Chapter

Use of Cardiac Troponin for the Diagnosis of Cardiac Pathology in Postmortem Samples Taken at Autopsy

David C. Gaze

Abstract

The diagnosis of acute cardiac pathology is a clinical challenge in both the living and in the postmortem setting. Cardiac troponin (cTn) T and cardiac troponin I released from the contractile apparatus of cardiomyocytes into the circulation can be detected by sensitive and specific immunoassays and are the gold standard biochemical test for diagnosis of acute coronary syndromes (ACS). Recently with the advent of more sensitive detection methods, elevation in non-ACS has become apparent causing clinical confusion. In most cases, these elevations are related to subclinical cardiac damage and often confer poor prognosis in cTn-positive patients. Biomarkers of cardiomyocyte damage may be of value in routine hospital and medico-legal autopsy. A significant body of evidence has emerged since the late 1990s, assessing the clinical utility of cardiac troponin in biological fluids or in immunohistochemical staining of cardiac tissue to aid in the diagnosis of acute cardiac pathology when standard microscopic evidence is inconclusive. This chapter reviews the extensive literature on the subject and details the disparity between pericardial fluid and serum for the use of cTn in the postmortem setting.

Keywords: cardiovascular disease, risk, diagnostics, therapeutic intervention, treatment, prediction

1. Introduction

Cardiac troponins (cTn) T (cTnT) and I (cTnI) are the gold standard biochemical markers used to identify acute cardiac pathologies in patients who present with typical and atypical chest pain to the emergency department. These muscle-associated proteins confer superior diagnostic and prognostic ability compared to conventional nonspecific muscle derived enzyme markers such as creatine kinase (CK), its MB isoform (CK-MB), or myoglobin. Cardiac troponin determination is central to the diagnosis of non-ST segment elevation acute myocardial infarction (NSTEMI), contributing to international guidelines for diagnosis and management of NSTEMI patients [1].

Recent advancement in laboratory technology driven both by clinical demand and the commercial *in vitro* diagnostic market has seen the emergence of highly analytically sensitive immunoassays for the termination of cTnT and cTnI in biological samples, mainly serum and plasma. The role out and increasing popularity of the sensitive methods have introduced new clinical challenges, notably defining acceptable reference intervals in the apparently healthy population, sex-specific cut-off values and novel clinical roles in non-acute cardiac diseases where often secondary underlying cardiac disease is present [2].

One area of interest has been the potential value of cTnT and cTnI in the postmortem setting and may provide insight into the cause of death. Troponin analysis in postmortem blood and pericardial fluid during autopsy investigations can potentially help medical examiners and forensic pathologists attribute what happened before, during, and after a death. This chapter will explore the use of cardiac troponin in the postmortem setting, from its application in routine hospital as well as medico-legal autopsy and forensics, assessing the usefulness in offering a clearer picture of an individual's final moments.

2. Clinical utility of cardiac troponin in myocardial damage

Cardiac-specific isoforms of the contractile protein complex troponin, namely cTnT and cTnI, are released into the bloodstream following damage to cardiomyocytes. The mechanism by which these structural proteins are released into the circulation has been debated significantly over many years. Initially, it was thought that cTn could only be released following overt cellular necrosis; however, recently it has been suggested that release can occur in ischemia without necrosis [3]. A review of the subject by Ragusa and colleagues suggest release mechanisms, including apoptosis, necroptosis, physiological cardiomyocyte renewal, and cellular wounding can contribute to cTn release as well as necrosis [4]. An immunohistochemical study using a canine model of coronary occlusion ranging from 30 minutes to 6 hours demonstrated variable loss of both cTnT and cTnI in paraffin-embedded left ventricular myocardial sections [5]. Loss was variable but more so for cTnT than for cTnI, and loss was greater at the periphery of the infarct area rather than the centralised region (**Figure 1**).

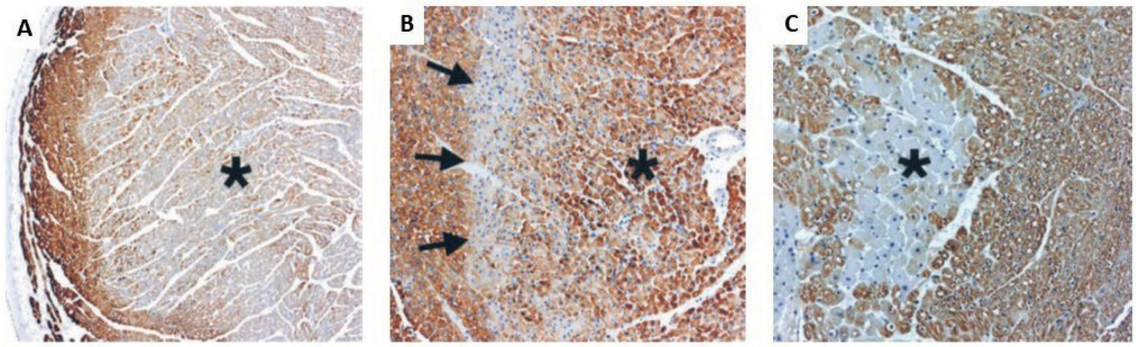

Figure 1.
Canine left ventricular myocardial tissue following 6 h of coronary artery occlusion. Immunohistochemical staining of (A) cTnI demonstrating decreased but non-uniform staining in the central necrotic area (asterisk); (B) cTnT demonstrating loss at edge of infarct zone (arrows) and (C) canine left ventricular myocardial tissue following 45 min of coronary artery occlusion demonstrating loss of cTnI in the zone of necrosis (asterisk) (source: Adapted from [5]).

