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Postmortem pericardial natriuretic peptides as markers of cardiac function in medico-legal autopsies

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Abstract Atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) in the blood are clinical markers for the diagnosis of cardiac failure. This study was a comprehensive analysis of the postmortem pericardial levels of the natriuretic peptides in serial medico-legal autopsy cases (n=263, within 72 h postmortem) to assess their validity in investigating cardiac function. There was no significant relationship of pericardial ANP or BNP levels with postmortem time or the age of the subjects. The ANP and BNP levels showed negative correlations with the pericardial cardiac troponin T level. The ANP level was significantly elevated in drowning cases. Pericardial BNP and the BNP/ANP ratio were significantly higher for chronic congestive heart disease. However, asphyxiation, sharp instrument injury, hyperthermia, and fatal MA poisoning cases showed lower levels for both markers. These observations suggest that elevations in the postmortem pericardial ANP and BNP may mainly depend on acute atrial overload and subacute or chronic cardiac failure, respectively, and may be reduced by advanced myocardial damage.

Keywords Atrial natriuretic peptide · Brain natriuretic peptide · Pericardial fluid · Cardiac function

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Introduction

Acute/chronic cardiac failure is involved in the process of death as a consequence of acute disease or trauma, or as a contributory factor due to preexisting diseases. It is, therefore, important to evaluate terminal cardiac function in forensic casework [1–3]. The postmortem diagnosis of cardiac insufficiency is usually based on the findings of cardiac dilation and visceral congestion involving the lungs, liver, and spleen. However, objective assessment of the severity is very difficult. In clinical practice, atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP), which are vasoactive hormones secreted by the heart, are routinely used for the diagnosis, management, and prognosis of heart diseases with cardiac insufficiency, and their pericardial levels are also useful for this purpose [4–15]. Although a number of reports have suggested that postmortem biochemistry is one of the most productive ancillary procedures available to forensic pathologists [16–19], there appears to be no published data on the natriuretic peptides in postmortem investigation.

In the present study, we comprehensively examined the postmortem ANP and BNP levels in the pericardial fluid as markers for investigating the cardiac function in serial medico-legal autopsy cases.

Materials and methods

Materials

Medico-legal autopsy cases (within 72 h postmortem) at our institutes (n=263) were examined. The cases comprised 195 males and 68 females, 24–94 years of age with a median of 61 years, and a postmortem interval of 3–72 h. The pericardial fluid was aseptically collected using syringes and subsequently stored at −20°C in tubes of polyethylene terephthalate to avoid the degradation of the natriuretic peptides [20], and measured within 7 days.

For all cases, the causes of death were classified based on complete autopsy, macromorphological, micropatholog-

ical, and toxicological examinations. The subjects involved in the present study were as follows: cardiac death groups (n=78) consisting of acute myocardial infarction (AMI, n=16), recurrent myocardial infarction (RMI, n=20), ischemic heart disease without any pathological evidence of infarction (IHD, n=23), chronic congestive heart disease (CHD, n=19), and control groups (n=185, survival time <24 h) consisting of cerebrovascular diseases (CVD, n=14: spontaneous cerebral hemorrhage, n=12; subarachnoid hemorrhage, n=2), blunt injury (n=58: head injury, n=30; others, n=28), sharp instrument injury (n=12), asphyxiation (n=23), including strangulation and hanging (n=11), aspiration of a foreign body or vomit (n=12), drowning (n=14: freshwater, n=8; saltwater, n=6), fire fatalities (n=44), including those of blood carboxyhemoglobin (COHb) 60% (n=26) and COHb>60% (n=18), hyperthermia (heat stroke, n=3), hypothermia (cold exposure, n=9), and fatal methamphetamine (MA) poisoning (n=8). For these groups, clearly representative cases were collected, excluding cases involving complications that may have influenced the death process, including advanced liver cirrhosis, renal failure, intoxication, and other traumas. For control cases, subjects without marked coronary stenosis (>50%), pulmonary fibrosis or pulmonary vascular lesions were included. The case profiles and autopsy data are shown in Tables 1 and 2.

