Diagnostic significance of myocardial stress markers in unnatural deaths

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Objective: To examine the diagnostic significance of myocardial stress markers in unnatural deaths treated in forensic medicine.

Materials and Methods: The materials were cardiac blood, femoral blood, and cerebrospinal fluid (CSF) from inspections or autopsies in the Department of Legal Medicine, Kitasato University School of Medicine for diagnostic purposes. We measured N-terminal pro-B-type natriuretic peptide (NT-proBNP) and troponin T using a Cobas h 232 (Roche Diagnostics K.K., Tokyo).

Results: Troponin T was useful in diagnosing ischemic heart diseases only in femoral blood. However, NT-proBNP indicated a relationship with the cause of death in all the materials. Moreover, combination of NT-proBNP and femoral blood troponin T measurements suggested a more accurate diagnosis of ischemic heart diseases.

Conclusions: Our data suggest that the NT-proBNP measurements strongly reflect the degree of load to the heart leading to death. This device is compact and readily operable and enables measurement results to be obtained at the scene during inspections or autopsies; therefore, this method revealed a considerably high, practical, diagnostic value.

Introduction

Currently, in the aging society that our country is facing, there has been an increasing tendency to see unnatural deaths in the elderly with scant medical histories, measures, and prevention becoming social issues.[1] In forensic medicine, it is extremely important to find the cause of death which should be revealed by autopsy. However, the bodies in many unnatural deaths do not undergo autopsies in Japan but are diagnosed by inspections. To not only elucidate the truth behind an individual's death, but also so as not to miss criminal deaths, it is ideal to reveal the cause of death by autopsies for all deaths, including the elderly; however, in Japan, where the percentage of consented autopsies is remarkably low (0.9%), compared to that in Western countries, the ideal situation is unrealistic. Yet, the diagnostic accuracy of inspections is still low and often misdiagnoses are made;[2] therefore, it is necessary to improve the percentage of consented autopsies as well as to improve diagnostic accuracy. Although various attempts such as biochemical tests of cadaveric materials and postmortem imaging tests have been made in order to improve diagnostic accuracy,[3-14] due to significant postmortem changes, the diagnostic values are poor even though those tests are useful in a clinical setting. Thus, in this study we focused on NT-proBNP which has recently been an index in the diagnosis of cardiac failure, determination of therapeutic effect, and determination of prognosis in a clinical setting,[15] and troponin T for which the postmortem diagnostic value remains doubtful[10-11] and examined the possibility of simpler measurements of NT-proBNP and troponin T and of improving the accuracy of postmortem diagnoses using cadaveric materials and an autoanalyzer, the Cobas h 232 (Roche Diagnostics K.K., Tokyo).

Materials and Methods

Materials

Subject materials were cardiac blood, femoral blood and CSF collected from 150 inspection or autopsy cases in the Department of Legal Medicine, Kitasato University School of Medicine, for diagnostic purposes (male, 88 cases; female, 62 cases; age, 14-98 years; mean age, 67.9 years). Based on the test results, the causes of deaths of the subjects were divided into an ischemic heart disease group (A) and other-causes-of-death group (B), and compared. The causes of deaths in Group B included cardiac tamponade caused by acute aortic dissection, pulmonary thromboembolism, deaths from diseases such as malignant tumors, and suicides, including hanging, acute carbon monoxide poisoning, and acute hydrogen sulfide poisoning.

Methods and Analysis

Measurements were made by immunochromatography, using the autoanalyzer, Cobas h 232 (Roche Diagnostics). This device is able to test 5 items: troponin T, NT-proBNP, myoglobin, CK-MB, and D-dimer, of which troponin T and NT-proBNP were measured in the present study. Among 150 cases, in relation to the sites possible for obtaining samples and collected volumes, the tested items differed in 3 samples (cardiac blood, femoral blood, and CSF). The numbers of samples for each test item are shown in the "Results."

NT-proBNP is a substance in which synthesis is facilitated by loads to the heart and released into the blood, which is considered to be a continuous variable. We, therefore, prepared the receiver operating characteristic (ROC) curve and tried to set the cutoff based on those measurements. Regarding troponin T,