Using monoclonal antibodies specific to the cardiac isoforms, immunoassay technologies can quantify the amount of cTnT or cTnI in a biological matrix [6]. Initially, early immunoassays utilised high clinical cut-off values (high specificity and low sensitivity) allowed the separation of patients with overt acute myocardial infarction (AMI) from apparently healthy persons who were deemed negative for cTn based on the equivalent cTnT or cTnI concentration to the then-used gold standard tests (CK or CK-MB). Subsequently, the large body of evidence demonstrating elevation of CK and CK-MB in the absence of an elevated cTn questioned the cardio-specificity of the enzyme markers, along with approximately 30% of patients ruled out with AMI

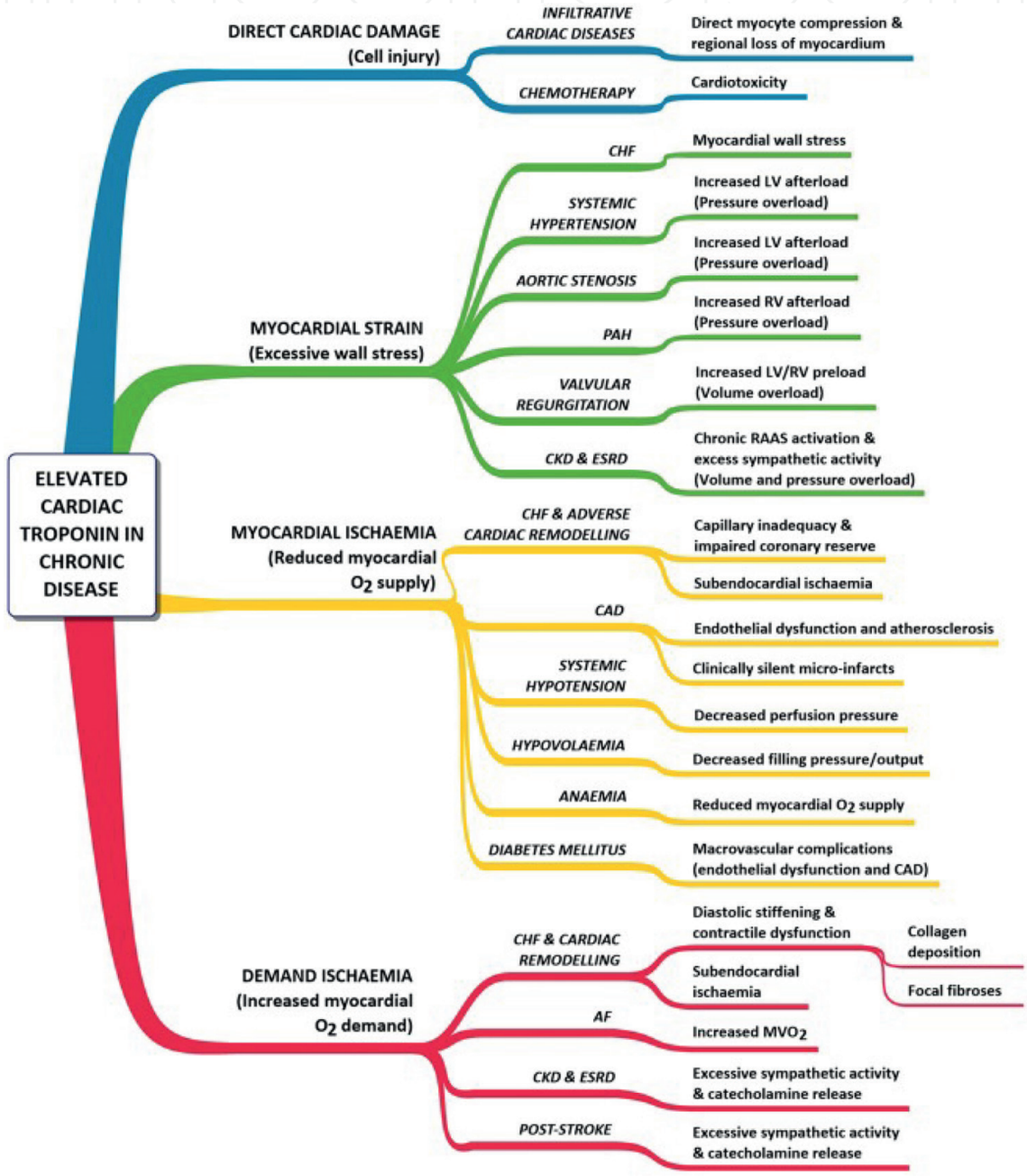

Figure 2.
Categories of cardiac troponin release in acute and chronic diseases. All conditions have documented evidence of elevated cTn. AF, atrial fibrillation; CAD, coronary artery disease; CHF, chronic heart failure; CKD, chronic kidney disease; ESRD end-stage renal disease; LV left ventricle; MVO2, myocardial oxygen consumption; PAH, pulmonary artery hypertension; RAAS, renin-angiotensin-aldosterone system; RV, right ventricle (source: [2], with authors permission).

demonstrating positive cTn which is associated with poor prognosis, resulted in the adoption of cTn as the gold standard test for diagnosis of AMI [6].