The cardiac death groups were diagnosed based on macro- and microscopical findings of heart diseases, without any evidence of death other than a cardiac attack. Histopathological investigation was done on the left anterior, left posterior, septal, and right lateral myocardial sections by routine hematoxylin–eosin (HE). The main histopathological findings were as follows: AMI, fresh localized myocardial damage (myocardial eosinophilic changes, necrosis with/without interstitial hemorrhage, or inflammatory infiltration) irrespective of preexisting old myocardial infarction; RMI, fresh localized myocardial damage with replacement of the necrotic fibers by dense collagenous scar; IHD, diffuse interstitial congestion, edema, patchy myocardial eosinophilic changes, in part accompanied by multiple hemorrhages and contraction bands; CHD, volume-overloaded ventricles with or without myocyte hypertrophy, and interstitial fibrosis. The cardiac pathological findings in cardiac death cases are shown in Table 3.

Biochemical analyses

The pericardial ANP and BNP concentrations were measured by radioimmunoassay using Shionoria RIA ANP and RIA BNP assay kits (Shionogi, Osaka), respectively. The ranges of measurement were <2,000 pg/ml for ANP and BNP [20]. Samples were diluted (×10 and ×100) to measure higher concentrations (>2,000 pg/ml), and measurements were performed in duplicate to exclude

Table 1 Case profiles (n=263)

Cause of death	n	Age (years)		Survival time (h)		PMI (h)	
		Male/Female	Range	Median	Range	Range	Median
AMI	16	12/4	51–88	67	<0.5–32	10–36	20.0
RMI	20	15/5	51–81	65	<0.5–24	6–72	21.4
IHD	23	16/7	37–87	61	<0.5–6	45–68	18.0
CHD	19	12/7	41–87	74	Unknown	5–70	18.4
Cerebrovascular diseases	14	10/4	45–72	58	<0.5–12	5–42	23.0
Blunt injury							
Head injury	30	26/4	24–77	60	0–48	3–38	15.7
Others	28	23/5	28–94	61	0–72	4–72	19.7
Sharp instrument injury	12	10/2	34–81	50	<0.5–5	12–50	18.8
Asphyxia							
Strangulation/hanging	11	6/5	34–71	53	<0.5	8–68	22.8
Aspiration	12	9/3	42–94	68	<0.5–2	12–72	20.6
Drowning							
Freshwater	8	6/2	47–76	60	<0.5	8–37	19.3
Saltwater	6	4/2	42–60	52	<0.5	8–18	15.1
Fire fatality							
COHb <60%	26	17/9	32–93	70	<0.5	7–29	15.3
COHb >60%	18	13/5	45–85	67	<0.5	5–49	15.7
Hyperthermia	3	2/1	51–72	65	1–5	17–23	21.5
Hypothermia	9	7/2	54–85	73	3–8	12–72	26.3
Fatal MA poisoning	8	7/1	30–52	37	3–24	6–70	25.8
Total	263	195/68	24–94	61	0–unknown	3–72	19.5

AMI Acute myocardial infarction, *RMI* recurrent myocardial infarction, *IHD* ischemic heart disease, *CHD* chronic congestive heart disease, *PMI* postmortem interval, *COHb* blood carboxyhemoglobin concentration, *MA* methamphetamine

Table 2 Autopsy data (mean±SD) in cardiac deaths in comparison with the other groups

Cardiac deaths						
	AMI	RMI	IHD	CHD	Drowning	Others
Cases number (male/female)	16 (12/4)	20 (15/5)	23 (16/7)	19 (12/7)	14 (10/4)	171 (130/41)
CPR	5	7	3	9	0	38
Body height (cm)						
Male	163±6.5	163±7.3	165±6.8	166±8.2	169±6.0	164±13
Female	146±10	151±3.8	147±6.9	148±7.9	153±11	154±7.2
Body weight (kg)						
Male	66±15	68±15	71±28	60±16	66±17	63±39
Female	42±1.3	48±5.8	49±13	56±14	55±15	48±13
Heart weight (g)						
Male	465±87	494±84	399±92	522±150	432±96	381±100
Female	285±64	395±99	321±67	430±144	343±31	329±87
Pericardial fluid (ml)	10±7	7±10	11±5	15±40	15±6	10±5
Range	2–25	1–30	2–25	5–150	6–35	0.5–35
Lung weight (g)						
Male						
Left	524±240	640±318	564±178	431±111	655±293	470±165
Right	573±255	740±283	635±192	568±127	766±263	564±195
Female						
Left	383±230	404±70	373±138	368±128	442±144	420±361
Right	515±332	511±95	515±290	512±121	552±38	425±132
Pleural effusion (ml)	0	8±43	0	25±37	213±536	0
Range (median)		0–150 (0)		0–400 (20)	0–1,750 (100)	
Liver weight (g)						
Male	1,608±432	1,750±385	1,350±334	1,495±396	1,520±265	1,416±307
Female	1,122±318	1,374±412	1,208±547	1,380±164	1,140±310	1,196±243
Kidney weight (g)						
Male	324±103	350±92	316±83	277±126	354±73	279±69
Female	213±70	215±87	228±68	268±57	234±63	234±44
Spleen weight (g)						
Male	148±64	182±82	153±92	128±63	102±52	124±57
Female	90±40	128±57	115±136	106±59	83±42	116±61

