Postmortem Specificity of Troponin for Acute Miocard Infarction Diagnosis throug Qualitative Dosing from Pericardial Fluid

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Cardiac disease is the leading cause of death, and sudden cardiac death occupies the first place in sudden deaths of natural causes. Sudden cardiac death due to lethal arrhythmia may be the first manifestation of a cardiac disease, such cases becoming suspect dead, thus forensic cases. The autopsy performed in such cases may reveal important cardiovascular disease but not obvious macroscopic or histological changes of acute myocardial infarction (IMA), except for cases of survival for several hours after the onset of the symptomatology. Biochemical markers were used to test for myocardial lesions in the absence of morphological changes. Methods for determining myoglobin, CK-MB, troponin T (cTn T), troponin I (cTn I) were introduced to the clinic to diagnose the condition of patients with chest pain as early as the 1990s. The lack of pathognomonic elements in corps investigations, where part of the analysis cannot be carried out, requires verification of the value of the investigations that can be carried out, with reference to the biochemical in the present case, in establishing the diagnosis with certainty.

Keywords: autopsy, sudden cardiac death, troponin I, acute myocardial infarction;

Sudden death is defined as a natural, rapid and unexpected event, occurring in an hour or less than an hour, from the onset of clinical occurrence, in people with unknown or known illnesses, in apparently healthy condition. The definition provided by the World Health Organisation extends the time period to 24 hours after the victim has been seen in a stable clinical condition [1]. From a clinical point of view, sudden death is an unexpected and potentially irreversible syncope [2, 3], which differentiates it from all causes of syncope, which are mostly reversible. This phenomenon records two incident peaks: a first peak in the first year of life, and a second peak in decades 4-7, and after 70 years there will be a decrease in incidence [4]. Simonin classifies the sudden death in five categories: the sudden organic death (with obvious pathological lesions at the autopsy), the sudden death with no detectable organic damage at the autopsy, the sudden functional death (the existence of a pre-morbid field, insufficient to cause death), the sudden reflexogenic death (sudden death through inhibition), sudden autopsy death [5].

Worldwide cardiovascular diseases are thought to be responsible for approximately 17 million deaths each year, with about 25% being suddenly dead [1].

Sudden cardiac death (SCD) due to lethal arrhythmia may be the first manifestation of a cardiac disease, such cases becoming suspected dead, and leading to forensic cases. Continuous ECG monitoring in coronary units identified ventricular fibrillation (62%) as the primary fatal arrhythmia. Ventricular tachycardia accounted for 7%, for various bradyarrhythmias and asistolia to account for 31% of cases [6]. Causes of sudden cardiac death include: coronary heart disease, hypertensive disease, cardiomyopathy, valvular disease, myocarditis, malformations of the excitotrophic system, and reflex causes [4, 7, 32]. Among the causes of coronary death, myocardial infarction records a general mortality of 35%, 25-50% being sudden death, 10-15% dying in the hospital, and 15-20% of survivors of the acute period, die in the first year of event. The most common cause is the thrombotic occlusion of the coronary arteries (mainly the anterior descending coronary artery - the so-called artery of sudden death). Tanatogenesis is explained by fatal arrhythmias, pump dysfunctions and embolism [2, 7].

The risk increases in the case of high risk subgroups [8-10]. For example, the incidence of SCD is 0.1-0.2 per 1000 per year in subjects with a high coronary risk profile, 0.5 per 1000 per year in those with an earlier coronary event, at 1.5 per 1000 on year in patients with congestive heart failure and ejection fraction (EF) <35% and 2.5 per 1000 per year in the case of heart failure survivors. A combination of early myocardial infarction, low ejection fraction and ventricular tachycardia is associated with an SCD risk of nearly 3.5 per 1000 per year [11].

Certain clinical difficulties in the exact diagnosis of an IMA required the establishment of criteria that can be defined, including ischemic symptoms, ECG changes and biochemical determinations, which is why the diagnosis of IMA can be viewed from several perspectives: clinical, ECG, anatomopathological and biochemical, diagnosis of IMAs having at least two changes of these clinical and paraclinical parameters [7,11-14].

If diagnosis of acute myocardial infarction in life-threatening patients does not generally raise problems, the same cannot be said for autopsy diagnosis. The coroners experience a real challenge in establishing the diagnosis, often autopsy and histopathological examination not being sufficient in this regard [4, 9,10, 18]. The explanation lies in the fact that microscopically visible lesions require 6 hours of survival after the acute coronary event. In order to be macroscopically detectable, a minimum survival of 12 h is required [16]. Microscopically, after the aforementioned time, clogging necrosis occurs, with the loss of striations, contraction strips, edema, haemorrhage and inflammatory infiltrates with neutrophils. After 18-24 h, the nuclei become

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picnotics, with marginal contraction strips, after 24-72 h full loss of nuclei and striations occurs, with inflammatory infiltration abundant with neutrophils. Incipient macrophage and mononuclear infiltrates and an incipient fibroblast reaction occurs after 10-21 days [5, 16].

