Rom J Leg Med [27] 230-238 [2019]
DOI: 10.4323/rjlm.2019.230

Biochemical markers and microbiology in post-mortem diagnosis of sepsis: a systematic review

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Abstract: In many circumstances the forensic pathologist can question whether the dead person experienced sepsis, which caused or contributed to death.

The autopsy of a death related to sepsis is a difficult task for the forensic pathologist due to the lack of typical pathological factors as well as clinical and circumstantial information about death. Several authors underlined how forensic biochemistry and microbiology could help in diagnosing a death related to sepsis.

The research we carried out analyses the main scientific studies in literature, primarily tracing biochemical markers evaluated to help diagnosing a death related to sepsis.

This review analyses the main problems linked to forensic microbiology investigations, whose results are burdened by heavy issues concerning their interpretation, above all when clinical and circumstantial data are lacking.

Key Words: Sepsis, forensic setting, biochemical markers, microbiology.

INTRODUCTION

Sepsis is a systemic inflammatory response to infections caused by a high morbidity and mortality, especially in elders [1, 2].

From an epidemiological point of view, it is considered one of the biggest public health issues, and the occurrence of sepsis in industrialized countries is of 50-95 cases per 100000 inhabitants. Furthermore, it is the cause of 2% of hospitalization in the USA, affecting about 700000 people per year, eliciting 210000 deaths. That is why sepsis is considered one of the first 10 causes of death [3].

Although the pathophysiological mechanism is still under study, this pathology is linked to a deregulation of the immune system response, as well as the metabolic response, which follows the infection [4-7].

It should be noted that a systemic inflammatory response, similar or equal to that happening during sepsis, may occur even when there is no infection, and can be related to a variety of severe clinical conditions, i.e. Major injuries, burns, severe hemorrhages, cardiac shock and pancreatitis [7-9]. In fact, in these cases, the term systemic inflammatory response syndrome (SIRS) is generally used to describe the triggering of the systemic inflammatory response independently of the cause [7,10].

In 2016, the Sepsis-3 conference defined sepsis as a "life-threatening organ dysfunction caused by a deregulated host response to infection", and septic shock as a "subset of sepsis in which underlying circulatory and cellular/metabolic abnormalities are profound enough to substantially increase mortality" [11].

Sepsis, severe sepsis and septic shock are the progressive worsening of the same pathology [12, 13].

Severe sepsis entails hypertension, organ dysfunction and hypoperfusion, whereas septic shock implies the persistence of hypertension despite fluid resuscitation, causing widespread perfusion alterations [10].

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The progression from sepsis to septic shock reflects the evolution of the systemic inflammatory response. During this process, a percentage of patients may develop ARDS (acute respiratory distress syndrome), DIC (disseminated intra vascular coagulation) and MOFS (multiple organ failure syndrome) [7].

Therefore, the approach of the forensic pathologist in cases of death related to sepsis must take into account the deregulation of the immune system that can involve several organs [14-15]. Thus, a complete post-mortem examination, together with in-depth histological, microbiological and biochemical analyses is needed.

Based on a critical and systematic analysis of literature, this review aims at summarizing the current scientific evidences on autopsy reports, as well as recapping all the principal methods and devices, which are helpful in post-mortem diagnoses of sepsis-related deaths.

MATERIALS AND METHODS

We conducted a comprehensive biomedical literature search focusing on sepsis-related death and post-mortem diagnosis.

Specifically, we used the PUBMED/MEDLINE (https://www.ncbi.nlm.nih.gov/pmc/) and SCOPUS (https://www.scopus.com) search engines to find indexed citations using the search terms: "sepsis", "post-mortem diagnosis of sepsis", “forensic biochemistry”, “forensic microbiology”, “septic biomarkers” and "forensic pathology". To increase specificity, our keywords were combined with the descriptors Restrict to MeSH Major Topic [MAJR] and Main Heading [MH] (in title and/or abstract fields of citation records) for articles published in English in the past 20 years. The resulting citations were then vetted with reference to post-mortem diagnosis of sepsis. The full-text articles of all relevant citations were retrieved and reviewed.