AMI Acute myocardial infarction, *RMI* recurrent myocardial infarction, *IHD* ischemic heart disease, *CHD* chronic congestive heart disease, *CPR* cardiopulmonary resuscitation

possible interference due to contaminants. The clinical serum reference range was 10–40 pg/ml for ANP and 2–20 pg/ml for BNP.

Cardiac troponin T (cTnT) was measured using an electrochemiluminescence immunoassay (Roche Diagnostics, USA) [21]. The cross-reactivity to skeletal muscle troponin T was less than 0.01% [22].

Toxicological analyses

The blood COHb concentration was determined using a CO-oximeter system for the fire fatalities [23, 24], and volatile chemicals, including alcohol, were analyzed using head-space gas chromatography for all cases. Drug analyses were performed by gas chromatography/mass spectrometry when the preliminary screening tests were positive.

Statistical analyses

The Pearson product–moment correlation coefficient was used to compare two parameters, including the ANP or BNP levels, the age of the subjects, and the postmortem interval. Comparisons between groups were performed using a nonparametric Mann–Whitney *U* test and the Scheffe test was used for analysis involving multiple comparisons. These analyses were performed using the software/StatView, version 5.0 (SAS Institute SAS Campus Drive Cary). A *P*-value of less than 0.05 was considered to indicate statistical significance. The sensitivity and specificity in distinguishing the two groups using cutoff ANP and BNP values were estimated by means of receiver operating characteristics (ROC) analysis; ROC curves were constructed by plotting sensitivity against (1-specificity). The area under the curves were calculated and analyzed by one-tailed test. The optimal

Table 3 Pathological findings in cardiac death cases (n=78)

	AMI	RMI	IHD	CHD
Case number	16	20	23	19
Macroscopic pathology				
Ventricular dilatation (left>right/right>left)				
No	2	5	8	0
Mild	2 (1/0)	5 (3/0)	12 (1/3)	0
Moderate	9 (5/0)	8 (4/2)	3 (0/0)	5 (2/2)
Marked	3 (2/0)	2 (1/0)	0	14 (4/0)
Coronary artery, >75% stenosis	12	19	14	7
Coronary thrombus	7	6	0	0
Interstitial fibrosis	7	16	6	9
Localized myocardial lesions				
Myocardial lesions >1/3 left ventricle wall	8	0	0	0
Fibrous scar >1/3 left ventricle wall	0	7	0	0
Microscopic finding				
Myocardial lesions				
Interstitial congestion, edema and patchy myocardial eosinophilic changes	0	0	23	19
Patchy interstitial hemorrhages (with contraction bands)	7 (2)	6 (1)	0	0
Localized necrosis (with inflammatory infiltration)	9 (4)	14 (8)	0	0

Details of heart weight shown in Table 2
AMI Acute myocardial infarction, *RMI* recurrent myocardial infarction, *IHD* ischemic heart disease, *CHD* chronic congestive heart disease

compromise between sensitivity and specificity was determined graphically [25, 26].

Results

Postmortem influence, age- and gender-dependency

The postmortem ranges for the pericardial fluid were ANP 0.1–280 pg/ml (mean/median 23.0/10.4) and BNP 2–17,700 pg/ml (mean/median 443/65.4). For all cases, both factors (y) showed no significant relationship to the postmortem interval (the time interval between death and autopsy) (x) (ANP, y=−0.19x+32.0, r=−0.131, P=0.0632; BNP, y=−2.5x+497, r=−0.024, P=0.7018). There was no significant relationship between the ANP and BNP levels and the age of the subjects, and no significant gender-differences. For all cases, there was a very mild postmortem time-dependent elevation in the pericardial cTnT levels (r=0.182, P=0.0129).

