Pathology and immunopathology of the lung in sepsis

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Abstract: A correct definition of sepsis is extremely hard due to an increased etiological and pathologic heterogeneity, a large number of medical specialties involved in the detection and treatment, etc. If, in clinical practice there are specific guidelines in diagnosing it in legal medicine the diagnostic is often very difficult to prove as the microbiological results are often misleading, a personal history is not always available, gross and pathological signs are often unspecific. Recently the use of immunohistochemistry and recent advances in tanatochemistry were proven to be able to increase the accuracy in diagnosing the sepsis at the autopsy. This review summarizes a few recent advances in diagnosing the sepsis at the autopsy focused on the lungs, the earliest and most frequently affected organ in sepsis.

Key Words: sepsis immunohistochemistry, sepsis lungs, lungs immunohistochemistry, lung sepsis immunohistochemistry, pulmonary immunopathology in sepsis

A correct definition of sepsis is extremely hard due to an increased etiological and pathologic heterogeneity, a large number of medical specialties involved in the detection and treatment, etc. Classically sepsis was defined by the presence/ the possibility of an infection associated with a generalized inflammatory response (SIRS). SIRS was characterized by the presence of minimum two of the following: temperature above 38°C or below 36°C, heart rate over 90 bpm (or PaCO2< 32 torr), leucocytes over 12000/mm3 or below 4000/mm3, or immature leucocytes over 10%[1]. Recently a panel of experts redefined sepsis and associated syndromes [2-4] as presented below. Infection was defined as a pathological process determined by the invasion of a normally sterile tissue, a body cavity or a body fluid with pathogen or conditional pathogen microorganisms. The main problems with this definition are: (1) infections may appear in normally non-sterile tissues like the colon (e.g. Clostridium difficile colitis), (2) the inflammatory response may be determined not by the microorganism but by the release of an exotoxin, (3) microorganisms can be found in normally sterile tissues without a pathological significance (e.g. asymptomatic bacteremia).

Bacteremia is neither enough nor sufficient to diagnose a septic process; there is transient bacteremia, without clinical consequences or septic processes without positive blood cultures. SIRS represents a clinical-biological syndrome determined by increased plasma cytokine levels as a response to either an infectious or non-infectious process (trauma, burns, acute pancreatitis, vascular ischemia, etc.). In order for SIRS to be positively associated with sepsis a causing infectious agent must be positively identified or a high suspicion must be raised. Sepsis is defined as a clinical syndrome characterized by an abnormally increased host response to an infection.
Severe sepsis is defined as a sepsis associated with organ dysfunction. Septic shock is defined as a septic process associated with persistent arterial hypotension (systolic blood pressure below 90 mmHg or under two standard deviation, or a medial arterial pressure below 60 mmHg, or a decrease in systolic pressure of more than 40 mmHg despite volemic repletion treatment, or (in children) tachycardia associated with signs of perfusion deficit).

The lung is the organ most often affected in sepsis mainly because (1) pneumonia is often the starting point of the septic process, (2) almost every disseminated infectious process is associated with a systemic inflammatory response (SIRS) in which the first organ to be affected is usually the lung and (3) there is an affected gas exchange and altered pulmonary hemodynamics, due to an increased capillary permeability and pulmonary pressure in the first stages of sepsis. Clinically sepsis related lung injury is easily quantifiable using the following panel of parameters: (1) acute onset, (2) bilateral opacities, (3) capillary pulmonary pressure under 18 mmHg (with normal left atrial pressure) and PaO2/FiO2<40 kPa (300mmHg). The presence of these criteria make the clinician able to diagnose an acute lung injury; if PaO2/FiO2 is below 200 mmHg acute respiratory distress syndrome (ARDS) is diagnosed [5-7].

Lungs are often the point of origin for sepsis; Sands, in a study conducted on over 10 million cases, found them to be at the origin of sepsis in 42% of cases[8]. In forensic practice a sepsis of a pulmonary origin is less frequent than in the general population (see the chart below), but it still account for more than 20% of all sepsis cases.

**Sepsis, point of origin**

![Figure 1. Sepsis, point of origin; comparison between the results obtained by Sands [8] and the results obtained in the National Institute of Legal Medicine Romania (personal, unpublished data)](image)

**Pulmonary pathology in sepsis**

Because lungs respond similarly to a wide variety of aggressions the morphological pattern associated with numerous pathological conditions is very similar. The lungs are usually red-bluish and heavy, due to edema, increased number of immune cells and pulmonary stasis secondary to a decrease in cardiac pump function; sectioning the lungs may reveal a discharge of black blood, or pink foam, if acute pulmonary edema is found, or a green-yellow puss (if a localized pulmonary process is present), but usually the discharged fluid is greyish, dirty, caused by excess parenteral hydration (if the patient was hospitalized). Sometimes pulmonary hemorrhages or pleural petechias are found, mainly caused by an associated coagulation disorder.