In these cases, the autopsy may reveal serious cardiovascular disease, but histological or macroscopic changes of an acute myocardial infarction (IMA) are not apparent, unless the person has survived for at least 6 h after the onset of the symptomatology [5]. Therefore, in the absence of obvious morphological changes, lesion detection is done by resorting to biochemical markers - sensitive and specific instruments, which even make it possible to determine the extent of myocardial cell damage. In case of investigations from corpses where part of the analysis can not be performed, the lack of pathognomonic elements requires the capitalization of other investigations [17-19], such as biochemical, in accurately establishing the diagnosis. Thus, biomarkers whose growth in the blood of IMA patients is explained by affecting the integrity of the cardiomyocyte membrane, with subsequent discharges into the interstitial fluid and subsequently in microcirculation of intracellular macromolecules that can be identified in the blood at different time intervals from the onset of necrosis. They are identified by serological analysis, based on their molecular weight, intracellular localization, local blood and lymph flow. In addition to markers of necrosis, markers of atheromatous plaque instability were also identified [7]. Cardiac Troponins T and I are currently the most commonly used markers of cardiac necrosis. If the serum troponins cannot be identified, creatine kinase MB isoform (CK-MB) is used.

Troponins. The first method for determining Tn I in serum was published in 1987 [20]; Bodor published an immunoenzymometric method using monoclonal antibodies in 1992 [21]. Numerous types of immunological analysis for Tn I are available; the antibodies may recognize certain epitopes of the Tn I molecule, and so by such methods various types of Tn I can be determined in the plasma, since the terminal regions are cleaved by proteases; the evaluation focuses on the region more resistant to local degradation [22]; an ideal assessment should recognize only epitopes from the stable part of the molecule and not be affected by the IC and ICT complexes (after myocardial injury, the troponin-ICT complex is gradually released into the blood where it degrades in the binary IC complex and free troponin) determinations of anti-cTn I antibodies have been reported [22, 23]; the Tn I molecule is less stable than Tn T. Cardiac troponins (TnT and I) are preferred biomarkers for the identification of necrotic myocardial lesions having an almost absolute specificity for heart muscle and high sensitivity, distinguishing between a myocardial and skeletal muscle lesion and even reflecting microscopic areas of necrosis [24]. Cardiac troponins typically increase 3 h after the onset of chest pain. In STEMI (transmural) heart attacks the level of this enzyme increases by more than 20 times the normal range and may remain elevated for up to 2 weeks after the onset of necrosis. In patients undergoing reperfusion of the obstructed coronary artery there is a rapid increase in serum troponin, indicating reperfusion of the infarcted area [7, 23].

The Universal Definition Guide of IMA recommends the dynamic determination of troponin, namely at presentation and after 3-6 h. An increased value of myocardial necrosis biomarkers is defined as exceeding the 99th percentile of values of a normal reference population using a high accuracy test (binding coefficient < = 20%). Currently there are sensitive tests (which detect cardiac troponin in 20-50% of healthy individuals) and high sensitivity (high sensitivity cardiac troponins, hs-cTn, which detects troponin in 50-90% of healthy individuals) [24]. The increase in dynamics (repeated determinations at 6-9 h) of this enzyme is necessary to demonstrate in order to differentiate the acute growth from chronic growth, the latter being encountered in patients with chronic renal disease and chronic heart failure. The consensus document of the 2007 American Academy of Biochemistry recommends a serum change of more than 20% between samples taken at 3-6 h intervals. Elevated values of cardiac troponin in the absence of clinical signs of ischemia may be justified by: myocarditis, aortic dissection, pulmonary thromboembolism, hypertrophic cardiomyopathy. Osuna et al. confirm the specificity of cTnI in LP evaluations (82 cases) postmortem [25]. Ellingsen and Hetland suggest, based on a 102-case study, that in the case of a white autopsy an increase in femoral blood serum cTnT could indicate a sudden cardiac death [23]. Zhu et al. find a link between cTnI and the severity of myocardial lesions, but not between CKMB and the severity of tissue destruction [27, 28].