RESULTS

Forensic microbiology – a brief overview: microbiological investigations can be extremely useful and sometimes essential in the diagnosis of sepsis-related deaths [16-20]. However, when medical history is not enough, clinical data are not available and autopsy results are ineffective, interpreting the data of forensic microbiology can be difficult. The main difficulty is understanding if a potential micro-organism is the expression of an ante-mortem infection or a post-mortem false positive result [16, 21-24].

Generally, blood and cerebrospinal fluid are the most frequently used body fluids used for bacterial culture [19, 25-26]. Furthermore, some samples from organs such as spleen and lungs can also be useful for the microbiological investigation [16, 19]. Blood and spleen are considered especially useful within 72 hours in cases of suspected sepsis-related death, whereas lung tissue culture is not indicated because of the high number of false positives [20]. When using liver and spleen, it must be noted that a positive culture can also be related to a local bilious or urinary infection. In these cases, results must be compared to histological data and to the data obtained from cultures from other sampling sites [20]. At least another two samples from different sites are necessary and should always be taken in suspected sepsis [20]. Usually, only one haemoculture is not enough; two are necessary and sufficient in order to exclude or track down a bacteremia when the micro-organism is not the common contaminant and probabilities of bacteremia are low or moderate (i.e. in cases of pneumonia or intra-abdominal infections); three should be taken to exclude bacteremia when its probability is high or continuous bacteremia is taken into consideration [19,27].

The results coming out of a forensic microbiological analysis are: lack of growth, growth of a single isolate o mixed growth [23]. Mixed growth can be the result of contamination, post-mortem translocation or agonal spread:

- According to Tsokos *et al.* [20], contamination represents the isolated or additional growth of micro-organisms in post-mortem cultures, which are not authentic pathogens and that can mask real pathogenic agents. The authors themselves highlight that the diffusion of resident micro-organisms through the body after death (due to the transportation of the body or to autopsy) might cause false positive results.

- Post-mortem translocation relates to bacterial growth after death and the ending of blood circulation. These bacteria can migrate within blood and tissues [23]. In human beings, bacterial translocation can be promoted by a series of pathologies such as haemorrhagic shock, abdominal surgery, neoplasms, cardiac insufficiency, intestinal inflammatory diseases, intestinal occlusions. The mechanisms, which foster bacterial translocation are: intestinal bacterial growth, increased permeability and damage of the intestinal mucosal barrier and the reduction in the efficiency of the immune system [16, 28-33].

- Agonal spread happens during the agonal period or during resuscitation and the artificial maintenance of blood circulation. This phenomenon might be caused to the weakening of mucosal integrity due to hypoxic phenomena during agony, which can lead to the circular spread of micro-organisms, including pathogens and commensal bacteria, during the agonal phase. This process may depend on factors, which are linked to the pathogenicity of micro-organisms and local factors. However, according to literature [23-24] the process of agonal spread is more theoretic than documented and could be even less common than what is thought.

On the other hand, there are no clear evidences and studies, which confirm that this phenomenon commonly happens in the forensic practice.

To avoid false positives, tissue samples and blood should be taken respectively within 15 and 48 hours after death [20].

Normally, even when applying all the necessary sterility measures during tissue and blood sampling, there is a contamination risk equal to 4-6% [17]. Post-mortem translocation does not affect cultures when samples are taken 24 hours after death or if the body is preserved at 4°C before autopsy. However, according to other authors sterility lowers from 80% to 60% 18 hours after death. And a false positive can be caused by post-mortem growth of a small number of microbes, which were already in the blood stream before death [23].

In fact, many bacteria can die, which makes it impossible to identify them via culture. For this reason, aside cellular culture methodologies, it can be useful to apply molecular techniques (PCR amplification) to locate the genome of possible pathogens [23].