Relationship to cardiac troponin T levels and pathology

Pericardial ANP and BNP levels showed no significant relationship with the pericardial cTnT levels for all cases (correlation coefficient, r=−0.105 to −0.118, P>0.05). However, significant negative correlations (r=−0.22 to −0.50, P<0.05) were observed for some specific causes of death, independently of the postmortem interval: cardiac deaths (AMI, RMI, IHD, and CHD), drowning, hyperthermia, hypothermia, and MA fatalities for ANP; RMI, CHD, CVD, sharp instrument injury, asphyxiation, fire fatalities, hyperthermia, and MA fatalities for BNP (Fig. 1a,b).

The BNP level (y) showed a weak positive correlation with heart weight (x_1), the heart weight/body height ratio (x_2) and heart weight/body weight ratio (x_3) for all cases (y=0.02x_1+384, r=0.276, P<0.0001, y=0.001x_2+2.35, r=0.291, P<0.0001 and y=0.002x_3+6.67, r=0.216, P<0.001, respectively), and the correlation was moderate for CHD (r=0.504, r=446, and r=0.354, P<0.05, respectively). The BNP level also correlated with the lung weight/body height ratio for CHD (r=0.701, P<0.01). However, the ANP level showed no significant correlation with those factors. For cardiac death cases, the BNP level was significantly higher for cases showing moderate to marked ventricular dilatation than for those showing normal or mildly enlarged ventricle (n=44 and n=34, respectively, P<0.01). However, the ANP and BNP levels were independent of the severity of coronary lesions, size of fresh or old myocardial lesions, or other histopathological findings. Furthermore, there was no significant relationship of the ANP and BNP levels with the volume of pericardial fluid or pleural effusion, the weight of liver, kidney, or spleen, and no significant difference between cases with and without cardiopulmonary resuscitation (n=62 and n=201, respectively).

Analyses with regard to the cause of death

For all cases, there was a mild positive correlation between the pericardial ANP (y) and BNP (x) levels (y=0.008x+22.1, r=0.387, P<0.0001). However, when analyzed with regard to the cause of death, the correlation was moderate to marked for AMI, RMI, CHD, sharp instrument injury, and drowning (r=0.508–0.686), whereas, it was insignif-

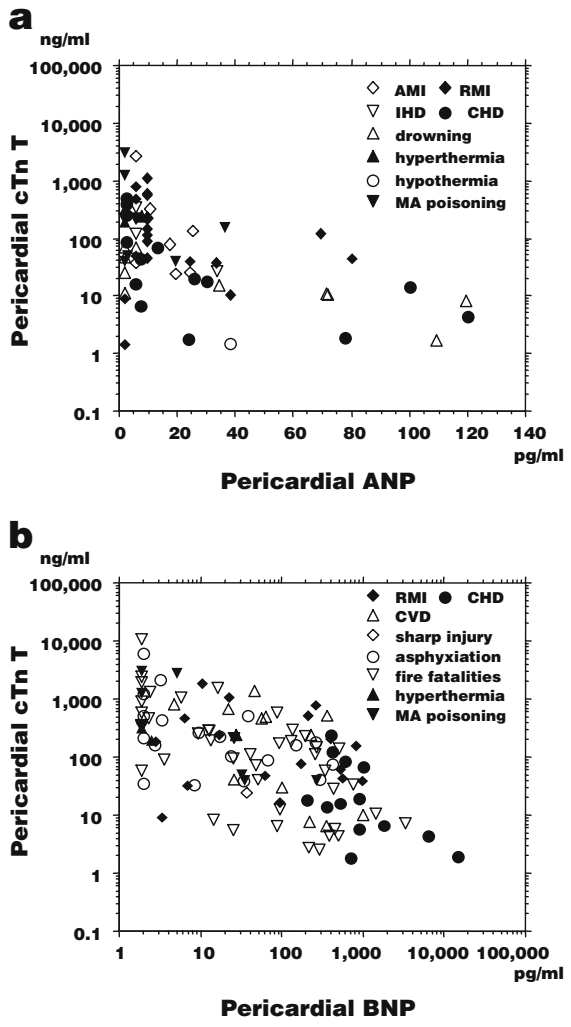

Fig. 1 The relationships of cardiac troponin T (cTnT) with atrial natriuretic peptide (ANP) (**a**) and brain natriuretic peptide (BNP) (**b**) in postmortem pericardial fluid, showing negative correlations

icant for IHD, CVD, and fire fatalities (r<0.2). Pericardial ANP and BNP showed different relationships with the cause of death, as shown in Table 4.

