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Authors: Cristian Palmiere, Camilla Tettamanti, Alessandro Bonsignore, Francesco De Stefano, Jessica Vanhaebost, Guillaume Rousseau, Maria Pia Scarpelli, Daniel Bardy



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Cardiac troponins and NT-proBNP in the forensic setting: overview of sampling site, postmortem interval, cardiopulmonary resuscitation, and review of the literature.

Cristian Palmiere (1)
Camilla Tettamanti (2)
Alessandro Bonsignore (2)
Francesco De Stefano (2)
Jessica Vanhaebost (3)
Guillaume Rousseau (4,5,6)
Maria Pia Scarpelli (1)
Daniel Bardy (7)

- 1) CURML
University Center of Legal Medicine
Lausanne University Hospital
Chemin de la Vulliette 4
1000
Lausanne 25
Switzerland
- 2) DISSAL
Departmental Section of Forensic and Legal Medicine
University of Genova
Via de Toni 12
16132
Genova
Italy
- 3) Service d'Anatomie Pathologique et Médecine Légale
Cliniques Universitaires Saint-Luc
Université Catholique de Louvain
1200
Brussels
Belgium
- 4) Lunam University, Angers, France
- 5) Department of Forensic Medicine, University Hospital - Angers, 49933, Angers Cedex 09, France
- 6) Biochemistry and Genetics Department, University Hospital - Angers, 49933, Angers Cedex 09, France
- 7) Laboratory of Clinical Chemistry
Lausanne University Hospital
Lausanne
Switzerland

Corresponding author:

Cristian Palmiere

CURML

Chemin de la Vulliette 4

1000

Lausanne 25

Switzerland

Telephone + 41 21 314 49 61

Mobile phone + 41 79 556 69 89

Fax + 41 21 314 70 90

cristian.palmiere@chuv.ch

Highlights

- Cardiac troponins and NT-proBNP may be reliably measured in postmortem serum from femoral blood
- Postmortem intervals over 48 h may cause increased levels of cardiac troponins in postmortem serum from femoral blood
- Cardiopulmonary resuscitation does not influence cardiac troponins and NT-proBNP levels

Abstract

The possible use of biochemical markers in the postmortem diagnosis of myocardial ischemia is well known in the forensic setting, though several issues have limited its widespread adoption. The study presented herein focuses on N-terminal pro-B-type natriuretic peptide, troponin T, and troponin I, and the possible influence due to sampling site chosen, postmortem interval elapsed, and cardiopulmonary resuscitation attempts. Comparisons were performed between antemortem serum levels of these markers and postmortem levels measured in pericardial fluid and postmortem serum samples obtained from different sampling sites ($n = 16$). Levels of these markers were also compared in cases characterized by various postmortem intervals ($n = 48$, consisting of 24 ischemic heart disease cases and 24 controls) as well as in cases with and without cardiopulmonary resuscitation ($n = 22$, consisting of 14 cases of hanging and 8 cases of drug intoxication). Our results indicate that N-terminal pro-B-type natriuretic peptide, troponin T, and troponin I values determined in postmortem serum from femoral blood (collected up to 24 h after death) do not differ significantly from those measured in venous blood antemortem serum samples (collected at the upper limbs). In addition, our results reveal that the time elapsed after death should always be taken into consideration when cardiac troponins are measured in postmortem samples. Lastly, our findings reveal the absence of statistically significant differences between levels of the tested biomarkers (in postmortem serum from femoral blood) in cases without cardiopulmonary resuscitation compared to cases with cardiopulmonary resuscitation (at least for postmortem intervals up to 24 h).

Keywords: cardiac troponins; NT-proBNP; postmortem biochemistry; postmortem interval; sampling site; resuscitation.

1 Introduction

The possible use of biochemical markers (cardiac troponins, natriuretic peptides, myoglobin and myosin as well as lactate dehydrogenase (LDH) and creatine kinase (CK) isoenzymes) in the postmortem diagnosis of myocardial ischemia, whether or not combined with complementary investigations (such as immunohistochemistry and radiological examinations with contrast material injection) is well known and surely not novel in the forensic setting [1-35].

Nevertheless, use of such analyses seems to have been confined to only some research teams over the years and has never actually spread among forensic pathologists. This lack of widespread use may be for a myriad of reasons. The first and most important is surely the challenge of identifying the most reliable combination of biomarkers and, above all, the most suitable biological sample(s) to collect at autopsy for their analysis. Choices of various possible specimens have broadened over time, including femoral vein blood, external iliac vein blood, left heart blood, right heart blood, vitreous humor as well as pericardial and cerebrospinal fluid, with results that may prove conflicting or inconclusive at times.

The second issue is undoubtedly the difficulty of determining ranges of plausible diagnostic values according to postmortem intervals and chosen biological samples, since the onset of decompositional changes might be characterized by unpredictable leakage into the circulation and/or extracellular fluid of numerous molecules from cells whose membrane integrity might have been damaged, compromised, or disrupted after death, including myocytes.