Micro-organisms, such as N. meningitidis, Neisseria gonorrhoeae, Haemophilus influenzae, Salmonella species, Staphylococcus aureus, Streptococcus pneumoniae, streptococco beta-emolitico, Klebsiella species, E. coli, Mycobacterium tuberculosis, Entorobacteriaceae e C. albicans should always be considered pathogens [16,35-36].

According to Sunagawa *et al.* [19], some bacteria, such as S. aureus, Streptococcus pneumoniae, Escherichia coli, Pseudomonas aeruginosa often indicate bacteremia. On the contrary, coagulase-negative staphylococci (that belong to the skin bacterial flora) indicate contamination during sampling.

In a recent work, Palmiere *et al.* provided a general approach to interpret autopsy data in suspected sepsis-related death. The authors themselves indicate 4 groups:

- Death non-related to infection: in these cases, autopsy, histological, biochemical and microbiological data are negative.
- True infection: in these cases, autopsy, histological, biochemical and microbiological data are positive.
- False positives: bacterial cultures appear positive but histological and autopsy data are negative, whereas biochemical results are within range. This group should be subdivided into:
- Contamination at sampling;
- Bacterial translocation where isolated micro-organisms are usually commensal pathogens of the digestive tract;
- Combined situations that can be related both to procedure contaminations or post-mortem translocations.
- Unspecified: there are mixed results among autopsy, histological, microbiological and biochemical results.

Forensic biochemistry: the search yielded the main scientific papers about the aid of forensic biochemistry in sepsis-related death and we identified main biochemical markers.

According to Tsokos *et al.* [7], autopsy blood samples taken for the determination of biochemical markers should be taken from the femoral vein. Blood should be drawn via sterile syringe from the femoral vein after the vessel is exposed by dissecting the thigh. Furthermore, it would be necessary to obtain at least 2 samples of corpse blood at two different post-mortem intervals, thus to estimate the concentration of the marker at time of death using a linear regression model when at least two post-mortem values above the detection limit have been measured [37].

After sampling, the blood sample must be immediately fractionated by centrifuging it at 3000 rpm for 10 minutes. After that, serum is separated thanks to a pipe and is transferred to the laboratory. Alternatively, the serum can be kept frozen at -80°C [7].

However, aside peripheral blood, several studies of forensic biochemistry [38-41] considered using cardiac blood and different body fluids (vitreous body, pericardial fluid, cerebrospinal fluid and urine).
The main biochemical markers studied were:

- Procalcitonin: procalcitonin (PCT) is a peptide precursor of the hormone calcitonin, composed of 116 amino acids [2,42-44]. PCT can be detected after 3-4 hours as a response to sepsis, whose peak is approximately after 6-8 hours and has a half-life of 24-30 hours [45-49]. In fact, during sepsis, PCT values usually exceed 2 ng/ml and can also reach 100 ng/ml in severe sepsis [7]. Compared to pro-inflammatory cytokines, PCT is more stable and has a higher half-file [7].
- The evaluation of procalcitonin in sepsis-related deaths was initially proposed by Tsokos *et al.* and then also studied by other authors [2,50-52], who generally confirmed the usefulness of this marker in septic deaths. In one of their first studies [37], the authors observed increased procalcitonin in every sepsis case. Furthermore, a decrease of the PCT value was observed as compared to its ante-mortem levels, which suggests that the post-mortem evaluation of such marker is possible up until 140 hours after death.

However, it should be taken into account that different pathologies can determine an increase of PCT values of around 2 ng/dl [2,53]. These include injuries, surgeries, thermal damage, cardiogenic shock [2,53-55]. According to some authors [56-58], PCT values above 10 ng/dl are an indication of sepsis. Ramsthaler *et al.* did not find false positives using 10 ng/ml as cut-off. Nevertheless, Bode-Janish *et al.* [2], in one of their studies, describe a case where there were no signs of sepsis or infection, but the PCT value was above 10 ng/ml.

