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# Searching for highly sensitive and specific biomarkers for sepsis: State-of-the-art in post-mortem diagnosis of sepsis through immunohistochemical analysis

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**Raffaele La Russa[1,2*], Aniello Maiese[1*], Rocco Valerio Viola[1], Alessandra De Matteis[1], Enrica Pinchi[1], Paola Frati[1,2] and Vittorio Fineschi[1,2]**

## Abstract

The autoptical observations commonly ascribed to sepsis deal with unspecific general and local signs of inflammation or ischemia, such as myocardial inflammation, pulmonary edema and infiltration, cerebral swallowing, and tubular necrosis in the kidney. In the two last decades, some studies have been carried out to implement immunohistochemical markers for post-mortem diagnosis. All of these target molecules are specifically up-regulated or down-regulated during systemic inflammatory responses, especially for infective causes. Among these, we found some antigens expressed on leukocyte surfaces (very late antigen-4 (VLA-4), cluster differentiation-15 (CD15)), enzyme contained in neutrophils granules (lysozyme (LZ), lactoferrin (LF)), endothelial markers and junctions (E-selectin, vascular endothelial cadherin (VE-cadherin)), and soluble factors (vascular endothelial growth factor (VEGF), tumor necrosis factor alpha (TNFα), procalcitonin (PCT), soluble triggering receptor expressed on myeloid cells-1 (s-TREM-1)). All of these showed potential reliability in differentiating sepsis cases from controls. Further studies are needed to provide a concrete validation for a combination of markers on specific organ samples in order to reach a post-mortem diagnosis of sepsis also in the absence of clinical records.

## Keywords

forensic pathology, immunohistochemistry, post-mortem diagnosis, sepsis

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## Introduction

Sepsis is a condition frequently diagnosed in the clinical setting, characterized by a very high mortality, up to 40%–70% in cases that progress to septic shock. Despite a constant improvement in the environmental hygiene of high-resource countries, even in these contexts, the measured incidence of the syndrome is constantly increasing, evidently due to the progressive aging of the population and, above all, the increased survival of subjects carrying multiple and severe comorbidities. In addition to the demographic factors, we must consider those directly related to healthcare, such as the increase of invasive diagnostic and therapeutic procedures performed and the extension of indications for surgery to such kinds of patients that were excluded until recently.

[1]Department of Anatomical, Histological, Forensic and Orthopaedic Sciences, Sapienza University of Rome, Rome, Italy
[2]IRCSS Neuromed Mediterranean Neurological Institute, Pozzilli, Italy

*Equal contribution.

**Corresponding author:**
Vittorio Fineschi, Department of Anatomical, Histological, Forensic and Orthopaedic Sciences, Sapienza University of Rome, Viale Regina Elena 336, 00161 Rome, Italy.
Email: vittorio.fineschi@uniroma1.it

In the European Union, the number of sepsis cases is estimated to be about 1.4 million per year, as well as up to 700,000 victims, due to an overall mortality rate of 28%–50%. The phenomenon affects all clinical and territorial contexts, but has a particular incidence in the hospital population, for the reasons previously mentioned. The economic costs are as huge as 5% of the total health expenditure in the United States in 2011. This is consistent with the fact that sepsis is considered the main cause of morbidity and mortality in the intensive care units. A topic of this chapter which, moreover, has recently been identified by the European authorities as one of the most relevant health problems for the present and the immediate future is precisely that of the antibiotic resistances that are found more and more frequently in the microbial species isolated into hospital wards.

A further indirect sign of how the theme is central to the international medical–scientific debate comes from the recent and multiple collegial efforts to standardize knowledge and methodologies both in the definition and in the diagnosis and in the early clinical approach to the patient.

### *Definition and diagnosis: from the clinical setting to the post-mortem examination*

Quite surprisingly, since sepsis is one of the most classic manifestations of infectious pathology and has always been described, its clinical definition has been the subject of discussion and has seen a recent revision. In the last decade of 20th century, it seemed that the definition of "systemic inflammatory response (SIRS) arising from infection" developed in 1991 by the joint efforts of the American College of Chest Physicians (ACCP) and the Society of Critical Care Medicine (SCCM) could work thoroughly to include all cases and discriminate the differential diagnoses. On the contrary, instead, the recent "Sepsis-3" Consensus Conference opted for the definition of a "life-threatening organ dysfunction caused by a dysregulated host response to infection," which is only apparently more generic.

The intention of the experts involved in this update was clearly that of focus on the two pivotal aspects, in addition to the implicit one of the presence of a microbial infection. The first one is the physiopathological drive of the disregulation of host's response to the infection, which is predominant rather than the intrinsic virulence of the agent. The second clinically relevant aspect focused by the new definition is the impairment of one or more organic functions and the consequent risk of death (Figure 1).

At the same time, both the criteria and diagnostic tools available to the clinician in the evaluation and treatment of patients affected from sepsis are contained within the definition provided. First, they can rely on all the findings suggestive of local and systemic infection, within which the clinical microbiological analysis and cultures are included. These will be correlated with physical, laboratorial, and instrumental signs commonly used to monitor organ functions. The fundamental issue to meet the needs of clinicians is to provide reliable, but above all rapidly accessible, tools, so as not to delay the necessary antibiotic therapy, as well as vital support treatments. This orientation, of course, leads to a reduction in specificity of the diagnostic response.

