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ORIGINAL ARTICLE

Elevation of NT-proBNP and cardiac troponins in sepsis-related deaths: a forensic perspective

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Abstract In the present study, the levels of NT-proBNP, troponin T, and troponin I were measured in postmortem serum from femoral blood in a series of sepsis-related fatalities that had undergone forensic autopsies. We aimed to assess whether a possible increase in the concentrations of these biomarkers was correlated to macroscopic or microscopic observations that suggest myocardial damage or cardiac dysfunction. Two study groups were retrospectively formed, a sepsis-related fatalities group and a control group. Both groups consisted of 16 forensic autopsy cases. Unenhanced computed tomography scan, autopsy, histological, toxicological, microbiological, and biochemical analyses were performed for all cases in both groups. Levels of procalcitonin, C-reactive protein, NT-proBNP, troponin T, and troponin I were systematically measured in postmortem serum from femoral blood. The preliminary results suggest that the postmortem serum troponin I, troponin T, and NT-proBNP levels are increased in sepsis-related deaths in the absence of any relevant coronary artery disease, myocardial ischemia, or signs of heart failure. These findings corroborate clinical data from previous studies pertaining to the usefulness of troponins and natriuretic peptides as indicators of toxic and inflammatory damage to the heart in cases of severe sepsis and septic shock without concomitant underlying coronary syndromes.

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Introduction

Sepsis and septic shock annually affect an estimated 750,000 people in the USA. Together, they are the leading causes of mortality in the non-cardiac intensive care unit [1–4]. Recent estimates have revealed that severe sepsis is recorded in about 2 % of patients admitted to hospitals in developed countries, with case-fatality rates ranging from 30 to 50 % [5].

Epidemiological studies performed in the USA have shown that the mortality rate due to severe sepsis has decreased from 39 to 27 % due to the increasing availability of evidence-based therapies. However, both severe sepsis and septic shock remain important clinical and economic burdens for healthcare systems and a major public health concern [2, 5–7].

The growing increase in sepsis incidence can be due to various reasons: first, the growing use of chemo- and immunotherapy responsible for immunosuppression in the patients receiving these therapies; second, the rapid surge in the emergence of antibiotic-resistant microorganisms over the past few years; and third, the increase in the percentage of ageing citizens who are at greater risk of sepsis because of the presence of numerous comorbidities [5, 8].

Cardiovascular abnormalities in cases of severe sepsis and septic shock are frequent complications that have been well documented. They may result in non-coronary artery disease-related myocardial injuries. Decreased left ventricular systolic function, reversible ventricular dilatation, and reduced responsiveness to fluid resuscitation and catecholamines characterize this dysfunction [1, 2, 7, 9].

Myocardial dysfunction associated with sepsis is a phenomenon that was first described decades ago though it has been

recognized as a more important factor only recently, following the widespread use of echocardiography in the intensive care unit. The pathophysiology is not completely understood. Cellular, extracellular, and molecular mechanisms have been postulated to explain the myocardial dysfunction occurring during sepsis. Some of these hypotheses include alterations in coronary blood flow, the action of circulating depressant factors, increased catecholamine levels, and calcium dysregulation [10–14].

Cardiac troponins I and T are specific to the myocardium. Elevation in their levels in sepsis has been shown to occur in the absence of coronary thrombosis. The reasons for the increase in troponin levels are still not clearly delineated, though several hypotheses have been proposed, including ischemia and direct myocardial damage due to substances released into the circulation by pathogens, cytokines, or reactive oxygen radicals released due to the infectious process [2–4, 15–18].

Type A natriuretic peptide (atrial natriuretic peptide, ANP), type-B natriuretic peptide (brain natriuretic peptide, BNP), and the N-terminal fragments (NT-proANP and NT-proBNP) of their pro-hormones (proANP and proBNP) are released from cardiomyocytes in response to increased transmural atrial and ventricular pressure. The levels of NT-proANP and NT-proBNP are elevated in proportion to the severity of heart failure. However, it has been observed that the increase in plasma levels of BNP, NT-proBNP, and NT-proANP is not specific to heart failure, and the circulating levels of these natriuretic peptides may be influenced by several cardiac and non-cardiac conditions, including sepsis, severe sepsis, and septic shock. The correlation between plasma levels of natriuretic peptides (and their N-terminal fragments) and cardiac filling pressures in sepsis and septic shock is still not significant. Thus, another hypothesis has been put forth, which proposes that the systemic inflammatory response alone may be responsible for the elevated levels of these compounds in patients with septic shock [9, 19, 20].

