Non-antibiotic treatment options in patients with sepsis and septic shock syndrome

Hradil, Marian Thomas

UNIVERSITY OF ZAGREB
SCHOOL OF MEDICINE

Marian Thomas Hradil

Non-Antibiotic Treatment Options In Patients With Sepsis And Septic Shock Syndrome

GRADUATION PAPER

Zagreb, 2021

This graduate thesis was made at the Department of Internal Medicine, Unit of Clinical Pharmacology, University Hospital Centre Zagreb, and School of Medicine, Zagreb, Croatia, mentored by Prof. dr. sc. Robert Likić and was submitted for evaluation in the academic year 2020/2021.

ABBREVIATIONS

AKI	Acute kidney injury
ARDS	Acute respiratory distress syndrome
DAMP	Danger-associated molecular pattern
DIC	Disseminated intravascular coagulation
FMT	Fecal microbiota transplantation
GM-CSF	Granulocyte-macrophage colony-stimulating factor
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
ICU	Intensive care unit
IL	Interleukin
IV	Intravenously
IVIG	Intravenous immunoglobulins
IVIgGM	IgM-enriched immunoglobulins
LPS	Lipopolysaccharide
MHC	Major histocompatibility complex
NP	Nanoparticles
PAMP	Pathogen-associated molecular pattern
SOFA	Sequential organ failure assessment
TLR	Toll-like receptor
TNF	Tumor necrosis factor

Table of Contents

Summary

Title: Non-antibiotic treatment options in patients with sepsis and septic shock syndrome

Author: Marian Thomas Hradil

Sepsis, currently defined as dysregulated systemic immune response of the host to an infectious insult with subsequent life-threatening organ dysfunction, is a common multifactorial disease. In conjunction with septic shock, the most severe form of sepsis characterized by profound circulatory and metabolic derangement, the two are one of the leading causes of mortality and critical illness worldwide. Despite improvements in the management of sepsis, including timely administration of antibiotic agents, optimization of intravascular volume status and supportive care, no specific treatment exists as of yet. However, an increased understanding of the pathophysiological mechanisms and the biphasic course consisting of pro and anti-inflammatory states, has paved the way for alternative treatment options. Active research has spawned candidate drugs including extracorporal blood purification, immune modulation with corticosteroids, intravenous immunoglobulins, mesenchymal stem cells, GM-CSF, rhIL-7, thymosin-α1 or nanoparticles. Vasopressin-agonists and beta-blocker are agents regulating the cardiovascular system. Promising strategies targeting other organ systems involve novel agents like alkaline phosphatase, thrombomodulin and the gut microbiome. Finally, micronutrients have been assessed as treatment alternatives. However, investigations on new efficient therapy agents are hampered by the broad spectrum of the syndrome, its definition and the heterogeneity of the patients affected. Lacking identification of sepsis subtypes limits the applicability of research results. The outcomes of studies conducted on alternative treatment agents for sepsis and septic shock distance themselves from one-size-fits-all, to focus on individualized medicine including careful treatment timing and clinical pheno- and genotyping. In this context, the mentioned agents are currently of particular interest in treatment for sepsis and give a promising outlook. Therefore, the aim should be stratification of disease and patient to identify who benefits most from therapy.

Keywords: Sepsis; Septic shock; Critically ill; Therapy

Sažetak

Naslov: Mogućnosti ne-antibiotskog liječenja bolesnika sa sepsom i sindromom septičkog šoka

Autor: Marian Thomas Hradil

Sepsa je česta multifaktorna bolest koja se trenutno definira kao neregulirani sustavni imunološki odgovor domaćina na infekciju s naknadnom disfunkcijom organa koja je opasna po život. Najteži oblik sepse je septički šok, kojeg karakterizira poremećaj cirkulacije i metabolizma. Zajedno predstavljaju jedan od vodećih uzroka smrtnosti i kritičnih bolesti u svijetu. Unatoč napretku postignutom u liječenju sepse, uključujući pravovremenu primjenu antibiotskih sredstava, optimizaciju stanja intravaskularnog volumena i potpornu njegu, ne postoji specifično liječenje. Veće razumijevanje patofizioloških mehanizama i dvofaznog tijeka koji se sastoji od pro i protuupalnih stanja, otvorilo je put alternativnim mogućnostima liječenja. Postojeća istraživanja rezultirali su pronalaskom mogućih lijekova, uključujući ekstrakorporalno pročišćavanje krvi, imunološku modulaciju kortikosteroidima, intravenske imunoglobuline, mezenhimske matične stanice, GM-CSF, rhIL-7, timozin-α1 ili nanočestice. Vasopresin agonisti i beta-blokatori su lijekovi koji djeluju na kardiovaskularni sustav. Obećavajuće strategije usmjerene na druge organske sustave uključuju nova terapija poput alkalne fosfataze, trombomodulina i crijevnog mikrobioma. Konačno, mikroelementi su procijenjeni kao alternative liječenju. Međutim, istraživanja novih učinkovitih terapijskih sredstava otežana su širokim spektrom sindroma, njegovom definicijom i heterogenošću pacijenata. Nedostatak identifikacije podtipova sepse ograničava primjenjivost rezultata istraživanja. Rezultati studija provedenih alternativnim terapijama za liječenje sepse i septičnog šoka, ukazuju na veću uspješnost individualnog pristupa pacijentu od univerzalnog. Takav pristup uključuje pažljivo određivanje vremena liječenja te kliničke feno- i genotipiziranje. Upravo zbog prethodno navedenog, spomenuta sredstva trenutno su od posebnog interesa za liječenje sepse i daju obećavajuće izglede stoga bi cilj trebao biti raslojavanje bolesti i pacijenta kako bi se utvrdilo tko će najvjerojatnije imati koristi od terapije.

Ključne riječi: Sepsa; Septički šok; Kritično bolestan; Terapija

Introduction

Recognizing sepsis as a global health priority in May 2017, the World Health Organization (WHO) adopted a resolution to improve diagnosis, prevention and management of this life-threatening disease. For centuries, the term “sepsis” had been used broadly, dating back to the time of Hippocrates who regarded it as a process of rotting flesh and wound fester (1). More recently, sepsis has been defined as life-threatening organ dysfunction caused by a dysregulated host response to infection. As a subset of sepsis and associated with a higher risk of mortality, septic shock describes the state of circulatory, cellular and metabolic dysfunction (2). Despite this long history and advances in medical care, sepsis remains one of the leading causes of critical illness and death globally. Indeed, mortality from septic shock is severly high, at nearly 35% to 40%. Overall, the worldwide incidence has increased over the past years and is expected to adhere to this trend, in light of the ageing population, increased chronic diseases and immunosuppressant and chemotherapy drug use (3). In light of its known impact, research focused on understanding the complex mechanisms of the immune responses underlying sepsis. However, despite substantial progress, there is no specific treatment for sepsis, and the basic elements of management have not changed for decades. The Surviving Sepsis Campaign guidelines summarize current recommendations. The timely initiation of broad-spectrum antibiotics is the mainstay of treatment. Still, there is a strong urgency for specific treatment options in sepsis and septic shock. The worldwide increasing frequency of antimicrobial resistant pathogen strains, antibiotic toxicity and superinfection risk further emphasizes this need (2). By presenting and discussing pathophysiological based starting points and current state of research, the aim of this review is to give an insight into possible non-antibiotic treatment options for sepsis and septic shock.

Pathophysiology

Describing the human response mechanism which underlies the state of sepsis is complex. Despite progress made in the comprehension of pathophysiology, it remains unclear why part of patient's immune response successfully fights infection, while others slide off into a dysregulated state. Contrary to previous assumptions, the septic course encompasses both, a state of excessive inflammation and immunosuppression, unable to return to homeostasis.
However, the most common starting point of this highly heterogeneous and multifaceted syndrome is infection of the lungs, comprising 64% of all cases (4). The subsequent response of the body is mediated by the innate and adaptive immune system. The former consists of pattern recognition receptor (PRR) bearing cells, including macrophages, polymorphonuclear leukocytes, dendritic cells and epithelial cells. The Toll-like receptor (TLR) family, cytoplasmic receptors and mannose receptors are able to recognize pathogen-associated molecular patterns (PAMPs). PAMPs are bacterial triggers that activate the innate immune system. Components found in the bacterial cell membrane, such as lipopolysaccharides (LPS), are the prototypical class of PAMPs. Flagellin, peptidoglycan, bacterial DNA and glycoproteins are further examples. PPRs can also recognize danger-associated molecular patterns (DAMPs), which are released during the inflammatory insult. The adaptive immunity, also known as acquired immune system, is mediated by cellular responses via T- and B-cells. Additionally, this type of immunity constitutes the immunological memory. Not only the bacterial insult but also failure of timely activation and cessation of immune activation leads to tissue damage. After binding of microbial components to the immune cell receptors, downstream signaling leads to release of proinflammatory cytokines (e.g. TNF-α, IL-1, IL-6), chemokines (e.g. ICAM-1, VCAM-1) and nitric oxide. Subsequently, more inflammatory cells, like leukocytes, get recruited. As mentioned before, the overall response is regulated by a mixed proinflammatory and anti-inflammatory balance of mediators. Although this state involves more signalling pathways, to explain these in detail would be beyond the scope of this review. Once the host response becomes dysregulated, including the so-called cytokine storm of uncontrolled proinflammatory mediator production, sepsis occurs. Furthermore, transition to an immunosuppressive state follows at variable times. Particularly in elderly people this state may dominate. The generalized immune reaction leads to extensive cell damage and as an end result to multiple organ dysfunction. The exact causative mechanisms are still unknown but possibly encompass tissue hypoxia, direct cell damage by mediators and products of inflammation, in addition to altered apoptosis. Part of the cumulative outcome is capillary leakage and a distorted microcirculation. In the end, sepsis is a syndrome involving all organ systems (5).

Fundamentals of Management

Opinions regarding the optimal treatment of patients with sepsis and septic shock are diverse up to now. Early administration of antibiotics, source identification and control, and resuscitation are widely accepted. Despite these, the Surviving Sepsis Campaign guidelines reflect the agreement on the fundamentals of treatment (2).

So as to reduce sepsis-related mortality, the “hour-1 bundle” was introduced, which highlights explicitly the importance of immediate initiation of management. Lactate levels should get obtained and if initial lactate is elevated (> 2 mmol/L), it should be remeasured within 2-4 hours. Prior to administration of antibiotic agents, blood cultures must be obtained to prevent sterilization. Furthermore, empiric broad-spectrum antibiotic therapy should be introduced. In most cases, this should be directed against gram-positive and gram-negative bacteria, and if there is the suspicion of an intra-abdominal process, anaerobic coverage has to be warranted. When the results of the pathogen identification including sensitivities are known, the therapy should be narrowed down. Fluid resuscitation is crucial, in order to address septic shock and tissue hypoperfusion. Moreover, guidelines recommend rapid administration of 30 ml/kg intravenous crystalloids (2). Since concerns of fluid overload and its harmful effects have been raised, continuous infusion beyond initial measures requires careful assessment (4). Lastly, for a hypotensive patient after or during fluid resuscitation, vasopressors should be applied to maintain the mean arterial pressure (MAP) ≥ 65 mm Hg. The Surviving Sepsis Campaign guidelines recommend norepinephrine as first-line agent (2). Over the entire course of sepsis, high-quality supportive care has to be warranted as it has been demonstrated to correlate with improved outcomes. Nursing, specific organic support, mechanical ventilation and nutritional support are the substance of this approach (5).

