VILNIUS UNIVERSITY
FACULTY OF MEDICINE

Final Thesis
May 2022

Cellular Hibernation and Immunoparalysis in the Context of Sepsis Induced Organ Dysfunction

Institute of Clinical Medicine, Clinic of Anesthesiology and Intensive Care

Written by:	Svea Wegener, VI Year, Group IV Born 21.08.1997 in Wedel, Germany
Supervisor:	Prof. (HP) Dr. Jurate Sipylaite
The Head of Department/Clinic:	Prof. (HP) Dr. Jurate Sipylaite

Dedicated to my beloved father.

Index

1. Abstract

Sepsis remains the leading cause of death in the intensive care unit. The limited effectivity of current therapies suggests that more research targeting the complex pathophysiologic processes of sepsis is essential to establish a comprehensive treatment with significant outcome improvement. This thesis is aimed to describe the counterintuitive pathophysiology of sepsis while focusing on the immunologic course of sepsis, the effects of cytokine storm, the cellular hibernation response to stress and their implication in organ dysfunction, the state of immune cell paralysis and potential treatment options.

2. Keywords

Sepsis, MODS, Cytokine Storm, Cellular Hibernation, Immunoparalysis, Immunotherapy

3. Introduction

Sepsis is defined as a dysregulated host immune response to infection, which can lead to life-threatening organ dysfunction (1).

The epidemiologic relevance was presented in 2020 by the World Health Organization in the "Global Report on the Epidemiology and Burden of Sepsis (2020)", stating that Sepsis accounts for approximately 20% of overall deaths worldwide and the mortality rate of septic patients is approximately 20%, while for those requiring treatment in the Intensive Care Unit it is up to 42%.

Research of the last decades changed the concept of systemic infection from pathogen centered to a mixed pathophysiology, in which the host response to infection plays a major role and lead to the first consensual definition of sepsis as "Systemic response to an infection" in 1992, diagnosed by the presence of a Systemic Inflammatory Response Syndrome (SIRS) plus proven or suspected infection (2). In 2016, a new definition was conceptualized, defining sepsis as "Life-threatening organ dysfunction due to a dysregulated host immune response to infection", along with new diagnostic criteria composed of a minimum 2 point elevation of the SOFA (Sepsis-related Organ Failure Assessment) Score plus proven or suspected infection (1), which allows the early detection of sepsis and timely

administration of bundle care, which has essential influence on the clinical course and mortality (1).

The mortality of septic patients is based on the clinical course and increases with the development of complications, such as septic shock and/or MODS (multi-organ dysfunction syndrome). The former is defined clinically as “persisting hypotension requiring vasopressors to maintain MAP >56 mmHg (Mean Arterial Pressure) plus a serum lactate concentration of >2 mmol/L despite adequate volume resuscitation” and increases mortality to 40% (1). The latter is assessed with the SOFA score (Sequential Organ Failure Assessment Score), while the mortality increases proportionally to the number of failing organs (1).

Despite intensive scientific research in the field of septic pathology, the clinical translation of experimental findings is often unsuccessful. This thesis is aimed to describe the current understanding of cellular hibernation and immunoparalysis which are important pathophysiologic factors of sepsis-induced organ dysfunction.

4. Literature Search Strategy

A Systematic literature search was conducted using the Database “Medline” with the search engine “PubMed”, which is maintained by the “National Center for Biotechnology and Information (NCBI)” at the “National Library of Medicine”. The selected time range of articles spans from 1986 to 2021. Main key words used: Sepsis, Septic Shock, Septic Organ Dysfunction, Cytokine Storm, Inflammatory Cytokines, Inflammatory Mediators, Disease Tolerance, Disease Resistance, Immunity, Immunoparalysis, Cellular Hibernation, Mortality in Sepsis, Immunotherapy, Blood purification techniques.

5. Sepsis: Course and Mortality

While many factors, such as pathogen (type, quantity) and patient factors (immune status, age, co-morbidities) influence the host response to an infection (3–5), special attention can be drawn to the epigenetic control of gene transcription and genetic polymorphism of sepsis-associated genes, of which many have been and continue to be identified leading to the understanding of the role of genotypic clusters involved in the pathway of host response (6–9). Especially cytokine receptor SNP’s (single nucleotide polymorphisms) and transcription

factor SNP's have been studied, outcome-correlated (6,10–12) and may be used as biomarkers of sepsis susceptibility and in the prognosis of the course of the disease (13).

In contrast to the normal response to an acute infection, which is proportional to the pathogen burden and terminated after elimination thereof, sepsis causes a specific pattern of an initial hyper inflammatory phase called SIRS (systemic inflammatory response syndrome) followed by a late hypo inflammatory phase called CARS (compensatory anti-inflammatory response syndrome) (4,5). The former is caused by PAMP (pathogen-associated molecular pattern) and DAMP (damage-associated molecular pattern) induced massive and uncontrolled activation of PRR's (pathogen recognition receptors), which are expressed on the surface of cells of the innate immune system, for instance TLR's (toll-like receptors), leading to the excessive release of pro-inflammatory cytokines (cytokine storm) (3,14), inducing pro-inflammatory cascades, with IL1β, IL-6, IL-12, IL-17 (Interleukins) being the most prominent (6). The latter is caused by the down-regulation of the primary effector cells (granulocytes, macrophages, monocytes, mast cells and natural killer cells) via anti-inflammatory cytokines, mainly IL-1 RA (receptor antagonist), IL-4 and IL-10 (6) with apoptotic and autophagic loss, as well as the formation of tolerance mechanisms by lymphocytes (15), resulting in a state of immunoparalysis.

The shift in the immune homeostasis correlates with distinct mortality phases, that can be divided into an early phase (day 1-5), late phase I (day 6-15) and late phase II (day 16-150) (16). Early phase coincides with the hyperinflammatory phase, where death is mainly caused by hyperinflammatory syndromes, such as toxic shock syndrome, refractory shock and severe metabolic disturbances (4) but may be anticipated by early recognition using the SOFA score with timely bundle treatment initiation (17–20) amidst a cumulative mortality of 10% (15). Late phase I and II are divided by different cumulative mortalities of 20-40% and 50-70% respectively (15) and marked by immunosuppression making the host vulnerable for a flare up of a not sufficiently cleared primary infection, reactivation of latent viruses or superinfection by opportunistic pathogens and hospital organisms (4,16). Thus, sepsis-induced immunosuppression is the major cause of high mortality and organ dysfunction in sepsis (13,21).

It is important to note that certain patient groups exhibit a different pattern of sepsis course. Patients with intrinsic or extrinsic immunosuppression may have an absent or diminished early pro-inflammatory phase (22,23) and elderly patients are, due to immunosenescence,

more likely to develop an early late phase with a pronounced immunoparalysis (22,24), which may alter the mortality pattern described above.

6. Disease Resistance vs. Disease Tolerance

Disease resistance describes the classical view of infectious response, defined as the ability of the immune system to recognize and eliminate pathogens, despite the possible causation of collateral damage caused by this process (25). In contrast, disease tolerance is the ability to limit tissue injury caused by the invading pathogen itself, or the inflammatory/immune response to them by utilizing tissue damage control mechanisms as the cellular stress and/or damage response (25–27).

Due to the collateral damage caused by the immune cascades activated in sepsis, the severity of the inflammatory responses has been associated with the development of multiple organ dysfunction (28). This led to the concept of disease tolerance in septic pathology, as an induction of tolerance may regulate or prevent the course of sepsis development, with the protective effect being presumable disease- and pathogen-specific (25,27,28). Multiple mechanisms of disease tolerance induction are under investigation, for example the low-dose treatment with cytostatic drugs (29).

7. Cytokine Storm

Cytokines are small signaling proteins (< 40 dKa) that bind to specific receptors acting in a autocrine, paracrine and endocrine manner to induce target cell activation, proliferation and migration, thereby encompassing an important immunomodulating function (6,30). They are produced predominantly by macrophages and T cells (31) and are classified into Interleukins, Chemokines, Interferons, Tumor Necrosis Factors and Growth Factors with Interleukins having the major impact on immune cells in the context of infectious disease (6). The excessive release of cytokines during the early stage of sepsis pathogenesis is termed "Cytokine Storm" in which cytokine cascades build a positive feedback loop with immune cells resulting in an overwhelming inflammatory response to infection (31).

Important potentiators and potentially therapeutic targets of the cytokine storm are transcription factors. Especially NFkB (nuclear transcription factor κ B), SP-1 (specific

Protein 1), hPNPase(old-35) (polynucleotide phosphorylase), AP-1 (activator protein 1) and PPARs (peroxisome proliferator-activated receptors) have been identified to play a role in upregulating genes coding for cytokines (32–34).

In this context NFkB is a transcription factor of major importance. It is activated by more than 400 stimuli, for example bacterial toxins, bacterial products and pro-inflammatory cytokines, by binding to specific subtypes of TLR's (toll-like receptors) and, in turn, upregulates the transcription of many pro-inflammatory genes encoding cytokines, cellular adhesion molecules and enzymes, such as COX-2 and iNOS (cyclooxygenase 2, inducible nitric oxide synthase) (31,35–37). Mycobacteria are identified to activate TLR1, gram-positive bacteria predominantly activate TLR 2 via PG (peptidoglycan) and gram-negative bacteria primarily bind to TLR-4 via LPS (lipopolysaccharide) (37–39).