Sepsis-related fatalities can be occasionally encountered in forensic casework. Postmortem diagnosis of sepsis mainly relies on the correlation of diverse findings that include medical history (when available) and circumstantial data as well as histological, microbiological, and biochemical analysis results. Apart from postmortem serum concentrations of procalcitonin, C-reactive protein, and other parameters of systemic inflammation, the complete biochemical profile of sepsis-related deaths is yet to be extensively investigated. Thus, to our knowledge, no published information exists to date pertaining to the behavior of troponins and natriuretic peptides in sepsis-related fatalities that have undergone forensic autopsies.

In the present study, NT-proBNP, troponin T, and troponin I levels were measured in postmortem serum from femoral blood in a series of sepsis-related fatalities. We aimed to assess the expression levels of these biomarkers and determine whether an increase, if any, in the concentrations of these biomarkers was correlated to macroscopic or microscopic findings suggestive of myocardial damage or cardiac dysfunction.

Materials and methods

Study design

The present study was conducted during 2010–2015 and was designed as a retrospective, single-center study. All cases collected for the study underwent medicolegal autopsies as requested by the inquiring authorities (the public prosecutor). Laboratory analyses, including measurement of NT-proBNP, troponin T, and troponin I levels, were performed as part of the medicolegal investigations.

Study populations

Two study groups were retrospectively formed: a sepsis-related fatalities group and a control group. The sepsis-related fatalities group consisted of 16 forensic autopsy cases (10 male subjects and 6 female subjects between 44 and 81 years of age) in which the cause of death was attributed to multiple-organ failure "due to sepsis" or "possibly related to sepsis" based on postmortem investigation results. All subjects included in the septic group underwent cardiopulmonary resuscitation attempts.

Eight of these subjects (multiple-organ failure due to sepsis) had been admitted to the intensive care units of local hospitals, where they subsequently died. All cases had a documented, clinical diagnosis of sepsis and septic shock in vivo. Sepsis was diagnosed based on evidence of pneumonia or abdominal infection, along with the presence of systemic inflammatory response syndrome (SIRS) according to the definition proposed by the American College of Chest Physicians/Society of Critical Care Medicine (ACCP/SCCM). All these patients received anti-inflammatory and antibiotic therapy during hospitalization. Treatment duration and hospitalization ranged from 24 to 96 h.

Eight other subjects (multiple-organ failure possibly related to sepsis) that had not been admitted to the intensive care units of local hospitals prior to death and had no documented diagnosis of clinically confirmed sepsis were selected. All these cases were transferred to the medicolegal center with histories of fever and respiratory or abdominal symptoms in the days prior to death suggesting diagnoses of respiratory or abdominal infections.

Within the limits of available information, none of these subjects suffered from preexisting known cardiac disease.

The control group consisted of 16 age-, race-, and gender-matched forensic autopsy cases (10 male subjects and 6 female subjects between 42 and 80 years of age). All cases selected for this group originated from forensic practice with deaths occurring outside the hospital. None of the subjects included in this group had a documented clinical diagnosis

of sepsis in vivo and none had been admitted to the hospital prior to death. All cases included in this group underwent cardiopulmonary resuscitation attempts.

As with the septic group and within the limits of available information, none of these subjects suffered from preexisting known cardiac disease.

Ethics

All relevant ethical issues were discussed with the local ethics committee. Since all biological samples were anonymized prior to analysis, ethics committee approval to perform biochemical analyses in the selected subjects was not necessary.