Corticosteroids

Dating back to the first proposal for sepsis treatment in the 1950s, corticosteroids have a long history of research (6). Agents like methylprednisolone, dexamethasone and hydrocortisone are known to suppress inflammation. Therefore, in various inflammatory conditions, like asthma and rheumatoid arthritis, they have been utilized successfully. Very recently, corticosteroids were tested as treatment option in critically ill COVID-19 patients, showing promising results in terms of mortality reduction (7). However, due to beneficial effects on blood vessels and hemodynamics, hydrocortisone is included in the international treatment guidelines if adequate fluid resuscitation and vasopressor therapy are ineffective to restore hemodynamic stability (2). Given the known catecholamine-sparing effects in vasodilatory shock known, research focused firstly on high dose corticosteroid administration (IV hydrocortisone of ≥ 400 mg/day). However, most results showed no valuable improvement in reversal of the pathophysiological shock state. Changing the aim, low dose corticosteroids (IV hydrocortisone < 400 mg/day) yielded benefits in reversal of shock. Contrary to assumptions, it was found that the hemodynamic effects were unrelated to the severity of illness (8). Currently, the surviving sepsis campaign guidelines suggest IV hydrocortisone at a dose of 200 mg per day (2). Furthermore, corticosteroids interfere with proinflammatory signaling pathways through inhibition of NF-kß. Additionally, neutrophil activation and production of inflammatory cytokines, such as IL-6 and IL-8, is decreased. Another reason for the impulse to administer corticosteroids is based on the relative corticosteroid deficiency due to inadequate adrenal response in critically ill patients (8). Notwithstanding the physiologic rationale, research data remained inconclusive regarding the therapeutic effects in sepsis and septic shock for a long time. This uncertainty is reflected in the strength of recommendation of the above-mentioned international guidelines, which refers to only a weak recommendation and low quality of evidence (2).

In the recent years, two larger randomized controlled trials were conducted regarding the use of corticosteroids (9,10). Both studies, published in the year 2018, showed different results. Annane et al. (9) found in their trial, which involved 1,241 patients receiving hydrocortisone plus fludrocortisone, a lower 90-day all-cause mortality if treatment was offered compared to placebo. Hydrocortisone, administered to 3,658 patients, did not result in lower mortality in contrast to the placebo group (10). Still, one should note that both trials differed in several respects, for example in the severity of illness, type and method (intermittent boluses vs continuous) of drugs administered. So as to assess the wide range of conducted studies and gain insight in the treatment of septic patients with corticosteroids, the most recent Cochrane meta-analysis evaluated the data from 61 randomized clinical trials (11). The promising results showed a slightly reduced 28-day mortality with moderate-certainty evidence.

Moreover, the patients treated with corticosteroids had a large reduction in ICU and length of hospital stay with high-certainty evidence. The positive trend in survival and length of stay was also reflected in similar meta-analyses (12,13). The same systematic reviews also report of vasopressor sparing effects and a faster shock reversal. One should remark that most evidence is based on studies using hydrocortisone as drug of choice. Besides these findings' suggestion of a beneficial role in the sepsis treatment, harmful effects have to be considered. Apprehensions of corticosteroids increasing the risk of superinfections, gastrointestinal bleeding, stroke, cardiac events and neuropsychiatric effects were unsubstantiated (11–13). However, the results of the mentioned meta-analyses coincided in a higher finding of hypernatraemia, hyperglycaemia and neuromuscular weakness.

To conclude, in light of the considerable amount of studies, corticosteroids seem promising in increasing survival and decreasing the length of stay in patients with sepsis and septic shock, while adverse effects compared to placebo or usual treatment are minimal. Furthermore, corticosteroids have been researched in combination therapy with ascorbic acid and thiamine. They have been proposed to be beneficial as treatment option. This will be discussed in detail in the following chapter on vitamin C.

Vitamin C

Ascorbic acid is a natural micronutrient with antioxidant effects that serves as an important cofactor in processes involving iron and copper-containing enzymes. It became prominent for its essential role in humans with James Lind's discovery of scurvy treatment in the 18th century (14). From thereon, the beneficial effects of vitamin C to prevent disease have often been proposed. The key physiologic role lies in its function as scavenger of free radicals. This gives rise to possible beneficial effects in the sepsis treatment by protecting the endothelial function and improving micro-vascular flow (15). Elimination of reactive oxygen species counteracts pathophysiological septic shock changes like endothelial cell apoptosis, smooth muscle mediated vasodilation and the permeability of vessels. The characteristic hypotension refractory to catecholamines may be alleviated by vasopressor sparing effects. Ascorbate enhances the endogenous synthesis of norepinephrine and vasopressin by acting as a cofactor of the respective enzymes (16). Furthermore, it has been stated that vitamin C has bacteriostatic activity (17). By complex interactions, such as preventing the activation of nuclear factor-κB leading to a reduction in inflammatory mediators and effects on the macrophage function, ascorbic acid serves as an immunoregulatory agent (18).

Previous studies showed that during critical illness, the plasma and intracellular levels of vitamin C are decreased (19). Findings by Carr et al. (20) reported that 40% of patients in the ICU with septic shock had serum levels close to the diagnosis of scurvy (< 11.3 u/mol/l).

Recently, the results of the meta-analysis by Langlois et al. (19) showed a possible tendency towards mortality reduction when administering vitamin C high dose intravenously. A large multicenter study by Fowler et al. (21), including 167 patients with sepsis and ARDS who received either a vitamin C infusion or a placebo every 6 hours for 96 hours, was designed to determine the effects of ascorbic acid on organ failure scores and inflammatory markers. Vitamin C did not significantly improve the Sequential Organ Failure Assessment (SOFA) score or inflammatory markers, but showed a lower 28-day mortality rate during the first 96 hours (29.8%) compared to the placebo group (46.3%). Three other systematic reviews and meta-analysis investigated the effects of vitamin C in critically ill subjects. In contrast to the prior results, the outcomes indicated that no significant reduction in the incidence of mortality took place. Still, a common finding was that the intravenous administration of ascorbic acid alone was associated with decreased duration of mechanical ventilator support, decreased length of ICU stay and a decreased need for vasopressors (22–24).
Despite these results, the evidence for the use of vitamin C alone still remains inconclusive and needs further research. Still, the treatment seems a promising option.

A new approach distances itself from the use of ascorbic acid as monotherapy, to the combination of this drug with corticosteroids and thiamine. The concept of these cheap and available drugs builds on their synergistic effects when administered together. These include a decreased oxidative stress, improved endothelial function, supported catecholamine synthesis, decreased immune suppression and improved aerobic processes. A more detailed insight of the physiologic effects is given in the chapters of each individual drug. It should be mentioned that, besides an ascorbic acid deficiency, septic patients showed a prevalence of thiamine deficiency as well. This ranged between 20-71% (25).
A study conducted by Marik et al. (15) in the year 2017 on 47 patients presented a positive outlook for this therapy. The four-day combination of hydrocortisone 50 mg IV every 6 hours, 1500 mg of vitamin C IV every 6 hours and thiamine 200 mg IV every 12 hours demonstrated significant effects. The hospital mortality was reduced from 40.4% to 8.5%. Moreover, a decreased SOFA-score and earlier weaning off vasopressors in the treatment group advocated the use of the combination treatment in sepsis patients. However, the small size, study design and no blinding are certain limitations of the scope. Another study by Byerly et al. (26) involving data of 146 patients concluded that the treatment yielded an increased survival and lactate clearance. However, these preliminary positive results did not get replicated further by following studies. Several randomized controlled trials conducted in the recent years following Marik et al. publication showed similarities with regard to a not significantly improved survival. Nevertheless, a decreased need of vasopressors, a better SOFA score and an improved lactate clearance were discovered (27–29). A retrospective

before-and-after cohort study with 229 septic shock patients further underlined these findings (30).

Although the current evidence does not fully support the direct effects of the combination therapy on a survival benefit, positive effects have been proven and the data will support further research and a better understanding of the role in the treatment of sepsis patients. In the future, other trials, like the large VICTAS trial (31), involving 501 patients, that terminated in October 2019 and still needs evaluation, will bring further insights. In the end, the combination treatment of the three drugs remains a promising approach with sufficient availability and cost efforts. If further research shows a safe and effective profile, the bundle will save billions of dollars and millions of life-years in the US (32).

Immunoglobulins

Immunoglobulins, secreted by differentiated B-cells, are a pivotal part of the defense against infection. In the humoral immunity, the classes of IgA, IgG and IgM play the most important role. Acting between the adaptive and innate immune system, immunoglobulins possess both pro- and anti-inflammatory properties. Intravenous immunoglobulins are prepared from a donor pool. This kind of treatment strategy has been already applied to hematological, immunological and neurological illnesses. The rationale backing up this application is the direct antibacterial, via recognition and removal of pathogens, anti-inflammatory, via inhibition of mediator gene transcription and anti cell-death effects. However, in sepsis a deficit of immunoglobulins is a common finding. This may affect phagocytosis justifying this treatment approach. Furthermore, IgG can block superantigens released by staphylococci and streptococci. Polyclonal IVIG contain autoantibodies that neutralize cytokines leading to anti-inflammatory impacts (33).

Trials investigating the administration of IgG in septic patients have shown disappointing results (34,35). The largest, a Phase III trial involving 653 patients with sepsis or septic shock, identified no effect on the 28-day mortality (35). As mentioned above, the most important constituents of the humoral immunity are IgA, IgG and IgM. However, the classical IVIG agents mainly contained IgG. More recently, different, more physiologic, formulations have been articulated. Pentaglobin contains 12% IgM, 12% IgA and 76% IgG. Trimodulin has an even further increased amount of IgM, with 23%. These two preparations are summarized under the term IgM-enriched immunoglobulins (33). The results of many studies and meta-analysis of these agents raises a more promising outlook, suggesting that IVIgGM is connected to an improved mortality rate (36–40). The most recent meta-analysis, by Cui et al. (40), evaluated nineteen studies comprising 1,530 patients. The treatment group experienced favorable outcomes regarding a reduced mortality risk and a shortened length of

mechanical ventilation. However, similar to the other meta-analysis, Cui et al. highlighted the drawback of heterogeneity, with the effects tending to be smaller if only high quality studies are considered. The CIGMA study (41), a double-blind Phase II study of patients receiving trimodulin or placebo, could not yield significant differences in mortality. Nevertheless, post hoc analyses performed in a subset group with either high C-reactive protein, procalcitonin or low IgM suggested a significant relative reduction of 54-68% in mortality. The results suggest that a patient profile with heightened inflammatory indicators has to be identified in future treatments to warrant beneficial effects. Besides ambiguities with regard to the subjects to whom to deliver IVIG, the timing is also of question. Overall, most studies are in favor of an early administration. In the CIGMA trial, IVIG was to be administered within 12h and Berlot et al.' study (42) of 355 patients reported a connection of early application with a more positive outcome.

In order to draw a full conclusion regarding the efficacy of IVIG in sepsis therapy, one must take into consideration safety and costs as well. Even though most of the discussed studies assume a relatively safe profile of IVIG, some serious adverse reactions have been reported. These include thromboembolism, cholestasis and renal failure, more common in patients with pre-existing risk factors (33). Further, one should bear in mind the high costs of the IVIG formulations (43). The cost-benefit ratio should be taken into account before treatment. The future path of IVIG seems one of the most promising in the treatment of sepsis and septic shock. Still, the Surviving sepsis guidelines (2) advises against the use of this agent due to low quality of evidence.

Alkaline Phosphatase

As the renal function is assessed in the SOFA score, so as to determine a patient's status and organ function in the state of sepsis, the presence of acute kidney injury affects the prognosis. In general, half of the sepsis cases are admitted to the ICU because of reduced or absent function of one or multiple organs (44). In a clinical setting, AKI is characterized by a decrease in urine output and an increase in creatinine in the serum. Over 40% of AKI cases in patients at the ICU are attributable to the presence of sepsis (45). The mortality increases with increased AKI severity and sepsis related mortality approximately doubles in the occurrence of end-stage renal disease (46). In light of these correlations, alkaline phosphatase posed as a promising treatment, even though it was initially thought to be a direct antisepsis drug due to the dephosphorylizing of LPS and DAMPs and thus detoxifying action. LPS belongs to the group of PAMPs, which are key initiators of the inflammatory process in the body via activation of TLRs. Through complex interplay, renal microcirculation suffers damage and cell death, as a result of impaired microcirculatory flow and hypoxia.

Moreover, this cell death leads to release of DAMPs, proceeding the inflammatory processes and impacts. Long-term effects are fibrosis and chronic kidney disease (47).

