

ORIGINAL ARTICLE

Post mortem troponin T analysis in sudden death: Is it useful?

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Abstract

Introduction: Cardiac-related diseases contributed approximately 50-60% of sudden natural death cases. This study aimed to describe the cardiac troponin T (cTnT) findings in post mortem subjects irrespective of the cause and manner of death, and the possible use of post mortem serum cTnT as a modality in investigating sudden natural death. **Methods:** The study samples comprised 140 subjects aged 18 to 50 years old, natural and unnatural causes of sudden death brought to the Department of Forensic Medicine, Hospital Sungai Buloh (HSgB) and Hospital Sultanah Aminah Johor Bahru (HSAJB) for a period of 12 months. The subjects were categorised into 5 groups: cardiovascular disease (CVD), sudden unexplained death (SUD), thoracic trauma (TT), non-thoracic trauma (NTT) and other diseases (OD). **Results:** Median troponin concentration in cases of CVD, SUD, TT, NTT, and OD were 0.51 µg/L, 0.17 µg/L, 0.62 µg/L, 0.90 µg/L and 0.51 µg/L respectively. We found no significant difference of troponin T level in different causes of death ($p \geq 0.05$). NTT has the highest median troponin concentration with 0.90 µg/L, SUD possessed the lowest median concentration with 0.17 µg/L. **Conclusion:** Troponin T is neither specific nor useful as cardiac biomarker for post mortem sample. Therefore, it may not be a useful diagnostic tool at autopsy.

Keywords: Sudden unexplained death, cardiac death, troponin T, post mortem biochemistry

INTRODUCTION

World Health Organization (WHO) defines sudden death as natural, nonviolent, unexpected death occurring within twenty four hours of the onset of symptoms.¹ Cardiac-related diseases contributed approximately 50-60% of the cases and common causes are coronary artery disease (CAD), cerebrovascular disease (CVD), cardiomyopathy, rheumatic heart disease and congenital heart disease.² A small proportion of these natural death cases however, show no gross or microscopic findings which could be used to determine the cause of death. This group of sudden unexpected death is widely speculated to be caused by lethal cardiac arrhythmia or ion channelopathies.²

In the case of sudden unexplained death, the absence of gross and microscopic findings especially on the heart, coupled with the possibility of lethal cardiac arrhythmia, cardiac

troponins are thought to be a sensitive and specific biomarker for myocardial injury.³ The troponin complex consists of three proteins (troponin T, I, and C). Troponin C is identical in skeletal muscle and myocardium, but troponin T and I are somewhat different from their respective counterpart in the skeletal muscle. Cardiac troponin I (cTnI) and T (cTnT) are examined in routine clinical investigations of myocardial damage, mainly for the diagnosis and management of acute coronary syndrome (ACS).⁴ Troponin is released from injured cardiomyocytes approximately three hours after ischaemic event, and the elevation may persist for several weeks. The peak concentration is also correlated with the degree of injury.⁵ Therefore, cardiac troponins are outstanding biomarkers for their degree of specificity and sensitivity, for the length of time available for diagnosis and for their provision of biochemical evidence of cardiac micronecrosis.^{3,6}

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Several studies have been performed to determine the correlation between post mortem and ante mortem levels of cardiac biomarkers and some of these have shown that post mortem levels of cardiac troponin are significantly higher in death due to myocardial ischaemia than other causes of death.³ On the other hand, serum cTnT could not be used directly with post mortem samples as a new cut-off value was necessary in these situations. The aim of this study is to describe the cTnT findings in post mortem subjects irrespective of the cause and manner of death, hence the possible use of post mortem serum cTnT as a biomarker in investigating the cause of sudden death.