the values below the lower detection limit of this device (0.03 ng/ml) were determined to be negative, while those above were positive.
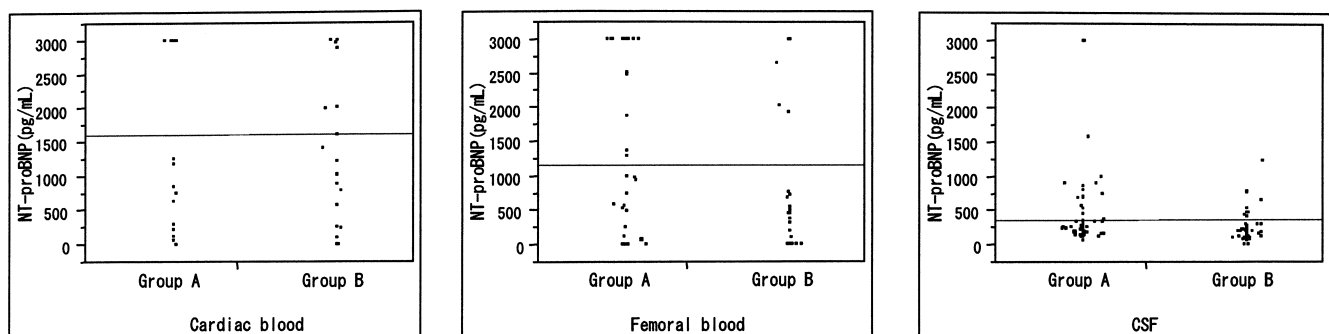
Results

NT-proBNP

The plots of NT-proBNP values for each sample divided in to Groups A and B based on the cause of death are shown in Figure 1. The numbers of samples were: 43 cardiac blood samples (Group A, 23; Group B, 20), 74 femoral blood samples (Group A, 39; Group B, 35), and 108 CSF samples (Group A, 58; Group B, 50).

As shown in Figure 1, the samples which showed >3,000 pg/mL exceeding the upper detection limit of this device were: cardiac blood (Group A, 12; Group B, 3), femoral blood (Group A, 14; Group B, 5), CSF (Group A, 2; Group B, 0), and many cases were clearly found in ischemic heart disease in Group A. Higher values in Group B showed malignant tumors, including lung cancer, inflammatory diseases, including pneumonia and peritonitis, and pulmonary thromboembolism. Meanwhile, in Group A, some cases showing lower values were subsequently revealed to be acute myocardial infarction as determined by autopsy. Moreover, in Group B, many cases which showed lower values were found to have resulted in acute outcomes of both intrinsic and extrinsic deaths such as cardiac tamponade based on acute aortic dissection, acute hydrogen sulfide poisoning, and hanging.

Receiver operating characteristic (ROC) curve of NT-proBNP

The ROC curve prepared based on the above is shown in Figure 2. The ROC curve is a plot graph of sensitivity
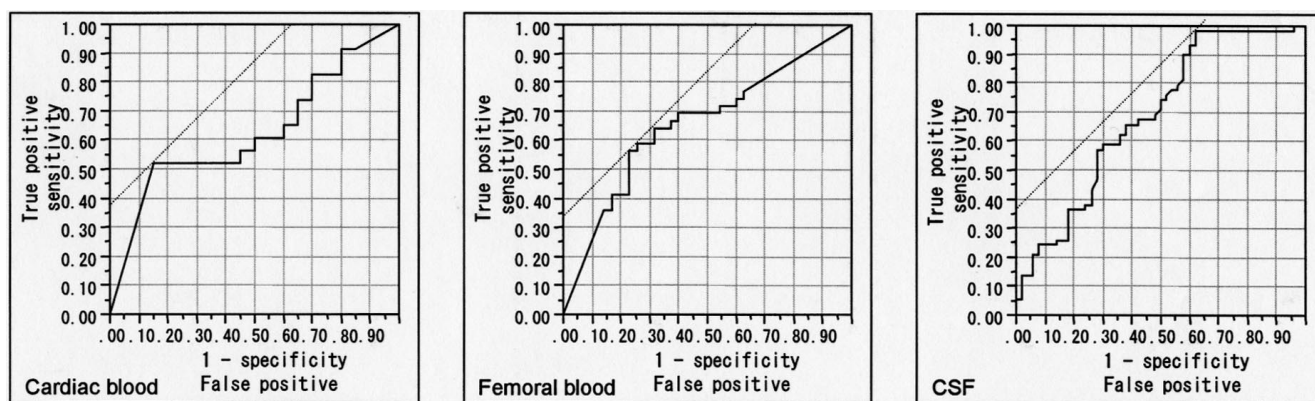

Figure 1. NT-proBNP measurement results for each sample

Group A: ischemic heart disease
Group B: various causes of death except ischemic heart disease
CSF, cerebrospinal fluid

Figure 2. ROC curve of each NT-proBNP sample

CSF, cerebrospinal fluid

Table 1. Cutoff obtained from an ROC curve and the sensitivity and specificity based on the cutoff in NT-proBNP

	Cardiac blood	Femoral blood	CSF
Cutoff (pg/mL)	3,000	928	116
Sensitivity (%)	52.2	56.4	98.3
Specificity (%)	85.0	77.1	38.0
Area under the curve	0.62609	0.65458	0.68569

CSF, cerebrospinal fluid

Table 2. The number of troponin T negative and positive cases based on the cause of death for each sample

