# Biochemical Markers of Sudden Cardiac Death

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*The possible use of biochemical markers in the postmortem diagnosis of sudden cardiac deaths is well known in the forensic setting, though several issues have limited its widespread adoption. The aim of the present study was to describe the discovery of cardiac troponin I (cTnI), myoglobin (MYO) and creatinine kinase MB (CK-MB) in femoral whole blood (FWB), pericardial fluid (PF) and vitreous humor (VH) samples, that were collected post-mortem, in various cases of death related to ischemic heart disease, using the rapid diagnostic test "Cardiac Markers Combo Rapid Test Device". The authors conducted a prospective study in which they analyzed a total of 100 autopsies performed at the Legal Medicine Clinical Service of Galați County. The results of the present study showed that cardiac markers can be determined using rapid diagnostic tests for blood from the femoral vein and pericardial fluid, but not for the vitreous humor. Using postmortem biochemical markers of myocardial ischaemia requires caution and flexibility, so that the forensic pathologist has to take into consideration, when interpreting the results, both the postmortem interval, and the type of biological sample.*

*Keywords: sudden death, cardiac, markers, troponin, forensic pathology*

Sudden cardiac death is defined as a death that occurs rapidly or within a maximum 24 hours after the onset of the symptoms, in apparent good health condition, determined by a well-established organic cause [1, 2]. The most common cause of sudden death is ischemic heart disease, which can lead to death from ventricular fibrillation, triggered by ischemia or myocardial infarction [3, 4]. Therefore, death from cardiovascular diseases, particularly myocardial infarction, is a public health problem representing a significant share of forensic casuistry [5, 6]. If death occurs immediately after the acute myocardial infarction, the forensic pathologist faces the lack of gross and histological changes that are typical for the condition [7]. Thus, some objective evidences for the diagnosis of sudden cardiac death are both imperative and urgent. Post-mortem biochemical analysis of multiple biomarkers or indicators, such as cardiac troponin I (cTnI), creatine kinase MB (CK-MB) and myoglobin (MYO), plays a significant role in the diagnosis of such cases [8].

However, in the literature there is a certain limitation on the use of such tests by only some of the research teams and poor spreading among researchers in forensic medicine [9, 10]. This discrepancy may occur for several reasons. The first and most important is certainly the challenge of identifying the most reliable combination of biomarkers and, in particular, the most suitable biological sample that can be drawn from the body and then be analyzed. The types of samples multiplied and diversified over time, so at the moment, they include: blood from the femoral vein, blood from the right or left heart cavities, vitreous humor, pericardial fluid and also cerebrospinal fluid. The second problem is, undoubtedly, the difficulty of determining the reference value ranges, according to the post-mortem interval and the chosen biological samples, as the onset of autolysis and putrefaction can be characterized by the unpredictable spread into circulation and/or into the extracellular fluid of many molecules from cells whose membrane integrity was damaged, compromised or disrupted after death [11, 12].

## Experimental part

*Material and method*

The aim of the present study was to describe the discovery of cardiac troponin I (cTnI), myoglobin (MYO) and creatinine kinase MB (CK-MB) in femoral whole blood (FWB), pericardial fluid (PF) and vitreous humor (VH) samples, that were collected post-mortem, in various cases of death related to ischemic heart disease, using the rapid diagnostic test "Cardiac Markers Combo Rapid Test Device", developed by "Ecotest".

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This rapid test detects myoglobin, CK-MB and Troponin I through visual interpretation of color development on the internal strip. Anti-myoglobin, anti-CK-MB and anti-cTnI antibodies are immobilized on the respective test regions of the membrane. During testing, the specimen reacts with the anti-myoglobin, anti-CK-MB and anti-cTnI antibodies conjugated to colored particles and precoated on the sample pad of the test. The mixture then migrates through the membrane by capillary action, and interacts with reagents on the membrane. If there are certain sufficient markers in the specimen, a colored band will form at the corresponding test region of the membrane, indicating a positive result for that marker.

