ORIGINAL ARTICLE

Sensitivity and specificity of NT-proBNP to detect heart failure at post mortem examination

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Abstract NT-proBNP, a marker of cardiac failure, has been shown to be stable in post mortem samples. The aim of this study was to assess the accuracy of NT-proBNP to detect heart failure in the forensic setting. One hundred sixty-eight consecutive autopsies were included in the study. NT-proBNP blood concentrations were measured using a chemiluminescent immunoassay kit. Cardiac failure was assessed by three independent forensic experts using macro- and microscopic findings complemented by information about the circumstances of body discovery and the known medical story. Area under the receiving operator curve was of 65.4% (CI 95%, from 57.1 to 73.7). Using a standard cut-off value of >220 pg/mL for NT-proBNP blood concentration, heart failure was detected with a sensitivity of 50.7% and a specificity of 72.6%. NT-proBNP vitreous humor values were well correlated to the ones measured in blood ($r^2=0.658$). Our results showed that NT-proBNP can corroborate the pathological findings in cases of natural death related to heart failure, thus, keeping its diagnostic properties passing from the ante mortem to the post mortem setting. Therefore, biologically inactive polypeptides like NT-proBNP seem to be stable enough to be used in forensic medicine as markers of cardiac failure, taking into account the sensitivity and specificity of the test.

Keywords NT-proBNP · Natriuretic peptides · Biomarkers · Heart failure · Forensic autopsy

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Introduction

NT-proBNP is the amino-terminal fragment of the prohormone of BNP (brain natriuretic peptide), which is secreted mainly by atrial or ventricular myocardial cells, essentially in response to ventricular wall stress. The biological actions of BNP include diuresis, vasodilatation, reduction of systemic and regional (renal and cardiac) sympathetic tone, myocardial relaxation, anti-hypertrophic and anti-fibrotic effects and cytoprotection of cardiomyocytes [1].

In clinical practice, BNP and NT-proBNP have been introduced because of their stability in biological specimens and their utility in the management of suspected or established heart failure (diagnosis, prognosis and guided medical treatment) [2–7].

Natriuretic cardiac peptides have also recently been evaluated as biomarkers of cardiac function in medicolegal practice. In the few previous studies on post mortem measurements of these peptides, attention was paid to BNP and ANP (atrial natriuretic peptide) [8]. NT-proBNP seems to be a more reliable biomarker than BNP and ANP because of its greater stability and longer half-life [6, 9–11].

This was confirmed in one post mortem study, where NT-proBNP has been shown to be stable over 24 days in blood and pericardial fluid sampled from bodies with a post mortem interval up to 24 h. In particular, after 24 days of storage at −20°C, NT-proBNP concentration in pericardial fluid decreased by no more than 16% [12]. NT-proBNP could therefore be useful in cases where routine autopsy evaluation is limited (putrefaction, severe trauma, etc.). However, the ability of this biomarker to identify cardiac failure at post mortem examination remains unknown. Therefore, the goal of this study was to estimate the diagnostic accuracy of NT-proBNP in assessing cardiac failure compared to standard post mortem investigations performed in bodies with a post mortem interval comparable to the one selected for the stability study [12], i.e. up to 24 h.

Materials and methods

Objectives

Our main objective was to measure sensitivity, specificity, positive and negative predictive values, likelihood ratios of NT-proBNP blood concentration to suspect cardiac failure at post mortem examination. Our secondary objective was to measure correlations between NT-proBNP blood concentrations and those measured in other available fluids (serum, pericardial fluid, vitreous humor). Moreover, we aimed to quantify the eventual influence of age, sex, BMI, other diseases known to modify the NT-proBNP levels and traumatic death on the sensitivity and specificity of NT-proBNP blood levels in detecting heart failure.