MATERIALS AND METHODS

Study population

The study sample comprised all natural and unnatural causes of sudden death cases which were brought to the Department of Forensic Medicine Hospital Sungai Buloh (HSgB) and Hospital Sultanah Aminah Johor Bahru (HSAJB) for a period of 12 months, from May 2013 until May 2014. These were medico-legal post mortem cases whereby the P 61 order for post mortem was issued by the investigating police officer. Male and female subjects of 18-50 years of age, refrigerated body with post mortem interval (PMI) of less than 24 hours and all ethnicities or nationalities were included in the study. PMI is defined as time that has elapsed between death of an individual and discovery of the body.⁷ Sudden unnatural deaths included road traffic accident fatalities, homicide or suicide victims within the inclusion criteria were also recruited. Exclusion criteria were decomposed, charred and skeletonized human remains. For every decedent, the following data were collected; autopsy findings, cause and manner of death. Cause of death was defined as disease or injury that triggered the chain of events producing death. Manner of death was defined as circumstances surrounding death; natural, accidental, suicide, homicide or undetermined.⁸

All subjects underwent routine autopsy procedures which included brain and thoraco-abdominal viscera dissection. Histology, microbiology and serology analysis were done accordingly. Routine alcohol and toxicology analysis were performed.

Biochemical analyses

At autopsy, blood was drawn from jugular/subclavian/femoral veins and centrifuged to

separate the serum. The serum was then stored at -20°C before analysis. cTnT was measured using electro-chemiluminescence immunoassay method on an automated analyser (Elecsys 2010, Roche Systems, Germany). The upper limit for clinical reference range was 0.01 µg/L. An elevated cTnT was defined as being greater than 0.01 µg/L. Precision was determined using protocol (EP5-A2) of the CLSI (Clinical and Laboratory Standard Institute). There were 2 runs per day in duplication each for 21 days (n = 84). The coefficient of variation (CV) of repeatability (within-run precision) for level 1 (0.017 ± 0.06 µg/L) and level 2 (0.194 ± 0.020 µg/L) is 2.6% and 1.7% respectively. The CV for intermediate precision for level 1 and level 2 is 3.4% and 2.0% respectively.

Statistical analysis

Non-parametric Kruskal–Wallis test was used to compare more than two groups and the non-parametric Mann–Whitney U test was used for comparison between two individual groups. Spearman's rank order correlation (r) was used to evaluate the relationship between pairs of parameters. Statistical analysis was performed using statistical SPSS (version 16) and p-value of less than 0.05 was considered as statistically significant.

RESULTS

A total of 140 autopsy cases were recruited with HSgB and HSAJB contributed 70 cases each respectively. Twenty two (22) samples were excluded due to severe haemolysis as interpretation of results would be impossible. The final sample consisted of 118 cases. In gender distribution, 107 (90.7%) of the subjects were male and the balance of 11 (9.3%) were females. Eight different ethnicities were identified; Malay (n=31), Chinese (n=28), Indian (n=23), Indonesia (n=10), Bangladesh (n=14), Nepal (n=6) and Myanmar (n=6).

The subjects were divided into five groups according to the gross and microscopic findings; cardiovascular disease (CVD, n=35), sudden unexplained death (SUD, n=15), thoracic trauma (TT, n=19), non-thoracic trauma (NTT, n=40), and other diseases (OD, n=9) (Table 1). The grouping was essentially meant to assess the value of cTnT in relation to the cardiac and non-cardiac cases.

Troponin T level in cases of CVD, SUD, TT, NTT, and OD were 0.51 µg/L, 0.17 µg/L, 0.62 µg/L, 0.9 µg/L and 0.51 µg/L respectively

TABLE 1: Classification of subjects according to autopsy findings

Categories of disease/injuries	Frequency (n)	Age (Years)
		Median (Range)
Non-thoracic trauma (NTT)	40	31 (21-50)
Cardiovascular disease (CVD)	35	38 (21-49)
Thoracic trauma (TT)	19	27 (19-48)
Sudden unexplained death (SUD)	15	35 (19-47)
Other disease (OD)	9	34 (20-47)

(Table 2). We found no significant difference of troponin T levels in different causes of death's group ($p \geq 0.05$). The highest median concentration was NTT with $0.90 \mu\text{g/L}$, while SUD possessed the lowest median of $0.17 \mu\text{g/L}$.