The use of alkaline phosphatase yielded positive results showing improved survival and diminished inflammation in several animal models (48,49). Following the encouraging results of the animal trials, a small Phase II clinical trial with 36 septic patients was done by Heemskerk et al. (50). In addition to no safety issues, better survival rates, reduced proximal tubule injury, improved endogenous creatinine clearance and thus an improved renal function were demonstrated. A recent systematic review and meta-analysis conducted by Tang et al. (51) analyzed four randomized clinical trials involving 392 patients with alkaline phosphatase therapy, including the above mentioned. They concluded that the patients with sepsis associated AKI receiving treatment showed protective effects. The endogenous creatinine clearance enhanced at days 7, 14 and 28 with a dose of 0.212 mg/kg. Mortality showed an improvement at days 28 and 90 with a dose of 1.6 mg/kg. These findings correlate with a late effect, possibly explained by the effects of alkaline phosphatase on inflammation induced fibrosis. In the short-run, only overall AKI biomarkers improved.

Nevertheless, the longer-term benefits on renal function by alkaline phosphatase treatment in sepsis patients advocate for a future treatment option and further studies. The better survival of treated patients in comparison to patients with placebo impressively underlines this conclusion. As an interesting side note regarding the long term benefits, a recently published study proposed a novel role for alkaline phosphatase as protector of the integrity of the blood-brain barrier. This may reveal a therapeutic approach to decrease long-term cognitive impairment symptoms in sepsis patients and possibilities of altering drug pharmacokinetics which affect the brain (52).

Granulocyte-macrophage colony-stimulating factor (GM-CSF)

GM-CSF belongs to the first pioneer agents in the young field of immunostimulatory therapy for septic patients. Up to now, it is one of the most studied immune-activating agents. It has been in use for several years in neutropenic hematologic patients or in autoimmune diseases like rheumatoid arthritis. GM-CSF is a hematopoietic growth factor that mobilizes neutrophils and monocytes from the bone marrow. Acting on the JAK-STAT, MAPK and PI3K pathway, the agent leads to increased cell survival and proliferation. Moreover, in vitro experiments showed that, following administration, monocyte HLA-DR expression is increased as well as pro-inflammatory cytokine production.

Considering these results, it has been postulated that therapy with GM-CSF is potentially immune-activating in patients with sepsis associated immunoparalysis. Therefore, it was hypothesized that the rate of nosocomial and secondary infection and the resulting morbidity

and mortality decreases if applied (53). The studies conducted in the last years are diverse, spanning from neonates to elderly. A randomized controlled trial of non-neutropenic children with immunoparalysis demonstrated a facilitated recovery of TNF-α and a protection from nosocomial infections (54). In the study of 56 septic neonates, GM-CSF therapy increased the number of monocyte HLA-DR (55). Similar to agents introduced in the previous chapters, one must find the ideal time span for appropriate treatment. Taking this as a major factor, a Phase II study addressed the problem by implementing HLA-DR expression as biomarker guidance for immunosuppression in the study population. In this study, Meisel et al. (56) provided encouraging results showing improved immunocompetence, a shorter mechanical ventilation time and decreased length of stay in hospital. The authors state, that biomarker guided GM-CSF therapy is safe in septic patients. Recently, a similar Phase IIa trial using the personalized medicine approach by selecting only critically ill patients with impaired neutrophil phagocytosis was conducted. Besides similar findings according to the safety profile, the study supports the beneficial effects on monocyte HLA-DR and neutrophil phagocytosis, albeit limited to the patients responding (57). Since most trials tend to be small in size, it is helpful to take a look at the meta-analysis of twelve randomized controlled trials, conducted by Bo et al. (58). Unfortunately, the data analysis could not show any direct impact of GM-CSF treatment on the mortality. However, one positive result was the earlier resolution of infection. Regarding the disappointing results, one should keep in mind that GM-CSF was not the sole therapy in this study.

Generally, most of the data is still insufficient for a full evaluation of survival, and the treatment group is often unselected and evaluated based on the stage of immunosuppression. It thus may appear that the treatment has not a significant impact on septic patients, while there might be a subset of patients who benefit from it. Overall, this heterogeneity of immune system and responses among septic patients gives reason for future studies investigating how to select the right patients and combination of treatment.

Recombinant human interleukin-7 (rhIL-7)

Until recently, the classic approach to sepsis was to focus on the excessive inflammatory response of the body to the intruding pathogen. Nowadays, research started focusing more on the subsequent phase, the overriding immunosuppression. Efforts to improve the outcomes are steadily carried forward by new data. Patients with sepsis have a high one-year mortality rate, attributable to opportunistic infections. More precisely, over 70% of deaths are actually occurring after several days to weeks after the onset of sepsis (59,60). Immunostimulatory therapy agents, like interleukin-7, pursue the goal to prevent immunosuppression. IL-7 arises mainly from stromal cells in organs like the thymus, liver,

skin, intestine and peripheral lymphoid organs. This multifunctional cytokine affects T- and B-cells, being indispensable for the proliferation of naïve and memory T-cells (61).

As demonstrated by Venet at al. (62), *ex vivo* treatment of lymphocytes from septic patients leads to restored T-cell proliferation and IFN-y secretion. Proliferative and anti-apoptotic effects are believed to be mediated by IL-7 through recruitment of the STAT transcription factors and the phosphoinositide 3-kinase pathway activation. Animal models supported these findings of restored lymphocyte function, which came to be associated with improved survival (61). However, many of the anticipated results for clinical use of this immunostimulatory agent were posed by Francois et al. (63) in the IRIS-7 randomized clinical trial. Twenty-seven patients received recombinant human IL-7, also known under the name CYT107, in this double-blind, placebo controlled study. The analysis yielded a good tolerance for the administered agent. Positively striking were the immune characteristic findings. The absolute lymphocyte count and the circulating CD4+ and CD8+ T-cells were three- to four-fold elevated and even persisted for 2-4 weeks following cessation of treatment. Moreover, the results were consistent with previous studies on other immune system related diseases regarding the long-lasting effects on the lymphocyte count (64). This reversal in loss of adaptive immune cells gives promising future prospects of IL-7 in the sepsis treatment, as it is one of the hallmarks and probably a main mechanism in its morbidity and mortality. Unfortunately, the mentioned clinical trial did not assess differences in the mortalities between groups. When searching for new treatment alternatives, one must also consider the safety profile. Immunotherapies have been connected to harmful side effects, as seen in past studies involving different agents for oncological treatment. Fortunately, current literature does not report of any life-threatening adverse effects after IL-7 therapy. The most common side effect is a skin rash at the injection site (64).

Despite lack of objections regarding the safety of IL-7, one can agree that the present data is still insufficient to fully confirm reliability. Finally, the promising approach regarding immunotherapy in sepsis requires an individual approach, similar to other agents mentioned. Present investigations are currently searching for biomarkers to stratify suitable patients and the right timing of treatment. Patients receiving IL-7 should be in the immunosuppressed stage of sepsis to profit. Moreover, due to the patients' variation in response to sepsis and treatment, the combination of several immunomodulatory agents may increase the chances of success. Aiming at different targets in the immune system is the rationale advocating combination treatment. Still, one needs to understand the underlying mechanisms and concentrate on data collection.

Micronutrients (Thiamine, Vitamin D, L-carnitine, Selenium)

Part and parcel of the septic course is a high energy expenditure. The metabolic demands are elevated and fundamental biochemical processes are distorted; the cellular damage and this hypermetabolic state lead to deficiencies in the micronutrients. So as to restore homeostasis and counteract destructive processes like the generation of free radicals, the use of micronutrient treatment is of help. Its attractiveness lies in the simple, safe and cheap attributes of this therapy. This chapter will provide a brief overview of the micronutrients thiamine, vitamin D, L-carnitine and selenium as treatment option in sepsis. Vitamin C is discussed in the according chapter.

Thiamine is needed by the body as an important cofactor for enzymatic processes like carbohydrate metabolism, energy production and preservation of the redox status. Humans are not able to endogenously produce thiamine. By obtaining thiamine through dietary intake of cereal grains, beans, nuts and meat, the body can store up to 30 mg in the tissue. Still, the quick turnover leads to a deficiency within 2 weeks in absence of intake. If deficient, syndromes such as cardiac beriberi or Wernicke's encephalopathy might develop (18).
Among septic patients, one can often measure a deficiency state ranging in prevalence up to 71% (25). In previous investigations of lactic acidosis and reversing shock, thiamine administration yielded inconclusive results (18). Moskowitz et al. (65) studied thiamine as monotherapy in 88 patients with septic shock. The results made clear that no overall benefits on mortality, shock reversal or lactate levels could be derived. Improvement was only found in those with baseline thiamine deficiency. A post-hoc analysis was conducted in accordance with this study. This time, results were more promising, showing decreased requirements for renal replacement therapy and thus risk reduction for AKI (66).
Considering the inconsistency of these results, the evidence for use of thiamine remains debatable. One could consider supplementation in septic patients being of risk for deficiency. However, combining thiamine with corticosteroids and ascorbic acid is a promising new approach and further discussed in the chapter of vitamin C.

Vitamin D is widely known for its effects on bone strength. Obtained through either synthesis in the skin with the help of ultraviolet (UV) light, or directly absorbed from the gastrointestinal tract, the active form of vitamin D is calcitriol. However, vitamin D was found to play a role in the regulation of the innate and adaptive immune system with the detection of its receptor on T-cells, B-cells, neutrophils and antigen-presenting cells. It is determined that CD4+ Th1 cell production of inflammatory cytokines gets blocked, while IL-4, IL-5 and Il-10 production by Th2 cells is increased resulting in anti-inflammatory properties of the agent (67).

3,000 critically ill patients were evaluated by Moromizato et al.(68), showing that vitamin D deficiency is a significant predictor of sepsis and determines a high increase in mortality. In light of the assumption of the beneficial role of vitamin D, its repletion has been a promising target. On the other hand, the clinical studies up to day, even when stressing benefits, remain heterogeneous in their conclusions. The meta-analysis of six randomized clinical trials by Langlois et al. (69) summarizes the state of research. Unfortunately, no reduction in mortality or length of stay in hospital or ICU was found. Nevertheless, one of the included trials, involving 475 critically ill patients, showed a significantly lower hospital mortality in the severe vitamin D deficiency subgroup (70). A post hoc analysis of the same study, after excluding the early deaths to allow more time for action of vitamin D, revealed a reduction of 28-day mortality (71).
It can be concluded that there is evidence for a high prevalence of vitamin D insufficiency in septic patients with a potential impact on the outcome. Screening and treatment of deficient patients could be advised. However, when applied to clinical practice as therapy, studies diverge and suggest only a marginal effect as treatment option.

L-Carnitine, a coenzyme needed for ß-oxidation of fatty acids, has been investigated by a manageable number of studies. After promising animal studies, two clinical studies assessed a potential profit (25). Firstly, a Phase I trial of 31 patients, found no reduction of the SOFA score in the treatment group. However, there was improvement of mortality (72). The second was a Phase II clinical trial, involving 250 patients. Again, a significant reduction of the SOFA score could not be seen (73). It has been proposed that interpatient variability of the levocarnitine levels and unidentified patient characteristics have led to these disappointing results (74). Further research is needed.

Selenium is important for the production of antioxidant enzymes. It reaches the body via the dietary intake of dairy products, meat, grains and vegetables. The state of research provides insight into a possible selenium deficiency in patients with sepsis. Furthermore, it was postulated that low levels of selenium might be associated with mortality (25). The recent meta-analysis, by Li et al. (75), analyzed the data of 13 randomized controlled trials comparing selenium and placebo in septic patients. Besides an effect on duration of vasopressor therapy, length of hospital stay and incidence of ventilator-associated pneumonia, the results failed to show a mortality benefit. Thus, even given a rationale for administering selenium, current evidence is not in favor of suggesting clinical use.

Extracorporal Blood Purification

In order to improve therapeutic efficacy and support sepsis treatment, extracorporal blood purification emerged. The idea is to attenuate the sepsis-related enormous inflammatory response by clearance of PAMPs and DAMPs including endotoxins and cytokines. Various techniques were developed with different types of membranes. One strategy is to remove the endotoxins, with polymixin B beads being one of the most widely used. This device binds and neutralizes LPS in the circulation. However, it has been found to be toxic if administered directly into the blood stream. Therefore, the blood of the patients is filtered through the extracorporal system with polymixin fibers. Another strategy focusses on cytokine reduction. High-volume hemofiltration and coupled plasma filtration and adsorption are examples thereof. Finally, some therapies involve both techniques (76).

