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Endocan measurement for the postmortem diagnosis of sepsis

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ABSTRACT

The vascular endothelium has been shown to play a pivotal role in the pathophysiology of sepsis through the expression of surface proteins and secretion of soluble mediators. Endocan (endothelial cell-specific molecule-1), a 50-kDa dermatan sulfate proteoglycan, is expressed by endothelial cells in lung and kidney and can be detected at low levels in the serum of healthy subjects. Increased concentrations were described in patients with sepsis, severe sepsis and septic shock compared to healthy individuals, with serum concentrations related to the severity of illness. In the present study, we investigated endocan, procalcitonin and C-reactive protein in postmortem serum from femoral blood in a series of sepsis-related fatalities and control individuals who underwent medicolegal investigations. Endocan was also measured in pericardial fluid. Two study groups were prospectively formed, a sepsis-related fatalities group and a control group. The sepsis-related fatalities group consisted of sixteen forensic autopsy cases with documented clinical diagnosis of sepsis in vivo. The control group consisted of sixteen forensic autopsy cases with various noninfectious causes of death. Postmortem serum endocan concentrations were significantly higher in the sepsis group, with values ranging from 0.519 ng/ml to 6.756 ng/ml. In the control group, endocan levels were undetectable in eleven out of sixteen cases. The results of the data analysis revealed similar endocan concentrations in the pericardial fluid of both studied groups. Endocan can be considered a suitable biological parameter for the detection of sepsis-related deaths in forensic pathology routine.

1. Introduction

Severe sepsis and septic shock remain leading causes of death in critically ill patients in intensive care units despite great advances of modern medicine [1–3]. Clinicians are still in need of accurate, rapid laboratory biomarkers in order to identify patients who would rapidly benefit from prompt empirical antibiotic therapy and other supportive treatment. An increased knowledge of the pathophysiology of sepsis could therefore have the potential of generating new diagnostic options and treatment modalities for this serious condition [4].

In the forensic field, despite improved methods of collecting blood and tissue samples for postmortem bacteriology and extensive research in biochemical and immunohistochemical investigations, the diagnosis of sepsis as the cause of death still remains challenging. Forensic pathologists have rarely full access to medical records before autopsy is performed. Microbiological investigations are part of the diagnostic work-up but may fail or be interfered by growth of multiple bacteria due to postmortem contamination. Macroscopic and microscopic findings can be elusive or nonspecific and often lack defined organ alterations. Finally, immunohistochemical investigations are not performed systematically [5,6].

C-reactive protein (CRP) and procalcitonin (PCT) have been shown to be stable in postmortem samples and are routinely measured for diagnostic purposes in forensic pathology routine as in clinical practice. However, other etiologies apart from bacterial infections can increase CRP and PCT levels. Hence, a vast number of molecules have been investigated in recent years in order to identify the most reliable combinations of biomarkers that could reliably discriminate between noninfectious and infectious inflammation in the forensic setting [7–11].

The vascular endothelium has been demonstrated as playing a critical role in sepsis pathogenesis by producing cytokines and chemotactic factors as well as expressing surface adhesion molecules that induce circulating leukocyte migration into tissues. Consequently, there is a strong, biological rationale for targeting markers of endothelial activation as biomarkers of sepsis [2,3].

Abbreviations: PCT, procalcitonin; CRP, C-reactive protein; EC, endothelial cells; SIRS, systemic inflammatory response syndrome.

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In clinical practice, a large number of molecules secreted by the endothelial cells (EC) have been investigated as potential biomarkers for the early diagnosis of sepsis. These included regulators of endothelial activation, adhesion molecules as well as mediators of coagulation, permeability and vasomotor tone [3].

Endocan (endothelial cell-specific molecule-1) is a soluble 50-kDa proteoglycan made up of a mature polypeptide of 165 amino acids and a single dermatan sulfate chain covalently linked to the serine residue at position 137. The molecule is expressed and secreted by the endothelium in the lungs and kidneys in response to pro-inflammatory cytokines and pro-angiogenic growth factors. Endocan was found freely circulating at low levels in the serum of healthy subjects and overexpressed by several types of human tumors [12–23]. Additionally, increased levels were described in patients with sepsis, severe sepsis and septic shock compared to healthy individuals, with concentrations related to the severity of the disease, thus suggesting that the molecule may represent a further diagnostic and prognostic marker of sepsis [2,24].

In the study herein described, endocan, PCT and CRP levels were measured in postmortem serum from femoral blood in a series of sepsis-related fatalities and control cases that underwent medicolegal investigations. All sepsis-related cases had a documented, clinical diagnosis of sepsis in vivo that was established during hospitalization. Endocan was also measured in pericardial fluid in both studied group. The first objective of this study was to assess the diagnostic potential of endocan for the postmortem identification of sepsis-related deaths compared to CRP and PCT. The second aim was to compare postmortem serum endocan levels to pericardial fluid endocan concentrations in order to explore the usefulness of using pericardial fluid as an alternative to postmortem serum for diagnostic purposes.