To achieve the goal of their study, the authors conducted a prospective study in which they analyzed a total of 100 autopsies performed at the Legal Medicine Clinical Service of Galați County. Before beginning the autopsy, blood from the femoral vein and vitreous humor were sampled, and after opening the pericardial cavity, pericardial fluid was collected. Forensic autopsies were performed were performed within 24 hours after death. All cases in which the pericardial fluid was contaminated with blood and the cases with obvious signs of autolysis were excluded from the study. Also, the cases with cardiopulmonary resuscitation maneuvers, chest trauma and other suspected causes of necrosis were excluded.

The sample analysis was carried out immediately after collection, using the rapid diagnostic test " Cardiac Markers Combo Rapid Test Device ". The reading was performed at 5, 10, 15 and 30 minutes after the beginning of the test. The collected data were statistically analyzed using SPSS (Version 20).

## Results and discussions

Causes of death were divided according to the results of toxicological and histopathological examinations as follows: ischemic heart disease (45 cases), including acute myocardial infarction (26 cases) and diffuse myocardial fibrosis with coronary atherosclerosis (19 cases) and non-cardiac causes of death (55 cases), of which craniocerebral trauma (16 cases), hanging (13 cases), acute poisoning with harmful substances (11 cases), drowning (9 cases) and incised or stab wounds (6 cases).

Table 1 presents the results of rapid testing of the cardiac markers, grouped by cause of death, determined at the autopsy. After analysis, it was found that vitreous humor gave negative results in all performed tests.

**Table 1**
„CARDIAC MARKER COMBO TEST DEVICE" RAPID DIAGNOSTIC TEST AND MICROSCOPIC CCORDING TO THE CAUSE OF DEATH

| Cause of death | Femoral whole blood (FWB) | | | Pericardial fluid (PF) | | | Vitreous humor (VU) | | |
|---|---|---|---|---|---|---|---|---|---|
| | MYO positive | CK-MB positive | cTnI positive | MYO positive | CK-MB positive | cTnI positive | MYO positive | CK-MB positive | cTnI positive |
| Acute myocardial infarction | 21 of 26 | 24 of 26 | 25 of 26 | 22 of 26 | 24 of 26 | 26 of 26 | 0 | 0 | 0 |
| Myocardial sclerosis | 17 of 19 | 15 of 19 | 16 of 19 | 16 of 19 | 18 of 19 | 18 of 19 | 0 | 0 | 0 |
| Head trauma | 5 of 16 | 6 of 16 | 1 of 16 | 1 of 16 | 1 of 16 | 1 of 16 | 0 | 0 | 0 |
| Hanging | 5 of 13 | 3 of 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Intoxications | 3 of 11 | 3 of 11 | 1 of 11 | 0 | 0 | 0 | 0 | 0 | 0 |
| Drowning | 2 of 9 | 2 of 9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Sharp object trauma | 1 of 6 | 2 of 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Table 2 and Table 3 show the sensitivity and specificity of the rapid diagnostic test, for each cardiac marker, to determine the cause of ischemic cardiac death, depending on the pathological product that was tested.

**Table 2**
RESULTS OF THE CARDIAC MARKERS USING FWB AND PF FOR DIAGNOSING CARDIAC ISCHEMIC DISEASE

| | | | | | |
|---|---|---|---|---|---|
| **Myoglobin** | **FWB** | 84.8% | 70.9% | 70.4% | 84.8% |
| | **PF** | 84.8% | 98.1% | 97.4% | 88.5% |
| **Creatine kinase MB** | **FWB** | 86.7% | 70.9% | 70.9% | 86.7% |
| | **PF** | 93.3% | 98.1% | 97.7% | 94.7% |
| **Cardiac troponine I** | **FWB** | 91.1% | 96.4% | 95.3% | 93% |
| | **PF** | 97.8% | 98.1% | 97.8% | 98.2% |