In light of the many different technologies and studies done on extracorporeal blood purification, meta-analysis can be of great help. Putzu et al. (77) conducted a meta-analysis recently, which assesses if this treatment option reduces mortality in sepsis and septic shock. Thirty-seven trials involving 2,499 patients were included. Hemoperfusion yielded a lower mortality, as did hemofiltration and plasmapheresis. However, unexpectedly hemoperfusion filters using polymixin coating did not result in a mortality difference. Unfortunately, as the flip side of these promising results, the quality of evidence is low.

Mixed results were also found by the meta-analysis conducted by Snow et al. (78). Only hemofiltration, endotoxin removal and nonspecific adsorption devices revealed a mortality benefit, not combined hemofiltration and adsorption or cytokine removal. However, after trial sequential analysis based on the number of existing patients recruited, this meta-analysis of 39 randomized clinical trials yielded no mortality benefit. Interestingly, both meta-analyses revealed geographical impacts on the outcome of the studies. Taking into consideration these differences in patients and health care systems, one could see that particularly Asian countries were connected to a survival benefit. However, excluding single center studies from Japan, no benefit was found any longer (77,78). Additionally, it may be debatable if by extracorporal blood purification techniques also potentially favorable molecules are cleared, including drugs. All in all, inadequate and inconclusive data leave room for a clear recommendation for use of this treatment option.

Vasopressin Agonists

Vasoplegia describes the pathological state of low systemic vascular resistance. The consequence is profound hypotension, often in the presence of a normal or increased cardiac output. Sepsis counts as one of the most prevalent etiologies of vasoplegia. The

Sepsis-3 consensus (79) states that septic shock patients are clinically identified by vasopressor requirements to maintain a mean arterial pressure of 65 mmHg (in the absence of hypovolaemia) and an elevated lactate level. As already mentioned, the current Surviving Sepsis Campaign guidelines (2) recommend that crystalloid fluids be used first, and if this strategy fails or mean arterial pressure falls below 65 mmHg, to administer vasopressors, with norepinephrine being the first choice. However, vasopressin is mentioned for additional use to norepinephrine at doses of 0.03 units/min and in lower doses for catecholamin-resistant shock. Moreover, it has been reported that patients with septic shock express a relative vasopressin deficiency (80). As also mentioned in previous chapters, since catecholamines may have deleterious adverse effects, for example myocardial cell damage, immune suppression, hypermetabolic state and coagulation alteration, the concept of vasopressor sparing therapy emerged over the last years. The main current focus is on using V1a receptor agonists, as other vasopressor may also activate V2 receptors leading to potential severe adverse effects like vasodilatation, impaired diuresis and thromboembolism. One of these is selepressin. Besides stimulating vasoconstriction, other beneficial effects different from norepinephrine are exerted by prevention of endothelial permeability. This is beneficial in terms of prevention of pulmonary capillary fluid leakage, often found in septic patients. However, this agent is still under investigation (81).

As already described, vasopressin is a catecholamine-sparing agent, which, by the current state of evidence, is still not considered to be used as a single agent. The VAAST study (82) was a multicenter randomized placebo controlled trial which evaluated the effects of vasopressin with norepinephrine compared to norepinephrine alone. It concluded that the 28-day mortality showed no difference between the study groups. Nonetheless, the study showed that patients with milder septic shock had a superior survival outcome with low dose administration. Additionally, the application appeared to be safe, since no differences in adverse events were found. The authors commented that norepinephrine doses were reduced as well. A Phase III trial, by Gordon et al. (83), aimed to define the outcomes of vasopressin as the first vasopressor therapy. The study yielded no hemodynamic differences between the groups, but with the norepinephrine requirements being greater in the norepinephrine group. Much like the trial mentioned before, mortality rates were similar. Taking a look at the renal failure free days, no differences could be discerned, however, fewer patients with vasopressin needed renal replacement therapy. A meta-analysis, conducted by Rhodes et al. (2) took into account data from nine trials and further supported the findings of the above-mentioned studies.

Regarding these results, one could question why vasopressin agents should not be prioritized over the use of norepinephrine. As mentioned, mainly the catecholamine sparing effect has been proven, while benefit on mortality seems uncertain. Additional large studies

in the future could create more clarity, also in the face of emerging novel agents like selepressin.

Thrombomodulin

So as to fight off infections, the body mobilizes host defense mechanisms like inflammation and coagulation. The term immunothrombosis describes this interplay between the innate immunity, the platelet activation and coagulation pathways in order to protect the host integrity (84). In a severe state of disease, this may lead to the occurrence of disseminated intravascular coagulation (DIC), manifested by the simultaneous formation of blood clots and an increased propensity of bleeding. Multiple organ failure is a feared outcome. About 35% of septic patients apply to the criteria of DIC. Generally, a high INR and low platelet counts are characteristic findings and are connected with mortality (85,86). Moreover, some patients exhibit a coagulopathic phenotype connected with a higher mortality in sepsis (86).

Regarding these data, investigation focused on this path of treatment in severe sepsis. Previous candidates did not yield success. Drotrecogin alfa, a human recombinant activated protein C, after showing great mortality improvements in the huge PROWESS trial (87), was first included in the Surviving Sepsis Campaign guidelines (88); at a later stage, it was withdrawn from the market.

A new encouraging alternative seems to be thrombomodulin. Downregulated in severe sepsis, it normally enhances the activation of protein C which proteolytically inactivates the coagulation factors Va and VIIIa. As a unique feature, anti-inflammatory action has been shown by suppression of leukocyte adhesion, complement activation and inactivation of DAMPs like HMGB1 (89). Thrombomodulin proved safety in a Phase I trial (90) and Phase II and III trials proposed beneficial effects and confirmed effectiveness (91–93). Yamakawa et al. (94) conducted a systematic review and meta-analysis including 12 studies, consisting of 3 randomized controlled trials and 9 observational studies. In the three randomized controlled trials, a 20% risk reduction of mortality was observed, but these results were statistically insignificant. The analysis of the observational studies yielded similar results. However, no risk differences of serious bleeding complications between the study groups could be noted. The authors suggested that the application of recombinant human thrombomodulin is associated with a trend in mortality reduction at 28-30 days in sepsis patients with DIC. They remarked that the effect with therapy increases with an increasing baseline risk.

Very recently, another systematic review and meta-analysis was conducted by Valeriani et al. (95). In summary, the analysis confirmed the uniformity of bleeding risk between the study groups and the higher 28-day mortality in patients with sepsis associated coagulopathy. In

addition, a by one-fourth decreased 28-day mortality in the thrombomodulin group was revealed. Lately, a study involving 800 patients with sepsis associated coagulopathy have complied with the mortality finding of the prior studies (96).
Concludingly, thrombomodulin in patients with sepsis associated coagulopathy seems to be a reasonable treatment option. Still, in patients without increased coagulation it may be of no benefit and the identification of the right phenotype of patient for the treatment is essential.

Thymosin alpha 1

Sepsis counts as complex immune disorder leading to critical illness and death. Among other factors, the complexity lies in the two stage course of hyperinflammation subsequently followed by immunosuppression (97). It is assumed that reinforcing the host immunity with appropriate timing might be beneficial for severely septic patients in early, immunosuppressed, stages. This rationale led to the research of immunomodulating agents like thymosin alpha1. To date, this approach involving thymosin alpha1 has been extensively investigated in similar immunodeficiency diseases, such as cancer, hepatitis virus infections and HIV (98). More recently, several studies tested and demonstrated its lymphocyte restoring effects in COVID-19 patients (99,100). The beneficial mechanism of this naturally in the thymus occurring peptide lies in its immune restoring and augmenting function. In addition to directly activating natural killer cells and CD8+ T-cells, T-cell maturation into CD4+/CD8+ T-cells is stimulated. Moreover, it also suppresses IL-1ß and TNF-α and increases the expression of MHC Class I, MHC class II which play a role in antigen presentation and recognition by the immune system. The immunomodulating action is exhibited through its interaction with TLRs (101).
Liu et al.‘ systematic review (102) analyzed the efficacy of thymosin alpha1 in sepsis patients incorporating 19 randomized clinical trials. Besides a good safety profile with a rate of adverse reactions of less than 1%, the results corresponded in a decrease of the mortality and a positive impact on the cytokine levels (IL-6, IL-10, TNF-α), as well as an increased level of HLA-DR, an immunologic indicator. Several studies pointed toward an association of a higher mortality in septic shock and increased secondary infections with a low expression of mHLA-DR (103,104). Alongside these promising results, the length of ICU stay, incidence of multiple organ failure and mechanical ventilation period remained unchanged. As mentioned by the authors, the limitations are found in the low quality of evidence, due to the small sample sizes and missing standardized reporting guidelines.
Next to these promising results, studies emerged which added another, already widely used drug in inflammatory states, ulinastatin, to the treatment. Ulinastatin is a urinary trypsin inhibitor arising in the liver and found in human urine. As a broad-spectrum serine protease

inhibitor, it affects inflammatory processes. It modulates chemokines and proinflammatory cytokines such as IL-1ß through the inhibition of the enhanced expression.Thus, it protects against the systemic inflammatory response (105–107). Wang et al. (108) analyzed and compared six randomized clinical trials in a systematic review and meta-analysis. The outcome is in accordance with the above-mentioned findings in thymosin alpha1 monotherapy and yielded optimistic results. The combination treatment conveyed an increased 28-day survival rate and CD4+T-cell expression, whilst the mean time of ICU stay and mechanical ventilation time was decreased. Another systematic review and meta-analysis of eight randomized clinical trials, conducted by Liu et al. (109), matches the findings except that the ICU stay was not decreased. Taking into consideration both treatment options, thymosin alpha1 alone and in combination with ulinastatin, there is a visible trend towards a lower mortality as demonstrated by a systematic review and meta-analysis of 12 randomized controlled trials (110). The mortality in the treatment groups was 28.5%, compared to 42.2% in the control groups. However, all the listed meta-analysis remarked the small sample sizes and insufficient quality of evidence of the included studies.
Still, in fact of the promising safety profile and great results regarding the mortality rate, the treatment with thymosin alpha1 as monotherapy or in combination seems a hopeful approach. The agent, if administered at a right time in the progression of the disease, can help to enhance the immune competence and target the immunosuppressed state. However, cautious interpretation is needed, since size and quality in the trials were their main limitations.

Microbiome

There have been ample attempts to understand the role of intestinal microbiome balance in disease states. Today, it is known that a healthy composition of the commensal bacteria holds stake in the finely tuned host immunity and its competence. However, septic patients present with a disturbed composition and low diversity. The etiologies of this dysbiosis are assigned to clinical interventions, for example parenteral feeding, mechanical ventilation and of course the use of antibiotics and other drugs. Moreover, the disease encompasses pathophysiological changes like decreased gastrointestinal motility, mucosal perfusion and cell integrity. Vice versa, a non-functioning gut microbiome is suspected to increase the chances of immunosuppression, sepsis and multiorgan dysfunction. The intestinal microbiome serves as activator of innate and adaptive immunity. In addition to metabolic significance and preservation of the epithelial barrier, short-chain fatty acids from dietary fibers possess immunomodulatory properties through activation of G-protein receptors,

cytokine production and regulatory T-cells. Further, there is a proposed emphasis on microbial products having an effect on brain receptors (111).

These immunomodulatory properties have been studied as therapeutic opportunity in septic patients. Here, the strategies of replenishing the pool of good microbes via probiotics and the total recolonization of the intestine with fecal microbiota transplantation come into play. The meta-analysis of 30 trials with 2,972 patients, conducted by Manzanares et al. (112), evaluated the overall efficacy of probiotics for clinical outcomes. The outcome was promising in decreasing infections, counting in ventilator-associated pneumonia. However, no effect on mortality or length of hospital stay was observed. Clinical recommendation is minor, given the significant heterogeneity of the studies. The variety of strains and the dosages are further conclusive limitations. Recently, concerns regarding the safety of probiotics arose. A study postulated the possibility that probiotic strains can lead to bacteremia (113). Investigating the safety, optimal choice of species and dosage of probiotics should be subjected to future research. In comparison to probiotics, FMT might show superiority. Apart from the higher number of transferred bacteria, bile acids, proteins and bacteriophages are included. Currently, FMT is an effective and commonly used treatment option in *Clostridium difficile* infections (111). Nevertheless, there are not many clinical studies which address this treatment option in sepsis. In four case reports, patients with different etiologies of sepsis received FMT. These showed improved organ function, improvement of sepsis and survival (114–116). Although these results are highly promising, FMT application in septic patients is still in its infant stages. Moreover, late reports raised caution, in light of infection with *E. coli* after FMT (117,118). Thus, careful donor screening is warranted. In the end, improving sepsis treatment by way of the addition of microbiome modulation needs larger trials to document the exact composition, dosages and patient characteristics so as to make further assumptions.