Integral to the adoption of cTn was the appropriate definition of cut-off to confer an abnormal concentration. This was subsequently defined as the 99th percentile value of an apparently healthy population. When adopted into routine clinical practice, this lowered the sensitivity of the assays allowing early diagnosis in the evolving infarction but at the cost of specificity. Initially, this caused clinical confusion with a larger number of patients presenting with low concentrations of cTn just above the AMI cut-off value, but further research of such patients found the presence of comorbid conditions (**Figure 2**) often with underlying cardiovascular pathophysiology [2].

3. Biochemical testing in assisting cause of death at postmortem

Biochemical testing in postmortem investigations (termed thanatochemistry, necrochemistry or the chemistry of death) was initially established in the early 1950s, and a great number of biochemical analytes have proved an adjunctive tool to assist the cause of death at postmortem [7]. Adoption of biochemical testing especially in the medico-legal forensic autopsy has often been limited. The determination of death may have significant impact on those directly or indirectly involved in the death of an individual and can carry a custodial sentence. Thus, the scientific evidence presented in court is intensely scrutinised both by the prosecution and defence counsels. Whilst no biochemical test is infallible, many are associated with the likelihood of a disease process rather than a definitive diagnosis of the disease. Often the barrier to use is the interpretation of results of biochemical assays from cadaveric sampling, hindered by the lack of reference normality in death; thus, results are compared to reference intervals generated in the living [7, 8] with few studies demonstrating corresponding histopathological findings to the biochemical results. Interpretation is further complicated by factors such as postmortem interference in the assay technology, appropriate sampling matrices, postmortem autolysis, microbial metabolism, fluid redistribution, and postmortem interval (PMI). Molecular biophysical properties such as molecular weight, structure, intracellular location, electrical charge, ionic strength, protein affinity, and cell membrane permeability may differ between life and death and can influence interpretation in both situations [7].

There are a number of fluid components which are suitable for cadaveric biochemical testing, namely vitreous humour from the posterior segment of the eye, cerebral spinal fluid (CSF), synovial fluid, pericardial fluid (PCF), venous femoral blood, venous jugular blood, peripheral blood sampling, urine, gastric contents and right ventricle heart whole blood [7–9]. Analytes and potential uses in postmortem samples are listed in **Table 1**.

4. Conventional cardiac biomarkers at postmortem

The importance of cardiac biomarkers assisting in postmortem diagnosis was highlighted in cases where a suspected myocardial lesion cannot be diagnosed by routine histological analysis. They were utilised initially for the determination of sudden cardiac death. Initially, CK and lactate dehydrogenase isoenzyme analysis of pericardial fluid was utilised [10, 11], followed by K:Na ratio [12]; CK isoenzymes,

Analyte	Sample matrix	Forensic utility
Adrenaline:Noradrenaline	Urine	Hypothermia
Acetone	Blood	Chronic alcohol abuse, hypothermia, diabetic ketoacidosis
Ammonia	Vitreous humour	Liver failure
Carbohydrate-deficient transferrin	Vitreous humour	Chronic alcohol abuse
Chloride	Vitreous humour	Saline poisoning, salt water drowning, dehydration
Chymase activity	Blood	Anaphylactic shock
Chromogranin A	Blood, CSF	Hypothermia
C-reactive protein	Blood	Recent infection, trauma, burns, ketoacidosis, malignancy, autoimmune diseases, inflammatory diseases, sepsis
Creatine Kinase & CK-MB	Blood	Cardiac pathology
Creatine Kinase-BB	CSF	Cerebral trauma, cerebral hypoxia
Creatinine	Vitreous humour	AKI, CKD, high-protein diet, large muscle mass (anabolic steroid abuse), heat shock
Ethyl glucuronide	Vitreous humour, Urine	Antemortem ethanol ingestion
Free fatty acids	Blood	Hypothermia
Fructoasmine	Vitreous humour	Diabetic ketoacidosis
Glucocortcoids	Blood	Hypothermia
Hypoxanthine	Vitreous humour	Time of death
Myoglobin	Blood, Urine	Hyperthermia, cardiac pathology
Neurone specific enolase	CSF	Cerebral traumatic injury, cerebral hypoxia
Potassium	Vitreous humour	Postmortem decomposition
S-100b	CSF	Cerebral injury
Thyroglobulin/fT3	Vitreous humour, Blood	Neck trauma, strangulation
Troponin	Pericardial fluid, Blood	Cardiac pathology
Tryptase	Blood	Anaphylactic shock
Urea	Vitreous humour	Renal dysfunction, upper GI haemorrhage

AKI, acute kidney injury; CKD, chronic kidney disease; CK-MB, creatine kinase-MB isoform, CK-BB; creatine kinase-BB isoform; CSF, cerebrospinal fluid.

