Jikeikai Med J 2019 ; 66 : 9-15

Utility of Biochemical Markers in the Postmortem Diagnosis of Ischemic Heart Disease

Shojiro TAKASU, Sari MATSUMOTO, Yuko KANTO, Saki KODAMA, and Kimiharu IWADATE

Department of Forensic Medicine, The Jikei University School of Medicine

ABSTRACT

Purpose : The postmortem diagnosis of ischemic heart disease (IHD) by means of biochemical markers, as performed in the field of forensic medicine, is a major concern because of postmortem changes of these markers. In the present study, we evaluated the use of these markers in body fluids obtained during medicolegal autopsies for diagnosing acute IHD postmortem.

Methods : In serum, pericardial fluid, and urine obtained from 152 cases (72 of IHD and 80 control cases), levels of these biochemical markers were measured : N-terminal pro-brain natriuretic peptide, heart-type fatty acid-binding protein, creatine kinase MB, myoglobin, and cardiac troponin T, and cardiac myosin light chain I.

Results : Significantly higher levels were observed in urine of N-terminal pro-brain natriuretic peptide and cardiac troponin T and in pericardial fluid of heart-type fatty acid-binding protein, myoglobin, cardiac troponin T, and creatine kinase MB in cases of acute IHD than in control cases ($P<0.05$).

Conclusion : Our results suggest that postmortem measurement of biochemical markers in pericardial fluid and urine is a useful postmortem tool for diagnosing acute IHD.

(Jikeikai Med J 2019 ; 66 : 9-15)

Key words : N-terminal pro-brain natriuretic peptide, heart-type fatty acid-binding protein, creatine kinase MB, myoglobin, cardiac troponin T

INTRODUCTION

Ischemic heart disease (IHD) is a common cause of sudden death in developed countries. Therefore, the diagnosis of IHD at medicolegal autopsies is a major concern in forensic medicine. Whether biochemical markers that are measures for clinical medicine can also be measured for forensic medicine is uncertain because the markers undergo postmortem changes. In forensic medicine, IHD is diagnosed mainly on the basis of macroscopic and microscopic findings. However, because the early stages of pathological myocardial necrosis are present 6 hours after the onset of myocardial ischemia, diagnosing IHD is challenging if death has occurred during the acute phase[1].

Biochemical markers that are reportedly elevated in living persons with IHD include N-terminal pro-brain natriuretic peptide (NT-proBNP), heart-type fatty acid-binding protein (hFABP), creatine kinase MB (CKMB), myoglobin, cardiac troponin (cTn) T, and cardiac myosin light chain I[2-9]. Although postmortem elevations of these mark-

Received : April 5, 2019 / Accepted : June 18, 2019
高須翔志郎，柗本　紗里，菅藤　裕子，児玉　　早，岩楯　公晴
Mailing address : Shojiro TAKASU, Department of Forensic Medicine, The Jikei University School of Medicine, 3-25-8 Nishi-Shimbashi, Minato-ku, Tokyo 105-8461, Japan.
E-mail : s-takasu@jikei.ac.jp

ers are a major concern, previous studies have suggested that their levels in serum and pericardial fluid are useful for diagnosing IHD during forensic autopsies[10-22].

We have recently reported that the level of soluble lectin-like oxidized low-density lipoprotein receptor 1 in pericardial fluid is a useful tool for diagnosing cases of IHD postmortem[23]. In the present study, we measured levels of biochemical markers in specimens obtained postmortem and evaluated their use for the diagnosis of acute IHD.

Materials and Methods

Case selection

The cases included in the present study were medicolegal autopsy cases examined at The Jikei University School of Medicine from January 2015 through March 2017. A total of 72 cases of death due to acute IHD (mean patient age, 61.6 years ; age range, 34 to 87 years) and 80 control cases of death (mean patient age, 61.8 years ; age range, 22 to 89 years) were examined. The postmortem interval (PMI) in all cases was 72 hours or less. This study was approved by the Ethics Committee of The Jikei University School of Medicine for Biochemical Research 27-218 (8103).

Number of samples and measurement methods

Samples of serum from cardiac blood, pericardial fluid, and urine were obtained as quickly as possible during autopsy and stored at −80°C until the biochemical markers were measured. The levels of biochemical markers were measured by SRL Inc. (Tokyo, Japan). The measuring methods were chemiluminescence enzyme immunoassay for CKMB and serum myoglobin, electrochemiluminescence immunoassay for NT-proBNP and cTnT, enzyme immunoassay for cardiac myosin light chain I, and a latex coagulating method for hFABP.