The point of view of the autoptical and medicolegal diagnosis will be clearly reversed, aiming to the highest possible accuracy as there are no risks in establishing a false negative diagnosis. More generally, professionals involved in the post-mortem assessment necessarily look for the tools able to provide the most accurate response, including the so-called "ancillaries techniques" compared to the traditional autopsy. At present, the scenario of the tools available to ascertain sepsis at autopsy as a cause of death is somewhat uncertain. On the whole, in fact, the signs that can be found in the external and internal examination of the corpse, as well as in the possibilities of histological observations with routine hematoxylin and eosin, are rather scarce and above all lack objectivity and specificity.

Among these, it is possible to include the finding of myocardial interstitial infiltration from mononuclear leukocytes. In the brain, however, it is possible to find the effects of a global ischemia secondary to the cardio-circulatory depression and septic shock. Consequently, one can observe swelling of the parenchyma, widening of gyrations, and narrowing of sulci. Based on the interval between insult and death, we observe the evolution of neuronal necrosis from red neurons, the late aspects of vascular proliferation, and reactive gliosis. According to a similar principle, it is possible to find acute tubular necrosis in kidney samples.

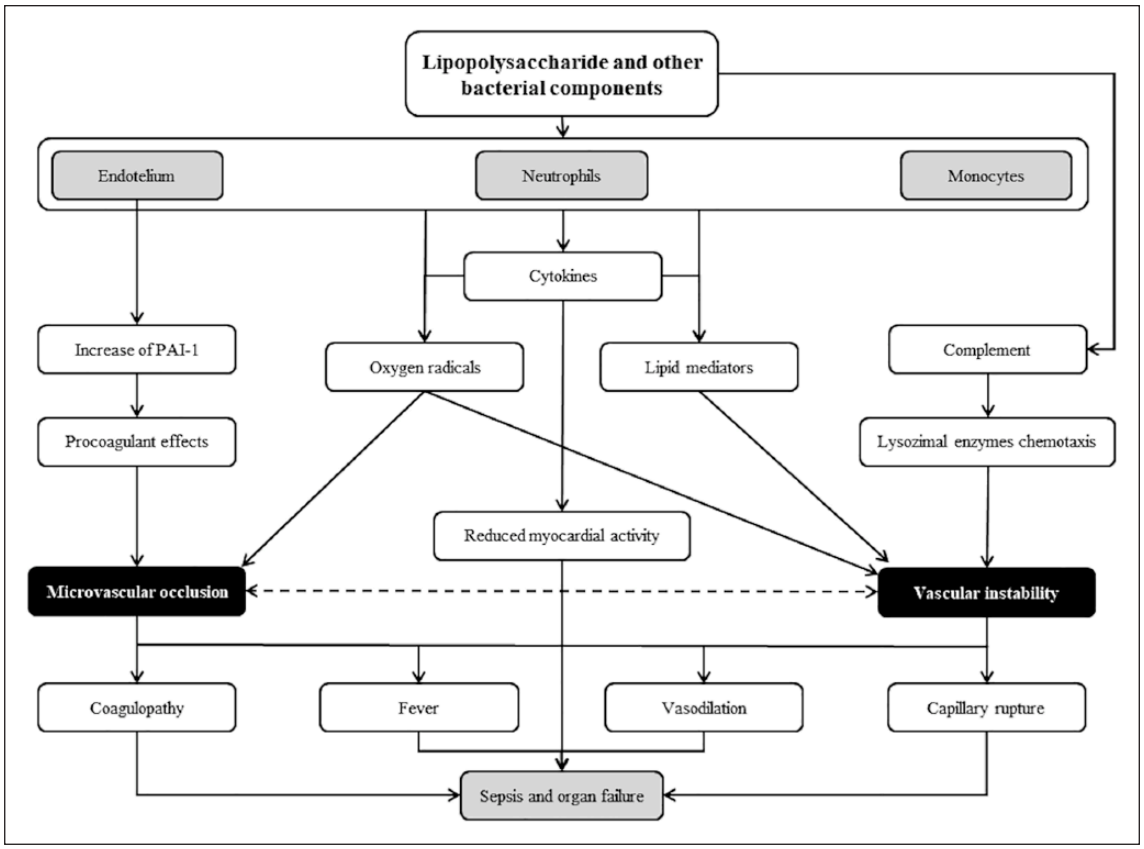


**Figure 1.** Physiopathological drive of the disregulation of host's response to the infection and the impairment of one or more organic functions and the consequent risk of death.

Macroscopically, organs may appear edematous with a poor corticomedullary differentiation.

The lungs are among the most involved organs, since for the establishment of an acute respiratory distress syndrome (ARDS), they could already be present at the macroscopic examination, characterized by red color, increased weight and consistency, heavy congestion, and edema. The related microscopy is that of the diffuse alveolar damage (DAD), always depending on the time interval until the exitus, making it possible to find a hyperacute stage (exudative, with alveolar collapse, hemorrhage, hyaline bronchiolar and ductular membranes, and neutrophilic margination in capillaries) or a post-acute stage (regenerative and, subsequently, reparative). It is evident that all the conditions that can produce an ARDS can result in a similar picture in addition to sepsis.