Table 1.
Biochemical analytes, sample matrices, and potential forensic utility.

aspartate aminotransferase and hydroxybutyrate dehydrogenase [13] and myosin and cathepsin D, a lysosomal aspartyl protease that degrades proteins [14, 15].

5. Cardiac troponin analysis at postmortem

The first reported use of cTn analysis was in 1998 by Osuna and colleagues who studied 89 cadavers with a mean age of 51.38 ± 2.04y [16]. Subjects were assigned

between four groups, MI (n = 25), asphyxia (n = 30), cranio/multiple trauma (n = 17), and other natural deaths (n = 17). MI was determined by H&E and acridine orange histological staining. The research group determined the concentration of myoglobin, myosin, CK-MB, and cTnI in femoral vein serum samples and PCF in each case. PCF concentrations for all makers were significantly different between each outcome group; however, only myoglobin and myosin demonstrated significance in serum. The PCF cTnI values were higher than serum samples when using the Sanofi Diagnostic Pasteur assay. Values in both matrices were higher in MI patients compared to the other three groups (mean (range) Pericardial cTnI [pg/L]): 2.4 (0.3–6.5); 1.7 (0.03–3.7); 1.1 (0.01–2.3); 0.4 (0.0–1.8) in each group, respectively.

Cina and colleagues [17] demonstrated the utility of cTnT using the then available commercial Cardiac Rapid T (cTnT) lateral flow test from Roche Diagnostics. This device allows testing in the autopsy suite at the time of postmortem with qualitative results (positive or negative test lines) at 15 minutes from sample application. In 40 autopsy cases, 20 were deemed cardiac deaths and 20 were controls (noncardiac related) deaths, diagnosed by gross pathology and histological analysis. 85% (n = 17) of subclavian or femoral blood samples in the cardiac death group were positive for cTnT which was significantly different to control group, where 30% (n = 6) of serum samples were positive for cTnT. The authors deemed these as false-positive results. In subjects aged over 50 years, sensitivity and specificity of cTnT for diagnosis of AMI were 91% and 86%, respectively. The authors noted however the assay was ineffective in frozen blood samples or those with significant haemolysis which was evident at a PMI of >24 h. A similar study of 100 autopsy cases of sudden unexplained death (SUD) was carried out in Chaing Mai, Thailand, and utilised the same rapid cTnT assay [18]. Fifty-two of the deaths were considered cardiac with 20 due to sudden MI and 32 with evidence of old infarction or arrhythmic fibrosis (n = 22), coronary atherosclerosis, >75% luminal without evidence of fibrosis or thrombosis (n = 3), cardiomegaly, and heart weight > 400 g (n = 7)) or related to cardiac injury as a result of toxic substances. Thus, subjects were assigned to other cardiac death (SCD), non-cardiac natural death (NCD) or non-natural death (NND). The percentage positivity rate was higher in subclavian blood than femoral blood samples in all three groups. Subclavian blood sensitivity and specificity for SUD were 87.5% and 47%, respectively. Similar to the findings of Cina and colleagues, false-positive rates were associated with increasing PMI.

Davies and colleagues were the first to compare antemortem (<48 h before death) and postmortem concentrations of cTnT (Roche Elecsys 3rd generation electrochemiluminescent assay) and cTnI (Stratus CS fluorometric assay, Dade-Behring [now Siemens healthineers]) in five hospital-based autopsies [19]. One patient suffered cardiac death (myocarditis) with the remaining four were non-cardiac, but moribund before death. Results obtained between antemortem and postmortem samples were erratic. Four of the five subjects (n = 80%) had elevated antemortem cTnT and cTnI samples. The authors concluded postmortem cTn analysis in blood was not suitable due to lack of correlation of cause of death; however, they suggested that elevated antemortem cTn was related to all-cause mortality in those at end of life. A similar conclusion was made by Rahimi and colleagues in 2018 after studying 140 natural and unnatural deaths in Malaysia [20]. Subjects were classified into five groups: cardiovascular death, sudden unexplained death, thoracic trauma, non-thoracic trauma, and other diseases. Median jugular/subclavian/femoral blood cTnT (Roche Elecsys 3rd generation electrochemiluminescent assay) concentrations were 0.51, 0.17, 0.62, 0.90,

and 0.51 μg/L, respectively, with no significant difference (p= > 0.05) in relation to cause of death. The authors concluded cTnT lacked specificity in postmortem sampling and is therefore not a useful tool.

Lai and colleagues also compared antemortem and postmortem blood sampling. Demonstrating in four cases, a marked proportionate rise in cTnT in postmortem samples compared to antemortem samples. Interestingly, the authors found cTnT values were higher (mean cTnT 5.32 μg/L) in non-cardiac deaths compared to 4.91 μg/L in cardiac-related deaths [21].