Studied population

One hundred sixty-eight out of 210 consecutive medico-legal autopsies performed at the University Centre of Legal Medicine of Lausanne in 2006 were included in this study. To be included, standard investigation procedures (autopsy, histology, toxicology) had to be made and subjects had to be older than 15 years. The post mortem interval ranged from <12 to 24 h. Bodies with a post mortem interval >24 h were excluded from the study because putrefaction, at any stage, would have rendered impossible or at least very difficult to identify signs of heart failure at autopsy and thus to compare them with the NT-proBNP values measured in various body fluids most of which, moreover, would have been unavailable in case of putrefaction. All autopsies were demanded by legal authorities. The sample reflected the variability of cases seen in forensic post mortem evaluations, as it is illustrated in Table 1.

Reference test

No gold standard is available in forensic setting. The reference test is the usual procedure used when all available investigations can be performed in usual forensic practice. The diagnosis of heart failure was based on autopsy findings including cardiac cavities dilatation with or without myocardial hypertrophy, visceral congestion of internal organs, lung and/or peripheral oedema. Moreover, the presence of referred or detected pre-existing pathologies known to be causally associated with this syndrome (as coronary artery disease, hypertension, obesity and valvular heart disease) was considered in corroborating the diagnosis of heart failure.

For each case, a complete autopsy was performed, followed by routine histological examination. Details for each case were extracted from the autopsy report and completed by complementary information (sequence of events, circumstances of body discovery, known medical story).

All autopsies were performed by seven physicians, following international recommendations [13]. Three of these physicians were senior residents with at least 10 years of experience in forensic medicine, and 4 of them were residents, always supervised by a senior. All these forensic pathologists were blinded to NT-proBNP lab results.

Two independent forensic pathologists assessed their appreciation of cardiac failure for each case using a four-item Likert scale (most unlikely, unlikely, very likely, certainly). For cases where experts disagreed, a third forensic pathologist gave his opinion and cases were discussed until a consensus was reached. In their evaluations, these examiners were blinded to NT-proBNP values. Subjects were considered as having suffered from cardiac failure if assessors agreed they were very likely or certainly affected by this condition.

Index test (NT-proBNP)

Index test was performed after the reference test (prospective). Femoral blood, serum, vitreous humor and pericardial fluid were collected during the autopsy. Serum was obtained by centrifugation of blood samples, immediately after collection. Samples were sent by the forensic examiner to the laboratory and the specimens were stored at −20°C until the time of analysis. Laboratory staff, who reported results, was completely blinded to information regarding cases, except for gender and age. NT-proBNP blood concentrations were measured using a chemiluminescent immunoassay kit (Elecsys 2010 analyser, Roche Diagnostic, Basel, Switzerland; Dimension Xpand plus, Dade Behring, Deerfield, IL, USA). The procedure's test performances were the following: CV inter-assay 9% (490±

Table 1 Characteristics of subjects ($n=168$)

Characteristics	Cardiac failure		
	With $n=73$	Without $n=95$	p value
Age/gender (n,%)			<0.001
Male <55 years	28 (38.4%)	54 (56.8%)	
Male ≥55 years	26 (35.6%)	5 (5.3%)	
Female <65 years	11 (15.1%)	30 (31.6%)	
Female ≥65 years	8 (11.0%)	6 (6.3%)	
BMI (kg/m^2)	26.5 (5.4)	25.2 (5.7)	0.147
Cause of death (n,%)			<0.001
Natural			
Cardiovascular system	31 (42.5%)	0 (0%)	
Other	10 (13.7%)	13 (13.7%)	
Violent death			
Trauma	13 (17.8%)	33 (34.7%)	
Other violent deaths	14 (19.2%)	45 (47.4%)	
Unknown	5 (6.8%)	4 (4.2%)	
Autopsy findings			
Heart weight; mean (SD), g	448 (104)	348 (80)	<0.001
Ventricles			
Right dilatation (n,%)	26 (35.6%)	18 (18.9%)	0.015
Right hypertrophy (n,%)	29 (39.7%)	3 (3.2%)	<0.001
Left dilatation (n,%)	29 (39.7%)	14 (14.7%)	<0.001
Left hypertrophy (n,%)	36 (49.3%)	2 (2.1%)	<0.001
Coronary arteries			
Stenosis >75% (n,%)	18 (24.7%)	1 (1.0%)	<0.001
Thrombosis (n,%)	11 (15.1%)	0 (0%)	<0.001
Haemorrhage (n,%)	9 (12.3%)	0 (0%)	<0.001
Macroscopy of heart			
Fibrosis (n,%)	3 (4.1%)	1 (1.0%)	0.198
Signs of infarction (n,%)	12 (16.4%)	5 (5.3%)	0.017
Valvular insufficiency (n,%)	2 (2.7%)	0 (0%)	0.105
Histological findings			
Acute ischemia/infarction (n,%)	36 (49.3%)	23 (24.2%)	0.001
Fibrosis/hypertrophy (n,%)	62 (84.9%)	45 (47.4%)	<0.001
Coronary wall thickness >30% (n,%)	35 (47.9%)	16 (16.8%)	<0.001
Other disease known to increase NT-proBNP	50 (68.5%)	38 (40%)	<0.001
Degenerative or vascular path CNS (n,%)	8 (11.0%)	5 (5.3%)	0.171
Pulmonary hypertension (n,%)	17 (23.3%)	8 (8.4%)	0.007
Pulmonary thromboembolism (n,%)	3 (4.1%)	1 (1.0%)	0.198
Chronic bronchitis (n,%)	15 (20.5%)	4 (4.2%)	0.001
Renal vascular pathology (n,%)	26 (35.6%)	15 (15.8%)	0.003
Liver steatofibrosis (n,%)	5 (6.8%)	8 (8.4%)	0.705
Severe arteriosclerosis (n,%)	41 (56.2)	24 (25.3%)	<0.001
Blood alcohol positivities[a] (n,%)	17 (23.3%)	30 (31.6%)	0.235