In view of all these reasons, it is understandable why authors most often consider the findings acquired through autopsy and routine histology unspecific and unconvincing, even in cases of circumstantial suspicion of a lethal sepsis. Even more so, when detailed and reliable clinical data are unavailable, the post-mortem diagnosis of sepsis can be completely impossible, resulting in a real unresolved or missed case if the medico-legal examination does not implement any of the ancillary tests specifically directed to recognize sepsis, which are available especially in the field of immunohistochemistry (Figure 2).

### *Immunohistochemical assays for post-mortem sepsis detection*

The application of immunohistochemical techniques to this field is based on the improvement of medical and experimental knowledge in the physiopathology of sepsis. Studies have clarified the correlations between systemic signal cascades and generalized inflammatory responses. Furthermore, in each organ, these pathological events determine specific functional impairments which, as previously argued, are currently included in the definition of the septic state itself. To explain the functional deficit, precise correlations between the

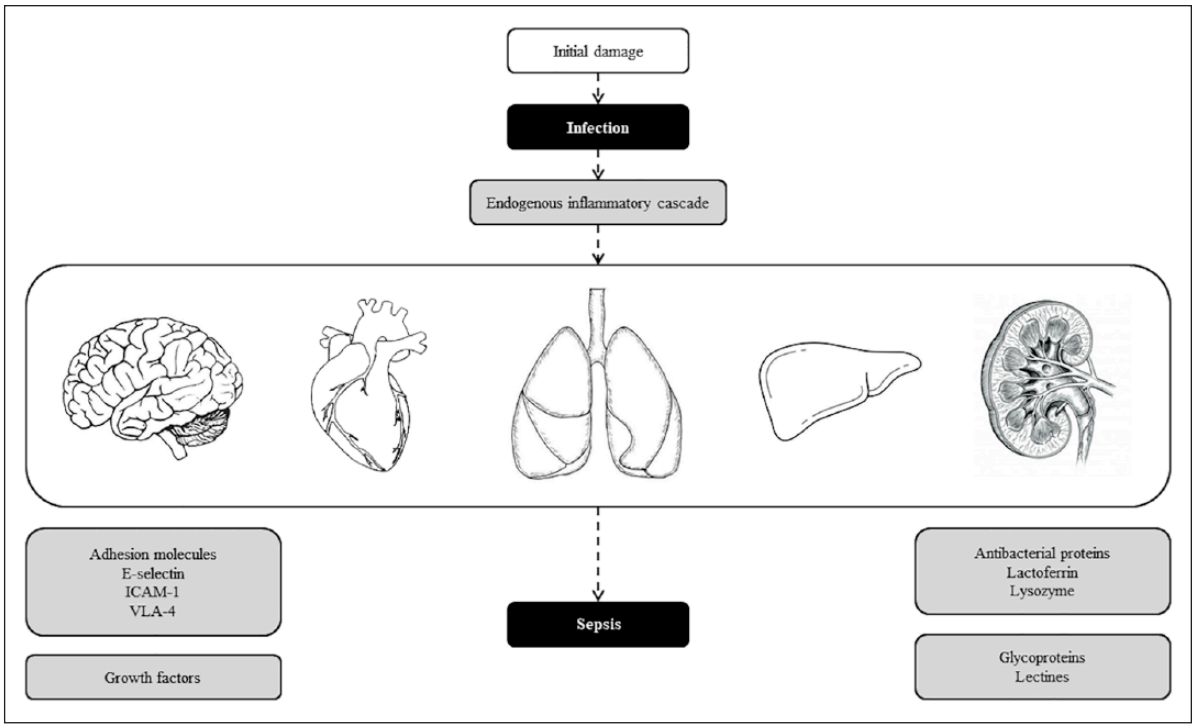


**Figure 2.** The application of immunohistochemical techniques to this field is based on the improvement of medical and experimental knowledge in the physiopathology of sepsis. To explain the functional deficit, precise correlations between the anatomical damage, mostly microscopical, and the molecular expression altered by systemic and local mediators that are freed during sepsis have been described.

anatomical damage, mostly microscopical, and the molecular expression altered by systemic and local mediators that are freed during sepsis have been described.

In this sense, what is considered the prevailing pathophysiological model in the course of sepsis is the establishment of a strong imbalance between pro-inflammatory and anti-inflammatory responses.[1] This condition can progress to a state of profound immunosuppression or, on the contrary, can produce a persistent massive inflammation. The principal pro-inflammatory mediators are the chemokines, the main ones responsible for chemotaxis, together with the cytokines which directly induce tissue and especially vascular endothelial activation. Through these processes, in addition to edema, the intravascular leukocyte margination is determined first, then the diapedesis and extravasation with the formation of more or less widespread cellular infiltrates in the tissues.[1]

If these events have always been known as pathognomonic correlates of local inflammation, in recent times, they have been extended to some target organs in case of sepsis and generalized inflammation. In the clinical setting, it is now agreed that sepsis can determine a true secondary cardiomyopathy. Septic cardiomyopathy is defined as a reversible myocardial depression that is established by activation of the tissue triggered by the cytokine storm in progress. This condition was described for the first time by Parker in 1984, but has been studied more in depth only thanks to the use of more advanced techniques for monitoring heart function. Even more recently, some phenotypic alterations and structural rearrangements of cardiomyocytes have been associated with the clinical finding. It is not yet possible, however, to identify a sensitive and specific molecular marker. It has also been hypothesized that myocardial dysfunction during sepsis reflects a condition similar to that of the so-called "hibernated myocardium" that occurs in the ischemic insult. In fact, similarities are observed in the metabolic and ultrastructural changes that are established in the two circumstances.

