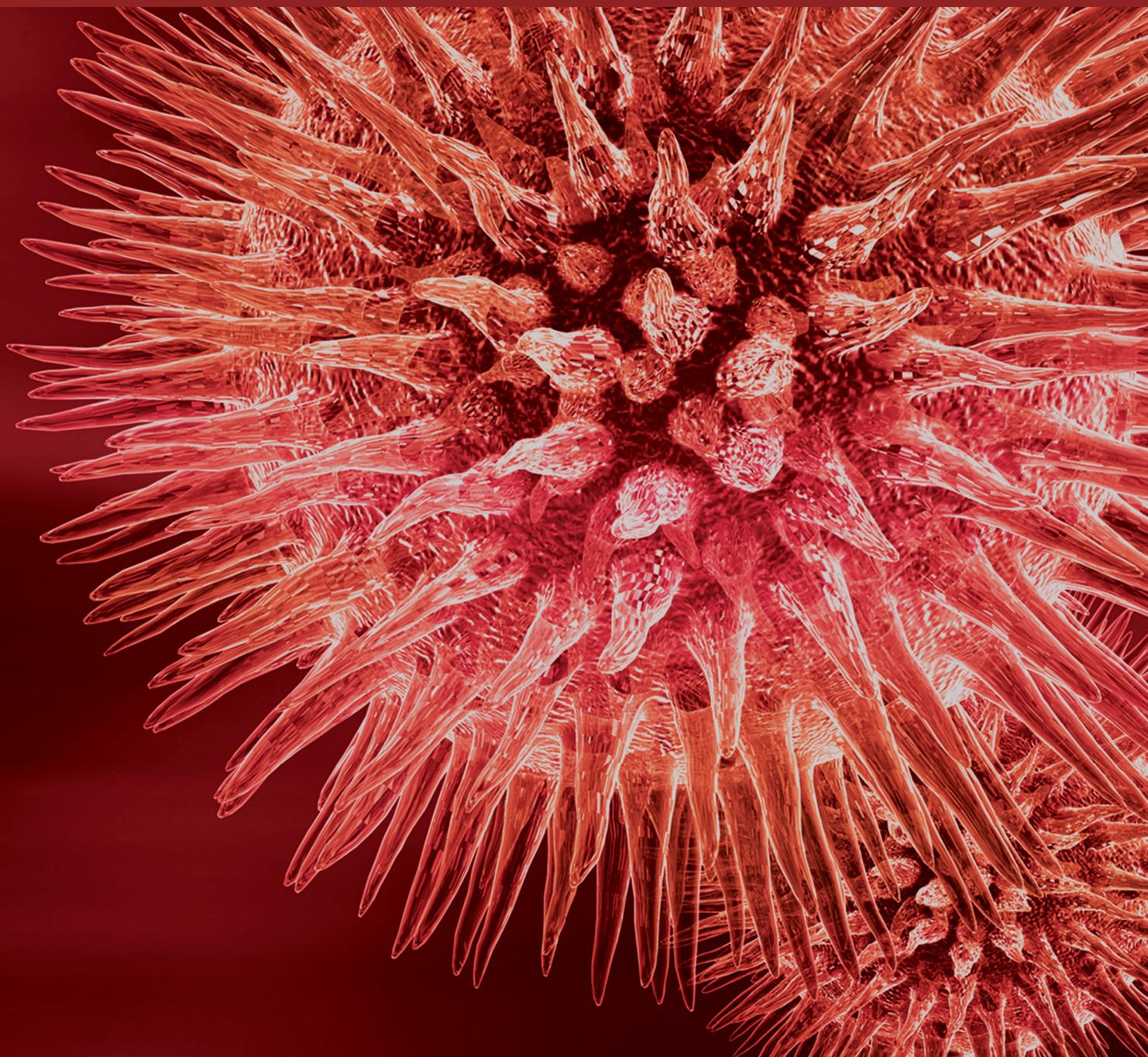


BioMed Research International

Sepsis: Diagnostic and Therapeutic Challenges

Guest Editors: Zsolt Molnár, Evangelos J. Giamarellos-Bourboulis, Anand Kumar, and Axel Nierhaus





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Editorial

Sepsis: Diagnostic and Therapeutic Challenges

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Intensive and critical care medicine has gone through unprecedented development over the last few decades. According to recent surveys, we now treat severalfold more critically ill patients in intensive care units (ICU) worldwide as compared to 10 years ago [1]. One of the most challenging tasks that intensive care specialists face is the treatment of serious infection-related multiple organ dysfunction, termed “sepsis” and “septic shock.” Sepsis has become a serious health economic issue around the world, with more patients dying due to sepsis related complications than breast and colorectal cancer together. According to recent data from the United States and Germany, sepsis is the single most expensive reason for hospitalization [2–4]. Large retrospective and prospective studies indicate that mortality of septic shock can still be as high as 45–55% and is associated with a 2- to 3-fold longer ICU and hospital stay [4, 5]. Accordingly, sepsis has become a serious health economic issue; hence, research of new frontiers in the diagnosis and treatment of sepsis has been a top priority in intensive care medicine.

The performance of sepsis research has had several difficulties. First of all, defining sepsis is a very difficult task as it is not a definitive disease [6]. Ever since the term “sepsis syndrome” was invented by Bone and coworkers, there has been a continuous search for appropriate, universally applicable definitions [7]. The latest consensus definitions have recently been published by an international task force as “Sepsis-3” [8]. Nonetheless, the problem lingers because sepsis is a very heterogeneous condition of different etiologies

and severity which can range from a mild form of one organ system dysfunction requiring only moderate support to a very severe multiple system organ failure needing invasive salvage therapies. This heterogeneity of the investigated patient populations may, at least in part, explain why clinical research of the last 30 years has often been regarded as a failure, since most studies either failed to show clear survival benefit, or positive results of single center studies were later contradicted by large multicenter trials [9].

In addition to the problems of defining sepsis, serious challenges in diagnostics also exist. In contrast to other specialties where diagnostic laboratory and/or radiological tests with high sensitivity and specificity exist, the diagnosis of sepsis is more complicated. There are two main elements to this problem. On the one hand, organ dysfunction has to be recognized early and resuscitation measures must be commenced without delay in order to stabilize the patient and to avoid any secondary organ damage. Simultaneously, the nature of the underlying infection has to be clarified. Unfortunately, conventional indicators of infection (fever, leukocytosis, etc.) have poor performance in the critically ill. Even new biomarkers have only 75–85% sensitivity and specificity to diagnose infection at best, mainly because of the fact that pathobiology varies considerably from one patient to another [6]. Therefore, any single test is inadequate to make the diagnosis of sepsis, and it is highly unlikely that there will ever be a particular laboratory parameter that can do the job. Hence, the competence and responsibility of the attending

physician are important beyond measure at present and may remain so for years to come.

Finally, it seems highly unlikely that a single comprehensive and specific “antiseptic” medication would appear on the scene. Treatment will always include nonspecific measures of organ support, such as oxygen therapy, mechanical ventilation, hemodynamic support, and renal replacement therapy, and antimicrobials. Of note, there seems to be a clear window of opportunity for most of these interventions to have an impact on survival: treatment has to be initiated as early as possible [10]. In addition, there is some rationale to apply adjunctive treatment and help the immune system in its deadly fight against the invading pathogens, by either reinforcing it or attenuating the inflammatory response [11]. However, this requires the introduction of novel markers of the immune response that enable the physician at the bedside to accurately gauge the actual state of the immune system and to tailor highly individualized interventions [12].

This issue tried to address some of these points. These new results and reviews, interesting as they are, may also serve as hypothesis generating for future research. In this special issue on sepsis, only a small bundle of the huge array of topics in novel sepsis research will be presented, but it nevertheless demonstrates the motivation and determination of the intensive care community in order to improve understanding and therapeutic modalities for our patients.

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References

- [1] K.-M. Kaukonen, M. Bailey, S. Suzuki, D. Pilcher, and R. Bellomo, “Mortality related to severe sepsis and septic shock among critically ill patients in Australia and New Zealand, 2000–2012,” *Journal of the American Medical Association*, vol. 311, no. 13, pp. 1308–1316, 2014.
- [2] D. F. Gaieski, J. M. Edwards, M. J. Kallan, and B. G. Carr, “Benchmarking the incidence and mortality of severe sepsis in the united states,” *Critical Care Medicine*, vol. 41, no. 5, pp. 1167–1174, 2013.
- [3] C. M. Torio and R. M. Andrews, “National Inpatient Hospital Costs: The Most Expensive Conditions by Payer, 2011: Statistical Brief #160,” Healthcare Cost and Utilization Project (HCUP) Statistical Briefs, Agency for Health Care Policy and Research, Rockville, Md, USA, 2006–2013.
- [4] S. Heublein, M. Hartmann, S. Hagel, R. Hutagalung, and F. M. Brunkhorst, “Epidemiology of sepsis in German hospitals derived from administrative databases,” *Infection*, vol. 17, article S71, 2013.
- [5] C. Engel, F. M. Brunkhorst, H.-G. Bone et al., “Epidemiology of sepsis in Germany: results from a national prospective multicenter study,” *Intensive Care Medicine*, vol. 33, no. 4, pp. 606–618, 2007.
- [6] I. László, D. Trásy, Z. Molnár, and J. Fazakas, “Sepsis: from pathophysiology to individualized patient care,” *Journal of Immunology Research*, vol. 2015, Article ID 510436, 13 pages, 2015.
- [7] R. C. Bone, C. J. Fisher Jr., T. P. Clemmer, G. J. Slotman, G. A. Metz, and R. A. Balk, “Sepsis syndrome: a valid clinical entity. Methylprednisolone Severe Sepsis Study Group,” *Critical Care Medicine*, vol. 17, no. 5, pp. 389–393, 1989.
- [8] M. Singer, C. S. Deutschman, C. W. Seymour et al., “The third international consensus definitions for sepsis and septic shock (Sepsis-3),” *Journal of the American Medical Association*, vol. 315, no. 8, pp. 801–810, 2016.
- [9] J.-L. Vincent, “We should abandon randomized controlled trials in the intensive care unit,” *Critical Care Medicine*, vol. 38, no. 10, pp. S534–S538, 2010.
- [10] A. Kumar, D. Roberts, K. E. Wood et al., “Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock,” *Critical Care Medicine*, vol. 34, no. 6, pp. 1589–1596, 2006.
- [11] R. S. Hotchkiss, G. Monneret, and D. Payen, “Immunosuppression in sepsis: a novel understanding of the disorder and a new therapeutic approach,” *The Lancet Infectious Diseases*, vol. 13, no. 3, pp. 260–268, 2013.
- [12] G. Monneret and F. Venet, “Sepsis-induced immune alterations monitoring by flow cytometry as a promising tool for individualized therapy,” *Cytometry Part B: Clinical Cytometry*, vol. 90, no. 4, pp. 376–386, 2016.

Research Article

Effect of Prophylactic Antifungal Protocols on the Prognosis of Liver Transplantation: A Propensity Score Matching and Multistate Model Approach

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Background. Whether routine antifungal prophylaxis decreases posttransplantation fungal infections in patients receiving orthotopic liver transplantation (OLT) remains unclear. This study aimed to determine the effectiveness of antifungal prophylaxis for patients receiving OLT. **Patients and Methods.** This is a retrospective analysis of a database at Chang Gung Memorial Hospital. We have been administering routine antibiotic and prophylactic antifungal regimens to recipients with high model for end-stage liver disease scores (>20) since 2009. After propensity score matching, 402 patients were enrolled. We conducted a multistate model to analyze the cumulative hazards, probability of fungal infections, and risk factors. **Results.** The cumulative hazards and transition probability of “transplantation to fungal infection” were lower in the prophylaxis group. The incidence rate of fungal infection after OLT decreased from 18.9% to 11.4% ($p = 0.052$); overall mortality improved from 40.8% to 23.4% ($p < 0.001$). In the “transplantation to fungal infection” transition, prophylaxis was significantly associated with reduced hazards for fungal infection (hazard ratio: 0.57, 95% confidence interval: 0.34–0.96, $p = 0.033$). Massive ascites, cadaver transplantation, and older age were significantly associated with higher risks for mortality. **Conclusion.** Prophylactic antifungal regimens in high-risk recipients might decrease the incidence of posttransplant fungal infections.

1. Introduction

Orthotopic liver transplantation (OLT) is the treatment of choice for patients with hepatocellular carcinoma, end-stage liver disease, and acute liver failure [1]. Despite advances in surgical techniques, availability of immunosuppressants, and evidence-based guidelines for perioperative management to improve the overall survival of transplant recipients, rejections and infections still affect early posttransplantation mortality. Although advances in immunosuppressants has decreased the incidence of organ rejections, recipients are at greater risk of infections [2]. The use of immunosuppressants

has been found to affect host immunity, causing recipients to become susceptible to viral and fungal infections, and subsequently death, after OLT [2, 3]. In addition, several lines of evidence reveal that intensive care unit conditions, surgical techniques, type of transplantation, type of anastomosis method, massive blood transfusion, and prophylactic antibiotics and immunosuppressants are associated with posttransplant fungal infections [4]. Despite the advances in surgical techniques leading to reductions in intraoperative blood transfusions and surgical time in recent years, the incidence of invasive fungal infection (IFI) still ranges from 5% to 20% [2, 5, 6].

IFI is the major cause of mortality in the early post-transplantation state. IFI-related mortality in organ transplantation has been found to cause up to 77% of deaths, one of the major causes of early posttransplantation mortality [6]. Among IFIs, *Candida* species are the most common pathogens, followed by *Aspergillus* species. Before 2009, antifungal prophylaxis was controversial owing to the lack of direct evidence that it improved survival. However, a prophylactic antifungal regimen for transplant recipients at high risk of fungal infection was suggested by the evidence-based guidelines of the Infectious Disease Society of America (IDSA) in 2009 [7]. Nevertheless, there is little direct evidence with regard to prognosis after such prophylactic strategies. Thus, we conducted a retrospective hospital-based cohort study to investigate whether routine antifungal prophylaxis regimens reduce the risk of fungal infections in patients receiving OLT. In addition, we conducted a multistate model to investigate transition-specific risk factors.

2. Patients and Methods

2.1. Study Cohort. Patients undergoing OLT between January 2005 and September 2014 at the Chang Gung Memorial Hospital, Linkou, were enrolled retrospectively and were followed up until December 2015. All patients receiving either deceased or living donor livers (LDLT) were enrolled, and routine screening of infections following OLT was conducted. Routine culture from ascites and catheter were conducted perioperatively and sputum culture was conducted routinely for patients under mechanical ventilation. Patients with fungal infection before transplantation were excluded to prevent overestimation of the incidence. Clinical data, including age, sex, type of hepatitis, status of liver cirrhosis, model for end-stage liver disease (MELD) score, indication for OLT, type of OLT, microbiological screening results, and status of ascites after OLT, were collected. Ethical approval was obtained from the Committee of Ethics in Biomedical Research of Chang Gung Memorial Hospital, and the study conformed to the ethical guidelines of the 1975 Declaration of Helsinki.

2.2. Prophylaxis Strategy. Our intervention group underwent routine antifungal prophylaxis. At our institution, since 2009, a prophylactic antifungal regimen was routinely provided to transplant recipients with a high (>20) MELD score before undergoing transplantation. Perioperative prophylaxis consisted of ceftriaxone (2000 mg/day) in two divided doses plus ampicillin sodium (1000 mg/q6 h) adjusted by renal function for patients with a lower MELD score (≤ 20) and vancomycin HCl (15 mg/kg/dose q12 h) adjusted by renal function plus Tienam (imipenem (500 mg)/cilastatin (500 mg); 500 mg/q6 h) adjusted by renal function for patients with a high MELD score (>20). We used echinocandins, either anidulafungin (100 mg/day) or micafungin (100 mg/day), for fungal prophylaxis to prevent drug interactions between the antifungal agents and the immunosuppressants (calcineurin inhibitors) [8].

2.3. Diagnosis of Fungal Infection. Our primary outcome was risk for fungal infection in patients with liver transplantation. The diagnosis of fungal infection was based on positive culture data after OLT, which revealed a specific fungus or positive findings of yeast in the blood, wound, urine, catheter, or sputum. A positive fungal culture from urine combined with clinical manifestations was identified as a fungal infection instead of colonization. Positive cultures from blood, urine, and sputum depended on the clinical manifestation to define it as an infection.

2.4. Statistical Analysis. Continuous variables were summarized as median with interquartile range, while categorical variables were presented as frequency and percentage. In univariate analysis, baseline characteristics were compared between the intervention group and nonintervention group using the chi-squared test, Fisher's exact test, or Wilcoxon's rank-sum test, as appropriate. To reduce selection and confounding biases, we conducted propensity score matching using the nearest neighbor matching method with a 1:1 ratio for the intervention and nonintervention groups [9]. Furthermore, we used a multistate model to model the "transplant to fungal infection transition," "transplant to death transition," and "fungal infection to death transition," occurring as a result of various reasons [10, 11]. First, death is a competing event with fungal infection occurrence. Second, we could simultaneously model all 3 transitions and estimate the cause-specific cumulative hazards, as well as cause-specific transition probability. In addition, we conducted cause-specific Cox models to investigate predictors of the 3 transitions. We performed model selection by Akaike information criterion (AIC) in a stepwise algorithm and substantive knowledge to find the parsimonious models [12]. In addition, we investigated the proportional hazards assumption using the modified Schoenfeld residuals test [13]. All reported confidence intervals (CIs) and tests were two-sided, with a 5% significance level. All analyses were performed using R software version 3.3.1 (R Foundation for Statistical Computing, Vienna, Austria) with contributed packages "MatchIt" [9], "MASS" [14], "mstate" [10, 11], and "survival" [13].

3. Results

A total of 561 patients were enrolled, of which 360 (64.2%) received the routine prophylactic antifungal regimen and 201 (35.8%) did not. After propensity score matching, a total of 402 patients were included for further analysis and the variables were comparable between the two groups. The demographic data before and after matching are presented in Table 1. After matching, the rate of fungal infection was 18.9% before routine prophylaxis and 11.4% after prophylactic treatment ($p = 0.052$), and the overall mortality rate of the recipients was 40.8% before the use of routine prophylaxis and 23.4% after ($p < 0.001$). Hepatitis B virus infection was dominant in the OLT recipients, followed by hepatitis C virus infection. The median time of fungal infection in the prophylaxis group was 27 days (interquartile range (IQR) 10.5–77.7 days), whereas it was

TABLE 1: Demographic data of the liver transplantation recipients (before and after matching).

	Before matching			After matching		
	No prophylaxis	Prophylaxis	<i>p</i> value	No prophylaxis	Prophylaxis	<i>p</i> value
<i>N</i>	201	360		201	201	
Age (median (IQR))	53.00 (47.00, 57.00)	55.00 (48.00, 60.00)	0.021	53.00 (47.00, 57.00)	54.00 (48.00, 59.00)	0.228
Age (%)						
≤55	71 (35.3)	123 (34.2)	0.048	71 (35.3)	69 (34.3)	0.953
>55–60	101 (50.2)	155 (43.1)		101 (50.2)	101 (50.2)	
>60	29 (14.4)	82 (22.8)		29 (14.4)	31 (15.4)	
Sex (%)						
Male	153 (76.1)	264 (73.3)	0.533	153 (76.1)	160 (79.6)	0.471
Female	48 (23.9)	96 (26.7)		48 (23.9)	41 (20.4)	
HCC (%)						
No	112 (55.7)	195 (54.2)	0.79	112 (55.7)	108 (53.7)	0.764
Yes	89 (44.3)	165 (45.8)		89 (44.3)	93 (46.3)	
Viral hepatitis (%)						
None	26 (12.9)	78 (21.7)	0.017	26 (12.9)	29 (14.4)	0.905
HBV	126 (62.7)	183 (50.8)		126 (62.7)	127 (63.2)	
HCV	38 (18.9)	84 (23.3)		38 (18.9)	33 (16.4)	
HBV + HCV	11 (5.5)	15 (4.2)		11 (5.5)	12 (6.0)	
Ascites (%)						
Mild/moderate (≤2000 mL)	139 (69.2)	256 (71.2)	0.484	139 (69.2)	138 (68.7)	1
Massive (>2000 mL)	62 (30.8)	104 (28.9)		62 (30.8)	63 (31.3)	
Living donor (%)						
No	64 (31.8)	72 (20.0)	0.002	64 (31.8)	58 (28.9)	0.588
Yes	137 (68.2)	288 (80.0)		137 (68.2)	143 (71.1)	
MELD score						
≤20	130 (64.7)	258 (71.7)	0.104	130 (64.7)	133 (66.2)	0.834
>20	71 (35.3)	102 (28.3)		71 (35.3)	68 (33.8)	
Fungal infection (%)						
No	163 (81.1)	315 (87.5)	0.054	163 (81.1)	178 (88.6)	0.052
Yes	38 (18.9)	45 (12.5)		38 (18.9)	23 (11.4)	
Mortality (%)						
No	119 (59.2)	272 (75.6)	<0.001	119 (59.2)	154 (76.6)	<0.001
Yes	82 (40.8)	88 (24.4)		82 (40.8)	47 (23.4)	
Propensity score (median (IQR))	NA	NA		0.40 (0.32, 0.46)	0.40 (0.32, 0.44)	0.690

IQR: interquartile range, HCC: hepatocellular carcinoma, and MELD: model for end-stage liver disease. Ascites was measured during the operation.

21 days (IQR 10–48.5 days) in the nonprophylaxis group. The transition matrix of the 3 states is summarized in the Supplemental Table 1 (see Supplementary Material available online at <http://dx.doi.org/10.1155/2016/6212503>). 61 of 402 (15%) patients developed “transplantation to fungal infection” transition. Among 61 patients with fungal infection, 36 (59%) patients died.

The species causing fungal infection are shown in Table 2. The most common fungal infection was by *Candida albicans*: 33.7% of infections before routine prophylaxis and 35.9% after. *Aspergillus* infection disappeared after initiating the routine prophylactic antifungal regimen. The incidence of *Candida glabrata* and *Candida tropicalis* increased after frequent echinocandin usage. Yeast was found in 32.3% and 28.2% of the cultures before and after prophylaxis,

respectively. Eleven patients in the infected group developed 2 kinds of fungal infections and 1 developed 3 kinds of fungal infections.

A multistate model was used to evaluate the cumulative hazards and transition probability after OLT for “transplantation to fungal infection,” “transplantation to death,” and “fungal infection to death” transitions. Figure 1 shows that the cumulative hazards of a “transplantation to fungal infection” transition were lower in the routine prophylaxis group compared to the nonprophylaxis group. Cumulative hazards for “transplantation to death” and “fungal infection to death” transitions were similar in the 2 groups. We estimated 1-year, 2-year, and 3-year transition probabilities among the four states including “transplantation,” “fungal infection,” “death with fungal infection,” and “death without fungal

TABLE 2: Species of fungus before and after the prophylactic anti-fungal protocol.

No prophylactic period			Prophylactic period		
Species	Number	%	Species	Number	%
<i>Candida albicans</i>	25	33.7	<i>Candida albicans</i>	28	35.9
<i>Candida glabrata</i>	7	9.9	<i>Candida tropicalis</i>	12	15.3
<i>Candida tropicalis</i>	5	6.7	<i>Candida glabrata</i>	5	6.4
<i>Candida parapsilosis</i>	4	5.4	<i>Candida parapsilosis</i>	3	3.8
<i>Candida krusei</i>	2	2.7	<i>Candida krusei</i>	1	1.3
<i>Aspergillus</i>	3	4.0	<i>Candida guilliermondii</i>	1	1.3
Mold	2	2.7	<i>Candida sp.</i>	1	1.3
<i>Penicillium sp.</i>	1	1.3	<i>Mucor sp.</i>	1	1.3
<i>Trichosporon sp.</i>	1	1.3	Mold	4	5.0
Yeast	23	32.3	Yeast	22	28.2

Note. Eleven patients developed 2 kinds of fungal infection and 1 developed 3 kinds of fungal infection. Mold and yeast species are not routinely identified without physician's requests.

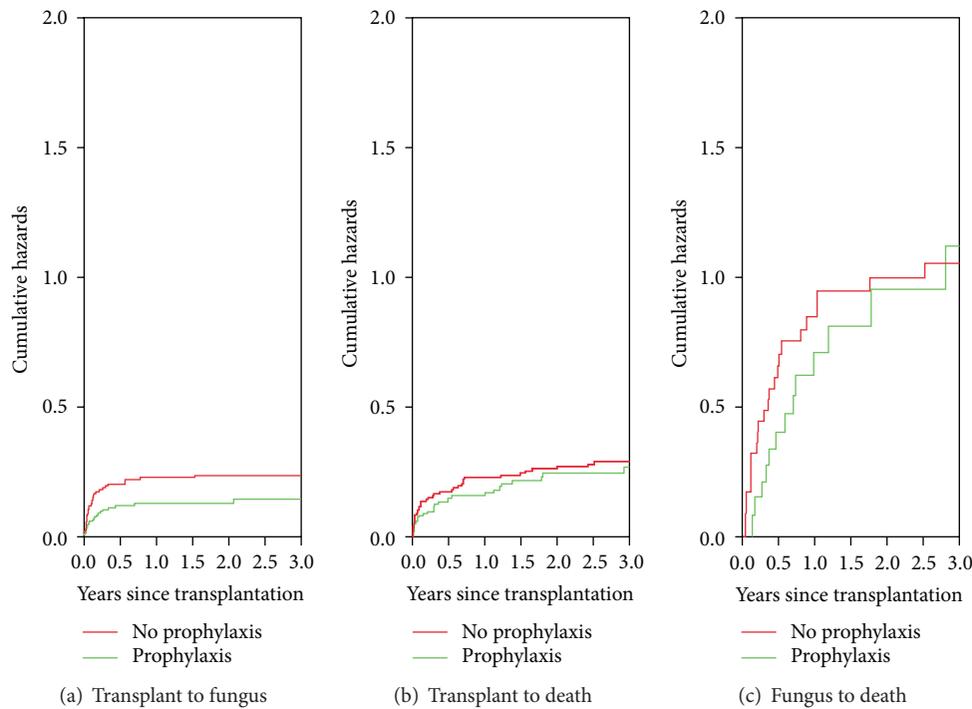


FIGURE 1: Nonparametric estimates of cumulative hazards of the multistate model stratified by transitions. (a) Cumulative hazards for the transition from liver transplantation to fungal infection demonstrate the fungal infection which occurred within the early period of transplantation and reached a plateau after 3 months. The routine prophylaxis group has lower cumulative hazards of “transplantation to fungal infection” transition. (b) Cumulative hazards for the transition from liver transplantation to death between the two groups were similar. (c) Cumulative hazards for the transition from fungal infection to death between the two groups were similar.

infection” (Table 3). The routine prophylaxis group had a lower probability of “fungal infection” and “death with fungal infection.” Notably, the sum of “fungal infection” and “death with fungal infection” probability did not obviously increase over time, indicating most fungal infections occurred in the early posttransplantation period.

We also investigated predictors in 3 transition-specific multivariable Cox models (Table 4). In the transition from “transplantation to fungal infection,” the routine prophylaxis group was significantly associated with reduced hazards for

fungal infection compared with the nonprophylaxis group (hazard ratio (HR): 0.57, 95% confidence interval (CI): 0.34–0.96, $p = 0.033$). In the transition from “transplantation to death,” patients with massive ascites were associated with a higher risk for mortality compared to patients with mild/moderate ascites (HR: 1.55, 95% CI: 1.02–2.36, $p = 0.042$). By checking the proportional hazards assumptions, LDLT was statistically significant associated with time-varying effects. Thus, we used the time point of 1.5 years after liver transplantation to model the LDLT effects (this

TABLE 3: The 1-year, 2-year, and 3-year transition probability among four states in the multistate model.

	State occupied probability (95% CI)		
	1-year	2-year	3-year
<i>Prophylaxis</i>			
Transplant	74.2 (67.9–80.5)	68.1 (60.8–75.3)	65.4 (57.6–73.2)
Fungus infection	6.3 (2.3–9.7)	4.8 (1.8–7.9)	5.0 (1.5–8.4)
Death with fungus infection	5.5 (2.3–8.6)	6.9 (3.3–10.5)	7.9 (4.0–11.8)
Death w/o fungus infection	14.0 (9.0–19)	20.2 (13.8–26.6)	21.7 (14.8–28.6)
<i>No prophylaxis</i>			
Transplant	62.6 (55.9–69.4)	59.5 (52.7–66.3)	58.5 (51.6–65.3)
Fungus infection	10.3 (6.1–14.4)	9.2 (5.3–13.2)	8.7 (4.9–12.6)
Death with fungus infection	8.7 (4.9–2.5)	10.2 (6.1–14.3)	10.7 (6.5–14.9)
Death w/o fungus infection	18.4 (13.0–23.8)	21.0 (5.4–26.7)	22.1 (16.3–27.8)

TABLE 4: Results of multivariable transition-specific Cox models.

