



Original communication

Usefulness of pericardial and pleural fluids for the postmortem diagnosis of sepsis



Cristian Palmiere*, Coraline Egger

University Center of Legal Medicine, Lausanne University Hospital, Rue du Bugnon 21, 1011 Lausanne, Switzerland

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ABSTRACT

The purpose of this study was to evaluate the postmortem distributions of procalcitonin (PCT), C-reactive protein (CRP), soluble triggering receptor expressed on myeloid cells-1 (sTREM-1) and soluble interleukin-2 receptor (sIL-2R) levels in postmortem serum from femoral blood, pericardial fluid and pleural fluid in a series of sepsis-related fatalities (12 subjects) and control cases (20 subjects) that underwent medico-legal investigations. Our aim was to assess the diagnostic potential of the results obtained from pericardial and pleural fluid analysis in identifying sepsis-related deaths. All sepsis-related cases had a documented, clinical diagnosis that was established in vivo during hospitalization. Pneumonia was the main infectious focus identified during autopsy and histology. *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Escherichia coli* were the most commonly identified bacteria in blood and lung tissue cultures. The preliminary results corroborate the usefulness of PCT, CRP, sTREM-1 and sIL-2R determination in postmortem serum for the identification of sepsis-related deaths. Moreover, the data suggest that, as far as PCT, CRP, sTREM-1 and sIL-2R measurements are concerned, pericardial and pleural fluids can be considered suitable alternatives to postmortem serum should femoral blood prove unavailable at autopsy.

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1. Introduction

The identification of sepsis-related deaths in forensic pathology routine may be extremely challenging for a myriad of reasons. Medical records are often unavailable when postmortem examinations are carried out and the results of blood and tissue cultures performed after death may be difficult to interpret. Moreover, autopsy and histology findings may lack defined organ alterations and be of infectious or non-infectious origin.^{1,2}

Procalcitonin (PCT), C-reactive protein (CRP) and other biomarkers have been shown to be measurable in postmortem serum obtained from femoral blood and be as reliable as in clinical practice. However, numerous situations frequently encountered in the forensic setting may force the pathologist to deal with blood insufficiency or unavailability during autopsy or to collect

postmortem serum of poor quality for biochemical purposes, limiting the ability to accurately diagnose sepsis-related deaths considerably.

Indeed, only small amounts of peripheral blood may be sampled in infant autopsy or bodies with significant decompositional changes. Not infrequently, blood specimens for analysis are allotted to toxicology in such cases. The identification of alternative biological fluids that can be reliably analyzed and whose results can be faithfully exploited to corroborate the hypothesis of sepsis-related death is therefore of utmost importance.^{2,3}

In the study herein described, PCT, CRP, soluble triggering receptor expressed on myeloid cells-1 (sTREM-1) and soluble interleukin-2 receptor (sIL-2R) levels were measured in postmortem serum from femoral blood as well as in pericardial and pleural fluids in a series of sepsis-related fatalities and control cases that underwent medico-legal investigations. All sepsis-related cases had a documented, clinical diagnosis that was established during hospitalization in vivo. Our aim was to evaluate the postmortem distributions of the tested parameters in the analyzed biological fluids and assess the diagnostic potential of the results obtained from pericardial and pleural fluid analysis in identifying sepsis-related deaths.

* Corresponding author. Tel.: +41 021 314 49 61, +41 79 556 69 89 (mobile); fax: +41 021 314 70 90.

E-mail addresses: cristian.palmiere@chuv.ch, palmierecristian@hotmail.com (C. Palmiere).

2. Material and methods

2.1. Study protocol

The present study was a prospective, single center study, performed during 2013. All cases collected for this study underwent medico-legal autopsies as requested by the inquiring authorities. Laboratory analyses were performed as part of the medico-legal investigations.

2.2. Study populations

Two study groups were prospectively formed, a sepsis-related fatalities group and a control group. The sepsis-related fatalities group consisted of 12 forensic autopsy cases (8 males and 4 females between 48 and 77 years of age). All patients had been admitted to the intensive care unit of local hospitals, where they subsequently died. All cases had a documented, clinical diagnosis of pneumonia and sepsis *in vivo* (duration of sepsis between 10 h and 52 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).⁴

Histology, neuropathology, toxicology, microbiology and biochemical investigations were performed in all cases. Specimens for microbiology were collected from at least two different sampling sites and always included cardiac blood and lung tissue cultures. Biochemistry was carried out in postmortem serum from femoral blood as well as in pericardial and pleural fluids collected during autopsy.