Another target organ of the mechanisms of progression and aggravation of sepsis is the lung. In the lungs, as anticipated, it is possible to find the

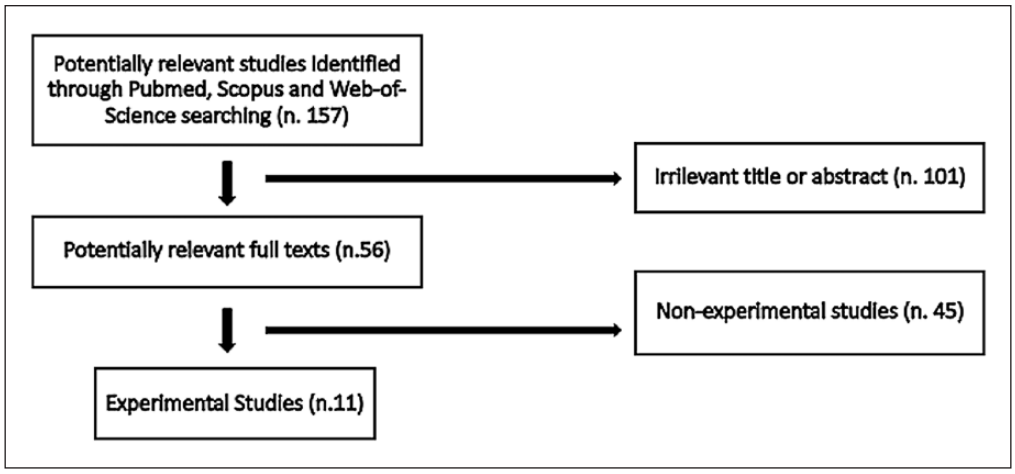


**Figure 3.** Methodology for the evaluation and selection of papers.

effects of a massive inflammatory activation, which occurs both in the vascular pole, due to the involvement of the alveolar capillaries, and in the epithelial compartment of the parenchyma with a dramatic reduction in the production of surfactant. The characteristic alterations of the capillary endothelium include the increased expression of transmembrane leukocyte adhesion molecules and, at the same time, the reduced expression of junctional intercellular molecular apparatus. In addition, the pro-inflammatory cascades induce the activation of the resident histiocyte populations (alveolar macrophages belonging to the myelomonocyte line) in addition to the local recall of immune-competent cells from the bloodstream.

In the case of the kidney, however, despite being one of the key affected organs for progression of severe sepsis to multiorgan failure syndrome (MOF), no ultrastructural rearrangements specifically attributable to sepsis have been identified, since the organ damage and lesions described up to now are always secondary to hypoperfusion mediated by reflex arteriolar vasoconstriction.

## Material and methods

Relevant scientific articles were identified from Pubmed, Scopus, and Web of Science until November 2018, using the following keywords: "*post-mortem diagnosis sepsis*" and "*immunohistochemistry sepsis*" were searched individually and in association with the other keywords.

The resulting 157 references were screened to exclude duplicates, which left 94 articles for further consideration. In addition, non-English papers were excluded and the following inclusion criteria were used: (1) original research articles, (2) reviews and mini-reviews, (3) documents and guidelines promulgated by scientific societies and international organizations, and (4) book chapters. These publications were carefully evaluated taking into account the main aims of the review (Figure 3). This evaluation left 56 scientific papers, distributed as original research articles, reviews and mini-reviews, documents published on institutional websites, and book chapters (Table 1).

## Results

To date, few experimental studies that evaluate the diagnostic usefulness of specific immunohistochemical assays in the diagnosis of sepsis as a cause of death have been published. The scientific evidence gathered so far on the subject in question will be presented below, by each specific antibody.

### *E-selectin*

This target is also detected as CD62E. It is a primary adhesion receptor which allows the rolling of leukocytes. It is expressed on luminal side of endothelial cells only when activated by cytokines or endotoxins (e.g. the bacterial gram-negative lipopolysaccharide). Its expression in the pulmonary alveoli in case of sepsis has been evaluated in one study.[2] They grouped 37 individuals into four groups: definite sepsis because of autoptical findings and medical records (6); possible sepsis, suspicion based on individual case history, and exclusion of other causes by

**Table 1.** Non-experimental studies.

| | Authors | Journal |
|---|---|---|
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autopsy (7); non-septic unnatural death (17); and non-septic natural death (7).

The authors found a positive significant association between incidence of positively stained assays from the definite sepsis group and all other groups. They particularly noted a negative immunohistochemical reaction also in cases with a documented inflammatory lung injury (bronchopneumonia and ab ingestis pneumonia). They concluded suggesting the use of detection of E-selectin in lung specimens as a valuable marker of sepsis in forensic post-mortem examination.

### Very late antigen-4

This molecule is also known as CD49d/CD29. Very late antigen-4 (VLA-4) is a dimeric transmembrane protein belonging to the integrins type. It is expressed on circulating leukocytes surfaces, like monocytes, eosinophils, basophils, and lymphocytes. It plays a crucial role in rolling and vascular endothelium adhesion of leukocytes when activated by chemotactic agents.