Variable	Category	HR (95% CI)	<i>p</i> value
<i>Transition: transplant to fungal infection</i>			
Treatment	No prophylaxis	1	0.033*
	Prophylaxis	0.57 (0.34–0.96)	
Ascites	Mild/moderate	1	0.058
	Massive	1.65 (0.98–2.76)	
Propensity score		0.15 (0.01–1.76)	0.132
<i>Transition: transplant to death</i>			
Ascites	Mild/moderate	1	0.042*
	Massive	1.55 (1.02–2.36)	
Living donor (within 1.5 years)	No	1	<0.001*
	Yes	0.41 (0.26–0.66)	
Living donor (after 1.5 years)	No	1	0.861
	Yes	1.08 (0.45–2.62)	
<i>Transition: fungal infection to death</i>			
Age (years)	≤50	1	0.029
	>50, ≤60	2.55 (1.10–5.93)	
	>60	1.80 (0.67–4.83)	
Ascites	Mild/moderate	1	0.035
	Massive	2.19 (1.06–4.52)	
Living donor	No	1	0.113
	Yes	0.57 (0.28–1.14)	

HR: hazard ratio and CI: confidence interval. * $p < 0.05$.

time point was indicated by the residual plots). LDLT was associated with a lower risk for short-term mortality (with 1.5 years) compared to patients receiving cadaver transplantation (HR: 0.41, CI: 0.26–0.66, $p \leq 0.001$). However, 1.5 years after liver transplantation, LDLT was not significantly associated with a lower risk for mortality. In the transition from “fungal infection to death,” older age and massive ascites were significantly associated with a higher risk of mortality.

4. Discussion

Our current study demonstrated that routine prophylactic antifungal regimens are associated with a lower risk for “transplantation to fungal infection” in patients receiving

OLT. Patients with massive ascites had a higher risk for “transplantation to death” and “fungal infection to death” transitions. Patients receiving LDLT had a lower risk for the “transplantation to death” transition within 1.5 years after OLT.

Because of the evolution of surgical techniques and improvements in post-OLT management, the 5-year survival rate after OLT has reached 72–77% in recent times [15]. However, IFI is still one of the major causes of early mortality in liver transplant recipients. The incidence of IFI ranges from 5% to 20% [2, 5, 6]. According to the literature, *Candida* and *Aspergillus* are the most common causal agents and are associated with high mortality in organ transplantation, accounting for 30–60% of the infections [5, 16].

The reported risk factors for IFI include retransplantation, dialysis, prolonged operation time, and prolonged broad-spectrum antibiotics use [2, 17]. Fungal infections most frequently occur in the first month after OLT, and antifungal prophylaxis could significantly reduce fungal infections in patients receiving OLT. In a recent meta-analysis, antifungal prophylaxis has been shown to reduce fungal infection-related mortality [18]. Among antifungal drugs, echinocandins, such as caspofungin, micafungin, and anidulafungin, have excellent *in vitro* activity against *Candida* species, with few side effects and minimal drug-drug interactions; in particular, they do not influence the clearance of calcineurin inhibitors, which are commonly used immunosuppressants. Moreover, dosage adjustment is not required in patients with impaired renal function or those under dialysis [19].

Although advances in perioperative management and surgical techniques enable improved survival of OLT recipients, an infection after OLT is a major cause of mortality. A prophylactic protocol was established at our institution since 2009, which includes empiric antibiotics based on the MELD score and a regimen of prophylactic antifungal treatment in high-risk patients. IDSA guidelines have suggested prophylactic antifungal treatment in patients with renal dysfunction, retransplantation, or reoperation; however, more recent guidelines suggest prophylaxis in high-risk patients with high MELD scores, choledochojejunostomy, bile leaks, and LDLT [17, 20, 21]. Our current study showed that routine antifungal regimens reduce the risk for a “transplantation to fungal infection” transition. In addition, our results suggest fungal infections mostly occurred within the initial 3 months after OLT, which is consistent with other reports.

Most infections, either bacterial or fungal, occur in the first month after OLT, causing early mortality after transplantation in the first year [22]. In the current study, 1-year, 2-year, and 3-year probability of fungal infection and death with fungal infection were reduced in the prophylaxis group. These results indicate prophylactic protocols might reduce the incidence of fungal infection and death with fungal infection. The IDSA guideline in 2009 recommended routine antifungal prophylaxis for OLT recipients [23]. Recently, Saliba et al. reported that a MELD score of >30 might be the most important risk factor for IFI [17]. Patients with a MELD score > 20 have a higher possibility of pretransplantation renal dysfunction and liver dysfunction; therefore, we use this threshold for prophylaxis.

Patients undergoing OLT can have several time-dependent outcomes during follow-up [24]. A multistate model has been applied to analyze competing risks in patients with liver cirrhosis [25]. By evaluating the transition-specific risk factors from the multistate model, we found that a routine prophylactic antifungal regimen in high-risk recipients prevents further fungal infection. In patients with fungal infections, *C. albicans* was the most common species before and after prophylaxis. The incidence of other species was reduced after prophylaxis. Such findings are consistent with the literature [26]. Intrinsic resistance or resistance induced by the prophylactic agent might account for these findings. In addition, acquired resistance to echinocandins

has been reported even in clinically relevant *Candida* spp. [27]. Acquired resistance species are associated with high mortality after fungal infections. Changing antifungal treatment from echinocandins to azoles or to liposomal amphotericin B should be considered if a positive fungal culture persists even after the antifungal regimen treatment. However, more data need to be collected to confirm this approach. In our institution, the initial positive culture of fungal infection revealed only yeast or molds, and further differentiation of the species required special cultures. If the patients' general condition improved after treatment or the condition became worse, we may not perform specific cultures for further differentiation and that is why almost one-third of the cultures revealed yeast only.

LDLT is associated with a lower short-term risk of “transplant to death” transition. The time-dependent effects might be associated with high-risk patients leaving the risk-set in the early period. LDLT might be associated with a shorter waiting time for the organ and prevention of the deterioration of liver function in the recipients. In addition, more reserve liver function and improved surgical techniques may improve long-term survival. Additionally, massive ascites indicates decompensated liver function and leads to about 50% mortality 2 years after patients present with uncontrolled ascites [28].

Our study has several advantages. First, we used propensity score matching to reduce selection and confounding biases in this observational study. Second, we used time to event outcomes in the current study. Such an approach allowed us to investigate time-varying treatment effects and adjust for competing risks (mortality is a competing risk for fungal infections, because it prevents the occurrence of fungal infection). If we did not adjust for competing risks, we would overestimate the cumulative incidence of fungal infection. Third, we used a multistate model to investigate the transition-specific risk factors.

Nevertheless, our present study has several limitations. First, unmeasured confounders could not be matched. For example, we could not match important biological data and donor-related factors. Second, time-lag bias can compromise the results in such a long-term observational study. For example, the reduced risk of fungal infection might be associated with more experience in surgical technique, improved intensive unit care, and better surgical facilities. Third, the small sample size in the current study limits us from thoroughly investigating predicting factors. Finally, this retrospective analysis of observational hospital-based cohort data might have information and performance biases.

5. Conclusion

We conclude that administering routine empiric antibiotic treatment and a prophylactic antifungal regimen to high-risk patients might reduce the incidence of fungal infection in the early stage after liver transplantation and prevent fungal infection-related mortality, which might lead to better long-term survival. *Candida* species remain the major cause of fungal infection despite prophylaxis. Further clinical trials are warranted to confirm our results.

Disclosure

This study is based in part on data from the database of liver transplantation in Chang Gung Memorial Hospital. The interpretations and conclusions contained herein do not represent those of the Chang Gung Memorial Hospital.

Competing Interests

The authors have no competing interests to declare.

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References

- [1] "National Institutes of Health Consensus Development Conference Statement: liver transplantation—June 20–23, 1983," *Hepatology*, vol. 4, no. 1, supplement, pp. 107S–110S, 1984.
- [2] M. J. Pacholczyk, B. Lagiewska, W. Lisik, D. Wasiak, and A. Chmura, "Invasive fungal infections following liver transplantation—risk factors, incidence and outcome," *Annals of Transplantation*, vol. 16, no. 3, pp. 14–16, 2011.
- [3] C.-H. Yang, X.-S. He, J. Chen et al., "Fungal infection in patients after liver transplantation in years 2003 to 2012," *Annals of Transplantation*, vol. 17, no. 4, pp. 59–63, 2012.
- [4] L. A. Collins, M. H. Samore, M. S. Roberts et al., "Risk factors for invasive fungal infections complicating orthotopic liver transplantation," *Journal of Infectious Diseases*, vol. 170, no. 3, pp. 644–652, 1994.
- [5] P. G. Pappas, B. D. Alexander, D. R. Andes et al., "Invasive fungal infections among organ transplant recipients: results of the transplant-associated infection surveillance network (TRANSNET)," *Clinical Infectious Diseases*, vol. 50, no. 8, pp. 1101–1111, 2010.
- [6] N. Singh, "Fungal infections in the recipients of solid organ transplantation," *Infectious Disease Clinics of North America*, vol. 17, no. 1, pp. 113–134, 2003.
- [7] P. G. Pappas and F. P. Silveira, "Candida in solid organ transplant recipients," *American Journal of Transplantation*, vol. 9, supplement 4, pp. S173–S179, 2009.
- [8] D. W. Denning, "Echinocandin antifungal drugs," *The Lancet*, vol. 362, no. 9390, pp. 1142–1151, 2003.
- [9] D. E. Ho, K. Imai, G. King, and E. A. Stuart, "MatchIt: nonparametric preprocessing for parametric causal inference," *Journal of Statistical Software*, vol. 42, no. 8, pp. 1–28, 2011.
- [10] H. Putter, M. Fiocco, and R. B. Geskus, "Tutorial in biostatistics: competing risks and multi-state models," *Statistics in Medicine*, vol. 26, no. 11, pp. 2389–2430, 2007.
- [11] L. C. de Wreede, M. Fiocco, and H. Putter, "mstate: an R package for the analysis of competing risks and multi-state models," *Journal of Statistical Software*, vol. 38, no. 7, pp. 1–30, 2011.
- [12] F. Kemp, "Modern applied statistics with S," *Journal of the Royal Statistical Society: Series D (The Statistician)*, vol. 52, no. 4, pp. 704–705, 2003.
- [13] T. M. Therneau and P. M. Grambsch, *Modeling Survival Data: Extending the Cox Model*, Statistics for Biology and Health, Springer Science & Business Media, 2000.
- [14] W. N. Venables and B. D. Ripley, *Modern Applied Statistics with S*, Springer, New York, NY, USA, 4th edition, 2002.
- [15] R. M. Merion, "Current status and future of liver transplantation," *Seminars in Liver Disease*, vol. 30, no. 4, pp. 411–421, 2010.
- [16] P. Muñoz, C. Rodríguez, E. Bouza et al., "Risk factors of invasive aspergillosis after heart transplantation: protective role of oral itraconazole prophylaxis," *American Journal of Transplantation*, vol. 4, no. 4, pp. 636–643, 2004.
- [17] F. Saliba, V. Delvart, P. Ichaï et al., "Fungal infections after liver transplantation: outcomes and risk factors revisited in the MELD era," *Clinical Transplantation*, vol. 27, no. 4, pp. E454–E461, 2013.
- [18] M. Cruciani, C. Mengoli, M. Malena, O. Bosco, G. Serpelloni, and P. Grossi, "Antifungal prophylaxis in liver transplant patients: a systematic review and meta-analysis," *Liver Transplantation*, vol. 12, no. 5, pp. 850–858, 2006.
- [19] C. A. Kauffman and P. L. Carver, "Update on echinocandin antifungals," *Seminars in Respiratory and Critical Care Medicine*, vol. 29, no. 2, pp. 211–219, 2008.
- [20] F. P. Silveira and S. Kusne, "Candida infections in solid organ transplantation," *American Journal of Transplantation*, vol. 13, supplement 4, pp. 220–227, 2013.
- [21] J. Fortún, P. Martín-Dávila, S. Moreno et al., "Risk factors for invasive aspergillosis in liver transplant recipients," *Liver Transplantation*, vol. 8, no. 11, pp. 1065–1070, 2002.
- [22] F. A. Romero and R. R. Razonable, "Infections in liver transplant recipients," *World Journal of Hepatology*, vol. 3, no. 4, pp. 83–92, 2011.
- [23] P. G. Pappas, C. A. Kauffman, D. Andes et al., "Clinical practice guidelines for the management of candidiasis: 2009 update by the Infectious Diseases Society of America," *Clinical Infectious Diseases*, vol. 48, no. 5, pp. 503–535, 2009.
- [24] P. K. Andersen, R. B. Geskus, T. de Witte, and H. Putter, "Competing risks in epidemiology: possibilities and pitfalls," *International Journal of Epidemiology*, vol. 41, no. 3, pp. 861–870, 2012.
- [25] P. Jepsen, H. Vilstrup, and P. K. Andersen, "The clinical course of cirrhosis: the importance of multistate models and competing risks analysis," *Hepatology*, vol. 62, no. 1, pp. 292–302, 2015.
- [26] B. J. Kullberg and M. C. Arendrup, "Invasive candidiasis," *The New England Journal of Medicine*, vol. 373, no. 15, pp. 1445–1456, 2015.
- [27] M. C. Arendrup and D. S. Perlin, "Echinocandin resistance: an emerging clinical problem?" *Current Opinion in Infectious Diseases*, vol. 27, no. 6, pp. 484–492, 2014.
- [28] P. Ginés, E. Quintero, V. Arroyo et al., "Compensated cirrhosis: natural history and prognostic factors," *Hepatology*, vol. 7, no. 1, pp. 122–128, 1987.

Review Article

Advanced Hemodynamic Management in Patients with Septic Shock

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In patients with sepsis and septic shock, the hemodynamic management in both early and later phases of these “organ dysfunction syndromes” is a key therapeutic component. It needs, however, to be differentiated between “early goal-directed therapy” (EGDT) as proposed for the first 6 hours of emergency department treatment by Rivers et al. in 2001 and “hemodynamic management” using advanced hemodynamic monitoring in the intensive care unit (ICU). Recent large trials demonstrated that nowadays protocolized EGDT does not seem to be superior to “usual care” in terms of a reduction in mortality in emergency department patients with early identified septic shock who promptly receive antibiotic therapy and fluid resuscitation. “Hemodynamic management” comprises (a) making the diagnosis of septic shock as one differential diagnosis of circulatory shock, (b) assessing the hemodynamic status including the identification of therapeutic conflicts, and (c) guiding therapeutic interventions. We propose two algorithms for hemodynamic management using transpulmonary thermodilution-derived variables aiming to optimize the cardiocirculatory and pulmonary status in adult ICU patients with septic shock. The complexity and heterogeneity of patients with septic shock implies that individualized approaches for hemodynamic management are mandatory. Defining individual hemodynamic target values for patients with septic shock in different phases of the disease must be the focus of future studies.

1. Introduction

A recent consensus report defines sepsis as “life-threatening organ dysfunction caused by a dysregulated host response to infection” [1]. Septic shock is defined as a “subset of sepsis in which particularly profound circulatory, cellular, and metabolic abnormalities are associated with a greater risk of mortality than with sepsis alone” [1]. Clinical indicators of septic shock are defined by a need for vasopressor administration to maintain a mean arterial pressure (MAP) of 65 mmHg or greater and a serum lactate level greater than 2 mmol/L in the absence of hypovolemia [1].

Complex disease syndromes such as septic shock require multimodal diagnostic and therapeutic approaches. Besides

the diagnosis of septic shock and early causal therapy one major challenge in its treatment remains the resuscitation and management of cardiocirculatory and respiratory dysfunction.

In this context, the hemodynamic management in both early and later phases of these syndromes is crucial. However, with regard to the management of cardiovascular dynamics in patients with sepsis and septic shock we still have more questions than answers.

In this article, we therefore aim to expand on the difference between “early goal-directed therapy” (EGDT) and “hemodynamic management” and propose an approach for goal-directed hemodynamic management in patients with septic shock.

2. Early Goal-Directed Therapy in Septic Shock

In 2001, Rivers et al. published their monocentric randomized controlled landmark study describing that EGDT during the first 6 hours of treatment markedly reduced mortality among patients admitted to the emergency department with severe sepsis or septic shock [2]. The 6-hour EGDT algorithm as proposed by Rivers et al. is a multimodal protocolized therapeutic approach targeting a central venous pressure (CVP) of 8–12 mmHg (by giving fluids), a MAP of 65–90 mmHg (by giving vasoactive agents), and a central venous oxygen saturation (ScvO₂) of ≥70% (by transfusion of red blood cells or administration of inotropic agents) [2]. Of note, in both the study and the control group, more than 94% of patients received immediate adequate antibiotic therapy [2]. This study essentially contributed to the notion that “optimization” of hemodynamics during the first hours of treatment can markedly lower mortality of patients with sepsis. The Surviving Sepsis Campaign (SSC) guidelines adopted those treatment goals in the recommendations for the “initial resuscitation” within the “6-hour bundles” [3].

The concept of EGDT as proposed by Rivers et al. has recently been questioned by three large multicenter randomized controlled trials—the ProCESS [4], ARISE [5], and PROMISE trial [6]—and an updated meta-analysis [7]. These trials demonstrated that nowadays protocolized EGDT for the first 6 hours (including monitoring of ScvO₂ and liberal red blood cell transfusion) seems not to be superior to “usual care” in terms of a reduction in mortality in emergency department patients with septic shock. It needs to be stressed, however, that, in all three trials, patients were identified early as having septic shock (in contrast to the Rivers trial) and promptly received antibiotic therapy and fluid resuscitation. In addition, the mortality observed in the control groups was markedly lower in the current trials compared with the Rivers study. Therefore, one can conclude that “usual resuscitation” has improved since the Rivers study and that the SSC guidelines increased the awareness for sepsis and its early recognition and its treatment with antibiotics and intravenous fluid [6, 8].

In addition, baseline characteristics of the patients of the three recent trials differed from those of the Rivers study with regard to severity of illness and timing of diagnosis of septic shock (lower lactate levels, higher ScvO₂, and lower APACHE II score) [9, 10]. Thus, the results of these trials might lack “external validity.” In particular, the question about potentially beneficial effects of goal-directed therapy in patients with very severe septic shock, in patients who do not respond to initial therapy, or in patients in whom the diagnosis of septic shock is established at a later point in time cannot be answered by these trials [9].

Nevertheless, in response to this new evidence, the SSC recently changed their bundle recommendations with regard to hemodynamic resuscitation within the first 6 hours [11]. Instead of targeting distinct values of CVP and ScvO₂ the guidelines now recommend to “re-assess volume status and tissue perfusion” by repeated “focused exam (...) including vital signs, cardiopulmonary, capillary refill, pulse, and skin findings” or “two of the following: measure CVP, measure

ScvO₂, bedside cardiovascular ultrasound, dynamic assessment of fluid responsiveness with passive leg raise or fluid challenge.” These updated recommendations reflect the recent evidence from the three large randomized controlled trials as well as the widely accepted importance of assessment of fluid responsiveness.

Key elements in the initial treatment of sepsis therefore remain the early recognition of sepsis and early antibiotic therapy and source control. Hemodynamic therapy should aim at the optimization of intravascular volume status, perfusion pressure, and blood flow to restore tissue perfusion. However, major questions regarding the optimization of cardiovascular dynamics remain as will be discussed in the next paragraphs.

3. The Difference between “Early Goal-Directed Therapy” and “Hemodynamic Management”

The concept of EGDT needs to be differentiated from “hemodynamic management” of patients with septic shock.

EGDT as described by Rivers et al. [2] and reassessed by the three large trials described above [4–6] only covers the first 6 hours of resuscitation of patients with sepsis and septic shock and is usually applied in emergency department patients presenting with suspected or confirmed sepsis or septic shock.

Therefore, EGDT is based on the basic hemodynamic variables CVP, MAP, and ScvO₂. From a pathophysiologic point of view the use of these hemodynamic targets to guide therapy with fluids, vasopressors, and inotropes can be questioned [12]. CVP has limited capabilities to reflect intravascular volume status and fluid responsiveness [13–15] and its use as a resuscitation goal in EGDT might lead to fluid overload [16, 17]. With regard to MAP, individual target values are not well described [18]. ScvO₂ is an unspecific parameter of the balance between oxygen delivery and oxygen consumption. It has been shown that ScvO₂ is below 70% in only about 27% of septic shock patients in the first hours after admission to the intensive care unit (ICU) [19]. Moreover, even in patients with high ScvO₂ a mismatch between oxygen delivery and consumption may be present.

Patients with septic shock regularly require intensive care for days or even weeks. “Hemodynamic management,” therefore, refers to the diagnostic and therapeutic approaches aiming to identify and resolve cardiocirculatory alterations during the complete course of septic shock—from initial differential diagnosis to early resuscitation and hemodynamic therapy of patients with septic shock associated with complex complications such as acute respiratory distress syndrome (ARDS), renal failure, abdominal compartment syndrome, or preexisting myocardial dysfunction.

“Hemodynamic management” can utilize advanced hemodynamic parameters (reflecting global blood flow, myocardial contractility, intravascular volume status, fluid responsiveness, and cardiac afterload) assessed with a variety of different techniques such as echocardiography, pulmonary artery catheterization and thermodilution, transpulmonary

thermodilution, and calibrated and uncalibrated pulse contour analysis. In addition, functional tests (passive leg raising test [20] and fluid challenge test [21]) are used to assess fluid responsiveness, that is, an increase in cardiac output (CO) after administration of fluid.

4. Hemodynamic Management of Patients with Septic Shock

Hemodynamic management comprises (a) making the diagnosis of septic shock (as one differential diagnosis of circulatory shock), (b) assessing the hemodynamic status (volume status, fluid responsiveness, need for vasopressor, or inotropic agent) including the identification of therapeutic conflicts (e.g., intravascular hypovolemia in the presence of pulmonary fluid overload), and (c) guiding therapeutic interventions.

The hemodynamic management of septic shock patients remains a complex challenge. There are no SSC guideline recommendations on the hemodynamic management for the period following the initial 6 hours of treatment in septic shock [3]. A consensus conference report of the European Society of Intensive Care Medicine (ESICM) can provide guidance on how to perform hemodynamic monitoring in critically ill patients with circulatory shock [22].

4.1. Differential Diagnosis of Cardiovascular Pathophysiology and Diagnosis of Septic Shock. In patients with circulatory shock, the identification of the type of shock is crucial to adequately guiding causal and supportive therapeutic approaches [22, 23]. Signs of poor tissue perfusion indicative for the presence of circulatory shock can be found on physical examination [24, 25]. However, using physical examination for identifying the underlying type of shock and the specific hemodynamic alterations is challenging [26–29]. Therefore, if physical examination does not lead to a clear diagnosis of the underlying type of shock, further hemodynamic assessment by echocardiography or—in complex patients—advanced hemodynamic monitoring (pulmonary artery catheter or transpulmonary thermodilution) is recommended [22]. In patients with septic (i.e., distributive) shock, increased CO (hyperdynamic circulatory failure), normal or decreased intravascular fluid status, and markedly decreased systemic vascular resistance are characteristic findings. However, in patients with impaired myocardial contractility (e.g., because of ischemic or septic cardiomyopathy) or hypovolemia, CO can also be decreased.

4.2. Hemodynamic Monitoring for the Assessment of the Hemodynamic Status. The serial assessment of the hemodynamic status of a patient with septic shock is crucial to identifying the therapeutic options to optimize perfusion pressure and global blood flow in order to restore and optimize tissue perfusion. Since both hypovolemia and hypervolemia are associated with unfavorable outcomes [17, 30], assessment of the hemodynamic status (including volume status and fluid responsiveness) remains a key challenge in the treatment of septic shock. While CVP, cardiac filling pressures, and static volumetric parameters of cardiac preload alone should not be

used to guide fluid therapy, fluid therapy based on more than one single hemodynamic variable and the use of dynamic parameters (pulse pressure variation and stroke volume variation that can only be used in patients with sinus rhythm and controlled mechanical ventilation) is recommended by the ESICM consensus report [22]. Because appropriate clinical hemodynamic endpoints to guide and titrate therapy with fluids are poorly defined, a careful titration of fluids especially in the presence of elevated filling pressures and extravascular lung water has been suggested [22]. To predict the patient's response to a fluid bolus, a passive leg raising test, that is, autotransfusion of blood from the lower extremities to the thoracic compartment, can be performed [31, 32]. The clinical gold standard test to evaluate fluid responsiveness is the actual administration of a fluid bolus and the continuous monitoring of CO to monitor the hemodynamic response during this fluid challenge test [21]. It has to be mentioned, however, that the concept of fluid responsiveness is based on pathophysiologic considerations and has not been rigorously evaluated in randomized controlled trials [33].

4.3. Hemodynamic Management in Septic Shock: Therapeutic Conflicts. If septic shock is complicated by ARDS, therapeutic conflicts between absolute or relative intravascular hypovolemia causing circulatory shock and pulmonary fluid overload remain key challenges in the hemodynamic management. Therefore, the ESICM expert consensus on circulatory shock and hemodynamic monitoring suggests the use of advanced hemodynamic monitoring in patients with severe shock (especially if complicated by ARDS) [22]. Pulmonary artery catheterization remains reasonable in septic shock patients with right ventricular failure or pulmonary artery hypertension [34]. However, the use of the pulmonary artery catheter has declined in the ICU setting during the recent years for a variety of reasons including its invasiveness and the availability of less-invasive hemodynamic monitoring technologies [35].

In septic shock accompanied by ARDS, hemodynamic management based on transpulmonary thermodilution can add additional valuable information about extravascular lung water index (EVLWI) [36] and pulmonary vascular permeability [37]. EVLWI gives useful prognostic information regarding mortality in critically ill patients in general, in patients with sepsis or septic shock, and in patients with ARDS [36]. Recent data demonstrate that EVLWI is of high prognostic value during fluid resuscitation in septic patients after initial resuscitation [38]. Because transpulmonary thermodilution allows the measurement of CO, volumetric cardiac preload parameters, and EVLWI and the calculation of systemic vascular resistance, its use might help to guide fluid therapy and therapy with vasopressors and inotropes in complex septic shock patients even in the context of pulmonary fluid overload. Transpulmonary thermodilution has therefore recently been suggested to be used in shock patients without ARDS not responding to initial therapy or in patients with shock and ARDS [39]. The combined application of transpulmonary thermodilution and calibrated pulse contour analysis additionally allows the continuous

estimation of CO during functional diagnostic tests aiming to assess fluid responsiveness.

4.4. Hemodynamic Management: Different Phases of Resuscitation and “Deresuscitation”. Although it is generally agreed upon that fluid administration and vasopressor therapy are key components in the hemodynamic management of patients with septic shock, basic questions about the timing of these therapeutic interventions largely remain unanswered or controversial. Although the SSC guidelines recommend “aggressive fluid resuscitation during the first 24 hours of management” [3], dosing and timing of intravenous fluid administration remain largely empirical [40–42].

Hypovolemia is associated with tissue hypoperfusion and organ failure [43]. The rationale behind fluid therapy in hemodynamically compromised patients is to increase oxygen delivery by increasing stroke volume (and thus CO) [40]. This is based on the physiologic relation of cardiac preload and stroke volume as described by the Frank-Starling cardiac function curve [40]. Since fluid loading transiently increases the stressed blood volume and venous return (by increasing the gradient between mean systemic filling pressure and right atrial pressure) fluid administration can result in an increase of stroke volume in patients on the ascending part of the Frank-Starling curve [43–45].

Despite this sound physiologic concept, it is important to consider that only about 50% of critically ill patients are in a hemodynamic state of fluid responsiveness [40, 45]. In addition, increasing CO by fluid loading is probably only justifiable if signs of tissue hypoperfusion are present [40].

Aggressive fluid resuscitation has been found to be independently associated with worse outcomes in critically ill patients including organ dysfunction and mortality [30, 46]. Excessive fluid resuscitation results in tissue edema impairing endothelial integrity, microcirculatory blood flow, and diffusion of oxygen and metabolites and finally results in impaired organ blood flow [30, 45]. A negative fluid balance, conversely, has been shown to be associated with survival in patients with sepsis [47] and with improved pulmonary organ function in patients with ARDS [48].

In the absence of definite evidence regarding the optimal timing of fluid administration in septic shock, concepts for hemodynamic management taking into account different phases of fluid resuscitation and “deresuscitation” have been proposed. An early transition to a conservative fluid management or even “late goal-directed fluid removal” following the initial resuscitation phase characterized by the liberal administration of fluids has been suggested [45]. Marik even suggested a primarily conservative approach of fluid bolus administration (in contrast to the SSC recommendations) [46]. With regard to factors that have to be considered during fluid therapy, Malbrain et al. emphasized the importance of 4 key elements: drug, dosing, duration, and deescalation [49].

However, these concepts and suggestions further need to be evaluated in observational and interventional clinical studies before they can be recommended for routine clinical practice.