The upper limits of serum clinical reference values in living patient at our institution were as follows : CKMB, <5.0 ng/ml ; myoglobin, <106 ng/ml ; NT-proBNP, <125 pg/ml ; cTnT, <0.1 ng/ml ; cardiac myosin light chain I, 2.5 ng/ml ; and hFABP, < 6.2 ng/ml.

From the 152 cases, the numbers of samples collected were serum, 150 ; pericardial fluid, 96 ; and urine, 60. Markers in serum were measured in 71 cases of acute IHD and 79 control cases. The control cases consisted of 10 cases of congestive heart failure (CHF) and 69 cases of death due to external causes (asphyxia, 23 ; drowning, 12 ; drug intoxication, 15 ; hypothermia, 8 ; carbon monoxide intoxication, 4 ; head trauma, 3 ; cervical cord trauma, 1 ; 2 hyperthermia, 1 thoracic injury). Markers in pericardial fluid were measured in 46 cases of acute IHD and 50 control cases. The control cases consisted of 10 cases of CHF and 40 cases of death due to external causes (asphyxia, 14 ; drowning, 11 ; drug intoxication, 3 ; hypothermia, 6 ; carbon monoxide intoxication, 1 ; head trauma, 2 ; cervical spinal cord injury, 1 ; hyperthermia, 1 ; and thoracic injury, 1). Markers in urine were measured in 26 cases of acute IHD and 34 control cases. The control cases consisted of 3 cases of CHF and 31 cases due to external causes (asphyxia, 7 ; drowning, 5 ; drug intoxication, 6 ; hypothermia, 7 ; carbon monoxide intoxication, 1 ; head trauma, 2 ; cervical spinal cord trauma, 1 ; hyperthermia, 2 ; and thoracic injury, 1). Because urine samples were of an insufficient volume, urine myoglobin could not be measured.

Diagnostic basis of IHD and CHF

Acute IHD was diagnosed on the basis of macroscopic and microscopic findings of coronary stenosis, coronary thrombosis/plaque rupture, or myocardial necrosis. The diagnosis of CHF was made with the existence of a heart condition (cardiomegaly, valvular disease), subcutaneous edema, pulmonary edema, pleural effusion, ascites, or congestion of the organs. Cases with a earlier myocardial infarctions were excluded.

Statistical analysis

Statistical analyses were performed with the software program STATA 13.0 (Stata Corp, College Station, TX, USA). Data are shown as means ± standard deviation (SD). The unpaired T-test was used to evaluate the differences in age between cases of acute IHD and control cases. The Mann-Whitney U test was used to evaluate differences in body mass index (BMI), heart weight, and marker concentrations. The Kruskal-Wallis equality-of-population rank test was used to evaluate the difference in marker concentrations according to PMI in each group. Receiver operating characteristic (ROC) analysis was performed for marker concentrations that differed significantly between the groups. The cutoff value was determined with the Youden Index. Spearman's rank correlation coefficient was performed to evaluate the correlation between the marker con-

centrations. In each case, p values less than 0.05 were considered statistically significant.

Results

No significant difference in age or PMI was found at baseline between cases of IHD and control cases (Table 1). However, both mean BMI and heart weight were significantly greater in cases of acute IHD than in control cases.

Significant differences between the acute IHD and control groups were observed for pericardial fluid levels of hFABP, myoglobin, cTnT, and CKMB and urine levels of cTnT and CKMB (Table 2). However, the urine CKMB level was significantly higher in the control group. The levels of NT-proBNP and myosin light chain I did not differ significantly between cases of acute IHD and control cases (data not shown).

In samples of pericardial fluid, hFABP showed an area under the curve (AUC) from ROC analysis of 0.69 (95% confidence interval [CI] = 0.58-0.79) and a cut-off value for diagnosing acute IHD of 13.4 μg/ml (sensitivity, 73.9% ; specificity, 52.0%), myoglobin showed an AUC of 0.66 (95% CI = 0.56-0.77) and a diagnostic cut-off value of 132.0 μg/ml (sensitivity, 76.1% ; specificity, 54.0%), cTnT showed an AUC of 0.65 (95% CI = 0.54-0.76) and a diagnostic cut-off value of 116 ng/ml (sensitivity, 67.4% ; specificity, 58.0%), and CKMB showed an AUC of 0.62 (95% CI = 0.51-0.73) and a diagnostic cut-off value of 4.4 μg/ml (sensitivity, 56.6% ; specificity, 64.0%). In samples of urine, cTnT showed an AUC of 0.67 (95% CI = 0.53-0.81) and a diagnostic cut-off value of 0.012 ng/ml (sensitivity, 53.9% ; specificity, 75.8%).