The histological landmark for ARDS are the diffuse alveolar lesions, with three main stages – exudative, proliferative, and fibrotic. In the exudative phase we can find capillary congestion, interstitial and alveolar edema, focal hemorrhages, neutrophil margination (determined by an increased adhesion between endothelial cells and neutrophils, with subsequent leucostasis, increased vascular diameter and total absence of an intra-alveolar inflammatory reaction, especially in cases of septic shock with a rapid evolution), interstitial inflammation and microvascular thrombi. After 72 hours hyaline membranes can be identified, due to
an increased production of fibrin deposits in the alveolar spaces, leading to the alteration of surfactant secretion and subsequently congestive atelectasis (Figure 2). The proliferative phase is characterized by the cuboidalization of the alveolar epithelium (rapid proliferation of type 2 pneumocytes in denuded epithelium), fibroblast proliferation, squamous metaplasia, thrombi and small peripheral infarcts. In the fibrotic phase are found interstitial fibrosis, sometimes with cystic spaces, alveolar spaces and bronchioles surrounded by fibrotic tissue, leading to a honeycomb appearance. Bronchopneumonia appears often as either a final event, secondary to blood dissemination from the entry point, or as a consequence of mechanic ventilation. Sometimes septic emboli can lead to vascular occlusions with subsequent pulmonary infarction.

**Pulmonary immunopathology in sepsis**

As neither classical histopathology nor postmortem microbiology are sensible enough to identify a septic process with a high level of certainty other methods have been proposed of which immunohistochemistry (sometimes associated with tanatochemistry) seem to be the most promising. In the following paragraphs we will summarize a few interesting researches in this area in sepsis.

C5a is a complement activator with important functions in the initial stages of sepsis by releasing granular enzymes from immune cells, increasing the production of superoxide dismutase in neutrophils, increasing the release of histamine from monocytes, having vasodilator effects, etc. Riedemann[9] found an increased expression of C5a receptor (C5aR) in septic lungs, mostly with an epithelial bronchiolar pattern, associated with positive diffuse immunostaining in liver (hepatocytes and sinusoidal cells), kidney (proximal and distal tubular epithelial cells but not in glomeruli) and heart (diffuse staining of cardiomyocytes, after 12 hours). Moreover plasma C5a levels were proven to be positively associated with increased TNFα and IL6 levels, association correlated with decreased survival [9].

Angiostatin, a proteolitic fragment from plasminogen, is one of the most potent anti-angiogenic proteins; it is produced by macrophages and neutrophils activated by IL-8, fMLP (f-methionin-leucin-prolin) and GROα (Growth Related Oncogene). Angiostatin binds to the surface of ATP-synthase and αβ3 integrin from the surface of endothelial cells inhibiting neutrophil proliferation and migration and inducing endothelial cell apoptosis. In sepsis angiostatin has a strong positive immune reaction in alveolar septa, alveolar macrophages, neutrophils and large vessels [10].

Lactoferrin is an iron binding glycoprotein, found in secondary granules from leucocytes, with important bacteriostatic and bactericide functions [11-13]. In lungs lactoferrin is found in alveolar, interstitial, and intravascular leucocytes and macrophages (the latter often neighboring the vascular endothelium. In sepsis lactoferrin immunopositivity is strong, and homogenous; non septic patients have a weak positive reaction, with a granular distribution in leucocytes and a vacuolar distribution in macrophages. [13] Increased lactoferrin levels are not however sepsis specific, being also found in asthma, chronic bronchitis, lung cancer, tuberculosis, etc. [14-16].

Angiotensin converting enzyme (ACE) is mainly expressed transmembranary in endothelial cells, some epithelial cells and non-epithelial cells, and has a very high concentration in pulmonary endothelium. ACE is known to influence arterial tension, cardiac remodeling, cellular proliferation, coagulation, cellular hypertrophy, etc. Normally the strongest ACE immune expression in lungs is present in alveolar capillaries, followed by arterioles, and arteries; endothelial cells of venules and veins have a very weak reaction; in sepsis ACE expression is significantly reduced in lungs and it was not dependent upon the postmortem interval[17]. Moreover in ARDS reduced levels of ACE are found in plasma and increased levels are found in bronchoalveolar lavage (possible due to alveolar cell damage)[18].

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**Figure 2.** Lungs, ARDS: hyaline membranes, alveolar edema, acute inflammatory reaction. HE 40x
iNOS is an inducible enzyme which catalyzes the synthesis of NO from L-arginin. Arginase II catalyzes the conversion of arginine to L-ornitin and urea, and is present in high concentration in the mammary gland, liver, lungs, and brain. Studies conducted on septic rats revealed an increase of pulmonary levels of iNOS and a decrease (up to zero after 16 hours) of arginase II, the most likely mechanism being competition for substrate [19].