Postmortem investigations

Unenhanced computed tomography (CT) scans were performed before any manipulation of the corpses in all cases included in the study. Postmortem CT angiographies were carried out after the CT scans and prior to autopsies. Autopsies were jointly performed by two forensic pathologists (at least one of whom was board certified) in accordance with both local standards and international guidelines for medicolegal cases. Conventional histology included hematoxylin-eosin (HE) staining of brain, heart, lung, liver, and kidney samples. HE staining was performed after tissue fixation in formaldehyde. Hearts were sectioned before or after fixation in 10 % neutral buffered formalin. The major epicardial coronary arteries were either serially sectioned at approximately 2-mm intervals or longitudinally sectioned intact on the heart. Histological sections of the coronary arteries were prepared at three different equally spaced levels to best identify plaque rupture sites. Routine histology staining analysis for coronary arteries included HE, Masson's trichrome, and Verhoeff van Gieson. Immunohistochemical investigations using antibodies against fibronectin and C5b-9 of both cardiac ventricles were also performed in some selected cases.

Systematic toxicological analysis included blood ethanol level determination as well as general screening for volatile and non-volatile drugs, poisons, and metabolites.

All subjects included in the septic group underwent complete medicolegal autopsies between 5 and 62 h after death. Histological, toxicological, microbiological, and biochemical studies were performed in all cases. Specimens for microbiological testing were collected from at least two different sampling sites and always included cardiac blood. No cases of neonatal sepsis were included in this group.

Sepsis and multiple-organ failure as causes of death was confirmed by postmortem investigations in all eight cases with a clinical diagnosis of sepsis in vivo, along with the exclusion of other causes of death.

The cause of death was attributed to multiple-organ failure possibly related to sepsis in the other eight cases based on postmortem investigation results. Underlying bacterial infections and sepsis were postulated as the causes of multiple-organ failure. Alternative causes of death were excluded based on autopsy and other investigation findings.

Acute peritonitis following surgical anastomosis dehiscence, intestinal necrosis, gastrointestinal perforation, or intra-abdominal abscess rupture (eight cases) and pneumonia (eight cases) were the infectious foci identified by means of autopsy and histology in the sepsis group.

Postmortem coronary angiographies showed normal coronary arteries. Postmortem investigations did not reveal the presence of acute coronary thrombosis, myocardial infarction, myocardial hypertrophy, or findings suggestive of heart failure. Inflammatory cell infiltration within the myocardium could not be demonstrated. Microscopic investigations documented the presence of unspecific myocardial changes including limited areas of elongated myocardial fibers, slight interstitial edema, small clusters of myocytes with condensed sarcoplasm, and preserved nuclei. Contraction band necrosis was noticed in 4 out of 16 cases. Interstitial fibrosis was documented in 3 out of 16 cases.

Levels of procalcitonin and C-reactive protein were systematically measured in postmortem serum from femoral blood and their increased levels were observed in all cases.

All subjects included in the control group underwent complete medicolegal autopsies between 12 and 48 h after death. Histological, toxicological, microbiological, and biochemical analyses were performed in all cases. Postmortem investigations failed to reveal evidence consistent with the existence of underlying bacterial infections or septic states.

The causes of death were determined as blunt trauma (four cases), sharp injury (four cases), gunshot wounds (four cases), and drug intoxication (four cases). Autopsies mainly revealed various degrees of coronary artery atherosclerosis with no evidence of significant luminal narrowing. As with the septic group, relevant coronary artery abnormalities were ruled out by postmortem coronary angiographies. Moreover, postmortem investigations did not show the presence of acute coronary thrombosis, myocardial infarction, myocardial hypertrophy, or findings suggestive of heart failure. Contraction band necrosis was noticed in 2 out of 16 cases (opiate intoxications). Interstitial fibrosis was documented in 4 out of 16 cases.

Sample collection

Peripheral blood from the femoral veins was systematically collected for toxicological and biochemical studies prior to autopsy. Femoral blood samples were collected by aspiration with sterile needles and syringes from the femoral vein(s). Blood samples were drawn after clamping the vein(s) at the proximal end and keeping the lower limb(s) raised for several minutes. Samples were stored in tubes containing sodium fluoride and preservative-free gel serum separator tubes. The

latter were centrifuged immediately post collection at 3000*g* for 15 min. After centrifugation, the separated supernatant (postmortem serum) was collected and stored in preservative-free tubes. No specimens were excluded due to insufficient sample volume. Postmortem serum samples were transferred to the laboratories immediately post collection. When analyses were delayed, samples were stored at −20 °C.