The third and not least significant reason is undeniably the difficulty of clearly defining the weight and role played by eventual cardiopulmonary resuscitation in inducing increases in cardiac biomarker levels (in blood and/or other biological fluids) exclusively due to the release of intracellular molecules following cardiac massage regardless of whether genuine myocardial ischemia was present or not.

Although various, promising investigations have been performed over the course of time in the forensic setting, the challenging issues aforementioned (biomarker type, sampling site, postmortem interval, and cardiopulmonary resuscitation influences) have never been analyzed simultaneously in the same study.

The purpose of the paper presented herein is threefold:

- to determine N-terminal pro-B-type natriuretic peptide (NT-proBNP), troponin T, and troponin I in a series of cases that underwent forensic investigations, with antemortem serum (from peripheral blood collected at the upper limbs at hospital admission) and a series of postmortem samples available for comparison, thereby investigating the postmortem distribution of these molecules after death and identifying the most suitable biological sample(s) to collect at autopsy,
- to determine the same molecules in a series of cases that underwent forensic investigations including ischemic heart disease (microscopically identified) deaths and controls (drug intoxication and hanging cases without ischemic heart disease) characterized by various postmortem intervals, thereby investigating the stability of these molecules according to the time elapsed after death,
- to measure the same molecules in a series of cases that underwent forensic investigations, with and without cardiopulmonary resuscitation (first aid cardiopulmonary resuscitation without defibrillation), thereby investigating the possible role of mechanical trauma on the chest due to

cardiopulmonary resuscitation in changing concentrations of these molecules regardless of the presence of authentic myocardial ischemia.

2 Materials and methods

2.1 Study design and study populations

The present study was performed during 2010-2017. All selected cases originated from forensic practice and underwent medicolegal investigations as requested by local inquiring authorities (the public prosecutor). Laboratory analyses were performed as part of the medicolegal investigations.

To address the first aim of the study, comparisons between antemortem and postmortem concentrations of NT-proBNP, troponin T, and troponin I were performed on samples collected from 16 individuals (11 males and 5 females, mean age 41.4 years) who had been admitted to hospital shortly prior to death.

Diagnosis on hospital admission consisted of sudden loss of consciousness in the presence of witnesses. Death was pronounced within 10 h of patient hospitalization. The corpses were transferred to the medicolegal centre for further investigations. Complete medicolegal autopsies were performed between 18 and 24 h after death. The causes of death were determined to be drug intoxication in all cases and were unrelated to chest trauma.

Antemortem samples consisted of serum obtained from venous blood collected from the upper limbs between 2 and 4 h prior to death.

Postmortem samples for comparison consisted of postmortem serum obtained from blood sampled at different sampling sites (right atrium, left atrium, right ventricle, left ventricle, ascending aorta, carotid artery, subclavian artery, jugular vein, subclavian vein, and femoral vein) as well as pericardial fluid.

Case inclusion criteria for this study group consisted of:

- availability of antemortem serum samples collected from the upper limb(s) at hospital admission,
- antemortem serum samples collected during hospitalisation between 2 and 4 h prior to death,
- interval between death and postmortem sample collection not exceeding 24 h,
- availability of all postmortem samples at autopsy,
- causes of death unrelated to chest trauma.

To address the second aim of the study, concentrations of NT-proBNP, troponin T, and troponin I were measured in postmortem serum from femoral blood (based on the results of the first aim of the study) in a series of cases of ischemic (coronary) heart disease (microscopically identified) and non-ischemic-heart-disease cases (including cases of hanging and drug intoxication) characterized by various postmortem intervals.

The ischemic (coronary) heart disease cases consisted of 24 individuals (18 males and 6 females, mean age 49.3 years), 8 of whom characterized by postmortem intervals ranging from 4 to 24 h, a further subgroup of 8 characterized by postmortem intervals ranging from 25 to 48 h, and the last subgroup by postmortem intervals ranging from 49 to 72 h.

The non-ischemic-heart-disease cases consisted of 24 individuals (20 males and 4 females, mean age 44.2 years, 10 cases of hanging and 14 of drug intoxication). 8 out of 24 cases were characterized by postmortem intervals ranging from 4 to 24 h, a further subgroup of 8 was characterized by postmortem

intervals ranging from 25 to 48 h, and the last subgroup of cases by postmortem intervals ranging from 49 to 72 h.

Complete medicolegal autopsies were performed between 4 and 72 h after death. In all cases, the cause of death was unrelated to chest trauma.

Case inclusion criteria for this study group consisted of:

- postmortem interval not exceeding 72 h,
- availability of postmortem serum from femoral blood at autopsy,
- causes of death unrelated to chest trauma,
- postmortem investigation findings supporting the hypothesis of ischemic heart disease death (for the first group) and the hypothesis of drug intoxication or mechanical asphyxia following external, mechanical force applied to the neck region (for the second group),
- postmortem investigation findings excluding the hypothesis of ischemic (coronary) heart disease death in the second group.