[a] Samples with >0.1 g/kg blood alcohol concentration measured by Head-Space-GC-FID, were considered as positive

SD standard deviation

44 pmol/L), CV intra-assay 7.3% (431±32 pmol/L) and 9.7% (1,400±135 pmol/L). No cross-reactivity for human BNP, ANP or N-terminal proANP. LOD 9.7 pmol/l [7]. As serum is not always available in forensic setting (haemolysis), we chose blood as a reference standard, and we defined the clinical serum value of >112 pg/mL as cut-off also for NT-proBNP blood concentration. To test whether NT-proBNP blood values are correlated to those measured in serum, pericardial fluid and vitreous humor, the same laboratory method was applied to these fluids.

Other measures

Myocardial specimens for the histological examination were derived from at least five different topographic locations, namely left anterior, left lateral, left posterior, septal and right lateral. They were stained with hematoxylin–eosin. For each case, alcohol concentration was assessed in peripheral blood. Toxicological analyses were performed, when requested, by GC/MS. The presence of common abuse substances was assessed, including cocaine and its metabolites, and the results were compared to published data [14].

Statistical analyses

Receiver operating curve and area under the curve (AUC) were calculated for different NT-proBNP values. In the clinical setting, the reference ranges for NT-proBNP vary depending on the assay method used and the nature of the control population [15, 16]. A suggested "normal" range for NT-proBNP is 68–112 pg/mL [5, 16]. Thus, we chose a cut-off value of >112 pg/mL to define NT-proBNP as positive or negative. Sensitivity, specificity, positive and negative predictive values, positive and negative likelihood ratios were then given with 95% confidence intervals (95% CI). Sensitivity and specificity were calculated for subpopulations (age and sex, BMI, presence of renal, pulmonary and central nervous system pathology, signs of trauma). Linear regression analysis was used to define association between NT-proBNP concentrations in blood and in other biological fluids. Transformations were used to test other associations than linear correlations, when required. R^2 from the regression was calculated to estimate the strength of the association between blood and other body fluids. Analyses were conducted using STATA 10.1 (College Station, TX 77845 USA).

Results

From 210 consecutive autopsies performed from January to December 2006, 168 were included in the analysis. Criteria for exclusions are given in Fig. 1.