2. Materials and methods

2.1. Subjects

Two study groups were prospectively formed, a sepsis-related fatalities group and a control group. The sepsis-related fatalities group consisted of sixteen forensic autopsy cases who had been admitted to the intensive care unit of the local hospitals, where they died. All cases had a documented clinical diagnosis of sepsis in vivo (duration between 20 and 90 h). Sepsis was diagnosed based on evidence of infection along with the presence of systemic inflammatory response syndrome (SIRS) according to the definition of the American College of Chest Physicians/Society of Critical Care Medicine (ACCP/SCCM) [25].

Medicolegal autopsies were performed between 6 and 58 h after death and were always preceded by unenhanced CT scans. Histology, neuropathology, toxicology, and microbiological investigations were performed in all cases of this group as well as biochemical investigations on postmortem serum from femoral blood and vitreous humor. Specimens for microbiology were collected from at least two different sampling sites.

Sepsis and multiple organ dysfunction syndrome (MODS) as the causes of death were confirmed by postmortem investigations in all these cases. Pneumonia, pleural empyema, pleuritis, tracheobronchitis, pericarditis, endocarditis, pyelonephritis, intra-abdominal abscesses, acute peritonitis and anastomosis dehiscence were the most common septic foci identified during autopsy. Postmortem findings suggesting MODS included myocardial ischemia, pulmonary edema, pleural effusions, shock lungs, hypoxic liver damage, mesenteric ischemia, kidney ischemia, pancreatic ischemia and brain edema. Alternative causes of death were excluded based on autopsy and further postmortem investigations. Descriptive characteristics of the studied subjects, laboratory results, and main postmortem macroscopic and microscopic findings are reported in Table 1.

The control group consisted of sixteen forensic autopsy cases. 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. As with the septic group, complete medicolegal autopsies were always preceded by unenhanced CT scans and were performed between 10 and 60 h after death. In selected cases, postmortem angiographies were also carried out. Histology, neuropathology, microbiology and toxicology were performed in all cases, as well as biochemical investigations on postmortem serum from femoral blood and vitreous humor. Specimens for microbiology were collected from at least two different sampling sites. The causes of death were determined to be cardiac death (3 cases), drug intoxication (3 cases), hypothermia (2 cases), coronary thrombosis (2 cases), traffic accident with survival time within 6 h (1 case), hanging (1 case), diabetic ketoacidosis (1 case), gunshot wounds (1 case), carbon monoxide intoxication (1 case) and hepatorenal insufficiency (1 case). Postmortem investigations failed to reveal findings consistent with the existence of underlying bacterial infections and sepsis despite positive microbiology results in some cases, which were attributed to postmortem contamination. Since all cases selected for the control group originated from forensic practice with deaths occurred outside the hospital in all cases, antemortem clinical data were not available. Descriptive characteristics of the control group subjects, laboratory results, main postmortem macroscopic and microscopic findings as well as causes of death are reported in Table 2.

2.2. Sample collection

2.2.1. Postmortem serum from femoral blood

Using a sterile needle and syringe, postmortem blood samples were collected by aspiration through the femoral vein(s) during autopsy. All blood samples were centrifuged immediately post-collection at 3000*g* for 15 min. After centrifugation, the separated supernatant (5 ml postmortem serum) was collected, stored in preservative-free tubes and frozen at −20 °C until analysis.

2.2.2. Pericardial fluid

Undiluted samples of pericardial fluid (5 ml) were collected immediately post pericardium incision during autopsy. All samples were immediately centrifuged at 3000*g* for 15 min. After centrifugation, the separated supernatant was collected, stored in preservative-free tubes and frozen at −20 °C until analysis.

2.2.3. Femoral blood, right cardiac blood and tissue samples for microbiology

The external side of the right atrium was sterilized by searing with a heated scalpel blade and cardiac blood was aspirated using a syringe. Once collected, the blood was stored in blood-culture bottles (aerobic and anaerobic) and immediately incubated at 37 °C. Lung, spleen and liver surface was sterilized by searing with a heated scalpel blade. Tissue samples were immediately transferred to the laboratory.

2.3. Laboratory assays

2.3.1. Determination of PCT concentrations

PCT was measured by commercially available immunoassays on the Roche Modular E170 analyzer (Roche Diagnostic, Mannheim, Germany) using postmortem serum from femoral blood. Results were expressed in μg/l. The analytical sensitivity was 0.1 μg/l, according to manufacturer information.