Creatine kinase isoenzymes. Dreyfus in 1960 used the CK for IM. In 1967 Rosalki improved the test and it became the standard for determining CK activity. The isoenzymes were then studied for a more specific diagnosis of IMA [29, 30]. The impossibility of detecting the troponin most indicated for the diagnosis of IMA is the dosage of creatine kinase MB (CK-MB). As with troponin, the dynamic evaluation of CK-MB isoforms released from skeletal muscles is necessary, and should be found in the blood for a longer period of time, at elevated levels compared to CK-MB myocardium. Some laboratories dosed this isoenzyme by immunological testing with increased sensitivity and specificity using antiCK-MB monoclonal antibodies [21]. CK-MB also increases in myocarditis, cardiac trauma, shock, catheterization and cardiac surgery. It should be emphasized that the use of cardiac troponins in the detection of cardiac necrosis is more sensitive than the use of CK-MB. The fact that troponins remain elevated in the serum of STEMI patients for a long time has the advantage of late diagnosis of IMA. This persistent growth has a disadvantage in the context of suspicion of reinfarction [7].

Although CK serum elevation is a sensitive myocardial necrosis marker, total CK is not recommended in the IMA diagnosis due to low specificity (wide distribution in the skeletal muscle). There is, therefore, a risk of a false positive diagnosis in the case of a muscular condition, skeletal trauma, excessive effort, ethanol poisoning, seizures, intramuscular injections, pulmonary thromboembolism [5, 24]. Myoglobin is rapidly released into blood from the affected cardiomyocytes and can be detected in the serum several hours after the IMA onset. The maximum is reached faster than the other biomarkers, returning to normal within the first 24 h [7].

In 1954 and 1955, LaDue and Karmen investigated biochemical markers in myocardial lesions in patients with chest pain. They measured TGO (ASAT-aspartate aminotransferase) and found it elevated in patients with IMA. In myocardial infarction was also studied myosin, cathepsin-D, lipoprotein fractions in pericardial fluid [30]. Differences between ischemic areas and non-ischemic myocardial areas have been observed (the output of potassium from the injured cells would alter the K + / Na + ratio, which would become overhead in the ischemic areas). The C5b-9 complement fraction could be identified

biomarkers	the time interval to blood appearance	the interval of time up to the maximum value*	time interval up to return to normal
CK-MB	3-12 h	24 h	48-72 h
TnI	3-12 h	24 h	5-10 days
TnT	3-12 h	12-48 h	5-14 days
Myoglobin	1-4 h	6-7 h	24 h
Isoforms CK-MB	2-6 h	18 h	-
Isoforms CK-MM	1-6 h	12 h	38 h

Table 1
MARKERS OF MYOCARDIAL ISCHEMIA

* in the case of early reperfusion, biomarkers reach the enzyme tip more rapidly, the highest reached value is higher and the serum concentration decreases rapidly.

in ischemic areas immediately after ischemia was set and would be resistant to autolysis; in ischemic areas could no longer be identified, by immunohistochemistry: Prealbumin, reactive C protein, ceruloplasmin, alpha 1 antitrypsin, myosin, myoglobin. [31].

Atrial natriuretic peptide (ANP) and cerebral natriuretic peptide (BNP) have been investigated for cardiac function evaluation [24]. Both peptides are small in size (28 and respectively 32 amino acids) and are synthesized and secreted by the atrial and ventricular myocardium. Atrial and ventricular myocytes produce proANP and proBNP which are then cleaved in the active forms ANP and BNP and the amino-terminal portion of NTproANP and NTproBNP.

Increase in cardiac pressure or volume may trigger the synthesis and release of natriuretic peptides. The concentration of ANP and NTproANP in circulation reflects an increase in presarkin [7, 24]. Increase in BNP and NTproBNP reflects in particular the increase in post-cardiac heart rate. Their production would be due to altered myocardial tissue; a Zhu study [28] finds a negative correlation of these markers with T-cardiac troponin (cTnT) in pericardial fluid.

An increase in ANP compared to BNP (low growth) was observed in drowning (preload - post-load). An increase in BNP relative to ANP would be present in congestive heart failure (348), and the BNP concentration would be correlated with ventricular dilatation, with pulmonary stasis (pulmonary weight) [32]. An increase in BNP could indicate in cases without a clear cause of death an involvement in the determinism of death of the evolutionary cardiac hypertrophy of pulmonary congestion, pulmonary edema due to cardiac dysfunction. Michaud shows that for the determination of natriuretic peptides, different samples (LP, femoral blood, femoral blood serum) can be used with the same value; results in vitreous humor appear to be worthless [30]. Other markers of myocardial cell lesions. Several enzymes or proteins have been studied, especially for the early stage IMA diagnosis, such as fatty acid binding proteins (FABP - fatty acid bindingprotein), glycogen phosphorylase, BB isoenzyme (GP-BB); myosin (light and heavy chains of myosin, myocardial structural protein) [33, 34].