In urine samples, the number of cases under the detection limit were 2 of acute IHD and 10 control for NT-proBNP, 1 of acute IHD and 4 control for cTnT, and 26 of acute IHD and 33 control for myosin light chain I.

When NT-proBNP was compared between control cases with CHF and those without CHF (Table 3), serum and pericardial fluid NT-proBNP was significantly higher in cases with CHF. Urine NT-proBNP levels were significantly

Table 1. Baseline characteristics

Variables	Cases of acute ischemic heart disease (n = 72)	Control cases (n = 80)	p value
Age (years)	61.6 ± 13.8	61.8 ± 18.5	0.82
Body mass index (kg/m^2)	24.0 ± 5.3	22.3 ± 5.4	< 0.05
Postmortem interval (hours)	31.8 ± 14.4	35.7 ± 13.2	0.08
Heart weight (g)	456.1 ± 108.9	373.6 ± 109.7	< 0.05

Table 2. Comparison of cases of acute ischemic heart disease and control cases

	Acute ischemic heart disease	Control	p value
hFABP (μg/ml)			
Serum	22.2 ± 16.4	23.1 ± 17.4	0.88
Pericardial fluid	39.0 ± 32.8	20.8 ± 21.1	< 0.05
Urine	0.120 ± 0.129	0.426 ± 1.16	0.63
Myoglobin (μg/ml)			
Serum	280.1 ± 225.0	361.2 ± 305.1	0.21
Pericardial fluid	371.8 ± 355.1	207.4 ± 216.9	< 0.05
Cardiac troponin T (ng/ml)			
Serum	301.0 ± 498.1	273.3 ± 416.1	0.38
Pericardial fluid	665.7 ± 1,024.7	290.3 ± 415.5	< 0.05
Urine	0.06 ± 0.20	0.01 ± 0.01	< 0.05
Creatine kinase MB (μg/ml)			
Serum	5.18 ± 4.76	4.83 ± 4.85	0.60
Pericardial fluid	8.10 ± 14.9	4.38 ± 5.04	< 0.05
Urine	0.005 ± 0.001	0.006 ± 0.001	< 0.05

hFABP, heart-type fatty acid-binding protein

Table 3. Comparison of N-terminal pro-brain natriuretic peptide and heart weight between control cases with or without congestive heart failure

	With congestive heart failure	Without congestive heart failure	*p* value
NT-proBNP (ng/ml)			
Serum	23.5 ± 21.3 (n = 10)	0.97 ± 1.21 (n = 69)	< 0.05
Pericardial fluid	44.0 ± 49.9 (n = 10)	3.41 ± 3.44 (n = 40)	< 0.05
Urine	2.34 ± 2.33 (n = 3)	0.29 ± 1.04 (n = 31)	0.06
Heart weight (g)	490.7 ± 93.6 (n = 10)	356.9 ± 101.8 (n = 69)	< 0.05

NT-proBNP, N-terminal pro-brain natriuretic peptide

Table 4. Comparison of N-terminal pro-brain natriuretic peptide levels and heart weight between cases of acute ischemic heart disease and control cases without congestive heart failure

	Acute ischemic heart disease	Control group without congestive heart failure	*p* value
NT-proBNP (ng/ml)			
Serum	9.38 ± 32.2 (n = 71)	0.97 ± 1.21 (n = 69)	0.07
Pericardial fluid	20.3 ± 53.6 (n = 46)	3.41 ± 3.44 (n = 40)	0.21
Urine	0.19 ± 0.31 (n = 26)	0.29 ± 1.04 (n = 31)	< 0.05
Heart weight (g)	456.1 ± 108.9 (n = 72)	356.9 ± 101.8 (n = 69)	< 0.05