To address the third aim of the study, concentrations of NT-proBNP, troponin T, and troponin I were measured in postmortem serum from femoral blood (based on the results of the first aim of the study) in a series of 22 forensic cases (15 males and 7 females, mean age 50.2 years) including:

- 7 cases of hanging with postmortem intervals up to 24 h and cardiopulmonary resuscitation attempts (first aid cardiopulmonary resuscitation, external heart massage, without defibrillation),
- 7 cases of hanging with postmortem intervals up to 24 h without cardiopulmonary resuscitation,
- 8 cases of drug intoxication with postmortem intervals up to 24 h without cardiopulmonary resuscitation.

Case inclusion criteria for this study group consisted of:

- postmortem intervals up to 24 h,
- availability of postmortem serum from femoral blood at autopsy,
- causes of death unrelated to chest trauma,
- postmortem investigation findings excluding the hypothesis of ischemic (coronary) heart disease death in all cases,
- postmortem investigation findings supporting the hypothesis of drug intoxication or mechanical asphyxia following external, mechanical force applied to the neck region.

2.2 Postmortem investigations and sample collection

Medicolegal autopsies, histology, toxicology and biochemical investigations were performed in all cases. Conventional histology included haematoxylin-eosin (HE) stains of brain, heart, lung, liver, and kidney. Hearts were sectioned before or after fixation in 10% neutral buffered formalin. Full thickness areas involving the left anterior, the lateral free wall, and left posterior ventricle as well as the interventricular septum and right anterior ventricle were systematically sampled. The major epicardial coronary arteries were either serially sectioned at approximately 2-mm intervals or longitudinally sectioned intact on the heart. In selected cases, coronary arteries were removed from the heart and retained for decalcification prior to dissection. In selected cases, histological sections of the coronary arteries were prepared at three different, equally spaced levels.

Systematic toxicological analysis included blood ethanol level determination as well as general screening for volatile and nonvolatile drugs, poisons, and metabolites.

Femoral blood samples for toxicological and biochemical investigations were collected prior to autopsy by aspiration with sterile needles and syringes from the femoral vein(s). Blood samples were drawn after clamping the vein(s) at the proximal end and keeping the lower limb(s) raised for several minutes. Samples were stored in tubes containing sodium fluoride and preservative-free gel serum separator tubes. The latter were centrifuged immediately post collection at 3000 *g* for 15 min. After centrifugation, the separated supernatant (postmortem serum) was collected and stored in preservative-free tubes. No specimens were excluded due to insufficient sample volume. Postmortem serum samples were transferred to the laboratories immediately post collection. When analyses were delayed, samples were stored at - 20°C.

Right atrium blood, left atrium blood, right ventricle blood, left ventricle blood, ascending aorta blood, carotid artery blood, subclavian artery blood, jugular vein blood, subclavian vein blood, and pericardial fluid samples were collected during autopsy by aspiration with sterile needles and syringes, stored immediately in preservative-free gel serum separator tubes and centrifuged immediately post collection at 3000 *g* for 15 min. After centrifugation, the separated supernatant was collected and stored in preservative-free tubes. No specimens were excluded due to insufficient sample volume. Postmortem samples were transferred to the laboratories immediately post collection. When analyses were delayed, samples were stored at - 20°C.

2.3 Laboratory assays

Cardiac troponin I was analyzed with the Access® AccuTnI™ Immunoassay on Access II (Beckman Coulter, Fullerton, CA, USA). Results were expressed in µg/l. The clinical reference value (according to the laboratory where the analysis was performed) was 0.03 µg/l (corresponding to 0.03 ng/ml).

Levels of cardiac troponin T were measured with hs-TnT reagents by electrochemiluminescence immunoassay (ECLIA). Results were expressed in ng/l. The clinical reference value (according to the laboratory where the analysis was performed) was 14 ng/l.

NT-proBNP levels were measured with the commercially available immunoassays on the Roche Modular E170 system (Roche Diagnostics GmbH, Mannheim, Germany). Results were expressed in ng/l. The clinical reference value (according to the laboratory where the analysis was performed) was 738 ng/l (corresponding to 738 pg/ml and 87 pmol/l).

2.4 Statistical analysis

Comparisons were performed using the non-parametric Mann-Whitney *U* test. Statistical significance was defined as a *P* value of less than 0.05. All statistical analyses were performed using GraphPad Prism 4.0 (GraphPad Software, La Jolla, CA, USA).

3 Results

3.1 Postmortem distribution of troponin T, troponin I, and NT-proBNP

No statistically significant differences were noticed between antemortem serum and postmortem serum (obtained from femoral blood) levels of all tested markers (*P* > 0.05 for all comparisons). On the contrary, statistically significant differences were found between antemortem serum and pericardial fluid levels of all analyzed molecules as well as between antemortem serum and postmortem serum

(from all other sampling sites) concentrations of all measured parameters ($P < 0.05$ for all comparisons in all fluids).

Levels of both troponins I and T in pericardial fluid were up to 100 times greater than those measured in postmortem serum from femoral blood. Analogously, pericardial fluid NT-proBNP concentrations were up to 35 times greater than those measured in postmortem serum from femoral blood.