Beta-Blocker

As discussed in previous chapters, sepsis is a multimodal disease which affects many different organ systems. The most commonly affected organ is the heart (119). The dysregulated host response leads to hemodynamic instability in many cases. The physiologic response is that of a hyperdynamic state with increased cardiac output and decreased systemic vascular resistance, leading to cardiac exhaustion at an early stage of sepsis. Bacterial toxins, inflammatory responses and oxidative damage further exaggerate the condition and frequently ends in septic cardiomyopathy (120). In order to ameliorate the risk of hypoperfusion of peripheral tissues and organ injury, aggressive fluid resuscitation and vasopressor use are recommended in the current guidelines. However, there is a downside

which affects especially the heart, which already suffered sustained damage in the beginning of sepsis. Not only the so far elevated endogenous catecholamine levels, but also the administration of exogenous catecholamine lead to sympathetic nerve overstimulation with detrimental effects. It stands to reason, that cardiac dysfunction induced in the early stage of sepsis influences the mortality rate and belongs to one of the major prognostic factors of sepsis (121). Beta-blockers have proven their productivity as common therapy for chronic heart failure and ischemic heart diseases. The application averts harmful effects on the sympathetic adrenergic nerves. Interestingly, selective beta1- blockade may not only modulate cardiovascular but also metabolic, immune and hemostatic factors. To name a few, ß-adrenergic blockade improves glucose maintenance, facilitates Th2 cell responses and decreases levels of circulating cytokines, therefore suppressing pro-inflammatory processes and reduces platelet activation (121).

The beneficial potential of this treatment gave reason for the meta-analysis by Liu et al. (122) to explore the efficacy of esmolol in sepsis and septic shock. Esmolol is especially suitable, due to its quick efficiency characteristic and high selectivity for ß1-receptors. Positive expectations gave the significantly increased survival rate. Secondary findings were a decreased heart rate and decreased levels of troponin I, a marker of myocardial injury. Counterintuitively and despite the decreased heart rate, no effects were observed on the mean arterial blood pressure and central venous pressure. These promising results were supported by a recently conducted meta-analysis by Li et al. (119) involving six studies. It was demonstrated that beta-blocker use is safe and reduces the 28-day mortality rate. Cardiac troponin and heart rate were reduced. The risk of reduced cardiac output and blood pressure resulting in hypoperfusion of vital organs, one of the main concerns, was defused as well. No observed difference in mean arterial and central venous pressure was found between the study groups. Lastly the lactate levels were indistinguishable.

Ultimately, circulatory failure is one of the main causes of death in sepsis patients, the beta-receptor antagonizing approach seems promising. Further, several non-cardiac related effects have been suggested. The present studies underline this positive course. Yet, clinical data is still sparse and future research is necessary. Subcategories, like individual patient characteristics and dosage studies, to customize the treatment for best results are points of investigation.

Mesenchymal stem cells

Mesenchymal stem cell therapy is a novel strategy of the fight against sepsis. These cells were described for the first time in the 1970s in guinea pig bone marrow (123). They delineate adult stem cells which are undifferentiated. Mesenchymal stem cells possess the

ability of self-renewal, by proliferating and differentiating into many types of cells, including for example osteoblasts, chondroblasts, adipocytes, macrophage-like cells and marrow stroma (124). Multiple advantages, compared to other stem cell types, such as easier and more efficient isolation from adult tissue, builds a steady premise for a promising new way of treatment (125). The therapeutic potential of mesenchymal stem cells lies in their immunomodulatory, anti-inflammatory, anti-bacterial, anti-apoptotic and differentiation properties (126). Due to their low expression of MHC I and II, they are immune privileged and there is no need for the use of immunosuppression. Also, they enhance preserving mechanisms which lead to better tissue repair and restoration after sepsis, further leading to faster function restoration in organs after multiple organ failure (125).

It is not surprising that a lot of research focused on this drug, which showed that mesenchymal stem cells can dampen inflammatory processes and improve survival rate. Unfortunately, most of the data up to day focuses on preclinical studies, experiments mainly conducted on animal models. A detailed meta-analysis of all related studies from the years 2009 to 2019, published by Sun et al. (127), summarizes the current status of research. The results obtained from 1,266 animals demonstrated a significantly lower mortality rate. Since this data mainly reflected the application in rodents, very recently a randomized controlled porcine study was designed which evaluated 32 pigs. The main outcome was that the treatment group with bone marrow-derived mesenchymal stem cells showed good tolerance, but there was no overlap in mortality with the other studies. No improvement of the sepsis outcome was found (128). Even though this invites caution, one should keep in mind the small sample size. Three Phase I studies, relating to sepsis patients, were conducted and raised a promising outlook with regard to the therapy with mesenchymal stem cells. In all studies the treatment was assessed as safe to administer to patients with sepsis (129–131). In general, a favorable safety profile, not limited to sepsis in particular, was underlined by the meta-analysis by Thompson et al. (132). Next to an increased risk of fever, no major adverse effects like infusion-related toxicity, infection, thrombotic or embolic events were identified. Interestingly, theoretical concerns of tumorgenicity had been raised, but could not be well-founded by the clinical studies. Eventually, there have been two recent Phase II clinical trials. The START study (133) conducted with ARDS patients, besides favorable endothelial biomarker changes, reported no improvement in survival. However, the high cell-viability in the cell batches could be held responsible for this. A larger study is necessary to draw conclusion on clinical benefits. Nevertheless, the RUMCESS study (134) finally yield encouraging results. Patients with mesenchymal stem cell therapy had a faster hemodynamic stabilization, vasopressor withdrawal, attenuation of respiratory failure and shortening of the neutropenia period.

Up to now, mesenchymal stem cells have not yet been investigated enough to draw an ultimate conclusion about their efficacy in sepsis patients, improving present treatment guidelines. Still, progress in revealing the mechanism and the beneficial effect has been made. The design of most of the studies is not uniform, varying a lot in the severity of illness, time of intervention, source of mesenchymal stem cells and their dosage. Additionally, patients may also have received conventional therapy, making it difficult to evaluate the efficacy. In the end, a lot of distortion of the studies may be based on the heterogeneity of each patient and should be kept in mind for further research and clinical application.

Nanoparticles

Given steady advancement in technologies, nowadays it is possible to engineer nanoscale particles. These nanoparticles have been proposed as profitable in the therapy of sepsis and septic shock. Usually, small molecular drugs have a high distribution profile, which leads to unwanted side effects and rapid clearance by the kidneys. Since NPs can be created with most favourable pharmacokinetic properties, relating to surface, composition and size, their half-life and biodistribution profile is enhanced. In the end, this leads to more time for developing effects like antimicrobial, before they get cleared from the circulation. In this sense, strategies involve other drugs that are combined with nanoparticles to improve poor solubility and bioavailability. Additionally, the high surface area compared to a lower volume might be used for better functionality in ligand interactions with other cells (135). Since these discoveries, NPs have been employed for diagnostic and therapeutic strategies. However, to focus on the diagnostic aspects is out of scope of this chapter. In order to provide an overview, NPs scope of application ranges from drug delivery, antibacterial, immune modulating, endotoxin antagonizing and bloodpurification mechanisms.

As before mentioned, unfavorable pharmacokinetic properties of many drugs has led to the idea of encapsulating antimicrobial agents with the help of NPs. There have been many studies investigating for example antibiotic drug combinations with NPs for a targeted delivery. It has been shown that this strategy also overcomes issues like biofilms or degrading enzymes produced by many bacteria. For other indications than sepsis, the US Food and Drug Administration approved nanoparticle powered drug delivery systems with amphotericin B. However, these have been restricted again due to toxicity issues recently (136). Silver (Ag) nanoparticles have been postulated to act directly as antibacterial agent. By toxic accumulation and generation of free radicals, they induce death in many gram-negative and positive bacteria. Additionally, it has been stated that resistance development against nanoparticles is unlikely. Even if studied in many in vitro experiments, in vivo trials

are largely absent due to safety concerns (137). However, there are many similar nanoscale agents constantly engineered and tested (135–137).

Besides acting directly on pathogens, immunomodulatory strategies have been pursued. The idea is to reprogram the immune system by suppressing cytokine production and enhancing pathogen elimination. Naturally secreted nanovesicles, called exosomes, are a suitable agent since they carry microRNAs and other proteins. Alexander et al. (138) made use of the anti-inflammatory properties of miR-146a, contained in exosomes released from bone marrow-derived dendritic cells. After administration to LPS-induced mice, TNF-α and IL-6 levels were reduced significantly. In a similar study by Wang et al. (139), miR-223 from mesenchymal stem cells, after injection in mice, lead to decreased proinflammatory cytokines and further protected against cardiac injury and death. However, careful identification and understanding is warranted since some exosomes can aggravate inflammation (138).

Nanoparticles can act as antagonists to toxins, as seen in a study using polymeric cores wrapped with cell membrane from macrophages. Behaving like a macrophage decoy, they neutralized endotoxins after binding. Further, proinflammatory cytokines got sequestered and terminated the downstream inflammation cascade. In a mouse *E. coli* bacteremia model the outcome was a decreased proinflammatory cytokine level, inhibited bacterial dissemination and enhanced survival (140).

As mentioned before, nanoparticles have been utilized in extracorporal blood cleansing devices. One example is the “biospleen” developed by Kang et al. (141). The idea was to mix blood in a septic individual with magnetic nanobeads coated with an engineered human opsonin. The mannose-binding lectin, capturing pathogens and toxins, was then passed through the device with a magnet pulling the bound complex from the blood. Testing in a rat model with *Staph. aureus* and *E. coli* in the blood removed 90% of pathogens in one hour. Moreover, inflammatory cytokines were removed. In a model of endotoxemic rats, substantial survival improvement was shown after a five-hour treatment period.

These promising results of nanoparticle based therapy uncover a multitude of treatment opportunities. Still, this field is in its infancy and needs trials to be collected in the future.

Conclusion

In summary, sepsis remains a profound and widespread health problem, connected to high mortality and morbidity. Identification and timely treatment are key strategies to ensure the highest chances of survival. Due to the strong need for a specific treatment, research investigated the pathophysiology of sepsis. However, supportive care of high quality, such as early administration of antibiotics and source control, remain the best propositions to improve the outcome of patients. The non-antibiotic treatment options described here all seem promising, but are not suitable for monotherapy given the current stages of research. Still, investigation has led to several hopeful starting points for adjunctive therapy in the fight against sepsis and septic shock.

Being already integrated in the international treatment recommendations, corticosteroids seem the most promising adjunctive treatment option. This drug has been extensively researched and offers not only benefits in the state of shock, but shows also direct effects against inflammatory processes in the body, leading finally to a positive outlook in terms of survival. Due to its long history in medical application, the side effect profile is well known and shows only minimal risks.

Even more interesting is the combination of this agent with two other, readily available and cheap drugs, namely vitamin C and thiamine. The recent data of this synergistic combination advocates for the possibility of application in the future treatment of septic patients. Therefore, if future studies prove fruitful, corticosteroids alone and combined could be integrated in the guidelines, apart from being just a vasopressor alternative.

Generally speaking, simple agents like ascorbic acid and micronutrients are easily and widely accessible drugs with a very good safety profile. The distorted homeostasis in septic patients is mostly accompanied by deficits in these substances and therefore should be taken into account when considering treatment.

Since sepsis affects the entire body, its mortality is attributable to the failure of vital organs. Protecting these organs from harmful effects of the septic state improves survival in the short and long-term. Alkaline phosphatase is the best example in the strategy of defending against these influences, in particular with regard to the fact that there has been no licensed drug yet for the prevention and treatment of sepsis-associated AKI. Considering the much higher mortality when accompanied by kidney damage, and the long-term impact on renal function even after the disease, the need for treatment is paramount.