Scharg *et al.* [51,59] reported promising and useful results when evaluating PCT values in vitreous body and pericardial fluid in cases of sepsis as compared to those of control cases. Specifically, the PCT measurement in pericardial fluid represents an alternative to post-mortem serum, which has a good sensitivity and a specificity [51]. Attia *et al.* report the usefulness of this marker when diagnosing a sepsis-related death and refer that tissue samples such as liver, kidney and brain can be used as an alternative or in association with serum for the measurement of PCT.

- IL-6: represents a mediator in the acute phase of inflammation during infection. Normally, IL-6 is not detectable in healthy subjects, showing no injuries, infections or other types of stress [45]. In a study about an autopsy [60], authors studied this marker in the serum in cases of sepsis and control cases. Results showed an increase in many samples included in the sepsis group, as compared to the other study group. Furthermore, it was described the increased concentration in post-mortem samples as compared to the ante-mortem values. This, according to the authors, was related to the cellular release caused by autolysis and putrefaction. IL-6 could be useful in the forensic practice and the values above 1500 pg/ml obtained in the first time period of the post-mortem interval is a strong indication of sepsis when there are no injuries, burns or other conditions that could prove SIRS. According to Schrag *et al.* measurement of interleukin-6 (alike interleukin-8, and tumor necrosis factor alpha) is non-optimal for post-mortem discrimination of cases with sepsis.

- Soluble interleukin-2 receptor (sIL-2R): IL-2 is released by the T-cells, which are activated during sepsis. When there is a high expression of the IL-2 receptor, the alpha subunit is released in the blood stream. Reichelt *et al.* observed an increase in this marker's levels in cases of sepsis as compared to control cases. Moreover, they described a post-mortem value higher than ante-mortem measurements. The authors concluded that this marker represents a useful tool in order to diagnose sepsis at autopsy.

- Lipopolysaccharide binding protein (LBP): LBP is a liver synthesised protein, which is released as protein in the blood stream during the acute phase of inflammation/infection. Physiological levels of plasma are between 5 and 15 μg/ml [45]. In a previous study [61], LBP was elevated in all sepsis cases whereas in 88% of control group cases, LBP levels were below 10 μg/ml. Moreover, the post-mortem time course of LBP serum concentrations indicated a constant increase in sepsis cases and control groups. According to the authors, LBP could be a useful marker in the post-mortem diagnosis of sepsis. Furthermore, they advise to take at least two measurements at different post-mortem intervals in order to better evaluate the marker value at time of death.

- C-reactive protein (CRP): is an inflammation and tissue damage systemic marker, constantly used in clinical practice. In healthy subjects, the median concentration is 0,8 mg/l, the 90th percentile is equal to 3 mg/l and the 99th to 10 mg/l. During inflammation/infection, the CRP value can reach and exceed 500 mg/l [45]. Hepatic production is quick, as it reaches 5 mg/l values after 4-5 hours, whereas plasma half-life is of around 19 hours [45,62-65]. Different studies [60, 66, 67] described how this marker increased in cases of sepsis, demonstrating there was an inflammatory process at time of death. Furthermore, a lower post-mortem value was reported, as compared to those ante-mortem, which can be linked to proteolysis mechanisms caused by autolysis and putrefaction. This is another case where it would be useful to take two post-mortem samples to apply a linear analysis of regression and to calculate the value at time of death [60]. Madea *et al.* reported low values of CPR in elderly and infants in cases of pneumonia, which is probably related to an age-dependent process, that sees a lower inflammatory response for these age groups. In another study CPR was measured in the pericardial fluid, conveying false positives both in the sepsis and control groups, with a sensitivity of 80% and a specificity of 95%.

According to the authors, this marker is not specific for sepsis cases and is less useful than PCT.