Studies have shown an elevation of NFkB in all organs upon exposure of bacterial toxins, as well as in PMN's (peripheral mononuclear cells) of septic patients, indicating a role in septic pathology (37,40–43). Further the NFkB level positively correlates with sepsis mortality and the APACHE Score (Acute Physiology and Chronic Health Evaluation Score) predicting hospital mortality in critically-ill patients (37,44,45). The resulting signaling cascades of NFkB activation eventually leads to tissue injury and contributes to the development of tissue injury and MODS (37).

Various NFkB inhibitors have been identified and tested clinically or in animal models to identify their therapeutic potential and are found to have a positive effect on septic mortality. An improved survival of patients with severe sepsis was be observed upon treatment with Human Recombinant Active Protein C (37,38), which has been approved by the EMA (European Medical Association) in 2002 after the PROWESS trial results indicated a significant improvement in the 28 day mortality rate (46) but was taken off-market in 2011 after further trials could not reproduce the positive effect of the drug. The outcome of endotoxemia in LPS-induced Shock is positively influenced by IL-10 and a deletion of the gene encoding for poly(ADP-ribose)polymerase-1 made mice resistant to endotoxic shock (37,47–49). Furthermore an endotoxin tolerance mechanism is observed with increased Iκ Bα, p50 or p52 expression manifesting with a diminished NFkB activity and expression of NFkB-dependent genes (37,50–52).

Until today, trials targeting specific cytokines with monoclonal antibodies or the use of apheresis to remove cytokines from the circulation have not shown a clear benefit in the

treatment of sepsis-associated cytokine storm, so that organ-supportive therapy and antibiotic control of pathogen burden remain the mainstay of treatment (53). Although certain patient groups may benefit from targeted immunotherapy, the lack of research on inclusion criteria for certain drugs does not allow a clear matching, making is necessary to focus on precision diagnostics in future research (53).

8. Mechanisms of MODS

Multiple organ dysfunction is the most fatal complication of sepsis. Causative molecular pathways lead to microcirculatory and mitochondrial dysfunction resulting in organ damage.

The six most relevant organ systems in septic organ pathology are the cardiovascular, respiratory, renal, hepatic, hematologic, nervous and gastrointestinal systems, in order of their sequential failure (13).

The release of pro-inflammatory cytokines, such as TNF-α (tumor necrosis factor alpha), IL-1, IL-6, IL-12, IL-18 and INF-γ (interferon gamma) trigger an acute phase response leading to symptoms such as pyrexia, tachypnea, tachycardia, metabolic alterations, specifically increased gluconeogenesis, muscle catabolism and altered lipid metabolism, activation of the coagulation and complement pathway, downregulation of natural anticoagulants, leukocytosis with neutrophilic predominance, and the production of acute phase proteins in the liver, namely C-reactive protein and serum amyloid A, induced by IL-1 and TNF-α plus fibrinogen and α-2 macroglobulin, induced by IL-6 (54,55,37,56). In turn they upregulate the NFKb pathway leading to a positive feedback loop of pro-inflammatory mediator production (37,53).

Increased expression of cellular adhesion molecules such as ICAM-1 (intracellular adhesion molecule 1) and VCAM-1 (vascular adhesion molecule 1) and chemokines such as IL-8, MIP-1/2 (macrophage inflammatory protein 1/2), MCP 1/2 (monocyte chemoattractant protein 1/2) and CINC (cytokine-induced neutrophil chemoattractant) are a milestone for neutrophilic infiltration into organs, their activation, and the subsequent release of ROS (reactive oxygen species), RNS (reactive nitrogen species) and proteolytic enzymes (13,37,57–61). This leads to the development of endotheliopathy, which is describing endothelial activation and injury followed by increased vascular permeability leading to

leakage of molecules and cells into tissue with resulting edema, hemorrhage, infection or ischemia (13,37,57–61).

A upregulated expression of COX-2 (cyclo-oxygenase 2), 5-LO (5 lipo-oxygenase) and FLAP (5-LO-activating protein) lead to the production of PG's (prostaglandins), LT's (leukotrienes) and TBXA2 (thromboxane A2), which have an effect on the cardiovascular system, contributing by vasodilation to systemic hypotension and may cause direct tissue injury by various mechanisms (37,38,62).

Furthermore increased iNOS (inducible nitric oxide synthase) expression leads to elevated levels of NO (nitric oxide), which acts, along with PG's on VSMC's (vascular smooth muscle cells) to cause vasodilation and consequent systemic hypotension and vascular hyporeactivity (37,38,63–67). This effect may be anticipated by utilization of iNOS inhibitors (37,65,66).

Increased levels of TF (tissue factor), PAI-1 (plasminogen activator inhibitor -1) and factor VIII (coagulation factor 8) caused by pro-inflammatory cytokines activate the coagulation system and impair fibrinolysis, which may be causative for the development of the DIC (disseminated intravascular coagulation) syndrome, characterized by an initial hypercoagulable state with the formation of organ-threatening microthrombi, which may result in microvascular occlusion and/or may embolize, followed by an hypocoagulable state characterized by thrombocytopenia caused by the consumption of platelets, fibrinogen and coagulation factors, eventually leading to severe internal hemorrhage (68–72,35,37). The treatment for the DIC syndrome is based on general control of the causative agent for sepsis plus anticoagulant therapy in the hypercoagulable state and plasma or coagulation factor concentrates and/or platelet transfusion according to the PT (prothrombin time), fibrinogen concentration and platelet count, for bleeding patients (68,73–77).

Cumulation of a diminished vascular tone caused by NO and PG's and the formation of microthrombi or hemorrhages caused by DIC, lead to microcirculatory dysfunction, tissue hypoperfusion and hypoxia, resulting, along with increased vascular permeability caused by endotheliopathy, in tissue injury, which may precipitate organ failure (visualized in Fig.1).

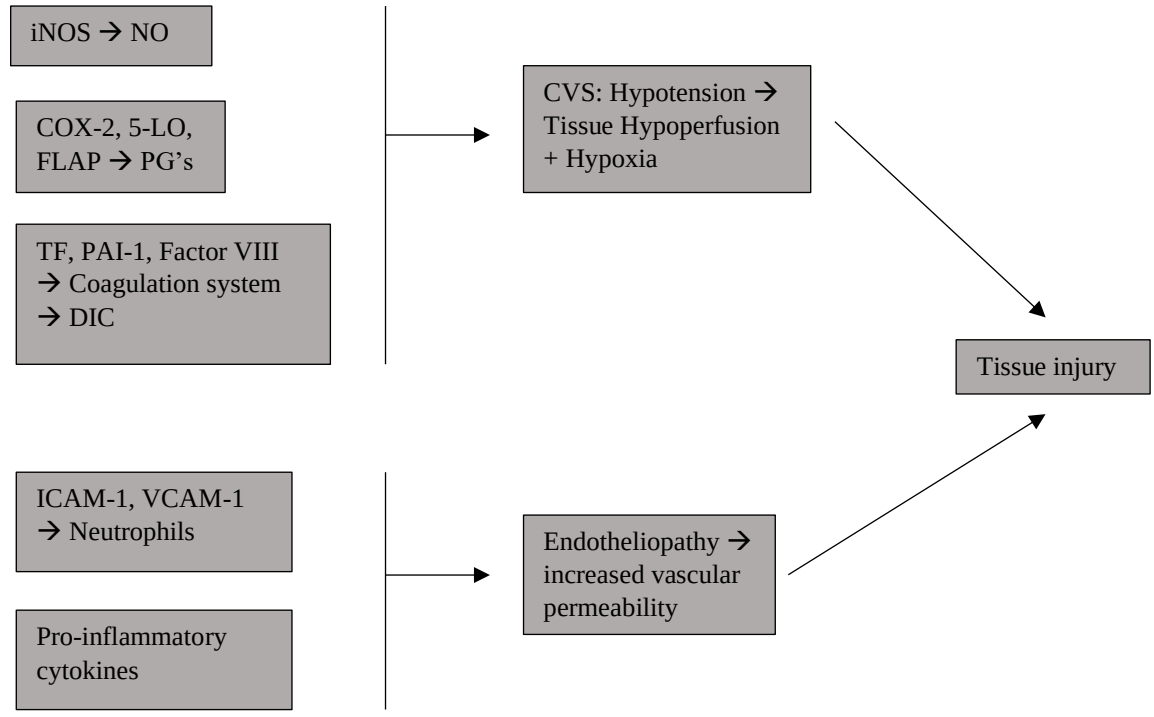

Fig. 1: Mechanisms of tissue injury in septic patients

Graphic adapted from: Liu SF, Malik AB. NF-kappa B activation as a pathological mechanism of septic shock and inflammation. Am J Physiol Lung Cell Mol Physiol. 2006 Apr;290(4):L622-L645.

