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Review

Histological changes in neonatal sepsis

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Proceedings

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The role of the clinical pathological dialogue in problem solving
Guest Editors: Gavino Faa, Vassilios Fanos, Peter Van Eyken

Abstract

One of the most significant causes of neonatal morbidity and mortality is represented by neonatal sepsis that often manifests itself as a systemic inflammatory response syndrome (SIRS). The progression of SIRS usually leads to multiple organ dysfunction, occasionally culminating in multiple organ failure (MOF).

The loss of endothelial barrier represents the unifying lesion of multiple organs in newborns affected by sepsis and the most important pathological change responsible for the evolution toward MOF in neonates.

The aim of this study is to present the most important pathological changes occurring in neonatal sepsis.

Keywords

Neonatal sepsis, MOF, endothelial damage, loss of podocytes, endothelial apoptosis.

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Introduction

One of the most significant causes of neonatal morbidity and mortality is the neonatal sepsis. Sepsis can be defined as a generalized inflammatory response of the organism and often manifests itself as a systemic inflammatory response syndrome (SIRS) [1]. The progression of SIRS usually leads to multiple organ dysfunction culminating in multiple organ failure (MOF) [2].

Neonatal sepsis has been categorized into two groups: early onset if it occurs within 3-6 days, and late onset after 4-7 days [3].

This pathology is due to a bacterial or fungal infection acquired during the perinatal period.

Many pathogens have been associated with sepsis in neonatal period: the most important cause of neonatal sepsis is the Group B *Streptococci* (GBS) [4], while *E. coli* is the most common cause of mortality. Current efforts toward maternal intrapartum antimicrobial prophylaxis have significantly reduced the rates of GBS disease but have been associated with increased rates of Gram-negative infections, expecially in very low birth weight infants [5]. Other pathogens of neonatal sepsis are *L. monocytogenes* and *S. aureus*. The birth canal, localized between the uterus and the outside of the vagina, harbours a polymicrobial community [6]. Next to nonpathogenic species, such as *Lactobacillus spp.*, potentially pathogenic bacteria including those previously mentioned are found in vagina in 1 out of 5 women. During birth, the aspiration by the neonate of bacteria is considered a major cause of neonatal sepsis in the first three to seven days of life (early-onset sepsis). Subsequent to aspiration, bacteria like GBS, can proliferate to striking densities in the neonatal lung, as shown in newborn primates with GBS pneumonia [7]. The antimicrobial quality of the local pulmonary environment, such as the concentration of surfactant, may be important for the metabolic activity in the bacterial community and therefore for the expression of bacterial virulence factors such as bacterial toxins [8].

Sepsis causes about a million neonatal deaths annually [9, 10]. Sepsis is a potentially fatal whole-body inflammation (a SIRS) caused by severe infection [11, 12]. Severe sepsis is sepsis complicated by organ dysfunction.

Septic shock is sepsis complicated by a high lactate level or by shock that does not improve after fluid resuscitation [13]. Bacteremia is the presence of viable bacteria in the blood. The term septicemia, the presence of microorganisms or their toxins in the blood, is no longer used by the consensus committee [12].

Endothelial dysfunction: a key element

The endothelium is a major target of sepsis-induced events and endothelial cell damage accounts for much of the pathology of septic-shock [1].

Vascular endothelial cells are among the first cells in the body that come into contact with circulating bacterial molecules. Endothelial cells have mechanisms that recognize structural patterns of bacterial pathogens and subsequently initiate the expression of inflammation mediators [14].

In sepsis and septic shock a series of immunological events alter endothelial function in the macrocirculation and microcirculation. Endothelial nuclear swelling, detachment, apoptosis, and complete loss of the endothelial barrier are the main pathological changes that are observed in blood vessels [15].

The disorders of the normal function of the endothelium include derangement of the vascular tone, increase of endothelium permeability, activation of the endothelial cells, production of various regulators and disorders of coagulation. Nitric oxide (NO) is the modulator that mediates the action of most vasodilators. The overproduction of NO during sepsis is possibly the most important cause of the vasopressor-resistant hypotension which characterizes septic shock [16] (**Fig. 1**). The endothelium is activated by the binding of LPS with Toll-like receptors or by binding of inflammatory mediators with various receptors. Other environmental factors such as hypoxia, the ipoperfusion, increasing temperature, acidosis and alterations in glycemia can affect endothelial function. The activation of endothelial cells involves the expression on their surface of adhesion molecules as VCAM-1 and ICAM-1 and the E-selectine that mediate the migration of leukocytes in inflammed tissues.

The interaction of these factors with their receptors activates signaling pathways downstream, influencing the transcription factors and altering cell function and/or gene expression. The cell adhesion