- Soluble CD14 subtype (sCD14-ST): CD14 is expressed in a series of cells (macrophages, neutrophils, monocytes) or it can be soluble (sCD14). Plasma protease can determine the cleavage of this protein with a sCD14-ST or presepsin formations. Yaegashi *et al.* observed increased sCD14-ST concentration in subjects affected by sepsis. In forensics, Palmiere *et al.* showed how this marker increased in the serum in sepsis-affected subjects, using a value between 600 and 1200 pg/ml as cut-off. However, sensitivity and specificity were not higher than PCT. Authors report that the combined use of PCT and sCD14-ST can increase diagnosis accuracy as compared to single markers taken singularly into account.

- Triggering receptor expressed on myeloid cell type 1 (sTREM-1): sTREM-1 is a receptor of the immunoglobulin family, expressed on the neutrophils and monocytes surface. It is upregulated in cases of bacterial sepsis. In a study by Palmiere *et al.* [71], they demonstrated that this marker increased in the serum in cases of sepsis, using 90 pg/ml as cut-off. Whereas, in control cases, the serum level was below 90 pg/ml. Furthermore, in case of sepsis, a sTREM-1 increase was also seen in urine and pericardial fluid. According to the authors, this marker alone does not have higher specificity and sensitivity as compared to procalcitonin. However, a combined sTREM-1 and PCT evaluation can improve accuracy in post-mortem biochemical investigations.

- Endocan-1: Endocan-1 is a 50-KDa dermatan sulphate proteoglycan, expressed in the endothelial cells of kidneys and lungs and whose concentration in the

serum is low in healthy subjects. A rise of its concentration was found in patients affected by sepsis, severe sepsis or septic shock. The concentration was proportionate to the severity of the disease [72]. Scherpereel *et al.* proposed a cut-off value equal to 1,2 ng/ml. In forensics, some authors demonstrated that this marker increased in the post-mortem serum in cases of sepsis with values between 0,519 ng/ml and 6,756 ng/ml. Whereas, Endocan-1 resulted undetectable in many control cases regardless of the post-mortem interval, showing that the molecule is not released in the blood stream after death due to autolysis and putrefaction. On the contrary, values above 1 ng/ml were found in control cases, which were characterised by a widespread vascular damage.

- Neopterin: is a molecule mainly produced by monocytes/macrophages when stimulated by interferon gamma, produced by T cells. Its biological function is not yet completely clear, but it is thought to have a toxic action against micro-organisms [45]. In forensics, this marker was studied by Ishikawa *et al.* and by Ambach *et al.* [75-76]. In cases of sepsis and traumatic brain injury-delayed deaths, the values of Neopterin were higher than 500 nmol/l in the post-mortem serum, which confirms the activation of monocytes/macrophages and the release of Neopterin during SIRS. Therefore, the specificity of this marker is really limited.

- NT-proBNP and Troponins: NT-proBNP is released by cardiomyocytes as a response to increased atrial and ventricular blood pressure. Plasma increase is not specific of cardiac insufficiency and can be affected by other pathologies, such as sepsis. Troponins are specific markers of the myocardium. The increase of their plasma level in sepsis was showed to happen also without coronary thrombosis. The increase of these markers during sepsis could be related to cardiac damage caused by cytokines, reactive oxygen radicals released during sepsis [77]. According to Tettamanti *et al.* there is a significant increase of those in the NT-proBNP, troponin I and troponin T serum in cases of sepsis as compared to those of control cases. Results suggested that the values of these markers in post-mortem serum increased in sepsis-related deaths lacking severe heart pathology, heart attack or any sign of cardiac insufficiency.

DISCUSSION

In many circumstances the forensic pathologist can question whether the dead person experienced sepsis, which caused or contributed to death [78].

The autopsy of a death related to sepsis is a difficult task for the forensic pathologist due to the lack of typical pathological factors as well as clinical and circumstantial information about death.