5. Advanced Hemodynamic Monitoring Using Transpulmonary Thermodilution in the Hemodynamic Management of Septic Shock

5.1. Transpulmonary Thermodilution. Transpulmonary thermodilution allows the determination of a variety of hemodynamic variables in patients equipped with a central venous catheter placed in the superior or inferior vena cava and a dedicated thermistor-tipped arterial catheter that is usually placed in the abdominal aorta through the femoral artery [50–53]. Single-indicator transpulmonary thermodilution techniques that are now commercially available from two different manufacturers [52, 54] have been developed based on the experience with double-indicator (thermodye) transpulmonary thermodilution [55]. When using single-indicator transpulmonary thermodilution, a thermal indicator (cooled saline) is injected in the central venous circulation and passes the right heart, the pulmonary circulation, and the left heart. Subsequently, the thermal indicator bolus is detected by the thermistor located at the tip of the arterial catheter and a curve reflecting the dilution of the cold indicator on its way through cardiopulmonary circulation is derived. Further analysis of this thermodilution curve allows the calculation of various hemodynamic parameters for the assessment of CO, myocardial contractility, cardiac preload, and EVLWI [52, 54, 56–60]. In short, CO is calculated from the thermodilution curve by applying a modified Stewart-Hamilton algorithm [61, 62]. Based on two further main parameters characterizing the thermodilution curve—that is, mean transit time (MTt) and the downslope time (DSt)—global end-diastolic volume index (GEDVI) and EVLWI can be computed as described in detail before [59, 63–68]. GEDVI can be used to estimate cardiac preload (volumetric cardiac preload parameter) and EVLWI that is elevated in patients with pulmonary edema or pneumonia is a marker of fluid outside of the pulmonary vasculature [59, 63–68].

5.2. Data on Transpulmonary Thermodilution in the Hemodynamic Management of Septic Shock Patients. Despite the pathophysiologic rationale for advanced hemodynamic management in septic shock patients and the expert consensus recommendations for its use described above [22], to date, definite algorithms to guide fluid therapy using advanced hemodynamic monitoring with transpulmonary thermodilution cannot be generally recommended based on the existing literature.

In general, hemodynamic monitoring per se will never influence patient outcome unless measured hemodynamic variables trigger meaningful and reasonable therapeutic interventions that are able to improve outcome [69].

In a two-center, randomized trial in septic and nonseptic shock patients, Trof et al. compared two hemodynamic management algorithms using predefined values of different hemodynamic parameters as upper resuscitation limits—transpulmonary thermodilution-derived values of EVLWI and GEDVI in one group and pulmonary artery occlusion pressure in the other group [70]. The authors observed no clinically relevant or statistically significant difference in the primary endpoints (ventilator-free days and ICU and hospital

length of stay), organ failure, and mortality. Therapy guided by GEDVI and EVLWI resulted in a more positive fluid balance. However, the study protocol used by the authors was repeatedly criticized for the endpoints chosen to serve as an upper limit to administer fluid [71–74].

Another example that illustrates the problem of questionable treatment algorithms is a prospective trial by Zhang et al. comparing hemodynamic treatment based on transpulmonary thermodilution-derived variables with CVP-based management in patients with septic shock with or without ARDS. The trial was stopped prematurely and did not show any statistically significant differences in the primary endpoint (28-day mortality) and secondary endpoints [75]. Again, major flaws in the study design and the hemodynamic treatment protocol might explain the authors' findings [76–78]; for example, applying the same hemodynamic algorithm to septic shock patients with and without ARDS seems to be counterintuitive and against basic pathophysiologic principles [76, 78]. In addition, questionable therapeutic interventions (low-molecular starch 130/0.4 and diuretics) were applied triggered by questionable cut-off values of transpulmonary thermodilution-derived variables [76].

In another study, a hemodynamic treatment algorithm to guide fluid administration in patients with septic shock was described which is based on functional cardiac preload parameters (pulse pressure variation if applicable or changes in stroke volume after a passive leg raising test) and transpulmonary thermodilution-derived cardiac index (CI) [79]. There was no difference in the time till resolution of shock (primary endpoint) between the study group and the control group in which fluid administration was guided by an algorithm primarily based on CVP. In the study group, however, the amount of fluids administered per day was lower compared to the control group.

Considering the results of these studies, the conclusion that advanced hemodynamic management is useless to improve outcome in septic patients is not justified. Those studies rather suggest that further trials investigating hemodynamic treatment strategies in septic shock patients are necessary. It must be the aim to develop treatment algorithms aiming at a rational optimization of appropriate pathophysiologically reasonable hemodynamic target variables. These algorithms then need to be thoroughly evaluated in randomized controlled trials in clearly characterized patient collectives using reasonable outcome measures.

5.3. Transpulmonary Thermodilution in the Hemodynamic Management of Septic Shock: Suggestion for a Treatment Algorithm. In the following we propose two hemodynamic treatment algorithms using transpulmonary thermodilution-derived variables aiming to optimize the cardiocirculatory and pulmonary status in adult ICU patients with septic shock as defined by the recent consensus definition [1]. An outline of the algorithms is given in Figures 1 and 2. These algorithms are based on pathophysiologic rationale, clinical experience, and available data from previous studies. The algorithms have not been tested in a randomized controlled trial and—in the absence of definite evidence on this topic—are meant to initiate a discussion on how advanced hemodynamic

monitoring using transpulmonary thermodilution might be performed in an algorithmic approach in septic shock.

According to the different phases of hemodynamic resuscitation described above we distinguish between an algorithm for the early phase, that is, during the first 24 hours of treatment (Algorithm 1) and an algorithm for further hemodynamic management (Algorithm 2).

Hemodynamic therapeutic interventions in Algorithm 1 include fluids (crystalloids), a vasopressor (norepinephrine), and an inotrope (dobutamine) titrated according to EVLWI and CI. Crystalloids are given as a bolus of 500 mL (fluid challenge) [46]. We define fluid responsiveness as an increase in CI of $\geq 15\%$ or an increase in MAP of $\geq 15\%$ or a cumulative increase in CI and MAP of $\geq 20\%$ (given that the dose of vasopressors is kept constant). EVLWI is complemented by the arterial partial pressure of oxygen (PaO_2)/fraction of inspired oxygen (FiO_2) ratio to account for the individual pulmonary function of the patient.

Algorithm 2 gives more general treatment recommendations based on CI, GEDVI, EVLWI (again complemented by the $\text{PaO}_2/\text{FiO}_2$ ratio), and MAP. During treatment according to Algorithm 2 all patients receive norepinephrine to maintain a MAP of ≥ 65 mmHg [46]. Algorithm 2 takes into account that, during later phases of treatment, in individual patients different treatment goals are necessary (negative fluid balance, positive fluid balance, or inotropic support). In addition, Algorithm 2 promotes individual treatment decisions in the light of therapeutic conflicts.

We deliberately did not include lactate measurements in our algorithms. Nevertheless, although elevated lactate is an unspecific marker of tissue hypoperfusion, serial measurements of lactate can help to assess the response of hemodynamic management strategies in patients with septic shock [22].

6. Hemodynamic Management in Septic Shock: Open Research Questions and Future Directions

When planning future studies on the impact of hemodynamic management on outcome in patients with septic shock various factors need to be considered.

First, although the new consensus definitions allow identifying patients with sepsis and septic shock in clinical practice, the complexity of sepsis and septic shock makes exact definitions of these “infection-related (multiple) organ dysfunction syndromes” extremely difficult. This is a problem in clinical interventional studies evaluating the effect of any therapeutic intervention in septic patients. Sepsis and septic shock are not distinct and well characterized “diseases” but rather complex syndromes that are often even accompanied by other syndromes of critical illness (such as ARDS); therefore, in clinical studies, therapeutic interventions are usually evaluated in a very heterogeneous group of septic patients [80]. This makes definite conclusions about the value of a certain intervention and the identification of distinct subgroups of septic patients who might benefit from it very challenging because possible positive or negative effects

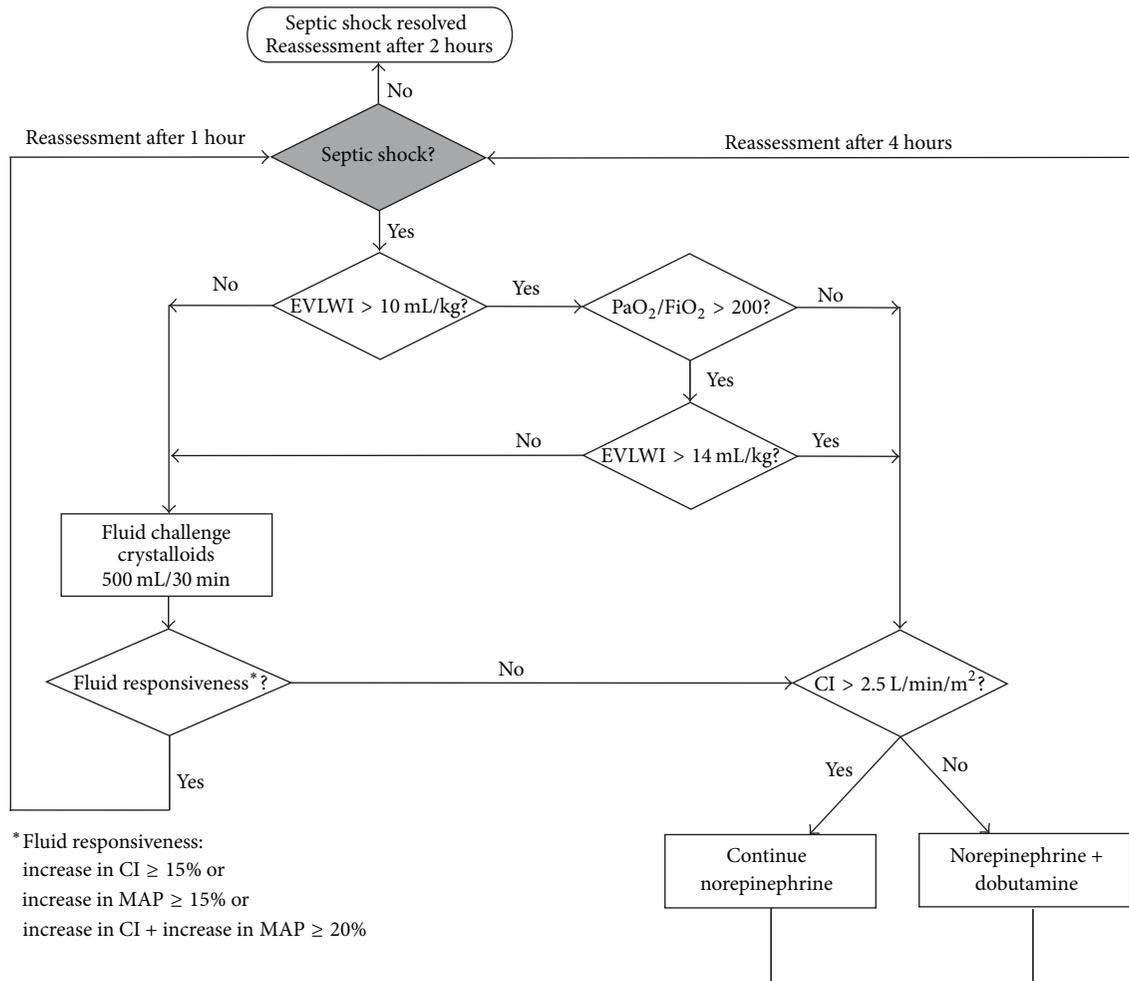


FIGURE 1: Algorithm 1—treatment algorithm for the first 24 hours of hemodynamic management. Hemodynamic therapeutic interventions in Algorithm 1 include fluids (crystalloids), a vasopressor (norepinephrine), and an inotrope (dobutamine) titrated according to extravascular lung water index (EVLWI) and cardiac index (CI). Crystalloids are given as a bolus of 500 mL (fluid challenge). We define fluid responsiveness as an increase in CI of ≥15% or an increase in mean arterial pressure (MAP) of ≥15% or a cumulative increase in CI and MAP of ≥20% (given that the dose of vasopressors is kept constant). EVLWI is complemented by the arterial partial pressure of oxygen (PaO₂)/fraction of inspired oxygen (FiO₂) ratio to account for the individual pulmonary function of the patient.

of a therapeutic approach might overlap [80, 81]. This is one reason why many randomized controlled clinical trials in septic patients fail to prove a beneficial effect of the studied intervention [80]. Therefore, future studies should precisely define the patient population studied (e.g., “patients with community-acquired septic shock of pulmonary origin” instead of “patients with sepsis or septic shock”).

In addition, complex disease syndromes require a multi-modal therapeutic approach. The difficulty to prove beneficial effects of a certain single therapeutic intervention in critically ill patients has been discussed before [80]. In complex critically ill patients, it might be a basic misconception to choose mortality as the primary outcome endpoint in interventional studies. Mortality is determined by a variety of different factors thus making it very difficult to prove a clinically relevant decrease in mortality by a single intervention. Endpoints reflecting an improvement in organ dysfunction might better

serve the purpose to evaluate beneficial effects of therapeutic approaches in ICU patients [80].

The complexity and heterogeneity of patients with septic shock implies individualized approaches for hemodynamic management. This includes individualized targets for hemodynamic resuscitation parameters [12, 18] and a definition of the terms “normal values” and “optimization” (in contrast to maximization). First studies on goal-directed therapy had proposed to target “supranormal” hemodynamic values in high-risk surgical patients [82]; however, this concept was later disproved in critically ill septic patients [83, 84]. In line, data from a recent study in pigs with severe acute pancreatitis as a paradigm for severe systemic infection showed that a “maximized” utilization of the cardiac preload reserve is not an “optimized” fluid management approach [85]. Normal values of hemodynamic variables show marked interindividual variability and are dependent on a variety of biometric and

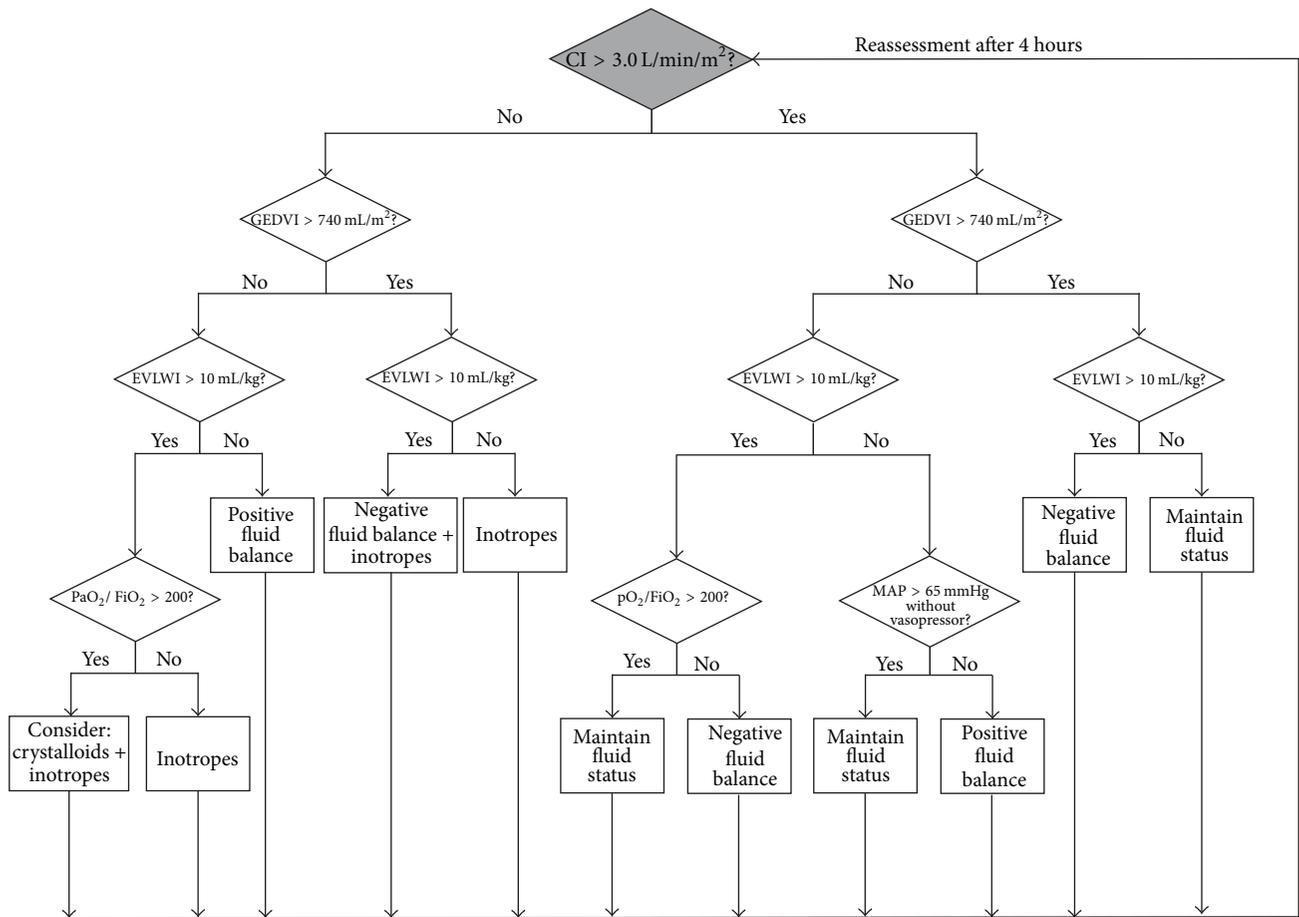


FIGURE 2: Algorithm 2—treatment algorithm for hemodynamic management during the intensive care unit stay following the initial 24 hours. Algorithm 2 gives treatment recommendations based on cardiac index (CI), global end-diastolic volume index (GEDVI), extravascular lung water index (EVLWI), and mean arterial pressure (MAP). EVLWI is complemented by the arterial partial pressure of oxygen (PaO₂)/fraction of inspired oxygen (FiO₂) ratio to account for the individual pulmonary function of the patient. During treatment according to Algorithm 2 all patients receive norepinephrine to maintain a MAP of ≥65 mmHg.

pathophysiologic factors [86–91]. Therefore, a “one size fits all” approach will always be deemed to fail in the hemodynamic management of critically ill patients [78]. Defining a septic patient’s individual optimal values of hemodynamic variables targeted during hemodynamic management in different phases of the disease will remain a highly complex challenge but must be the focus of future research in this field.

Finally, the ultimate goal of hemodynamic management strategies based on advanced global hemodynamic variables must be to improve microcirculatory perfusion. Different technologies for the bedside assessment of the microcirculatory perfusion are available today. However, these technologies are not recommended to guide therapy outside of clinical studies [22]. The link between alterations in global hemodynamics and the microcirculation in septic shock is still not fully elucidated [12]. Therefore, therapeutic concepts for the improvement of microcirculatory perfusion are not clinically established. Future hemodynamic management concepts should therefore integrate global hemodynamic variables and variables reflecting microcirculatory perfusion.

7. Summary

In patients with sepsis and septic shock, the hemodynamic management in both early and later phases of these “organ dysfunction syndromes” is a key therapeutic component.

It needs however to be differentiated between EGDT as proposed for the first 6 hours of emergency department treatment by Rivers et al. in 2001 and “hemodynamic management” using advanced hemodynamic monitoring in the ICU.

Recent large trials demonstrated that nowadays protocolized EGDT does not seem to be superior to “usual care” in terms of a reduction in mortality in emergency department patients with early identified septic shock who promptly receive antibiotic therapy and fluid resuscitation.

“Hemodynamic management” comprises (a) making the diagnosis of septic shock as one differential diagnosis of circulatory shock, (b) assessing the hemodynamic status including the identification of therapeutic conflicts, and (c) guiding therapeutic interventions.

We propose two algorithms for hemodynamic management using transpulmonary thermodilution-derived variables aiming to optimize the cardiocirculatory and pulmonary status in adult ICU patients with septic shock.

The complexity and heterogeneity of patients with septic shock implies that individualized approaches for hemodynamic management are mandatory. Defining individual hemodynamic target values for patients with septic shock in different phases of the disease must be the focus of future studies.

Competing Interests

Bernd Saugel, Wolfgang Huber, and Daniel A. Reuter collaborate with Pulsion Medical Systems SE (Feldkirchen, Germany) as members of the medical advisory board. For all other authors there are no competing interests to disclose.

References

- [1] M. Singer, C. S. Deutschman, C. W. Seymour et al., "The third international consensus definitions for sepsis and septic shock (Sepsis-3)," *The Journal of the American Medical Association*, vol. 315, no. 8, pp. 801–810, 2016.
- [2] E. Rivers, B. Nguyen, S. Havstad et al., "Early goal-directed therapy in the treatment of severe sepsis and septic shock," *The New England Journal of Medicine*, vol. 345, no. 19, pp. 1368–1377, 2001.
- [3] R. P. Dellinger, M. M. Levy, A. Rhodes et al., "Surviving sepsis campaign: international guidelines for management of severe sepsis and septic shock: 2012," *Critical Care Medicine*, vol. 41, no. 2, pp. 580–637, 2013.
- [4] D. M. Yealy, J. A. Kellum, D. T. Huang et al., "A randomized trial of protocol-based care for early septic shock," *The New England Journal of Medicine*, vol. 370, no. 18, pp. 1683–1693, 2014.
- [5] S. L. Peake, A. Delaney, M. Bailey et al., "Goal-directed resuscitation for patients with early septic shock," *The New England Journal of Medicine*, vol. 371, no. 16, pp. 1496–1506, 2014.
- [6] P. R. Mouncey, T. M. Osborn, G. S. Power et al., "Trial of early, goal-directed resuscitation for septic shock," *The New England Journal of Medicine*, vol. 372, no. 14, pp. 1301–1311, 2015.
- [7] D. C. Angus, A. E. Barnato, D. Bell et al., "A systematic review and meta-analysis of early goal-directed therapy for septic shock: the ARISE, ProCESS and ProMISe Investigators," *Intensive Care Medicine*, vol. 41, no. 9, pp. 1549–1560, 2015.
- [8] B. Saugel and D. A. Reuter, "Goal-directed resuscitation in septic shock," *The New England Journal of Medicine*, vol. 372, no. 2, pp. 189–191, 2015.
- [9] D. De Backer and J. L. Vincent, "Early goal-directed therapy: do we have a definitive answer?" *Intensive Care Medicine*, vol. 42, no. 6, pp. 1048–1050, 2016.
- [10] R. G. Gupta, S. M. Hartigan, M. G. Kashiouris, C. N. Sessler, and G. M. L. Bearman, "Early goal-directed resuscitation of patients with septic shock: current evidence and future directions," *Critical Care*, vol. 19, no. 1, article 286, 2015.
- [11] http://www.survivingsepsis.org/SiteCollectionDocuments/SSC_Bundle.pdf.
- [12] B. Saugel, C. J. Trepte, K. Heckel, J. Y. Wagner, and D. A. Reuter, "Hemodynamic management of septic shock: is it time for 'individualized goal-directed hemodynamic therapy' and for specifically targeting the microcirculation?" *Shock*, vol. 43, no. 6, pp. 522–529, 2015.
- [13] P. E. Marik, M. Baram, and B. Vahid, "Does central venous pressure predict fluid responsiveness? A systematic review of the literature and the tale of seven mares," *Chest*, vol. 134, no. 1, pp. 172–178, 2008.
- [14] P. E. Marik and R. Cavallazzi, "Does the central venous pressure predict fluid responsiveness? An updated meta-analysis and a plea for some common sense," *Critical Care Medicine*, vol. 41, no. 7, pp. 1774–1781, 2013.
- [15] T. G. Eskesen, M. Wetterslev, and A. Perner, "Systematic review including re-analyses of 1148 individual data sets of central venous pressure as a predictor of fluid responsiveness," *Intensive Care Medicine*, vol. 42, no. 3, pp. 324–332, 2016.
- [16] D. J. Kelm, J. T. Perrin, R. Cartin-Ceba, O. Gajic, L. Schenck, and C. C. Kennedy, "Fluid overload in patients with severe sepsis and septic shock treated with early goal-directed therapy is associated with increased acute need for fluid-related medical interventions and hospital death," *Shock*, vol. 43, no. 1, pp. 68–73, 2015.
- [17] J. H. Boyd, J. Forbes, T.-A. Nakada, K. R. Walley, and J. A. Russell, "Fluid resuscitation in septic shock: a positive fluid balance and elevated central venous pressure are associated with increased mortality," *Critical Care Medicine*, vol. 39, no. 2, pp. 259–265, 2011.
- [18] P. Asfar, F. Meziani, J.-F. Hamel et al., "High versus low blood-pressure target in patients with septic shock," *The New England Journal of Medicine*, vol. 370, no. 17, pp. 1583–1593, 2014.
- [19] T. Boulain, D. Garot, P. Vignon et al., "Prevalence of low central venous oxygen saturation in the first hours of intensive care unit admission and associated mortality in septic shock patients: a prospective multicentre study," *Critical Care*, vol. 18, no. 6, article 609, 2014.
- [20] F. Cavallaro, C. Sandroni, C. Marano et al., "Diagnostic accuracy of passive leg raising for prediction of fluid responsiveness in adults: systematic review and meta-analysis of clinical studies," *Intensive Care Medicine*, vol. 36, no. 9, pp. 1475–1483, 2010.
- [21] M. Cecconi, A. K. Parsons, and A. Rhodes, "What is a fluid challenge?" *Current Opinion in Critical Care*, vol. 17, no. 3, pp. 290–295, 2011.
- [22] M. Cecconi, D. De Backer, M. Antonelli et al., "Consensus on circulatory shock and hemodynamic monitoring. Task force of the European Society of Intensive Care Medicine," *Intensive Care Medicine*, vol. 40, no. 12, pp. 1795–1815, 2014.
- [23] J. L. Vincent and D. De Backer, "Circulatory shock," *The New England Journal of Medicine*, vol. 370, pp. 582–583, 2014.
- [24] J.-L. Vincent, C. Ince, and J. Bakker, "Clinical review: circulatory shock—an update: a tribute to Professor Max Harry Weil," *Critical Care*, vol. 16, article 239, 2012.
- [25] H. Ait-Oufella and J. Bakker, "Understanding clinical signs of poor tissue perfusion during septic shock," *Intensive Care Medicine*, 2016.
- [26] R. Vazquez, C. Gheorghe, D. Kaufman, and C. A. Manthous, "Accuracy of bedside physical examination in distinguishing categories of shock: a pilot study," *Journal of Hospital Medicine*, vol. 5, no. 8, pp. 471–474, 2010.
- [27] B. Saugel, S. Ringmaier, K. Holzapfel et al., "Physical examination, central venous pressure, and chest radiography for the