The two initial assessors agreed on the presence of cardiac failure from the results given in the autopsy report for 135 cases (80.4%) showing a substantial agreement (kappa=0.61) using Landis and Koch's interpretation of kappa values [17]. Total agreement among the three experts was easily reached for the remaining 33 cases (19.6%). Cardiac failure was considered as certain for 27 cases (15.9%), very likely for 46 cases (27.4%), unlikely for 46 cases (27.4%) and most unlikely for 49 cases (29.2%).
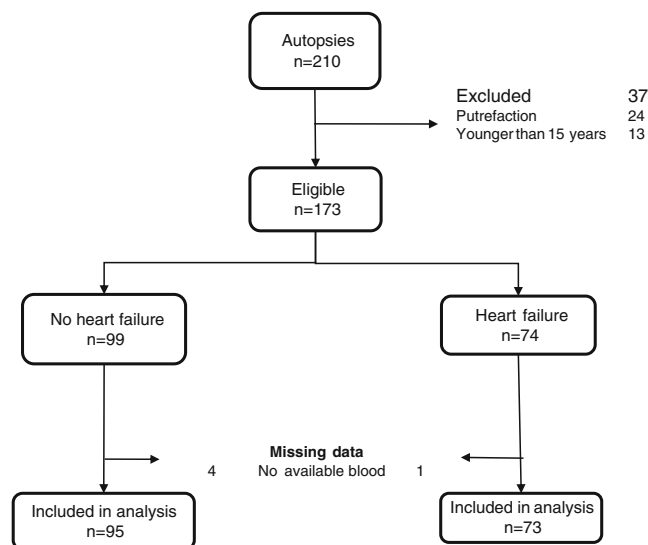
Fig. 1 Flow chart indicating the studied population and the excluded cases

Characteristics of cases with or without heart failure and levels of significance (p values) are reported in Table 1. Subjects with heart failure were older than those without this condition and they were more often of male gender. Among them, death was more recurrently natural, related to cardiovascular diseases. Furthermore, subjects with heart failure were more likely to present other diseases known to influence NT-proBNP values ($p<0.001$). Finally, victims who died from traumatic causes were two times less likely to suffer from heart failure than other deceased (28.3% vs. 49.2%).

Validity of NT-proBNP for predicting heart failure

We verified that NT-proBNP blood concentration could be used instead of serum in forensic settings (Fig. 3a). Concentrations were logged and regression was used to compare findings. In forensic settings, 58.5% of the variance of blood values can be explained from serum values using the following equation: $NT\text{-}proBNP_{serum}=e^{0.91 \cdot \ln(NT\text{-}proBNPblood)+1.1}$. Therefore, the standard serum cut-off value of >112 pg/mL of NT-proBNP is equivalent to >220 pg/mL of NT-proBNP blood concentration for our sample.

Using a standard cut-off value of >220 pg/mL of NT-proBNP$_{blood}$ to define heart failure, 37/73 (50.7%) subjects with this condition were detected positive (sensitivity), whereas 26/95 (72.6%) individuals without heart failure had a NT-proBNP$_{blood}$≤220 pg/mL (specificity).

Area under the receiving operator curve (Fig. 2) was of 65.4%, with a confidence interval of 95% (CI 95%), from 57.1 to 73.7. An optimal diagnosis test would have an area under the curve of 100%, whereas a test which has no predictive diagnosis ability would have an area of 50%