9. Cellular Hibernation

Contrary to the assumption that septic organ dysfunction is a result of direct tissue injury followed by cellular apoptosis or necrosis, post-mortem histological examination of cells taken from patients who died from sepsis or septic shock showed a quantitative lack of apoptotic and necrotic cells sufficient to impair organ dysfunction (78). This finding lead to the theory of cellular hibernation describing the rearrangement of cellular metabolism in response to hypoxia and oxidative stress to a low-energy consumption state to preserve long-term viability (79–81).

The energy required for all cellular processes is ATP (adenosine triphosphate), which can be cleaved to ADP (adenosine diphosphate) and Pi (inorganic phosphate), thereby releasing free energy. The production of ATP occurs in the mitochondria by either glycolysis or the OXPHOS (oxidative phosphorylation) process, that couples the tricarboxylic acid cycle with the electron transport chain to produce ATP. In the tricarboxylic acid cycle, proteins, fatty

acids and carbohydrates are oxidized and electrons are transferred by NADH (nicotinamide adenine dinucleotide) and FADH2 (flavin adenine dinucleotide) to the electron transport chain, where the oxidation of the coenzymes yields ATP in a series of reactions by creation of a proton gradient across the inner mitochondrial membrane. Oxygen is essential for this process of energy production. Other factors such as the bio-availability of NADH and FADH2 and the integrity of the numerous enzymes involved in the process of OXPHOS may also influence the efficiency (80).

Tissue injury caused by a change in mitochondrial respiration can be described in the concept of cytopathic hypoxia - a diminished oxygen utilization by cells due to mitochondrial dysfunction (82). Multiple theories about the mechanism behind the development of cytopathic hypoxia in septic pathology have been established – the inhibition of pyruvate dehydrogenase, the nitric-oxide-mediated inhibition of cytochrome a,a3, the peroxynitrite-mediated inhibition of mitochondrial enzymes and the poly(ADP-ribose) polymerase hypothesis (82).

Pyruvate dehydrogenase is an enzyme catalyzing the reaction from pyruvate, the end product of glycolysis, to acetyl CoA (Acetyl Coenzyme A), which subsequently enters the tricarboxylic acid cycle whose activity is regulated by negative feedback and enzyme phosphorylation mediated by the pyruvate dehydrogenase kinase family. Studies have shown increased activity of the pyruvate dehydrogenase kinases (82–84) and an increased ratio of the inactive to active form of the pyruvate dehydrogenase complex has been found in animal models of chronic sepsis (82,85,86), limiting the utilization of the tricarboxylic acid cycle coupled to the electron transport chain to produce ATP, leading to increased lactate production and the possibility of metabolic acidosis.

As described earlier, sepsis leads to upregulation of iNOS enzymes, and subsequent increased production of NO. Cytochrome a,a3 is the terminal complex of the electron transport chain and subject to competition between NO, which acts as an inhibitor, and oxygen (O2), which acts as an activator. In conditions of a raised NO concentration (> ~1 mcM) or in a state of low pO2 (partial oxygen pressure), NO reversibly inhibits cytochrome a,a3 and thereby prevents ATP production (82,87–92).

Furthermore, NO forms ONOO- (peroxynitrite) by a reaction with O2-. Increased production of O2- by mitochondria was found in hypoxic conditions or in a state of cytochrome a,a3 inhibition (82,93,94). Combined with raised NO levels this may lead to a substantial increase

in ONOO- production , which causes irreversible inhibition of mitochondrial energy production in in-vitro conditions by inhibition of mitochondrial F0F1ATPase responsible for ATP production from ADP (95), inhibition of complexes of the electron transport chain (95) and inhibition of the aconitase enzyme of the tricarboxylic acid cycle (96).

Poly(ADP-ribose) polymerase 1 (PARP-1) is a nuclear enzyme activated by single-strand breaks in nuclear DNA, that catalyzes the production of ADP-ribose from NAD+ and polymerization thereof (82,97,98). ROS like ONOO- produced in sepsis may induce PARP-1 by DNA damage and lead to a depletion of cellular NAD+/NADH content which is essential for the OXPHOS process to generate ATP (82,99).

In total, the altered macro- and microcirculation in early sepsis prior resuscitation results in a decreased PO2 in tissues (100) with changes in the microcirculation remaining even post macrocirculatory resuscitation, which leads to sustained tissue hypoxia causing cellular hibernation in response to low PO2, and mechanisms of cytopathic hypoxia, and other direct insults caused by inflammatory mediators with resulting organ dysfunction (101). Due to the diminished O2 utilization by mitochondria, a decreased tissue VO2 stands in contrast to the rising tissue PO2, which is sending a negative feedback to the microcirculation causing vasoconstriction, thereby further potentiating the process of cellular hibernation (102) (visualized in figure 2).

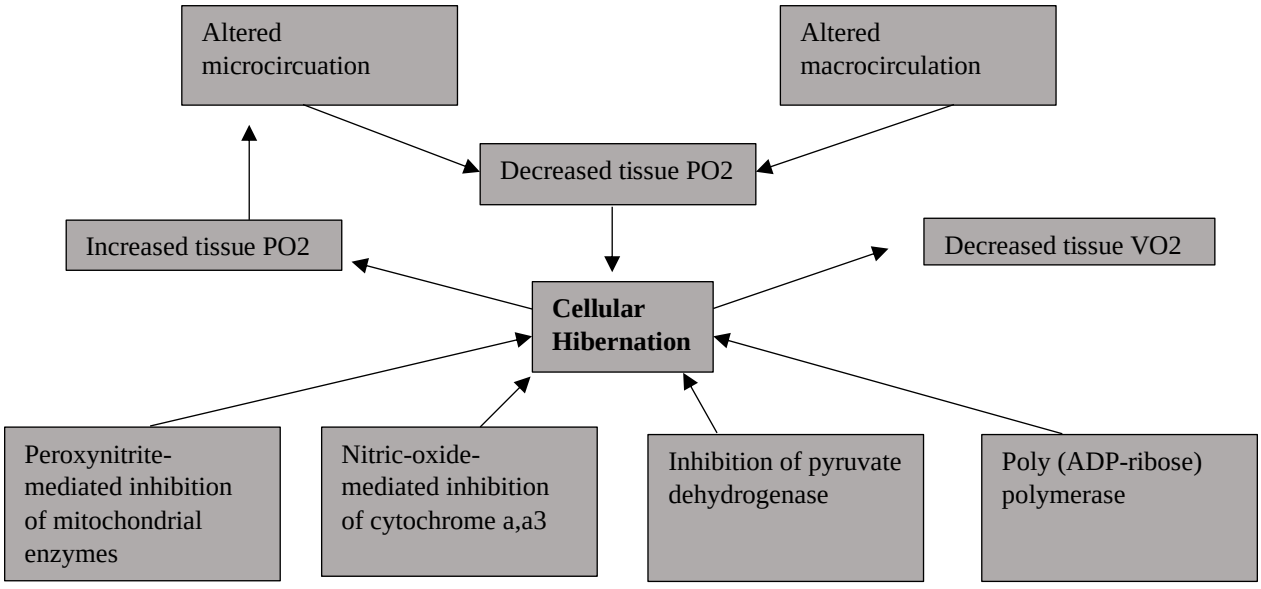

Fig. 2: Mechanisms of Cellular Hibernation

10. Immunoparalysis

Beside the quantitative loss of immune cells (15) during late phase of sepsis, namely T- cells, B- cells, macrophages and dendritic cells, as shown in post-mortem analysis (78,103–106), several mechanisms are suggested to cause a qualitative loss in innate and adaptive immune cell function, termed immunoparalysis, resulting in insufficient clearance of septic foci and the potential for superinfections and reactivation of latent viruses (22,107,108). Although it is thought to occur in the late phase of sepsis during CARS, some evidence also suggests that a certain degree of immunoparalysis may exist from the onset of sepsis (22,16,109,110). The mortality associated with the immunoparalytic state (15) suggests the importance of biologic markers to assess the degree of immunoparalysis and potentially aid in prediction of the disease course and outcome (22,111,4,112). Moreover they could be decisive for the therapeutic induction of immunocompetence by the use of immunomodulatory medications (22,111,4,112).

Monocytes have been shown to exhibit an impairment in antigen presentation and pro-inflammatory mediator production due to decreased expression of mHLA-DR (monocyte human leukocyte antigen DR isotype), which is the most researched biomarker for immunoparalysis to date and has been positively correlated with mortality (22,113–116).

Further, several receptors that initiate pro-inflammatory cascades upon stimulation have been shown to be downregulated in immunoparalytic conditions (117). Together with upregulation of negative costimulatory molecules located on lymphocytes and monocytes, PD-1 (programmed cell death protein 1) and PD-L1 (programmed cell death protein ligand 1), whose binding induces a IL-10 mediated down-regulation of inflammatory functions, this leads to a diminished immune functional capacity (22,107,118).

An upregulation of Treg's (T regulatory lymphocytes) is associated with immunoparalysis and actively down-regulate the adaptive immune response (22,119–121) by TGF-β (transforming growth factor β) and IL-10 secretion and attenuation of pro-inflammatory cytokines, as well, as induction of cytotoxic T-cells and Monocyte down-regulation (22,121–124).

Epigenetic regulation plays a major role in the development of immunoparalysis by modulation of pro- and anti-inflammatory gene expression (125) through multiple mechanisms such as histone deacetylation or methylation (125–127). An example for epigenetic modification is the shift of macrophages to the M2 phenotype (128).