In 2006, Zhu and co-workers published two seminal papers in *Legal Medicine* examining the value of postmortem cTnT in cardiac, peripheral blood and PCF in relation to traumatic causes of death [22] and sudden cardiac death pathology [23] in medicolegal autopsies. In traumatic death (n = 405) due to blunt/sharp instrument injury, asphyxiation, drowning, fatal fire, hyper and hypothermia, and toxic poisoning are due to metamphetamine or carbon monoxide. Cardiac blood and PCF cTnT values were lower where PMI was <12 h compared cases where PMI ranged from 12 to 48 h. Elevated cTnT was associated, however, with histological evidence of advanced myocardial damage involving swelling and liquefactive necrosis [22]. In their cohort of sudden cardiac death autopsies (n = 96), 35% were due to AMI, 24% due to recurrent MI, 25% ischemic heart disease without infarction and 16% due to other cardiac causes. The comparative control group (n = 75) consisted of 47% asphyxiation, 36% drowning and 17% cerebrovascular diseases. In agreement with their first study, pericardial cTnT concentrations were higher than blood samples. Differences in cTnT concentrations related to pathological evidence of cardiac damage differed between early (<12 h) and late (12–48 h) postmortem period, concluding that elevations in blood and PCF are dependent not only on the severity of myocardial damage (determined by infarct size, intensity of lesions, interstitial haemorrhage and necrosis) but also by the PMI [22].

Remmer and colleagues [24] focused on postmortem serum and PCF cTnT in relation to PMI from 101 forensic autopsy cases in Estonia. PMI ranged from 8 h to 141 h. Although differences in cTnT were observed between five groups of cause of death (cardiovascular disease; other disease; poisoning; asphyxia; drowning; hypothermia; thoracic trauma, other trauma and fatal fires), significant attention to PMI (**Figure 3**) is important rather than comorbid cardiovascular disease.

In addition to the effects of PMI as demonstrated above, Kumar et al. used SDS-PAGE and Western blotting of cardiac tissue extracts from 10 medicolegal autopsies of burns cases [25]. The authors demonstrated a pattern of cTnT degradation in a time-dependent manner at room temperature (7.3 h, 18.2 h, 30.3 h, 41.2 h, 41.4 h, 54.3 h, 65.2 h and 88.4 h), demonstrating the disappearance of intact cTnT protein and the increasing presence of low-molecular-weight bands related to time (**Figure 4a**). Furthermore, the groups have examined degradation patterns according to the cause of death (**Figure 4b–d**) [26].

A study of 20 autopsies of sudden cardiac death and 8 controls (violent non-cardiac deaths) demonstrated significantly higher cTnT and cTnI concentrations in pulmonary venous blood. Mean ± SD cTnT were 1826 ± 363 μg/L versus 65 ± 11 μg/L, respectively, and for cTnI were 28 ± 3 mg/L versus 0.14 ± 0.02 μg/L; however, it should be noted that the PMI in all cases was 8 h [27].

The value of cTn as a biochemical marker in relation to sudden cardiac death has been the subject of a systematic review [28] and formal meta-analysis [29]. Whilst both reviews demonstrate the elevation of cTn to be higher in pericardial fluid compared to blood sampling in the postmortem setting (**Figure 5**), blood is

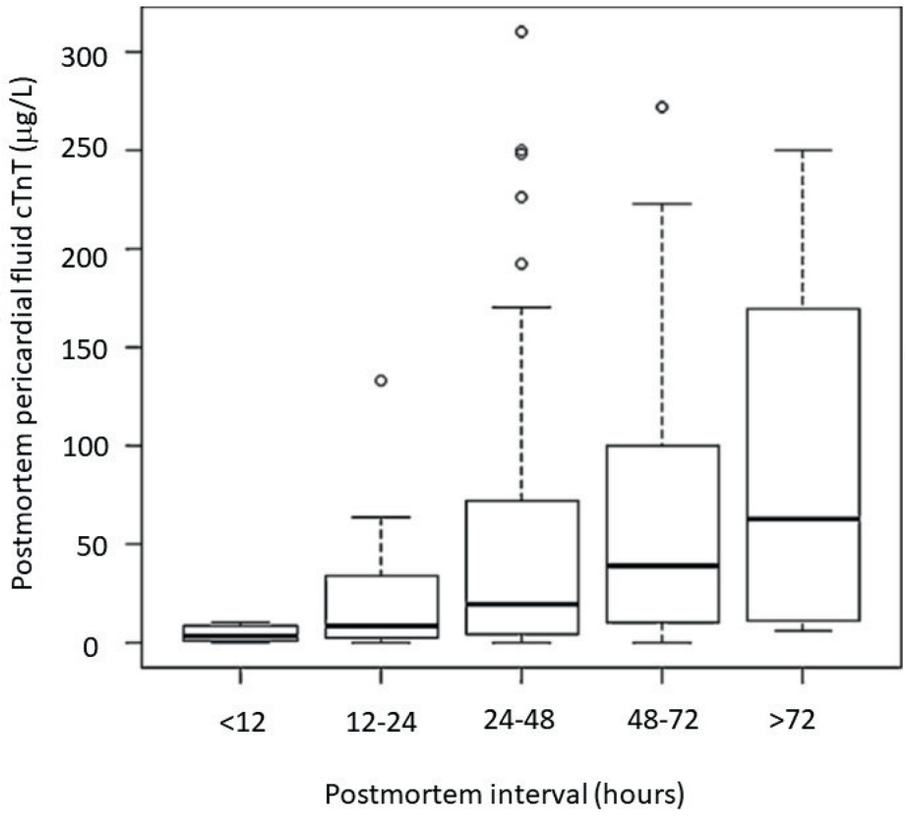

Figure 3.
Cardiac troponin T (cTnT) concentration in relation to postmortem interval in 101 medicolegal autopsies. Cause of death were due to cardiovascular disease, other disease, poisoning, asphyxia, drowning, hypothermia, thoracic and non-thoracic trauma and fatal fires. (source: Adapted from [24]).

susceptible to the effects of haemolysis, postmortem interval, autolysis, and potential bacterial interferences. Pericardial fluid is therefore the preferred sample of choice.