In fact, at autopsy it is rare to track down macroscopic signs of an ongoing septic process (abscesses or valvular vegetations in the endocardium), whereas other signs, such as widespread haemorrhagic petechiae, early livor mortis and pulmonary clumping can be non-specific signs) [79].

Furthermore, pathological signs can be related to sepsis complications, such as organ hypoperfusion or disseminated intra vessel blood coagulation. In these cases, other non-specific signs, such as heart hypoxia, brain and lungs oedema, splenic infarct, acute tubular injury (ATI) or intestinal haemorrhages, thrombotic vascular occlusions and cerebral hypoxic ischemic damage [3, 80]. Astrocytes and microglia proliferations are also very common in sepsis, but are a reflection of several pathologies, among which ischemia [7,15].

Specific attention should be paid to lungs that are one of the most affected organs when it comes to sepsis-induced damage. The histologic examination can show a leukocyte invasion in alveolar spaces, due to the activation of inflammatory cytokines [80]. In other cases, we could witness ARDS (acute respiratory distress syndrome), which is nonetheless non-specific for sepsis.

Aside pulmonary alterations, Tsokos *et al.* describe a granulocyte infiltration of the portal tracts that are specific for sepsis. A recent study [2] describes this result in 68% of sepsis cases and in 68% of control cases, which shows it is non-specific after all.

Therefore, from a histological point of view, it is extremely difficult to track down specific signs, above all at the beginning of the septic process [2].

From a microbiological point of view, several difficulties arise when interpreting culture results or those related to molecular analysis with PCR. The latter guarantees rapider results with good sensitivity and specificity, but its use in the forensic practice is really limited [50,82-83] and still today, samples culture is the most used microbiological mean.

According to Sunagawa *et al.* the bacteremia diagnosis criterion should entail the analysis of a couple of blood samples taken from different sites, and the recovery of a single, bacteriological species of obligate anaerobe.

The real risk of altered results is due to multiple factors, which must be considered. One data to be taken into account is the cadaverous state. In fact, putrefaction at room temperature can foster the bacterial translocation process and lead to false positives [16, 84]. The proliferation of resident pathogens and blood relocation is a normal part of standard post-mortem changes [16]. This is why samples are needed to obtain a microbiological examination as soon as possible.

Another factor to be considered is the handling of organs prior samples, which can lead to increased positive cultures [16]. Thus, samples should be taken at the beginning of autopsies, disinfecting the sample area and by using sterile tools.

Yet another factor to be considered is the fact that finding only one pathogen does not mean there is a

real infection. In fact, according to some authors also an isolated growth can be expression of contamination and translocation.

Another element of the debate is the number of samples: Fernandenz-Rodriguez suggested to analyse 5 samples from different tissues and, should the same pathogen be found, the result would indicate a real infection.

According to us, and coherent with some authors [19], unless there are specific conditions (i.e. Fulminating bacteremia) 2-3 samples from different sites (e.g. Femoral and heart blood) are enough.

Furthermore, multiple data should be taken into account, such as pre-existing pathologies, previous hospitalizations, treatments undertaken while alive [16]. In this sense, some pathologies (such as intestinal ulcers, heart attack, extended shock status) can foster bacterial translocation phenomena. Whereas the administration of an antibiotic therapy right before death could decrease the bacterial count in the blood and lead to false positives. So, from another perspective, the lack of positive cultures does not exclude bacteremia.

However, due to the major difficulty in diagnosing sepsis-related death at post-mortem many biochemistry studies have been suggested to help with the post-mortem diagnosis of sepsis.

In the last years, many scientific studies have proposed using biochemical markers to help in post-mortem sepsis-related diagnoses.

Notwithstanding the progresses made by scientific research, currently, there is no ideal marker, which clearly allows to establish whether death was caused by a bacterial infection.