The highest levels of troponins I and T were found (in the order) in postmortem serum obtained from left ventricle blood, ascending aorta blood, carotid artery blood, subclavian artery blood, right ventricle blood, right atrium blood, and left atrium blood. Comparable concentrations of troponins I and T were measured in postmortem serum obtained from right atrium blood, jugular vein blood, and subclavian vein blood.

3.2 Stability of troponin T, troponin I, and NT-proBNP according to postmortem intervals

The measured values of troponin T, troponin I, and NT-proBNP in all studied groups and subgroups are reported in Table 1.

As shown, postmortem intervals greater than 48 h were characterized by troponin T and I values in postmortem serum from femoral blood over 1000 ng/l and 100 $\mu\text{g/l}$, respectively, regardless of an ischemic or non-ischemic cause of death.

No statistically significant differences were noticed in troponin T, troponin I, and NT-proBNP levels between the 4-24 h and the 25-48 h subgroups of both ischemic and non-ischemic causes of death, which would suggest postmortem stability of all these molecules in postmortem serum from femoral blood up to 48 h after death.

(Table 1)

3.3 Role of cardiopulmonary resuscitation

No statistically significant differences were noticed among levels of all tested biomarkers (measured in postmortem serum from femoral blood) in hanging and drug intoxication cases without cardiopulmonary resuscitation versus hanging cases with cardiopulmonary resuscitation and postmortem intervals up to 24 h ($P > 0.05$ for all comparisons).

These findings would indicate that cardiopulmonary resuscitation (external heart massage without defibrillation) is not associated with postmortem increases in troponin T, troponin I, and NT-proBNP levels in postmortem serum from femoral blood (in those cases that had postmortem intervals up to 24 h) possibly mimicking ischemia-induced increased concentrations of these molecules.

4 Discussion

In the realm of forensic pathology, numerous studies have been carried out over time in order to assess whether cardiac proteins (troponins, natriuretic peptides, myosin, myoglobin, cathepsin D, as well as lactate dehydrogenase and creatine kinase isoenzymes) measured in various biological fluids collected at autopsy might be reliably used for the postmortem diagnosis of myocardial ischemia (Table 2) [1-35].

(Table 2)

Most of these studies focused on the analysis of cardiac proteins and enzymes in blood and pericardial fluid, whereas comparisons between antemortem and postmortem blood/serum concentrations were undertaken exceptionally.

Davies et al. [17] have been among the few authors to measure cardiac troponin levels in blood taken from patients shortly before death (antemortem samples retrieved from hospital clinical biochemistry laboratory) and compare them to postmortem values obtained in blood sampled from different sites (heart, superior vena cava, iliac and femoral veins) and at different intervals during the early postmortem period. Though erratic results bearing little or no relation to antemortem troponin levels were obtained for all involved individuals, these researchers still highlighted the potential usefulness of cardiac troponin measurement in the postmortem setting to support the diagnosis of cardiac death in selected cases.

The review of pertinent papers covering the value of cardiac protein determination in various postmortem samples has shown that a large majority of authors identified pericardial fluid as the (postmortem) biological specimen with the highest (or one of those with the highest) diagnostic reliability to support a postmortem diagnosis of myocardial ischemia [1-4, 6-9, 15, 16, 18-20, 23, 27-29,32,34].

At the same time, a portion of researchers expressed concerns with using (postmortem) pericardial fluid to confirm the diagnosis of myocardial ischemia arising from the fact that troponin concentrations in this sample appeared to be significantly higher (of several orders of magnitude) than those expected in blood or serum (and higher than the concentrations obtained in pericardial fluid sampled from patients undergoing coronary artery bypass surgery) [12]. The alleged reason for the elevated troponin levels in pericardial fluid would be linked to the close proximity of the myocardium to the pericardium and the consequent, possible direct leakage of myocardial proteins into pericardial fluid in the early postmortem period [12, 34].

An analogous consideration (the adjacency of the left ventricle myocardium to ventricular blood and eventual egress of myocardial proteins directly into heart blood after death due to the onset of decompositional changes) could also account for the fact that, in our study, the highest levels of troponins and NT-proBNP were found in postmortem serum obtained from left ventricle, ascending aorta, carotid artery blood, and subclavian artery blood.

It must also be highlighted that Gonzalez-Herrera et al. [34], who are authors of one of the most recent articles on this topic based on the use (for the first time) of a highly sensitive cardiac troponin assay, found that average levels of cardiac troponin T in postmortem serum from femoral blood were markedly higher than those measured in clinical serum using the same highly sensitive assay.

The results of our own investigations tend to indicate that NT-proBNP, troponin T, and troponin I values determined in postmortem serum from femoral blood (collected up to 24 h after death) do not differ significantly from those measured in venous blood antemortem serum samples (collected at the upper limbs).