Both reviews also address the issue of cut-off values demonstrating significant difference to cut-off values in the living. Non-cardiac deaths often demonstrate significantly positive cTn values in PCF and blood, thus questioning the sensitivity and specificity at postmortem. Barberi and van den Hondel suggest that more work is required to determine the appropriate cut-off values at postmortem [28].

6. Correlation between postmortem cardiac troponin and histological evidence of cardiomyocyte necrosis

At postmortem, the diagnosis of myocardial infarction is typically assessed by gross macroscopic anatomy further confirmed by microscopic histology and immunohistochemical analysis. Defining AMI in a medico-legal autopsy is a clinical challenge for the forensic pathologist as detection can only be made 4–6 hours after the onset of cardiac ischemia. Histological changes indicative of AMI include oedema, congestion, haemorrhage, inflammation cytoplasmic vacuoles, contraction band alterations, fibrosis and necrosis. Immunohistochemical analysis at postmortem has focused on a number of markers of cellular damage, including C5b-9, myoglobin, CK-MB, fibronectin myosin heart-type fatty acid binding protein and desmin [30].

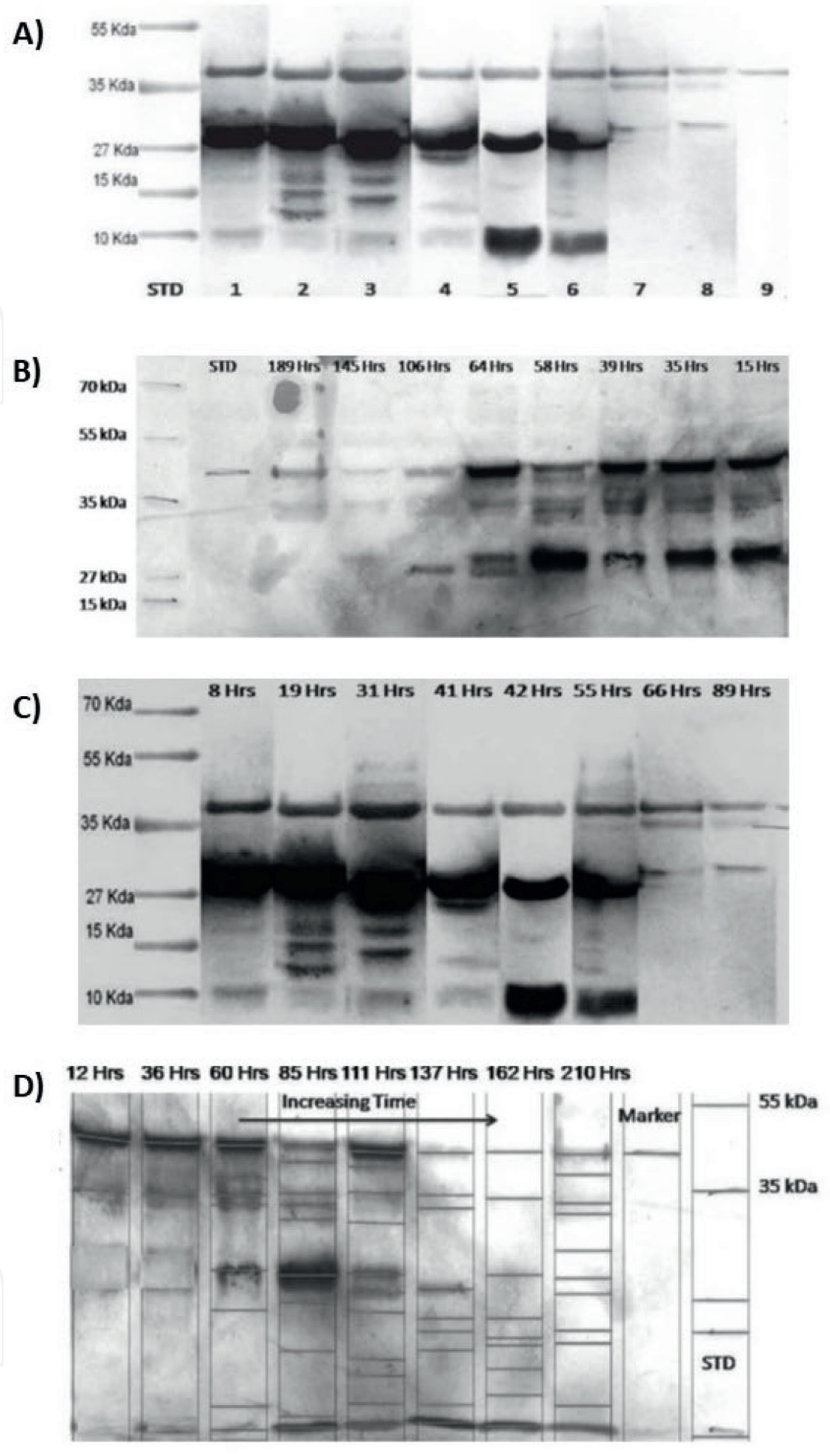

Figure 4.
Cardiac troponin T (cTnT) degradation patterns (Western blotting) in (a) fatal burn; (b) myocardial infarction; (c) electrocution; (d) asphyxiation (source: Adapted from (a) [25]; b–d [26]).