The external side of the right atrium was sterilized by searing with a heated scalpel blade, and the cardiac blood was aspirated using sterile needles and syringes prior to any other manipulation of either thoracic or abdominal organs. Once collected, the cardiac blood was injected into blood culture bottles, transported promptly to the laboratory for incubation at 37 °C, and cultured for the presence of aerobic and anaerobic microorganisms.

Tissue samples for microbiology were obtained from the spleen, liver, and lungs by the conventional approach by searing a small surface area of the organs to dryness with a red hot metal instrument and removing tissue blocks with sterile instruments. Once collected, these samples were immediately transported to the laboratory and cultured for aerobic and anaerobic microorganisms.

Laboratory assays

Levels of procalcitonin and C-reactive protein were determined using previously described techniques [21]. Results were expressed in micrograms per liter and milligrams per liter, respectively. The analytical sensitivity was 0.1 μg/l and 0.15 mg/l, respectively, according to manufacturer information. Increased postmortem serum procalcitonin and C-reactive protein levels suggestive of generalized inflammations and bacterial infections were selected based on the forensic literature and former medicolegal investigation results (2 μg/l and 10 mg/l, respectively) [21].

The level of C-reactive protein (immunoturbidimetric Tinaquant CRP) was determined with the Roche standard methods using the Roche Modular P system (Roche Diagnostics GmbH, Mannheim, Germany). The level of NT-proBNP and procalcitonin were measured with the commercially available immunoassays on the Roche Modular E170 system (Roche Diagnostics GmbH, Mannheim, Germany).

Results for NT-proBNP were expressed in nanograms per liter. The clinical reference value (according to the laboratory where the analysis was performed) was 738 ng/l (corresponding to 738 pg/ml and 87 pmol/l).

Cardiac troponin I was analyzed with the Access® AccuTnI™ assay on Access II (Beckman Coulter, Fullerton, CA, USA). Results were expressed in micrograms per liter. The clinical reference value (according to the laboratory where the analysis was performed) was 0.03 μg/l (corresponding to 0.03 ng/ml).

Levels of postmortem serum cardiac troponin T were measured with hs-TnT reagents by electrochemiluminescence immunoassay (ECLIA). Results were expressed in nanograms per liter. The clinical reference value (according to the laboratory where the analysis was performed) was 14 ng/l.

Toxicology consisted of ethanol determination and general unknown screening for common drugs and illegal substances by gas chromatography-mass spectrometry (GC-MS) using commercial mass spectrum libraries, high-performance liquid chromatography with diode-array detection (HPLC-DAD), and headspace-gas chromatography flame ionization detection (HS-GC-FID).

Statistical analysis

Data are reported as mean concentrations and ranges. Comparisons of NT-proBNP, troponin I, and troponin T levels between the sepsis and the control cases were performed using the non-parametric Mann-Whitney *U* test. Spearman's rank correlation for non-parametric data was used to test the correlation between NT-proBNP and troponin I as well as that between NT-proBNP and troponin T in septic cases. Statistical significance was defined as a p value of less than 0.05. All statistical analyses were performed using GraphPad Prism 4.0 (GraphPad Software, La Jolla, CA, USA).

Laboratory results

Multiple microorganisms including one (or more than one) member(s) of the *Enterobacteriaceae* family in association with one (or more than one) member(s) of the enterococcus group were typically identified in cases of death following abdominal infections.

Klebsiella pneumoniae and *Streptococcus pneumoniae* accounted for the majority of identified bacteria in patients with respiratory infections.

Postmortem microbiological results correlated with antemortem results in seven out of eight cases that had been admitted to the intensive care units of local hospitals.

As expected, postmortem serum procalcitonin and C-reactive protein concentrations were significantly higher in the sepsis group (means 4.31 μg/l and 125 mg/l, respectively) than in the control group (means 0.09 μg/l and 8 mg/l, respectively). In addition, significantly higher concentrations of troponin I, troponin T, and NT-proBNP were found in postmortem serum in septic cases than in control individuals.

All septic patients had increased postmortem serum troponin I levels. The mean value was 3.98 μg/l (range 0.99–9.16 μg/l). Only 2 of the 16 control cases (both opiate intoxications) had postmortem serum troponin I levels higher than the clinical reference values (0.42 and 0.37 μg/l).