Sepsis was confirmed as the cause of death based on macroscopic, microscopic, biochemical and bacteriological findings. In order to avoid false positive cases due to postmortem blood contamination, positive bacterial cultures from both cardiac blood and lung tissue for one microorganism (or more than one microorganism but at least one potentially responsible for infection) were considered diagnostic. Alternative causes of death were excluded based on autopsy and postmortem investigation results. Intervals after death ranged between 8 and 50 h.

The control group consisted of 20 forensic autopsy cases (10 males and 10 females between 19 and 78 years of age). None of these subjects had a documented, clinical diagnosis of sepsis *in vivo*. All cases selected for this group originated from forensic practice with deaths that had occurred outside hospital. Laboratory results pertaining to the period immediately preceding death were therefore unavailable. The cause of death was determined to be natural cardiac death in all cases (arrhythmia with no evidence of coronary thrombosis or myocardial infarction). Postmortem investigations failed to reveal findings consistent with the existence of underlying bacterial infections. Intervals after death ranged between 10 and 42 h.

Histology, neuropathology, toxicology, microbiology and biochemical investigations were performed in all cases. Specimens for microbiology were collected from at least two different sampling sites and always included cardiac blood and lung tissue cultures. Biochemistry was carried out in postmortem serum from femoral blood as well as in pericardial and pleural fluids collected during autopsy.

2.3. Sample collection

Peripheral blood was collected by aspiration through the femoral vein(s) prior to autopsy using a sterile needle and syringe. Blood was centrifuged immediately post collection at 3000 g for 15 min. After centrifugation, the separated supernatant

(postmortem serum) was collected, stored in preservative-free tubes and frozen at -20°C until analysis. No specimens were excluded due to insufficient sample volume.

Undiluted samples of pericardial and pleural fluid were collected immediately post pericardium incision and pleural cavity opening during autopsy. All samples were immediately centrifuged at 3000 g for 15 min. After centrifugation, the separated supernatants were collected, stored in preservative-free tubes and frozen at -20°C until analysis. No specimens were excluded due to insufficient sample volume.

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, cardiac blood was injected into blood culture bottles, transported promptly to the laboratory for incubation at 37°C and cultured for aerobic and anaerobic microorganisms. Tissue samples for microbiology were obtained from the lungs in the usual way 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. Cultures were performed by current standard accepted procedures.

2.4. Laboratory assays

PCT, CRP and sTREM-1 levels were determined in all sampled fluids according to the techniques previously described.⁵ Results were expressed in $\mu\text{g/l}$, mg/l and pg/ml , respectively.

sIL-2R was quantified in postmortem serum, pericardial and pleural fluids with a commercialized specific enzyme-linked immunosorbent assay (ELISA) kit, according to the manufacturer protocol. Results were expressed in ng/ml .

2.5. Statistical analysis

Pathological PCT, CRP, sTREM-1 and sIL-2R levels in postmortem serum suggesting the presence of generalized inflammation and bacterial infections were chosen according to the available literature (2 $\mu\text{g/l}$, 10 mg/l , 90 pg/ml and 5 ng/ml , respectively).^{2,5,6} Pericardial fluid cutoff levels for PCT, CRP and sTREM-1 were set at 2 $\mu\text{g/l}$, 10 mg/l and 135 pg/ml , respectively, based on the results of former medico-legal investigations.^{5,7} PCT, CRP, sTREM-1 and sIL-2R concentrations in the pleural fluid suggesting generalized inflammation and bacterial infections were not preliminarily identified due to the unavailability of previous studies on postmortem material for comparison.

Nonparametric tests were used throughout the study. The Mann–Whitney *U* test was used to evaluate the difference between the two groups. For all tests, statistical significance was set at $p < 0.05$. Graphpad Prism 4.0 (Graphpad Software, La Jolla, CA, USA) was used for statistics.

2.6. Ethics

All relevant ethical issues were identified and discussed with the local Ethical Committee. All cases collected for this study underwent medico-legal autopsies as requested by the public prosecutor. Postmortem serum from femoral blood and pericardial fluid are systematically collected in our facility prior to or during autopsy. Pleural fluid is collected during autopsy in selected cases. Biochemical investigations are systematically performed in all suspected sepsis-related deaths as well as all sudden unexpected deaths as part of medico-legal investigations. All specimens were anonymized prior to analysis. No further ethical approval was

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