NT-proBNP, N-terminal pro-brain natriuretic peptide

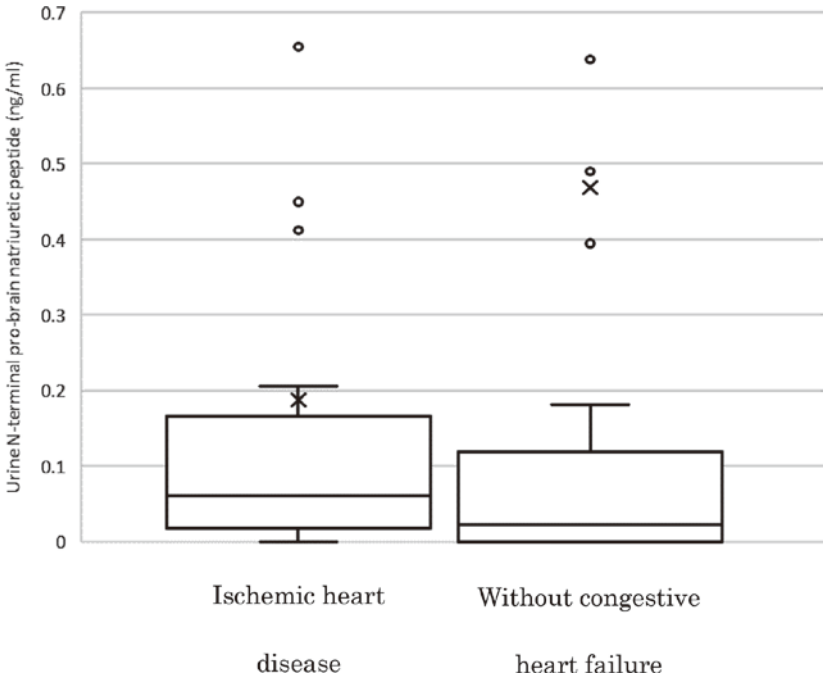

Fig. 1. Box chart comparing urine N-terminal pro-brain natriuretic peptide between cases of acute ischemic heart disease and control cases without congestive heart failure. × = mean value.

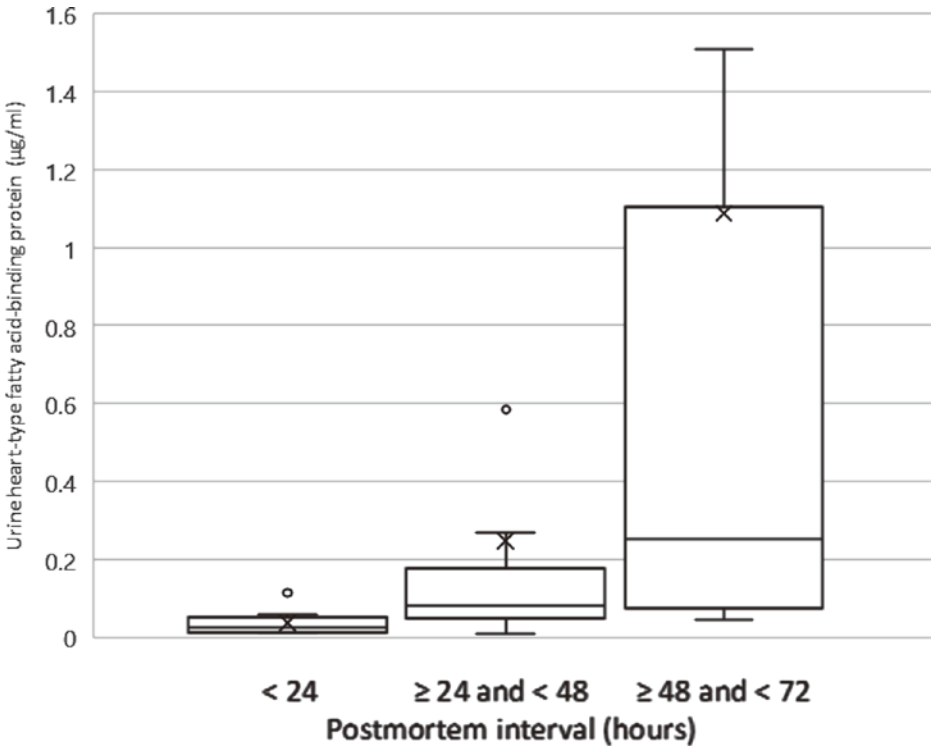

Fig. 2. Box chart comparing urine heart-type fatty acid-binding protein between the lengths of postmortem intervals in control cases. × = mean value.

higher in cases of acute IHD than in control cases without CHF (Table 4, Fig. 1), had an AUC for predicting acute IHD of 0.58 (95% CI = 0.46-0.70) and a cut-off value for diagnosing acute IHD of 1.92 ng/ml (sensitivity, 60.1% ; specificity, 50.0%). However, urine NT-proBNP levels in control cases without CHF did not differ significantly between cases with a heart weight greater than or less than 350 g (data not shown). A significant PMI-dependent elevation of a marker level was observed only for urine hFABP (Fig. 2).