Apart from the grouping done based on the gross disease or injury noted on the subjects, further analysis was performed to look at the actual causes within the cardiovascular disease category. Coronary artery disease (CAD) and ischaemic heart disease (IHD) formed slightly more than 70% of the causes of cardiac death. Other causes included hypertensive heart disease (HHD), acute myocardial infarction (AMI), cardiomyopathy, intramyocardial bridging, cardiomegaly, atrial septal defect (ASD) and coronary anomalies with valvular heart disease (Table 3).

Within the sudden cardiac death group, the level of cTnT in the subcategory of acute myocardial infarction is exceptionally high (median= $7.61 \mu\text{g/L}$). This finding supported the hypothesis that troponin T is sensitive in detecting grossly visible AMI which would be 3-7 days old. The severity of myocardial damage, together with length of time before death, would contribute to the high cTnT level.⁸

An analysis was performed to see the difference of troponin T levels based on gender and ethnics. However, the results showed that

the troponin T levels based on gender ($p=0.607$, Mann-Whitney) and ethnics ($p=0.10$, Kruskal Wallis) were not significant. The fact that female subjects contributed only 9.3% of the total subjects, an actual reflection of gender differences could not be ascertained. According to Spearman's rank order correlation test, there is no statistically significant correlation between age and troponin levels.

DISCUSSION

The goals of this study were to assess the correlation of cardiac biomarkers and deaths according to different categories of disease or injuries based on the gross autopsy findings and microscopic examination. We also would like to determine the usability of cTnT as a diagnostic indicator for sudden cardiac death.

Firstly, under routine conditions, almost all bodies presented at autopsy had some degree of autolysis and haemolysis.³ In order to reduce this interference, an inclusion criterion of post mortem interval of less than 24 hours was put in place. All testing for this study was performed in the same laboratory and on the same instrument as the clinical samples received from living patients. A common finding in our study and that of previous studies was that the cardiac biomarkers were elevated above

TABLE 2: Serum cTnT levels according to the different categories of disease or injuries

Categories of disease/injuries	Frequency (n)	Troponin T level ($\mu\text{g/L}$)	<i>p-value</i>
		Median (Interquartile Range)	(Kruskal-Wallis)
Non-thoracic trauma (NTT)	40	0.90 (6.118)	<i>p=0.875</i>
Thoracic trauma (TT)	19	0.62 (8.983)	
Cardiovascular disease (CVD)	35	0.51 (5.220)	
Other diseases (OD)	15	0.51 (0.590)	
Sudden unexplained death (SUD)	9	0.17 (2.265)	

TABLE 3: Serum cTnT levels according to the different causes of sudden cardiac death

CARDIOVASCULAR DISEASE	Frequency (n)	Percentage (%)	Median/Troponin range ($\mu\text{g/L}$)
Coronary artery disease	18	51.4	0.75 (0.003-15.11)
Ischaemic heart disease	7	20.0	0.88 (0.020-9.10)
Acute myocardial infarction	2	5.7	7.61 (0.47-14.7)
Hypertensive heart disease	3	8.6	0.15 (0.07-4.17)
Others:	5	14.3	0.046 (0.072-11.6)
(1) Myocardial bridging (n=1)			
(2) Cardiomyopathy (n=1)			
(3) Atrial septal defect (n=1)			
(4) Cardiomegaly (n=1)			
(5) Coronaries anomalies with valvular heart disease (n=1)			
Total	35	100	

the clinical value in almost all cases. While haemolysis is known to interfere with the assay, in clinical settings, haemolysis and free haemoglobin actually had a negative effect on the assay, causing the cTnT level to be lower than it actually was.³ However, in post mortem samples, despite the presence of haemolysis, the values were all above the normal limit for living subjects. Some authors speculated the cause to be due to non-specific myocardial damage as a result of hypoxia during agonal period as well as cardio-respiratory resuscitation.^{9,10,11} We tend to disagree as the cTnT is known to be released in the peripheral blood approximately 3 hours after the onset of the myocardial injury. In one post mortem study, in a group of subjects with elevated troponin, only 50% showed contraction band necrosis, thus indicating that the troponin released was most probably as a result of leakage from the myocytes membrane, rather than an actual myocyte necrosis.¹² Indeed, there are other factors involved and thus warrants further studies.⁵ The scenario of troponin elevation in the absence of myocardial injury is also observed in critically ill patients. The mechanism is not yet understood. Hypotheses were made to explain the situation and that involved microvascular dysfunction and increased myocardial cell permeability in the situation of oxygen supply and demand mismatch.¹²