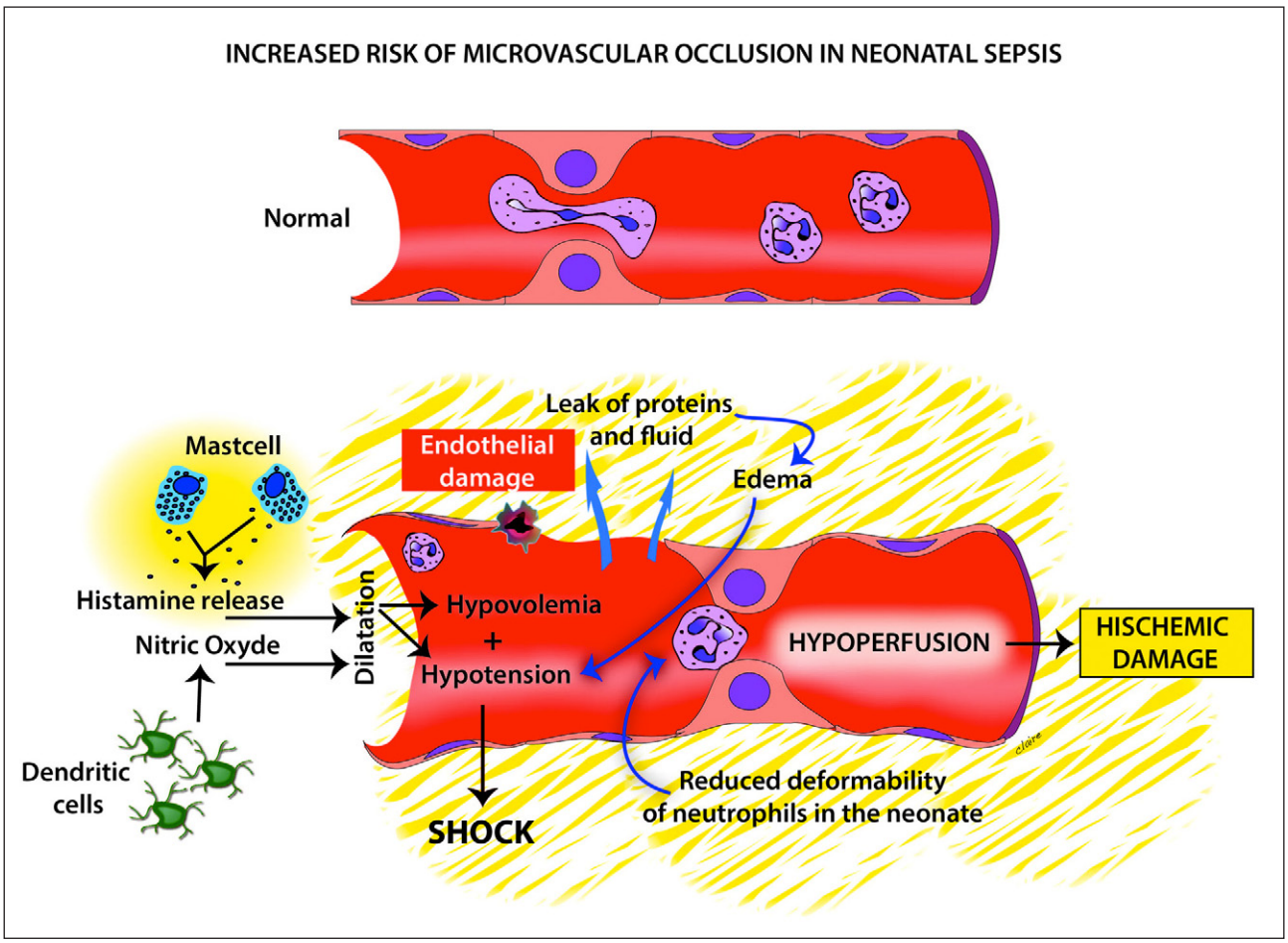

Figure 1. Endothelial damage, causing increase of permeability, activation of endothelial cells, and the overproduction of nitric oxyde during sepsis, might be the most important cause of hypotension which characterizes septic shock.

molecules undergo an up-regulation and this fact results in an increase in the rolling, adhesion and transmigration of leukocytes, a process that is increased by cytokines and cell recruitment. The activation of the coagulation cascade, as a result of endothelial damage, leading to the production of thrombin and therefore to the formation of fibrin [17]. However the activation of the coagulation may be result in the appearance of focal ischemia [18, 19].

Pathological features in sepsis

Endothelium

Vascular pathological changes were mainly detected in capillaries and, in general, in small vessels, but endothelial lesions were also found in medium-sized arteries and veins. Endothelial damage is characterized by nuclear swelling, detachment, apoptosis, and complete loss of the endothelial barrier: these endothelial lesions are the main pathological changes that are observed in blood vessels often associated with adhesion of inflammatory cells and micro thrombosis (**Fig. 2** and **Fig. 3**).

Kidney

The first aspect that has been considered is represented by the endothelial damage, characterized by endothelial swelling, detachment and apoptosis. Following the activation of the inflammatory cascade and coagulation, we can observe congestion, pieces of fibrin in the vascular lumen and thrombosis. In a untreated and a more advanced stage of sepsis we can observe a common blood extravasation into the interstitium, caused by the rupture of the vessel wall (**Fig. 4**, **Fig. 5**, **Fig. 6**). Glomeruli presented loss of podocytes and this fact causes the adhesion of the glomerular hank at the capsule. We can observe also acute tubular necrosis

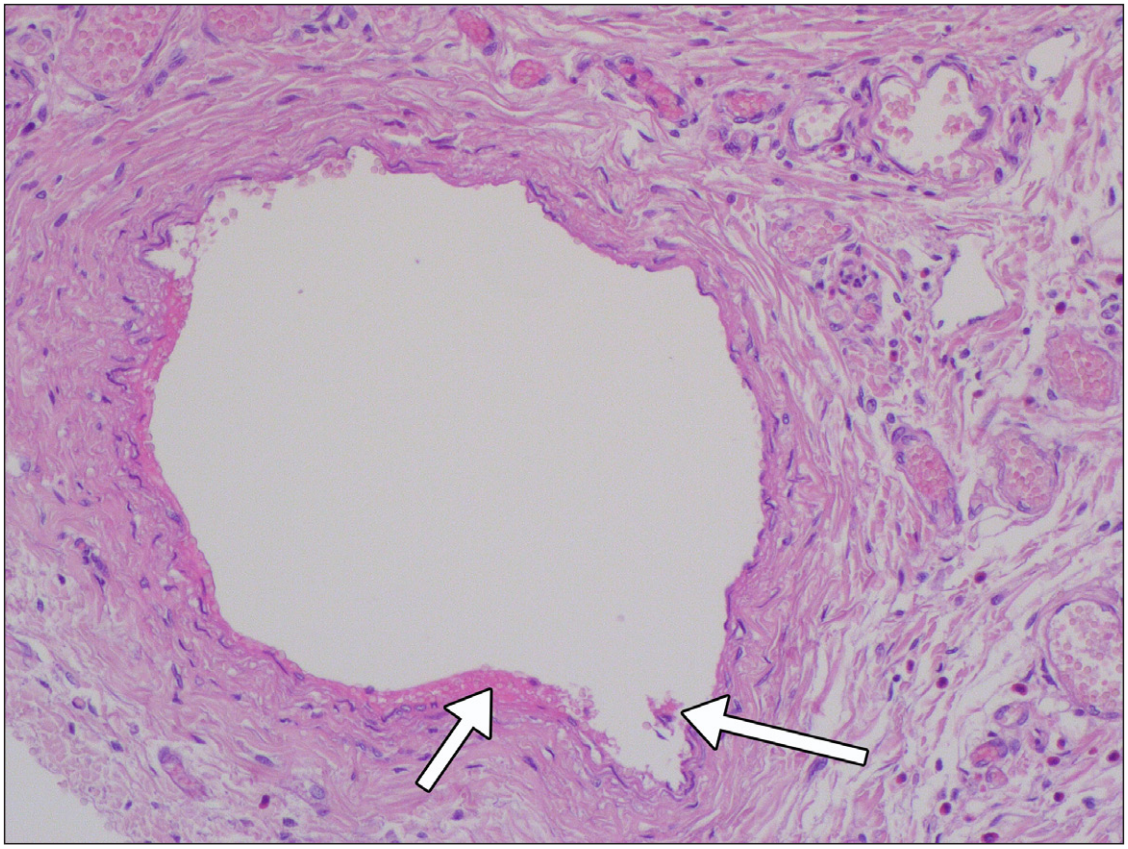

Figure 2. Pieces of fibrin into coronary artery (arrows). Eosinophil granulocytes are scattered in the interstitium.