Discussion

In the present study, the mean serum levels of all biochemical markers were higher in both cases than clinical reference values. Postmortem increases in these markers might be explained by the autolysis of myocardium and skeletal muscles[3,6,9,24-27]. Although several studies have shown that postmortem serum levels of biomarkers are useful for diagnosing acute myocardial infarction[10-12,14-16], the present study found no significant differences in serum levels of any biochemical marker between cases of acute IHD and control cases. Possible reasons of this discrepancy between the present study and previous studies are the use of cardiac blood serum, the longer PMI, and the choice to use cases of acute IHD in the present study. Shorter PMI and peripheral blood serum were often used in previous studies ; therefore, the results were likely to be less affected by the autolysis of the myocardium. Included in the present study as non-control cases were not clinically diagnosed cases of acute myocardial infarction but cases of sudden death suspected to have been due to acute IHD. Thus, in many cases of the present study, microscopic myocardial necrosis was not obvious. Therefore, the antemortem myocardial damage and concentrations of biochemical markers may have been lower than in cases of acute myocardial infarction.

Postmortem levels of NT-proBNP in cases of CHF, high heart weight, and myocardial infarction are reportedly high[11,12]. Although the serum NT-proBNP level was significantly higher in control cases with CHF, we observed no difference in these levels between cases of acute IHD and control cases. Moreover, no difference was observed between control cases with heart weight higher than or less than 350 g.

Because pericardial fluid is an ultrafiltrate of the serum, biochemical marker levels in pericardial fluid might reflect the serum levels in living patients[28]. Some studies in forensic medicine have reported the utility of measuring biochemical markers in pericardial fluid obtained postmortem[10,13,17-22]. Although the present study found that levels of hFABP, myoglobin, cTnT, and CKMB in pericardial fluid were significantly higher in cases of acute IHD than in control cases, the concentrations of these markers in both types of cases were much higher than the serum clinical reference levels in living patients, and the elevation was observed after a short PMI. Substances existing in the myocardium might pass through the epicardium owing to myocardium autolysis that occurs postmortem. The biochemical markers measured in the present study exist in the myocardium and are known to be elevated in conditions with myocardial damage, such as IHD. Therefore, the effect of postmortem autolysis is a major concern, and the result of these biochemical markers in the postmortem pericardial fluid was controversial.

Although the present study found that NT-proBNP levels in samples of pericardial fluid were significantly higher in control cases with CHF, no significant difference was suggested between cases of acute IHD and control cases (Tables 4). A possible reason that levels of NT-proBNP in pericardial fluid did not differ significantly is molecular weight. The molecular weight of NT-proBNP is 8.5 kDa and is the lowest of the markers measured in this study ; thus, NT-proBNP is likely to pass through the epicardium at the earlier stage of PMI.

In living patients, the renal clearance of hFABP and NT-proBNP has been reported in several studies to be important[4,5,29,30]. The authors of these studies suggested that low-molecular-weight substances, such as NT-proBNP (8.5 kDa) and hFABP (15 kDa), are more likely than cTnT (37 kDa) to pass through the glomerular membrane and, as such, the kidney plays a large role in the excretion.

In the present study, NT-proBNP and hFABP were detected in urine (Table 2). Because of their low molecular weight, these substances might be released soon after myocardial damage and pass through the glomerular filtration membrane. Therefore, increases in urine levels of these markers are likely to reflect their serum levels. However, because high -molecular-weight markers, such as cTnT and CKMB, were detected in the urine, the contribution of postmortem autolysis should be considered. The molecular weight of NT-proBNP is lower than that of hFABP ; thus, the survival time after onset of myocardial ischemia might not have been long enough for hFABP to be released from the myocardium, to be excreted in the urine, and to show a significant difference between the cases of acute IHD and control cases.

Of the control cases with CHF in the present study, urine samples were obtained from only 3. Therefore, although the urine NT-proBNP levels were higher in control cases with CHF than in other control cases, owing to the small sample size no significant difference was observed.