There was no significant difference in the pericardial ANP level between cardiac and noncardiac death cases. The ANP level was significantly higher for drowning compared with that for other groups, except for CHD and hypothermia, using the Mann–Whitney U test, whereas the ANP level was low for asphyxiation and sharp instrument injury. There was a mild increase for AMI and fire fatalities. In blunt injury cases, the ANP level was higher for head injury than nonhead injury (median 17.0 and 8.4 pg/ml, respectively, P<0.05). There was no significant difference between groups with survival time <0.5 (n=158) and >0.5 h (n=105) (P=0.4954). When analyzed by ROC, the sensitivity and specificity for ANP in distinguishing drowning and asphyxiation were 0.71 and 0.83 at a cutoff value of 30 pg/ml, respectively. The ANP levels above this cutoff value were detected in 23.5% of all cases (62/263), being relatively frequent for CHD (n=7/19, 36.8%), RMI (n=5/20, 25.0%), head injury (n=11/30, 36.7%), and IHD (n=7/23, 26.9%) besides drowning (n=10/14, 71.4%), and infrequent in cases of sharp instrument injury, hyperthermia, and MA fatalities (n=2/24, 8.3%).

The BNP level and BNP/ANP ratio were markedly higher for cardiac deaths compared with those for noncardiac death cases (P<0.01 and P<0.001, respectively). For all groups, a significantly higher BNP level was observed for CHD compared with that in other groups except hypothermia, whereas the BNP level was very low for IHD, asphyxiation, sharp instrument injury, hyperthermia, and MA fatalities (Table 4). The pericardial BNP level and BNP/ANP ratio were significantly higher for fire fatalities with lower (<60%) COHb than for those with higher (>60%) COHb (P<0.05). Furthermore, the BNP level and BNP/ANP ratio were markedly elevated in cases showing longer survival times (>0.5 h, n=105) compared to those of cases showing short survival times (<0.5, n=158) (P<0.01 and P<0.001, respectively). ROC analysis showed that the sensitivity and specificity in distinguishing CHD and IHD were 1.00 and 0.96 for BNP at a cutoff value of 150 pg/ml, and 0.86 and 0.87 for the BNP/ANP ratio at a cutoff value of 9.0, respectively. For hypothermia, AMI and RMI, the BNP levels above this cutoff value were seen in 100% (n=9/9), 37.5% (n=6/16), and 45.0% (n=9/20), respectively. Differences in the pericardial ANP or BNP level between freshwater and saltwater drowning cases and between strangulation/hanging and aspiration were not significant.

Discussion

ANP and BNP are small peptides consisting of 28 and 32 amino acid residues, which are synthesized and secreted from the atrial and ventricular myocardium, respectively [5]. Clinical studies have suggested that ANP is secreted from the atrial wall in normal subjects and that the elevation mainly depends on pulmonary hypertension and atrial overload [27, 28]. It is also secreted from the left ventricular wall in patients with left and right ventricular dysfunction [12]. BNP is mainly derived from the left ventricular wall, and is increased in patients with left and/or right ventricular dysfunction [5–7, 27, 28]. These peptides are also secreted in the pericardial fluid [8, 9]. A previous clinical study showed that ANP and BNP levels in the pericardial fluid were correlated with and higher than the respective blood levels, showing more marked increases due to cardiac failure [8]. Thus, pericardial ANP and BNP may be more suitable and useful as other biochemical markers for cardiac function.

In the present study, there was no significant postmortem influence on pericardial ANP and BNP levels within 72 h after death. These peptides showed negative correlations with cardiac troponin T levels in the pericardial fluid, suggesting that their production by cardiac tissue may be reduced depending on the severity of myocardial damage.