These preliminary results seem to suggest alternative conclusions to those reported by Davies et al. [17] (absence of relationship between antemortem and postmortem serum troponin levels) and Gonzalez-Herrera et al. [34], (higher troponin T levels in postmortem serum from femoral blood compared to antemortem serum) and would confirm, on the contrary, one of the statements in the study by Batalis et al. [27]. These authors identified the combination cardiac troponin I-femoral vein blood as one of the sampling site-cardiac protein combinations with a statistically significant difference between ischemic heart disease cases and control cases.

Another point that deserves emphasis is that Gonzalez-Herrera et al. [34] identified a troponin T cut-off point of 3200 ng/l in pericardial fluid, and 250 ng/l in postmortem serum from femoral blood, for the diagnosis of sudden cardiac death. This latter value is very close to the highest troponin T concentrations that we found in postmortem serum from femoral blood in those subjects deceased from myocardial

ischemia with postmortem intervals up to 48 h (41-242 ng/l in the ischemia group with postmortem intervals up to 24 h and 46-236 ng/l in the ischemia group with postmortem intervals up to 48 h, with no statistically significant differences between the two groups).

The reason why our analyses have shown uninterpretable troponin T and I values in postmortem serum from femoral blood (over 1000 ng/l and 100 µg/l, respectively) in both ischemic-heart-disease and non-ischemic-heart-disease cases with postmortem intervals over 48h might basically be the consequence of postmortem change occurrence, with repercussions also on troponin levels in postmortem serum from femoral blood.

These results would indicate that the time elapsed after death should be taken into consideration when cardiac troponins are measured in postmortem samples, since decompositional change onset involving myocytes might cause disruption of cell membrane integrity and thus release intracellular proteins into circulation or extracellular fluid, regardless of whether genuine myocardial ischemia is present or not.

On the other hand, the possible influence of postmortem interval on troponin concentrations in postmortem samples has been repeatedly investigated in the past, with erratic results at times. For instance, Zhou et al. [19,20,23], Remmer et al. [30], and Chen et al. [33] observed postmortem interval-dependent differences and elevations in troponin levels in various postmortem biological fluids, whereas Pérez-Carceles et al. [15] and Peter et al. [22] failed to notice significant correlations between cardiac troponins (measured in pericardial fluid and blood from femoral veins) and postmortem interval. It is worth noting, however, that according to the results of Peter et al. [22], troponin T measured in cardiac blood (unspecified sampling site) showed a significant positive correlation with autolysis time, troponin I a positive though not significant correlation with autolysis time, and both troponins no correlation with autolysis time when measured in femoral blood.

A final point that deserves mentioning pertains to the absence of statistically significant differences between levels of all tested biomarkers (measured in postmortem serum from femoral blood) in cases without cardiopulmonary resuscitation and cases with cardiopulmonary resuscitation (at least for postmortem intervals up to 24 h).

These findings are in agreement with those previously reported by Dressler et al. [11], Cina et al. [13], Pérez-Carceles et al. [15], and Ben Khalifa et al. [21] and do not differ substantially from those described in two clinical studies, thereby confirming the reliability of these postmortem results.

Indeed, Polena et al. [36] observed that the duration of cardiopulmonary resuscitation was positively associated with troponin I elevation in 38 out of 68 selected patients (mean cardiopulmonary resuscitation duration 19.8 minutes), whereas 30 out of 68 patients (mean cardiopulmonary resuscitation duration 12.2 minutes) had negative troponin I levels after successful reanimation. In this study, troponin I concentrations were measured prior to, during and at least 8 hours following cardiopulmonary resuscitation.

Lin et al. [37] found that cardiopulmonary resuscitation, even in the absence of defibrillation, can inflict cardiac injury, as evidenced by increased blood troponin I levels that peaked transiently around 16 h, though most patients had blood troponin I levels below 2 ng/ml immediately after spontaneous circulation return.

Globally considered, these results would indicate that measurements of cardiac troponins in individuals who had been reanimated and for whom spontaneous circulation failed to return or did not return long enough after cardiopulmonary resuscitation (which may correspond to clinical troponin measurements “during reanimation”) fail to show increased troponin levels, possibly due to the fact that cardiocirculatory function was not (or not long enough) restored.

This finding is significant because it might imply that increased troponin levels measured after death in individuals deceased after several hours of successful cardiopulmonary resuscitation might be difficult to distinguish from those potentially provoked by genuine myocardial ischemia, whereas they would be in

the clinical setting in view of the specific kinetic pattern of serum troponin after resuscitation and in myocardial infarction.

Some points of our study deserve to be stressed. The first is the fact that, as shown, in forensic cases with postmortem intervals over 48 h, the diagnostic weight of troponin T and I determination is limited, and the measured concentrations in postmortem serum do not reliably indicate the presence of antemortem myocardial ischemia. In practice, most forensic autopsies are performed more than 48 h after death and often the postmortem interval is unknown. Consequently, histology and immunohistochemistry remain irreplaceable basic tools for the diagnosis of myocardial ischemia in these cases.