Troponin T	Cardiac blood		Femoral blood		CSF	
	Group A	Group B	Group A	Group B	Group A	Group B
Positive	58	51	51	29	14	12
Negative	1	1	10	31	61	55

CSF, cerebrospinal fluid

Table 3. Sensitivity, specificity, and positive/negative predictive value of each sample in troponin T

	Cardiac blood	Femoral blood	CSF
Sensitivity (%)	98.3	83.6	18.7
Specificity (%)	1.9	51.7	82.1
Positive predictive value (%)	53.2	63.8	53.8
Negative predictive value (%)	50.0	75.6	47.4

CSF, cerebrospinal fluid

and "1-specificity." The more the curve is located in the upper left of the graph, the more useful the test is. The area under the curve and the optimum cutoff obtained from the graph, and the sensitivity and specificity based on the cutoff, are shown in Table 1. Sensitivity was lower in cardiac blood and femoral blood, and specificity was lower in CSF.

Troponin T
The number of positive and negative cases based on the cause of death for each sample is shown in Table 2. Furthermore, based on those results, sensitivity and specificity were calculated, and the results are shown in Table 3. There were 111 cardiac blood samples (Group A, 59; Group B, 52), 121 femoral blood samples (Group A, 61; Group B, 60), and 142 CSF samples (Group A, 75; Group B, 67). Remarkably lower values were noted in specificity for cardiac blood and sensitivity for CSF; moreover, the positive and negative predictive values were both as low as around 50% for cardiac blood and CSF. On the other hand, femoral blood showed the highest values among the 3 samples as sensitivity was approximately 84% and the negative predictive value was 76%.

Discussion

NT-proBNP is a byproduct produced when BNP is made from proBNP, in which the synthesis pre-proBNP is immediately started when the heart is pressure loaded, cleaved to proBNP, and then to BNP and NT-proBNP.[16,17] NT-proBNP is considered to undergo renal clearance and thus supposed to be largely influenced by renal function and is known to change its value by various factors.[18-20] However, it is extremely difficult to temper all of these for diagnosis; therefore, clinically there have been a number of studies to propose the values by only considering age and preparation of present guidelines.[21-26] NT-proBNP is highly stable compared to BNP,[27] and half life is 20 minutes for BNP and 120 minutes for NT-proBNP in living bodies. Moreover, Michaud et al.[28] demonstrated the stability of NT-proBNP in cadaveric materials (blood and pericardial fluid). Thus, we also considered that NT-proBNP may be extremely useful for the diagnosis of ischemic heart disease in cadaveric materials and, therefore, initiated this study.

Generally, if the cause of death was ischemic heart disease, NT-proBNP was considered to increase in all cases because ischemia leads to cardiac failure. However, from our results, NT-proBNP did not always show higher values in Group A, and higher values were also observed in Group B (Figure 1). When we investigated lower values (<60 pg/ml) in femoral blood in Group A in autopsy cases, 2 of 3 cases had a thrombus in the coronary artery and were diagnosed as acute myocardial infarction. Because blood BNP starts to increase after several hours from myocardial ischemia,[29] the cases with acute courses leading to death before biosynthesis and release were considered to show low values even though it was ischemic heart disease. In addition, there was a constant tendency for higher values in Group B. Many of those were in the terminal state of cancer, inflammatory diseases such as pneumonia and peritonitis, and pulmonary thromboembolism in which a substantial load to the heart is inferred. Moreover, among Group B, there was also a constant tendency in suicides based on cause of death. Regarding NT-proBNP in CSF, 2 of 3 acute carbon monoxide poisoning cases indicated high values, while both acute hydrogen sulfide poisoning cases and all 3 hanging cases showed low values. This was considered because acute carbon monoxide poisoning requires some time from occurrence to death, though both acute sulfide poisoning and hanging lead to an extremely short time from occurrence to death. Namely, not limited to intrinsic diseases like ischemic heart disease, but also in extrinsic diseases, NT-proBNP increases in diseases in which load to the heart is great, and with less load (when cardiac arrest occurred in a short time) the value was considered to be low. Therefore, it is natural that results that were relatively not useful were obtained by preparing the ROC curve based on the results including diseases in which load to the heart is great (Figure 2, Table 1). However, in Group B, granted that diseases in which the load to the heart is considered to be great (those with a mostly high NT-proBNP value) were excluded; it goes without saying that the results will be more useful. In either case, cadavers in which disease history and other situations are completely unknown are often subjected to inspection or autopsy in forensic medicine, and a substance like NT-proBNP that strongly reflects the course from the occurrence of damage to death (cardiac failure) was useful for the assumption of the cause of death.