VE-cadherin is an endothelial cell-cell adhesion molecule identifiable exclusively in endothelial intercellular junctions, with a high immunopositivity in arteries, followed by arterioles, and capillaries. In sepsis VE-cadherin staining was reduced in arteries and arterioles, and a slight decrease was noticed when increasing the postmortem interval, but the latter was not statistically significant[17].

αβ3 integrin is a vitronectin and angiostatin receptor normally found in activated endothelial cells in almost all organs, in non-activated endothelial cells in the lungs (where the binding of vitronectin leads to an increase in vascular permeability), in neutrophils (increasing their motility), monocytes (increasing their adhesivity to activated endothelial cells), alveolar epithelium and great pulmonary vessels endothelium(subunit α only).

In sepsis αβ3 integrin expression increases significantly in epithelial bronchial cells, alveolar epithelial cells and alveolar macrophages (and not in septal macrophages)[18].

VEGF is a multipurpose cytokine mainly synthetized by endothelial cells and having important functions in angiogenesis and vascular permeability. It is normally found in lungs in alveolar epithelium, bronchial epithelium, alveolar macrophages, and smooth muscle cells from small vessels. In sepsis in glandular cells and epithelial alveolar cells the immunoreactivity is significantly decreased, most likely due to mechanical ventilation (increased oxygen levels lead to an inhibition of VEGF gene transcription) [20]. On the other side, in macrophages, smooth muscle cells and activated pericytes stimulated by LPS have an increased VEGF immunopositivity and increased mRNA levels (they are starting to increase two hours after LPS stimulation) [21].

E-selectin is only present on the surface of endothelial cells if they are activated by the presence of IL-1β, TNFα or LPS. During inflammation E-selectin is important especially for recruiting leucocyte in affected areas. Leucocytes have a low-affinity ligand for E-selectin; when it is expressed it generates a smooth rolling of the leucocytes on the endothelial surface; when the leucocytes became activated the bonds become tighter by upregulating CD1b and neutrophilic ligand for ICAM-1. E-selectin must be synthetized de novo; therefore its expression in sepsis only appears a few hours after endothelial activation [22-25]. Tsokos et al [25] found increased E-selectin levels in the lungs of septic patients. The reaction was homogenous positive in all pulmonary lobes, in endothelial cells, arterioles, arteries, capillaries, postcapillary venules and pulmonary veins, in 1-4 days after death. In the control group only one weak positive reaction was found in a 83 years old patient died from drowning. Plasma E-selectin was also found to be increased in septic patients, especially in Gram negative sepsis [26]. Unlike endothelial E-selectin which has roles in favoring leucocyte adherence to endothelial cells, plasma E-selectin decreases leucocyte adherence by occupying its binding sites on the leucocyte surface. Plasma E-selectin has highly increased values in sepsis when associated with disseminated intravascular coagulation (DIC) and is a more sensitive marker for endothelial activation than TNFα or IL6[27]. Plasma values of E-selectin in sepsis with DIC are 114.6 ± 77.9 ng/ml and in sepsis without DIC are 54.5 ± 53.1 ng/ml[27].

VLA-4 (very late antigen – 4, CD49d/CD29) a surface protein found on monocytes, eosinophils, basophils, and lymphocytes, binds to fibronectin and VCAM-1. In sepsis VCAM-1 has a strong positive reaction in intravascular, interstitial and intraalveolar leucocytes, reaction which is homogenous and independent upon the postmortem interval. In non-septic patients only a slightly positive immune reaction is found within the interstitium [28].

ICAM-1 (CD54) is a surface antigen with a low expression on the pulmonary endothelium, lymphocytes, neutrophils and macrophages. A recent study revealed that an increased ICAM-1 expression in pulmonary microcirculation is associated with a 114% increase in neutrophil-endothelium adhesivity. Various studies showed the utility of ICAM-1 and sICAM-1 in sepsis (1) as an indicator of ARDS[29], as an indicator for severe evolution of sepsis in both adults and children (sICAM-1) [30], in identifying the patients at risk for developing a hepatic dysfunction in sepsis[31], as an indicator for DIC in politrauma patients (sICAM-1), etc. In septic patients ICAM-1 is positive on endothelial cells from pulmonary arteries, arterioles, alveolar capillaries, postcapillary venules, veins, and also on macrophages and lymphocytes; the reaction is homogenous, independent upon the postmortem interval[28].
References


Figure 3. VE-cadherin immunostaining in (a) regular lung tissue (with strong expression in arterioles and capillaries), and (b) in septic lung, 48 hours after death, revealing a faint expression in arterioles and capillaries (after Muller et al, with permission[17])