The specific antibody against VLA-4 was tested in a study on lung tissues from 30 dead individuals,[3] 8 of whom had a confirmed post-mortem diagnosis of sepsis and 22 controls. The results reported a significative difference at the semi-quantitative analysis of immunohistochemical staining of sepsis group, which showed a strong expression in intravascular, interstitial, and intra-alveolar leukocytes. Authors concluded that anti-VLA-4 is a useful marker in the forensic setting both for confirming or ruling out a suspected case of sepsis.

### Intercellular adhesion molecule-1

This cell surface glycoprotein is also identified as CD54. It is an integrin ligand expressed on endothelial and immune system cells to mediate extravasation and recruitment into sites of inflammation. The main pro-inflammatory cytokines, notably interleukin-1 (IL-1) and TNFα, up-regulate its expression.

We found implementation of specific immunohistochemical stainings in two studies. Tsokos and Fehlauer[3] compared lung specimens of eight cases with ascertained diagnosis of sepsis with a control group. The positive reaction of endothelium in all pulmonary vessels, alveolar macrophages, and lymphocytes was significantly stronger and wider in cases than controls.

Subsequently, Galassi et al.[4] tried the application of the antibody on myocardial specimens with the same purpose but found no relevant differences between cases and controls. The only interesting observation was an intense immunoreactivity corresponding to the vascular endothelium around mycetes embolus into the myocardium. The authors then argued that reliability of intercellular adhesion molecule-1 (ICAM-1) as a marker for sepsis assessed on lung specimen is not extendable to heart histopathology.

### Lactoferrin

Lactoferrin (LF) is a globular protein similar to plasmatic transferrin (transferring family). It can be found in tears, saliva, and other fluids, but it is specifically expressed in the secondary granules of neutrophils. With neutrophils degranulation, LF performs a dual immune function as it exerts a direct antibacterial action and promotes leukocyte chemotaxis by increasing the adhesiveness of the circulating cells with the endothelial surface.

LF antibodies have been tested for post-mortem diagnosis of sepsis in two studies. The first one[5] found significative differences in disposition and expression of positively stained cells in lung sections between cases (13; all with ascertained diagnosis of sepsis) and controls (14 with ascertained cause of death other than sepsis). In the second study, heart samples were tested, comparing 56 cases of ascertained sepsis with a control group.[4] The authors reported positive reaction in more than half of the cases (56%), concluding that implementation of the immunohistochemical assay could increase sensitivity in detection of myocardial inflammation at light microscopy (Figure 4(a)).

Based on these experimental results, we can proceed to immunohistochemical assay for LF to corroborate a diagnosis of sepsis on both lung and myocardial sections.

### Lysozyme

Lysozyme (LZ) is a low molecular weight enzyme. Its expression is quite similar to that of LF. It is abundant in secretions (tears, saliva, and mucus) and is present in primary and secondary granules of macrophages and neutrophils. LZ forms part of innate immune response because of its bacteriostatic and bactericidal activity: it catalyzes the hydrolysis of the peptidoglycan, which composes the bacterial wall.

LZ antibodies have been tested for post-mortem diagnosis of sepsis in the same study that mentioned about LF,[5] as it was intended to be as useful as the other one in revealing and measuring leukocytic infiltrates. Nevertheless, authors could not find significative differences between cases and controls. They considered that the immunohistochemical application of LZ antibody in the elucidation of sepsis as cause of death is limited by the wide range