Some authors suggested using a combination of biochemical markers, as compared to a single laboratory parameter, in order to obtain higher sensitive and specific results. However, is it not clear how truthfully useful could this combined evaluation be in forensic practice, considering that a multi-parameter evaluation could also cause interpreting doubts (e.g. in case of disagreement) making diagnosing even more difficult.

One of the main issues concerns the concentration alterations happening after death, which are caused by autolysis and putrefaction. As described in the results part, literature suggests taking two samples at different post-mortem times to establish (with a linear regression) the marker's value at time of death. However, this also leads to doubts: the autolysis and putrefaction processes do not always follow a linear path and at moments, based on circumstantial factors (i.e. weather changes as hours go by), can speed up or slow down the putrefaction process. Consequently, tissue, cellular and protein decomposition do not follow a constant trend. This is to say that we are not sure whether a linear regression (which evaluates two samples at different post-mortem times) can allow an ever correct and reliable value. Where possible, blood samples should be taken as soon as possible, in order to reduce possible interpreting mistakes. This is not always possible though. The sample quality should be tested with a routine analyses of hemolysis, as well as serum protein contents and fractions, which can be indicators of contamination and decomposition involving autolysis and putrefaction [38].

The most studied and, currently, the most reliable marker is undoubtedly PCT, which can be evaluated on blood or (alternatively) on pericardial fluid [51]. In fact, many studies [2, 7, 37, 50] showed that this marker is very useful in sepsis diagnosing, even post-mortem. It also seems to be more stable in relation to autolysis phenomena. However, also for this marker we need to consider some limitations: PCT can increase some pathological conditions (such as injuries, surgeries, cardiogenic shock, etc.) Therefore, a careful analysis of clinical and circumstantial data is necessary, as well as the autopsy, to identify possible coexisting conditions that can imply an increase of this marker in non-related sepsis cases. That is why a cut-off equal to 2 ng/ml was associated with a high negative prediction [2, 33].

According to other studies [2,56-58], values above 10 ng/ml imply sepsis and, by using this cut-off, Ramsthaler did not find false positives. However, other scientific works [50,86] describe cases where PCT was above 10 ng/ml but there were no other signs of sepsis. According to us, a very high cut-off could allow a very high specificity (minimising false positives) but on the other hand could lead to an important underestimation of the biochemical results, due to the difficulties in interpreting the cases where PCT is between 2 and 10 ng/ml in daily forensic practice.

Notwithstanding the complexities at examination, forensic biochemistry (mainly through PCT study) represents an essential and fundamental tool for post-mortem diagnosing of sepsis. Obviously, biochemical data (whose interpretation is difficult when considered alone) need to be considered in combination with clinical, circumstantial (where present), macroscopic, histological and microbiological data.

A limitation brought by biochemical studies is that in subjects affected by immunodeficiency, the inflammatory response can be very mild and thus also micro-organisms, which have a low level of pathogenicity, can lead to death. In these cases, sometimes the process is very quick, and death occurs without showing an obvious inflammatory response [79], which can be examined via immunochemical and biochemical investigations. In those cases, biochemical investigations could bring false positives although the obvious septicaemic status.

In conclusion, still today, the diagnosis of sepsis-related death can be particularly problematic, mainly in those cases where clinical and circumstantial ante-mortem data are missing. In suspected sepsis, it is necessary to observe the indications relative to sampling

to avoid contaminations during microbiological investigations. Both for forensic microbiology and biochemistry, samples should be taken as soon as possible to minimise alterations due to autolysis and putrefaction. At the current state, PCT is a useful and essential biochemical marker for post-mortem diagnosis of sepsis. Only a multidisciplinary evaluation, which takes into account clinical, circumstantial (where present), macroscopic, histological and microbiological and biochemical data, will make it possible, by following sufficiently rigorous scientific criteria, to reach a post-mortem diagnosis of sepsis.

Conflict of interest. The authors declare that there is no conflict of interest.

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