Similarly, all individuals in the septic group had pathologically elevated postmortem serum troponin T levels. The mean

value was 59 ng/l (range 21–95 ng/l). Only 2 of the 16 control cases (both opiate intoxications) had postmortem serum troponin T levels higher than the clinical reference values (26 and 31 ng/l). Significantly, the same subjects had increased postmortem serum troponin I levels.

Moreover, all septic cases had postmortem serum NT-proBNP concentrations higher than clinical reference values. The mean concentration was 5536 pg/ml (corresponding to 653 pmol/l). The range was 2678–10,680 pg/ml (316–1260 pmol/l). Only 1 of the 16 control cases (opiate intoxication) had NT-proBNP concentration higher than the clinical reference values (1256 pg/ml, corresponding to 148 pmol/l), along with increased levels of both troponin I and troponin T (0.42 μg/l and 26 ng/l).

A highly significant correlation was found between NT-proBNP and troponin I levels in postmortem serum ($r=0.71$, $p<0.05$). A significant positive correlation was also found between NT-proBNP and troponin T concentrations ($r=0.68$, $p<0.05$).

Overall, these results suggest that postmortem serum levels of troponin I, troponin T, and NT-proBNP are increased in sepsis-related deaths, in the absence of any relevant coronary artery disease, myocardial ischemia, or signs of heart failure. These findings would therefore confirm former clinical data pertaining to the usefulness of troponins and natriuretic peptides as indicators for toxic and inflammatory damage to the heart in cases of severe sepsis and septic shock without concomitant underlying coronary syndromes.

Discussion

Sepsis is a clinical syndrome caused by inefficient homeostatic mechanisms and infection containment. It is characterized by the signs and symptoms of a systemic inflammatory reaction to infection as well as those of organ dysfunction resulting from alterations in microcirculation [22].

The cardiovascular abnormalities associated with sepsis largely account for the life-threatening nature of the syndrome. The extent of cardiac abnormalities associated varies with the time course of the illness and its severity. The heart undergoes diverse physiological and metabolic changes during sepsis that usually normalize within 7–10 days in survivors. Physiological changes include ventricular dilatation, ejection fraction depression, and generalized or regional left ventricular wall hypokinesia, as well as systolic and diastolic dysfunction. Contribution of the right ventricle in sepsis-related myocardial dysfunction is not known, though similar sepsis-related physiological changes are believed to affect both ventricles [9, 15, 23, 24].

The hemodynamic pattern in human septic shock is generally characterized by a hyper-circulatory state including decreased systemic vascular resistance and a markedly increased cardiac index after adequate fluid resuscitation. However, numerous studies have demonstrated intrinsically depressed left ventricular performance in patients with septic shock [7].

The exact mechanism underlying this myocardial dysfunction/depression is not clearly delineated, though the proposed hypotheses mostly support a prominent role for functional rather than anatomical abnormalities [25].

Earlier studies in animals had suggested that myocardial ischemia caused by myocardial hypoperfusion is a possible mechanism by which sepsis causes cardiac abnormalities. However, studies in humans with septic shock did not confirm these studies, since instead of reduced coronary blood flow, which characterizes myocardial hypoperfusion, coronary blood flow rate is either maintained or even increased in patients with septic shock. Similarly, the hypothesis implicating abnormal cellular metabolism in the pathogenesis of myocardial dysfunction has not been proven. The presence of myocardial depressants in circulation was demonstrated by an in vitro assay using spontaneously beating myocardial cells in rats. The mechanism of action of these myocardial depressants was subsequently shown to be attributable to a synergistic effect of tumor necrosis factor-alpha and interleukin-1 beta. Direct cardiac myotoxic effect of these circulating molecules released by pathogens (e.g., endotoxins) has also been postulated as possibly involved in provoking myocardial depression and cardiac dysfunction. Cytokine-mediated changes, including adrenergic response attenuation at the cardiomyocyte level, intracellular calcium trafficking alterations, and blunted calcium sensitivity of contractile proteins, have been suggested as major mechanisms underlying sepsis-induced cardiac dysfunction [2, 7, 9, 12, 13, 22–26].

Patients with sepsis are known to have increased troponin levels even in the absence of coronary artery disease. The pathophysiology and mechanisms of increase in the level of cardiac troponins in sepsis have been studied intensively and remain to be fully understood [3, 17, 23, 27–29].