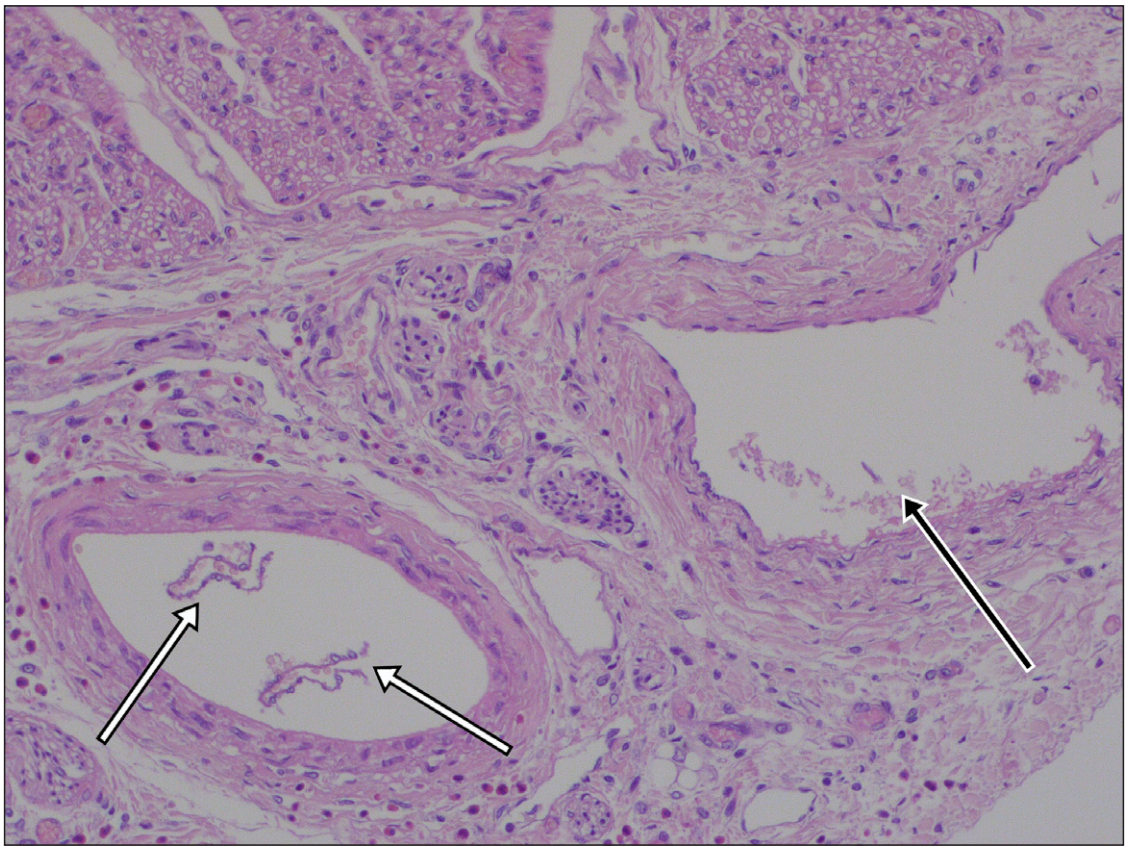

Figure 3. Two coronary vessels showing endothelial detachment (white arrows) and pieces of fibrin (black arrow).

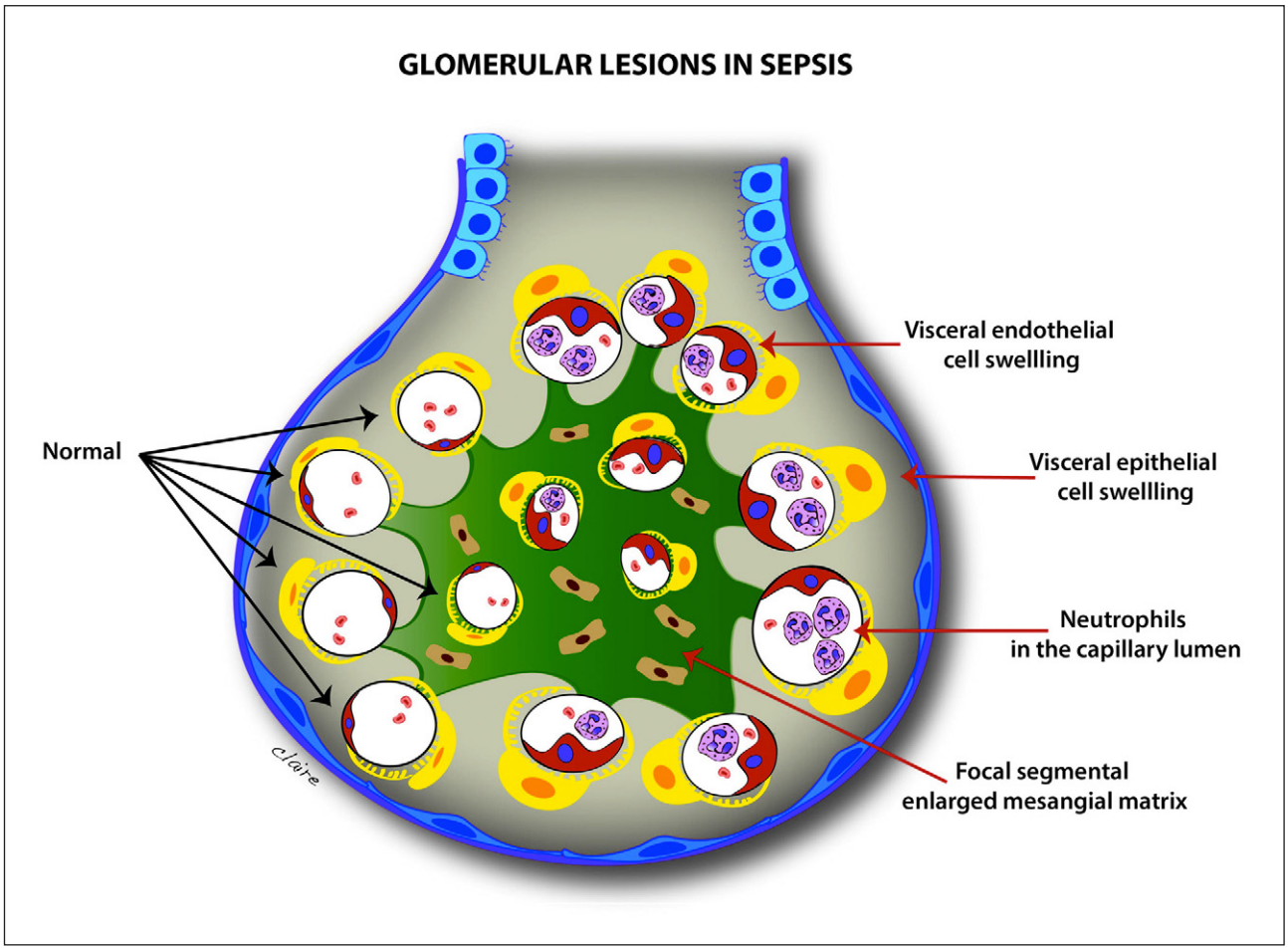

Figure 4. During sepsis glomeruli might show many pathological changes: endothelial and epithelial swelling, presence of neutrophils in the capillaries and focal segmental enlarged mesangial matrix.