Experimental part

Considering the clinical practice preference for troponin dosage in the IMA diagnosis due to increased sensitivity and specificity, a comparison of troponin I doses was realised, measured in the pericardial fluid, depending on the cause of death, was performed to assess its specificity as a marker of myocardial ischemia and acute myocardial infarction. Pericardial fluid was preferred to blood because of postmortem haemolysis of the latter. Doses were made in 21 consecutive forensic cases over a period of time. Troponin I was determined in LP using the Immulite 1000 equipment; dosing of troponin I is a chemiluminescent immunometric study with solid phase enzyme labeling; the solid phase is taped with mouse monoclonal antibodies, antitroponin I; the liquid phase consists of alkaline phosphatase conjugated to goat anti-troponin I polyclonal antibodies; the patient sample and the reagent (conjugate) are incubated with solid phase for 30 min; At this time troponin I in the patient sample forms a sandwich complex with mouse monoclonal antibodies and antibodies from the liquid phase; the unbound particles of the patient sample and the enzyme conjugate are then removed by centrifugal washing; finally the chemiluminescent substrate is added to the test unit (containing the solid phase) and the signal generated is proportional to the troponin I in the sample. The kit is factory calibrated and checked at opening with low and high calibration. Verification is repeated every two weeks. Controls run before samples.

Results and discussions

Two cases with very likely IMA diagnosis have very high cTnI values, one with 21.3% and the other with 9.5%, suggesting differences in specificity. The highest elevations of cTnI (fig. 1) were recorded in acute myocardial infarction, resulting in a specificity of this enzyme in postmortem. Growths of troponin are observed in postprandial growth, drowning, asphyxia by hanging, bronchial asthma with pneumothorax, pulmonary embolism.

The possible influence of external factors (temperature, humidity, etc.) has not been studied.

The 95% confidence interval is between 2054 U / L and 5153 U / L.

With values above 5153 U / L there were only 12 cases, sepsis, four cases, vertebral artery rupture, one case, hanging, one case, hypothermia, one case, coronary bridging with myocardial ischaemia, one case, acute myocardial infarction, probed, two cases and two cases without clear cause of death, but most likely acute myocardial infarction, amid cardiomyopathy, hypertrophic and dilated).

Table 2
CTnI CASES (21 CASES)

Nr.	CTnI (U/L)	Anatomopathological and / or toxicological diagnosis
1	450	Chronic etymology; ketoacidosis
2	128	Drowning
3	1227	Bronchopneumonia
4	15088	ATS; IMA
5	230	Acute ethyl poisoning
6	1992	Intoxication with CO. ICC.
7	18000	Sepsis. putrefaction
8	179	Hepatic cirrhosis; hepatic coma.
9	159	Hanging; Ethyl poisoning.
10	18000	Hanging; putrefaction
11	214	ATS ; Ethyl poisoning
12	4990	Bronchopneumonia
13	30,6	Asthma. pneumothorax.
14	725	Hypovolemic shock. Haemoperitoneum.
15	542	**Cardiomyopathy**
16	305	Aspiration of regurgitated food
17	135	ICC
18	90	Drowning
19	15656	ATS, **IMA**
20	345	ATS
21	3552	Pulmonary embolism

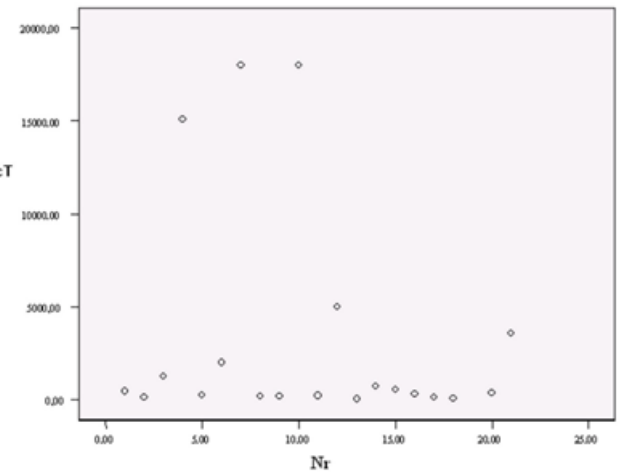

Fig. 1 Dispersion of cTnI by number of case

Conclusions

For cardiac troponin I (cTnI), there are increased levels in IMA, but also in cases of increased cardiac stasis (drowning death, hanging, bronchial asthma with pneumothorax, pulmonary embolism), but differential diagnosis is easy to do with these groups; although investigating the ratio of CKMB / CK enzymes in LP and investigating the cTnI value in LP appears to be of use in forensic practice (with caution) remains a long way to go through to an ideal marker of myocardial lesion, which should be found in high concentrations in the myocardium, be specific, not be found in other tissues; should be rapidly and fully released as a result of myocardial lesion so as to provide for the diagnostic a quantitative assessment of the lesion; should also be persistent in the analysis environment for an interval of hours to be useful to the diagnosis, but not too much so that recurrent lesions can be identified [1]; this latter feature is not of interest to postmortem investigations, but could be translated into the analysis of autolithic order.

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