A second point that deserves to be emphasized is that, to address the second aim of the study, we included hanging and drug intoxication cases as control individuals. The choice of drug intoxication cases might appear problematic, considering that some of these individuals might have died after suffering from prolonged agony, gradual failure of the heart, and insufficient oxygen supply.

It must be highlighted, however, that our study aimed to investigate the stability of NT-proBNP, troponin T, and troponin I in forensic cases having various postmortem intervals, but regardless of the hypoxic mechanism (ischemic or non-ischemic) responsible for the leakage of these compounds into the systemic circulation. In this regard, the choice of drug intoxication cases as control cases did not affect the general outcome of the study.

To conclude, we believe that postmortem use of the biochemical markers of myocardial ischemia requires a fair amount of caution and flexibility, considering the utmost importance of postmortem interval duration before biological fluid collection and sampling site choice when interpreting obtained results.

On the other hand, numerous researchers have emphasized that elevated troponin levels (in both postmortem serum and pericardial fluid) reflect myocardial damage severity from various causes and do not correlate specifically, necessarily or univocally with myocardial ischemia. This information must always be taken into account in order to avoid jumping to categorical conclusions in the absence of detailed arguments from all postmortem investigation findings.

It is always worth remembering that pathologists should never yield to the temptation of establishing direct links between a single (postmortem) biochemical result and a probable cause of death. This is because postmortem biochemistry is a complementary discipline and appears completely useless when considered in isolation from the rest, though it can indeed offer a wealth of information when integrated and interpreted in the context of global findings.

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Table 1

Table 1 summarizes the measured values of troponin T, troponin I, and NT-proBNP in both studied groups (ischemic heart disease, non-ischemic-heart-disease) and subgroups (according to postmortem intervals)

Group / Biomarker	Ischemia 4-24 h	Ischemia 25-48 h	Ischemia 49-72 h	Controls 4-24 h	Controls 25-48 h	Controls 49-72 h
Troponin T (hs-TnT, ng/l)	41-242	46-236	> 1.000	3-44	3-51	> 1.000
Troponin I (µg/l)	0.16-9.16	0.21-8.87	> 100	0.03-0.37	0.03-0.42	> 100
NT-proBNP (ng/l)	< 50 -10.700	< 50 -21.000	< 50 -16.000	< 50 -11.200	< 50 -22.500	< 50 -21.000

Table 2

Table 2 summarizes the main results of former studies performed on this topic

Study (author(s) and year)	Nature of the study	Nature of the analyzed sample	Analyzed biomarker(s)	Main finding(s)
Luna et al. 1982 [1]	Biochemical analysis in postmortem samples (several study groups including myocardial infarction)	Pericardial fluid	CK, LDH and their isoenzymes	CK levels in the pericardial fluid significantly increased in myocardial infarction
Luna et al. 1983 [2]	Biochemical analysis in postmortem samples (several study groups including myocardial infarction)	Pericardial fluid	CK isoenzymes	The highest CK-MB values were found in the pericardial fluid in the myocardial infarction group
Stewart et al. 1984 [3]	Biochemical analysis in postmortem samples (four study groups including cardiac deaths, resuscitation group and no resuscitation group)	Pericardial fluid	CK, LDH and their isoenzymes	Measurements of cardiac enzymes in pericardial fluid may prove useful in establishing the postmortem diagnosis of acute myocardial injury
Osuna et al. 1990 [4]	Biochemical analysis in postmortem samples (cardiac deaths)	Postmortem serum from femoral vein blood Pericardial fluid Vitreous humor	CK CK-MB LDH Myoglobin Myosin Cathepsin D	The sample material of choice for biochemical tests (diagnosis of myocardial infarction) is the pericardial fluid. The most informative markers are myoglobin, myosin, CK-MB and

				cathepsin D.
Burns et al. 1992 [5]	Biochemical analysis in postmortem samples (three study groups including death from myocardial infarction)	Blood from right atrium Blood from a peripheral vein Pericardial fluid	CK and CK isoenzymes	Early myocardial infarction can be identified measuring CK isoenzymes
Perez-Carceles et al. 1995 [6]	Biochemical analysis in postmortem samples (several study groups including myocardial infarction)	Postmortem serum from femoral vein blood Pericardial fluid Vitreous humor Postmortem serum from femoral vein blood Pericardial fluid Pericardial fluid	Myoglobin CK CK-MB LDH Myosin Cathepsin D	The highest levels of CK, CK-MB, and LDH were observed in the pericardial fluid in cases with morphological evidence of myocardial ischemia
Perez-Carceles et al. 1995 [7]	Biochemical analysis in postmortem samples (several study groups including myocardial infarction)	Postmortem serum from femoral blood Pericardial fluid	CK-MB Myosin	The highest levels of myosin were observed in serum and pericardial fluid in subjects who showed morphological evidence of myocardial ischemia
Osuna et al. 1998 [8]	Biochemical analysis in postmortem samples (several study groups including myocardial infarction)	Pericardial fluid and postmortem serum from femoral vein blood	cTnI Myosin Myoglobin CK-MB	The highest levels of all studied markers were observed in the pericardial fluid in subjects who had died from myocardial infarction
Osuna et al. 1998 [9]	Biochemical analysis in postmortem samples (several study groups including myocardial infarction)	Pericardial fluid and postmortem serum from femoral vein blood	Myoglobin Myosin CK-MB	The highest levels of myosin pericardial fluid were found in cases with morphological signs of myocardial infarction. The highest ration of pericardial fluid to serum myosin and myoglobin concentrations was found in the group of subjects who died of myocardial infarction