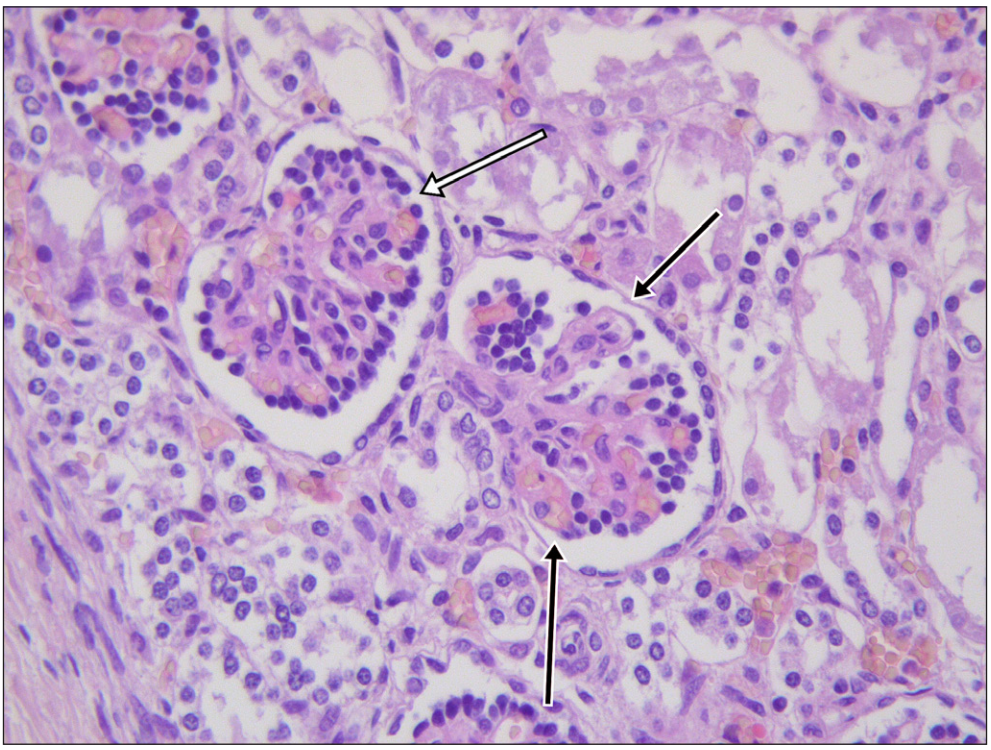

Figure 5. Glomerulus with standard podocyte number (white arrow). Glomerulus with focal loss of podocytes (black arrows).

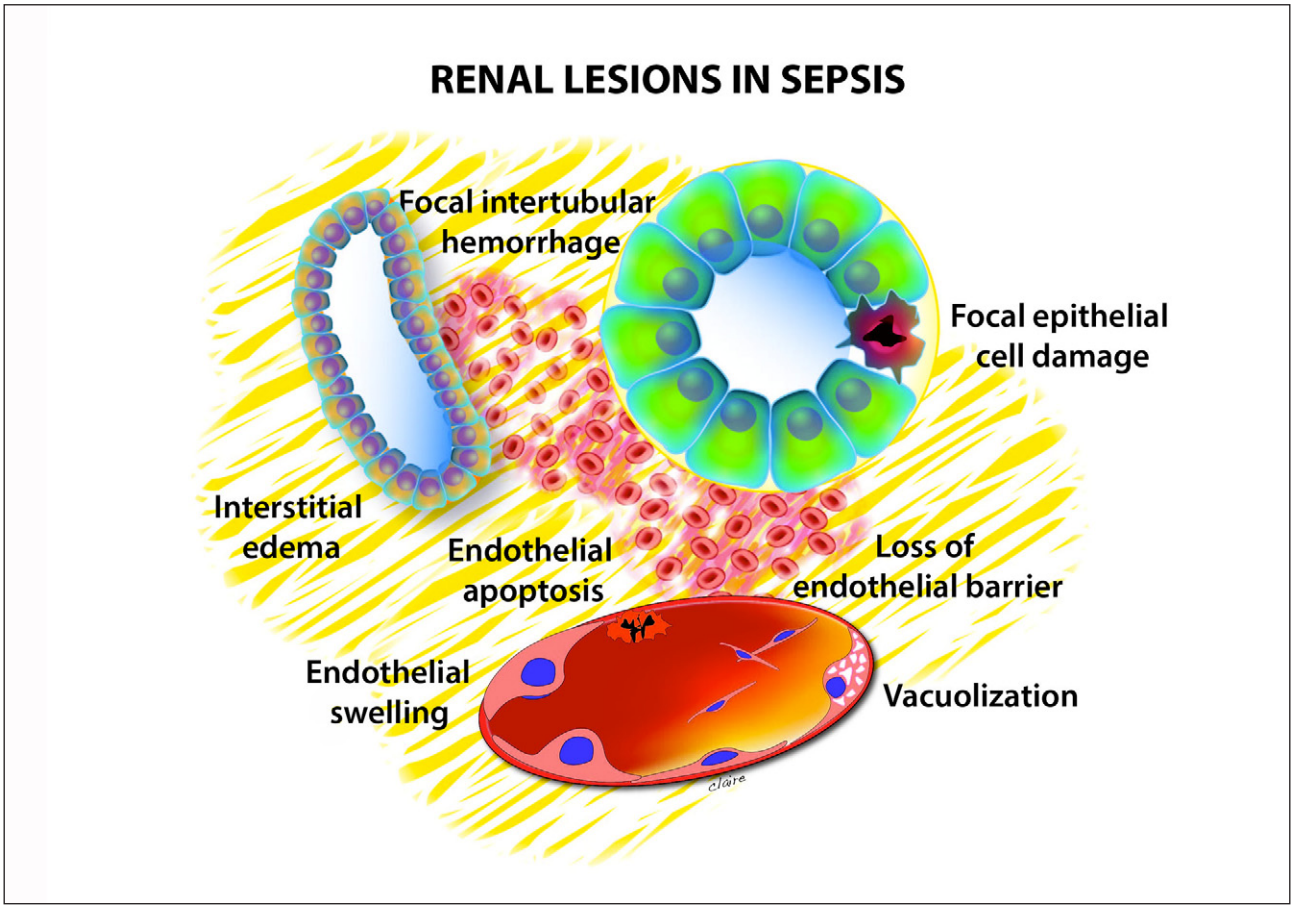

Figure 6. During sepsis endothelial damage causes the loss of the endothelial barrier and the exit of serum in the interstitium (interstitial edema), accordingly. The endothelial changes causes hypoxia, responsible for the acute tubular necrosis of the proximal tubules.

of the proximal tubules and, in an advanced stage of sepsis, a blood extravasation into the interstitium.

Lung

Next to the endothelial damage, we may observe alveolar edema, focal presence of granulocytes and erythrocytes within the alveolar spaces, such as an alveolar pneumonia. Apoptosis of the superficial respiratory epithelium is a consequence of blood vessels damage, represented by endothelial loss, and congestion (**Fig. 7**, **Fig. 8**, **Fig. 9**).

Heart

Cardiomyocytes show intracellular edema with vacuolization of the cytoplasm. Intercellular edema is also present. We may observe eosinophilic globules of cardiomyocytes apoptosis and endothelial damage of the coronary arteries and veins that occurs with endothelial swelling, detachment and thrombosis. Cardiomyocytes might showing focal wave, direct consequence of acute hypoxia or going to myocytolysis (**Fig. 10** and **Fig. 11**).