Since troponin T drew attention as a clinically useful substance for diagnosis of myocardial infarction, there have been a number of reports on its usefulness and issues for demanding the diagnostic value in cadaveric materials in forensic medicine as well.[8-14] In our results (Table 3), many cases indicated high values in cardiac blood, regardless of the cause of death; therefore, its diagnostic value was low. Also, in CSF, although the specificity was high, the sensitivity was remarkably low; and, as a

Table 4. Cutoff, sensitivity, and specificity found from ROC curve after correction in NT-proBNP

	Cardiac blood	Femoral blood	CSF
Cutoff (pg/mL)	224	73	122
Sensitivity (%)	73.9	69.2	82.8
Specificity (%)	75.0	77.1	62.0
Area under the curve	0.74565	0.74799	0.7231

CSF, cerebrospinal fluid

result, the diagnostic value of CSF was also low. Comparably, femoral blood displayed appropriately 84% sensitivity and 76% in the negative hitting ratio, which were the best among 3 samples, and is useful for the diagnosis of ischemic heart disease (Table 4). Sensitivity here means the percentage of determined subjects with ischemic heart disease to be troponin T positive, and the negative hitting ratio is the percentage of determined subjects with a cause of death other than ischemic heart disease when the test result is negative. Troponin T is a protein that constructs cardiac muscle; and, because it is released into the blood by myocardial damage, including ischemic heart disease, it is of high clinical diagnostic value. However, particularly in cadaveric materials treated in forensic medicine, cardiac blood comes under a strong influence of postmortem changes; and, therefore, it is natural that there will be a large number of materials that indicate high values, regardless of the cause of death. This can be a major reason that, conventionally, useful results for a postmortem diagnosis cannot be obtained by biochemical tests using cadaveric cardiac blood.

In the meantime, biosynthesis of BNP is facilitated by load to the heart (ischemic and pressure load) and released into the blood, i.e., if the course did not exist, NT-proBNP, a byproduct, is unlikely to increase by postmortem changes; therefore, it was useful for the diagnosis of cardiac failure in cardiac blood and other cadaveric materials. Therefore, based on this data, we wondered if there was a way to improve the diagnostic value using cadaveric materials. As mentioned, troponin T is originally a protein that constructs cardiac muscle, and its value may increase by postmortem changes.[8-11] Namely, postmortem influence cannot be ignored when the results are positive; however, when the results are negative, its influence is said to be small, up to 75 hours postmortem.[14] We, therefore, considered this result significant. If we look at troponin T of the femoral blood, when it is negative, it should indicate that a cause of death other than ischemic heart disease is highly likely, if myocardial infarction of the hyperacute phase can be excluded. Also, as mentioned, when the NT-proBNP value is high, it is likely to be cardiac failure. But because the value is also high in various other disease conditions, ischemic heart disease cannot be diagnosed by NT-proBNP alone. Thus, in order to improve the diagnostic precision, we largely classified the test results of NT-proBNP and troponin T into 4 groups.

(a) If troponin T in femoral blood is positive and NT-proBNP is high, it is highly likely to be ischemic heart disease.

(b) If troponin T in femoral blood is positive and NT-proBNP is low, it is highly likely to be ischemic heart disease of a relatively acute phase, including acute myocardial infarction.

(c) If troponin T in femoral blood is negative and NT-proBNP is high, it is highly likely to be a disease strain to the heart, other than ischemic heart disease.

(d) If troponin T in femoral blood is negative and NT-proBNP is also low, it is highly likely to be a disease which takes an acute outcome other than acute myocardial infarction or acute cardiac disease in the hyperacute phase.

If we look carefully at (c) here, a similar tendency to the cases with higher NT-proBNP was shown in Group B. That is, many malignant tumors, inflammatory diseases, and pulmonary thromboembolisms showed higher values of NT-proBNP and negative or lower values of troponin T. As an attempt, we determined that when troponin T of femoral blood is negative it is not ischemic heart disease, and we prepared an ROC curve and found the cutoff, sensitivity, and specificity in the same manner as previously reported (Table 4). In comparison of these results with those in Table 1, the diagnostic values of ischemic heart disease are clearly increased for all 3 samples, which was also practically useful.

If obvious suicide and cases in which the cause of death is clear from the medical history and the antemortem information is excluded, (c) and (d) were suspected to be the diseases which result in some chronic or acute outcome, and investigation of the cause of death

by autopsy is highly desirable. Although (b) has less load to the heart, this suggests an injury to the cardiac muscle and is also considered to require an investigation of the cause of death by autopsy. It is suggested that the possibility of using cadaveric materials not only to improve the presumption of the cause of death but also to determine the necessity of autopsy by combination of these two data. The possibility of preparation of further useful cutoff levels, and the establishment of precise diagnostic criteria by accumulating data from cadaveric materials, is feasible.

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