Cina et al. 1998 [10]	Biochemical analysis in postmortem samples (cardiac-related deaths vs non-cardiac-related deaths)	Subclavian venous blood	CK-MB cTnI	Statistically significant differences between cardiac and non-cardiac causes of death in postmortem serum levels of both markers (cTnI concentrations > 40 ng/ml only in cardiac deaths)
Dressler et al. 1998 [11]	Biochemical analysis in postmortem samples (heart contusion vs resuscitation by heart massage)	Whole blood and serum	cTnT (qualitative quick test analysis + quantitative ELISA analysis)	Statistically significant differences between heart contusion vs heart massage
Cina et al. 1999 [12]	Biochemical analysis in postmortem samples (cardiac-related deaths vs non-cardiac-related deaths)	Pericardial fluid	cTnI	cTnI levels in the pericardial fluid up to six orders of magnitude above levels expected in serum. Cardiopulmonary resuscitation did not influence pericardial fluid cTnI levels
Cina et al. 2001 [13]	Biochemical analysis in postmortem samples (cardiac deaths vs control cases)	Subclavian or femoral vein blood sampled at the time of external examination	cTnT (rapid assay)	Positive results for cTnT in most cardiac cases. Cardiopulmonary resuscitation did not appear to result in false positive cases.
Ellingsen and Hetland 2004 [14]	Biochemical analysis in postmortem samples (forensic and non-forensic cases including acute myocardial damage cases)	Postmortem serum from femoral blood	cTnT	cTnT may serve as a valuable tool to support the diagnosis of cardiac-related deaths in cases with inconclusive postmortem morphological findings
Pérez-Carceles et al. 2004 [15]	Biochemical analysis in postmortem samples (ischemic heart disease vs control cases)	Pericardial fluid and postmortem serum from femoral venous blood	cTnI myoglobin CK-MB	Statistically significant differences in pericardial fluid for all the biochemical markers in ischemic heart disease cases. No statistically significant correlations between the levels of the analyzed biomarkers and the

				postmortem interval or the use of cardiopulmonary resuscitation.
Martinez-Sanchez and Rodriguez-Vicente 2004 [16]	Biochemical analysis in postmortem samples (three sudden death study groups including myocardial infarction)	Plasma Pericardial fluid Vitreous humor	LDH CK-NAC CK-MB	LDH, CK-NAC and CK-MB measurement in plasma, pericardial fluid and vitreous did not provide valuable additional information in cases of sudden death. CK-NAC and CK-MB values in pericardial fluid were more elevated in cases of atheromatous occlusive coronary artery disease without myocardial infarction. CK-NAC and CK-MB values in vitreous were more elevated in cases of myocardial infarction
Davies et al. 2005 [17]	Comparison antemortem – postmortem	Antemortem samples vs postmortem blood samples (superior vena cava, iliac veins, heart, femoral veins)	cTnT cTnI	Erratic results bearing little or no relation between antemortem and postmortem levels
Martinez-Diaz et al. 2005 [18]	Postmortem study: biochemistry, histology, immunohistochemistry	Pericardial fluid Postmortem serum from femoral veins	cTnI Myoglobin CK-MB	Statistically significant differences for all the studied markers in the pericardial fluid. The highest levels in the group of cases who have died from myocardial infarction
Zhu et al. 2006 [19]	Biochemical analysis in postmortem samples (several study groups including acute myocardial infarction)	Pericardial fluid Blood from left and right heart chambers as well as external iliac vein	cTnT	Elevated postmortem serum and pericardial cTnT levels occur depending on the severity of myocardial damage from various causes, survival time and postmortem interval