Macrophages are thought to be the key mediator of shifting the cytokine balance to the anti-inflammatory site by polarizing into M2 phenotype, which is secreting high levels of diverse anti-inflammatory molecules, such as IL-10 and IL-1-RA (IL-1 receptor antagonist), thereby down-regulating pro-inflammatory cytokine release (129). In vitro experiments also suggest a generalized diminished capacity of pro-inflammatory cytokine secretion by monocytes without changes in anti-inflammatory cytokine secretion capability (130).

Further pathophysiologic mechanisms causative for sepsis-induced immunoparalysis are under investigation and provide scientists with several potential therapeutic targets of sepsis.

11. Clinical implications

Despite the establishment of timely bundle care with focus on infection source control and organ-supportive therapy, sepsis remains the leading cause of death in the ICU.

To anticipate deterioration of a septic patient, the pathophysiological processes described in the previous chapters need to be taken into consideration when searching for further treatment options. Until today many scientists are focused on finding ways to target the immunopathology of sepsis, with only a limited number of successful translations of in-vitro to in-vivo conditions.

Regarding the cytokine storm as initiator of the septic sequelae, either a decreased cytokine and inflammatory mediator production or an increased elimination can be targeted. The former implies the investigation of immunotherapeutic drugs, with many concepts from oncology being utilized for research, while for the latter, the effectivity of extracorporeal blood purification techniques in the context of sepsis is investigated.

Extracorporeal blood purification therapies implicate convection, adsorption, combination and further therapies, as listed in table 1 (131). Although evidence for their potential in extracting inflammatory mediators and bacterial toxins from blood has been established, there is a lack of high-quality trials in septic patients (132).

Convection techniques include HVFT (high volume hemofiltration therapy), CCRT (continuous renal replacement therapy) and HCO (high-cut-off) membrane therapy. In HVFT, an increased convective target dose of > 35 ml/kg/h is used, compared to CCRT, leading to mediator elimination and the effectivity of different convective target dosages and

application modes (continuous vs. intermittent) for certain septic patient groups have been investigated (133,134), but a comprehensive conclusion cannot be drawn for all sepsis patient groups. Similar to HVFT, the utilization of HCO membrane therapy resulted in increased removal of mediators in a septic patient subgroup, but was associated with concomitant loss of albumin (135), while further trials were not completed (132).

TPE (therapeutic plasma exchange) is thought to be beneficial not only by clearing the body from mediators and toxins, but also by providing instant replenishment of plasma molecules and the utilization is currently under investigation (131,136)

The combination of filtration and adsorption, CPFA (coupled filtration and adsorption) did not show any benefit in clinical trials (131,137,138).

Adsorption techniques include hemoperfusion through PMX (polymyxin B immobilized) fiber columns, for which the data is contradictory until today (131,139–141), and Hemadsorption using CytoSorb, which is able to absorb many cytokines and mediators, but also free Hb (Hemoglobin), Myoglobin, Bilirubin, Bile acids, bacterial toxins, activated components of the complement system and drugs, which might be especially beneficial for septic patients when utilized in early phase (131,142–144). Recent studies using CytoSorb in patients with sepsis and/or septic shock suggest a decreased vasopressor requirement, increased lactate clearance and the resolution of septic shock (145,146) and an improved 28-day mortality rate (147), making CytoSorb the most promising blood purification technique up to this date.

Convection Therapies	CCRT – Continuous Renal Replacement Therapy HVFT – High Volume Hemofiltration Therapy HCO – High-cut-off Membrane Therapy
Adsorption Therapies	PMX – Immobilized Polymyxin B CytoSorb - Hemoadsorption
Combination Therapies	CPFA – Coupled Plasma Filtration Adsorption CFA - Combined Filtration and Adsorption
Further Therapies	TPE- Therapeutic Plasma Exchange RAD - Renal Assist Device SCD – Selective Cytopheretic Device

Table 1 – Overview of blood purification techniques

Graphic adapted from: Jarczak D, Kluge S, Nierhaus A. Sepsis-Pathophysiology and Therapeutic Concepts. Front Med (Lausanne). 2021;8:628302

Immunomodulatory treatment targeting innate and adaptive immune cell function and cytokine production may aid in restoration of immune homeostasis in a septic context and is currently highly investigated. Up until today there is not much clear evidence of beneficence, mainly due to the lack of clinical translation of in-vivo or animal models, uncompleted trials, or negative trial results.

The previously described down-regulation of immune cell function by upregulation of PD-1 and PD-L1, representing a “checkpoint” in the negative costimulatory pathway of a normal T-cell response, lead to the investigation of monoclonal antibodies blocking either PD-1 or PD-L1, thus inhibiting the binding ability, which lead to an unwanted increase in cytokine production and secretion by T cells and monocytes in ex-vivo studies (148). A phase I trial using Nivolumab, a monoclonal antibody targeting PD-1, in 38 sepsis and/or septic shock patients resulted in no increase in inflammatory cytokines and did not result in unexpected safety findings and stimulated INF-γ, an anti-inflammatory cytokine, production by T cells (131,149), making Nivolumab a potential candidate to target immune cell function without increasing cytokine production, yet further trials on larger patient groups are needed.

IL-7, also called the “maestro of the immune system”, has an important role in the development, maturation, expansion and homeostasis of B and T cells (150) with studies demonstrating an increase in absolute lymphocyte count and the number of circulating CD4+ and CD8+ T cells without increased pro-inflammatory response or organ function deterioration and increased INF-γ production by T-cells upon treatment with recombinant human IL-7 in patient with septic shock and severe lymphopenia (131,151), making recombinant human IL-7 also a potential therapeutic candidate.

Polyvalent immunoglobins have been shown to be able to neutralize toxins, interact with complement factors and increase opsonization of pathogens leading to increased phagocytosis (131,152–155). Until today no immunoglobin therapy is recommended for treatment in septic patients due to lack of definitive data due to inconsistency of study protocols, heterogeneity of patients and alterations of laboratory parameter spectrums, but more RCT’s (randomized controlled trials) are on the way to further clarify utility (131,156).

12. Conclusion

Many advances in the understanding of septic pathophysiology have been made and are continued to be made, hence allowing the identification of the main therapeutic targets in sepsis treatment. Beside source-control and organ support, the immunologic nature of sepsis needs to be taken into consideration, namely regulation of the cytokine production and secretion, as well, as immune cell function. Many molecular pathways are targeted in research in vitro and in vivo, with some having promising results. Nevertheless, more research is needed to provide a comprehensive therapy for patients in sepsis and septic shock.

13. List of Abbreviations

List of Abbreviations

SIRS – systemic inflammatory response syndrome

SOFA – sepsis-related organ failure assessment

MODS – multiple organ dysfunction syndrome

MAP – mean arterial pressure

NCBI – national center for biotechnology and information

SNP – single nucleotide polymorphisms

CARS – compensatory anti-inflammatory response syndrome

PAMP – pathogen-associated molecular pattern

DAMP – damage-associated molecular pattern

PRR – pathogen recognition receptor

TLR – toll-like receptor

IL– interleukin

NK-cell - natural killer cell

RA- receptor antagonist

DNA – deoxyribonucleic acid

NFkB – nuclear transcription factor kappa B

AP – activator protein

PPAR – peroxisome proliferator-activated receptors

COX – cyclooxygenase

iNOS – inducible nitric oxide synthase

PG – peptidoglycan

LPS – lipopolysaccharide

PMN – peripheral mononuclear cells

APACHE – acute physiology and chronic health evaluation

EMA – European medical association

P – protein

INF – interferon

TNF – tumor necrosis factor

ICAM – intracellular adhesion molecule

VCAM – vascular adhesion molecule

MIP – macrophage inflammatory protein

MCP – monocyte chemoattractant protein

CINC - cytokine-induced neutrophil chemoattractant

ROS – reactive oxygen species

RNS – reactive nitrogen species

LO – lipoxygenase

PG – prostaglandin

FLAP – 5-LO-activating protein

LT – leukotrienes

TBX – thromboxane

NO – nitric oxide

VSMC – vascular smooth muscle

TF – tissue factor

PAI – platelet activator inhibitor

DIC – disseminated intravascular coagulation

PT – prothrombin time

ATP – adenosine triphosphate

ADP – adenosine diphosphate

Pi – inorganic phosphate

OXPHOS – oxidative phosphorylation

NADH/NAD+ – nicotinamide adenine dinucleotide

FADH – flavin adenine dinucleotide

CoA – coenzyme A

O2 – oxygen

pO2 – partial oxygen pressure

ONOO- – peroxynitrite

PARP – poly (ADP-ribose) polymerase

VO2 – Volume of oxygen

mHLA-DR – monocyte human leukocyte antigen DR isotype

PD – programmed cell death protein

PD-L – programmed cell death protein ligand

Treg – T regulatory lymphocytes

TGF-β – transforming growth factor beta

IL-RA- interleukin receptor antagonist

HVFT – high volume hemofiltration therapy

CCRT – continuous renal replacement therapy

HCO – high-cut-off

TPE – therapeutic plasma exchange

CPFA – coupled filtration and adsorption

PMX – polymyxin B immobilized

Hb – hemoglobin

14. References

1. Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA. 2016 Feb 23;315(8):801–10.