A number of studies have evaluated postmortem cTn concentrations in relation to evidence of cardiomyocyte necrosis (**Figure 6**). Ortmann and colleagues identified antigen depletion in the detection of early ischemic cardiac lesions in 8 cases of AMI, 8 cases of sudden cardiac death and 12 cases of acute exogenic hypoxia due to hanging or carbon monoxide poisoning. Strong evidence of immunohistochemical depletion

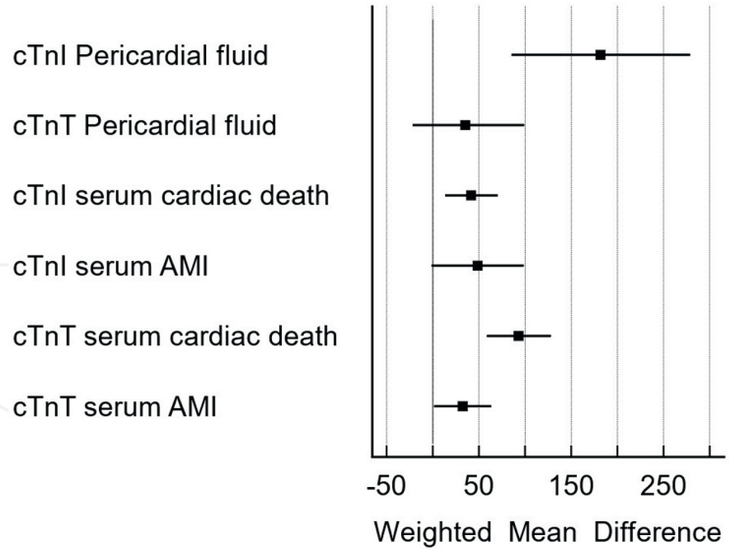

Figure 5.
Meta-analysis of pericardial fluid and serum cardiac troponin I, cTnI; or cardiac troponin T, cTnT; in the investigation of cardiac death and acute myocardial infarction, AMI (source: data extracted from [28]).

of cTnT was evident in all eight cases of AMI, in 50% of sudden cardiac death and in 1 (8%) of acute exogenic hypoxia, with 42% demonstrating weak loss and 50% demonstrating negative results (no loss of cTnT staining) [30].

Martinez Diaz and colleagues have demonstrated immunohistological changes in cTn in AMI or multiple trauma compared to other causes of death. PCF cTnI, myoglobin and CKMB were all significantly higher in AMI or multiple trauma cases compared to other causes of death. Serum concentrations of cTnI myoglobin and CKMB were not significantly different between the two groups [31]. Immunohistological analysis was performed by the authors with the analysis of troponin C and cTnT staining. 86% of cases demonstrated strong positive TnC with expression differing in isolated cells demonstrating contraction band necrosis but with significantly less intensity in the area of the infarction. cTnT staining was less evident in only 46% of cases in focal areas of the tissue.

Campobasso et al. [34] compared 4 immunohistochemical markers as early indicators of myocardial ischemia in 18 sudden cardiac deaths (4 AMI,4 coronary deaths, 8 acute cardiac deaths compared to 6 cases of acute traumatic death gunshot wounds with immediate lethal head injury). The authors stained paraffin-embedded myocardial tissue and immunohistochemically stained the tissue for C5b-9, fibronectin, myoglobin and cTnI. Diffuse depletion of cTnI was evident in all AMI deaths, in 75% of acute cardiac deaths, with 50% of coronary deaths demonstrating limited cellular foci depletion and normal distribution in all six cases of acute traumatic death. Whilst the staining patterns were significantly different between the cardiac and non-cardiac deaths, the authors concluded that no single marker was able to detect early myocardial ischemia and the combination of all four markers was useful in demonstrating evidence of myocardial ischemia and/or necrosis [34].

More recently, Amin and co-workers stained histological sections from ischemic and non-ischemic cardiac tissue for cTnT, myoglobin and caspase-3, demonstrating cTnT detection in normal myocardium and loss in necrotic tissue. The loss of cTnT was non-uniform with greater loss at the periphery compared to the central regions of infarcted tissue [35].