With regard to the cause of death, a significant elevation in the pericardial ANP level, in contrast to mildly elevated BNP, was observed in drowning cases, irrespective of fresh

Table 4 Postmortem pericardial atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) levels (mean±SD; range, median) with regard to the cause of death

Cause of death	*n*	ANP (pg/ml)			BNP (pg/ml)			BNP/ANP		
AMI	16	21.4±24.7	0.1–81	15.0	280±394	2.0–1,210	103	248±912	0.11–3,670	11.6
RMI	20	17.8±26.9	0.1–80	11.2	256±331	2.0–1,060	82.0	129±327	0.87–1,375	17.0
IHD	23	24.1±30.2	0.2–110	12.4	27.6±43.2	2.0–190	10.7	5.1±13.3	0.04–64.5	0.59
CHD	19	40.2±70.3	1.5–280	19.0	2,326±3,708	204–14,600	882[a]	770±1,608	3.5–5,270	76.8[b]
Cerebrovascular diseases (CVD)	14	15.7±14.2	0.1–48	11.0	200±288	5.0–1,050	65.4	56.5±133	0.36–489	3.7
Blunt injury (I-B)										
Head injury	30	27.2±22.9	0.7–84	17.0	379±438	2.0–1,890	301	24.8±53.9	1.2–283	10.0
Others	28	13.8±19.3	0.2–100	8.4	296±436	2.0–1,500	73.0	27.7±45.7	0.2–174	8.7
Sharp instrument injury (I-S)	12	6.7±8.8	0.3–32	3.7[c]	54.9±58.6	2.0–159	36.1	58.6±148	0.3–520	4.4
Asphyxia (A)	23	13.0±14.9	0.2–58	5.1[d]	71.5±114	2.0–416	17.3	12.0±25.8	0.3–118	2.0
Drowning (D)	14	38.5±43.2	0.5–200	26.0[e]	154±198	2.0–572	21.6	3.9±3.9	0.2–13.5	2.9
Fire fatality (F)										
COHb <60%	26	26.2±46.7	0.3–240	17.0	349±743	2.0–3,540	99.0	197±619	0.03–2,646	5.5
COHb >60%	18	25.6±28.1	0.8–110	11.6	125±188	2.0–532	16.8	6.4±10.3	0.1–28.0	1.0
Hyperthermia (H)	3	7.1±5.5	1.2–12	8.1	11.1±15.2	2.0–28.6	2.6	1.5±1.0	0.3–2.4	1.7
Hypothermia (C)	9	48.0±63.9	0.3–120	19.2	3,289±5,916	276–17,700	1,084[f]	648±1,403	0.2–4,233	58.7[g]
Fatal MA poisoning (MA)	8	10.3±12.7	0.2–37	6.0	48.2±93.6	2.0–277	16.5	174±489	0.15–1,385	1.9
Total	263	23.0±34.6	0.1–280	10.4	443±1,578	2.0–17,700	65.4	136±607	0.03–5,270	5.5

ANP: [c,d,e]In I-B cases, the ANP level was significantly higher for head injury than for nonhead injury according to the Mann–Whitney *U* test ($P<0.05$). BNP: Significantly high ($P<0.05$)[a,f]. In addition, IHD showed a lower level than AMI and RMI ($P<0.05$), and the level was significantly higher for fire fatalities with lower (<60%) COHb than for those with higher (>60%) COHb according to the Mann–Whitney *U* test ($P<0.05$). BNP/ANP: Significantly high ($P<0.05$)[b,g]. In addition, IHD showed a lower level than AMI and RMI ($P<0.05$), and the ratio was significantly higher for fire fatalities with lower (<60%) COHb than for those with higher (60%) COHb with the Mann–Whitney *U* test ($P<0.05$)

AMI Acute myocardial infarction, *RMI* recurrent myocardial infarction, *IHD* ischemic heart disease, *CHD* chronic congestive heart disease, *COHb* blood carboxyhemoglobin concentration, *MA* methamphetamine

[a](BNP) CHD vs other groups, except for H and C according to the Scheffe test

[b](BNP/ANP) CHD vs RMI, IHD, I-B, I-S, A, D, and F using the Scheffe test

[c](ANP) Significantly low for sharp instrument injury compared with AMI, CHD, I-B, and fire fatalities

[d](ANP) Significantly low for asphyxiation compared with CHD and fire fatalities

[e](ANP) Significantly high for drowning and with other groups, except for CHD and hypothermia

[f](BNP) C vs other groups except for CHD using Mann–Whitney *U* test

[g](BNP/ANP) C vs other groups, except for CHD according to the Mann–Whitney *U* test

or saltwater drowning and independent of the lung weight and volume of pleural effusion at autopsy. These findings suggest that the elevation in pericardial ANP level for drowning may depend on multiple factors, including cardiac dysfunction due to electrolyte disturbance, acute atrial overload involving increased wall tension, pulmonary hypertension caused by water aspiration during fresh and saltwater drowning, or stretched atrial chambers due to increased circulatory blood volume especially for freshwater drowning [29, 30], and relatively mild myocardial damage [18, 19].