2. Bone RC, Sibbald WJ, Sprung CL. The ACCP-SCCM consensus conference on sepsis and organ failure. Chest. 1992 Jun;101(6):1481–3.

3. Arina P, Singer M. Pathophysiology of sepsis. Curr Opin Anaesthesiol. 2021 Apr 1;34(2):77–84.

4. Hotchkiss RS, Monneret G, Payen D. Sepsis-induced immunosuppression: from cellular dysfunctions to immunotherapy. Nat Rev Immunol. 2013 Dec;13(12):862–74.

5. Hotchkiss RS, Monneret G, Payen D. Immunosuppression in sepsis: a novel understanding of the disorder and a new therapeutic approach. Lancet Infect Dis. 2013 Mar;13(3):260–8.

6. Chousterman BG, Swirski FK, Weber GF. Cytokine storm and sepsis disease pathogenesis. Semin Immunopathol. 2017 Jul;39(5):517–28.

7. Sutherland AM, Walley KR. Bench-to-bedside review: Association of genetic variation with sepsis. Crit Care. 2009;13(2):210.

8. Arcaroli J, Fessler MB, Abraham E. Genetic polymorphisms and sepsis. Shock. 2005 Oct;24(4):300–12.

9. Texereau J, Pene F, Chiche JD, Rousseau C, Mira JP. Importance of hemostatic gene polymorphisms for susceptibility to and outcome of severe sepsis. Crit Care Med. 2004 May;32(5 Suppl):S313-319.

10. Mira JP, Cariou A, Grall F, Delclaux C, Losser MR, Heshmati F, et al. Association of TNF2, a TNF-alpha promoter polymorphism, with septic shock susceptibility and mortality: a multicenter study. JAMA. 1999 Aug 11;282(6):561–8.

11. Arnalich F, López-Maderuelo D, Codoceo R, Lopez J, Solis-Garrido LM, Capiscol C, et al. Interleukin-1 receptor antagonist gene polymorphism and mortality in patients with severe sepsis. Clin Exp Immunol. 2002 Feb;127(2):331–6.

12. Wurfel MM, Gordon AC, Holden TD, Radella F, Strout J, Kajikawa O, et al. Toll-like Receptor 1 Polymorphisms Affect Innate Immune Responses and Outcomes in Sepsis. Am J Respir Crit Care Med. 2008 Oct;178(7):710–20.

13. Chen J, Wei H. Immune Intervention in Sepsis. Front Pharmacol. 2021;12:718089.

14. Chen XH, Yin YJ, Zhang JX. Sepsis and immune response. World J Emerg Med. 2011;2(2):88–92.

15. Venet F, Monneret G. Advances in the understanding and treatment of sepsis-induced immunosuppression. Nat Rev Nephrol. 2018 Feb;14(2):121–37.

16. Otto GP, Sossdorf M, Claus RA, Rödel J, Menge K, Reinhart K, et al. The late phase of sepsis is characterized by an increased microbiological burden and death rate. Crit Care. 2011 Jul 28;15(4):R183.

17. Rhodes A, Evans LE, Alhazzani W, Levy MM, Antonelli M, Ferrer R, et al. Surviving Sepsis Campaign: International Guidelines for Management of Sepsis and Septic Shock: 2016. Intensive Care Med. 2017 Mar;43(3):304–77.

18. Rhodes A, Phillips G, Beale R, Cecconi M, Chiche JD, De Backer D, et al. The Surviving Sepsis Campaign bundles and outcome: results from the International Multicentre Prevalence Study on Sepsis (the IMPreSS study). Intensive Care Med. 2015 Sep;41(9):1620–8.

19. Levy MM, Rhodes A, Phillips GS, Townsend SR, Schorr CA, Beale R, et al. Surviving Sepsis Campaign: association between performance metrics and outcomes in a 7.5-year study. Intensive Care Med. 2014 Nov;40(11):1623–33.

20. Damiani E, Donati A, Serafini G, Rinaldi L, Adrario E, Pelaia P, et al. Effect of performance improvement programs on compliance with sepsis bundles and mortality: a systematic review and meta-analysis of observational studies. PLoS One. 2015;10(5):e0125827.

21. Delano MJ, Ward PA. Sepsis-induced immune dysfunction: can immune therapies reduce mortality? J Clin Invest. 2016 Jan;126(1):23–31.

22. Hamers L, Kox M, Pickkers P. Sepsis-induced immunoparalysis: mechanisms, markers, and treatment options. Minerva Anestesiol. 2015 Apr;81(4):426–39.

23. Iskander KN, Osuchowski MF, Stearns-Kurosawa DJ, Kurosawa S, Stepien D, Valentine C, et al. Sepsis: multiple abnormalities, heterogeneous responses, and evolving understanding. Physiol Rev. 2013 Jul;93(3):1247–88.

24. Reber AJ, Chirkova T, Kim JH, Cao W, Biber R, Shay DK, et al. Immunosenescence and Challenges of Vaccination against Influenza in the Aging Population. Aging Dis. 2012 Feb;3(1):68–90.

25. Schneider DS, Ayres JS. Two ways to survive infection: what resistance and tolerance can teach us about treating infectious diseases. Nat Rev Immunol. 2008 Nov;8(11):889–95.

26. Soares MP, Gozzelino R, Weis S. Tissue damage control in disease tolerance. Trends in Immunology. 2014 Oct;35(10):483–94.

27. Soares MP, Teixeira L, Moita LF. Disease tolerance and immunity in host protection against infection. Nat Rev Immunol. 2017 Feb;17(2):83–96.

28. Toro J, Manrique-Caballero CL, Gómez H. Metabolic Reprogramming and Host Tolerance: A Novel Concept to Understand Sepsis-Associated AKI. J Clin Med. 2021 Sep 16;10(18):4184.

29. Figueiredo N, Chora A, Raquel H, Pejanovic N, Pereira P, Hartleben B, et al. Anthracyclines induce DNA damage response-mediated protection against severe sepsis. Immunity. 2013 Nov 14;39(5):874–84.

30. Dinarello CA. Historical insights into cytokines. Eur J Immunol. 2007 Nov;37 Suppl 1:S34-45.

31. Sygitowicz G, Sitkiewicz D. Molecular mechanisms of organ damage in sepsis: an overview. Braz J Infect Dis. 2020 Dec;24(6):552–60.

32. Meftahi GH, Jangravi Z, Sahraei H, Bahari Z. The possible pathophysiology mechanism of cytokine storm in elderly adults with COVID-19 infection: the contribution of “inflame-aging.” Inflamm Res. 2020 Sep;69(9):825–39.

33. Sarkar D, Fisher PB. Molecular mechanisms of aging-associated inflammation. Cancer Lett. 2006 May 8;236(1):13–23.

34. Lavrovsky Y, Chatterjee B, Clark RA, Roy AK. Role of redox-regulated transcription factors in inflammation, aging and age-related diseases. Exp Gerontol. 2000 Aug;35(5):521–32.

35. Pahl HL. Activators and target genes of Rel/NF-kappaB transcription factors. Oncogene. 1999 Nov 22;18(49):6853–66.

36. Rajapakse N, Kim MM, Mendis E, Kim SK. Inhibition of inducible nitric oxide synthase and cyclooxygenase-2 in lipopolysaccharide-stimulated RAW264.7 cells by carboxybutyrylated glucosamine takes place via down-regulation of mitogen-activated protein kinase-mediated nuclear factor-kappaB signaling. Immunology. 2008 Mar;123(3):348–57.

37. Liu SF, Malik AB. NF-kappa B activation as a pathological mechanism of septic shock and inflammation. Am J Physiol Lung Cell Mol Physiol. 2006 Apr;290(4):L622–45.

38. Brown MA, Jones WK. NF-kappaB action in sepsis: the innate immune system and the heart. Front Biosci. 2004 May 1;9:1201–17.

39. Takeuchi O, Sato S, Horiuchi T, Hoshino K, Takeda K, Dong Z, et al. Cutting edge: role of Toll-like receptor 1 in mediating immune response to microbial lipoproteins. J Immunol. 2002 Jul 1;169(1):10–4.

40. Kelly A, Vereker E, Nolan Y, Brady M, Barry C, Loscher CE, et al. Activation of p38 plays a pivotal role in the inhibitory effect of lipopolysaccharide and interleukin-1 beta on long term potentiation in rat dentate gyrus. J Biol Chem. 2003 May 23;278(21):19453–62.

41. Liu SF, Ye X, Malik AB. Pyrrolidine dithiocarbamate prevents I-kappaB degradation and reduces microvascular injury induced by lipopolysaccharide in multiple organs. Mol Pharmacol. 1999 Apr;55(4):658–67.

42. Pocock J, Gómez-Guerrero C, Harendza S, Ayoub M, Hernández-Vargas P, Zahner G, et al. Differential activation of NF-kappa B, AP-1, and C/EBP in endotoxin-tolerant rats: mechanisms for in vivo regulation of glomerular RANTES/CCL5 expression. J Immunol. 2003 Jun 15;170(12):6280–91.