Complications

MOF has been defined as a syndrome consisting of the sequential failure of two or more organ systems in patients with clinical signs of sepsis. Respiratory dysfunction usually is the first apparent organ failure. The other organ systems involved are the hepatic, renal, cardiovascular, nervous, hematologic and gastrointestinal system [20, 21]. There are two main mechanisms involved in sepsis: the arterial vasodilation and inotropic cardiac dysfunction [22]. Regarding the arterial vasodilation during sepsis are produced large quantities of substances, including vasoconstrictor and vasodilator agents, however, the body breaks a resistance to the action of the vasoconstrictor substance. Among the substances vasodilatory stands out the role of NO, whose production is increased by cytokines released during the septic episode; NO is capable of causing

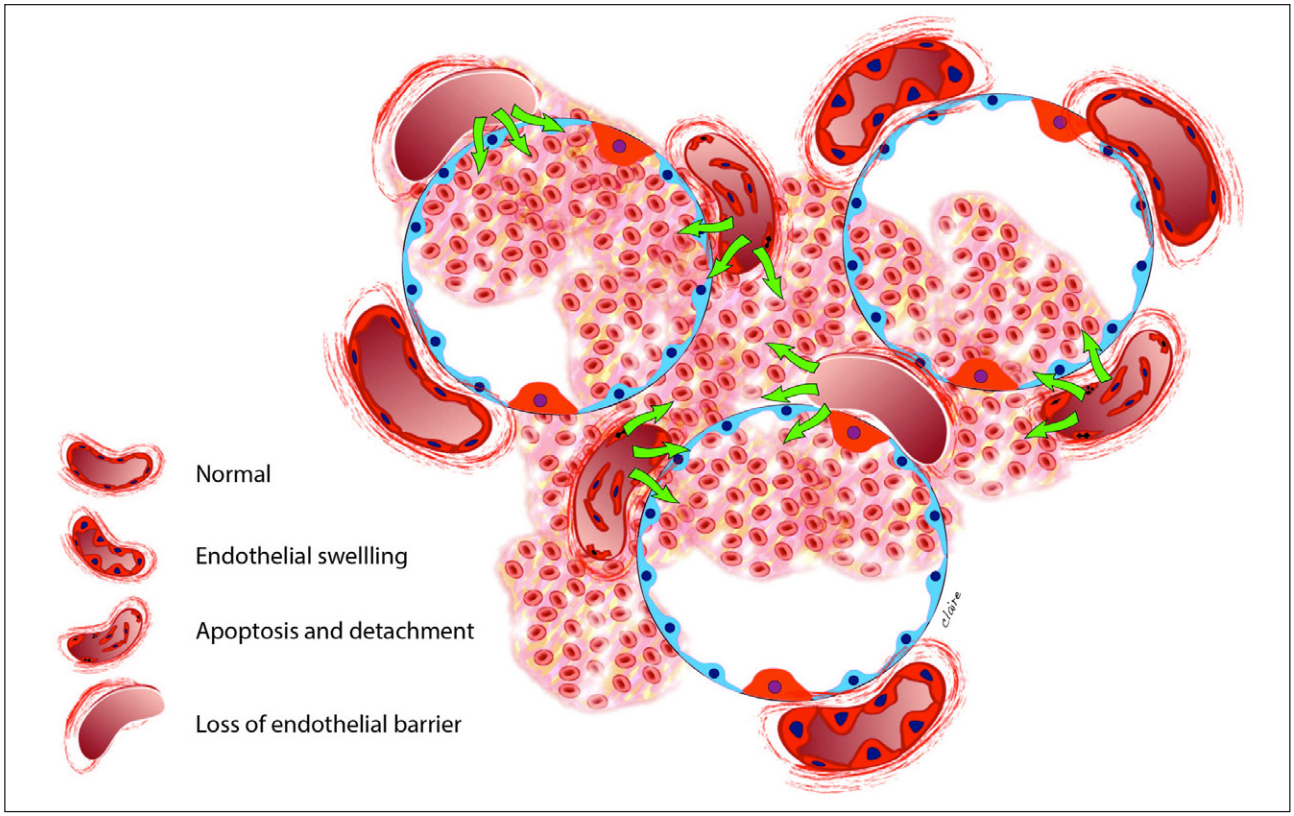

Figure 7. During sepsis the loss of endothelial barrier causes the transition of blood from vessel lumen into alveolar spaces.

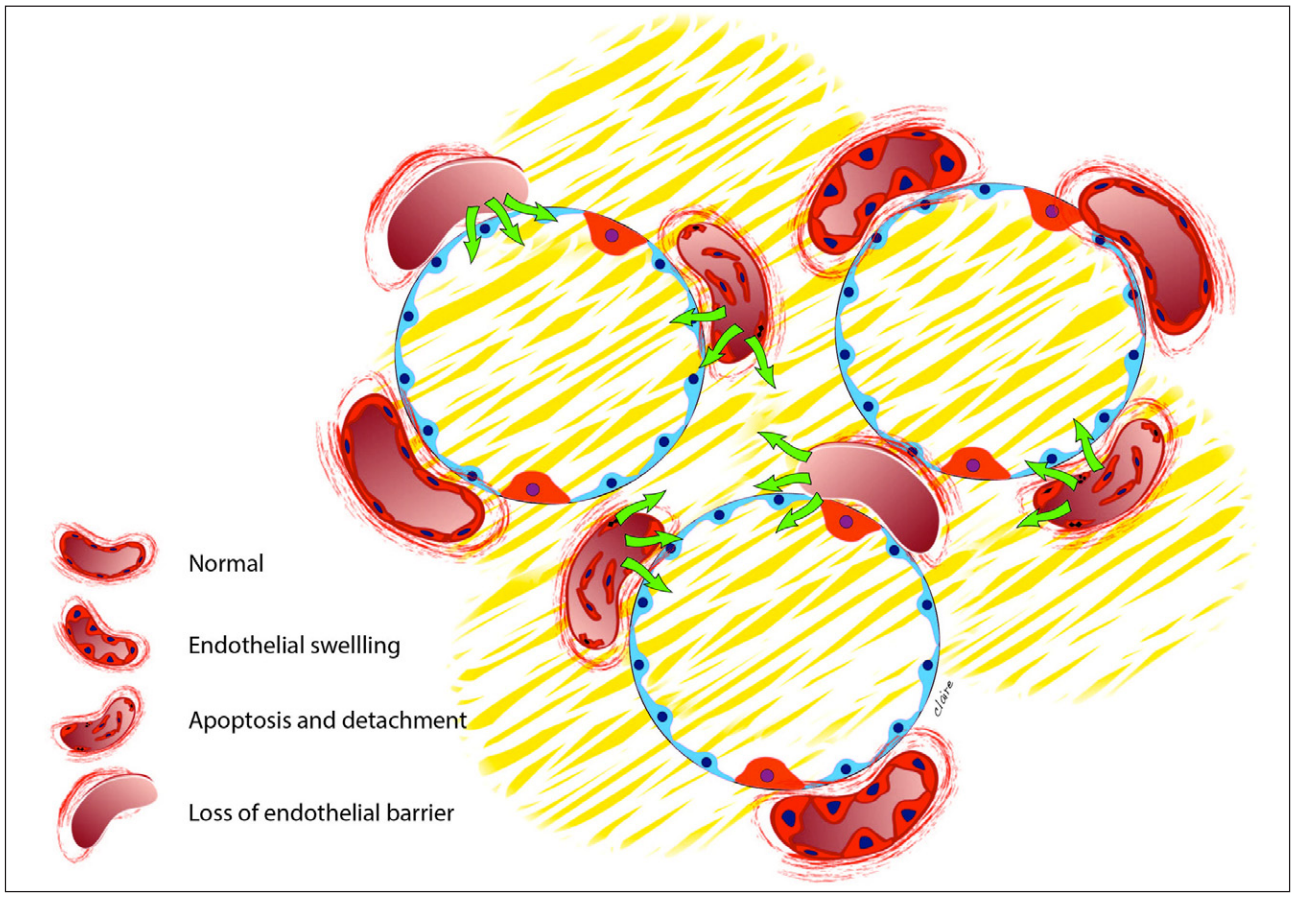

Figure 8. During sepsis the loss of endothelial barrier is responsible for interstitial and alveolar edema.