- prediction of transpulmonary thermodilution-derived hemodynamic parameters in critically ill patients: a prospective trial," *Journal of Critical Care*, vol. 26, no. 4, pp. 402–410, 2011.
- [28] B. Saugel, S. V. Kirsche, A. Hapfelmeier et al., "Prediction of fluid responsiveness in patients admitted to the medical intensive care unit," *Journal of Critical Care*, vol. 28, no. 4, pp. 537.e1–537.e9, 2013.
- [29] A. Perel, B. Saugel, J.-L. Teboul et al., "The effects of advanced monitoring on hemodynamic management in critically ill patients: a pre and post questionnaire study," *Journal of Clinical Monitoring and Computing*, 2015.
- [30] P. E. Marik, "Iatrogenic salt water drowning and the hazards of a high central venous pressure," *Annals of Intensive Care*, vol. 4, article 21, 2014.
- [31] X. Monnet and J.-L. Teboul, "Passive leg raising," *Intensive Care Medicine*, vol. 34, no. 4, pp. 659–663, 2008.
- [32] X. Monnet and J.-L. Teboul, "Passive leg raising: five rules, not a drop of fluid!," *Critical Care*, vol. 19, article 18, 2015.
- [33] A. S. Saleh, "Is the concept of fluid responsiveness evidence-based?" *Intensive Care Medicine*, vol. 42, no. 7, pp. 1187–1188, 2016.
- [34] C. Richard, X. Monnet, and J. L. Teboul, "Pulmonary artery catheter monitoring in 2011," *Current Opinion in Critical Care*, vol. 17, no. 3, pp. 296–302, 2011.
- [35] H. B. Gershengorn and H. Wunsch, "Understanding changes in established practice: pulmonary artery catheter use in critically III patients," *Critical Care Medicine*, vol. 41, no. 12, pp. 2667–2676, 2013.
- [36] M. Jozwiak, J.-L. Teboul, and X. Monnet, "Extravascular lung water in critical care: recent advances and clinical applications," *Annals of Intensive Care*, vol. 5, no. 1, article 38, pp. 1–13, 2015.
- [37] X. Monnet, N. Anguel, D. Osman, O. Hamzaoui, C. Richard, and J.-L. Teboul, "Assessing pulmonary permeability by transpulmonary thermodilution allows differentiation of hydrostatic pulmonary edema from ALI/ARDS," *Intensive Care Medicine*, vol. 33, no. 3, pp. 448–453, 2007.
- [38] H. Wang, N. Cui, L. Su et al., "Prognostic value of extravascular lung water and its potential role in guiding fluid therapy in septic shock after initial resuscitation," *Journal of Critical Care*, vol. 33, pp. 106–113, 2016.
- [39] M. Jozwiak, X. Monnet, and J. L. Teboul, "Monitoring: from cardiac output monitoring to echocardiography," *Current Opinion in Critical Care*, vol. 21, no. 5, pp. 395–401, 2015.
- [40] P. E. Marik, X. Monnet, and J.-L. Teboul, "Hemodynamic parameters to guide fluid therapy," *Annals of Intensive Care*, vol. 1, article 1, 2011.
- [41] T. Boulain, J. Boisrame-Helms, S. Ehrmann et al., "Volume expansion in the first 4 days of shock: a prospective multicentre study in 19 French intensive care units," *Intensive Care Medicine*, vol. 41, no. 2, pp. 248–256, 2014.
- [42] M. Cecconi, C. Hofer, J.-L. Teboul et al., "Fluid challenges in intensive care: the FENICE study. A global inception cohort study," *Intensive Care Medicine*, vol. 41, no. 9, pp. 1529–1537, 2015.
- [43] A. Perner and D. De Backer, "Understanding hypovolaemia," *Intensive Care Medicine*, vol. 40, no. 4, pp. 613–615, 2014.
- [44] A. C. Guyton, A. W. Lindsey, B. N. Kaufmann, and J. B. Abernathy, "Effect of blood transfusion and hemorrhage on cardiac output and on the venous return curve," *American Journal of Physiology*, vol. 194, no. 2, pp. 263–267, 1958.
- [45] M. L. N. G. Malbrain, P. E. Marik, I. Witters et al., "Fluid overload, de-resuscitation, and outcomes in critically ill or injured patients: a systematic review with suggestions for clinical practice," *Anaesthesiology Intensive Therapy*, vol. 46, no. 5, pp. 361–380, 2014.
- [46] P. E. Marik, "Early management of severe sepsis: concepts and controversies," *Chest*, vol. 145, no. 6, pp. 1407–1418, 2014.
- [47] F. Alsous, M. Khamiees, A. DeGirolamo, Y. Amoateng-Adjepong, and C. A. Manthous, "Negative fluid balance predicts survival in patients with septic shock: a retrospective pilot study," *Chest*, vol. 117, no. 6, pp. 1749–1754, 2000.
- [48] H. P. Wiedemann, A. P. Wheeler, G. R. Bernard et al., "Comparison of two fluid-management strategies in acute lung injury," *The New England Journal of Medicine*, vol. 354, no. 24, pp. 2564–2575, 2006.
- [49] M. L. Malbrain, N. Van Regenmortel, and R. Owczuk, "It is time to consider the four D's of fluid management," *Anaesthesiology Intensive Therapy*, vol. 47, pp. 1–5, 2015.
- [50] Z. Friedman, H. Berkenstadt, N. Margalit, E. Segal, and A. Perel, "Cardiac output assessed by arterial thermodilution during exsanguination and fluid resuscitation: experimental validation against a reference technique," *European Journal of Anaesthesiology*, vol. 19, no. 5, pp. 337–340, 2002.
- [51] M. S. G. Goepfert, D. A. Reuter, D. Akyol, P. Lamm, E. Kilger, and A. E. Goetz, "Goal-directed fluid management reduces vasopressor and catecholamine use in cardiac surgery patients," *Intensive Care Medicine*, vol. 33, no. 1, pp. 96–103, 2007.
- [52] K. Bendjelid, R. Giraud, N. Siegenthaler, and F. Michard, "Validation of a new transpulmonary thermodilution system to assess global end-diastolic volume and extravascular lung water," *Critical Care*, vol. 14, article R209, 2010.
- [53] B. Saugel, A. Umgelter, T. Schuster, V. Phillip, R. M. Schmid, and W. Huber, "Transpulmonary thermodilution using femoral indicator injection: a prospective trial in patients with a femoral and a jugular central venous catheter," *Critical Care*, vol. 14, no. 3, article R95, 2010.
- [54] N. Kiefer, C. K. Hofer, G. Marx et al., "Clinical validation of a new thermodilution system for the assessment of cardiac output and volumetric parameters," *Critical Care*, vol. 16, article R98, 2012.
- [55] S. G. Sakka, C. C. Rühl, U. J. Pfeiffer et al., "Assessment of cardiac preload and extravascular lung water by single transpulmonary thermodilution," *Intensive Care Medicine*, vol. 26, no. 2, pp. 180–187, 2000.
- [56] D. A. Reuter, T. W. Felbinger, C. Schmidt et al., "Stroke volume variations for assessment of cardiac responsiveness to volume loading in mechanically ventilated patients after cardiac surgery," *Intensive Care Medicine*, vol. 28, no. 4, pp. 392–398, 2002.
- [57] S. G. De Hert, D. Robert, S. Cromheecke, F. Michard, J. Nijs, and I. E. Rodrigus, "Evaluation of left ventricular function in anesthetized patients using femoral artery dP/dtmax," *Journal of Cardiothoracic and Vascular Anesthesia*, vol. 20, no. 3, pp. 325–330, 2006.
- [58] E. Fernández-Mondéjar, R. Rivera-Fernández, M. García-Delgado, A. Touma, J. Machado, and J. Chavero, "Small increases in extravascular lung water are accurately detected by transpulmonary thermodilution," *The Journal of Trauma*, vol. 59, no. 6, pp. 1420–1424, 2005.

- [59] T. Tagami, S. Kushimoto, Y. Yamamoto et al., "Validation of extravascular lung water measurement by single transpulmonary thermodilution: human autopsy study," *Critical Care*, vol. 14, no. 5, article R162, 2010.
- [60] F. Michard, S. Alaya, V. Zarka, M. Bahloul, C. Richard, and J.-L. Teboul, "Global end-diastolic volume as an indicator of cardiac preload in patients with septic shock," *Chest*, vol. 124, no. 5, pp. 1900–1908, 2003.
- [61] T. W. Felbinger, D. A. Reuter, H. K. Eltzschig, J. Bayerlein, and A. E. Goetz, "Cardiac index measurements during rapid preload changes: a comparison of pulmonary artery thermodilution with arterial pulse contour analysis," *Journal of Clinical Anesthesia*, vol. 17, no. 4, pp. 241–248, 2005.
- [62] O. Goedje, K. Hoeke, M. Lichtwarck-Aschoff, A. Faltchauser, P. Lamm, and B. Reichart, "Continuous cardiac output by femoral arterial thermodilution calibrated pulse contour analysis: comparison with pulmonary arterial thermodilution," *Critical Care Medicine*, vol. 27, no. 11, pp. 2407–2412, 1999.
- [63] D. A. Reuter, T. W. Felbinger, K. Moerstedt et al., "Intrathoracic blood volume index measured by thermodilution for preload monitoring after cardiac surgery," *Journal of Cardiothoracic and Vascular Anesthesia*, vol. 16, no. 2, pp. 191–195, 2002.
- [64] C. Wiesenack, C. Prasser, C. Keyl, and G. Rödig, "Assessment of intrathoracic blood volume as an indicator of cardiac preload: single transpulmonary thermodilution technique versus assessment of pressure preload parameters derived from a pulmonary artery catheter," *Journal of Cardiothoracic and Vascular Anesthesia*, vol. 15, no. 5, pp. 584–588, 2001.
- [65] M. Lichtwarck-Aschoff, R. Beale, and U. J. Pfeiffer, "Central venous pressure, pulmonary artery occlusion pressure, intrathoracic blood volume, and right ventricular end-diastolic volume as indicators of cardiac preload," *Journal of Critical Care*, vol. 11, no. 4, pp. 180–188, 1996.
- [66] R. Katzenelson, A. Perel, H. Berkenstadt et al., "Accuracy of transpulmonary thermodilution versus gravimetric measurement of extravascular lung water," *Critical Care Medicine*, vol. 32, no. 7, pp. 1550–1554, 2004.
- [67] V. V. Kuzkov, M. Y. Kirov, M. A. Sovershaev et al., "Extravascular lung water determined with single transpulmonary thermodilution correlates with the severity of sepsis-induced acute lung injury," *Critical Care Medicine*, vol. 34, no. 6, pp. 1647–1653, 2006.
- [68] G. S. Martin, S. Eaton, M. Mealer, and M. Moss, "Extravascular lung water in patients with severe sepsis: a prospective cohort study," *Critical Care*, vol. 9, no. 2, pp. R74–R82, 2005.
- [69] M. R. Pinsky, "Hemodynamic evaluation and monitoring in the ICU," *Chest*, vol. 132, no. 6, pp. 2020–2029, 2007.
- [70] R. J. Trof, A. Beishuizen, A. D. Cornet, R. J. de Wit, A. R. J. Girbes, and A. B. J. Groeneveld, "Volume-limited versus pressure-limited hemodynamic management in septic and nonseptic shock," *Critical Care Medicine*, vol. 40, no. 4, pp. 1177–1185, 2012.
- [71] J. Takala, "Hemodynamic support: focus on monitoring tools or treatments?" *Critical Care Medicine*, vol. 40, pp. 1359–1360, 2012.
- [72] J. Teboul, X. Monnet, and A. Perel, "Results of questionable management protocols are inherently questionable," *Critical Care Medicine*, vol. 40, no. 8, pp. 2536–2537, 2012.
- [73] M. L. Malbrain and D. A. Reuter, "Hemodynamic treatment algorithms should follow physiology or they fail to improve outcome," *Critical Care Medicine*, vol. 40, no. 10, pp. 2923–2925, 2012.
- [74] M. H. Hooper and P. E. Marik, "Transpulmonary thermodilution: the jury is out," *Critical Care Medicine*, vol. 40, no. 11, p. 3109, 2012.
- [75] Z. Zhang, H. Ni, and Z. Qian, "Effectiveness of treatment based on PiCCO parameters in critically ill patients with septic shock and/or acute respiratory distress syndrome: a randomized controlled trial," *Intensive Care Medicine*, vol. 41, no. 3, pp. 444–451, 2015.
- [76] K. H. Polderman, B. Bein, S. Kluge, and B. Saugel, "Guiding fluid resuscitation in critically ill patients: how to evaluate the available tools?" *Intensive Care Medicine*, vol. 41, no. 5, pp. 962–964, 2015.
- [77] W. Huber, B. Henschel, R. M. Schmid, and B. Haller, "Comments on Zhang et al.: Effectiveness of treatment based on PiCCO parameters in critically ill patients with septic shock and/or acute respiratory distress syndrome: a randomized controlled trial," *Intensive Care Medicine*, vol. 41, no. 7, pp. 1389–1390, 2015.
- [78] T. Boulain and M. Cecconi, "Can one size fit all? The fine line between fluid overload and hypovolemia," *Intensive Care Medicine*, vol. 41, no. 3, pp. 544–546, 2015.
- [79] J.-C. Richard, F. Bayle, G. Bourdin et al., "Preload dependence indices to titrate volume expansion during septic shock: a randomized controlled trial," *Critical Care*, vol. 19, article 5, 2015.
- [80] J. L. Vincent, "We should abandon randomized controlled trials in the intensive care unit," *Critical Care Medicine*, vol. 38, supplement 10, pp. S534–S538, 2010.
- [81] J.-L. Vincent, "Evidence-based medicine in the ICU: important advances and limitations," *Chest*, vol. 126, no. 2, pp. 592–600, 2004.
- [82] W. C. Shoemaker, P. L. Appel, H. B. Kram, K. Waxman, and T.-S. Lee, "Prospective trial of supranormal values of survivors as therapeutic goals in high-risk surgical patients," *Chest*, vol. 94, no. 6, pp. 1176–1186, 1988.
- [83] L. Gattinoni, L. Brazzi, P. Pelosi et al., "A trial of goal-oriented hemodynamic therapy in critically ill patients. SvO₂ Collaborative Group," *The New England Journal of Medicine*, vol. 333, no. 16, pp. 1025–1032, 1995.
- [84] M. A. Hayes, A. C. Timmins, E. H. S. Yau, M. Palazzo, D. Watson, and C. J. Hinds, "Oxygen transport patterns in patients with sepsis syndrome or septic shock: influence of treatment and relationship to outcome," *Critical Care Medicine*, vol. 25, no. 6, pp. 926–936, 1997.
- [85] K. H. Wodack, A. M. Poppe, T. Lena et al., "Individualized early goal-directed therapy in systemic inflammation: is full utilization of preload reserve the optimal strategy?" *Critical Care Medicine*, vol. 42, no. 12, pp. e741–e751, 2014.
- [86] S. Wolf, A. Riess, J. F. Landscheidt, C. B. Lumenta, P. Friederich, and L. Schürer, "Global end-diastolic volume acquired by transpulmonary thermodilution depends on age and gender in awake and spontaneously breathing patients," *Critical Care*, vol. 13, article R202, 2009.
- [87] S. Wolf, A. Riess, J. F. Landscheidt, C. B. Lumenta, L. Schürer, and P. Friederich, "How to perform indexing of extravascular lung water: a validation study," *Critical Care Medicine*, vol. 41, no. 4, pp. 990–998, 2013.
- [88] W. Huber, S. Mair, S. Q. Götz et al., "Extravascular lung water and its association with weight, height, age, and gender: a study

in intensive care unit patients,” *Intensive Care Medicine*, vol. 39, no. 1, pp. 146–150, 2013.

- [89] V. Eichhorn, M. S. Goepfert, C. Eulenburg, M. L. N. G. Malbrain, and D. A. Reuter, “Comparison of values in critically ill patients for global end-diastolic volume and extravascular lung water measured by transcadiopulmonary thermodilution: a metaanalysis of the literature,” *Medicina Intensiva*, vol. 36, no. 7, pp. 467–474, 2012.
- [90] B. Saugel, S. Mair, S. Q. Götz et al., “Indexation of cardiac output to biometric parameters in critically ill patients: a systematic analysis of a transpulmonary thermodilution-derived database,” *Journal of Critical Care*, vol. 30, no. 5, pp. 957–962, 2015.
- [91] W. Huber, S. Mair, S. Q. Götz et al., “A systematic database-derived approach to improve indexation of transpulmonary thermodilution-derived global end-diastolic volume,” *Journal of Clinical Monitoring and Computing*, 2016.

Review Article

The Endothelial Glycocalyx: New Diagnostic and Therapeutic Approaches in Sepsis

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Sepsis is defined as a life-threatening organ dysfunction caused by a dysregulated host response to infection. The endothelial glycocalyx is one of the earliest sites involved during sepsis. This fragile layer is a complex network of cell-bound proteoglycans, glycosaminoglycan side chains, and sialoproteins lining the luminal side of endothelial cells with a thickness of about 1 to 3 μm . Sepsis-associated alterations of its structure affect endothelial permeability and result in the liberation of endogenous damage-associated molecular patterns (DAMPs). Once liberated in the circulatory system, DAMPs trigger the devastating consequences of the proinflammatory cascades in sepsis and septic shock. In this way, the injury to the glycocalyx with the consecutive release of DAMPs contributes to a number of specific clinical effects of sepsis, including acute kidney injury, respiratory failure, and septic cardiomyopathy. Moreover, the extent of glycocalyx degradation serves as a marker of endothelial dysfunction and sepsis severity. In this review, we highlight the crucial role of the glycocalyx in sepsis as a diagnostic tool and discuss the potential of members of the endothelial glycocalyx serving as hopeful therapeutic targets in sepsis-associated multiple organ failures.

1. Introduction

Defined as a “life-threatening organ dysfunction caused by a dysregulated host response to infection”, sepsis represents a severe disorder with a devastating mortality exceeding for septic shock in hospitals of about 40% [1]. Although recognized as a disease of modern-day hospitals and critical care medicine, already Hippocrates of Kos mentioned the term sepsis ($\eta\psi\iota\varsigma$), which was further illuminated by his succeeding fellows, that is, Semmelweis and Pasteur [2, 3]. Since the new millennium, the definition for sepsis has changed several times; concurrently, the research community demonstrates rapidly new insight into this complex disease [4–6]. The alternating definitions also demonstrate the difficulty in a comprehensive understanding of the complex pathophysiology. The current sepsis definition focuses on organ dysfunction, which is associated with the high mortality [1]. The origin of this organ dysfunction is based on the dysregulated interaction of host response to an infection [1]. Generally, host defence begins with the recognition of pathogens via a set of receptors recognizing pathogen-associated molecular patterns

(PAMPs). However, during the last decades, also endogenous ligands have been described as causative agents of tissue injury and cell damage [7]. In contrast to the pathogen-associated molecular patterns (PAMPs), the origin of damage-associated molecular patterns (DAMPs) lies within the host, as tissue damage after major surgery or burns causes liberations of degradation products of the endothelial glycocalyx, such as heparan sulfates [7–10]. Acting as highly potent DAMPs, these glycocalyx fragments trigger the devastating consequences of the proinflammatory cascades in sepsis and septic shock [10]. The present review focuses on the endothelial compartment during sepsis, highlights the important role of the glycocalyx as a diagnostic tool, and discusses the potential of members of the endothelial glycocalyx serving as hopeful therapeutic targets in sepsis-associated multiple organ failures.

2. The Endothelial Glycocalyx

2.1. Structure. Endothelial cells line the luminal side of blood vessels, thereby modulating the microvascular environment.

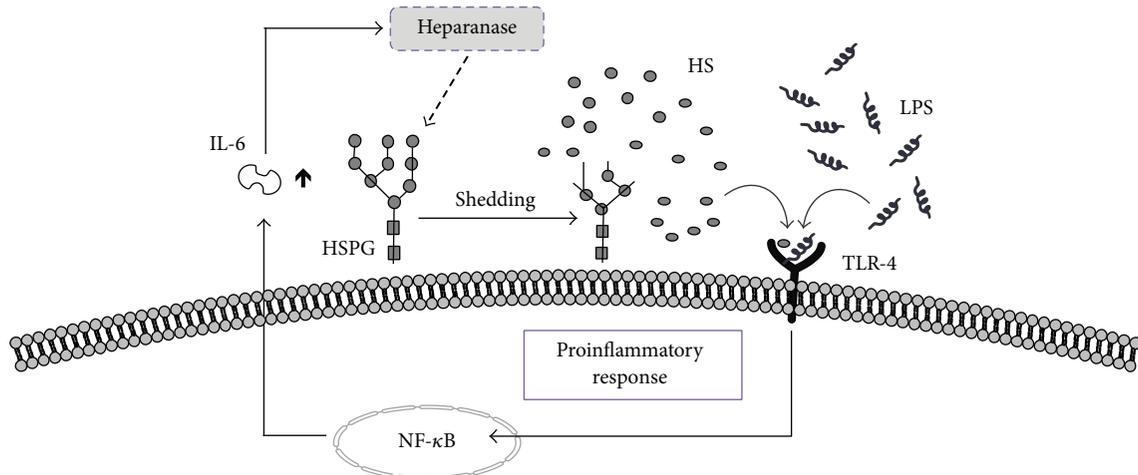


FIGURE 1: Model of proinflammatory response induced by heparanase. Heparanase cleaves and solubilizes heparan sulfate (HS) fragments from their proteoglycan (HSPG) within highly sulfated regions. Analogue to LPS, HS fragments then signal through MyD88-dependent receptors, of which TLR-4 is one, and this leads to NF-kappaB cleavage and activation. NF-kappaB-dependent upregulation leads to the release of cytokine production including interleukin-6 (IL-6). Cytokines are involved in activating heparanase, thereby enhancing the devastating circle of an inflammatory response.

Towards the tissue, endothelial cells connect to the basement membrane. Endoluminal, the endothelial glycocalyx, coats the endothelial cells and interacts with the blood, thereby regulating microcirculatory flow [12]. This fragile endothelial surface layer has a thickness from 1 to 3 μm and consists of proteoglycans, glycoproteins, glycosaminoglycans (GAGs), and associated plasma proteins, including albumin. GAGs consist of a core membrane-bound protein of the syndecan or glypican families with attached heparan or chondroitin sulfate side chains [13]. Moreover, hyaluronan, a nonsulfated, uncharged GAG, is attached to cell-surface proteins (CD44) and stabilises the glycocalyx structure by exhibiting water-retaining characteristics [14].

2.2. Physiological Role of the Endothelial Glycocalyx. The endothelial glycocalyx specifically exhibits crucial roles in the mediation of shear-stress and the associated production of nitric oxide as well as the housing of vascular protective enzymes (e.g., superoxide dismutase) and a wide range of anticoagulant factors (e.g., antithrombin, protein C, and tissue factor pathway inhibitor) [15]. Moreover, the endothelial glycocalyx modulates the inflammatory response by mediating the leukocyte adhesion as well as binding of several inflammatory mediators, such as chemokines, cytokines, and growth factors [12]. Beside these modulating assignments, the endothelial glycocalyx is crucial for maintenance of the vascular barrier [16]. In 1896, Starling described the chain of endothelial cells as an impermeable membrane for proteins. According to this principle, the endothelial cells separate the interstitium, a plasma layer low in proteins, from the protein-rich intravascular space. However, this principle did not consider the reduction in fluid extravasation by the endothelial glycocalyx. In conclusion, with the current knowledge about the role of the endothelial glycocalyx, the Starling

principle has to be revised, considering the following aspects: venous reabsorption, the amount of capillary filtration, and the opposition to capillary filtration [16].

2.3. Alteration of the Endothelial Glycocalyx during Sepsis. Alteration in the composition of the glycocalyx after exposure to an inflammatory insult is one of the earliest features during sepsis. Destruction of the glycocalyx leads to capillary leakage, accelerated inflammation, platelet aggregation, coagulation, and loss of vascular tonus [17]. By expressions of adhesion molecules like intercellular adhesion molecule 1 (ICAM1) or vascular cell adhesion molecule 1 (VCAM1), endothelial cells enable leukocyte adherence, rolling, and migration [18]. Notably, neutrophil granulocytes are recognized as Janus-faced actors during sepsis. They have a fundamental role in the clearance of pathogens, but neutrophil activation is also associated with tissue damage. In this setting, secretion and activation of sheddases harm tissue integrity by degradation of the extracellular matrix and components of neutrophil extracellular traps (NET) act as DAMPs [19, 20]. Heparanase represents one of these enzymes and is activated by proinflammatory cytokines, for example, reactive oxygen species [21]. Today, only one human form is known, the heparanase-1 [22]. This highly specific enzyme is an endo- β -glucuronidase, which sheds heparan sulfate side chains from their proteoglycan within highly sulfated regions [22] (Figure 1). Thereby, after cleavage of the 65-kDa heparanase to its active 50-kDa, heparanase liberates circulating heparan sulfates, which act as highly potent DAMPs [10, 23]. Recently, we showed that circulating heparan sulfate in the serum of septic shock patients induces a strong proinflammatory response in cardiomyocytes, hence causing cardiac mitochondrial dysfunction [9, 10].

Besides enabling leukocyte migration, endothelial activation enhances the inducible nitric oxide synthase, which causes peripheral blood pooling by vasodilatation. Additionally, blood coagulation is shifted towards a procoagulatory state, and vascular permeability is increased due to tight-junction loosening, causing extravasation of tissue and plasma proteins into the surrounding tissue [24, 25]. These alterations seem locally reasonable, as they allow for bottling pathogens up for immunological clearance. Systemically, this reaction has serious implications on circulation and hence on tissue nourishment and oxygenation. In conclusion, the injury of the endothelial glycocalyx causes the clinical appearance of critically ill septic patients, who present generalized oedema and concurrent intravascular hypovolemia, low blood pressure, and high pulse frequency.

3. Markers of Glycocalyx Degradation as Diagnostic Tools

As a result of its unique position directly between the blood and the vessel wall, the endothelial glycocalyx plays a pivotal role in microvascular physiology, in particular by regulating vascular endothelial permeability, vascular tone, and coagulation [26]. During inflammation caused by sepsis or major trauma, the glycocalyx becomes “activated,” which appears to be directly involved in a widespread of endothelial damage, hence contributing to microvascular dysfunction [12]. Thus, there is a strong pathophysiological rationale for targeting markers of endothelial damage during sepsis. Up to now, over 1.200 original articles as well as several reviews have been published evaluating markers of endothelial activation in critically ill patients [27]. The following paragraph aims to discuss the potential of the newly investigated but promising markers of endothelial damage, such as syndecan-1, heparan sulfates, heparanase, endocan, and angiopoietins as diagnostic tools in sepsis.

3.1. Syndecan-1. Circulating levels of syndecan-1 are related to endothelial damage and glycocalyx degradation. Rehm and colleagues investigated syndecan-1 levels in arterial blood of patients undergoing surgery of the ascending aorta. During early reperfusion after global ischemia with circulatory arrest, they reported a transient 42-fold increase in syndecan-1 [28]. Furthermore, electron microscopy in guinea pigs showed a shedding of the glycocalyx with a consecutive loss of syndecan-1 after ischemia and reperfusion (I/R) [28]. In fact, in addition to these findings, syndecan-1 correlates with coagulopathy and increased mortality in sepsis patients [29]. In this study, levels of syndecan-1 have been evaluated in 104 patients suffering from severe sepsis or septic shock, in 28 patients after major abdominal surgery and in 18 healthy young volunteers without any signs of infection. Levels of syndecan-1 were markedly elevated in the sepsis and the surgery group, compared with the control group. Notably, septic patients showed significantly higher levels than patients belonging to the surgery group [29]. Moreover, there was a strong correlation between levels of IL-6 and syndecan-1 in both sepsis and surgery group [29]. In addition, another study with 20 patients shows a significant increase

in circulating syndecan-1 on sepsis onset [30]. However, in the plasma of nine healthy male volunteers undergoing endotoxemia (0.5 ng/kg/hour infusion of *E. coli* LPS), syndecan-1 plasma levels did not increase after 4 and 6 hours. The authors conclude that endothelial disruption and damage observed in patients with severe sepsis cannot be fully reproduced in human experiments, since unsafe and ethically unacceptable doses of LPS would therefore be needed [30]. However, the applied endotoxemia did influence the endothelium as evidenced by an early decline in protein C and a late increase in tPA [30]. Likewise, another prospective observational study with 20 patients suffering from septic shock and 20 healthy adults volunteers likewise showed a significant increase in syndecan-1 content in plasma of septic patients, compared to controls [31].

3.2. Heparanase. The elevated expression of heparanase has been reported in several studies evaluating human malignancies. Heparanase expression correlates with enhanced local and distant metastatic spread, increased vascular density, and reduced postoperative survival [32, 33]. Moreover, heparanase levels are elevated in the urine and plasma of patients with diabetes and correlate with blood glucose levels [34]. Two studies indicate that heparanase expression is elevated during sepsis-associated pulmonary [35] and renal [36] failure. However, these measurements are limited to tissue levels in certain organs [35]. Therefore, we recently measured heparanase level and activity in the plasma from 18 patients suffering from Gram-negative ($n = 10$) or Gram-positive ($n = 8$) septic shock as well as in healthy humans ($n = 10$). We found a significantly higher level and activity of plasma heparanase in septic shock patients compared to healthy volunteers (Figure 2). Of note, there was a significant difference of heparanase levels between the strains of infection, with a significant higher heparanase level and activity in patients with Gram-negative septic shock [11]. As shown in Figure 3, these findings accompanied with significant higher levels of circulating heparan sulfates in patients with Gram-negative septic shock [10].

3.3. Heparan Sulfate. Several studies identified elevated levels of circulating heparan sulfate fragments in critically ill patients [9, 10, 29, 31, 37, 38]. Nelson and colleagues measured heparan sulfate levels in plasma obtained from patients admitted to the intensive care unit with septic shock as well as from matched control patients scheduled for neurosurgery. Median levels of heparan sulfates were fourfold increased in septic shock and were threefold higher in nonsurvivors (90 days study period). Thereby, levels of heparan sulfate correlated with levels of interleukin-6 and interleukin-10. Similarly, the already mentioned study with 104 patients suffering from severe sepsis or septic shock, 28 patients after major abdominal surgery, and 18 healthy controls shows higher levels of heparan sulfate in the sepsis group and the surgery group, compared to the control group [29]. Surprisingly, in comparison to the syndecan-1 levels (see above), the heparan sulfate levels were higher in the surgery group, compared to the sepsis group [29]. Recently, we identified a difference in heparan sulfate levels according

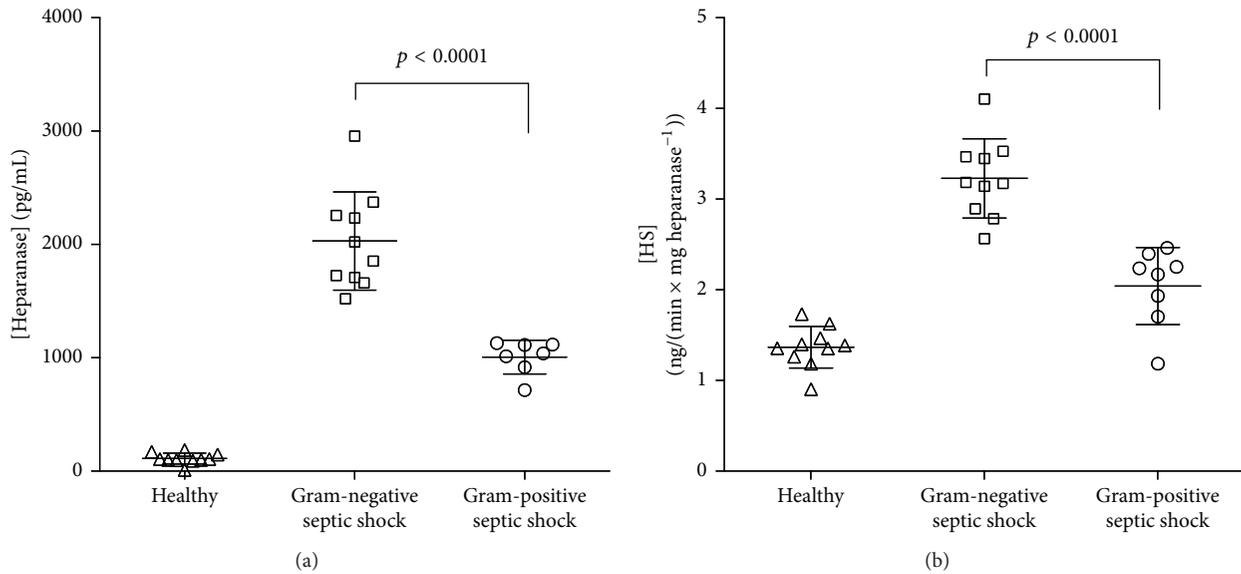


FIGURE 2: Heparanase level (a) and activity (b) in human sepsis ($n = 18$) and healthy volunteers ($n = 10$). Patients with Gram-negative ($n = 10$) septic shock show higher levels of heparanase and higher heparanase activity, compared to those suffering from Gram-positive ($n = 8$) septic shock (modified from [11]).