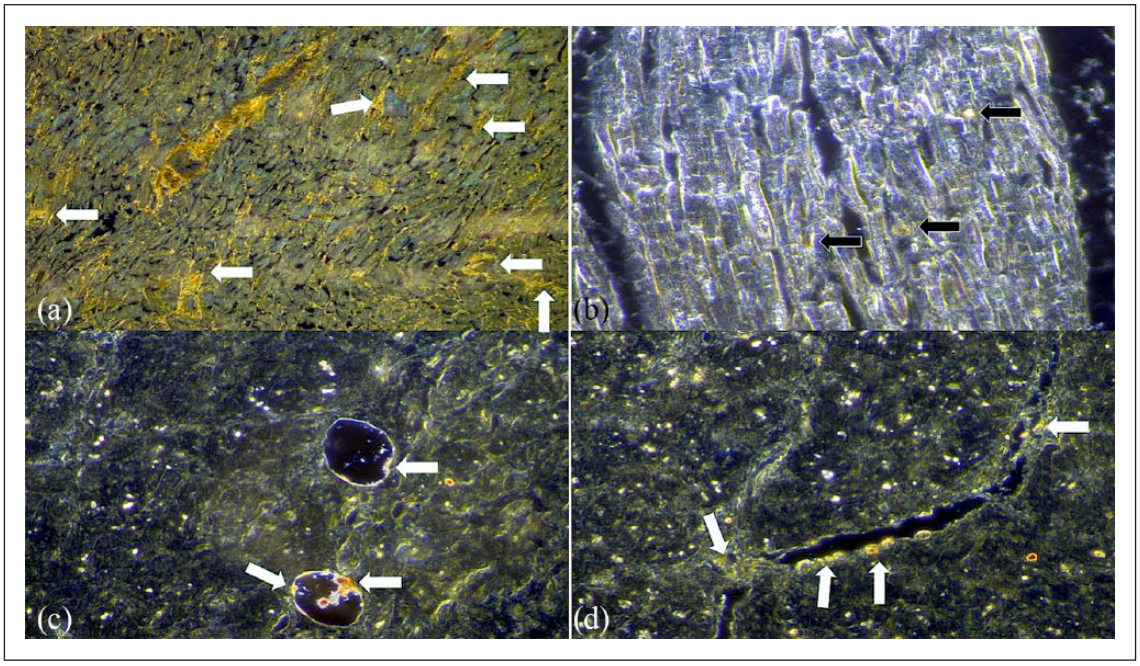


**Figure 4.** (a) Lactoferrin positive reaction demonstrating increased sensitivity in detection of myocardial inflammation at light microscopy (arrows; 20×). (b) TNFα positive macrophages (40×). (c) Specific vascular pattern expression of VE-cadherin among venules, obtaining a globally reduced immunoreactivity of the specific endothelium pattern of positivity (60×). (d) Immunohistochemistry reaction demonstrating the infiltration of mononuclear cells in septic cases. The fact of an active recruitment of myelomonocytic immune cells in specific targets during sepsis was easily expectable. The results highlighted that this phenomenon reaches such a degree that is clearly detectable at immunohistochemistry (60×).

of the specific immunoreactivity and the interindividual variability of leukocytic expression.

To date, there are no evidence supporting the implementation of LZ antibody by immunohistochemical staining to differentiate sepsis and other causes of death in the post-mortem setting.

### *Vascular endothelial growth factor*

Vascular endothelial growth factor (VEGF) is the first cytokine that has been studied by immunohistochemical assays in the post-mortem detection of sepsis. It is a small protein synthesized for extracellular release in response to some stimuli, mainly related to hypoxic conditions. It represents the pivotal mediator of vasculogenesis and angiogenesis and presents specific patterns of up-regulation both in carcinogenesis and reparative phases of inflammation.

Experimental evidence on variability in VEGF expression during different type of lung injury already existed. Tsokos et al.[6] used immunostaining for this cytokine on lung sections in a wider case-control study that evaluated its pulmonary expression during ARDS due to sepsis. They reported an expression pattern in line with previous data in the control group of 10 individuals who died by natural and unnatural causes other than sepsis. It consisted of a preponderant immunoreaction in alveolar epithelium, attributable to type II pneumocytes, and also in bronchial epithelial and glandular cells and in activated alveolar macrophages. Conversely, the eight septic cases showed strong positivity only on activated alveolar macrophages; the immunoreactivity of bronchial epithelium and glandular cells of bronchi and bronchioli lacked in the sepsis group. We need to highlight that all cases underwent mechanical ventilation during the last 4 days of their life.

The results suggested a marked reduction in lung expression of VEGF in patients suffering from ARDS in sepsis and mechanically ventilated before death. This condition seems to be associated to an alteration in the cellular-specific immunoreactivity for VEGF at immunohistochemistry, losing most of the normal or strong positivity, especially at bronchial epithelium.

### Tumor necrosis factor alpha

Tumor necrosis factor alpha (TNFα) is a circulating protein well known for its pleiotropic biological effects. It was originally ascribed to innate anti-tumor mechanisms but now is considered as the main pro-inflammatory cytokine with IL-1 beta. It also plays a predominant pathogenetic role in some auto-immune diseases like rheumatoid arthritis and Crohn's disease.

Considering previous studies on post-mortem analytical assays of biological fluids, Miyashita et al.[7] tested lung specimens for specific immunoreactivity with anti-TNFα antibody. They performed a morphometrical semi-quantitative analysis comparing the sepsis group of nine cases with a non-sepsis control group (eight individuals). They obtained a mean ratio of the number of positive macrophages to the total number of macrophages per each microscopic field. It was significantly higher in sepsis than in control group. Based on these results, the authors estimated a minimum cut-off value of the aforementioned ratio to make a diagnosis of sepsis equal to 20% (Figure 4(b)).

### Angiotensin-I converting enzyme

Angiotensin-I converting enzyme (ACE) is a transmembrane enzyme expressed by endothelial cells. It catalyzes the conversion of the pro-hormone angiotensin I into the active form of angiotensin II, which regulates blood pressure inducing systemic arteriolar vasoconstriction and modulating glomerular filtration rate (GFR). ACE also recognizes other substrates like kinins being involved in microcirculatory regulation during acute and post-acute inflammatory responses.

Experimental evidence showed reduced serum activity in individuals with ARDS caused by sepsis, which is consistent with lung injury associated to this pathological condition, as the pulmonary level is the main site of normal ACE expression and activity. Müller et al.[8] used immunostaining for this enzyme on lung sections in a human case-control study that evaluated its pulmonary expression in the post-mortem setting. They observed a significative reduction of endothelial immunoreactivity in 19 people who died after ARDS during sepsis (cases), comparing with sections obtained from tumor-free specimens of 20 surgical tumor lobectomies (controls).

These results indicate that the ACE antibody can have a role as a diagnostic marker of death caused by sepsis when an ante-mortem lung injury took place, revealing the patent loss of normal immunoreactivity of normal pulmonary endothelium.

### Vascular endothelial cadherin

Vascular endothelial cadherin (VE-cadherin) is a key element of the multiprotein complex that forms the adherens junction of basal cell-to-cell surfaces in epithelial tissues. Through variation of representation and density of these structures, the vascular endothelium can regulate permeability of the blood–tissue barrier; pro-inflammatory mediators determine a marked increase in the microcirculatory permeability to serum, solutes, and circulating immune system cells.