The great depth of the septic pathophysiology and steady advances in technology gave rise to more complex and more targeted drugs. Nanoparticles and intravenous immunoglobulins reflect the powerful possibilities of today's technologies. The scope of application is wide and is only just being discovered. Up to now, more research is needed in this young field of

treatment agents, and costs are still one of the main limiting factors. However, the future prospect seems exciting and promising.

Finally, the gained knowledge in the last years has uncovered the principle of the biphasic disease course in sepsis and septic shock. Additionally, considering the high variability in clinical response to immunotherapy and the heterogeneity of the sepsis affected population, the pivotal approach of patient centered and individualized care gained recognition and is going to establish a new field in medicine. Drugs that intervene with stimulating or depressing the inflammatory state, like GM-CSF, rhIL-7 and corticosteroids may reveal very promising treatment options if timing and patient's biological characteristics are taken into consideration. Progress in this field is slow as of yet, but has already defined potential biomarkers for identification of the right phase of disease and subset of patients that could benefit from immunomodulatory drugs. Moreover, this may help to improve accuracy of the diagnosis, reduce delayed treatment while ensuring the best treatment, and overall to limit unnecessary tests and interventions.

.

References

1. Reinhart K, Daniels R, Kissoon N, Machado FR, Schachter RD, Finfer S. Recognizing Sepsis as a Global Health Priority — A WHO Resolution. N Engl J Med. 2017;377(5):414–7.

2. 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 Medicine. 2017;43(3):304–377.

3. Vincent JL, Jones G, David S, Olariu E, Cadwell KK. Frequency and mortality of septic shock in Europe and North America: A systematic review and meta-analysis. Crit Care. 2019;23(1):1–11.

4. Font MD, Thyagarajan B, Khanna AK. Sepsis and Septic Shock – Basics of diagnosis, pathophysiology and clinical decision making. Med Clin North Am. 2020;104(4):573–85.

5. Taeb AM, Hooper MH, Marik PE. Sepsis: Current definition, pathophysiology, diagnosis, and management. Nutr Clin Pract. 2017;32(3):296–308.

6. Hahn EO, Houser HB, Rammelkamp CH Jr, Denny FW WL. Effect of cortisone on acute streptococcal infections and poststreptococcal complications. J Clin Invest. 1951;30(3):274–81.

7. Sterne JAC, Murthy S, Diaz J V., Slutsky AS, Villar J, Angus DC, et al. Association between Administration of Systemic Corticosteroids and Mortality among Critically Ill Patients with COVID-19: A Meta-analysis. JAMA - J Am Med Assoc. 2020;324(13):1330–41.

8. Buckley MS, Barletta JF, Smithburger PL, Radosevich JJ, Kane-Gill SL. Catecholamine Vasopressor Support Sparing Strategies in Vasodilatory Shock. Pharmacotherapy. 2019;39(3):382–98.

9. Annane D, Renault A, Brun-Buisson C, Megarbane B, Quenot J-P, Siami S, et al. Hydrocortisone plus Fludrocortisone for Adults with Septic Shock. N Engl J Med. 2018;378(9):809–18.

10. Venkatesh B, Finfer S, Cohen J, Rajbhandari D, Arabi Y, Bellomo R, et al. Adjunctive Glucocorticoid Therapy in Patients with Septic Shock. N Engl J Med. 2018;378(9):797–808.

11. Annane D, Bellissant E, Bollaert PE, Briegel J, Keh D, Kupfer Y, et al. Corticosteroids for treating sepsis in children and adults. Cochrane Database Syst Rev. 2019;2019(12).

12. Rochwerg B, Oczkowski SJ, Siemieniuk RAC, Agoritsas T, Belley-Cote E, D'Aragon F, et al. Corticosteroids in Sepsis: An Updated Systematic Review and Meta-Analysis. Crit Care Med. 2018;46(9):1411–20.

13. Fang F, Zhang Y, Tang J, Lunsford LD, Li T, Tang R, et al. Association of Corticosteroid Treatment with Outcomes in Adult Patients with Sepsis: A Systematic Review and Meta-analysis. JAMA Intern Med. 2019;179(2):213–23.

14. Carpenter KJ. The discovery of vitamin c. Ann Nutr Metab. 2012;61(3):259–64.

15. Marik PE, Khangoora V, Rivera R, Hooper MH, Catravas J. Hydrocortisone, Vitamin C, and Thiamine for the Treatment of Severe Sepsis and Septic Shock: A Retrospective Before-After Study. Chest. 2017;151(6):1229–38.

16. Carr AC, Shaw GM, Fowler AA, Natarajan R. Ascorbate-dependent vasopressor synthesis: A rationale for vitamin C administration in severe sepsis and septic shock? Crit Care. 2015;19(1):1–8.

17. Mandl J, Szarka A, Bánhegyi G. Vitamin C: Update on physiology and pharmacology. Br J Pharmacol. 2009;157(7):1097–110.

18. Obi J, Pastores SM, Ramanathan L V., Yang J, Halpern NA. Treating sepsis with vitamin C, thiamine, and hydrocortisone: Exploring the quest for the magic elixir. J Crit Care. 2020;57:231–9.

19. Langlois PL, Manzanares W, Adhikari NKJ, Lamontagne F, Stoppe C, Hill A, et al. Vitamin C Administration to the Critically Ill: A Systematic Review and Meta-Analysis. J Parenter Enter Nutr. 2019;43(3):335–46.

20. Carr AC, Rosengrave PC, Bayer S, Chambers S, Mehrtens J, Shaw GM. Hypovitaminosis C and vitamin C deficiency in critically ill patients despite recommended enteral and parenteral intakes. Crit Care. 2017;21(1):1–10.

21. Fowler AA, Truwit JD, Hite RD, Morris PE, Dewilde C, Priday A, et al. Effect of Vitamin C Infusion on Organ Failure and Biomarkers of Inflammation and Vascular Injury in Patients with Sepsis and Severe Acute Respiratory Failure: The CITRIS-ALI Randomized Clinical Trial. JAMA - J Am Med Assoc. 2019;322(13):1261–70.

22. Hemilä H, Chalker E. Vitamin C can shorten the length of stay in the ICU: A meta-analysis. Nutrients. 2019;11(4):1–30.

23. Putzu A, Daems AM, Lopez-Delgado JC, Giordano VF, Landoni G. The effect of Vitamin C on clinical outcome in critically ill patients: A systematic review with meta-analysis of randomized controlled trials. Crit Care Med. 2019;47(6):774–83.

24. Zhang M, Jativa DF. Vitamin C supplementation in the critically ill: A systematic review and meta-analysis. SAGE Open Med. 2018;6:1–12.

25. Belsky JB, Wira CR, Jacob V, Sather JE, Lee PJ. A review of micronutrients in sepsis: The role of thiamine, l-carnitine, Vitamin C, selenium and Vitamin D. Nutr Res Rev. 2018;31(2):281–90.

26. Byerly S, Parreco JP, Soe-Lin H, Parks JJ, Lee EE, Shnaydman I, et al. Vitamin C and thiamine are associated with lower mortality in sepsis. J Trauma Acute Care Surg. 2020;89(1):111–7.

27. Wani SJ, Mufti SA, Jan RA, Shah SU, Qadri SM, Khan UH, et al. Combination of vitamin C, thiamine and hydrocortisone added to standard treatment in the management of sepsis: results from an open label randomised controlled clinical trial and a review of the literature. Infect Dis (Auckl). 2020;52(4):271–8.

28. Fujii T, Luethi N, Young PJ, Frei DR, Eastwood GM, French CJ, et al. Effect of Vitamin C, Hydrocortisone, and Thiamine vs Hydrocortisone Alone on Time Alive and Free of Vasopressor Support among Patients with Septic Shock: The VITAMINS Randomized Clinical Trial. JAMA - J Am Med Assoc. 2020;323(5):423–31.

29. Chang P, Liao Y, Guan J, Guo Y, Zhao M, Hu J, et al. Combined Treatment With Hydrocortisone, Vitamin C, and Thiamine for Sepsis and Septic Shock: A Randomized Controlled Trial. Chest. 2020;158(1):174–82.

30. Shin TG, Kim Y-J, Ryoo SM, Hwang SY, Jo IJ, Chung SP, et al. Early Vitamin C and Thiamine Administration to Patients with Septic Shock in Emergency Departments: Propensity Score-Based Analysis of a Before-and-After Cohort Study. J Clin Med. 2019;8(1):102.

31. Hager DN, Hooper MH, Bernard GR, Busse LW, Ely EW, Fowler AA, et al. The Vitamin C, Thiamine and Steroids in Sepsis (VICTAS) Protocol: A prospective, multi-center, double-blind, adaptive sample size, randomized, placebo-controlled, clinical trial. Trials. 2019;20(1):1–16.

32. Blythe R, Cook D, Graves N. Scepticaemia: The impact on the health system and patients of delaying new treatments with uncertain evidence; A case study of the sepsis bundle. F1000Research. 2018;7(May):1–11.

33. Jarczak D, Kluge S, Nierhaus A. Use of intravenous immunoglobulins in sepsis therapy—a clinical view. Int J Mol Sci. 2020;21(15):1–17.

34. Werdan K, Pilz G, Müller-Werdan U, Enriquez MM, Schmitt D V., Mohr FW, et al. Immunoglobulin G treatment of postcardiac surgery patients with score-identified severe systemic inflammatory response syndrome-The ESSICS study. Crit Care Med. 2008;36(3):716–23.

35. Werdan K, Pilz G, Bujdoso O, Fraunberger P, Neeser G, Schmieder RE, et al. Score-based immunoglobulin G therapy of patients with sepsis: The SBITS study. Crit Care Med. 2007;35(12):2693–701.

36. Alejandria MM, Lansang MAD, Dans LF, Mantaring JB. Intravenous immunoglobulin for treating sepsis, severe sepsis and septic shock. Cochrane Database Syst Rev. 2013;2013(9).

37. Cavazzuti I, Serafini G, Busani S, Rinaldi L, Biagioni E, Buoncristiano M, et al. Early therapy with IgM-enriched polyclonal immunoglobulin in patients with septic shock. Intensive Care Med. 2014;40(12):1888–96.

38. Giamarellos-Bourboulis EJ, Tziolos N, Routsi C, Katsenos C, Tsangaris I, Pneumatikos I, et al. Improving outcomes of severe infections by multidrug-resistant pathogens with polyclonal IgM-enriched immunoglobulins. Clin Microbiol Infect. 2016;22(6):499–506.

39. Busani S, Damiani E, Cavazzuti I, Donati A, Girardis M. Intravenous immunoglobulin in septic shock: Review of the mechanisms of action and meta-analysis of the clinical effectiveness. Minerva Anestesiol. 2016;82(5):559–72.

40. Cui J, Wei X, Lv H, Li Y, Li P, Chen Z, et al. The clinical efficacy of intravenous IgM-enriched immunoglobulin (pentaglobin) in sepsis or septic shock: a meta-analysis with trial sequential analysis. Ann Intensive Care. 2019;9(1).

41. Welte T, Dellinger RP, Ebelt H, Ferrer M, Opal SM, Singer M, et al. Efficacy and safety of trimodulin, a novel polyclonal antibody preparation, in patients with severe community-acquired pneumonia: a randomized, placebo-controlled, double-blind, multicenter, phase II trial (CIGMA study). Intensive Care Med. 2018;44(4):438–48.

42. Berlot G, Vassallo CM, Busetto N, Nieto Yabar M, Istrati T, Baronio S, et al. Effects of the timing of administration of IgM- and IgA-enriched intravenous polyclonal immunoglobulins on the outcome of septic shock patients. Ann Intensive Care. 2018;8(1).

43. Aubron C, Berteau F, Sparrow RL. Intravenous immunoglobulin for adjunctive treatment of severe infections in ICUs. Curr Opin Crit Care. 2019;25(5):417–22.

44. Derek C. Angus, MD, MPH, FCCM; Walter T. Linde-Zwirble; Jeffrey Lidicker, MA; Gilles Clermont, MD; Joseph Carcillo, MD; Michael R. Pinsky, MD F. Epidemiology of sepsis. Crit Care Med Div Dep Anesthesiol Crit Care Med Cent Res Heal Care (DCA, GC, JC, MRP), Univ Pittsburgh, Pittsburgh, PA; Heal Process Manag (WTL-Z, JL), Inc, Doylestown,. 2001;15(1):148–50.