Apart from ischemia, numerous factors may contribute to microinjury and minimal myocardial cell damage in sepsis. One hypothesis is that it is a possible, direct cardiac myocytotoxic effect of endotoxins, inflammatory cytokines, and/or reactive oxygen radicals that are produced due to infection by activated neutrophils, macrophages, and endothelial cells. Tumor necrosis factor-alpha has been show to increase endothelial monolayer permeability to macromolecules and lower molecular weight solutes. It is therefore likely that similar permeability alterations may also occur at the myocyte cell membrane level, thus causing troponin release [2, 9, 14, 15, 17, 23, 27, 30].

Another hypothesis is that myocardial injury during sepsis is caused by microvascular thrombosis. A close relationship between the presence of inflammatory cytokines and a procoagulant state in patients with severe sepsis is well known. Inflammatory cytokines including tumor necrosis

factor-alpha, interleukin-1 beta, and interleukin-6 are capable of activating coagulation and inhibiting fibrinolysis. Thus, the possibility of small-vessel thrombosis with subsequent myocardial microinfarction and troponin release is possible. Ischemia and reperfusion injury associated with microvascular dysfunction may also be involved [3, 23, 27, 31]. However, a study by Altmann et al. [16] found no difference in coagulation parameters analyzed with rotational thromboelastometry between troponin I-positive and troponin I-negative patients with sepsis and septic shock. They, therefore, concluded that pathophysiological mechanisms other than thrombus-associated myocardial damage might play a major role in causing increased troponin levels in sepsis.

Some studies have also showed that increased cardiac filling pressure and increased wall stress in sepsis activate an intracellular signaling cascade leading to cardiac myocyte apoptosis, myocyte damage, and micronecrosis leading to troponin elevation [15, 23, 30].

Other factors than can potentially contribute to troponin elevation in sepsis are aggressive inotropic treatment use, severe hypotensive episodes due to septic shock, and prolonged resuscitation attempts, which could contribute to myocardial cell injury [17, 23, 27].

Natriuretic peptides (NPs) play an important role in cardiovascular homeostasis and fluid volume regulation. Increased plasma levels of natriuretic peptide hormones have been identified as predictors of cardiac dysfunction in numerous critical care settings including congestive heart failure, acute coronary syndromes, valvular heart disease, and septic shock. Atrial wall stretching induced by volume load is the primary regulator of ANP release. Left ventricular stretching and end-diastolic pressure and volume elevations regulate BNP release from the cardiac ventricle [9, 19, 20, 32–35].

The biological actions of both ANP and BNP are mediated through membrane-bound guanylyl cyclase/natriuretic peptide receptor-A, which, in response to hormone binding, activates a cyclic guanosine monophosphate-dependent signaling cascade [36].

BNP is secreted into the blood as a pro-hormone and it is cleaved into active BNP and inactive NT-proBNP. BNP and NT-proBNP are secreted in equimolar amounts though they are removed from the blood circulation by different mechanisms, making plasma concentrations unequal. Renal excretion is considered as the main mechanism by which NT-proBNP is cleared from the circulation, while BNP is cleared by specific clearance receptors and enzyme neutral endopeptidase. NT-proBNP has a longer half-life than does BNP (120 vs. 22 min). Renal failure seems to increase both BNP and NT-proBNP concentrations, though renal function alterations have a lesser effect on BNP than on NT-proBNP levels. The advantage of NT-proBNP over BNP, however, is its stability during sampling, transportation, and storage [9, 19, 37, 38].

Several research studies have shown that blood NT-proBNP levels are frequently increased in patients with severe sepsis and septic shock. The exact cause of increased NT-proBNP under these conditions is still not clearly delineated. Possible mechanisms include myocardial depression and other hemodynamic stimuli such as increased wall stress, ventricular dilatation, and right heart strain induced by acute respiratory distress syndrome. However, elevated NT-proBNP values measured in patients with severe sepsis and septic shock do not always correspond to left ventricular dysfunction. Furthermore, increasing evidence suggests that other mechanisms besides hemodynamic factors can enhance NT-proBNP secretion. Some of these might be relevant in sepsis. Proinflammatory cytokines tumor necrosis factor-alpha and interleukin-1 beta can increase BNP secretion in vivo. Cardiomyocyte stimulation with interleukin-6 can also lead to increased BNP and ANP secretion. Lipopolysaccharides from gram-negative bacteria can upregulate the expression of the BNP gene. Proinflammatory cytokine levels are significantly increased in the early phases of sepsis and this might enhance NPs secretion. Furthermore, tumor necrosis factor-alpha and interleukin-1 beta can also contribute to myocardial depression in patients with severe sepsis and septic shock. Lastly, neurohormones angiotensin II and endothelin-1 cause a significant increase in BNP gene expression, and the levels of these compounds are higher during sepsis [38–40].