43. Pritts TA, Moon MR, Wang Q, Hungness ES, Salzman AL, Fischer JE, et al. Activation of NF-kappaB varies in different regions of the gastrointestinal tract during endotoxemia. Shock. 2000 Aug;14(2):118–22.

44. Arnalich F, Garcia-Palomero E, López J, Jiménez M, Madero R, Renart J, et al. Predictive value of nuclear factor kappaB activity and plasma cytokine levels in patients with sepsis. Infect Immun. 2000 Apr;68(4):1942–5.

45. Böhrer H, Qiu F, Zimmermann T, Zhang Y, Jllmer T, Männel D, et al. Role of NFkappaB in the mortality of sepsis. J Clin Invest. 1997 Sep 1;100(5):972–85.

46. Bernard GR, Vincent JL, Laterre PF, LaRosa SP, Dhainaut JF, Lopez-Rodriguez A, et al. Efficacy and safety of recombinant human activated protein C for severe sepsis. N Engl J Med. 2001 Mar 8;344(10):699–709.

47. Fan J, Ye RD, Malik AB. Transcriptional mechanisms of acute lung injury. Am J Physiol Lung Cell Mol Physiol. 2001 Nov;281(5):L1037-1050.

48. Howard M, Muchamuel T, Andrade S, Menon S. Interleukin 10 protects mice from lethal endotoxemia. J Exp Med. 1993 Apr 1;177(4):1205–8.

49. Oliver FJ, Ménissier-de Murcia J, Nacci C, Decker P, Andriantsitohaina R, Muller S, et al. Resistance to endotoxic shock as a consequence of defective NF-kappaB activation in poly (ADP-ribose) polymerase-1 deficient mice. EMBO J. 1999 Aug 16;18(16):4446–54.

50. Bohuslav J, Kravchenko VV, Parry GC, Erlich JH, Gerondakis S, Mackman N, et al. Regulation of an essential innate immune response by the p50 subunit of NF-kappaB. J Clin Invest. 1998 Nov 1;102(9):1645–52.

51. Shames BD, Meldrum DR, Selzman CH, Pulido EJ, Cain BS, Banerjee A, et al. Increased levels of myocardial IkappaB-alpha protein promote tolerance to endotoxin. Am J Physiol. 1998 Sep;275(3):H1084-1091.

52. Medvedev AE, Kopydlowski KM, Vogel SN. Inhibition of lipopolysaccharide-induced signal transduction in endotoxin-tolerized mouse macrophages: dysregulation of cytokine, chemokine, and toll-like receptor 2 and 4 gene expression. J Immunol. 2000 Jun 1;164(11):5564–74.

53. Fajgenbaum DC, June CH. Cytokine Storm. Longo DL, editor. N Engl J Med. 2020 Dec 3;383(23):2255–73.

54. Sriskandan S, Altmann DM. The immunology of sepsis. J Pathol. 2008 Jan;214(2):211–23.

55. Moshage H. Cytokines and the hepatic acute phase response. J Pathol. 1997 Mar;181(3):257–66.

56. Amaral A, Opal SM, Vincent JL. Coagulation in sepsis. Intensive Care Med. 2004 Jun;30(6):1032–40.

57. Raeburn CD, Calkins CM, Zimmerman MA, Song Y, Ao L, Banerjee A, et al. ICAM-1 and VCAM-1 mediate endotoxemic myocardial dysfunction independent of neutrophil accumulation. Am J Physiol Regul Integr Comp Physiol. 2002 Aug;283(2):R477-486.

58. Rahman A, Anwar KN, True AL, Malik AB. Thrombin-induced p65 homodimer binding to downstream NF-kappa B site of the promoter mediates endothelial ICAM-1 expression and neutrophil adhesion. J Immunol. 1999 May 1;162(9):5466–76.

59. Graciano AL, Bryant DD, White DJ, Horton J, Bowles NE, Giroir BP. Targeted disruption of ICAM-1, P-selectin genes improves cardiac function and survival in TNF-alpha transgenic mice. Am J Physiol Heart Circ Physiol. 2001 Apr;280(4):H1464-1471.

60. Aird WC. The role of the endothelium in severe sepsis and multiple organ dysfunction syndrome. Blood. 2003 May 15;101(10):3765–77.

61. Xiong Y, Hla T. S1P Control of Endothelial Integrity. In: Oldstone MBA, Rosen H, editors. Sphingosine-1-Phosphate Signaling in Immunology and Infectious Diseases [Internet]. Cham: Springer International Publishing; 2014 [cited 2021 Apr 13]. p. 85–105. (Current Topics in Microbiology and Immunology; vol. 378). Available from: http://link.springer.com/10.1007/978-3-319-05879-5_4

62. Fletcher JR. Eicosanoids. Critical agents in the physiological process and cellular injury. Arch Surg. 1993 Nov;128(11):1192–6.

63. Chauhan SD, Seggara G, Vo PA, Macallister RJ, Hobbs AJ, Ahluwalia A. Protection against lipopolysaccharide-induced endothelial dysfunction in resistance and conduit vasculature of iNOS knockout mice. FASEB J. 2003 Apr;17(6):773–5.

64. Hollenberg SM, Broussard M, Osman J, Parrillo JE. Increased microvascular reactivity and improved mortality in septic mice lacking inducible nitric oxide synthase. Circ Res. 2000 Apr 14;86(7):774–8.

65. Kilbourn RG, Szabó C, Traber DL. Beneficial versus detrimental effects of nitric oxide synthase inhibitors in circulatory shock: lessons learned from experimental and clinical studies. Shock. 1997 Apr;7(4):235–46.

66. Parratt JR. Nitric oxide. A key mediator in sepsis and endotoxaemia? J Physiol Pharmacol. 1997 Dec;48(4):493–506.

67. Liaudet L, Soriano FG, Szabó C. Biology of nitric oxide signaling. Crit Care Med. 2000 Apr;28(4 Suppl):N37-52.

68. Giustozzi M, Ehrlinder H, Bongiovanni D, Borovac JA, Guerreiro RA, Gąsecka A, et al. Coagulopathy and sepsis: Pathophysiology, clinical manifestations and treatment. Blood Rev. 2021 Jun 25;100864.

69. Levi M, van der Poll T. Coagulation and sepsis. Thromb Res. 2017 Jan;149:38–44.

70. Levi M, van der Poll T. Disseminated intravascular coagulation: a review for the internist. Intern Emerg Med. 2013 Feb;8(1):23–32.

71. Hou B, Eren M, Painter CA, Covington JW, Dixon JD, Schoenhard JA, et al. Tumor necrosis factor alpha activates the human plasminogen activator inhibitor-1 gene through a distal nuclear factor kappaB site. J Biol Chem. 2004 Apr 30;279(18):18127–36.

72. Oeth PA, Parry GC, Kunsch C, Nantermet P, Rosen CA, Mackman N. Lipopolysaccharide induction of tissue factor gene expression in monocytic cells is mediated by binding of c-Rel/p65 heterodimers to a kappa B-like site. Mol Cell Biol. 1994 Jun;14(6):3772–81.

73. Levi M, Scully M. How I treat disseminated intravascular coagulation. Blood. 2018 Feb 22;131(8):845–54.

74. Franchini M, Lippi G. Fibrinogen replacement therapy: a critical review of the literature. Blood Transfus. 2012 Jan;10(1):23–7.

75. Levi M, Toh CH, Thachil J, Watson HG. Guidelines for the diagnosis and management of disseminated intravascular coagulation. British Committee for Standards in Haematology. Br J Haematol. 2009 Apr;145(1):24–33.

76. Köhler M. Thrombogenicity of prothrombin complex concentrates. Thromb Res. 1999 Aug 15;95(4 Suppl 1):S13-17.

77. Levi M, Ten Cate H. Disseminated intravascular coagulation. N Engl J Med. 1999 Aug 19;341(8):586–92.

78. Hotchkiss RS, Swanson PE, Freeman BD, Tinsley KW, Cobb JP, Matuschak GM, et al. Apoptotic cell death in patients with sepsis, shock, and multiple organ dysfunction. Crit Care Med. 1999 Jul;27(7):1230–51.

79. Abraham E, Singer M. Mechanisms of sepsis-induced organ dysfunction. Crit Care Med. 2007 Oct;35(10):2408–16.

80. Fink MP, Evans TW. Mechanisms of organ dysfunction in critical illness: report from a Round Table Conference held in Brussels. Intensive Care Med. 2002 Mar;28(3):369–75.

81. Buck LT, Hochachka PW, Schön A, Gnaiger E. Microcalorimetric measurement of reversible metabolic suppression induced by anoxia in isolated hepatocytes. Am J Physiol. 1993 Nov;265(5 Pt 2):R1014-1019.

82. Fink MP. Bench-to-bedside review: Cytopathic hypoxia. Crit Care. 2002 Dec;6(6):491–9.

83. Vary TC, Hazen S. Sepsis alters pyruvate dehydrogenase kinase activity in skeletal muscle. Mol Cell Biochem. 1999 Aug;198(1–2):113–8.

84. Vary TC. Increased pyruvate dehydrogenase kinase activity in response to sepsis. Am J Physiol. 1991 May;260(5 Pt 1):E669-674.