VE-cadherin antibodies have been tested for post-mortem diagnosis of sepsis in the same study that mentioned about ACE,[8] as authors expected to find an appreciable reduction of its expression in the same conditions in consideration of previous experimental evidences. They performed the same case-control analysis described before, implementing the different immunostaining methods. The observations made showed similar reduction in specific immunoreactivity. These findings have been confirmed in a further study by Herwig et al.,[9] in which, very similar methods have been applied with a larger control group (surgical lobectomies from 41 live oncological patients). Authors described a marked reduction of VE-cadherin expression in all pulmonary endothelia. Not different from the case of ACE, they always described a substantial maintenance of specific vascular pattern expression among different kind of vessels (arterioles, capillaries, major veins, and venules). This involves that when considering the strength of immunoreactions, it is needed to ascribe it to a specific vascular type (Figure 4(c)).

Similar to ACE, VE-cadherin antibodies can help in recognizing sepsis cases by obtaining a globally reduced immunoreactivity of the specific pulmonary endothelium pattern of positivity.

### C-C chemokine receptor type 2

This marker is also numbered as a cluster differentiation with CD192. As the whole name suggests, it consists of a transmembrane receptor, more

precisely a G protein-coupled one, activated by the chemokine monocyte chemoattractant protein-1 (MCP-1), also referred to as C-C motif ligand-2 (CCL2). Among immune system cells, C-C chemokine receptor type 2 (CCR2) is permanently expressed on a subset of circulating monocytes (CD14+/CD16 negative ones) and is crucial in the specific recruitment pattern in case of local inflammation.[9]

Considering the importance of chemoattraction mechanisms in lung injury during inflammation and previous findings of elevated serum and pulmonary levels of MCP-1 associated with fatal sepsis, An et al.[10] performed a study to evaluate the infiltration of mononuclear cells in septic lungs by immunohistochemistry. They assessed the specific immunostaining on nine cases with ascertained diagnosis of sepsis as cause of death and on eight controls dead from natural and unnatural causes other than sepsis. The morphometrical analysis (semi-quantitative evaluation) revealed a significantly higher representation of CCR2-positive mononuclear cells in the sepsis group rather than in controls (Figure 4(d)).

The fact of an active recruitment of myelomonocytic immune cells in lungs during sepsis was easily expectable. The results highlighted that this phenomenon reaches such a degree that is clearly detectable at immunohistochemistry.

### *CX3C chemokine receptor 1*

This marker is strictly analogous to the previous CCR2. Like the other one, it is a transmembrane receptor, always a G protein-coupled one, but it is specific for a different chemokine, the fractalkine (or neurotactin), also called CXC3CL1. CX3C chemokine receptor 1 (CX3CR1) is a constitutional surface marker of the other subset of circulating monocytes, the CD14+/CD16+ one.[9]

Not by chance it has been tested in the same study aforementioned,[10] for the same reasons and purposes. The morphometrical comparative analysis of CX3CR1-positive mononuclear cells in lung sections treated with the immunohistochemical assay showed a significative difference between the sepsis and control groups again.

Both CX3CR1 and CCR2 can be considered as potential markers to detect and quantify pulmonary mononuclear infiltrates in septic individuals, differentiating them from causes of death other than sepsis.

### *Procalcitonin*

Procalcitonin (PCT) is a 116 amino-acids peptide precursor constitutively produced by the neuroendocrine cells of the thyroid gland as the specific pro-hormone. During generalized infective states, all differentiated cells express the same molecule and release it into the bloodstream. Over the last decade, PCT has emerged as a useful diagnostic biomarker for sepsis and is routinely used to monitor the clinical course of such affection, mainly in case of bacterial or fungal infections.

In a recent paper, Maiese et al.[11] tried, for the first time, the usefulness of this molecule as a specific target for immunohistochemical assays, investigating the implementation of such a test for forensic purposes in different organs. The authors selected a cases group of people with an ascertained clinical diagnosis of sepsis before death. The control group consisted of five individuals with natural and unnatural cause of death, all characterized by suddenness. They compared the specific immunostaining for brain, heart, lung, liver, and kidney between groups, obtaining a variable, but always positive, immunoreactivity in all the specimens from sepsis group, while the control individuals were proven systematically negative. More in detail, the staining positive pattern was mainly intravascular in each organ, except for myocardial samples where cardiomyocytes also showed a nuclear reaction.

Aside with considerations about new perspectives on role of PCT during sepsis, this study proposes the immunohistochemical assay with PCT antibodies as an effective means to the post-mortem diagnosis of sepsis as cause of death.

### *Cluster differentiation-15*

Cluster differentiation-15 (CD15) is a cellular surface protein classified as an immunologically significant molecule as it differentiates neutrophils from other subtypes of normal leukocytes which do not express this antigen. It is an adhesion protein, and the immune functions that it promotes are both chemotaxis and phagocytosis. For clinical purposes, it is of paramount importance in immunohistochemical diagnosis of Hodgkin's lymphoma.