However, both ANP and BNP levels were low for asphyxiation, hyperthermia, and fatal MA abuse. With respect to this, ANP and BNP levels may be reduced depending on the severity of myocardial damage as described above. Our previous study showed increased serum and pericardial cTnT levels not only for myocardial infarction/ischemia cases but also for noncardiac death including hyperthermia, fatal MA poisoning, CO poisoning, and asphyxiation depending on the severity of myocardial damage [18, 19]. Thus, for asphyxiation, hyperthermia, and fatal MA abuse, massive myocardial damage due to advanced hypoxia may contribute to the low ANP and BNP levels.

The postmortem pericardial BNP level and BNP/ANP ratio were markedly elevated for CHD compared with the other groups, including myocardial infarction (AMI and RMI), while the elevation in ANP was as mild as that for ischemic heart diseases (AMI, RMI, and IHD). For CHD, the BNP level correlated with the grade of ventricular dilatation, heart, and lung weights at autopsy, suggesting a relationship with gradually developing cardiac hypertrophy, terminal pulmonary congestion, and edema as a consequence of fatal cardiac dysfunction without evident acute myocardial damage. Clinical investigations have shown that the concentration of BNP in the blood of normal subjects is lower than that of ANP, and that an elevation in BNP for AMI and chronic heart failure increases the BNP/ANP ratio, depending on the severity and duration of ventricular dysfunction [7, 31–33]. In a similar manner, a high postmortem pericardial BNP level and BNP/ANP ratio may be indicative of the severity and

duration of cardiac insufficiency before death. This suggests that elevation in the pericardial BNP level and BNP/ANP might mainly be due to subacute or chronic ventricular overload (cardiac failure). These findings may also be useful for evaluating cardiac insufficiency in other causes of death that occur without massive myocardial damage. With respect to this, hypothermia showed findings similar to those for CHD, suggesting the contribution of cardiac failure in the process of death and milder myocardial damage.

For ischemic heart diseases, myocardial infarction (AMI and RMI) showed mild elevation in the pericardial ANP and BNP levels. Elevation in the BNP level was observed in about 40% of the cases, suggesting a substantial duration of terminal cardiac failure caused by localized myocardial damage. Previous clinical studies have suggested the serum BNP level increases in the early phase after the onset of myocardial infarction/ischemia (<4.0 h), reflecting cardiac failure [1, 7]. In the present study, IHD showed a low pericardial BNP level and a mild ANP elevation, which is suggestive of acute atrial overload due to left ventricular dysfunction possibly associated with early fatal arrhythmia [34, 35].

Fire fatalities showed a mild increase in the pericardial ANP level and the BNP level and BNP/ANP ratio were significantly lower in cases with higher (>60%) COHb than in those with lower (<60%) COHb levels. This finding may be explained as a consequence of a shorter duration of cardiac failure or survival and more severe myocardial damage due to carbon monoxide intoxication [18, 36]. However, in head injury cases, a higher ANP level for head injury compared with nonhead injury suggests the influence of noncardiogenic pulmonary edema causing pulmonary hypertension [37, 38]. Furthermore, low pericardial ANP and BNP levels for sharp instrument injury may be due to blood loss and in part due to myocardial damage. Similar findings for hyperthermia and MA fatality may be attributed to massive myocardial damage, as observed for asphyxiation [18].

These observations suggest that postmortem pericardial ANP and BNP levels may be elevated due to acute overload and subacute or chronic cardiac failure, respectively. These markers may be useful for determining the cause of death due to chronic heart diseases, drowning, and hypothermia, and also for investigating cardiac dysfunction in other causes of death involving ischemic heart disease, blunt injury, and fire fatalities, when myocardial markers are used in combination.

In conclusion, the present study did not show any significant postmortem influence on pericardial ANP and BNP levels within 72 h after death. Both markers showed elevation depending on the cause of death, suggesting that elevation in the postmortem pericardial ANP and BNP may depend mainly on acute atrial overload and subacute or chronic cardiac failure, respectively, and may be reduced by advanced myocardial damage. The combined analysis of ANP, BNP, and other myocardial markers in the pericardial fluid may be useful for investigating cardiac dysfunction.

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