition for the release of cTn I (as occurs in myocardial infarction), while anoxic or ischaemic suffering does not provoke its appearance in pericardial fluid.

## Discussion

According to Adams et al. (1993 a) an ideal marker of myocardial injury should be found in high concentrations in the myocardium, be specific and not be found in other tissues, even in trace amounts or under pathological conditions. It should be released rapidly and completely only in response to a myocardial cellular lesion, provide a reliable basis for diagnosis and quantitative assessment of the lesion and persist in plasma for several hours in order to provide a convenient diagnostic time window but not so long that recurrent injury would not be identified. This last characteristic would be of interest in clinical practice but not in postmortem diagnosis, when any marker should also be free of interference arising during the postmortem interval and from contamination caused by adjacent fluids.

In clinical practice cTn I appears to be ideally suited for the detection of myocardial damage in complex situations (Adams et al. 1993 a, b; Mair et al. 1996; Mair 1997).

cTn I was 100% sensitive and 100% specific in detecting MI in patients with acute and chronic skeletal muscle injury, those suffering from chronic renal failure and in marathon runners (Cummins et al. 1987; Adams et al. 1993b; Bodor 1994).

With regards to the release kinetics, cTn I is released within 6 h after the onset of chest pain. It peaks at 12 h and remains high for at least 144 h after the onset of symptoms (Bodor et al. 1992; Larue et al. 1993). The release of MB-CK and myoglobin follows a very similar pattern, although myoglobin is released earlier (4 h after the onset of symptoms). Fragments of myosin heavy chains may be detected from the second day after infarction and levels may remain high for 8–10 days (Leger et al. 1990).

We have been unable to find similar studies on the use of cTn I in postmortem diagnosis to compare our results. The absence of any correlation between the postmortem interval and the markers analysed should be noted since this implies no interference from autolytic phenomena. We concluded that at least in our study, this factor did not affect the results. We found a statistically significant correlation between the diagnostic group and the levels of cTn I in serum and pericardial fluid. In pericardial fluid levels of cTn I are correlated with the concentrations of other markers but most closely with myoglobin, suggesting a similarity between both markers with regards their molecular weight and release kinetics. In serum, on the other hand, only myoglobin and troponin were correlated with the diagnostic group. This confirms the usefulness of pericardial fluid for biochemical tests in the postmortem diagnosis of myocardial infarction.

The differences observed in the myosin, cTn I and MBCK concentrations in the pericardial fluid of the group of subjects who died of myocardial infarction (in which we found the highest concentrations) and the other groups, point to their usefulness as markers of myocardial injury. The behaviour of myoglobin, on the other hand, which shows great differences between group 4 (other natural deaths, lowest levels) and the other groups confirms the negative predictive capacity of this biochemical marker.

Using 1 ng/ml as reference value in our study, cTn I shows a high diagnostic specificity (96%). The pericardial fluid levels of cTn I were lower than 1 ng/ml in only two subjects who died of myocardial infarction.

One characteristic of the troponin molecule is its early release into the different fluids. This, together with its structural characteristics and, especially, its extraordinary specificity show the diagnostic potential of determining its level in pericardial fluid alongside other more widely used markers.

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