Fig. 2 Receiving operator's curve illustrating NT-proBNP's ability to detect heart failure
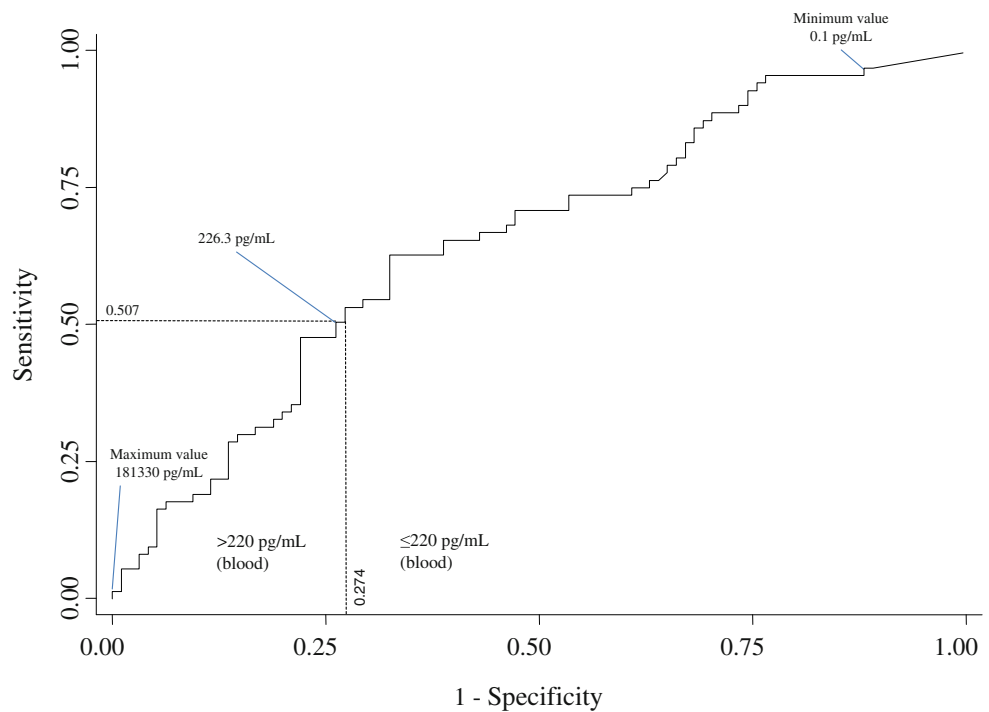
[18]. When including only cases for which heart failure was either considered certain ($n=27$), or most unlikely ($n=49$) the area under the receiving operator curve was of 67.8 (CI 95% 55.1 to 80.5). Sensitivity, specificity, predictive values and likelihood ratios of NT-proBNP for detecting heart failure are given with CI 95% in Table 2.

Correlation of NT-proBNP values in different body fluids

Body fluids other than blood were available for 89 autopsies. Correlation was highest between blood and vitreous humor ($R^2=0.658$; CI 95% 0.52 to 0.76), then between blood and pericardial fluid ($R^2=0.397$; CI 95% 0.21 to 0.56). R^2 corresponds to the proportion of variance explained by the correlated variable (identical to the squared Pearson's correlation value) and ranges from 0 to 1, where 1 is a perfect correlation. We also observed that

NT-proBNP values were most often much higher in pericardial fluid than in other fluids (Fig. 3b, c).

Sensitivity and specificity of NT-proBNP for sub-populations

Sensitivity and specificity of NT-proBNP across studied sub-populations are given in Table 3. BMI and presence of other diseases known to influence NT-proBNP levels do not seem to influence sensitivity and specificity of the test (Table 3). We, however, observed a lower specificity for subjects who died from natural events, compared to those who died from traumatic causes (23.1 vs. 82.1). There was also a trend towards age and gender differences with higher specificity for older men compared to lower specificity for older women (100 vs. 16.7).

Discussion

We have investigated the ability of NT-proBNP blood values to detect heart failure at post mortem examination by measuring its sensitivity, specificity, positive and negative predictive values and likelihood ratios as well.

NT-proBNP blood concentrations can corroborate the pathological findings in cases of natural death related to heart failure, as demonstrated by the substantial agreement (kappa=0.61) reached among the three experts who evaluated the autopsy reports. This is an important finding, which shows that NT-proBNP retains its diagnostic value, already well-established in the clinical setting [2–4, 7, 19–

Table 2 Validity of NT-proBNP to detect heart failure at post mortem examination

	Values	CI 95%
Sensitivity	50.7%	38.8% to 62.5%
Specificity	72.6%	62.4% to 81.0%
Predictive values		
Positive	58.7%	45.6% to 70.8%
Negative	65.7%	55.7% to 74.5%
Likelihood ratio		
Positive	1.9	1.2 to 2.8
Negative	0.68	0.53 to 0.86

