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Cardiac biomarkers in blood, and pericardial and cerebrospinal fluids of forensic autopsy cases: A reassessment with special regard to postmortem interval

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ABSTRACT

Previous studies suggested possible application of postmortem biochemistry of myocardial biomarkers to the investigation of sudden cardiac death; however, differences from clinical findings should be considered in autopsy materials. The present study involved a comprehensive investigation of cardiac troponin T and I (cTnT and cTnI), and creatine kinase MB (CK-MB) in cardiac and peripheral external iliac venous blood, pericardial fluid (PCF) and cerebrospinal fluid (CSF) for reassessment, with special regard to the estimated postmortem interval in relation to the cause of death, reviewing a large number of forensic autopsy cases ($n = 1923$). These cardiac biomarkers showed cause-of-death- and postmortem-time-dependent differences: blood and PCF levels of each marker were higher in hyperthermia (heatstroke), bathwater drowning and chronic congestive heart disease in cases of postmortem interval (PMI) <12 h. After 12 h postmortem, these markers were also higher in fatal drug abuse, spontaneous cerebral/subarachnoid bleeding, electrocution and pulmonary embolism. In addition, most other causes of death, including ischemic heart disease, showed substantial elevations, while these markers remained low in acute hemorrhagic death from sharp instrument injury, hypothermia (cold exposure) and sea-/freshwater drowning during PMI of <48 h. CSF cTnI and CK-MB showed similar findings. There was no difference between myocardial infarction and other causes of death to be discriminated, including asphyxiation, drowning and fire fatality. These findings are similar to clinical observations in critical ill patients, suggesting that elevated cardiac biomarkers cannot be a specific finding for death from acute ischemic heart disease, but indicate the severity of myocardial injury in postmortem investigation.

1. Introduction

Serum cardiac biomarkers, including cardiac troponin T and I (cTnT and cTnI), as well as creatine kinase MB (CK-MB), are routinely used for the clinical diagnosis of myocardial infarction [1–4]. Previous studies suggested possible application of these markers to the investigation of myocardial injury in autopsy cases; however, differences from clinical findings should be carefully considered in the evaluation of postmortem data with regard to the influences of fatal insults and agony during survival [5,6]. In addition, postmortem interference should be considered for these markers in autopsy materials [7–14]. Such postmortem interference can greatly depend on the site of sampling; however, insufficient data are available on sampling-site differences in the practical application of cardiac biomarkers in postmortem investigation.

The present study was a comprehensive investigation of cTnT, cTnI and CK-MB in heart and peripheral external iliac venous blood, pericardial fluid (PCF) and cerebrospinal fluid (CSF) using substantial forensic autopsy data for a reassessment, with special regard to the cause of death and estimated postmortem interval.

2. Materials and methods

2.1. Materials

A series of medicolegal autopsy cases ($n = 1923$; 3–48 h postmortem, with a median of 20 h) at our institute were examined.

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The cases comprised 1366 males and 557 females, between 1 and 100 (median, 61) years of age. Blood was drawn aseptically from bilateral cardiac chambers and the external iliac vein using syringes at autopsy. PCF and CSF (cerebellomedullar cisterna) were also collected aseptically using syringes after opening the pericardial and cranial cavities, respectively, carefully avoiding contamination. These samples were immediately analyzed after centrifugation.

The causes of death were classified on the basis of autopsy examination, including macromorphological, histological and toxicological analyses. Details are shown in Table 1. Cardiac deaths included those due to acute ischemic heart disease with pathological evidence of myocardial infarction without and with old infarction (acute and recurrent myocardial infarction; AMI and RMI), those without focal myocardial necrosis, but without any evidence of a cause of death other than a cardiac attack (acute ischemic heart disease, AIHD), and chronic heart disease with pathological evidence of congestive heart failure (chronic congestive heart disease, CHD). Fatal hemorrhage due to sharp instrument injury was subdivided into acute and subacute deaths with an estimated survival time (ST) of <0.5 and 0.5–24 h, respectively. For these groups, clearly accountable cases without any other complications that may have contributed to the death were collected; cases having significant injury were excluded from non-injury groups. The present study investigated cases in which death was witnessed and/or circumstantial evidence had been well-established to confirm ST and the postmortem interval (PMI) estimated based on pathological findings [14]. The range of error for estimated PMI depended on the time elapsed after death: within around 1–2 h in cases within 18 h postmortem, and within about 4–6 h in cases within 18–48 h postmortem, as described previously [14].