45. Bellomo R, Kellum JA, Ronco C. Acute kidney injury. Lancet. 2012;380(9843):756–66.

46. Hoste EAJ, Bagshaw SM, Bellomo R, Cely CM, Colman R, Cruz DN, et al. Epidemiology of acute kidney injury in critically ill patients: the multinational AKI-EPI study. Intensive Care Med. 2015;41(8):1411–23.

47. Peters E, Heemskerk S, Masereeuw R, Pickkers P. Alkaline phosphatase: A possible treatment for sepsis-associated acute kidney injury in critically Ill patients. Am J Kidney Dis. 2014;63(6):1038–48.

48. Su F, Brands R, Wang Z, Verdant C, Bruhn A, Cai Y, et al. Beneficial effects of alkaline phosphatase in septic shock. Crit Care Med. 2006;34(8):2182–7.

49. Beumer C, Wulferink M, Raaben W, Fiechter D, Brands R, Seinen W. Calf Intestinal Alkaline Phosphatase, a Novel Therapeutic Drug for Lipopolysaccharide (LPS)-Mediated Diseases, Attenuates LPS Toxicity in Mice and Piglets. J Pharmacol Exp Ther. 2003;307(2):737–44.

50. Heemskerk S, Masereeuw R, Moesker O, Bouw MPWJM, Van Der Hoeven JG, Peters WHM, et al. Alkaline phosphatase treatment improves renal function in severe sepsis or septic shock patients. Crit Care Med. 2009;37(2):417–23.

51. Tang W, Huang J, Huang X, Han X, Tang W, Ke G, et al. Effect of alkaline phosphatase on sepsis-associated acute kidney injury patients: A systematic review and meta-analysis. Med (United States). 2020;99(4):0–7.

52. Nwafor D, Brown C. A novel role for tissue-nonspecific alkaline phosphatase at the blood-brain barrier during sepsis. Neural Regen Res. 2021;16(1):99–100.

53. Mathias B, Szpila BE, Moore FA, Efron PA, Moldawer LL. A review of GM-CSF therapy in sepsis. Med (United States). 2015;94(50):1–10.

54. Hall MW, Knatz NL, Vetterly C, Tomarello S, Wewers MD, Volk HD, et al. Immunoparalysis and nosocomial infection in children with multiple organ dysfunction syndrome. Intensive Care Med. 2011;37(3):525–32.

55. Drossou-Agakidou V, Kanakoudi-Tsakalidou F, Sarafidis K, Tzimouli V, Taparkou A, Kremenopoulos G, et al. In vivo effect of rhGM-CSF and rhG-CSF on monocyte HLA-DR expression of septic neonates. Cytokine. 2002;18(5):260–5.

56. Meisel C, Schefold JC, Pschowski R, Baumann T, Hetzger K, Gregor J, et al. Granulocyte-macrophage colony-stimulating factor to reverse sepsis-associated immunosuppression: A double-blind, randomized, placebo-controlled multicenter trial. Am J Respir Crit Care Med. 2009;180(7):640–8.

57. Pinder EM, Rostron AJ, Hellyer TP, Ruchaud-Sparagano MH, Scott J, MacFarlane JG, et al. Randomised controlled trial of GM-CSF in critically ill patients with impaired neutrophil phagocytosis. Thorax. 2018;73(10):918–25.

58. Bo L, Wang F, Zhu J, Li J, Deng X. Granulocyte-colony stimulating factor (G-CSF) and granulocyte-macrophage colony stimulating factor (GM-CSF) for sepsis: A meta-analysis. Crit Care. 2011;15(1):R58.

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

60. 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;15(4):R183.

61. Patil NK, Bohannon JK SE. Immunotherapy: A Promising Approach to Reverse Sepsis- Induced Immunosuppression. Pharmacol Res. 2016;688–702.

62. Venet F, Foray A-P, Villars-Méchin A, Malcus C, Poitevin-Later F, Lepape A, et al. IL-7 Restores Lymphocyte Functions in Septic Patients. J Immunol. 2012;189(10):5073–81.

63. 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;3(5):1–18.

64. de Roquetaillade C, Monneret G, Gossez M, Venet F. IL-7 and its beneficial role in sepsis-induced T lymphocyte dysfunction. Crit Rev Immunol. 2018;38(6):433–51.

65. Donnino MW, Andersen LW, Chase M, Berg KM, Tidswell M, Giberson T, Wolfe R, Moskowitz A, Smithline H, Ngo L CM. Randomized, Double-Blind, Placebo-Controlled Trial of Thiamine as a Metabolic Resuscitator in Septic Shock: A Pilot Study. Crit Care Med. 2016;44(2):360–7.

66. Moskowitz A, Andersen LW, Cocchi MN, Karlsson M, Patel P V., Donnino MW. Thiamine as a renal protective agent in septic shock a secondary analysis of a randomized, double-blind, placebo-controlled trial. Ann Am Thorac Soc. 2017;14(5):737–41.

67. Baeke F, Takiishi T, Korf H, Gysemans C, Mathieu C. Vitamin D: Modulator of the immune system. Curr Opin Pharmacol. 2010;10(4):482–96.

68. Moromizato T, Litonjua AA, Braun AB, Gibbons FK, Giovannucci E, Christopher KB. Association of low serum 25-hydroxyvitamin D levels and sepsis in the critically ill. Crit Care Med. 2014;42(1):97–107.

69. Langlois PL, Szwec C, D'Aragon F, Heyland DK, Manzanares W. Vitamin D supplementation in the critically ill: A systematic review and meta-analysis. Clin Nutr. 2018;37(4):1238–46.

70. Amrein K, Schnedl C, Holl A, Riedl R, Christopher KB, Pachler C, et al. Effect of high-dose vitamin D3on hospital length of stay in critically ill patients with vitamin D deficiency: The VITdAL-ICU randomized clinical trial. JAMA - J Am Med Assoc. 2014;312(15):1520–30.

71. Martucci G, McNally D, Parekh D, Zajic P, Tuzzolino F, Arcadipane A, et al. Trying to identify who may benefit most from future vitamin D intervention trials: A post hoc analysis from the VITDAL-ICU study excluding the early deaths. Crit Care. 2019;23(1):1–11.

72. Puskarich MA, Kline JA, Krabill V, Claremont H JA. Preliminary Safety and Efficacy of L-carnitine Infusion for the Treatment of Vasopressor-Dependent Septic Shock: A Randomized Control Trial. JPEN J Parenter Enter Nutr. 2014;38(6):736–43.

73. Jones AE, Puskarich MA, Shapiro NI, Guirgis FW, Runyon M, Adams JY, et al. Effect of Levocarnitine vs Placebo as an Adjunctive Treatment for Septic Shock: The Rapid Administration of Carnitine in Sepsis (RACE) Randomized Clinical Trial. JAMA Netw open. 2018;1(8):e186076.

74. Jennaro TS, Puskarich MA, McCann MR, Gillies CE, Pai MP, Karnovsky A, et al. Using l-Carnitine as a Pharmacologic Probe of the Interpatient and Metabolic Variability of Sepsis. Pharmacotherapy. 2020;40(9):913–23.

75. Li S, Tang T, Guo P, Zou Q, Ao X, Hu L, et al. A meta-analysis of randomized controlled trials. Medicine (Baltimore). 2019;98.

76. Monard C, Rimmelé T, Ronco C. Extracorporeal blood purification therapies for sepsis. Blood Purif. 2019;47(Suppl3):2–15.

77. Putzu A, Schorer R, Lopez-Delgado JC, Cassina T, Landoni G. Blood Purification and Mortality in Sepsis and Septic Shock: A Systematic Review and Meta-analysis of Randomized Trials. Anesthesiology. 2019;131(3):580–93.

78. Snow TAC, Littlewood S, Corredor C, Singer M, Arulkumaran N. Effect of Extracorporeal Blood Purification on Mortality in Sepsis: A Meta-Analysis and Trial Sequential Analysis. Blood Purif. 2020;1–11.

79. Singer, M., Deutschman, C. S., Seymour, C. W., Shankar-Hari, M., Annane, D., Bauer, M., Bellomo, R., Bernard, G. R., Chiche, J. D., Coopersmith, C. M., Hotchkiss, R. S., Levy, M. M., Marshall, J. C., Martin, G. S., Opal, S. M., Rubenfeld, G. D., van der P DC. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA - J Am Med Assoc. 2016;315(8):801–19.

80. Landry DW, Levin HR, Gallant EM, Ashton RC Jr, Seo S, D'Alessandro D, Oz MC OJ. Vasopressin deficiency contributes to the vasodilation of septic shock. Circulation. 1997;95(5):1122–5.

81. Saad AF, Maybauer MO. The role of vasopressin and the vasopressin type V1a receptor agonist selepressin in septic shock. J Crit Care. 2017;40:41–5.

82. Russell JA, Walley KR, Singer J, Gordon AC, Hébert PC, Cooper DJ, Holmes CL, Mehta S, Granton JT, Storms MM, Cook DJ, Presneill JJ AD. Vasopressin versus Norepinephrine Infusion in Patients with Septic Shock. N Engl J Med. 2008;358(9):877-87.

83. Gordon AC, Mason AJ, Thirunavukkarasu N, Perkins GD, Cecconi M, Cepkova M, et al. Effect of early vasopressin vs norepinephrine on kidney failure in patients with septic shock: The VANISH randomized clinical trial. JAMA - J Am Med Assoc. 2016;316(5):509–18.

84. Engelmann B, Massberg S. Thrombosis as an intravascular effector of innate immunity. Nat Rev Immunol. 2013;13(1):34–45.

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

86. Lyons PG, Micek ST, Hampton N, Kollef MH. Sepsis-associated coagulopathy severity predicts hospital mortality. Crit Care Med. 2018;46(5):736–42.

87. Bernard GR, Vincent JL, Laterre PF, LaRosa SP, Dhainaut JF, Lopez-Rodriguez A, Steingrub JS, Garber GE, Helterbrand JD, Ely EW FCJ. Efficacy and safety of recombinant human activated protein C for severe sepsis. N Engl J Med. 2001;344(10):699-709.

88. Dellinger RP, Levy MM, Carlet JM, Bion J, Parker MM, Jaeschke R, et al. Surviving sepsis campaign: International guidelines for management of severe sepsis and septic shock: 2008. Critical Care Medicine. 2008;36(1):296–327.

89. Watanabe-Kusunoki K, Nakazawa D, Ishizu A, Atsumi T. Thrombomodulin as a Physiological Modulator of Intravascular Injury. Front Immunol. 2020;11(September):1–12.

90. Moll S, Lindley C, Pescatore S, Morrison D, Tsuruta K, Mohri M, et al. Phase I study of a novel recombinant human soluble thrombomodulin, ART-123. J Thromb Haemost. 2004;2(10):1745–51.

91. Vincent JL, Ramesh MK, Ernest D, Larosa SP, Pachl J, Aikawa N, et al. A randomized, double-blind, placebo-controlled, phase 2b Study to evaluate the safety and efficacy of recombinant human soluble thrombomodulin, ART-123, in patients with sepsis and suspected disseminated intravascular coagulation. Crit Care Med. 2013;41(9):2069–79.

92. Saito H, Maruyama I, Shimazaki S, Yamamoto Y, Aikawa N, Ohno R, et al. Efficacy and safety of recombinant human soluble thrombomodulin (ART-123) in disseminated intravascular coagulation: Results of a phase III, randomized, double-blind clinical trial. J Thromb Haemost. 2007;5(1):31–41.

93. Aikawa N, Shimazaki S, Yamamoto Y, Saito H, Maruyama I, Ohno R, et al. Thrombomodulin alfa in the treatment of infectious patients complicated by disseminated intravascular coagulation: Subanalysis from the phase 3 trial. Shock. 2011;35(4):349–54.

94. Yamakawa K, Aihara M, Ogura H, Yuhara H, Hamasaki T, Shimazu T. Recombinant human soluble thrombomodulin in severe sepsis: A systematic review and meta-analysis. J Thromb Haemost. 2015;13(4):508–19.