Possible limitations of the present study were the small sample size and differences of baseline characteristics between cases of acute IHD and control cases. Among the baseline characteristics, BMI and heart weight were significantly higher in acute IHD cases. Therefore, the amount of the biochemical markers in the myocardium was likely to be larger in cases of acute IHD than in control cases. Moreover, the contact surface of the heart to the pericardial fluid might also be larger and be more affected by postmortem autolysis.

In the present study, we measured the levels of biochemical markers that are used to diagnose acute IHD in specimens obtained from forensic autopsy cases. The results suggest that pericardial fluid and urine are useful diagnostic tools for the postmortem diagnosis of acute IHD and that NT-proBNP is useful for diagnosing CHF in cases with a PMI of 72 hours or less. However, further large-scale studies are needed.

Conflict of interest.

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

REFERENCES

1. Lodge-Patch I. The ageing of cardiac infarcts, and its influence on cardiac rupture. Br Heart J. 1951 ; 13 : 37-42.
2. Staub D, Jonas N, Zellweger MJ, Nusbaumer C, Wild D, Pfisterer ME, et al. Use of N-terminal pro-B-type natriuretic peptide to detect myocardial ischemia. Am J Med. 2005 ; 118 : 1287.
3. Ishii J, Wang JH, Naruse H, Taga S, Kinoshita M, Kurokawa H, et al. Serum concentrations of myoglobin vs human heart-type cytoplasmic fatty acid-binding protein in early detection