A potential confounding factor in raised cTnT concentration is cardiopulmonary resuscitation (CPR) procedure such as mechanical chest compression and external defibrillation. A study by Polena *et al.* has shown that raised cardiac

biomarkers were observed in association with increased duration of CPR.¹³ Other studies have revealed that creatine kinase MB (CKMB) concentrations were increased during CPR and independently associated with AMI. Remarkably, based on its kinetic pattern of levels measured at periodic intervals, raised cardiac troponin T and I concentrations were distinct for AMI and not associated with CPR.^{14,15} These studies however, were performed on the living subjects. In our study, CPR could not be determined to have been commenced as most of the subjects were brought directly to the mortuary as death was pronounced at home or at the scene. Therefore, the effect of this potential confounding factor could not be assessed with full certainty.

Post mortem cTnT values of above the clinical setting leads to the longstanding, problematic issues in interpretation of the obtained results. In the absence of databases with sufficient numbers of cases to establish the expected range, researchers independently suggested a cut-off value for post mortem cTnT. Ellingsen and Hetland suggested the serum cTnT level of 0.6 $\mu\text{g/L}$ as the cut off value between evident/possible cardiac deaths and negative controls. Zhu *et al.* suggested 0.2 $\mu\text{g/L}$ for serum from external iliac venous blood.¹⁶ In our study, 77.1% of the cases within the CVD group showed cTnT value above 0.75 $\mu\text{g/L}$. Furthermore, the cTnT levels were found to be above 0.5 $\mu\text{g/L}$ for the 4 groups; NTT, TT, CVD and OD.

In the subjects with morphological changes consistent with recent myocardial injury (n=2), the median serum cTnT level was 7.61 $\mu\text{g/L}$

compared with subjects with non-cardiac cause natural death (n=15) serum cTn level of 0.51 $\mu\text{g/L}$. The observed differences were statistically significant ($P < 0.0001$). This finding confirms that cTnT is sensitive at detecting AMI.

In the case of sudden unexplained death, the median of cTnT level was 0.17 $\mu\text{g/L}$. No elevation of cTnT supports the theory that death was most probably attributed to sudden lethal cardiac arrhythmia. Elevated serum cTnT in such a case supports the diagnosis of sudden cardiac death, but normal or low cTnT does not exclude a cardiac event, due to the three-hour window of onset of myocardial injury and detection of cTnT in the peripheral venous blood.⁵

Study limitation

This study was limited by factors including variance of PMI, body refrigeration time and blood sample haemolysis. A small sample size for females as well as some ethnic groups also posed a limitation to statistical interpretation in this study. Virtually in all circumstances, some degree of autolysis has already taken place at autopsy. While PMI influences cardiac troponin levels, conversely, studies had demonstrated that cardiac troponins degradation may actually be used for PMI estimation.^{17,18} However, PMI was not taken into consideration in the current paper due to difficulties in obtaining an accurate data.

CONCLUSION

Elevated cTnT is seen in almost all categories of death irrespective of presence or absence of myocardial injury. The findings from our study confirmed that troponin T is a sensitive marker in detection of AMI at autopsy, however, it is not specific and therefore limiting its role as an adjunct tool of investigation. In the case of sudden unexplained death, the low level of cTnT may be a strong indicator that death was instantaneous, most possibly caused by lethal arrhythmia.

Conflict of interest: The authors declare that they have no conflict of interest.

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Ethical approval: The protocol was approved by the Research Ethics Committee, Universiti Teknologi MARA and Medical Research & Ethics Committee, Ministry of Health, Malaysia.

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