Fig. 3 Correlations between NT-proBNP blood values and those measured in other body fluids
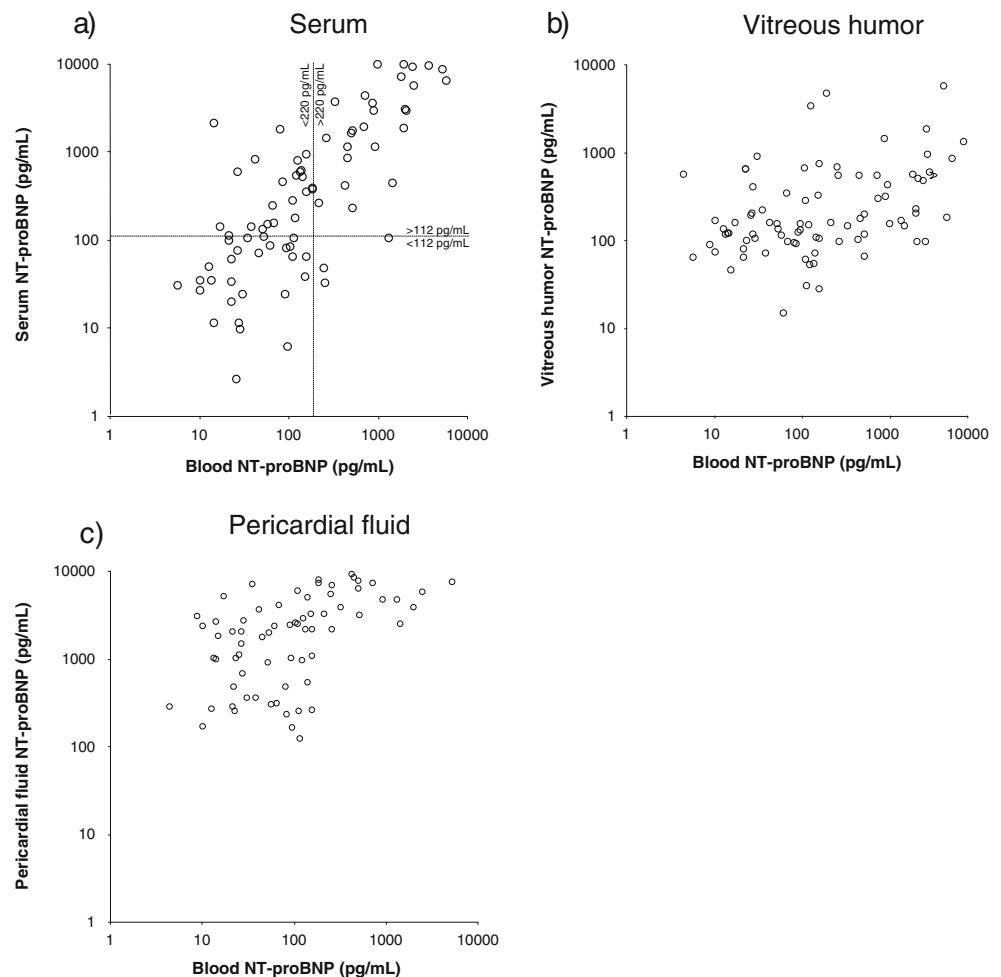
24]. Combining our observations with the results of a previous study demonstrating the stability of this biomarker in post mortem samples [12], we have shown—for the first time up to now—that a biomarker keeps its diagnostic features unaltered passing from the ante mortem to the post mortem setting. This differentiates NT-proBNP from other markers of cardiac pathologies, whose diagnostic validity using post mortem material is controversial. For example,

Table 3 Sensitivity and specificity of NT-proBNP to detect heart failure for different sub-populations

	Sensitivity	Specificity
All cases	50.7 (38.8 to 62.5)	72.6 (62.4 to 81.0)
Age/gender		
Men under 55 years	39.3 (22.1 to 59.3)	85.2 (72.3 to 92.9)
Men 55 years or over	53.8 (33.7 to 72.9)	100 (46.3 to 100)
Women under 65 years	45.5 (18.1 to 75.4)	56.7 (37.7 to 74.0)
Women 65 years or over	87.5 (46.7 to 99.3)	16.7 (0.1 to 63.5)
BMI		
≤30 kg/m^2	51.9 (38.0 to 65.5)	73.3 (61.7 to 82.6)
>30 kg/m^2	47.4 (25.2 to 70.5)	70.0 (45.7 to 87.2)
Pathologies		
With other pathologies[a]	58.0 (43.3 to 71.5)	65.8 (48.6 to 79.9)
Without other pathologies[a]	34.8 (17.2 to 57.2)	77.2 (63.8 to 86.8)
Cause of death		
Natural deaths	46.3 (31.0 to 62.4)	23.1 (6.2 to 54.0)
Violent death with trauma	59.3 (39.0 to 77.0)	82.1 (71.4 to 89.5)