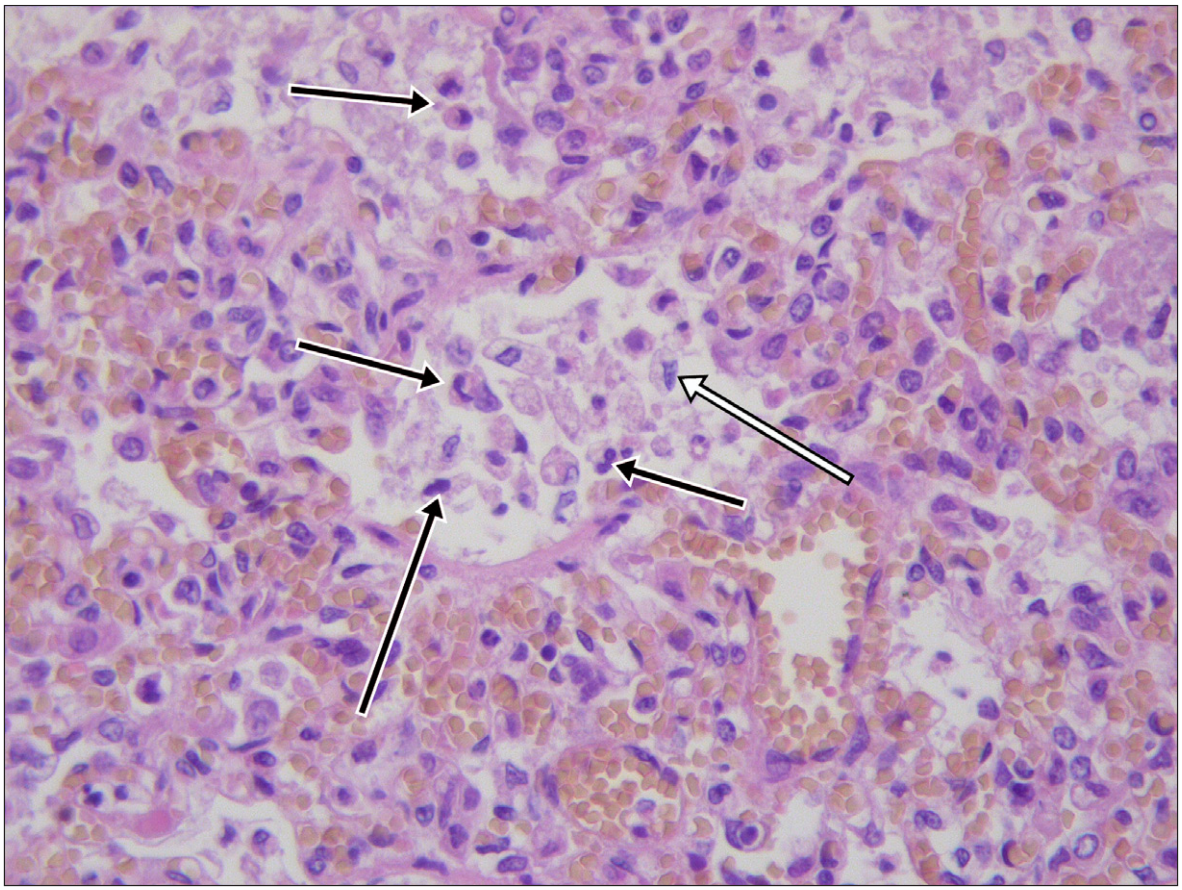

Figure 9. During sepsis, endothelial damage is responsible for alveolar edema, focal presence of granulocytes and erythrocytes within the alveolar spaces (black arrows) and loss of respiratory epithelial cells (white arrow).

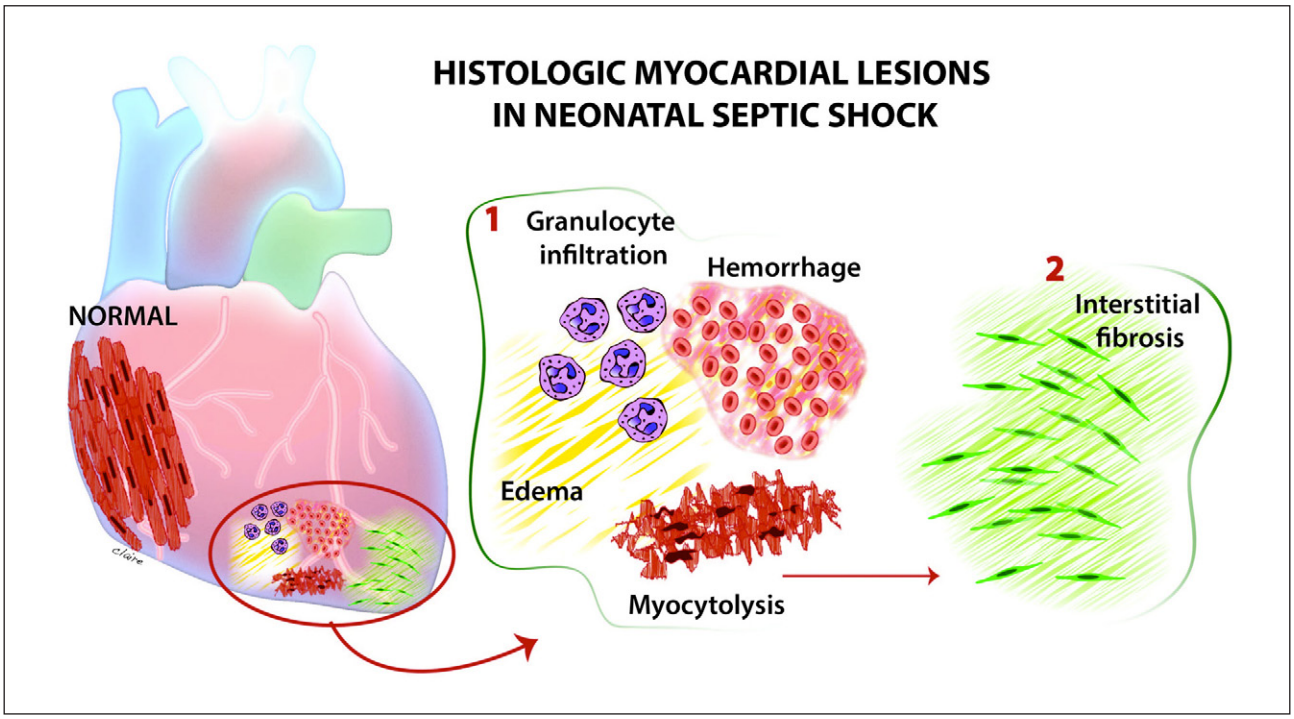

Figure 10. During sepsis hypoxia caused by endothelial damage and thrombosis is responsible for intercellular and intracellular edema, granulocyte infiltration in the interstitium, hemorrhage, focal wave arrangement of cardiomyocytes, myocytolysis that causes later interstitial fibrosis.