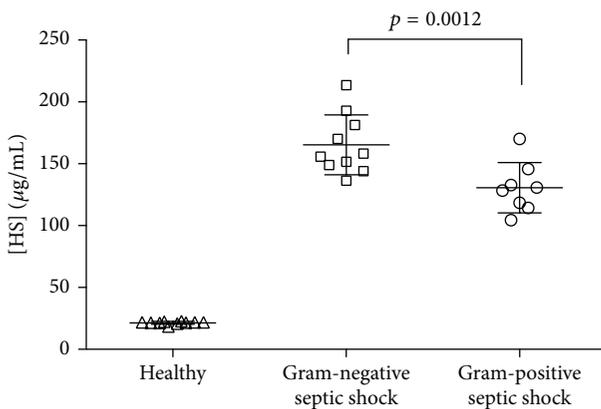


FIGURE 3: Heparan sulfate levels in human sepsis ($n = 18$) and healthy volunteers ($n = 10$). Patients with Gram-negative ($n = 10$) septic shock show higher levels of heparan sulfates, compared to those suffering from Gram-positive ($n = 8$) septic shock (modified from [10]).

to the type of bacterial infection (Figure 3) [10]. We sampled serum from 18 patients suffering from Gram-negative ($n = 10$) or Gram-positive ($n = 8$) septic shock as well as from healthy humans ($n = 10$). As expected, heparan sulfate levels were significantly higher in patients with septic shock compared to healthy volunteers (Figure 3). Notably, there was a significant difference of heparan sulfate levels between the strains of infection, with significantly higher heparan sulfate levels in patients with Gram-negative septic shock [10].

3.4. Endocan. Endocan is a soluble endothelial proteoglycan, known to be released during inflammatory response [39]. As such, endocan is considered as a promising biomarker

of endothelial dysfunction in sepsis [12]. In 150 patients suffering from sepsis or septic shock, endocan plasma levels showed a highly predictive value to diagnose patients with sepsis and septic shock and revealed prognostic information for 30-day and 6-month all-cause mortality [40]. Using venous occlusion plethysmography, Cox and colleagues showed that endocan is related to endothelial dysfunction in humans in vivo [41]. They investigated the endothelial function in 17 healthy male volunteers before and 4 h after the administration of 2 ng/kg LPS. Plasma levels of endocan significantly increased after LPS administration. Furthermore, there was a significant correlation between the increase in plasma endocan levels and the attenuation of vasodilatory responses to acetylcholine [41]. Similarly, another study with 78 patients showed that endocan plasma levels at day 0 are in patients with bacteremia compared to those without bacteremia, but neither CRP levels nor PCT levels at day 0 are different between the two groups [42]. Moreover, endocan levels < 2.54 ng/mL at admission seem to be highly predictive of a respiratory failure presence at day 3 after admission [43]. Using another threshold of 6.2 ng/mL in 63 patients admitted to the intensive care unit with sepsis, the sensitivity and specificity of endocan for predicting mortality were 75% and 84%, respectively. Measurement of endocan at intensive care unit admission revealed higher levels in nonsurvivors than in patients still alive 10 days later [44]. The results of the studies mentioned above suggest that, in septic patients, endocan blood levels are related to the severity of illness and the outcome of the patient and may represent a useful marker of endothelial dysfunction in sepsis and septic shock.

3.5. Angiopoietins. Angiopoietins (Angs) belong to a novel class of angiogenetic growth factors, playing several roles during inflammatory response [45]. Ang-1 is crucial for

the stability of blood vessels, whereas Ang-2 destabilizes vascular integrity and increases vascular permeability [45]. In this way, Ang-2 reflects the breakdown of the vascular barrier in critically ill patients [27]. Up to now, more than 10 studies investigating Ang-2 as a novel biomarker in sepsis and septic shock have been published [27]. Overall, these studies show that Ang-2 is increased in septic shock [46]. Notably, Kümper and colleagues report an independent association of circulating Ang-2 levels with 30-day survival after adjustment for APACHE II score, SOFA score, and serum lactate levels [47]. Moreover, Ricciuto and colleagues observed that serial measurements of Ang-2 are associated with 28-day mortality and multiple organ dysfunction (MOD) score [48]. In this study, sepsis survivors had lower daily levels of Ang-2 than nonsurvivors [48]. However, up to now, a cut point or threshold of circulating Ang-2 allowing differentiation of patients with infection or sterile inflammation or stratification of patients with respect to sepsis severity based on baseline or serial serum Ang-2 concentrations remains still uninvestigated [27].

4. Therapeutic Strategies

As discussed above, the endothelial glycocalyx is extensively involved in sepsis-related inflammatory response and organ dysfunction. Thus, strategies aiming at protecting or repairing glycocalyx damage reveal promising therapeutic targets in sepsis therapy [12, 15]. In this context, especially hydrocortisone, albumin, and adequate fluid resuscitation have been investigated during the last decades. These studies have shown that the therapeutic potential of these drugs may be, at least partly, based on the protection of the endothelial glycocalyx damage [17, 49]. Thus, the following subsection aims to outline established and experimental therapies to protect or repair glycocalyx damage during sepsis.

4.1. Hydrocortisone. Hydrocortisone is known to exhibit strong anti-inflammatory effects in several pathophysiological settings including I/R injury [16]. Thereby, glucocorticoids attenuate glycocalyx degradation by suppressing cytokine and chemokine release as well as reducing the migration of inflammatory cells and mast cell degranulation [16]. Furthermore, hydrocortisone exhibits protective effects against I/R injury by mediating nontranscriptional activation of eNOS [50]. Since the first step of endothelial injury after ischemia consists in a disruption of the glycocalyx [51], Chappell and colleagues investigated the role of hydrocortisone in shedding of the endothelial surface layer after I/R in an isolated heart model [52]. The administration of hydrocortisone reduced shedding of syndecan-1, heparan sulfate, and hyaluronan and consecutively attenuated postischemic oxidative stress and transudate formation. Moreover, electron microscopy revealed a mostly intact glycocalyx after hydrocortisone treatment [52]. A prospective study with 91 patients undergoing cardiac surgery showed that perioperative stress doses of hydrocortisone attenuate systemic inflammation and improve early outcome [53]. However, these findings seem to be limited to a predefined risk group of cardiac surgery patients, since a recent randomized controlled trial with 4494

patients reports no benefit of the use of dexamethasone, regarding the 30-day incidence of major adverse events, compared with placebo [54]. Similarly, the role of hydrocortisone in sepsis therapy remains controversial. Although clinical evidence exists that glucocorticoids improve vasopressor efficacy, it is uncertain whether patients benefit regarding the outcome [55]. Except a postulated protective effect of corticosteroids on glomerular glycocalyx [56], up to now, no data exist on the impact of steroids on the glycocalyx during sepsis.

4.2. Fluid Resuscitation. Fluid resuscitation is one of the fundamental principles for the management of sepsis [1]. However, there is emerging evidence that the type and dose of fluid crucially affect the outcome [57]. Several clinical studies have shown that hypervolemia has detrimental influences on patient outcome, including cardiopulmonary complications, anastomotic insufficiency, and mortality [56, 58]. A pilot study with elective surgery patients shows that hypervolemia increases the release of atrial natriuretic peptide (ANP) and causes enhanced shedding of the endothelial glycocalyx [49]. ANP is known to induce rapid shifts of intravascular fluid into the interstitium. Thereby, elevations of ANP preceded those of cytokines and coincided with or even preceded shedding of the glycocalyx in patients undergoing heart surgery [59]. Indeed, the integrity of the glycocalyx and its interaction with plasma-derived proteins, in particular albumin, is mainly influenced by the perioperative fluid management [16]. Ex vivo investigations showed that albumin prevents fluid extravasation in the heart more effectively than crystalloid or artificial colloid. Notably, this effect is independent of colloid osmotic pressure, rather based on an interaction of albumin with the endothelial glycocalyx [60]. In this way, even very low concentrations of albumin maintain endothelial barrier function [61]. Despite the supposed beneficial effect of albumin in experimental studies, in patients with severe sepsis, albumin replacement in addition to crystalloids did not improve outcome [62]. These negative results may be partly explained by the fact that a colloid only behaves, as first predicted by Starling, if the glycocalyx is undamaged and there is a volume deficit [63]. In fact, if the endothelial glycocalyx is damaged, oncotic pressure gradients play a minimal role because a large amount of protein-rich plasma translocate into the interstitial space, thereby minimizing the oncotic pressure gradient [64].

4.3. Heparanase Inhibition. Heparanase, a heparan sulfate-specific glucuronidase, mediates the onset of renal dysfunction and lung injury during sepsis [35, 36]. The structure of unfractionated heparin (UFH) is comparable to heparan sulfate but has higher N- and O-sulfate contents [65]. UFH is known as potent heparanase inhibitor [66]; however, the potent anticoagulative activity limits the therapeutic use as anti-inflammatory drug [65]. Schmidt and colleagues studied in a model of sepsis-induced renal and pulmonary injury the potential of nonanticoagulant N-desulfated re-N-acetylated heparin (NAH) as a competitive heparanase inhibitor [35, 36]. Heparanase inhibition by NAH prevented endotoxemia-associated glycocalyx loss and neutrophil adhesion and, accordingly, attenuated sepsis-induced acute lung and renal

injury and improves survival in mice subjected to polymicrobial sepsis [35, 36]. Notably, heparanase inhibition seems to be protective also after sepsis onset. Delayed heparanase inhibition 24 h after the onset of sepsis attenuated pulmonary endothelial hyperpermeability, suggesting that heparin is a lung-protective intervention even in established sepsis [35]. Heparin and its derivatives can bind histones through electrostatic interaction, which show pivotal inflammatory mediators in sepsis-associated acute lung injury [67]. In an aspiration model of ALI, induced by intratracheal instillation of hydrochloric acid (HCl), NAH improved the lethality rate, blood gas, MPO activity, lung oedema, and pathological score. However, UFH tended to aggravate the injury due to haemorrhagic complications [67]. These reported data suggest that heparanase inhibition, especially with NAH, may be a promising therapeutic approach in sepsis therapy. However, more experimental and clinical studies are needed to verify this strategy.

4.4. Synthetic Antimicrobial Peptides. The growing relevance of antimicrobial peptides is because of their promising capacity to act as additive drugs in times of increasing antibiotic resistance [68]. Thereby, antimicrobial peptides decrease inflammatory response, kill bacteria, and stimulate innate immunity [69]. Synthetic antimicrobial peptides have been designed based on the limulus-anti-LPS-factor to bind the lipid A-moiety of LPS [70]. However, in addition to a protection against Gram-negative bacteria, they attenuate inflammation and improve survival in Gram-positive bacterial, viral, and mixed infections in vitro and in experimental settings in vivo [10, 71, 72]. Of note, cellular attachment of enveloped viruses was shown to be decreased by a strong interaction between these synthetic antimicrobial peptides and glycocalyx-bound heparan sulfates [71]. Thus, peptide binding to and neutralization of circulating highly potent heparan sulfates may be the underlying mechanism for controlling inflammation [10]. In this way, synthetic antimicrobial peptides offer the unique opportunity to cope with both, PAMPs and DAMPs, and exhibit their activity in both infectious and sterile inflammation, by this dual characteristic [10]. The underlying mechanism seems to be a charge-dependent alteration in the secondary structure of both, PAMPs and DAMPs [11, 71, 73]. Moreover, we recently reported that the treatment of septic mice with the synthetic antimicrobial peptide 19–2.5 lowers levels of plasma heparanase and circulating heparan sulfate and reduces heparanase activity, compared to untreated control animals [11]. Additionally, mRNA levels of heparanase in several organs are downregulated in septic mice treated with peptide 19–2.5, compared to untreated control animals [11]. We also tested the ex vivo addition of peptide 19–2.5 to plasma of septic shock patients. As already mentioned above, plasma heparanase level and activity are elevated in septic shock and the addition of peptide 19–2.5 decreases heparanase activity [11]. These findings have been underlined by isothermal titration calorimetry, which revealed a strong exothermic reaction between peptide 19–2.5 and heparanase, indicating a direct Coulomb interaction between the positive charges of the peptide and the negative

groups or heparanase [11]. Thus, synthetic antimicrobial peptides seem to be a potential anti-inflammatory agent in sepsis by interaction with members of the endothelial glycocalyx.

Competing Interests

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References

- [1] M. Singer, C. S. Deutschman, C. W. Seymour et al., “The third international consensus definitions for sepsis and septic shock (Sepsis-3),” *The Journal of the American Medical Association*, vol. 315, no. 8, pp. 801–810, 2016.
- [2] D. C. Angus and T. Van Der Poll, “Severe sepsis and septic shock,” *The New England Journal of Medicine*, vol. 369, no. 9, pp. 840–851, 2013.
- [3] G. Majno, “The ancient riddle of sigma eta psi iota sigma (sepsis),” *Journal of Infectious Diseases*, vol. 163, no. 5, pp. 937–945, 1991.
- [4] B. Goldstein, B. Giroir, and A. Randolph, “International pediatric sepsis consensus conference: definitions for sepsis and organ dysfunction in pediatrics,” *Pediatric Critical Care Medicine*, vol. 6, no. 1, pp. 2–8, 2005.
- [5] P. Bentzer, J. A. Russell, and K. R. Walley, “Advances in sepsis research,” *Clinics in Chest Medicine*, vol. 36, no. 3, pp. 521–530, 2015.
- [6] R. P. Dellinger, M. M. Levy, J. M. Carlet et al., “Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock: 2008,” *Intensive Care Medicine*, vol. 34, no. 1, pp. 17–60, 2008.
- [7] M. E. Bianchi, “DAMPs, PAMPs and alarmins: all we need to know about danger,” *Journal of Leukocyte Biology*, vol. 81, no. 1, pp. 1–5, 2007.
- [8] H. Wang, H. Yang, C. J. Czura, A. E. Sama, and K. J. Tracey, “HMGB1 as a late mediator of lethal systemic inflammation,” *American Journal of Respiratory and Critical Care Medicine*, vol. 164, no. 10 I, pp. 1768–1773, 2001.
- [9] L. Martin, C. Peters, S. Schmitz et al., “Soluble heparan sulfate in serum of septic shock patients induces mitochondrial dysfunction in murine cardiomyocytes,” *Shock*, vol. 44, no. 6, pp. 569–577, 2015.
- [10] L. Martin, S. Schmitz, R. De Santis et al., “Peptide 19-2.5 inhibits heparan sulfate-triggered inflammation in murine cardiomyocytes stimulated with human sepsis serum,” *PLoS ONE*, vol. 10, no. 5, Article ID e0127584, 2015.
- [11] L. Martin, R. De Santis, P. Koczera et al., “The synthetic antimicrobial peptide 19-2.5 interacts with heparanase and heparan sulfate in murine and human sepsis,” *PLoS ONE*, vol. 10, no. 11, Article ID e0143583, 2015.
- [12] C. Chelazzi, G. Villa, P. Mancinelli, A. R. De Gaudio, and C. Adembri, “Glycocalyx and sepsis-induced alterations in vascular permeability,” *Critical Care*, vol. 19, no. 1, article 26, 2015.

- [13] M. Nieuwdorp, M. C. Meuwese, H. Vink, J. B. L. Hoekstra, J. J. P. Kastelein, and E. S. G. Stroes, "The endothelial glycocalyx: a potential barrier between health and vascular disease," *Current Opinion in Lipidology*, vol. 16, no. 5, pp. 507–511, 2005.
- [14] L. N. Broekhuizen, H. L. Mooij, J. J. P. Kastelein, E. S. G. Stroes, H. Vink, and M. Nieuwdorp, "Endothelial glycocalyx as potential diagnostic and therapeutic target in cardiovascular disease," *Current Opinion in Lipidology*, vol. 20, no. 1, pp. 57–62, 2009.
- [15] A. Burke-Gaffney and T. W. Evans, "Lest we forget the endothelial glycocalyx in sepsis," *Critical Care*, vol. 16, no. 2, article 121, 2012.
- [16] C. S. Alphonsus and R. N. Rodseth, "The endothelial glycocalyx: a review of the vascular barrier," *Anaesthesia*, vol. 69, no. 7, pp. 777–784, 2014.
- [17] B. F. Becker, D. Chappell, D. Bruegger, T. Annecke, and M. Jacob, "Therapeutic strategies targeting the endothelial glycocalyx: acute deficits, but great potential," *Cardiovascular Research*, vol. 87, no. 2, pp. 300–310, 2010.
- [18] K. Ley, "Molecular mechanisms of leukocyte recruitment in the inflammatory process," *Cardiovascular Research*, vol. 32, no. 4, pp. 733–742, 1996.
- [19] M. A. Kovach and T. J. Standiford, "The function of neutrophils in sepsis," *Current Opinion in Infectious Diseases*, vol. 25, no. 3, pp. 321–327, 2012.
- [20] M. J. Kaplan and M. Radic, "Neutrophil extracellular traps: double-edged swords of innate immunity," *Journal of Immunology*, vol. 189, no. 6, pp. 2689–2695, 2012.
- [21] G. Chen, D. Wang, R. Vikramadithyan et al., "Inflammatory cytokines and fatty acids regulate endothelial cell heparanase expression," *Biochemistry*, vol. 43, no. 17, pp. 4971–4977, 2004.
- [22] R. Goldberg, A. Meirovitz, N. Hirshoren et al., "Versatile role of heparanase in inflammation," *Matrix Biology*, vol. 32, no. 5, pp. 234–240, 2013.
- [23] K. J. Goodall, I. K. H. Poon, S. Phipps, and M. D. Hulett, "Soluble heparan sulfate fragments generated by heparanase trigger the release of pro-inflammatory cytokines through TLR-4," *PLoS ONE*, vol. 9, no. 10, Article ID e109596, 2014.
- [24] S. E. Gill, M. Rohan, and S. Mehta, "Role of pulmonary microvascular endothelial cell apoptosis in murine sepsis-induced lung injury in vivo," *Respiratory Research*, vol. 16, no. 1, article 109, 2015.
- [25] T. Van Der Poll, J. D. D. De Boer, and M. Levi, "The effect of inflammation on coagulation and vice versa," *Current Opinion in Infectious Diseases*, vol. 24, no. 3, pp. 273–278, 2011.
- [26] S. Weinbaum, J. M. Tarbell, and E. R. Damiano, "The structure and function of the endothelial glycocalyx layer," *Annual Review of Biomedical Engineering*, vol. 9, pp. 121–167, 2007.
- [27] K. Xing, S. Murthy, W. C. Liles, and J. M. Singh, "Clinical utility of biomarkers of endothelial activation in sepsis—a systematic review," *Critical Care*, vol. 16, no. 1, article R7, 2012.
- [28] M. Rehm, D. Bruegger, F. Christ et al., "Shedding of the endothelial glycocalyx in patients undergoing major vascular surgery with global and regional ischemia," *Circulation*, vol. 116, no. 17, pp. 1896–1906, 2007.
- [29] J. Steppan, S. Hofer, B. Funke et al., "Sepsis and major abdominal surgery lead to flaking of the endothelial glycocalyx," *The Journal of Surgical Research*, vol. 165, no. 1, pp. 136–141, 2011.
- [30] S. R. Ostrowski, R. M. G. Berg, N. A. Windeløv et al., "Coagulopathy, catecholamines, and biomarkers of endothelial damage in experimental human endotoxemia and in patients with severe sepsis: a prospective study," *Journal of Critical Care*, vol. 28, no. 5, pp. 586–596, 2013.
- [31] M. Sallisalmi, J. Tenhunen, R. Yang, N. Oksala, and V. Pettilä, "Vascular adhesion protein-1 and syndecan-1 in septic shock," *Acta Anaesthesiologica Scandinavica*, vol. 56, no. 3, pp. 316–322, 2012.
- [32] O. Ben-Izhak, V. Kaplan-Cohen, N. Ilan, S. Gan, I. Vlodaysky, and R. Nagler, "Heparanase expression in malignant salivary gland tumors inversely correlates with long-term survival," *Neoplasia*, vol. 8, no. 10, pp. 879–884, 2006.
- [33] I. Vlodaysky, P. Beckhove, I. Lerner et al., "Significance of heparanase in cancer and inflammation," *Cancer Microenvironment*, vol. 5, no. 2, pp. 115–132, 2012.
- [34] I. Shafat, N. Ilan, S. Zoabi, I. Vlodaysky, and F. Nakhoul, "Heparanase levels are elevated in the urine and plasma of type 2 diabetes patients and associate with blood glucose levels," *PLoS ONE*, vol. 6, no. 2, Article ID e17312, 2011.
- [35] E. P. Schmidt, Y. Yang, W. J. Janssen et al., "The pulmonary endothelial glycocalyx regulates neutrophil adhesion and lung injury during experimental sepsis," *Nature Medicine*, vol. 18, no. 8, pp. 1217–1223, 2012.
- [36] M. I. Lygizos, Y. Yang, C. J. Altmann et al., "Heparanase mediates renal dysfunction during early sepsis in mice," *Physiological Reports*, vol. 1, no. 6, Article ID e00153, 2013.
- [37] A. Nelson, I. Berkestedt, and M. Bodelsson, "Circulating glycosaminoglycan species in septic shock," *Acta Anaesthesiologica Scandinavica*, vol. 58, no. 1, pp. 36–43, 2014.
- [38] T. Annecke, J. Fischer, H. Hartmann et al., "Shedding of the coronary endothelial glycocalyx: effects of hypoxia/reoxygenation vs ischaemia/reperfusion," *British Journal of Anaesthesia*, vol. 107, no. 5, pp. 679–686, 2011.
- [39] S. Sarrazin, M. Lyon, J. A. Deakin et al., "Characterization and binding activity of the chondroitin/dermatan sulfate chain from Endocan, a soluble endothelial proteoglycan," *Glycobiology*, vol. 20, no. 11, pp. 1380–1388, 2010.
- [40] D. Pauly, S. Hamed, M. Behnes et al., "Endothelial cell-specific molecule-1/endocan: diagnostic and prognostic value in patients suffering from severe sepsis and septic shock," *Journal of Critical Care*, vol. 31, no. 1, pp. 68–75, 2016.
- [41] L. A. E. Cox, L. T. van Eijk, B. P. C. Ramakers et al., "Inflammation-induced increases in plasma endocan levels are associated with endothelial dysfunction in humans in vivo," *Shock*, vol. 43, no. 4, pp. 322–326, 2015.
- [42] K. Seo, T. Kitazawa, Y. Yoshino, I. Koga, and Y. Ota, "Characteristics of serum endocan levels in infection," *PLoS ONE*, vol. 10, no. 4, Article ID e0123358, 2015.
- [43] A. Palud, E. Parmentier-Decrucq, J. Pastre, N. De Freitas Caires, P. Lassalle, and D. Mathieu, "Evaluation of endothelial biomarkers as predictors of organ failures in septic shock patients," *Cytokine*, vol. 73, no. 2, pp. 213–218, 2015.
- [44] A. Scherpereel, F. Depontieu, B. Grigoriu et al., "Endocan, a new endothelial marker in human sepsis," *Critical Care Medicine*, vol. 34, no. 2, pp. 532–537, 2006.
- [45] H. Kranidioti, S. E. Orfanos, I. Vaki et al., "Angiopietin-2 is increased in septic shock: evidence for the existence of a circulating factor stimulating its release from human monocytes," *Immunology Letters*, vol. 125, no. 1, pp. 65–71, 2009.
- [46] J. M. Siner, V. Bhandari, K. M. Engle, J. A. Elias, and M. D. Siegel, "Elevated serum angiopietin 2 levels are associated with increased mortality in sepsis," *Shock*, vol. 31, no. 4, pp. 348–353, 2009.

- [47] P. Kümpers, A. Lukasz, S. David et al., "Excess circulating angiotensin-2 is a strong predictor of mortality in critically ill medical patients," *Critical Care*, vol. 12, no. 6, article R147, 2008.
- [48] D. R. Ricciuto, C. C. Dos Santos, M. Hawkes et al., "Angiotensin-1 and angiotensin-2 as clinically informative prognostic biomarkers of morbidity and mortality in severe sepsis," *Critical Care Medicine*, vol. 39, no. 4, pp. 702–710, 2011.
- [49] D. Chappell, D. Bruegger, J. Potzel et al., "Hypervolemia increases release of atrial natriuretic peptide and shedding of the endothelial glycocalyx," *Critical Care*, vol. 18, no. 5, article 538, 2014.
- [50] A. Hafezi-Moghadam, T. Simoncini, Z. Yang et al., "Acute cardiovascular protective effects of corticosteroids are mediated by non-transcriptional activation of endothelial nitric oxide synthase," *Nature Medicine*, vol. 8, no. 5, pp. 473–479, 2002.
- [51] A. W. Mulivor and H. H. Lipowsky, "Inflammation- and ischemia-induced shedding of venular glycocalyx," *American Journal of Physiology—Heart and Circulatory Physiology*, vol. 286, no. 5, pp. H1672–H1680, 2004.
- [52] D. Chappell, M. Jacob, K. Hofmann-Kiefer et al., "Hydrocortisone preserves the vascular barrier by protecting the endothelial glycocalyx," *Anesthesiology*, vol. 107, no. 5, pp. 776–784, 2007.
- [53] E. Kilger, F. Weis, J. Briegel et al., "Stress doses of hydrocortisone reduce severe systemic inflammatory response syndrome and improve early outcome in a risk group of patients after cardiac surgery," *Critical Care Medicine*, vol. 31, no. 4, pp. 1068–1074, 2003.
- [54] R. Bellomo, "Perioperative statins in cardiac surgery and acute kidney injury," *Journal of the American Medical Association*, vol. 315, no. 9, pp. 873–874, 2016.
- [55] G. P. Patel and R. A. Balk, "Systemic steroids in severe sepsis and septic shock," *American Journal of Respiratory and Critical Care Medicine*, vol. 185, no. 2, pp. 133–139, 2012.
- [56] S. Rinaldi, C. Adembri, S. Grechi, and A. R. De Gaudio, "Low-dose hydrocortisone during severe sepsis: effects on microalbuminuria," *Critical Care Medicine*, vol. 34, no. 9, pp. 2334–2339, 2006.
- [57] J. A. Myburgh and M. G. Mythen, "Resuscitation fluids," *The New England Journal of Medicine*, vol. 369, no. 13, pp. 1243–1251, 2013.
- [58] B. Brandstrup, H. Tønnesen, R. Beier-Holgersen et al., "Effects of intravenous fluid restriction on postoperative complications: comparison of two perioperative fluid regimens: a randomized assessor-blinded multicenter trial," *Annals of Surgery*, vol. 238, no. 5, pp. 641–648, 2003.
- [59] D. Bruegger, L. Schwartz, D. Chappell et al., "Release of atrial natriuretic peptide precedes shedding of the endothelial glycocalyx equally in patients undergoing on- and off-pump coronary artery bypass surgery," *Basic Research in Cardiology*, vol. 106, no. 6, pp. 1111–1121, 2011.
- [60] M. Jacob, D. Bruegger, M. Rehm, U. Welsch, P. Conzen, and B. F. Becker, "Contrasting effects of colloid and crystalloid resuscitation fluids on cardiac vascular permeability," *Anesthesiology*, vol. 104, no. 6, pp. 1223–1231, 2006.
- [61] A. P. Stevens, V. Hlady, and R. O. Dull, "Fluorescence correlation spectroscopy can probe albumin dynamics inside lung endothelial glycocalyx," *American Journal of Physiology—Lung Cellular and Molecular Physiology*, vol. 293, no. 2, pp. L328–L335, 2007.
- [62] P. Caironi, G. Tognoni, S. Masson et al., "Albumin replacement in patients with severe sepsis or septic shock," *The New England Journal of Medicine*, vol. 370, no. 15, pp. 1412–1421, 2014.
- [63] M. G. Mythen, "The glycocalyx and colloids: startling news or sigma by another name?" *Journal of the Intensive Care Society*, vol. 13, no. 3, pp. 191–192, 2012.
- [64] A. Lira and M. R. Pinsky, "Choices in fluid type and volume during resuscitation: impact on patient outcomes," *Annals of Intensive Care*, vol. 4, article 38, 2014.
- [65] B. Casu and U. Lindahl, "Structure and biological interactions of heparin and heparan sulfate," *Advances in Carbohydrate Chemistry and Biochemistry*, vol. 57, pp. 159–206, 2001.
- [66] S. Chen, X. Zhang, Y. Sun, Z. Hu, S. Lu, and X. Ma, "Unfractionated heparin attenuates intestinal injury in mouse model of sepsis by inhibiting heparanase," *International Journal of Clinical and Experimental Pathology*, vol. 8, no. 5, pp. 4903–4912, 2015.
- [67] Y. Zhang, Z. Zhao, L. Guan et al., "N-acetyl-heparin attenuates acute lung injury caused by acid aspiration mainly by antagonizing histones in mice," *PLoS ONE*, vol. 9, no. 5, Article ID e97074, 2014.
- [68] T. Schuerholz, K. Brandenburg, and G. Marx, "Antimicrobial peptides and their potential application in inflammation and sepsis," *Critical Care*, vol. 16, no. 2, article 207, 2012.
- [69] L. Martin, A. van Meegern, S. Doemming, and T. Schuerholz, "Antimicrobial peptides in human sepsis," *Frontiers in Immunology*, vol. 6, article 404, 2015.
- [70] L. Heinbockel, S. Sánchez-Gómez, G. M. De Tejada et al., "Pre-clinical investigations reveal the broad-spectrum neutralizing activity of peptide pep19-2.5 on bacterial pathogenicity factors," *Antimicrobial Agents and Chemotherapy*, vol. 57, no. 3, pp. 1480–1487, 2013.
- [71] M. Krepstakies, J. Lucifora, C.-H. Nagel et al., "A new class of synthetic peptide inhibitors blocks attachment and entry of human pathogenic viruses," *The Journal of Infectious Diseases*, vol. 205, no. 11, pp. 1654–1664, 2012.
- [72] T. Schuerholz, S. Doemming, M. Hornef et al., "The anti-inflammatory effect of the synthetic antimicrobial peptide 19-2.5 in a murine sepsis model: a prospective randomized study," *Critical Care*, vol. 17, no. 1, article R3, 2013.
- [73] T. Gutschmann, I. Razquin-Olazarán, I. Kowalski et al., "New anti-septic peptides to protect against endotoxin-mediated shock," *Antimicrobial Agents and Chemotherapy*, vol. 54, no. 9, pp. 3817–3824, 2010.