2.2. Biochemical analyses

Cardiac biomarkers were measured as part of routine laboratory investigation [14,15]. For cTnT assay, an electro-chemiluminescence immunoassay (Roche Diagnostics, USA) [16] was used; cross-reactivity to skeletal muscle troponin T was less than 0.01% [17]. Hemoglobin contamination at <0.5 g/dl did not interfere with the measurements. The clinical reference serum range was 0.1 ng/ml. cTnI and CK-MB were measured using an automated analyzer, the Stratus CS system (Dade Behring, Inc., Newark, IL, USA) [5,16,17]. Hemolysis or hemoglobin contamination (<1.35 g/dl), jaundice (bilirubin < 29.2 mg/dl) or lipemia (triglycerides < 7.0 mmol/L) did not interfere with the measurement [16]. Because of the specific monoclonal antibodies used in this system, cross-reaction between cTnI and skeletal troponin I or between CK-MB and CK-BB as well as the influence of a spectrum of other biological substances and drugs was negligible (manufacturer's instructions and information). Clinical serum reference ranges were <0.10 ng/ml for cTnI and <5.0 ng/ml for CK-MB [5,16]. The samples with hemoglobin imbibition due to hemolysis, which were often seen in fire fatality and freshwater drowning, were not excluded in the present study; the interference was assessed by dilution. These sample collections and analyses described above were performed within the framework of our routine casework, following the autopsy guidelines (2009) and ethical guidelines (2002 and 2013) of the Japanese Society of Legal Medicine, and data analysis based on autopsy and laboratory findings was approved by our institutional ethics committee.

2.3. Statistical analyses

Regression equation analysis was used to examine the relationship between pairs of parameters, including gender, age, ST, PMI and each cardiac marker at each site. The Kruskal–Wallis test was used for non-parametric multiple comparisons to classify the cause-of-death groups into higher-, intermediate- and lower-level groups according to measured cardiac marker levels, and the homogeneity of the classified groups was examined using the Kruskal–Wallis test again and the non-parametric Mann–Whitney *U* test, followed by multiple pairwise comparisons of the three above-mentioned groups using the Steel–Dwass test.

Table 1
Case profiles (n = 1923).

Cause of death	n	Male/female	Age (years)		Survival time (h)		With/without CPR	Postmortem interval (h)	
			Range	Median	Range	Median		Range	Median
Sharp instrument injury-1	44	36/8	19–73	47	<0.5	–	17/27	6–42	16.1
Sharp instrument injury-2	33	27/6	22–91	55	0.5–24	3.0	16/17	7–41	16.2
Blunt injury	485	388/97	1–100	57	0.1–496	3.5	242/243	3–48	19.9
Asphyxiation	136	79/57	1–94	55	0.1–10.5	0.5	35/101	6–47	21.3
Bathwater drowning	98	55/43	1–96	74	0.5–16	0.5	37/61	4–46	18.5
Sea- and freshwater drowning	74	51/23	3–89	60	0.1–8.5	0.5	14/60	7–48	22.5
Fatal methamphetamine abuse	35	28/7	20–62	39	0.5–144	3.0	12/23	6–39	25.8
Sedative-hypnotics intoxication	36	16/20	14–74	39	2.5–196	3.0	8/28	6–38	27.5
Fatal alcohol abuse	8	4/4	29–69	51	3–72	3.0	3/5	20–39	27.0
Other intoxications	32	20/12	18–87	51	0.1–36	3.0	11/21	4–44	25.0
Fire fatality	324	232/92	1–95	65	0.1–108	0.5	41/283	5–48	16.5
Hyperthermia (heatstroke)	37	22/15	1–95	65	1.5–108	3.0	11/26	8–47	25.3
Hypothermia (cold exposure)	37	25/12	26–87	65	3–24	3.0	5/32	8–45	26.6
Acute myocardial infarction	110	83/27	30–94	65	0.5–336	0.5	62/48	6–42	19.9
Recurrent myocardial infarction	90	73/17	45–94	66	0.5–60	0.5	59/31	5–44	19.5
Acute ischemia heart disease	86	61/25	17–97	68	0.3–96	0.5	51/35	4–45	19.0
Chronic congestive heart disease	39	32/7	16–91	59	Unknown	–	18/21	6–41	19.5
Hemopericardium	54	33/21	1–91	71	0.5–3	0.5	35/19	7–41	20.8
Right ventricular cardiomyopathy	10	1/9	45–88	68	0.5–108	3.0	4/6	14–33	21.0
Pulmonary embolism	13	7/6	26–80	54	0.4–10	0.5	6/7	5–36	24.5
CVD	44	27/17	23–88	62	0.5–624	3.0	15/29	5–39	23.1
Electrocution	7	7/0	23–66	55	<0.5	–	6/1	23–28	27.0
Malnutrition	8	2/6	21–92	51	Unknown	–	2/6	17–44	30.8
Pneumonia	83	57/26	1–97	59	0.5–672	72.0	35/48	6–42	20.3
Total	1923	1366/557	1–100	61	0.1–672	0.5	745/1178	3–48	20.0

Sharp instrument injury-1, acute hemorrhagic death (survival time, <0.5 h); sharp instrument injury-2, subacute hemorrhagic death (survival time, 0.5–24 h); CVD, spontaneous cerebral/subarachnoid bleeding; CPR, unsuccessful cardiopulmonary resuscitation at the time of death.