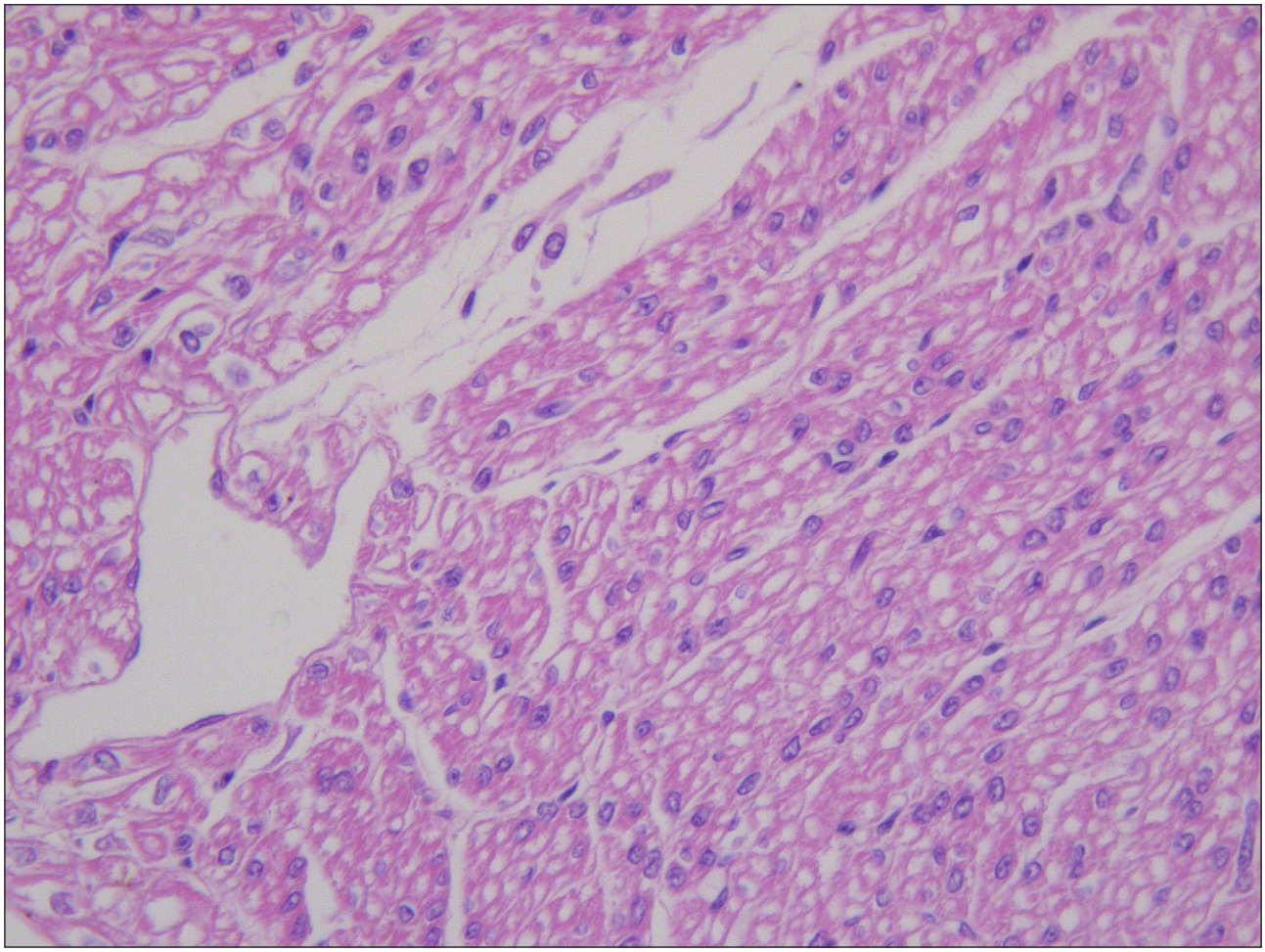

Figure 11. During sepsis cardiomyocytes show intracellular edema with vacuolization of the cytoplasm. Intercellular edema is also present.

significant systemic arterial vasodilation resistant to the action of norepinephrine and angiotensin II. Moreover, other activated mechanisms that maintain vasodilation: the increased plasma concentration of hydrogen ions and lactate, the depletion of ATP in the cells of the vascular tunica muscularis and the activation of ATP-sensitive potassium channels. It follows that potassium ions continuously entry into the cell, closing calcium channels. This mechanism inhibits the vasoconstrictive action of norepinephrine and angiotensin II which are strictly dependent from opening of calcium channels. The severe reduction in peripheral vascular resistance causes a severe discrepancy between the abnormal vascular capacitance and the decrease in arterial filling [23]. The myocardial depression in septic patients can be referred to the action of proinflammatory cytokines, especially TNF-alpha and IL-1 beta. In fact, myocardial function improves after the use of antibodies directed against TNF-alpha [24]. During sepsis, the kidneys are damaged and as a result is manifested acute renal failure caused by the action of inflammatory cytokines. The synthesis of TNF, produced by renal mesangial and endothelial cells, is responsible for glomerular apoptosis. Another mediator of renal damage is the PAF, produced by endothelial cells and mesangial cells. PAF and endothelin are vasoconstrictors that increase the afferent and efferent arteriolar resistance, followed by the reduction of glomerular plasma flow, perfusion pressure which is associated with a reduction glomerular permeability. Organ damage is aggravated by thrombi, secondaries to the coagulation cascade. During sepsis, kidneys are damaged and manifested acute renal failure caused by the action of the hemodynamic failure and inflammatory cytokines [25]. Pulmonary lesions are represented by adult respiratory distress syndrome (ARDS), that is characterized by an increased permeability of the alveolar-capillary membrane. This is due to alteration of endothelial and alveolar epithelial barrier that allows the passage of plasma,

erythrocytes and polymorphonuclear cells in the interstitium and within the alveolar spaces. The damage of the endothelium and alveolar epithelial cells activates the production of inflammatory cytokines and the expression of adhesion molecules in neutrophil and favoures their chemotaxis and diapedesis into the interstitium. Many events caused lung damage, as the activation of the complement, coagulation with microvascular embolization, degranulation of neutrophils with release of proteolytic enzymes and local production of endoperoxides. All these factors are responsible for interstitial edema and the passage of fluid into the alveoli [26]. The alveolar edema and the activity of the proteolytic enzymes of neutrophils damage the alveolar epithelial cells type 1 with secondary reduction of alveolar surfactant. This events alter the exchange of oxygen (hypoxia and hypercapnia) with the clinical picture of severe respiratory failure [27].

Conclusions

The analysis of the pathological data observed in this newborn affected by neonatal sepsis allows us to suggest a simple scheme for a complete histopathological examination in cases of neonatal sepsis (**Tab. 1**). Endothelial damage – represented by nuclear swelling, detachment, apoptosis, and complete loss of the endothelial barrier – represents the most important feature in the pathological analysis of the organs. Perivascular interstitial edema and microthrombosis may be considered the typical consequences of the endothelial damage. The finding of inflammatory cells in the alveolar spaces is the typical lesion indicating sepsis as the common cause of the observed pathological changes. Acute tubular necrosis of proximal tubules and loss of podocytes (acute podocytopathy) represent the typical renal lesions in newborn affected by sepsis. Wave arrangement of cardiomyocytes and vacuolization of their cytoplasm are, according with our data, the typical lesions of the newborn heart.