[a] Concerns pathologies known to influence NT-proBNP values (renal, pulmonary and central nervous system pathologies)

troponins (I and T) are known to be the preferred markers for the laboratory diagnosis of myocardial infarction in the clinical setting [25, 26]. Conversely, in the post mortem context, not only they are not specific for myocardial injury, as they increase also in other conditions [27, 28], but also their levels are influenced by the post mortem interval [28–30]. Some authors even pointed out that post mortem blood is an unsuitable sample to measure cardiac troponins [31].

Our results are in accordance with clinical data regarding the factors influencing the increase of NT-proBNP levels [1–3, 5, 6]. Likewise, subjects who were diagnosed with heart failure at post mortem examination were shown to be more likely to present other diseases known to influence NT-proBNP values, as renal, pulmonary and central nervous system pathologies, thus still confirming the clinical data. In fact, it is known that the levels of natriuretic peptides may rise in some pathological situations, such as renal pathologies, disorders associated with diastolic dysfunction, primary pulmonary hypertension, cor pulmonale, pulmonary embolism, congenital heart diseases and arrhythmogenic right-ventricular dysplasia [3]. This can also explain why we observed a lower specificity of NT-proBNP to detect heart failure for subjects who died from natural causes compared to those who died from traumatic events. Our finding that victims who died from traumatic causes were two times less likely to suffer from heart failure than other deceased (28.3% vs. 49.2%) can be related to the short agonal period which characterised most of these traumatic deaths.

In this study, we have also evaluated the correlations of NT-proBNP blood values with different body fluids, namely serum, pericardial fluid and vitreous humor. We found out that the correlation was highest between blood and vitreous humor ($R^2=0.658$), and then between blood and pericardial fluid ($R^2=0.397$). This is in discordance with the results of a previous study [12], which found a weak correlation with vitreous humor. In the mentioned study, however, values were not transformed before testing correlations. Our log-transformed values confirm the vitreous humor as a good substrate for post mortem biochemical testing [32–37]. Furthermore, the ascertainment that blood levels correlate well with those of vitreous humor and pericardial fluid is important, because in forensic practice, the latter body fluids might not be available, like in putrefied corpses. This means that in such cases where the autopsy investigation is obviously limited, an increased NT-proBNP blood value may give indication about the natural origin of death.

Beside this, we observed that NT-proBNP values were most often much higher in pericardial fluid than in other fluids. A possible explanation for this is that biochemical markers reach the pericardial fluid through passive diffusion and ultrafiltration due to pressure gradient, so they are detectable in higher concentration in pericardial fluid before they reach the other fluids [38–41].

In conclusion, our study shows that NT-proBNP has similar diagnostic properties in the post mortem setting as it has in the clinical one. However, a systematic dosage of this biomarker in body fluids of fresh corpses is unjustified because it has a low validity (with a sensitivity of 50.7% and a specificity of 72.6%) to detect heart failure. This is not due to an inappropriate choice of a cut-off point set at 220 pg/mL, as the area under the receiver operating curve also shows low predictive ability (AUC=65.4%). For this reason, it is suggested to use the standard macro- and microscopical methods to detect heart failure at post mortem examination, eventually validated by immunohistochemical studies [42, 43]. Conversely, in putrefied bodies, where autopsy is limited, increased NT-proBNP blood levels can give indication about the (natural) manner of death, in the absence of traumatic lesions during radiological examination.

Limitations

The study does not have enough power to truly identify clinically significant differences in sensitivity or specificity in sub-populations. Sensitivity and specificity of the reference test we used (autopsy findings) remain unknown. Linearity of NT-proBNP concentrations in vitreous humor and pericardial fluid was not tested.

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