Zhu et al. 2006 [20]	Biochemical analysis in postmortem samples (several study groups including acute myocardial infarction)	Pericardial fluid Blood from left and right heart chambers as well as external iliac vein	cTnT	Elevation in postmortem serum and pericardial fluid cTnT levels in sudden death may depend on the severity of ischemic myocardial damage, and on the postmortem interval for heart blood and pericardial fluid levels
Ben Khalifa et al. 2006 [21]	Biochemical analysis in postmortem samples (sudden cardiac deaths vs non-cardiac death without resuscitation)	Subclavian venous blood	cTnT	Increased cTnT levels in all the cases with myocardial infarction. Differences between resuscitated and non-resuscitated cases were not statistically significant
Peter et al. 2006 [22]	Biochemical analysis in postmortem samples (cardiac contusion and control cases)	Cardiac blood and blood from femoral veins	Troponin I, T and C	Troponin values in cardiac blood much higher than those measured in the venous blood. Significant positive correlation autolysis – cardiac blood troponin T value. No correlation (femoral) autolysis - venous blood troponin values.
Zhu et al. 2007 [23]	Biochemical analysis in postmortem samples (several study groups including myocardial infarction and ischemic heart disease)	Pericardial fluid Blood from left and right heart chambers as well as external iliac vein	cTnI CK-MB	Postmortem time-dependent elevation of both markers in cardiac blood, peripheral blood, and pericardial fluid. Elevated cTnI and CK-MB levels in blood and pericardial fluid are related to ischemic, hypoxic, and/or cytotoxic myocardial damage
Sidlo e al. 2008 [24]	Biochemical analysis in postmortem samples (sudden cardiac deaths vs control cases)	Blood from left cardiac ventricle and right femoral vein	cTnI pro-ANP	Blood cTnI and pro-ANP levels are not useful for the postmortem diagnosis of myocardial damage
Vargas et al. 2008 [25]	Biochemical analysis in postmortem samples (four study groups including recent myocardial infarction,	Postmortem serum from femoral blood	cTnI	cTnI did not correlate specifically with ischemia or infarction. Levels appeared to correlate significantly

	chronic ischemic heart disease, and no heart disease cases)			with intramyocardial injury
Maeda et al. 2009 [26]	Biochemical analysis in postmortem samples (several study groups including acute myocardial infarction and ischemic heart disease)	Cerebrospinal fluid	cTnI cTnT CK-MB	Cerebrospinal fluid levels of cTnT and cTnI are useful for investigating process and duration of myocardial damage in the death process. CK-MB marker of persistent hypoxic myocardial damage before death
Batalis et al. 2010 [27]	Biochemical analysis in postmortem samples (ischemic heart disease vs control cases)	Pericardial fluid and blood from multiples sites (femoral veins, subclavian veins, aorta, right ventricle, left ventricle)	CK CK-MB cTnI	Three anatomic site/analyte combinations were statistically significant: cTnI-femoral vein blood, CK-MB-right ventricle blood, CK-MB-pericardial fluid
Wang et al. 2011 [28]	Biochemical analysis in postmortem samples (several study groups including acute cardiac deaths)	Pericardial and cerebrospinal fluids	CK-MB cTnI Myoglobin	Combined analysis of CK-MB, cTnI and myoglobin in pericardial and cerebrospinal fluids are helpful for evaluating the severity of myocardial/skeletal muscle damage in death process
Sapouna et al. 2013 [29]	Biochemical analysis in postmortem samples (ischemic heart disease vs control cases)	Pericardial fluid	cTnI	Statistically significant differences in pericardial fluid cTnI in ischemic heart disease cases
Remmer et al. 2013 [30]	Biochemical analysis in postmortem samples (several study groups including cardiovascular disease)	Pericardial fluid Postmortem serum from femoral/iliac veins	cTnT	Overlapping values between the studied groups making difficult to apply results in individual cases. Postmortem time-dependent elevations in cTnT levels have to be taken into consideration
Ghormade et al. 2014 [31]	Biochemical analysis in postmortem samples (several study groups	Pericardial fluid	CK-MB LDH	The role of CK-MB in pericardial fluid as a biochemical marker for

	including sudden cardiac death due to ischemic heart disease)			definite detection of myocardial infarction in cases of sudden cardiac death is limited. LDH is not useful for postmortem diagnosis of myocardial infarction
Ghormade et al. 2014 [32]	Biochemical analysis in postmortem samples (several study groups including sudden cardiac death due to ischemic heart disease)	Pericardial fluid	CK-MB	The highest levels of CK-MB in pericardial fluid were noted in deaths due to ischemic heart disease
Chen et al. 2015 [33]	Biochemical analysis in postmortem samples (several study groups including acute myocardial infarction)	Cardiac blood Peripheral external iliac venous blood Pericardial fluid Cerebrospinal fluid	CK-MB cTnI cTnT	Cause of death and postmortem time dependent differences. No differences between myocardial infarction and other causes of death. Elevated levels are not a specific finding for ischemic heart disease death but indicate the severity of myocardial injury.
Gonzalez-Herrera et al. 2016 [34]	Biochemical analysis in postmortem samples (several study groups including sudden cardiac death)	Postmortem serum from femoral blood Pericardial fluid	cTnT (highly sensitive assay)	cTnT levels stable in both postmortem serum from femoral blood and pericardial fluid for up to 34 h after death. Markedly higher levels in both postmortem samples than in <i>in vivo</i> samples. Low cTnT levels in pericardial fluid may exclude cardiac damage.
Carvajal-Zarrabal et al. 2017 [35]	Biochemical analysis in postmortem samples (sudden cardiac death cases vs control cases)	Blood sampled from the pulmonary vein	CK-MB myoglobin cTnI cTnT LDH	Postmortem levels of cTnI and cTnT are useful to support a sudden cardiac death diagnosis in the first 12 h after death