In conclusion our data suggest that the loss of the endothelial barrier represents the most important feature in neonatal sepsis and the unifying lesion affecting multiple organs and systems causing the evolution toward MOF.

Declaration of interest

The Authors declare that there is no conflict of interest.

References

1. Morrison DC, Ulevitch RJ. The effects of bacterial endotoxins on host mediation systems. A review. Am J Pathol. 1978;93(2): 526-617.
2. Beal AL, Cerra FB. Multiple organ failure syndrome in the 1990s. Systemic inflammatory response and organ dysfunction. JAMA. 1994;19;271(3):226-33.
3. Fanos V, Caboni P, Corsello G, Stronati M, Gazzolo D, Noto A, Dessì A, Giuffrè M, Lacerenza S, Serraino F, Garofoli F, Serpero LD, Liori B, Carboni R, Atzori L. Urinary (1)H-NMR and GC-MS metabolomics predicts early and late onset neonatal sepsis. Early Hum Dev. 2014;90(Suppl 1)1:S78-83.

Table 1. Check list of histopathological features of neonatal sepsis.

Endothelium of blood vessel of small-medium size	• Endothelial damage: nuclear swelling, detachment, apoptosis • Perivascular edema • Congestion • Intravascular coagulation • Thrombosis
Kidney	• Endothelial damage • Interstitial edema • Blood extravasation into the interstitium • Glomerular damage • Loss of podocytes • Acute tubular necrosis of proximal tubules
Lung	• Endothelial damage • Alveolar damage • Neutrophil leukocytes, erythrocytes and edema into alveolar spaces • Apoptosis of respiratory epithelium
Heart	• Endothelial damage of coronary arteries and veins • Intercellular edema • Thrombosis • Cardiomyocytes lesions • Cytoplasmic vacuolization (intracellular edema) • Focal wave arrangement

4. Schröder H, Paust H.Group B streptococci: the most common cause of neonatal septicemia. Monatsschr Kinderheilkd. 1979;127(12):720-3.
5. Simonsen KA, Anderson Berry AL, Delair SF, Davies HD. Early-onset neonatal sepsis. 2014;27(1):21-47.
6. Verstraelen H, Swidsinski A. The biofilm in bacterial vaginosis:impilications for epidemiology, diagnosis and treatment. Curr Opin Infect Dis. 2013;26(1):86-9.
7. Rubens CE, Raff HV, Jackson JC, Chi EY, Bielitzki JT, Hillier SL. Pathophysiology and histopathology of group B streptococcal sepsis in Manaca nemestrina primates induced after intramniotic inoculation: evidence for bacterial cellular invasion. J Infect Dis. 1991;164(2):320-30.
8. Herting E, Gan X, Rauprich P, Jarstrand C, Robertson B. Combined treatment with surfactant and specific immuno-globulin reduces bacterial proliferation in experimental neonatal group B streptococcal pneumonia. Am J Respir Crit Care Med. 1999;159(6):1862-7.
9. Black RE, Cousens S, Johnson HL, Lawn JE, Rudan I, Bassani DG, Jha P, Campbell H, Walker CF, Cibulskis R, Eisele T, Liu L, Mathers C; Child Health Epidemiology Reference Group of WHO and UNICEF. Global, regional, and national causes of child mortality in 2008: a systematic analysis. Lancet. 2010;375(9730):1969-87.
10. Dellinger RP, Carlet JM, Masur H, Gerlach H, Calandra T, Cohen J, Gea-Banacloche J, Keh D, Marshall JC, Parker MM, Ramsay G, Zimmerman JL, Vincent JL, Levy MM; Surviving Sepsis Campaign Management Guidelines Committee. Surviving Sepsis Campaign guidelines for management of severe sepsis and septic shock. Crit Care Med. 2004;32(3):858-73.
11. Levy MM, Fink MP, Marshall JC, Abraham E, Angus D, Cook D, Cohen J, Opal SM, Vincent JL, Ramsay G; SCCM/ESICM/ACCP/ATS/SIS. 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. Crit Care Med. 2003;31(4):1250-6.
12. Bone RC, Balk RA, Cerra FB, Dellinger RP, Fein AM, Knaus WA, Schein RM, Sibbald WJ. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. Chest. 1992;101(6):1644-55.
13. Angus DC, van der Poll T. Severe Sepsis and Septic Shock. N Engl J Med. 2013;369(9):840-51.
14. Schmittinger CA, Dünser MW, Torgersen C, Luckner G, Lorenz I, Schmid S, Joannidis M, Moser P, Hasibeder WR, Halabi M, Steger CM. Histologic pathologies of the myocardium in septic shock: a prospective observational study. Shock. 2013;39(4):329-35.
15. Henneke P, Golenbock DT. Innate immune recognition of lipopolysaccharide by endothelial cells. Crit Care Med. 2002;30(5 Suppl):S207-13.
16. Beck GC, Rafat N, Yard B, Hanusch C. [The role of endothelial progenitor cells in sepsis]. [Article in German]. Anaesthesist. 2007;56(5):423-8.
17. Kotsovolis G, Kallaras Hippokratia K . The role of endothelium and endogenous vasoactive substances in sepsis. 2010;14(2): 88-93.
18. Drexler H. Nitric oxide synthases in the failing human heart: a doubled-edged sword? Circulation. 1999;99(23):2972-5.
19. Vallet B. Bench-to-bedside review: endothelial cell dysfunction in severe sepsis: a role in organ dysfunction? Crit Care. 2003;7(2):130-8.
20. Aird WC. The role of the endothelium in severe sepsis and multiple organ dysfunction syndrome. Blood. 2003; 15;101(10):3765-77.
21. Goris RJA. Sepsis and multiple organ failure: The result of whole body inflammation. In: Faist F, Meakins JL, Schildberg FW (Eds.). Host Defense Dysfunction in Trauma, Shock and Sepsis. Berlin: Springer Berlin Heidelberg, 1993, pp. 161-170.
22. Faa G, Fanni D, Gerosa C, Nemolato S, Faa A, Obinu E, Puxeddu E, Fraschini M, Iacovidou N, Zaffanello M, Fanos V. Multiple organ failure syndrome in the newborn: morphological and immunohistochemical data. J Matern Fetal Neonatal Med. 2012;25(Suppl 5):68-71.
23. Parrillo JE, Parker MM, Natanson C, Suffredini AF, Danner RL, Cunnion RE, Ognibene FP. Septic shock in humans. Advances in the understanding of pathogenesis, cardiovascular dysfunction, and therapy. Ann Intern Med. 1990;113(3):227-42.
24. Schrier RW, Abraham WT. Hormones and hemodynamics in heart failure. N Engl J Med. 1999;341(8):577-85.
25. Sessa WC. The nitric oxide synthase family of proteins. J Vasc Res. 1994 ;31(3):131-43.
26. Schor N. Acute renal failure and the sepsis syndrome. Kidney Int. 2002;61(2):764-76.
27. Fink MP, Delude RL. Epithelial barrier dysfunction: a unifying theme to explain the pathogenesis of multiple organ dysfunction at the cellular level. Crit Care Clin. 2005;21(2):177-96.
28. Gattinoni L, Chiumello D, Cressoni M, Valenza F. Pulmonary computed tomography and adult respiratory distress syndrome. Swiss Med Wkly. 2005;135(11-12):169-74.