85. Vary TC, Siegel JH, Nakatani T, Sato T, Aoyama H. Effect of sepsis on activity of pyruvate dehydrogenase complex in skeletal muscle and liver. Am J Physiol. 1986 Jun;250(6 Pt 1):E634-640.

86. Vary TC. Sepsis-induced alterations in pyruvate dehydrogenase complex activity in rat skeletal muscle: effects on plasma lactate. Shock. 1996 Aug;6(2):89–94.

87. Borutaité V, Brown GC. Rapid reduction of nitric oxide by mitochondria, and reversible inhibition of mitochondrial respiration by nitric oxide. Biochem J. 1996 Apr 1;315 (Pt 1):295–9.

88. Cassina A, Radi R. Differential inhibitory action of nitric oxide and peroxynitrite on mitochondrial electron transport. Arch Biochem Biophys. 1996 Apr 15;328(2):309–16.

89. Takehara Y, Kanno T, Yoshioka T, Inoue M, Utsumi K. Oxygen-dependent regulation of mitochondrial energy metabolism by nitric oxide. Arch Biochem Biophys. 1995 Oct 20;323(1):27–32.

90. Nishikawa M, Sato EF, Kuroki T, Inoue M. Role of glutathione and nitric oxide in the energy metabolism of rat liver mitochondria. FEBS Lett. 1997 Oct 6;415(3):341–5.

91. Giuffrè A, Sarti P, D'Itri E, Buse G, Soulimane T, Brunori M. On the mechanism of inhibition of cytochrome c oxidase by nitric oxide. J Biol Chem. 1996 Dec 27;271(52):33404–8.

92. Torres J, Darley-Usmar V, Wilson MT. Inhibition of cytochrome c oxidase in turnover by nitric oxide: mechanism and implications for control of respiration. Biochem J. 1995 Nov 15;312 (Pt 1):169–73.

93. Du G, Mouithys-Mickalad A, Sluse FE. Generation of superoxide anion by mitochondria and impairment of their functions during anoxia and reoxygenation in vitro. Free Radic Biol Med. 1998 Dec;25(9):1066–74.

94. Poderoso JJ, Carreras MC, Lisdero C, Riobó N, Schöpfer F, Boveris A. Nitric oxide inhibits electron transfer and increases superoxide radical production in rat heart mitochondria and submitochondrial particles. Arch Biochem Biophys. 1996 Apr 1;328(1):85–92.

95. Radi R, Rodriguez M, Castro L, Telleri R. Inhibition of mitochondrial electron transport by peroxynitrite. Arch Biochem Biophys. 1994 Jan;308(1):89–95.

96. Castro L, Rodriguez M, Radi R. Aconitase is readily inactivated by peroxynitrite, but not by its precursor, nitric oxide. J Biol Chem. 1994 Nov 25;269(47):29409–15.

97. Lautier D, Lagueux J, Thibodeau J, Ménard L, Poirier GG. Molecular and biochemical features of poly (ADP-ribose) metabolism. Mol Cell Biochem. 1993 May 26;122(2):171–93.

98. D'Amours D, Desnoyers S, D'Silva I, Poirier GG. Poly(ADP-ribosyl)ation reactions in the regulation of nuclear functions. Biochem J. 1999 Sep 1;342 (Pt 2):249–68.

99. Khan AU, Delude RL, Han YY, Sappington PL, Han X, Carcillo JA, et al. Liposomal NAD(+) prevents diminished O(2) consumption by immunostimulated Caco-2 cells. Am J Physiol Lung Cell Mol Physiol. 2002 May;282(5):L1082-1091.

100. De Backer D, Creteur J, Preiser JC, Dubois MJ, Vincent JL. Microvascular blood flow is altered in patients with sepsis. Am J Respir Crit Care Med. 2002 Jul 1;166(1):98–104.

101. Dyson A, Rudiger A, Singer M. Temporal changes in tissue cardiorespiratory function during faecal peritonitis. Intensive Care Med. 2011 Jul;37(7):1192–200.

102. De Blasi RA, Palmisani S, Alampi D, Mercieri M, Romano R, Collini S, et al. Microvascular dysfunction and skeletal muscle oxygenation assessed by phase-modulation near-infrared spectroscopy in patients with septic shock. Intensive Care Med. 2005 Dec;31(12):1661–8.

103. Hotchkiss RS, Tinsley KW, Swanson PE, Grayson MH, Osborne DF, Wagner TH, et al. Depletion of dendritic cells, but not macrophages, in patients with sepsis. J Immunol. 2002 Mar 1;168(5):2493–500.

104. Felmet KA, Hall MW, Clark RSB, Jaffe R, Carcillo JA. Prolonged lymphopenia, lymphoid depletion, and hypoprolactinemia in children with nosocomial sepsis and multiple organ failure. J Immunol. 2005 Mar 15;174(6):3765–72.

105. Hotchkiss RS, Tinsley KW, Swanson PE, Schmieg RE, Hui JJ, Chang KC, et al. Sepsis-induced apoptosis causes progressive profound depletion of B and CD4+ T lymphocytes in humans. J Immunol. 2001 Jun 1;166(11):6952–63.

106. Peck-Palmer OM, Unsinger J, Chang KC, McDonough JS, Perlman H, McDunn JE, et al. Modulation of the Bcl-2 family blocks sepsis-induced depletion of dendritic cells and macrophages. Shock. 2009 Apr;31(4):359–66.

107. Boomer JS, To K, Chang KC, Takasu O, Osborne DF, Walton AH, et al. Immunosuppression in patients who die of sepsis and multiple organ failure. JAMA. 2011 Dec 21;306(23):2594–605.

108. Torgersen C, Moser P, Luckner G, Mayr V, Jochberger S, Hasibeder WR, et al. Macroscopic postmortem findings in 235 surgical intensive care patients with sepsis. Anesth Analg. 2009 Jun;108(6):1841–7.

109. Osuchowski MF, Welch K, Siddiqui J, Remick DG. Circulating cytokine/inhibitor profiles reshape the understanding of the SIRS/CARS continuum in sepsis and predict mortality. J Immunol. 2006 Aug 1;177(3):1967–74.

110. Tamayo E, Fernández A, Almansa R, Carrasco E, Heredia M, Lajo C, et al. Pro- and anti-inflammatory responses are regulated simultaneously from the first moments of septic shock. Eur Cytokine Netw. 2011 Jun;22(2):82–7.

111. Leentjens J, Kox M, van der Hoeven JG, Netea MG, Pickkers P. Immunotherapy for the adjunctive treatment of sepsis: from immunosuppression to immunostimulation. Time for a paradigm change? Am J Respir Crit Care Med. 2013 Jun 15;187(12):1287–93.

112. Hotchkiss RS, Opal S. Immunotherapy for sepsis--a new approach against an ancient foe. N Engl J Med. 2010 Jul 1;363(1):87–9.

113. Landelle C, Lepape A, Voirin N, Tognet E, Venet F, Bohé J, et al. Low monocyte human leukocyte antigen-DR is independently associated with nosocomial infections after septic shock. Intensive Care Med. 2010 Nov;36(11):1859–66.

114. Monneret G, Lepape A, Voirin N, Bohé J, Venet F, Debard AL, et al. Persisting low monocyte human leukocyte antigen-DR expression predicts mortality in septic shock. Intensive Care Med. 2006 Aug;32(8):1175–83.

115. Monneret G, Venet F, Pachot A, Lepape A. Monitoring immune dysfunctions in the septic patient: a new skin for the old ceremony. Mol Med. 2008 Feb;14(1–2):64–78.

116. Wolk K, Döcke W, von Baehr V, Volk H, Sabat R. Comparison of monocyte functions after LPS- or IL-10-induced reorientation: importance in clinical immunoparalysis. Pathobiology. 1999;67(5–6):253–6.

117. Nolan A, Kobayashi H, Naveed B, Kelly A, Hoshino Y, Hoshino S, et al. Differential role for CD80 and CD86 in the regulation of the innate immune response in murine polymicrobial sepsis. PLoS One. 2009 Aug 12;4(8):e6600.

118. Keir ME, Butte MJ, Freeman GJ, Sharpe AH. PD-1 and its ligands in tolerance and immunity. Annu Rev Immunol. 2008;26:677–704.

119. Gabrilovich DI, Nagaraj S. Myeloid-derived suppressor cells as regulators of the immune system. Nat Rev Immunol. 2009 Mar;9(3):162–74.

120. Pillay J, Kamp VM, van Hoffen E, Visser T, Tak T, Lammers JW, et al. A subset of neutrophils in human systemic inflammation inhibits T cell responses through Mac-1. J Clin Invest. 2012 Jan;122(1):327–36.

121. Kessel A, Bamberger E, Masalha M, Toubi E. The role of T regulatory cells in human sepsis. J Autoimmun. 2009 Jun;32(3–4):211–5.

122. Sakaguchi S. Naturally arising CD4+ regulatory t cells for immunologic self-tolerance and negative control of immune responses. Annu Rev Immunol. 2004;22:531–62.

123. Taams LS, van Amelsfort JMR, Tiemessen MM, Jacobs KMG, de Jong EC, Akbar AN, et al. Modulation of monocyte/macrophage function by human CD4+CD25+ regulatory T cells. Hum Immunol. 2005 Mar;66(3):222–30.