Clinical Study

The Effect of Esmolol on Tissue Perfusion and Clinical Prognosis of Patients with Severe Sepsis: A Prospective Cohort Study

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Purpose. This study was aimed at investigating the effect of esmolol on tissue perfusion and the clinical prognosis of patients with severe sepsis. **Materials and Methods.** One hundred fifty-one patients with severe sepsis were selected and divided into the esmolol group ($n = 75$) or the control group ($n = 76$), who received conventional antiseptic shock treatment. The esmolol group received a continuous infusion of esmolol via a central venous catheter, and their heart rate (HR) was maintained at 70–100 bpm over 72 hours. **Results.** The HR of all patients reached the target level within 72 hours of treatment for both groups. The effect of esmolol on PvaCO₂ was only significant at 48 hours ($P < 0.05$). ScvO₂ increased in the esmolol group and decreased in the control group ($P < 0.01$). Lac showed a linear downward trend over the treatment time, but the reduction was more significant in the control group at 48 hours ($P < 0.05$) between the two groups. Kaplan-Meier analysis showed a significantly shorter duration of mechanical ventilation in the esmolol group than in the control group ($P < 0.05$). **Conclusions.** Esmolol reduced the duration of mechanical ventilation in patients with severe sepsis, with no significant effect on circulatory function or tissue perfusion.

1. Introduction

Sepsis is a systemic inflammatory response syndrome due to suspected or confirmed infection and can develop into severe sepsis, septic shock, and multiple organ dysfunction syndrome (MODS) [1]. Excessive activation of the sympathetic nervous system and a substantial increase in catecholamine secretion are important causes of cardiac dysfunction in patients with serious infections and septic shock; thus, the suppression of sympathetic nerve activation is a novel target for treating sepsis. Previously, researchers believed that β -blockers had negative inotropic effects and lowered blood pressure and thus were generally not suitable for treating septic shock. However, an increasing body of recent clinical and basic studies has shown that, for patients with septic shock, β -blockers not only effectively control HR but also

protect cardiac function and improve the clinical prognosis. This study aimed to investigate the effect of esmolol on the hemodynamics, tissue perfusion, and clinical prognosis of patients with severe sepsis and to explore the value of esmolol in the clinic.

2. Materials and Methods

2.1. Clinical Information. We conducted a prospective cohort clinical trial. One hundred ninety patients with severe sepsis were treated in the Department of Critical Care Medicine (Intensive Care Unit [ICU]), Fujian Provincial Hospital, from January 2010 to January 2013. Of these patients, after esmolol treatment, three patients had a decreased HR (<70 bpm), two patients had severe arrhythmia, four patients had low blood pressure, 21 patients were hospitalized for less than

72 hours because of rapid progression of the condition, and nine patients and their families declined to participate in this study. Therefore, these patients were excluded from this study. Accordingly, 151 patients met the inclusion criteria and were enrolled as study subjects. These patients were assigned into the esmolol group ($n = 75$) or the control group ($n = 76$) according to esmolol usage. There were 107 male patients and 44 female patients.

2.2. Inclusion Criteria. Inclusion criteria were as follows: (1) age > 18 years; (2) severe sepsis (all infections were eligible, including pneumonia, peritonitis, and intracranial infection) diagnosis according to the *Campaign to Save Septic Patients: 2008 Treatment Guidelines for Severe Sepsis and Septic Shock*; (3) mechanical ventilation via endotracheal intubation with a tidal volume of 6 mL/kg; and (4) satisfactory sedation and analgesic treatment, with HR > 100 bpm.

2.3. Exclusion Criteria. Exclusion criteria were as follows: (1) preexisting cardiac dysfunction, valvular heart disease, high-degree atrioventricular block; (2) acute or chronic pulmonary heart disease; (3) history of serious asthma; (4) chronic renal insufficiency; (5) cancer, autoimmune diseases, or contraindications for deep venous catheter placement; and (6) insulin-dependent diabetes.

This study complied with medical ethics standards and obtained approval from the Ethics Committee of our hospital (K2010-001-01). Moreover, because all the patients were intubated, we obtained informed consent from the patients' family members, who signed the informed consent form before the study.

2.4. Groups and Treatment. The 151 patients with severe sepsis were assigned into the esmolol group or the control group. All of the patients continued to receive routine treatment, including anti-infective treatment, respiratory and circulatory support, sedation and analgesic treatment, and nutritional support. In addition, patients in the esmolol group received a continuous infusion of esmolol via a micropump through a catheter placed in the superior vena cava. The initial dose was 0.05 mg/kg/min and was adjusted based on heart rate (HR) (target HR: 70 bpm < HR < 100 bpm within 72 hours). In case of low blood pressure, norepinephrine or dopamine was adjusted as necessary to maintain a mean arterial pressure (MAP) ≥ 65 mmHg. The infusion rate was adjusted based on the central venous pressure (CVP) to maintain the CVP at 10 to 15 mmHg. The control group also received natural saline via a micropump; in the same way, the esmolol group received esmolol.

2.5. Measures. We collected data on hemodynamic metrics (MAP, CVP, and HR), tissue perfusion indicators (central venous oxygen saturation [ScvO₂], venous-arterial carbon dioxide partial pressure [P(va)CO₂], and arterial blood lactate [Lac]), vasoactive-inotropic score (IS) [2], and fluid intake before and after 24 hours, 48 hours, and 72 hours of treatment in the two groups. The duration of the ICU stay

TABLE 1: Rank sum test of age (median) between the two groups.

Base value	Esmolol group ($n = 75$)	Control group ($n = 76$)	M-W U value	P
Age, median, years	58 (41-66)	59 (43-69)	2701	0.579

(days) and the duration of mechanical ventilation (days) were also recorded.

$$IS = \text{dopamine (mcg/kg/min)} + \text{dobutamine (mcg/kg/min)} + 100 \times \text{epinephrine (mcg/kg/min)} + 100 \text{ norepinephrine (mcg/kg/min)}.$$

2.6. Statistical Analysis. SPSS 19.0 software was used for the statistical analysis. Measurement data are expressed as the mean \pm standard deviation, and a two-independent-sample t -test was performed for group comparisons. One-way analysis of variance with repeated measures was performed to analyze changes in continuous variables from baseline between the two groups. A chi-square test was performed to analyze the 28-day mortality rate. A log-rank test was performed to analyze the ICU stay and the duration of mechanical ventilation. $P < 0.05$ was considered statistically significant.

3. Results

Group Comparison of Baseline Data. No significant difference was observed between the two groups at study entry with respect to age (Table 1), gender, Acute Physiology and Chronic Health Evaluation II (APACHE-II) score, hemodynamics (MAP, CVP, and HR), tissue perfusion indicators (ScvO₂, P(va)CO₂, and Lac), or vasoactive-inotropic score (IS) (Table 2) (all $P > 0.05$), suggesting that the baseline data were balanced and comparable between the two groups.

Fluid intake was significantly lower in the esmolol group than in the control group at 24 hours, 48 hours, and 72 hours after treatment (all $P < 0.01$). After treatment, the HR trended downward in both groups, with a significant difference between the two groups at all time-points (all $P < 0.01$). For the esmolol group, the HR of all the patients reached the target level within 72 hours of treatment. No significant difference between the two groups was observed for MAP, CVP, and IS at any time-point (all $P > 0.05$), as shown in Table 3.

For both groups, compared with the baseline measurement, a significant change was observed in Lac and PvaCO₂ at 24 hours, 48 hours, and 72 hours after treatment (all $P < 0.05$); however, no significant change was observed in ScvO₂ after treatment ($P > 0.05$). In both groups, compared with the baseline, PvaCO₂ showed a linear downward trend over the treatment time, and the effect of esmolol on PvaCO₂ was only significant at 48 hours ($P < 0.05$), with no difference in the other time-points ($P > 0.05$). ScvO₂ increased over the treatment time in the esmolol group and decreased over the treatment time in the control group ($P < 0.01$). Lac showed a linear downward trend over the treatment time, but the reduction was more significant in the control group at 48

TABLE 2: Chi-square test and *t*-test of the baseline characteristics of the patients (mean).

Base value	Esmolol group (<i>n</i> = 75)	Control group (<i>n</i> = 76)	<i>t</i> / χ^2	<i>P</i>
Male, <i>n</i> (%)	54 (72.0)	53 (69.7)	0.094	0.760
APACHE-II score, mean \pm standard deviation	24.20 \pm 7.66	25.46 \pm 7.83	-0.999	0.319
HR, mean \pm standard deviation	125.04 \pm 13.28	127.21 \pm 13.88	-0.982	0.328
MAP, mean \pm standard deviation	74.71 \pm 7.28	75.89 \pm 6.61	-1.051	0.295
CVP, mean \pm standard deviation	12.45 \pm 3.16	11.99 \pm 3.35	0.881	0.380
IS, mean \pm standard deviation	10.99 \pm 2.08	11.11 \pm 1.81	-0.374	0.709
Lac, mean \pm standard deviation	8.98 \pm 3.09	9.74 \pm 4.05	-1.301	0.195
P(va)CO ₂ , mean \pm standard deviation	9.54 \pm 3.89	10.12 \pm 3.52	-0.947	0.345
ScvO ₂ , mean \pm standard deviation	0.77 \pm 0.06	0.78 \pm 0.06	-0.537	0.592

Note: APACHE-II: Acute Physiology and Chronic Health Evaluation II; HR: heart rate; MAP: mean arterial pressure; CVP: central venous pressure; IS: vasoactive-inotropic score; Lac: arterial blood lactate; P(va)CO₂: venous-arterial carbon dioxide partial pressure; ScvO₂: central venous oxygen saturation.

TABLE 3: The effect of esmolol on hemodynamics in patients with severe sepsis.

(Time, H)	Esmolol group (<i>n</i> = 75) Mean \pm standard deviation	Control group (<i>n</i> = 76) Mean \pm standard deviation	<i>t</i>	<i>P</i>
<i>Fluid intake</i>				
24 h	4373.36 \pm 571.86	4841.75 \pm 658.89	-4.663	<0.01
48 h	4189.61 \pm 515.52	4720.74 \pm 648.60	-5.566	<0.01
72 h	3991.08 \pm 486.73	4553.20 \pm 591.72	-6.371	<0.01
<i>HR</i>				
Base value	125.04 \pm 13.28	127.21 \pm 13.88	-0.982	0.328
24 h	101.96 \pm 7.36	110.12 \pm 8.59	-6.272	<0.01
48 h	93.04 \pm 4.52	102.57 \pm 6.91	-10.039	<0.01
72 h	84.17 \pm 6.26	94.47 \pm 7.91	-8.861	<0.01
<i>MAP</i>				
Base value	74.71 \pm 7.28	75.89 \pm 6.61	-1.051	0.295
24 h	68.65 \pm 9.72	68.54 \pm 7.69	0.080	0.936
48 h	71.00 \pm 11.80	68.39 \pm 7.53	1.615	0.109
72 h	70.91 \pm 10.57	68.14 \pm 7.73	1.829	0.069
<i>CVP</i>				
Base value	12.45 \pm 3.16	11.99 \pm 3.35	0.881	0.380
24 h	10.04 \pm 1.72	10.04 \pm 1.71	0.002	0.998
48 h	10.08 \pm 1.43	9.83 \pm 1.23	1.158	0.249
72 h	9.77 \pm 1.60	9.91 \pm 1.64	-0.510	0.611
<i>IS</i>				
Base value	10.99 \pm 2.08	11.11 \pm 1.81	-0.374	0.709
24 h	10.92 \pm 1.39	10.74 \pm 1.40	0.794	0.428
48 h	9.56 \pm 1.18	9.51 \pm 1.44	0.243	0.808
72 h	9.09 \pm 1.24	9.29 \pm 1.23	-0.957	0.340

hours ($P < 0.05$), with no difference in the other time-points between the two groups ($P > 0.05$), as illustrated in Table 4.

One-way analysis of variance with repeated measures between the two groups (Table 5) (significance level: $\alpha = 0.05$) showed a significant difference in PvaCO₂ ($P < 0.005$), HR ($P < 0.001$), ScvO₂ ($P < 0.001$), and fluid intake ($P < 0.001$). No significant difference was observed for MAP, CVP, IS, or Lac between the groups (all $P > 0.05$).

The group comparison of prognostic indicators (Table 6) indicated that no significant difference was observed in the

28-day mortality rate between the two groups (5.3% [esmolol group] versus 7.9% [control group], $P = 0.760$). A rank sum test showed a significant difference between the two groups with respect to ICU stay ($P = 0.035$) and duration of mechanical ventilation ($P = 0.002$). Survival analysis (Kaplan-Meier analysis) showed no significant difference between the two groups in length of ICU stay ($P = 0.058$), as shown in Figure 1(a). However, the duration of mechanical ventilation was significantly shorter in the esmolol group than in the control group ($P < 0.05$), as shown in Figure 1(b).

TABLE 4: The effect of esmolol on tissue perfusion indicators in patients with severe sepsis.

(Time, H)	Esmolol group (n = 75)	Control group (n = 76)	t	P
Mean ± standard deviation				
<i>Lac, mmol/L</i>				
Base value	8.98 ± 3.09	9.74 ± 4.05	-1.301	0.195
24	6.62 ± 2.43	6.82 ± 2.43	-0.522	0.603
48	3.87 ± 1.89	3.10 ± 1.9	2.493	0.014
72	2.32 ± 0.98	2.41 ± 1.07	-0.534	0.594
<i>P(va)CO₂, mmHg</i>				
Base value	9.54 ± 3.89	10.12 ± 3.52	-0.947	0.345
24	6.71 ± 3.29	7.26 ± 3.34	-1.019	0.310
48	5.11 ± 2.10	5.94 ± 2.38	-2.285	0.024
72	2.73 ± 1.08	3.06 ± 1.73	-1.415	0.160
<i>ScvO₂</i>				
Base value	0.7711 ± 0.0566	0.7762 ± 0.0592	-0.537	0.592
24	0.8000 ± 0.0529	0.7679 ± 0.0510	3.798	0.000
48	0.7932 ± 0.0441	0.7657 ± 0.0569	3.318	0.001
72	0.7957 ± 0.0362	0.7636 ± 0.0551	4.227	0.000

TABLE 5: One-way analysis of variance with repeated measures between the two groups.

Group	Base value	Mean ± standard deviation			P
		24 h	48 h	72 h	
<i>HR, bpm</i>					
Esmolol group	125.04 ± 13.28	101.96 ± 7.36	93.04 ± 4.52	84.17 ± 6.26	<0.001
Control group	127.21 ± 13.88	110.12 ± 8.59	102.57 ± 6.91	94.47 ± 7.91	
<i>MAP, mmHg</i>					
Esmolol group	74.71 ± 7.28	68.65 ± 9.72	71.00 ± 11.80	70.91 ± 10.57	0.277
Control group	75.89 ± 6.61	68.54 ± 7.69	68.39 ± 7.53	68.14 ± 7.73	
<i>CVP, mmHg</i>					
Esmolol group	12.45 ± 3.16	10.04 ± 1.72	10.08 ± 1.43	9.77 ± 1.60	0.385
Control group	11.99 ± 3.35	10.04 ± 1.71	9.83 ± 1.23	9.91 ± 1.64	
<i>IS</i>					
Esmolol group	10.99 ± 2.08	10.92 ± 1.39	9.56 ± 1.18	9.09 ± 1.24	0.881
Control group	11.11 ± 1.81	10.74 ± 1.40	9.51 ± 1.44	9.29 ± 1.23	
<i>Fluid intake, mL/24 h</i>					
Esmolol group		4373.36 ± 571.86	4189.61 ± 515.52	3991.08 ± 486.73	<0.001
Control group		4841.75 ± 658.89	4720.74 ± 648.60	4553.20 ± 591.72	
<i>Lac, mmol/L</i>					
Esmolol group	8.98 ± 3.09	6.62 ± 2.43	3.87 ± 1.89	2.32 ± 0.98	0.705
Control group	9.74 ± 4.05	6.82 ± 2.43	3.10 ± 1.90	2.401 ± 1.07	
<i>P(va)CO₂, mmHg</i>					
Esmolol group	9.54 ± 3.89	6.71 ± 3.29	5.11 ± 2.10	2.73 ± 1.08	0.017
Control group	10.12 ± 3.52	7.26 ± 3.34	5.94 ± 2.38	3.06 ± 1.73	
<i>ScvO₂</i>					
Esmolol group	0.7712 ± 0.0566	0.8000 ± 0.0529	0.7932 ± 0.0441	0.7957 ± 0.0362	<0.001
Control group	0.7762 ± 0.0592	0.7679 ± 0.0510	0.7657 ± 0.0569	0.7636 ± 0.0551	

TABLE 6: Comparison of prognostic indicators between the two groups.

Result	Esmolol group (n = 75)	Control group (n = 76)	χ^2 /M-W U value	P
28-day mortality rate, n (%)	4 (5.3)	6 (7.9)	0.093	0.760
ICU stay, median (d)	13 (10-17)	15 (11-19)	2285.5	0.035
Duration of mechanical ventilation, median (d)	8 (6-11)	10 (8-14)	2002.0	0.002

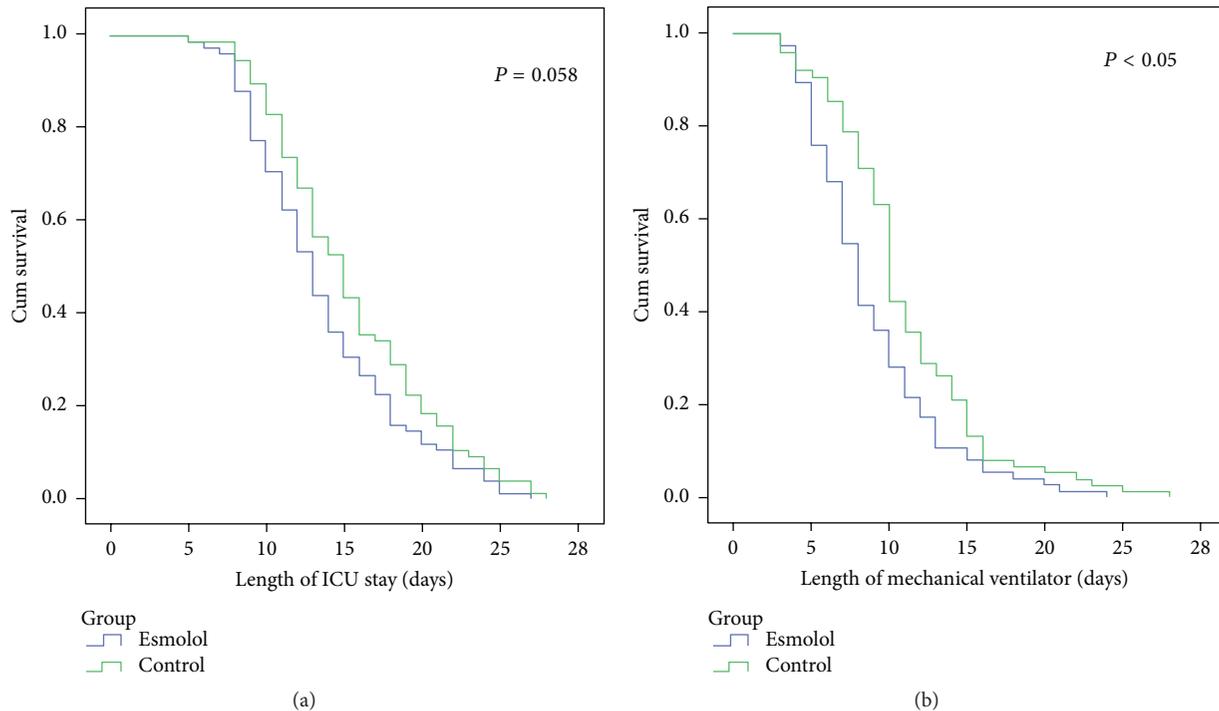


FIGURE 1: The survival analysis of length of ICU stay (a) and length of mechanical ventilation (b) based on the Kaplan-Meier analysis.

4. Discussion

β -Blockers have been investigated in studies of sepsis treatment for five decades. In the 1960s, Berk et al. [3] used a dog model to demonstrate that excessive β -adrenergic stimulation induced sepsis and that propranolol improved blood pressure and blood pH; this treatment even improved survival. Suzuki et al. [4] used a septic mouse model to inject esmolol, a selective β_1 -adrenoreceptor antagonist, and found that esmolol reduced the HR, blood pressure, and serum TNF- α levels in the experimental group. No significant effect on the Lac level was observed, suggesting that esmolol did not increase oxygen consumption in tissues. β -Blockers significantly reduce HR and time-course HR variability, thereby harmonizing cardiac force and frequency and reducing myocardial oxygen consumption [5]. Moreover, β -blockers reduce the expression of chemokines and inflammatory cytokines during cardiac dysfunction [6] and play a cardioprotective role in acute myocardial stunning [7]. With respect to septic shock, β -blockers also stabilize the circulation and improve myocardial injury [8, 9]. Therefore, β -blockers play an important role and show promise for the treatment of sepsis.

In contrast with experimental animals and basic research, which have demonstrated promise for β -blockers, clinical trials and studies of β -blockers for sepsis have produced inconsistent results. Schmittinger et al. [10] studied 40 patients with septic shock who required fluid resuscitation and vasoactive drugs (including norepinephrine, milrinone, and vasopressin). This study found that, after metoprolol treatment, in 39 patients, the HR was controlled at 65–95 bpm

with no MAP decrease, the stroke volume index (SVI) increased, the cardiac index remained stable, and the Lac level decreased significantly. The present study showed that after 72 hours of esmolol treatment, the HR was controlled at ≤ 100 bpm with a significant decrease in MAP, no significant change in CVP, and no significant increase in IS. These results suggest that for patients with severe sepsis, esmolol is effective in controlling HR, with no decrease in blood pressure. Moreover, no increase in the dose of vasoactive drugs is required. Therefore, esmolol is safe and feasible in clinical practice.

Small clinical studies and recent large retrospective studies have shown favorable results for the application of β -blockers. Christensen et al. [11] conducted a cohort study to analyze 8,087 adult cases aged ≥ 45 years who were treated from 1999 to 2005. The results showed that for patients who were on long-term β -blockers (>125 days) before ICU admission (β -blocker group), the 30-day mortality rate after ICU admission was 25.7%, which was significantly lower than that of the control group (31.4%) (odds ratio [OR] 0.74 and 95% confidence interval [CI] 0.63–0.87; $P < 0.05$). These results suggest that the use of β -blockers before ICU admission reduced mortality. Italian researchers Morelli et al. [12] conducted a randomized controlled clinical trial of 154 patients with septic shock. The patients were randomly assigned into one of two groups: one receiving continuous intravenous infusion of esmolol to maintain the HR at 80 to 94 bpm and another (control group) receiving routine treatment. The results showed that in the esmolol group, the HR of all of the patients was controlled within the target range; these results are consistent with those of the present

study. Moreover, in the study by Morelli et al., the 28-day mortality rate was 49.4% in the esmolol group and 80.5% in the control group (OR 0.39, 95% CI 0.26–0.59; $P < 0.001$). Therefore, these authors concluded that esmolol effectively controlled the HR and improved the survival of patients with sepsis. In another study, Morelli et al. [13] found that esmolol favorably controlled the HR of patients with septic shock without increasing Lac and mixed SvO₂, thereby having no adverse effect on tissue perfusion. This study also showed no significant difference in Lac reduction between the two groups ($P > 0.05$); thus, we conclude that esmolol has no effect on tissue perfusion in patients with sepsis.

Shock, in essence, is tissue ischemia and hypoxia; thus, to treat septic shock, it is important to implement active resuscitation and to improve tissue perfusion while administering anti-infective treatment. The presence of Lac is a delayed manifestation of tissue hypoperfusion, and ScvO₂ and P(va)CO₂ are two early clinical indicators of tissue perfusion. Studies have shown that P(va)CO₂ and ScvO₂ are related [14–16]. In the present study, one-way analysis of variance with repeated measures showed that Lac was slowly cleared in both groups, with no significant difference between the two groups. Morelli et al. [13] found that esmolol had no effect on SV and significantly increased the microcirculation in patients with sepsis. The present study also found that PvaCO₂ decreased over time in the esmolol group; thus, we believe that esmolol increases circulation, which is consistent with previous research. Moreover, ScvO₂ increased slightly in the esmolol group, with a significant difference between the two groups. However, more research is necessary to verify whether this effect is due to an esmolol-induced reduction in tissue oxygen consumption. Furthermore, we found that, in the esmolol group, fluid intake was reduced, with no significant changes in IS and CVP. Esmolol, therefore, reduced the adverse effects of excessive fluid intake. It was also observed that the HR was reduced in the esmolol group compared with the control group, with no significant change in MAP, suggesting that esmolol effectively controlled HR within the target range, without significant effects on cardiac systolic function. The duration of mechanical ventilation was shortened in the experimental group, an effect that was likely related to reduced HR, fluid intake, and pulmonary fluid. The 28-day mortality rate was 5.3% in the esmolol group and 7.9% in the control group, with no significant difference between the two groups ($P = 0.760$). No significant difference in ICU stay was observed between the two groups, likely due to the small sample size (this being a single-center study) and bias in the exclusion criteria.

Multiple basic and clinical studies of severe sepsis have shown a positive outlook for β -blockers. In particular, the cardioprotective effect and clinical outcomes of ultra-short-acting β -4-blockers in patients with septic shock are especially encouraging. Nevertheless, many questions remain unanswered, such as the timing of the treatment and dosage, potential synergistic effects between different types of β -blockers, and the relationship between the efficacy of β -blockers and the type of pathogens and infected sites. Hence, large clinical studies are necessary.

5. Limitation

This clinical trial lacks the measurement of tissue bacterial growth and of cytokines such as tumor necrosis factor alpha, as in the experimental study by Dimopoulos et al. [17]. This fact constitutes the limitation of the present investigation.

6. Conclusion

In summary, esmolol, an ultra-short-acting β -blocker, significantly controlled HR and reduced the duration of mechanical ventilation in patients with severe sepsis, with no significant effect on circulatory function or tissue perfusion. The observed decrease in PvaCO₂ may be related to the increased microcirculation associated with esmolol; the mechanism of increased ScvO₂ is not clear and requires further research.

Competing Interests

The authors declare that there are no competing interests regarding the publication of this paper.