95. Valeriani E, Squizzato A, Gallo A, Porreca E, Vincent JL, Iba T, et al. Efficacy and safety of recombinant human soluble thrombomodulin in patients with sepsis-associated coagulopathy: A systematic review and meta-analysis. J Thromb Haemost. 2020;18(7):1618–25.

96. Levi M, Vincent JL, Tanaka K, Radford AH, Kayanoki T, Fineberg DA, et al. Effect of a Recombinant Human Soluble Thrombomodulin on Baseline Coagulation Biomarker Levels and Mortality Outcome in Patients with Sepsis-Associated Coagulopathy. Crit Care Med. 2020;48(8):1140–7.

97. Hotchkiss RS, Monneret G PD. Disorder and a New Therapeutic Approach. Lancet Infect Dis 2013. 2013;13(3):260–8.

98. Dominari A, III DH, Pandav K, Matos W, Biswas S, Reddy G, et al. Thymosin alpha 1: A comprehensive review of the literature. World J Virol. 2020;9(5):67–78.

99. Yu K, He J, Wu Y, Xie B, Liu X, Wei B, et al. Dysregulated adaptive immune response contributes to severe COVID-19. Cell Res. 2020;30(9):814–6.

100. Liu Y, Pan Y, Hu Z, Wu M, Wang C, Feng Z, Mao C, Tan Y, Liu Y, Chen L, Li M, Wang G, Yuan Z, Diao B, Wu Y CY. Thymosin alpha 1 (Tα1) reduces the mortality of severe COVID-19 by restoration of lymphocytopenia and reversion of exhausted T cells. Clin Infect Dis 2020 Nov. 2020;1:1–27.

101. Li J, Liu CH, Wang FS. Thymosin alpha 1: Biological activities, applications and genetic engineering production. Peptides. 2010;31(11):2151–8.

102. Liu F, Wang HM, Wang T, Zhang YM, Zhu X. The efficacy of thymosin α1 as immunomodulatory treatment for sepsis: A systematic review of randomized controlled trials. BMC Infect Dis. 2016;16(1).

103. 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;32(8):1175–83.

104. 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;36(11):1859–66.

105. Inoue KI, Takano H, Yanagisawa R, Yoshikawa T. Protective effects of urinary trypsin inhibitor on systemic inflammatory response induced by lipopolysaccharide. J Clin Biochem Nutr. 2008;43(3):139–42.

106. Inoue KI, Takano H, Shimada A, Yanagisawa R, Sakurai M, Yoshino S, et al. Urinary trypsin inhibitor protects against systemic inflammation induced by lipopolysaccharide. Mol Pharmacol. 2005;67(3):673–80.

107. Linder A, Russell JA. An exciting candidate therapy for sepsis: Ulinastatin, a urinary protease inhibitor. Intensive Care Med. 2014;40(8):1164–7.

108. Wang FY, Fang B, Qiang XH, Yu TO, Zhong JR, Cao J, et al. The Efficacy and Immunomodulatory Effects of Ulinastatin and Thymosin α 1 for Sepsis: A Systematic Review and Meta-Analysis. Biomed Res Int. 2016;2016.

109. Liu D, Yu Z, Yin J, Chen Y, Zhang H, Xin F, et al. Effect of ulinastatin combined with thymosin alpha1 on sepsis: A systematic review and meta-analysis of Chinese and Indian patients. J Crit Care. 2017;39(438):259–66.

110. Li C, Bo L, Liu Q, Jin F. Thymosin alpha1 based immunomodulatory therapy for sepsis: A systematic review and meta-analysis. Int J Infect Dis. 2015;33:e90–6.

111. Nakov R, Segal JP, Settanni CRS, Bibb S, Barrini AG, Cammarota G, et al. Microbiome: What intensivists should know. Minerva Anestesiol. 2020;86(7):777–85.

112. Manzanares W, Lemieux M, Langlois PL, Wischmeyer PE. Probiotic and synbiotic therapy in critical illness: A systematic review and meta-analysis. Crit Care. 2016;20(1):262.

113. Yelin I, Flett KB, Merakou C, Mehrotra P, Stam J, Snesrud E, et al. Genomic and epidemiological evidence of bacterial transmission from probiotic capsule to blood in ICU patients. Nat Med. 2019;25(11):1728–32.

114. Li Q, Wang C, Tang C, He Q, Zhao X, Li N, et al. Therapeutic modulation and reestablishment of the intestinal Microbiota with fecal Microbiota transplantation resolves sepsis and diarrhea in a patient. Am J Gastroenterol. 2014;109(11):1832–4.

115. Li Q, Wang C, Tang C, He Q, Zhao X, Li N, et al. Successful treatment of severe sepsis and diarrhea after vagotomy utilizing fecal microbiota transplantation: A case report. Crit Care. 2015;19(1):1–12.

116. Wei Y, Yang J, Wang J, Yang Y, Huang J, Gong H, et al. Successful treatment with fecal microbiota transplantation in patients with multiple organ dysfunction syndrome and diarrhea following severe sepsis. Crit Care. 2016;20(1):1–9.

117. DeFilipp Z, Bloom PP, Torres Soto M, Mansour MK, Sater MRA, Huntley MH, et al. Drug-Resistant E. coli Bacteremia Transmitted by Fecal Microbiota Transplant . N Engl J Med. 2019;381(21):2043–50.

118. Food and Drug Administration. Fecal Microbiota for Transplantation: Safety Alert - Risk of Serious Adverse Events Likely Due to Transmission of Pathogenic Organisms | FDA [Internet]. 2020 [cited 2021 Feb 1]. Available from: https://www.fda.gov/safety/medical-product-safety-information/fecal-microbiota-transplantation-safety-alert-risk-serious-adverse-events-likely-due-transmission

119. Li J, Sun W, Guo Y, Ren Y, Li Y, Yang Z. Prognosis of β-adrenergic blockade therapy on septic shock and sepsis: A systematic review and meta-analysis of randomized controlled studies. Cytokine. 2020;126(July 2019).

120. Sweeney, T. E., & Khatri P. Septic Cardiomyopathy: Getting to the Heart of the Matter. Crit Care Med. 2017;45(3):556–7.

121. Suzuki T, Suzuki Y, Okuda J, Kurazumi T, Suhara T, Ueda T, et al. Sepsis-induced cardiac dysfunction and β-adrenergic blockade therapy for sepsis. J Intensive Care. 2017;5(1):1–10.

122. Liu P, Wu Q, Tang Y, Zhou Z, Feng M. The influence of esmolol on septic shock and sepsis: A meta-analysis of randomized controlled studies. Am J Emerg Med. 2018;36(3):470–4.

123. Friedenstein AJ, Chailakhjan RK LK. The development of fibroblast colonies in monolayer cultures of guinea-pig bone marrow and spleen cells. Cell Tissue Kinet. 1970;3:1–6.

124. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, et al. Multilineage potential of adult human mesenchymal stem cells. Science (80-). 1999;284(5411):143–7.

125. Keane C, Jerkic M LJ. Stem Cell-based Therapies for Sepsis. Anesthesiology. 2017;(6):1017–34.

126. Matthay MA, Pati S, Lee JW. Concise Review: Mesenchymal Stem (Stromal) Cells: Biology and Preclinical Evidence for Therapeutic Potential for Organ Dysfunction Following Trauma or Sepsis. Stem Cells. 2017;35(2):316–24.

127. Sun XY, Ding XF, Liang HY, Zhang XJ, Liu SH, Bing-Han, et al. Efficacy of mesenchymal stem cell therapy for sepsis: A meta-analysis of preclinical studies. Stem Cell Res Ther. 2020;11(1):1–10.

128. Horak J, Nalos L, Martinkova V, Tegl V, Vistejnova L, Kuncova J, et al. Evaluation of Mesenchymal Stem Cell Therapy for Sepsis: A Randomized Controlled Porcine Study. Front Immunol. 2020;11(February):1–13.

129. McIntyre LA, Stewart DJ, Mei SHJ, Courtman D, Watpool I, Granton J, et al. Cellular immunotherapy for septic shock: A phase I clinical trial. American Journal of Respiratory and Critical Care Medicine. 2018;197(3):337–347.

130. He X, Ai S, Guo W, Yang Y, Wang Z, Jiang D, et al. Umbilical cord-derived mesenchymal stem (stromal) cells for treatment of severe sepsis: aphase 1 clinical trial. Transl Res. 2018;199:52–61.

131. Schlosser K, Wang JP, Dos Santos C, Walley KR, Marshall J, Fergusson DA, et al. Effects of Mesenchymal Stem Cell Treatment on Systemic Cytokine Levels in a Phase 1 Dose Escalation Safety Trial of Septic Shock Patients. Crit Care Med. 2019;47(7):918–25.

132. Thompson M, Mei SHJ, Wolfe D, Champagne J, Fergusson D, Stewart DJ, et al. Cell therapy with intravascular administration of mesenchymal stromal cells continues to appear safe: An updated systematic review and meta-analysis. EClinicalMedicine. 2020;19.

133. Matthay MA, Calfee CS, Zhuo H, Thompson BT, Jennifer G, Levitt JE, et al. Treatment with allogeneic mesenchymal stromal cells for moderate to severe acute respiratory distress syndrome (START study): a randomised phase 2a safety trial. Lancet Respir Med. 2020;7(2):154–62.

134. Gennadiy G, Polina M, Elena P, Larisa K, Vera T, Eduard G, et al. The Results of the Single Center Pilot Randomized Russian Clinical Trial of Mesenchymal Stromal Cells in Severe Neutropenic Patients with Septic Shock (RUMCESS). Int J Blood Res Disord. 2018;5(1):1–8.

135. Yuk SA, Sanchez-Rodriguez DA, Tsifansky MD, Yeo Y. Recent advances in nanomedicine for sepsis treatment. Ther Deliv. 2018;9(6):435–50.

136. Papafilippou L, Claxton A, Dark P, Kostarelos K, Hadjidemetriou M. Nanotools for Sepsis Diagnosis and Treatment. Adv Healthc Mater. 2021;10(1):1–25.

137. Pant A, Mackraj I, Govender T. Advances in sepsis diagnosis and management: a paradigm shift towards nanotechnology. J Biomed Sci. 2021;28(1):1–30.

138. Alexander M, Hu R, Runtsch MC, Kagele DA, Mosbruger TL, Tolmachova T, et al. Exosome-delivered microRNAs modulate the inflammatory response to endotoxin. Nat Commun. 2015;6.

139. Wang X, Gu H, Qin D, Yang L, Huang W, Essandoh K, et al. Exosomal MIR-223 Contributes to Mesenchymal Stem Cell-Elicited Cardioprotection in Polymicrobial Sepsis. Sci Rep. 2015;5(February):1–16.

140. Thamphiwatana S, Angsantikul P, Escajadillo T, Zhang Q, Olson J, Luk BT, et al. Macrophage-like nanoparticles concurrently absorbing endotoxins and proinflammatory cytokines for sepsis management. Proc Natl Acad Sci U S A. 2017;114(43):11488–93.

141. Kang JH, Super M, Yung CW, Cooper RM, Domansky K, Graveline AR, et al. An extracorporeal blood-cleansing device for sepsis therapy. Nat Med. 2014;20(10):1211–6.

Biography

I was born on the 22nd of May 1996 in Bergisch Gladbach, in the Cologne/Bonn Region of North Rhine-Westphalia, Germany. I grew up in a small village close to Bensberg, where I finished my primary and secondary education by the year 2014.
After completing high school, I took one year off to orientate myself in relations to my future career path. During that time, I completed the prerequisites for studying mechanical engineering, as well as, participating in the Lufthansa pilot selection process. However, my interest in the field of medicine sparked after I started my paramedic training. I decided to study for the entrance exam for Zagreb Medical Studies in English in the hopes of fulfilling my dream of becoming a doctor. The exam was written in Madrid, Spain and I moved to Zagreb in 2015.
I have always felt connected to Croatia because of my childhood memories and family roots. My father was born in Zagreb and my grandfather studied medicine in the School of Medicine at the University of Zagreb.

During my studies in Zagreb between 2015 and 2021, I can contently say I have enjoyed learning and preparing for my chosen career path. Life apart from university in Croatia was delightful, I have made many good friendships. In my semester breaks I gained experience in various specializations via internships in Cologne and surrounding areas. In 2021 I finished my medical school by doing my clinical rotations in Germany and Austria.