In the forensic setting, CD15 antibodies are common markers for neutrophils infiltrates as well as LF antibody. Incidentally, different from the latter ones, CD15 had not been tested for post-mortem diagnosis of sepsis before the recent study

**Table 2.** Summary of the 11 studies published for immunohistochemical assays in post-mortem diagnosis of sepsis as cause of death.

| First author | Publication year | Number of cases | Number of controls | Antibody target | Organ samples tested |
|---|---|---|---|---|---|
| Tsokos et al.[2] | 2000 | 6 | 31 | E-selectin | Lung |
| Tsokos[3] | 2001 | 8 | 22 | VLA-4, ICAM-1 | Lung |
| Tsokos[5] | 2001 | 13 | 14 | LF, LZ | Lung |
| Miyashita et al.[7] | 2006 | 9 | 8 | TNFα | Lung |
| Müller et al.[8] | 2008 | 19 | 20 | VE-cadherin, ACE | Lung |
| An et al.[10] | 2009 | 9 | 8 | CCR2, CX3CR1 | Lung |
| Herwig et al.[9] | 2013 | 20 | 41 | VE-cadherin | Lung |
| Maiese et al.[11] | 2017 | 10 | 5 | PCT | Brain, lung, heart, liver, kidney |
| Galassi et al.[4] | 2018 | 56 | 25 | CD15, LF, α-Smooth Muscle Antigen, fibronectin, MMP-9, ICAM-1, Caspase-3 | Heart |
| Maiese et al.[12] | 2019 | 28 | 14 | TREM-1 | Brain, lung, heart, liver, kidney |

VLA-4: very late antigen-4; ICAM-1: intercellular adhesion molecule-1; LF: lactoferrin; LZ: lysozyme; TNFα: tumor necrosis factor alpha; VE-cadherin: vascular endothelial cadherin; ACE: angiotensin-I converting enzyme; CCR2: C-C chemokine receptor type 2; CX3CR1: CX3C chemokine receptor 1; PCT: procalcitonin; CD15: cluster differentiation-15; MMP-9: matrix metallopeptidase-9; TREM-1: triggering receptor expressed on myeloid cells-1.

from Galassi et al.[4] The authors performed the specific immunostaining in the same case-control study on myocardial sections that already mentioned about LF, obtaining almost stackable results. They found no particular advantages in the application of CD15 rather than LF antibodies.

According to these experimental findings, we can consider CD15 as a reliable marker like LF for post-mortem detection of lethal sepsis cases.

### *Triggering receptor expressed on myeloid cells-1*

Triggering receptor expressed on myeloid cells-1 (TREM-1) is a complex protein belonging to the superfamily of immunoglobulins. It is expressed as a transmembrane protein on circulating immune cells (neutrophils and mature monocytes) and recognizes as specific ligand the enhancer binding protein called C/EBPε, which is expressed on myeloid cell line surfaces. The interaction between ligand and receptor is increased by TREM-1 up-regulation, which is induced directly by exposure to bacterial lipopolysaccharides or other microorganism components. During acute infections, TREM-1 is secreted in the soluble form (s-TREM-1) and becomes highly detectable in serum so that it is used as a biomarker for sepsis in the clinical setting.

The same research group which mentioned before about PCT published even more recently a similar immunohistochemical study using s-TREM-1 antibodies.[12] They applied the same approach to larger experimental populations of cases (28) and controls (14); all the sepsis cases had an ascertained ante-mortem clinical diagnosis, and all controls were characterized by a sudden death from natural or violent causes. They obtained similar results as PCT antibodies, detecting a 100% rate of positivity on all cases organs, with either cellular or intravascular pattern or both, compared to 100% negativity in the controls.

The illustrated findings indicate that immunohistochemical assays for s-TREM-1 in sections from multiple organ samples (brain, heart, lung, liver, and kidney) could enable post-mortem diagnosis of sepsis with good sensitivity and specificity (Table 2).

## Discussion

In this article, we reviewed all the 11 published studies about post-mortem diagnosis of sepsis by means of immunohistochemical assays. All the studies used antibodies directed against molecules that are attributed a specific behavior in response to infectious stimuli. All but one used lung tissue as target tissue, selecting ante-mortem diagnosed events such as ARDS in the cases groups. Myocardium has been studied to a lesser extent (3 of 11), while research has rarely been extended to the brain, kidney, or liver (2 of 11). Moreover, the molecules involved are often markers of leukocyte

cells, especially of the myelomonocyte line, which is the case of CD15, LF, LZ, CX3CR1, CCR2, and VLA-4. Other molecules are variously expressed by vascular endothelium and, rarely, by specialized cells (myocardiocytes, hepatocytes, etc.), such as ICAM-1, E-selectin, VE-cadherin, and ACE. The remaining ones are soluble factors that can be found in intravascular lumen or immobilized on cellular surfaces and interstitium, like TNFα, PCT, VEGF, and s-TREM-1. In all cases, these mediators or antigens respond to specific stimuli mediated by systemic cytochemical cascades and circulating cytokines. Furthermore, the most recent studies that have been reviewed use PCT and s-TREM-1 as target molecules, which have become, in recent years, highly sensitive and specific biomarkers for sepsis in clinical practice, because they are able to distinguish secondary inflammatory events at infectious stimuli compared to non-infectious ones.

Even though all the evidences available come from preliminary case-control studies, each one of the highlighted marker could prove useful in confirmation or ruling out a diagnosis of sepsis in the post-mortem examination, especially in the forensic setting. An important comparison, then, should be made between sepsis and other natural causes of death, determining a systemic inflammatory state from a non-infectious origin.

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### ORCID iD

Vittorio Fineschi https://orcid.org/0000-0002-1686-3236

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