124. Venet F, Pachot A, Debard AL, Bohe J, Bienvenu J, Lepape A, et al. Human CD4+CD25+ regulatory T lymphocytes inhibit lipopolysaccharide-induced monocyte survival through a Fas/Fas ligand-dependent mechanism. J Immunol. 2006 Nov 1;177(9):6540–7.

125. El Gazzar M, Yoza BK, Chen X, Hu J, Hawkins GA, McCall CE. G9a and HP1 couple histone and DNA methylation to TNFalpha transcription silencing during endotoxin tolerance. J Biol Chem. 2008 Nov 21;283(47):32198–208.

126. Lyn-Kew K, Rich E, Zeng X, Wen H, Kunkel SL, Newstead MW, et al. IRAK-M regulates chromatin remodeling in lung macrophages during experimental sepsis. PLoS One. 2010 Jun 16;5(6):e11145.

127. Carson WF, Cavassani KA, Ito T, Schaller M, Ishii M, Dou Y, et al. Impaired CD4+ T-cell proliferation and effector function correlates with repressive histone methylation events in a mouse model of severe sepsis. Eur J Immunol. 2010 Apr;40(4):998–1010.

128. Carson WF, Cavassani KA, Dou Y, Kunkel SL. Epigenetic regulation of immune cell functions during post-septic immunosuppression. Epigenetics. 2011 Mar;6(3):273–83.

129. Benoit M, Desnues B, Mege JL. Macrophage polarization in bacterial infections. J Immunol. 2008 Sep 15;181(6):3733–9.

130. Rigato O, Salomao R. Impaired production of interferon-gamma and tumor necrosis factor-alpha but not of interleukin 10 in whole blood of patients with sepsis. Shock. 2003 Feb;19(2):113–6.

131. Jarczak D, Kluge S, Nierhaus A. Sepsis-Pathophysiology and Therapeutic Concepts. Front Med (Lausanne). 2021;8:628302.

132. Honoré PM, Jacobs R, Boer W, Joannes-Boyau O, De Regt J, De Waele E, et al. New Insights Regarding Rationale, Therapeutic Target and Dose of Hemofiltration and Hybrid Therapies in Septic Acute Kidney Injury. Blood Purif. 2012;33(1–3):44–51.

133. Zhang P, Yang Y, Lv R, Zhang Y, Xie W, Chen J. Effect of the intensity of continuous renal replacement therapy in patients with sepsis and acute kidney injury: a single-center randomized clinical trial. Nephrology Dialysis Transplantation. 2012 Mar 1;27(3):967–73.

134. Joannes-Boyau O, Honoré PM, Perez P, Bagshaw SM, Grand H, Canivet JL, et al. High-volume versus standard-volume haemofiltration for septic shock patients with acute kidney injury (IVOIRE study): a multicentre randomized controlled trial. Intensive Care Med. 2013 Sep;39(9):1535–46.

135. Morgera S, Slowinski T, Melzer C, Sobottke V, Vargas-Hein O, Volk T, et al. Renal replacement therapy with high-cutoff hemofilters: impact of convection and diffusion on cytokine clearances and protein status. American Journal of Kidney Diseases. 2004 Mar;43(3):444–53.

136. David S, Bode C, Putensen C, Welte T, Stahl K, The EXCHANGE study group, et al. Adjuvant therapeutic plasma exchange in septic shock. Intensive Care Med. 2021 Mar;47(3):352–4.

137. Livigni S, Bertolini G, Rossi C, Ferrari F, Giardino M, Pozzato M, et al. Efficacy of coupled plasma filtration adsorption (CPFA) in patients with septic shock: A multicenter randomised controlled clinical trial. BMJ Open. 2014 Jan;4(1):e003536.

138. Giménez-Esparza C, Portillo-Requena C, Colomina-Climent F, Allegue-Gallego JM, Galindo-Martínez M, Mollà-Jiménez C, et al. The premature closure of ROMPA clinical trial: mortality reduction in septic shock by plasma adsorption. BMJ Open. 2019 Dec;9(12):e030139.

139. Klein DJ, Foster D, Schorr CA, Kazempour K, Walker PM, Dellinger RP. The EUPHRATES trial (Evaluating the Use of Polymyxin B Hemoperfusion in a Randomized controlled trial of Adults Treated for Endotoxemia and Septic shock): study protocol for a randomized controlled trial. Trials. 2014 Dec;15(1):218.

140. Dellinger RP, Bagshaw SM, Antonelli M, Foster DM, Klein DJ, Marshall JC, et al. Effect of Targeted Polymyxin B Hemoperfusion on 28-Day Mortality in Patients With Septic Shock and Elevated Endotoxin Level: The EUPHRATES Randomized Clinical Trial. JAMA. 2018 Oct 9;320(14):1455.

141. Klein DJ, Foster D, Walker PM, Bagshaw SM, Mekonnen H, Antonelli M. Polymyxin B hemoperfusion in endotoxemic septic shock patients without extreme endotoxemia: a post hoc analysis of the EUPHRATES trial. Intensive Care Med. 2018 Dec;44(12):2205–12.

142. Kellum JA, Venkataraman R, Powner D, Elder M, Hergenroeder G, Carter M. Feasibility study of cytokine removal by hemoadsorption in brain-dead humans*: Critical Care Medicine. 2008 Jan;36(1):268–72.

143. Houschyar KS, Pyles MN, Rein S, Nietzschmann I, Duscher D, Maan ZN, et al. Continuous Hemoadsorption with a Cytokine Adsorber during Sepsis – a Review of the Literature. Int J Artif Organs. 2017 May;40(5):205–11.

144. Honoré PM, De Bels D, Spapen HD. An update on membranes and cartridges for extracorporeal blood purification in sepsis and septic shock: Current Opinion in Critical Care. 2018 Dec;24(6):463–8.

145. Friesecke S, Stecher SS, Gross S, Felix SB, Nierhaus A. Extracorporeal cytokine elimination as rescue therapy in refractory septic shock: a prospective single-center study. J Artif Organs. 2017 Sep;20(3):252–9.

146. Kogelmann K, Jarczak D, Scheller M, Drüner M. Hemoadsorption by CytoSorb in septic patients: a case series. Crit Care. 2017 Dec;21(1):74.

147. Friesecke S, Träger K, Schittek GA, Molnar Z, Bach F, Kogelmann K, et al. International registry on the use of the CytoSorb® adsorber in ICU patients: Study protocol and preliminary results. Med Klin Intensivmed Notfmed. 2019 Nov;114(8):699–707.

148. Patera AC, Drewry AM, Chang K, Beiter ER, Osborne D, Hotchkiss RS. Frontline Science: Defects in immune function in patients with sepsis are associated with PD-1 or PD-L1 expression and can be restored by antibodies targeting PD-1 or PD-L1. Journal of Leukocyte Biology. 2016 Dec;100(6):1239–54.

149. Hotchkiss RS, Colston E, Yende S, Crouser ED, Martin GS, Albertson T, et al. Immune checkpoint inhibition in sepsis: a Phase 1b randomized study to evaluate the safety, tolerability, pharmacokinetics, and pharmacodynamics of nivolumab. Intensive Care Med. 2019 Oct;45(10):1360–71.

150. Sprent J, Surh CD. Interleukin 7, maestro of the immune system. Seminars in Immunology. 2012 Jun;24(3):149–50.

151. Francois B, Jeannet R, Daix T, Walton AH, Shotwell MS, Unsinger J, et al. Interleukin-7 restores lymphocytes in septic shock: the IRIS-7 randomized clinical trial. JCI Insight. 2018 Mar 8;3(5):e98960.

152. Rieben R, Roos A, Muizert Y, Tinguely C, Gerritsen AF, Daha MR. Immunoglobulin M–Enriched Human Intravenous Immunoglobulin Prevents Complement Activation In Vitro and In Vivo in a Rat Model of Acute Inflammation. Blood. 1999 Feb 1;93(3):942–51.

153. Wand S, Klages M, Kirbach C, Warszawska J, Meybohm P, Zacharowski K, et al. IgM-Enriched Immunoglobulin Attenuates Systemic Endotoxin Activity in Early Severe Sepsis: A Before-After Cohort Study. Eller K, editor. PLoS ONE. 2016 Aug 9;11(8):e0160907.

154. Walpen AJ, Laumonier T, Aebi C, Mohacsi PJ, Rieben R. Immunoglobulin M-enriched intravenous immunoglobulin inhibits classical pathway complement activation, but not bactericidal activity of human serum. Xenotransplantation. 2004 Mar;11(2):141–8.

155. Nachbaur D, Herold M, Gächter A, Niederwieser D. Modulation of alloimmune response *in vitro* by an IgM-enriched immunoglobulin preparation (Pentaglobin). Immunology. 1998 Jun 14;94(2):279–83.

156. Kalvelage C, Zacharowski K, Bauhofer A, Gockel U, Adamzik M, Nierhaus A, et al. Personalized medicine with IgGAM compared with standard of care for treatment of peritonitis after infectious source control (the PEPPER trial): study protocol for a randomized controlled trial. Trials. 2019 Dec;20(1):156.