References

- [1] R. P. Dellinger, M. M. Levy, A. Rhodes et al., “Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock, 2012,” *Intensive Care Medicine*, vol. 39, no. 2, pp. 165–228, 2013.
- [2] G. Wernovsky, D. Wypij, R. A. Jonas et al., “Postoperative course and hemodynamic profile after the arterial switch operation in neonates and infants: a comparison of low-flow cardiopulmonary bypass and circulatory arrest,” *Circulation*, vol. 92, no. 8, pp. 2226–2235, 1995.
- [3] J. L. Berk, J. F. Hagen, W. H. Beyer, M. J. Gerber, and G. R. Dochat, “The treatment of endotoxin shock by beta adrenergic blockade,” *Annals of Surgery*, vol. 169, no. 1, pp. 74–81, 1969.
- [4] T. Suzuki, H. Morisaki, R. Serita et al., “Infusion of the β -adrenergic blocker esmolol attenuates myocardial dysfunction in septic rat,” *Critical Care Medicine*, vol. 33, no. 10, pp. 2294–2301, 2005.
- [5] O. Joulin, S. Marechaux, S. Hassoun, D. Mouton, S. Lancel, and R. Neviere, “Cardiac force-frequency relationship and frequency-dependent acceleration of relaxation are impaired in LPS-treated rats,” *Critical Care*, vol. 13, no. 1, article R14, 2009.
- [6] K. Werdan, A. Oelke, S. Hettwer et al., “Septic cardiomyopathy: hemodynamic quantification, occurrence, and prognostic implications,” *Clinical Research in Cardiology*, vol. 100, no. 8, pp. 661–668, 2011.
- [7] A. R. Lyon, P. S. C. Rees, S. Prasad, P. A. Poole-Wilson, and S. E. Harding, “Stress (Takotsubo) cardiomyopathy—a novel pathophysiological hypothesis to explain catecholamine-induced acute myocardial stunning,” *Nature Clinical Practice Cardiovascular Medicine*, vol. 5, no. 1, pp. 22–29, 2008.
- [8] J. Aboab, V. Sebille, M. Jourdain et al., “Effects of esmolol on systemic and pulmonary hemodynamics and on oxygenation in pigs with hypodynamic endotoxin shock,” *Intensive Care Medicine*, vol. 37, no. 8, pp. 1344–1351, 2011.
- [9] R. Silasi-Mansat, H. Zhu, N. I. Popescu et al., “Complement inhibition decreases the procoagulant response and confers organ protection in a baboon model of *Escherichia coli* sepsis,” *Blood*, vol. 116, no. 6, pp. 1002–1010, 2010.

- [10] C. A. Schmittinger, M. W. Dünser, M. Haller et al., “Combined milrinone and enteral metoprolol therapy in patients with septic myocardial depression,” *Critical Care*, vol. 12, no. 4, article R99, 2008.
- [11] S. Christensen, M. B. Johansen, E. Tønnesen et al., “Preadmission beta-blocker use and 30-day mortality among patients in intensive care: a cohort study,” *Critical Care*, vol. 15, no. 2, article R87, 2011.
- [12] A. Morelli, C. Ertmer, M. Westphal et al., “Effect of heart rate control with esmolol on hemodynamic and clinical outcomes in patients with septic shock: a randomized clinical trial,” *Journal of the American Medical Association*, vol. 310, no. 16, pp. 1683–1691, 2013.
- [13] A. Morelli, A. Donati, C. Ertmer et al., “Microvascular effects of heart rate control with esmolol in patients with septic shock: a pilot study,” *Critical Care Medicine*, vol. 41, no. 9, pp. 2162–2168, 2013.
- [14] K. M. Gaidukov, A. I. Len’kin, V. V. Kuz’kov, E. V. Fot, A. A. Smetkin, and M. I. Kirov, “Central venous blood oxygen saturation and venous to arterial PCO₂ difference after combined heart valve surgery,” *Anesteziologija i Reanimatologija*, no. 3, pp. 19–21, 2011.
- [15] J. Cuschieri, E. P. Rivers, M. W. Donnino et al., “Central venous-arterial carbon dioxide difference as an indicator of cardiac index,” *Intensive Care Medicine*, vol. 31, no. 6, pp. 818–822, 2005.
- [16] G. G. Tsaousi, K. A. Karakoulas, E. N. Amaniti, I. D. Soultati, M. D. Zouka, and D. G. Vasilakos, “Correlation of central venous-arterial and mixed venous-arterial carbon dioxide tension gradient with cardiac output during neurosurgical procedures in the sitting position,” *European Journal of Anaesthesiology*, vol. 27, no. 10, pp. 882–889, 2010.
- [17] G. Dimopoulos, M. Theodorakopoulou, A. Armaganidis et al., “Esmolol: immunomodulator in pyelonephritis by *Pseudomonas aeruginosa*,” *Journal of Surgical Research*, vol. 198, no. 1, pp. 175–184, 2015.

Clinical Study

Bioartificial Therapy of Sepsis: Changes of Norepinephrine-Dosage in Patients and Influence on Dynamic and Cell Based Liver Tests during Extracorporeal Treatments

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Purpose. Granulocyte transfusions have been used to treat immune cell dysfunction in sepsis. A granulocyte bioreactor for the extracorporeal treatment of sepsis was tested in a prospective clinical study focusing on the dosage of norepinephrine in patients and influence on dynamic and cell based liver tests during extracorporeal therapies. *Methods and Patients.* Ten patients with severe sepsis were treated twice within 72 h with the system containing granulocytes from healthy donors. Survival, physiologic parameters, extended hemodynamic measurement, and the indocyanine green plasma disappearance rate (PDR) were monitored. Plasma of patients before and after extracorporeal treatments were tested with a cell based biosensor for analysis of hepatotoxicity. *Results.* The observed mortality rate was 50% during stay in hospital. During the treatments, the norepinephrine-dosage could be significantly reduced while mean arterial pressure was stable. In the cell based analysis of hepatotoxicity, the viability and function of sensor-cells increased significantly during extracorporeal treatment in all patients and the PDR-values increased significantly between day 1 and day 7 only in survivors. *Conclusion.* The extracorporeal treatment with donor granulocytes showed promising effects on dosage of norepinephrine in patients, liver cell function, and viability in a cell based biosensor. Further studies with this approach are encouraged.

1. Introduction

Severe sepsis and septic shock remain a major cause of morbidity and mortality in critically ill patients and the treatment of these patients is very expensive [1–4]. The impairment of hemodynamics and liver function are major problems in patients with severe sepsis [5, 6]. In patients with septic shock

a liver dysfunction or liver failure occurred in nearly 19% and lead to a poor prognosis of these patients [6, 7].

Extracorporeal therapies have been suggested to influence successfully immune imbalances and subsequently the clinical course of multiorgan failure and sepsis [8]. Some studies showed hemodynamic stabilization of patients during extracorporeal treatment of sepsis; however, no clear impact

on survival was seen [8, 9]. The influence of extracorporeal therapies of sepsis on liver function has not been investigated yet, but it may be an important tool for the improvement of outcome in this high-risk cohort population with liver dysfunction or liver failure [6].

Extracorporeal bioreactors were studied in the treatment of liver failure and acute renal failure associated with sepsis using hepatocytes or renal tubular cells; the proper choice of the cell-source was of central importance [10–14]. The use of immune cells (leukocytes) to treat sepsis in an extracorporeal setting was reported from our group [15]. With regard to cellular immunocompetence, functional impairment of neutrophils and monocytes is associated with increased mortality in advanced stages of sepsis [16–23]. Therefore, we developed an extracorporeal granulocyte bioreactor system [24, 25]. The rationale for such an approach is that on one hand the plasma-modifying capacity of human phagocytes can be used (e.g., to remove antigenic material from the circulation) while on the other hand control over these cells can be maintained (e.g., retention of the cells and their release and break-down products, preventing local tissue effects; 15). In *in vitro* studies and two large animal models for septic shock, we were able to show the proof of principle and promising survival data [15, 24, 26]. Additionally, the granulocyte bioreactor was studied in a pilot phase I trial with ten septic shock patients and showed safety and compatibility of this complex therapy [25]. During extracorporeal granulocyte treatments, the dosage of norepinephrine could be significantly reduced, as blood pressure was stable in the treated septic patients.

The focus of the current work was to document the exact impact of the extracorporeal granulocyte treatment on the dosage of norepinephrine in patients and the liver function using extended hemodynamic monitoring with the PiCCO-System and dynamic measurement of the liver function with the LiMON-System [27, 28]; moreover, in this second clinical study, cell based analysis of hepatotoxicity of plasma of patients [29, 30] with severe sepsis or septic shock patients was done. Based on the results of the second study, a first controlled study with this new therapy should be designed.

2. Materials and Patients

The study was conducted in accordance with the Helsinki Declaration, received ethics approval from the local research ethics committee (reg.-no: II HV 28/2003), and was notified to the state authorities according to German pharmaceutical and medical device law. The trial was registered <https://clinicaltrials.gov/> (reg.-no: NCT00818597). Written informed consent was obtained from all participants or from the patients' representatives if direct consent could not be received.

2.1. Patients. Between January 2010 and November 2011, ten patients of one medical and one surgical intensive care units of a tertiary care university hospital were enrolled in the study. During a 22-month period, all patients were screened for the parameters of severe sepsis or septic shock as defined by international consensus criteria [31]. Definitions of organ

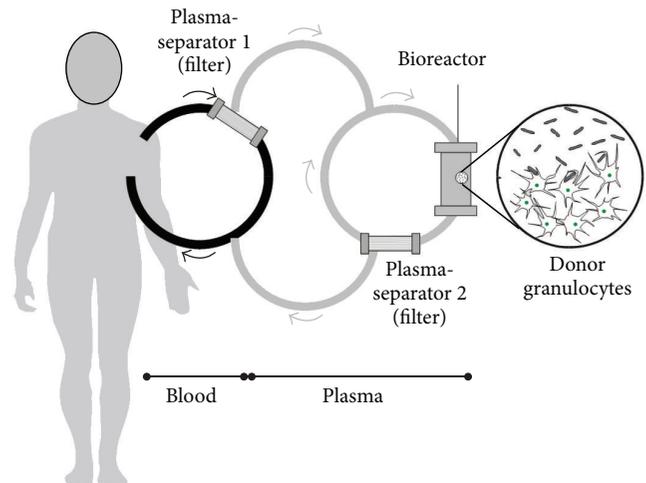


FIGURE 1: Schematic drawing of the extracorporeal plasma separation and cell perfusion model.

dysfunctions were adopted from the PROWESS study [32] with the difference that liver failure was not an exclusion criterion in this current study. The exclusion criteria were age under 18 years, hepatitis C, HIV infection, and active bleeding or contraindications against systemic heparinization.

2.2. Procedures. After inclusion of a patient, a healthy blood donor for obtaining an ABO-compatible granulocyte concentrate was identified and stimulated with corticosteroids (each 8 mg p.o. methylprednisolone, Sanofi-Aventis Deutschland GmbH, Frankfurt, Germany) and lenograstim (Granocyte, each 1.5 $\mu\text{g}/\text{kg}$ s.c., Chugai Pharma Deutschland, Frankfurt, Germany) 16 h before donation. Granulocytes were collected by extracorporeal density gradient centrifugation using hydroxyethyl starch (HES 200/0.5 6%, Fresenius Kabi AG, Bad Homburg, Germany) and citrate in a cell separator (COBE Spectra, Gambro BCT, Planegg-Martinsried, Germany) according to standard procedures. Prior to the treatment, the inclusion criteria were reconfirmed and the patients were treated for up to six hours with an extracorporeal bioreactor (Figure 1) consisting of plasma separation and plasma perfusion through the cell compartment containing the donor cells. Blood access was venovenous via a Shaldon catheter. Plasma separation was carried out by a dialysis monitor (BM25, Edwards Lifesciences GmbH, Unterschleißheim, Germany) using a 0.5 μm pore-size plasma filter (PF 1000N, Gambro Hospal GmbH, Planegg-Martinsried, Germany). The plasma was infused into a continuously recirculating donor cell compartment that was prefilled with hemofiltration solution HF-BIC 35-410 (Fresenius Medical Care, Bad Homburg, Germany). Plasma reflux to the patient was done through a second PF 1000N plasma filter to withhold the donor cells from being infused into the patient. Total extracorporeal volume was 400 mL. The blood flow rate was 110–150 mL/minute with a plasma separation rate of 16.7–33.3 mL plasma/minute using the BM 25 monitor. The MARS-Monitor 1 TC (Gambro Rostock GmbH, Rostock,

TABLE 1: Patients characteristics, illness severity, premorbidity, and clinical outcome for the study cohort ($n = 10$).

Patient	Major diagnoses at inclusion	Source of infection	Organ failure	Premorbidity	Age (years)	Sex (m)	Hospital survival
1	SS, ALI	Pneumonia, bacteremia, infection of foot, and thoracic empyema	ARF, ALI	Diabetes mellitus, chronic heart failure	66	m	Died (day 62)
2	SS, ALI, and endocarditis	Bacteremia, infection of sternum	ARF, ALI, and liver failure	Diabetes mellitus	72	m	Died (day 2)
3	Severe sepsis, thoracic empyema	Pneumonia, bacteremia	ALI	Alcohol abuse	33	m	Survived
4	SS, colitis	Peritonitis, colitis	ARF, ALI, DIC, and liver failure	Chronic heart failure	78	m	Died (day 4)
5	SS, ALI	Pneumonia, urinary tract infection	ARF, ALI	IHD, immunosuppression	59	m	Survived
6	SS, spondylodiscitis	Pneumonia, bacteremia, spondylodiscitis, and infection of arm	Liver failure, ALI, and ARF	COPD	67	m	Died (day 1)
7	SS, urosepsis	Pneumonia, bacteremia, and urinary tract infection	ALI, ARF, and DIC	Diabetes mellitus, IHD	76	m	Survived
8	SS, peritonitis	Bacteremia, pneumonia, and peritonitis	ALI	COPD, alcohol abuse	62	m	Died (day 22)
9	SS, perforated aortic aneurysm	Bacteremia, pneumonia, and peritonitis	ARF, ALI	IHD, cancer	83	m	Survived
10	SS, after urgent ACB surgery	Pneumonia	ALI	Diabetes mellitus, COPD, and IHD	57	m	Survived

ACB: aortocoronary bypass.

ALI: acute lung injury.

ARF: acute renal failure.

COPD: chronic obstructive pulmonary disease.

DIC: disseminated intravascular coagulation.

IHD: ischemic heart disease.

m: male.

SS: septic shock.

Germany) was used for the recirculating bioreactor circuit at a rate of 200 mL/minute and to maintain the temperature in the cell compartment at 37°C. Unfractionated heparin (40 IU/kg, Roche, Grenzach-Wyhlen, Germany) was given at the beginning of the extracorporeal treatment followed by a continuous infusion into the circuit. Heparin administration was adjusted to maintain activated clotting time (ACT) within 180–200 seconds. Following safety assessment of the first treatment, all patients were treated a second time 48 hours after the first treatment, again for up to six hours with granulocytes from the same donor.

2.3. Measurements. We recorded basic demographic information, illness severity (APACHE II, SOFA, MODS, and SAPS II scores), microbiological results, premorbidity, and clinical outcome for study cohort (Tables 1 and 2). Patients were followed up for 28 days and hospital survival. At the days “inclusion”, 1–8, 10, 12, 14, 21, 28, and before/after an extracorporeal bioreactor-treatment, the patients were screened for clinical and immunological data: hemodynamic, inflammation, coagulation, hemolysis, temperature, organ function blood parameters, cytokines, complement (C3, C4), and number of HLA-DR molecules per monocyte surface. “Day 1”

was defined as the day of the first bioreactor-treatment. At inclusion, at days 1 to 7 and before and after extracorporeal treatment, hemodynamic monitoring was done with the PiCCO-System (Table 4, PULSION Medical Systems, Feldkirchen, Germany; 27). The LiMON-System (based on the indocyanine green plasma disappearance rate, PULSION; 28) was used for the dynamic measurement of the liver function before and after extracorporeal treatment and on day 7.

2.4. Cell Based Analysis of Hepatotoxicity: Cell Cultures and Test Methods. Before and after extracorporeal treatment, 10 mL plasma was drawn from each patient for testing with our hepatotoxicity test (biosensor; 29, 30). The method to determine the toxicity of patient plasma used the human hepatocytes cell line HepG2/C3A obtained from the American Type Culture Collection (ATCC CRL-10741). The cells were cultivated in Dulbecco’s modified Eagle’s Medium (GIBCO Life Technologies, Eggenstein, Germany). HepG2/C3A cells were seeded in 24-well cell culture plates in a density of 250.000 cells/well; then, the cells were cultured for three days with 1 mL heparinized plasma from subjects. Subsequently, cells were rinsed once with medium and incubated with fresh medium (1 mL) for three days. Cells, respectively, cell culture

TABLE 2: Age, scores, and results of laboratory parameters, of hemodynamic monitoring (PiCCO-System), and of dynamic measurement of the liver function (LiMON-System) at inclusion or on day 1 (before granulocyte therapy) of survivors and nonsurvivors (median/0.25–0.75 quartile).

	Survivors (<i>n</i> = 5)	Nonsurvivors (<i>n</i> = 5)	Statistical significance (<i>p</i>)
Age (years)	59 (57–76)	67 (66–72)	n.s.
Surgery/no surgery	4/1	5/0	
APACHE II at ICU arrival/at inclusion	20.5 (17.8–25)/22 (22–34)	27 (27–33)/30 (28–32)	n.s.
SOFA at inclusion	8 (6–10)	14 (13–14)	n.s.
SAPS II at inclusion	47 (44–62)	74 (73–78)	0.032
MODS at inclusion	8 (7–12)	12 (11–13)	n.s.
Bilirubin ($\mu\text{mol/L}$)	12 (11–17)	44 (30–145)	0.016
ALAT (U/L)	32 (20–45)	37 (30–219)	n.s.
ASAT (U/L)	62 (44–74)	105 (52–669)	n.s.
Ammonia (mmol/L)	36 (32–62)	39 (38–61)	n.s.
PCT (ng/mL)	7.3 (0.5–43)	10.4 (7.4–14.6)	n.s.
Leukocytes (GpT/L)	18.1 (14.8–18.1)	16.2 (13.9–22.2)	n.s.
Thrombocytes (GpT/L)	159 (89–278)	125 (47–269)	n.s.
Prothrombin time as INR	1.09 (1.01–1.1)	1.32 (1.11–1.33)	n.s.
Activated partial thromboplastin time (s)	36 (33–49)	48 (45–54)	n.s.
Creatinine ($\mu\text{mol/L}$)	143 (79–238)	178 (165–213)	n.s.
Urea (mmol/L)	15.7 (5.7–16)	14.7 (13.8–24.9)	n.s.
Lactate (mmol/L)	1.0 (0.9–1.8)	2.0 (1.6–2.1)	n.s.
Complement C3 (g/L)	1.1 (1.0–1.8)	0.7 (0.5–1.0)	n.s.
Complement C4 (g/L)	0.5 (0.4–0.9)	0.4 (0.2–0.5)	n.s.
HLA-DR/CD-14 positive cells (expression/cell)	6413 (5890–6970)	8980 (7640–9590)	n.s.
Cardiac index ($\text{L/m}^2/\text{min}$)	3.5 (3.3–4.0)	2.5 (2.5–3.4)	n.s.
Stroke volume index (mL/m^2)	48 (43–51)	29 (28–39)	n.s.
MAP (mmHg)	92 (84–93)	71 (69–85)	n.s.
Norepinephrine ($\mu\text{g/kg/min}$)	0.13 (0.06–0.21)	0.36 (0.29–0.51)	0.032
ICG-PDR on day 1 (%) / ICG-PDR/CI	14.4 (13.3–17.2)/5.6 (4.9–5.7)	5.3 (2.5–11.9)/2.9 (1.1–2.9)	0.048/0.05

ALAT: alanine aminotransferase.

APACHE: Acute Physiology and Chronic Health Evaluation.

ASAT: aspartate aminotransferase.

CI: cardiac index.

HLA: human leukocyte antigen.

ICG-PDR: indocyanine green plasma disappearance rate.

MAP: mean arterial pressure.

MODS: multiorgan dysfunction syndrome (score).

n.s.: not (statistically) significant.

PCT: procalcitonin.

SAPS: simplified acute physiology score.

SOFA: sequential organ failure assessment (score).

supernatants, were tested for viability (XTT-test: dehydrogenases activity in the mitochondria, cell-count and vitality with Trypan blue-staining), synthesis of microalbumin, and cytochrome 1A2 activity. Each test batch with plasma from test persons was duplicated (+medium control) and each measurement was taken twice. Microalbumin was determined nephelometrically from cell culture medium supernatant (Image 800, Beckmann Coulter GmbH, Krefeld,

Germany). The XTT-test was carried according to the protocol of Scudiero et al. [33]. The activity of cytochrome P450 1A2 was determined by means of O-demethylation of 7-ethoxyresorufine to resorufin. The measurement was carried according to the protocol of Kelly and Sussman [34].

2.5. Statistical Analysis. The Statistical Package for the Social Sciences (SPSS) was used to conduct nonparametric analyses

TABLE 3: Cytokines values before the first extracorporeal granulocyte therapy, after the second extracorporeal granulocyte therapy, and after 7 days (median/0.25–0.75 quartile).

Cytokine	Before first extracorporeal therapy (day 1) ($n = 10$, pg/mL)	After second extracorporeal therapy (day 3) ($n = 8$, pg/mL)	After 7 days (day 8) ($n = 7$, pg/mL)
IL-6	57 (41–159)	78 (45–154)	28 (26–62) [#]
IL-8	40 (28–53)	57 (38–118)	30 (29–36)
TNF-alpha	30 (22–40)	22 (19–26)	15 (13–17) ^{*#}
IL-10	15 (8–20)	11 (8–13)	3 (3–8)

* Statistically significant ($p = 0.028$) compared to day 1 (before first extracorporeal treatment).

[#] Statistically significant ($p < 0.05$) compared to the end of second extracorporeal treatment.

IL: interleukin.

TNF: tumor necrosis factor.

using Friedman test and Wilcoxon test for the comparison of parameters in the course of disease; statistical significance between the survivors and nonsurvivors was analyzed with the Kruskal-Wallis one-way and the two-tailed Mann-Whitney U test. The results are expressed as the median with 0.25–0.75 quartile. Differences were considered significant at $p < 0.05$. Box Plots were used for graphics. The horizontal line within the boxes represents the median, whereas the upper part represents the 75th and the lower part the 25th percentiles. The whiskers represent the range of the values, whereas the circles and the asterisks show the outliers (extreme values that deviate from the rest of the sample).

3. Results

3.1. Clinical Characteristics of Patients and Survival. Nine patients with septic shock and one patient with severe sepsis were included in the study (all male, 9 out of 10 surgical patients). Details concerning diagnoses, source of infection, organ failure, age, sex, premorbidity, and survival are shown in Table 1. Nine out of ten patients had positive microbial tests; 18 Gram-positive bacteria, 12 Gram-negative bacteria, and 4 positive cultures with fungi were found.

The observed mortality rate was 40% within 28 days and 50% during stay in hospital. Five patients could be discharged from the hospital in stable condition. Patients 2 and 4 died after reduction of therapy on palliative care (on the very same day). During the first extracorporeal treatment, patient 6 died; an autopsy revealed an advanced and longer existing ischemia of the bowel. The time between beginning of shock and beginning of extracorporeal treatment was 5.5 (4.3–7.5) days. In Table 2, age, scores, and results of laboratory parameters, of hemodynamic monitoring (PiCCO-System), and of dynamic measurement of liver function (LiMON-System) at inclusion or at day 1 of survivors and nonsurvivors are shown. The dosage of norepinephrine, the values of bilirubin, and the median SAPS II were significantly higher and the indocyanine green plasma disappearance rate (ICG/PDR)/ICG-PDR cardiac index ratio was significantly lower in nonsurvivors than in survivors. Three patients developed a liver failure in the course of disease (Table 1, all nonsurvivors).

3.2. Extracorporeal Treatment. All extracorporeal treatments were carried out for 6 h without technical problems. Eight

patients were treated twice within 72 h with an extracorporeal bioreactor containing 12.3 (10.4–14.4) $\times 10^6$ granulocytes from healthy donors. On average, 11.7 (10.3–12.0) liters of separated plasma were treated by the therapeutic donor cells. To test whether the donor cells were still functional, every two hours cells from the cell circuit were evaluated for viability and functionality. For the whole treatment, the cells showed a viability of more than 90% and unimpaired cellular functions like phagocytosis and oxidative burst (data not shown).

There was no significant change in coagulations markers (platelet counts, antithrombin, prothrombin time, and fibrinogen) within 12 h after the extracorporeal circulation. D-dimers did not increase significantly during the extracorporeal treatment (data not shown). No hemorrhages and no signs of hemolysis were observed. Haptoglobin remained within the normal range and no significant change in values of lactatedehydrogenase and potassium was seen during the treatments (data not shown). Moreover, no allergic reactions were recognized.

3.3. Inflammation. During the six-hour treatment, a dramatic increase in the number of leukocytes was observed (before: 14.9 (13.8–19.4); after 6 hours: 18.2 (14.6–24.8); $\times 10^9/l$; $p = 0.002$); after 12 hours, however, a decrease to baseline was seen. This increase during extracorporeal treatment was not due to changes in a particular subset of WBC, the ratio of segmented to banded neutrophils remained unchanged. During the extracorporeal treatments, the complement factors C3 and C4 decreased slightly but were even in normal ranges during the whole observation time (data not shown). The values of procalcitonin decreased significantly during the six-hour treatment (before: 6.9 (0.3–11.8); after 6 hours: 6.2 (0.3–9.4); ng/mL; $p = 0.003$) and between days 3 and 28 (all time points) compared with day 1 (before extracorporeal treatment, $p < 0.05$, data not shown). The expression per cell of HLA-DR on monocytes increased significantly from inclusion [6974 (5888–9119)] to day 4 [9278 (6394–12526)] and between inclusion and days 5 to 28 (all time points, $p < 0.05$, data not shown). Between day 1 (before extracorporeal treatment) and day 3 after extracorporeal treatment, the values of IL-6 and IL-8 increased; these changes, however, were not significant and the values normalized on day 8 (Table 3). The values of IL-10 and TNF-alpha were lower and decreased between day 1 and day 8 (Table 3).

TABLE 4: Hemodynamic parameters, central venous oxygen saturation, dynamic measurement of the liver function (LiMON-System), and lactate before and after the extracorporeal granulocyte therapy (median/0.25–0.75 quartile) measured with the PiCCO-System (under significant reduction of norepinephrine; see Figure 2).

	Before extracorporeal therapy (<i>n</i> = 9)	After extracorporeal therapy (<i>n</i> = 8)	Statistical significance
CI (L/m ² /min)	3.1 (2.3–3.8)	3.5 (2.9–3.7)	n.s.
SVI (mL/m ²)	42 (33–56)	45 (40–52)	n.s.
MAP (mmHg)	73 (65–83)	74 (66–79)	n.s.
Heart rate (beat/min)	73 (67–95)	76 (70–85)	n.s.
SVRI (dyne × sec × cm ⁻⁵ /m ²)	1510 (1150–1730)	1290 (1130–1760)	n.s.
ITBVI (mL/m ²)	1060 (930–1160)	1000 (960–1120)	n.s.
EVLWI (mL/kg)	7.6 (6.8–9.4)	7.5 (6.5–8.3)	n.s.
cvSpO ₂ (%)	73.5 (68.4–76.5)	71.5 (68.6–79.1)	n.s.
Lactate (mmol/L)	1.5 (1.2–3.0)	1.3 (1.0–2.7)	n.s.
ICG-PDR (%)/ICG-PDR/CI	13.8 (11.9–15.6.)/4.4 (3.1–5.1)	14.2 (12.4–17.1)/4.6 (3.1–4.8)	n.s./n.s.

CI: cardiac index.

cvSpO₂: central venous oxygen saturation.

EVLWI: extravascular lung water index.

ICG-PDR: indocyanine green plasma disappearance rate.

ITBVI: intrathoracic blood volume index.

MAP: mean arterial pressure.

n.s.: not (statistically) significant.

SVI: stroke volume index.

SVRI: systemic vascular resistance index.

3.4. Hemodynamic Parameters during Extracorporeal Treatment. The dosage of norepinephrine could be reduced significantly during the extracorporeal treatments (Figure 2). Additionally, during the extracorporeal treatments, the mean arterial pressure, the heart rate, the parameters of hemodynamic monitoring using the PiCCO-System, the central venous oxygen saturation, and values of lactate showed no significant changes; but the cardiac index and stroke volume index increased (Table 4). After the second extracorporeal treatments of patients, the use of norepinephrine could reduce continue in the course of the observation time (data not shown).