**Table 3**
SENSITIVITY, SPECIFICITY, POSITIVE PREDICTIVE VALUE (PPV) AND NEGATIVE PREDICTIVE VALUE (NPV) OF CARDIAC MARKERS FROM FEMORAL WHOLE BLOOD (FWB) AND PERICARDIAL FLUID (PF)

| | MYO | | CK-MB | | cTnI | | MYO | | CK-MB | | cTnI | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | P | N | P | N | P | N | P | N | P | N | P | N |
| **Cardiac ischemic disease** | 38 | 7 | 39 | 6 | 41 | 4 | 38 | 7 | 42 | 3 | 44 | 1 |
| **Other causes of death** | 16 | 39 | 16 | 39 | 2 | 53 | 1 | 54 | 1 | 54 | 1 | 54 |
| **Total** | 54 | 46 | 55 | 45 | 43 | 57 | 39 | 61 | 43 | 57 | 45 | 55 |

The results of the present study showed that cardiac markers can be determined using rapid diagnostic tests for blood from the femoral vein and pericardial fluid, but not for the vitreous humor. Moreover, pericardial fluid compared with blood has greater sensitivity and specificity, which can be explained by the anatomical relationship of proximity between the pericardium and myocardium and the existence of possible infiltration of cardiac biomarkers directly in the pericardial fluid, immediately after death [13].

In clinical practice, it has been seen that cTnT and cTnI measurement is more precise than conventional CK-MB quantification [14, 15]. Furthermore, it has been suggested that cTnI can be useful in determining the cause of death in post-mortem examination, as a qualitative diagnostic test [16]. There is a general consensus that serum cTnI is a highly specific marker for myocardial ischemia. Also, Rossen and Hansen suggested that cTnI testing from the blood collected during the autopsy is a sensitive test in early diagnosis of myocardial infarction [17]. Ebell et al. [18] conducted a review of the literature, that was focused on the accuracy of cTnT and cTnI for the diagnosis of acute myocardial infarction in the emergency department and had concluded that the sensitivity of cTnT and cTnI increased from 10% to 45% within 1 hour of the onset of pain (depending on the cutoff) to more than 90% after 8 or more hours. The specificity decreased gradually from 87% to 80% for cTnT, from 1 to 12 hours after the onset of chest pain and for cTnI the specificity was about 95%.

Chen et al. [19] investigated comprehensively the cardiac markers from different samples taken from the body in a number of 1932 cases and showed that the level of each marker was higher in the blood collected from the bilateral heart chambers and in pericardial fluid than in blood from the iliac veins and it was even lower in the cerebrospinal fluid. Zhu et al. [20] also determined the level of cTnT in cardiac cavities blood, peripheral blood and pericardial fluid, and from the total of 405 cases, 57 were diagnosed as acute myocardial infarction. Again, in most cases, the pericardium fluid levels of the markers were higher than those of the right and left heart cavities blood and the external iliac vein blood levels were proved to be the lowest.

Most often, in the forensic medicine practise, the samples from the body can have different degrees of haemolysis [21, 22]. The use of frozen samples for biochemical post-mortem analysis should be avoided because freezing can cause severe hemolysis and can also affect the concentration of biochemical markers in different biological specimens. Barberi and van den Hondel [23] suggested that the bodies should be refrigerated, as this can delay the autolysis of the body and at the same time it can reduce the degree of hemolysis.

Several authors analyzed the importance of cardiac biomarkers in the post-mortem diagnosis of the acute myocardial infarction, and concluded that it is worth noticing that increased levels of these markers can also be seen in other causes of death in the practice of forensic medicine, such as hyperthermia, methamphetamine abuse, carbon monoxide poisoning, electrocution, pulmonary embolism, end-stage chronic renal disease, and cerebrovascular disease [24-27]. In addition, normal levels of cardiac biomarkers can not exclude the possibility of sudden cardiac death, because they can not be detected earlier than approximatively 3 hours after the myocardial injury [28].

## Conclusions

In conclusion, the results of this study showed not only that the pericardial fluid can be used in rapid diagnostic tests for acute myocardial infarction, but also that this sample has the highest specificity and sensitivity.

Using postmortem biochemical markers of myocardial ischaemia requires caution and flexibility, so that the forensic pathologist has to take into consideration, when interpreting the results, both the postmortem interval, and the type of biological sample.

Last but not least, it is important to remember that the forensic pathologist should not succumb to the temptation to establish direct links between only one biochemical result and the probable cause of death, these must be integrated and interpreted in the context of the overall findings.

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