The diagnostic potential of the levels of natriuretic peptides (and their N-terminal fragments) as well as troponins in postmortem serum and other biological fluids (mainly pericardial fluid and vitreous humor) collected at autopsy have been investigated to some extent in the forensic setting in situations of myocardial ischemia and heart failure [41].

Studies performed by Tanaka et al. [42] and Zhu et al. [43] showed that ANP and BNP can be measured in both blood and pericardial fluid and that the levels in the pericardial fluid are correlated with and higher than their respective levels in the blood. These studies did not report any significant effect of the postmortem interval on the levels of ANP and BNP in the pericardial fluid (at least within 72 h after death) and concluded that both biomarkers were useful for diagnosing acute atrial overload (ANP) and subacute or chronic heart failure (BNP). Subsequent studies that focused on NT-proBNP showed that its concentration was stable in frozen samples of postmortem serum and pericardial fluid, thus corroborating its diagnostic value in detecting heart failure after death [44–46].

Troponin I and troponin T levels have also been measured in various studies in postmortem serum obtained from blood sampled at different sampling sites, including femoral veins, iliac veins, subclavian veins, aorta, right heart, and left heart as well as in pericardial and cerebrospinal fluids. Femoral blood postmortem serum and pericardial fluid troponin levels have been shown to be useful in investigating the severity of myocardial damage due to various causes of death. Troponin

concentrations measured after death appeared to correlate with the severity and increase of ischemic myocardial damage, depending on postmortem intervals [47–65].

The results of the study presented herein tend to be in agreement with those reported in former investigations in the clinical setting. They indicate that postmortem serum troponin I, troponin T, and NT-proBNP levels are increased in sepsis-related deaths in the absence of macroscopic and microscopic findings suggestive of myocardial necrosis or cardiac failure. These data would therefore confirm the conclusions of clinical investigations performed in patients with sepsis and septic shock, which found increased blood troponin levels in the absence of coronary artery disease and higher blood NT-proBNP concentrations unrelated to left ventricular dysfunction.

This is the first study, to our knowledge, to investigate the biochemical profile of sepsis-related deaths with specific regard to troponins and NT-proBNP in a series of cases that had undergone forensic investigations including postmortem angiography, histology, and biochemistry. We were unable to find similar studies pertaining to postmortem serum troponin and NT-proBNP levels in the forensic setting with which to compare our results. In the clinical field, only Amman et al. [17] and ver Elst et al. [30] reported autopsy findings and biochemical data pertaining to sepsis-related deaths. However, in these studies, coronary angiography was not performed systematically, autopsy was carried out exclusively in a few cases, and biochemical investigations were limited to troponin I and T (in the study performed by ver Elst et al.) and troponin I only (in the study performed by Amman et al.).

Our present study has some limitations. The most important limitation is the relatively small number of studied cases, which may limit the accuracy of our research. However, precise selection criteria were applied during the recruitment process in all study groups and subgroups to minimize heterogeneity in the study populations. Prospective investigations including a greater number of subjects are therefore needed to confirm our findings.

Thus, even though further studies are required to confirm these preliminary observations, our results seem to indicate that the biochemical profile of sepsis-related deaths is characterized by increased troponin and NT-proBNP levels in postmortem serum from femoral blood in the absence of any other underlying significant macroscopic and microscopic observations consistent with myocardial ischemia or heart failure. Accordingly, postmortem serum troponin and NT-proBNP concentrations in sepsis-related fatalities should always be interpreted cautiously and in combination with data obtained from other investigations to ensure that the magnitude of myocardial damage is not overestimated and the formulation of unsupported conclusions regarding the cause of death is avoided.

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