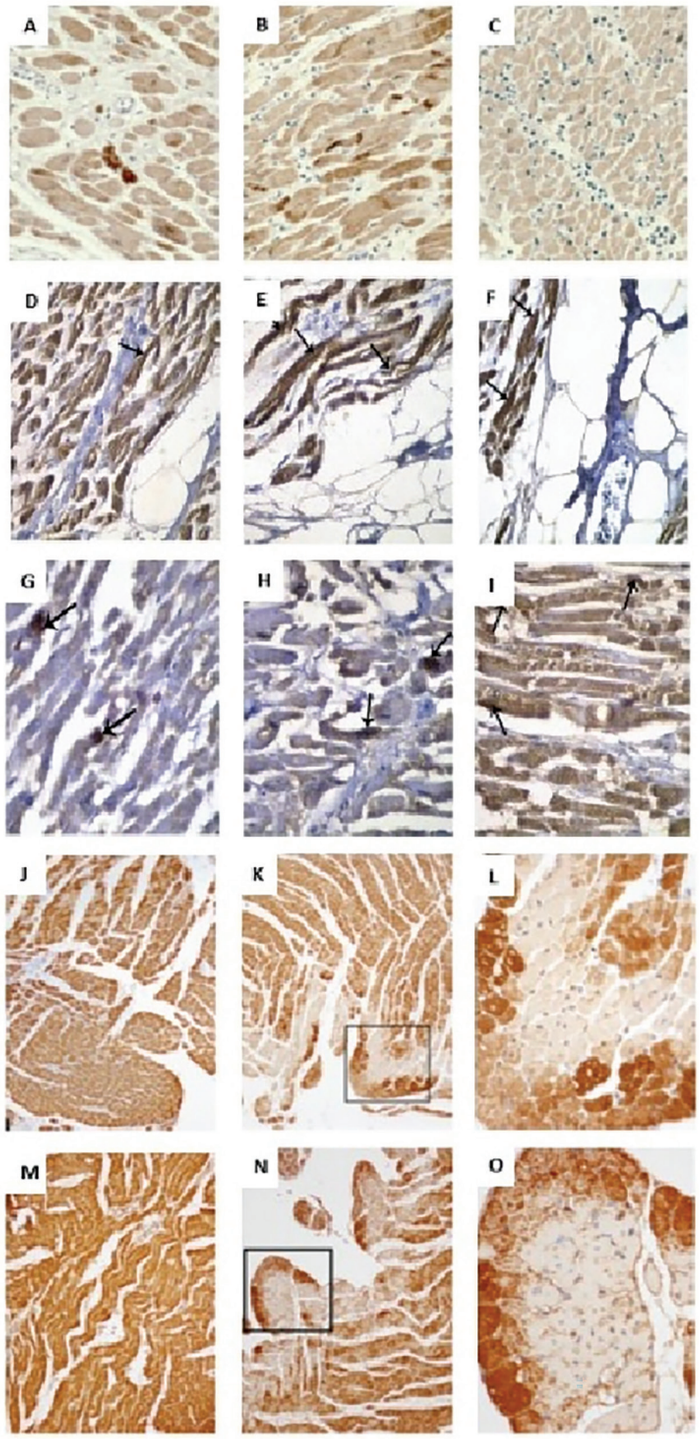

Figure 6
Immunohistochemical staining of cardiac troponin: (A) TnC in isolated cells with evidence of necrosis ×325; (B) TnC in contraction band necrosis ×300; (C) TnC in infarction zone ×200; (D–F) TnC in myocardium from sudden cardiac death due to coronary atherosclerosis. Brown expression (arrow) increases with PMI where (D) 1st PMI, (E) 2nd PMI, (F) 3rd PMI; (G–I) TnC in myocardium from sudden cardiac death due to myocardial infarction. Brown expression (arrow) increases with PMI where (G) 1st PMI, (H) 2nd PMI, (I) 3rd PMI; (J–L) cTnI immunohistochemical staining of the anteriolateral right ventricle in (J) non-ischemic cardiac tissue demonstrating no depletion in cTnI ×10; (K) 1 hour of LAD ligation demonstrating cTnI depletion in the subendocardial region [square box] ×10; (L) magnified box area from section K ×40. (M–O) cTnT immunohistochemical staining of the anteriolateral right ventricle in (M) non-ischemic cardiac tissue demonstrating no depletion in cTnT ×10; (N) 1 hour of LAD ligation demonstrating cTnT depletion in the subendocardial region [square box] ×10; (O) magnified box area from section N ×40 (sources: adapted from (A–C) [31]; (D–I) [32]; (J–O) [33]).

7. Conclusions

This chapter summarises the extensive literature base examining the clinical utility of cardiac troponin when tested in the postmortem setting. Whilst there is overwhelming evidence to support the superior value of pericardial fluid cTn rather than blood sampling due to significant interferences with the latter, there remains the issue of clinically validated cut-off concentrations in postmortem sampling. The effect of autolysis and increasing concentrations of cTn in fluid analysis correlated with increasing postmortem interval of significance. These features therefore suggest that cTn analysis is more suited as a rule out of cardiac involvement in sudden death rather than a rule in diagnostic aid. The diagnostic utility should be limited to the hospital autopsy rather than the medico-legal postmortem where these factors and interferences could provide scope for counter arguments by the defence counsel. Further work is required in the medico-legal setting to establish appropriate diagnostic cut-off values for cTnT and cTnI in postmortem samples, and clinical pathological guidelines should be written to provide support to the forensic teams to correctly interpret the evidence from this large selection of published literature.

Author details

David C. Gaze
Chemical Pathology, University of Westminster, London, United Kingdom

*Address all correspondence to: d.gaze@westminster.ac.uk

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