of acute myocardial infarction. Clin Chem. 1997 ; 43 : 1372–8.
4. Tanaka T, Hirota Y, Sohmiya K, Nishimura S, Kawamura K. Serum and urinary human heart fatty acid-binding protein in acute myocardial infarction. Clin Biochem. 1991 ; 24 : 195–201.
5. Tsuji R, Tanaka T, Sohmiya K, Hirota Y, Yoshimoto K, Kinoshita K, et al. Human heart-type cytoplasmic fatty acid-binding protein in serum and urine during hyperacute myocardial infarction. Int J Cardiol. 1993 ; 41 : 209–17.
6. Plebani M, Zaninotto M. Diagnostic strategies using myoglobin measurement in myocardial infarction. Clin Chim Acta. 1998 ; 272 : 69–77.
7. Wagner GS, Roe CR, Limbird LE, Rosati RA, Wallace AG. The importance of identification of the myocardial-specific isoenzyme of creatine phosphokinase (MB form) in the diagnosis of acute myocardial infarction. Circulation. 1973 ; 47 : 263–9.
8. Mair J, Artner-Dworzak E, Lechleitner P, Smidt J, Wagner I, Dienstl F, et al. Cardiac troponin T in diagnosis of acute myocardial infarction. Clin Chem. 1991 ; 37 : 845–52.
9. Katus HA, Yasuda T, Gold HK, Leinbach RC, Strauss HW, Waksmonski C, et al. Diagnosis of acute myocardial infarction by detection of circulating cardiac myosin light chains. Am J Cardiol. 1984 ; 54 : 964–70.
10. Wang Q, Michiue T, Ishikawa T, Zhu BL, Maeda H. Combined analyses of creatine kinase MB, cardiac troponin I and myoglobin in pericardial and cerebrospinal fluids to investigate myocardial and skeletal muscle injury in medicolegal autopsy cases. Leg Med. 2011 ; 13 : 226–32.
11. Zhu BL, Ishikawa T, Michiue T, Li DR, Zhao D, Tanaka S, et al. Postmortem pericardial natriuretic peptides as markers of cardiac function in medico-legal autopsies. Int J Leg Med. 2007 ; 121 : 28–35.
12. Michaud K, Augsburger M, Donze N, Sabatasso S, Faouzi M, Bollmann M, et al. Evaluation of postmortem measurement of NT-proBNP as a marker for cardiac function. Int J Leg Med. 2008 ; 122 : 415–20.
13. Meng X, Ming M, Wang E. Heart fatty acid binding protein as a marker for postmortem detection of early myocardial damage. Forensic Sci Int. 2006 ; 160 : 11–6.
14. Osuna E, Perez-Carceles MD, Vieira DN, Luna A. Distribution of biochemical markers in biologic fluids : application to the postmortem diagnosis of myocardial infarction. Am J Forensic Med Pathol. 1998 ; 19 : 123–8.
15. Perez-Carceles MD, Noguera J, Jimenez JL, Martinez P, Luna A, Osuna E. Diagnostic efficacy of biochemical markers in diagnosis post-mortem of ischaemic heart disease. Forensic Sci Int. 2004 ; 142 : 1–7.
16. Zhu BL, Ishikawa T, Michiue T, Li DR, Zhao D, Bessho Y, et al. Postmortem cardiac troponin I and creatine kinase MB levels in the blood and pericardial fluid as markers of myocardial damage in medicolegal autopsy. Leg Med. 2007 ; 9 : 241–50.
17. Batalis NI, Marcus BJ, Papadea CN, Collins KA. The role of postmortem cardiac markers in the diagnosis of acute myocardial infarction. J Forensic Sci. 2010 ; 55 : 1088–91.
18. Ghormade PS, Kumar NB, Tingne CV, Keoliya AN. Distribution & diagnostic efficacy of cardiac markers CK-MB & LDH in pericardial fluid for postmortem diagnosis of ischemic heart disease. J Forensic Leg Med. 2014 ; 28 : 42–6.
19. Ellingsen CL, Hetland O. Serum concentrations of cardiac troponin T in sudden death. Am J Forensic Med Pathol. 2004 ; 25 : 213–5.
20. Zhu BL, Ishikawa T, Michiue T, Li DR, Zhao D, Oritani S, et al. Postmortem cardiac troponin T levels in the blood and pericardial fluid. Part 1. Analysis with special regard to traumatic causes of death. Leg Med. 2006 ; 8 : 86–93.
21. Zhu BL, Ishikawa T, Michiue T, Li DR, Zhao D, Kamikodai Y, et al. Postmortem cardiac troponin T levels in the blood and pericardial fluid. Part 2 : analysis for application in the diagnosis of sudden cardiac death with regard to pathology. Leg Med. 2006 ; 8 : 94–101.
22. Gonzalez-Herrera L, Valenzuela A, Ramos V, Blazquez A, Villanueva E. Cardiac troponin T determination by a highly sensitive assay in postmortem serum and pericardial fluid. Forensic Sci Med Pathol. 2016 ; 12 : 181–8.
23. Takasu S, Matsumoto S, Kanto Y, Iwadate K. Utility of soluble lectin-like oxidized low-density lipoprotein receptor-1 (sLOX-1) in the postmortem diagnosis of ischemic heart disease. J Forensic Leg Med. 2018 ; 55 : 45–51.
24. Hunt PJ, Richards AM, Nicholls MG, Yandle TG, Doughty RN, Espiner EA. Immunoreactive amino-terminal pro-brain natriuretic peptide (NT-PROBNP) : a new marker of cardiac impairment. Clin Endocrinol. 1997 ; 47 : 287–96.
25. McMahon CG, Lamont JV, Curtin E, McConnell RI, Crockard M, Kurth MJ, et al. Diagnostic accuracy of heart-type fatty acid-binding protein for the early diagnosis of acute myocardial infarction. Am J Emerg Med. 2012 ; 30 : 267–74.
26. Van Nieuwenhoven FA, Kleine AH, Wodzig WH, Hermens WT, Kragten HA, Maessen JG, et al. Discrimination between myocardial and skeletal muscle injury by assessment of the plasma ratio of myoglobin over fatty acid-binding protein. Circulation. 1995 ; 92 : 2848–54.
27. Frearson N, Perry SV. Phosphorylation of the light-chain components of myosin from cardiac and red skeletal muscles. Biochem J. 1975 ; 151 : 99–107.
28. Ben-Horin S, Shinfeld A, Kachel E, Chetrit A, Livneh A. The composition of normal pericardial fluid and its implications for diagnosing pericardial effusions. Am J Med. 2005 ; 118 : 636–40.
29. Nayashida N, Chihara S, Tayama E, Akasu K, Kai E, Kawara T, et al. Influence of renal function on serum and urinary heart fatty acid-binding protein levels. J Cardiovasc Surg. 2001 ; 42 : 735–40.
30. Bjurman C, Petzold M, Venge P, Farbemo J, Fu ML, Hammarsten O. High-sensitive cardiac troponin, NT-proBNP, hFABP and copeptin levels in relation to glomerular filtration rates and a medical record of cardiovascular disease. Clin Biochem. 2015 ; 48 : 302–7.