3.5. Liver Function Markers and Tests. The dynamic measurement of liver function used the LiMON-System (based on the indocyanine green plasma disappearance rate, ICG-PDR) and the ICG-PDR cardiac index ratio which both on day 1 showed significantly lower values in nonsurvivors than in survivors (Table 2). During the extracorporeal treatments, no significant increases of PDR-values and of ICG-PDR cardiac index ratio could be observed (Table 4). The PDR-values increased significantly ($p = 0.027$) between day 1 [11.9 (5.3–13.8)] and day 7 [23.3 (20.2–23.8)] only in surviving patients.

At inclusion, the values of bilirubin were significantly lower in survivors than in nonsurvivors (Table 2). In the course of disease, no significant differences of bilirubin-values were seen between the survivors and nonsurvivors and during extracorporeal treatments.

In the cell based analysis of hepatotoxicity (biosensor), the HepG2/C3A cells were incubated with plasma of the patients before and after each extracorporeal granulocyte

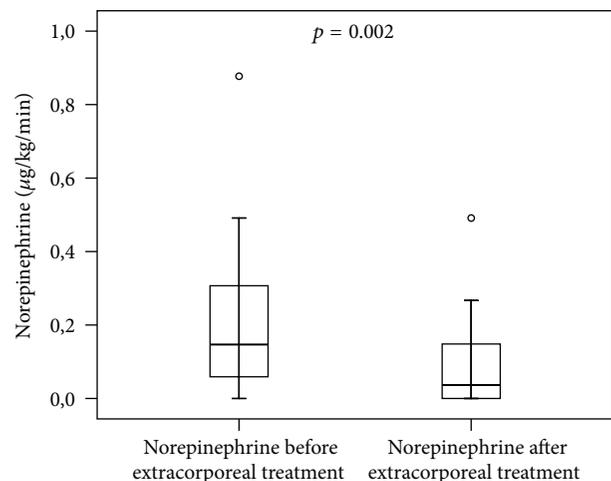


FIGURE 2: The dosage of norepinephrine ($\mu\text{g}/\text{kg}/\text{min}$) could be reduced significantly during the extracorporeal granulocyte treatments (Mann-Whitney U test; $n = 10$; median/0.25–0.75 quartile).

treatment. The cell-count and vitality (Figure 3), the synthesis of microalbumin (Figure 4), and the activities of cytochrome 1A2 and mitochondrial dehydrogenases (XTT-test, Figure 5) increased significantly during extracorporeal treatment. In addition, the values of lactatedehydrogenase (LDH) were significantly lower after extracorporeal treatment than the values of LDH before extracorporeal treatment (Figure 4). Only in survivors were significant increases of all biosensor parameters observed between day 1 (before extracorporeal

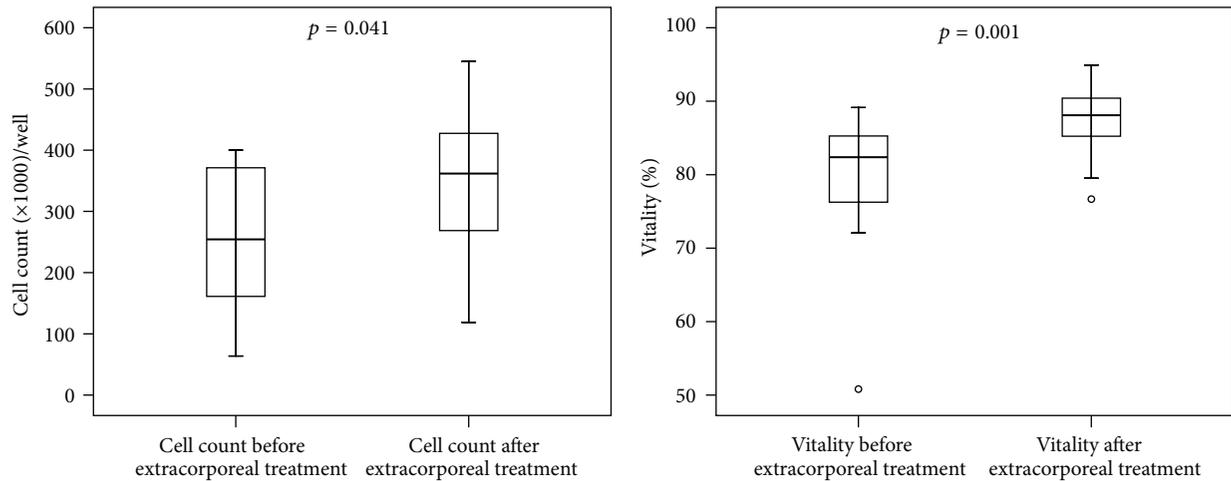


FIGURE 3: Cell-count and vitality (Trypan blue-staining) of HepG2/C3A cells (biosensor-test) incubated with plasma from septic patients before extracorporeal treatments compared to plasma from patients after extracorporeal granulocyte treatments (Mann-Whitney *U* test; *n* = 10; median/0.25–0.75 quartile).

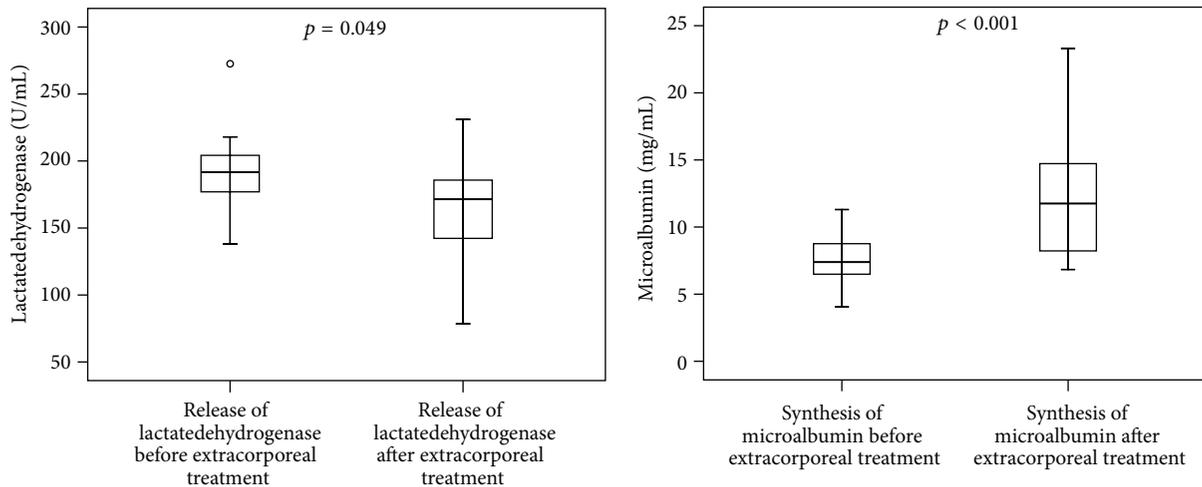


FIGURE 4: Release of lactatedehydrogenase and synthesis of microalbumin of HepG2/C3A cells (biosensor-test) incubated with plasma from septic patients before extracorporeal treatments compared to plasma from patients after extracorporeal granulocyte treatments (Mann-Whitney *U* test; *n* = 10; median/0.25–0.75 quartile).

treatment) and day 3 after extracorporeal granulocyte treatment ($p < 0.05$, data not shown), with the exception of LDH (significant decrease in survivors).

4. Discussion

The therapy with the extracorporeal granulocyte bioreactor system led to a reduction of norepinephrine dosage; moreover, the liver cell function and the viability in the biosensor-test were improved. We showed as before in the first clinical study [25] a good compatibility of the system. No significant changes in coagulation markers or any hemorrhages and signs of hemolysis were observed.

The extracorporeal granulocyte therapy influenced the immune system of the treated patients. During the six-hour treatment, an increase in the number of leukocytes

and a decrease of procalcitonin were observed in the sera of the patients. The expression per cell of HLA-DR on monocytes increased significantly from inclusion to day four; additionally, between day 1 (before extracorporeal treatment) and day 3 after extracorporeal treatment, the values of IL-6 and IL-8 increased slowly. Immunomodulation has been introduced as a supportive therapy to overcome immune system dysfunction and could show positive impact on survival of patients with severe sepsis in some studies [35] but failed in a number of other studies [36, 37]. Extracorporeal blood detoxification methods have also been suggested to successfully influence immune imbalances and subsequently clinical course and outcome of multiorgan failure and sepsis [8, 9, 38, 39], because immunosuppression seems to be the main course of mortality of patients with sepsis and multiple organ failure [40].

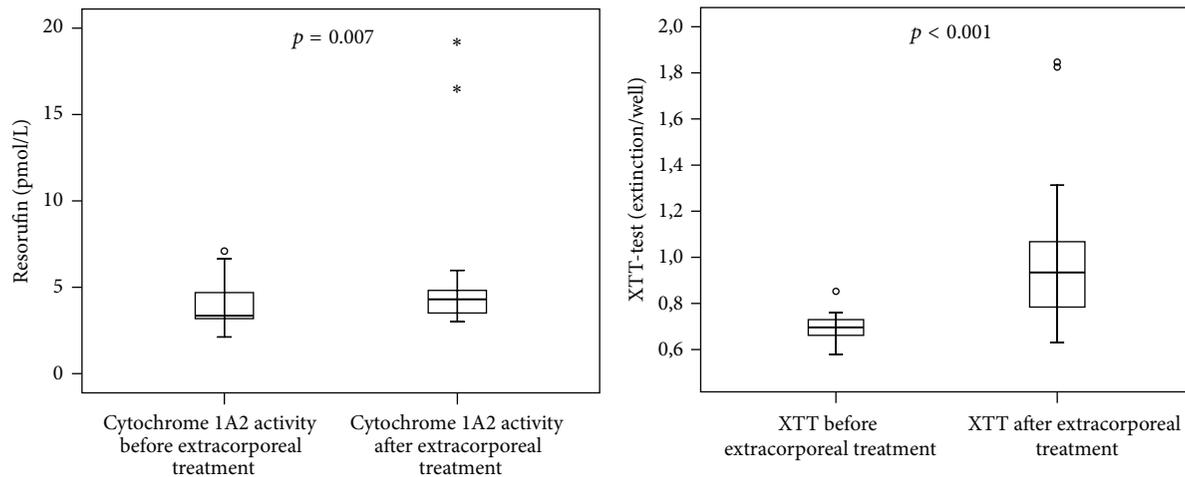


FIGURE 5: Activity of cytochrome 1A2 (metabolism of ethoxyresorufin to resorufin) and the XTT-test (dehydrogenases activity in the mitochondria) of HepG2/C3A cells (biosensor-test) incubated with plasma from septic patients before extracorporeal treatments compared to plasma from patients after extracorporeal granulocyte treatments (Mann-Whitney U test; $n = 10$; median/0.25–0.75 quartile). * Whisker.

The transfusion of granulocyte preparations (GTx) failed to improve survival in sepsis and neutropenic patients [41, 42]. However, there is some indication that steroid- or G-CSF-stimulated high-yield granulocyte donations might result in better survival in severe infections associated with neutropenia and cancer [42, 43]. In order to deploy the beneficial features of neutrophils such as phagocytosis of cellular debris, antigenic material, or pathogens and at the same time to circumvent the possible damaging local effects of systemically transfused neutrophils, a bed-side bioreactor was built, which uses granulocytes in a strictly extracorporeal mode. The bioreactor-cells are retained in the extracorporeal system and discarded after the treatment.

The 28-day mortality was 40% in the studied patients, consisting of nine patients with septic shock and one patient with severe sepsis, and is comparable with the results of other studies [1, 2]. Patients 2 and 4 died after reduction of therapy on palliative care (on the very same day and during the observation time). During the first extracorporeal treatment, patient 6 died; an autopsy revealed an advanced and longer existing ischemia of the bowel. No conclusions about survival can be drawn based on this (uncontrolled) study. The aim of the study was to document the impact of the extracorporeal granulocyte treatment on the dosage of norepinephrine in patients and the liver function using extended hemodynamic monitoring, dynamic measurement of the liver function with the LiMON-System, and, additionally, cell based analysis of hepatotoxicity of patient's plasma. Both need of vasopressors and the occurrence of liver dysfunction or failure are well known to impair the prognosis of patients with severe sepsis [5–7, 44]. The general idea of this work was to postulate that a new therapy of severe sepsis, for example, extracorporeal treatments, should be proven for a positive influence of hemodynamics and the liver in the septic organism.

Under reduction of vasopressor administration, the mean arterial pressure was stable and the cardiac index and the stroke volume index increased slightly during treatments

measured with a dynamic monitoring system (PiCCO, 27). These results are the second report of a decreasing dosage of norepinephrine in patients using this extracorporeal granulocyte treatment system [25].

In this study, different parameters of liver function and hepatotoxicity were measured. The static parameter bilirubin was significantly lower in survivors than in nonsurvivors at the beginning of the extracorporeal treatment. During the observation time of 28 days, however, no significant differences of bilirubin-values were seen between the survivors and nonsurvivors and during extracorporeal treatments. Bilirubin is known as a good prognostic parameter for liver dysfunction or failure. In contrast, for acute changes in liver function, bilirubin seems to be not a valuable parameter [45, 46].

For the measurement of acute impairment of the liver function, dynamic measurement with tracer-substances was introduced [28]. We used the LiMON-System based on the indocyanine green plasma disappearance rate (PDR) and calculated the ICG-PDR cardiac index ratio. In accordance with other studies [47, 48] we found significant lower values in nonsurvivors than in survivors on day 1 before extracorporeal treatment and an increase of PDR from day 1 to day 7 only in surviving patients. With regard to clinical characteristics, three patients of the nonsurvivor group developed a (sepsis-induced) liver failure in the course of the disease; therefore, the time between beginning of shock and beginning of extracorporeal treatment was 5.5 days. During the extracorporeal treatments, however, no significant increases of PDR-values or ICG-PDR cardiac index ratio were observed. The cause of this could be the low number of patients. Patients with markedly decreased PDR-values (<8%) did not respond well to the extracorporeal granulocyte treatment. The PDR of indocyanine green provides information for the assessment of mortality and morbidity in critically ill patients, in patients with severe sepsis, and in patients underlying cardiac surgery [47–50]. Limitations of the LiMON-System

are the nonexistent discrimination between liver function and perfusion of the splanchnic tract and false data in cases of hyperdynamic sepsis and hyperbilirubinaemia [51, 52].

In the cell based analysis of hepatotoxicity (biosensor; 29, 30), the HepG2/C3A hepatocyte cell line was incubated with plasma of the patients before and after each extracorporeal granulocyte treatment. During the extracorporeal treatments, a significant increase of vitality and function of the test cells was seen. These results suggest a positive impact of the extracorporeal granulocyte treatment on the liver cell vitality and function measured in this indirect cytotoxicity test. The reasons for this are still not clear but may be a result of reduction of toxins and drugs and the influence of chemokines and cytokines of the patients by the use of the extracorporeal bioartificial therapy. These factors are known to impair the cell functions and viability in HepG2/C3A cells [53–56]. Proinflammatory cytokines, for instance, are known to cause a dysfunction of mitochondria [50], to downregulate albumin synthesis [54], and to diminish function of some P450 cytochromes like CYP 1A2, CYP 2E1, and CYP 7A1 [55, 56].

Using the cell based analysis of hepatotoxicity of patient's plasma, only in survivors were significant increases of all biosensor parameters observed between day 1 (before extracorporeal treatment) and day 3 after extracorporeal granulocyte treatment. These data suggest a prognostic property of the biosensor in patients with severe sepsis, similarly to the PDR of indocyanine green [47]. In a previous prospective clinical study, the hepatotoxicity of plasma from septic and nonseptic patients was tested with this biosensor [29]. We found that the plasma of patients with septic shock impaired cellular functions and viability of HepG2/C3A cells. These values of biosensor parameters were increased only in survivors compared to nonsurvivors in this study. Additionally, only in the septic shock group were negative correlations found between cytochrome 1A2 activity and synthesis of albumin with APACHE II and SOFA scores (correlation coefficient: <0.7 , $p < 0.05$).

5. Conclusions

In summary, there are three significant findings based on the results of the present study: (a) extracorporeal granulocyte treatment led to immunomodulation and a reduction of norepinephrine-dosage in patients during extracorporeal therapy, (b) patients with markedly decreased indocyanine green PDR-values did not respond well to the extracorporeal granulocyte treatment and an increase of PDR was only seen in survivors, and (c) a positive impact on the viability and function of hepatocyte sensor cells (hepatotoxicity test of patient's plasma) were seen during the extracorporeal treatments.

Further studies should focus on changes of the hemodynamic system and the liver function in septic patients because of the prognostic value of these organ systems. Additionally, studies investigating the role of extracorporeal cell-therapies for sepsis are encouraged and should also address the influence on the survival of patients with severe sepsis.

Abbreviations

ACT:	Activated clotting time
ALT:	Alanine transaminase
AST:	Aspartate transaminase
APACHE:	Acute Physiology and Chronic Health Evaluation (Score)
G-CSF:	Granulocyte-colony stimulating factor
GTx:	Granulocyte transfusion
HLA-DR:	Human leukocyte antigen DR
IL:	Interleukin
INR:	International Normalized Ratio
MAP:	Mean arterial pressure
MODS:	Multiple Organ Dysfunction Score
PCT:	Procalcitonin
PDR:	Plasma disappearance rate
SAPS:	Simplified Acute Physiology Score
SOFA:	Sequential Organ Failure Assessment (Score)
TNF:	Tumor necrosis factor.

Competing Interests

Jens Altrichter and Steffen R. Mitzner have filed patents on the technology of extracorporeal cell perfusion technology and own shares of and work as part-time consultants for Artcline GmbH, a university spin off that now owns the patent rights. Martin Sauer, Thomas Wild, and Fanny Doß work as a part-time consultants for Artcline GmbH. Martin Sauer has filed patents on the technology of the hepatocyte based biosensor. Cristof Haubner, Annette Pertschy, Thomas Mencke, Maren Thomsen, Johannes Ehler, Jörg Henschel, Sandra Doß, Stephanie Koch, Georg Richter, and Gabriele Nöldge-Schomburg declare that they have no competing interests.

Authors' Contributions

Martin Sauer, Jens Altrichter, and Steffen R. Mitzner participated in the design of the study. Martin Sauer did the regulatory work and coordinated the preparation of the paper. Martin Sauer, Cristof Haubner and Georg Richter did the data analysis. Martin Sauer, Annette Pertschy, Jörg Henschel, Thomas Mencke, Maren Thomsen, Johannes Ehler, Georg Richter, Gabriele Nöldge-Schomburg, and Steffen R. Mitzner were clinical investigators of the study and performed the treatments. Fanny Doß, Sandra Doß, and Stephanie Koch analyzed the clinical probes from patients and samples from the extracorporeal circuit.

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References

- [1] D. C. Angus, W. T. Linde-Zwirble, J. Lidicker, G. Clermont, J. Carcillo, and M. R. Pinsky, "Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care," *Critical Care Medicine*, vol. 29, no. 7, pp. 1303–1310, 2001.
- [2] C. Engel, F. M. Brunkhorst, H.-G. Bone et al., "Epidemiology of sepsis in Germany: results from a national prospective multicenter study," *Intensive Care Medicine*, vol. 33, no. 4, pp. 606–618, 2007.
- [3] A. Schmid, H. Burchardi, J. Clouth, and H. Schneider, "Burden of illness imposed by severe sepsis in Germany," *European Journal of Health Economics*, vol. 3, no. 2, pp. 77–82, 2002.
- [4] H. Lee, C. J. Doig, W. A. Ghali, C. Donaldson, D. Johnson, and B. Manns, "Detailed cost analysis of care for survivors of severe sepsis," *Critical Care Medicine*, vol. 32, no. 4, pp. 981–985, 2004.
- [5] J. Takala, "Should we target blood pressure in sepsis?" *Critical Care Medicine*, vol. 38, no. 10, pp. S613–S619, 2010.
- [6] L. Kramer, B. Jordan, W. Druml, P. Bauer, and P. G. H. Metnitz, "Incidence and prognosis of early hepatic dysfunction in critically ill patients—a prospective multicenter study," *Critical Care Medicine*, vol. 35, no. 4, pp. 1099–1104, 2007.
- [7] J. Bakker, R. Grover, A. McLuckie et al., "Administration of the nitric oxide synthase inhibitor NG-methyl-L-arginine hydrochloride (546C88) by intravenous infusion for up to 72 hours can promote the resolution of shock in patients with severe sepsis: results of a randomized, double-blind, placebo-controlled multicenter study," *Critical Care Medicine*, vol. 32, no. 1, pp. 1–12, 2004.
- [8] T. Rimmelé and J. A. Kellum, "Clinical review: blood purification for sepsis," *Critical Care*, vol. 15, no. 1, article 205, 2011.
- [9] A. M. Shukla, "Extracorporeal therapy in sepsis: are we there yet," *Kidney International*, vol. 81, no. 4, pp. 336–338, 2012.
- [10] A. J. Ellis, R. D. Hughes, J. A. Wendon et al., "Pilot-controlled trial of the extracorporeal liver assist device in acute liver failure," *Hepatology*, vol. 24, no. 6, pp. 1446–1451, 1996.
- [11] I. M. Sauer, K. Zeilinger, N. Obermayer et al., "Primary human liver cells as source for modular extracorporeal liver support—a preliminary report," *International Journal of Artificial Organs*, vol. 25, no. 10, pp. 1001–1005, 2002.
- [12] H. D. Humes, D. A. Buffington, L. Lou et al., "Cell therapy with a tissue-engineered kidney reduces the multiple-organ consequences of septic shock," *Critical Care Medicine*, vol. 31, no. 10, pp. 2421–2428, 2003.
- [13] H. D. Humes, W. F. Weitzel, R. H. Bartlett, F. C. Swaniker, and E. P. Paganini, "Renal cell therapy is associated with dynamic and individualized responses in patients with acute renal failure," *Blood Purification*, vol. 21, no. 1, pp. 64–71, 2003.
- [14] J. Tumlin, R. Wali, W. Williams et al., "Efficacy and safety of renal tubule cell therapy for acute renal failure," *Journal of the American Society of Nephrology*, vol. 19, no. 5, pp. 1034–1040, 2008.
- [15] S. R. Mitzner, J. Freytag, M. Sauer et al., "Use of human pre-conditioned phagocytes for extracorporeal immune support: introduction of a concept," *Therapeutic Apheresis*, vol. 5, no. 5, pp. 423–432, 2001.
- [16] V. Caille, J.-D. Chiche, N. Nciri et al., "Histocompatibility leukocyte antigen-D related expression is specifically altered and predicts mortality in septic shock but not in other causes of shock," *Shock*, vol. 22, no. 6, pp. 521–526, 2004.
- [17] I. Kaufmann, A. Hoelzl, F. Schliephake et al., "Polymorphonuclear leukocyte dysfunction syndrome in patients with increasing sepsis severity," *Shock*, vol. 26, no. 3, pp. 254–261, 2006.
- [18] A. Pachot, G. Monneret, A. Brion et al., "Messenger RNA expression of major histocompatibility complex class II genes in whole blood from septic shock patients," *Critical Care Medicine*, vol. 33, no. 1, pp. 31–38, 2005.
- [19] B. M. Tavares-Murta, M. Zapparoli, R. B. Ferreira et al., "Failure of neutrophil chemotactic function in septic patients," *Critical Care Medicine*, vol. 30, no. 5, pp. 1056–1061, 2002.
- [20] M. Keel, U. Ungethüm, U. Steckholzer et al., "Interleukin-10 counterregulates proinflammatory cytokine-induced inhibition of neutrophil apoptosis during severe sepsis," *Blood*, vol. 90, no. 9, pp. 3356–3363, 1997.
- [21] G. Egger, R. Aigner, A. Glasner, H. P. Hofer, H. Mitterhammer, and S. Zelzer, "Blood polymorphonuclear leukocyte migration as a predictive marker for infections in severe trauma: comparison with various inflammation parameters," *Intensive Care Medicine*, vol. 30, no. 2, pp. 331–334, 2004.
- [22] L. Lehmann, U. Novender, S. Schroeder et al., "Plasma levels of macrophage migration inhibitory factor are elevated in patients with severe sepsis," *Intensive Care Medicine*, vol. 27, no. 8, pp. 1412–1415, 2001.
- [23] C. Wensich, P. Parschalk, M. Hasenhüdl, A. Griesmacher, and W. Graninger, "Polymorphonuclear leukocyte dysregulation in patients with gram-negative septicemia assessed by flow cytometry," *European Journal of Clinical Investigation*, vol. 25, no. 6, pp. 418–424, 1995.
- [24] M. Sauer, J. Altrichter, H. J. Kreutzer et al., "Extracorporeal cell therapy with granulocytes in a pig model of Gram-positive sepsis," *Critical Care Medicine*, vol. 37, no. 2, pp. 606–613, 2009.
- [25] J. Altrichter, M. Sauer, K. Kaftan et al., "Extracorporeal cell therapy of septic shock patients with donor granulocytes: a pilot study," *Critical Care*, vol. 15, no. 2, article R82, 2011.
- [26] M. Sauer, J. Altrichter, H.-J. Kreutzer et al., "Safety evaluation for a cell-based immune support system in an ex vivo rat model of gram-negative sepsis," *Therapeutic Apheresis and Dialysis*, vol. 13, no. 5, pp. 444–450, 2009.
- [27] E. Liton and M. Morgan, "The PiCCO monitor: a review," *Anaesthesia and Intensive Care*, vol. 40, no. 3, pp. 393–409, 2012.
- [28] S. G. Sakka, "Assessing liver function," *Current Opinion in Critical Care*, vol. 13, no. 2, pp. 207–214, 2007.
- [29] M. Sauer, "The use of human hepatocytes to determine liver function and liver regeneration," *PCT/EP 2007/001047; DE/2006 102006005526*.
- [30] M. Sauer, C. Haubner, T. Mencke et al., "Impaired cell functions of hepatocytes incubated with plasma of septic patients," *Inflammation Research*, vol. 61, no. 6, pp. 609–616, 2012.
- [31] "American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference: definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis," *Critical Care Medicine*, vol. 20, pp. 864–874, 1992.
- [32] G. R. Bernard, J.-L. Vincent, P.-F. Laterre et al., "Efficacy and safety of recombinant human activated protein C for severe sepsis," *New England Journal of Medicine*, vol. 344, no. 10, pp. 699–709, 2001.

- [33] D. A. Scudiero, R. H. Shoemaker, K. D. Paull et al., "Evaluation of a soluble tetrazolium/formazan assay for cell growth and drug sensitivity in culture using human and other tumor cell lines," *Cancer Research*, vol. 48, no. 17, pp. 4827–4833, 1988.
- [34] J. H. Kelly and N. L. Sussman, "A fluorescent cell-based assay for cytochrome P-450 isozyme 1A2 induction and inhibition," *Journal of Biomolecular Screening*, vol. 5, no. 4, pp. 249–253, 2000.
- [35] K. G. Kreymann, G. de Heer, A. Nierhaus, and S. Kluge, "Use of polyclonal immunoglobulins as adjunctive therapy for sepsis or septic shock," *Critical Care Medicine*, vol. 35, no. 12, pp. 2677–2685, 2007.
- [36] P.-Y. Bochud and T. Calandra, "Science, medicine, and the future: pathogenesis of sepsis: new concepts and implications for future treatment," *British Medical Journal*, vol. 326, no. 7383, pp. 262–266, 2003.
- [37] S. A. Nasraway, "The problems and challenges of immunotherapy in sepsis," *Chest*, vol. 123, no. 5, pp. 451–459, 2003.
- [38] C. Ronco, P. Inguaggiato, V. D'Intini et al., "The role of extracorporeal therapies in sepsis," *Journal of Nephrology*, vol. 16, supplement 7, pp. S34–S41, 2003.
- [39] R. Bellomo, P. Honoré, J. Matson, C. Ronco, and J. Winchester, "Extracorporeal blood treatment (EBT) methods in SIRS/Sepsis," *International Journal of Artificial Organs*, vol. 28, no. 5, pp. 450–458, 2005.
- [40] J. S. Boomer, K. To, K. C. Chang et al., "Immunosuppression in patients who die of sepsis and multiple organ failure," *The Journal of the American Medical Association*, vol. 306, no. 23, pp. 2594–2605, 2011.
- [41] P. Mohan and P. Brocklehurst, "Granulocyte transfusions for neonates with confirmed or suspected sepsis and neutropaenia," *Cochrane Database of Systematic Reviews*, vol. 4, Article ID CD003956, 2004.
- [42] S. J. Stanworth, E. Massey, C. Hyde et al., "Granulocyte transfusions for treating infections in patients with neutropenia or neutrophil dysfunction," *Cochrane Database of Systematic Reviews*, no. 3, Article ID CD005339, 2005.
- [43] A. Safdar, H. A. Hanna, M. Boktour et al., "Impact of high-dose granulocyte transfusions in patients with cancer with candidemia: retrospective case-control analysis of 491 episodes of candida species bloodstream infections," *Cancer*, vol. 101, no. 12, pp. 2859–2865, 2004.
- [44] M. W. Dünser, E. Ruokonen, V. Pettilä et al., "Association of arterial blood pressure and vasopressor load with septic shock mortality: a post hoc analysis of a multicenter trial," *Critical Care*, vol. 13, no. 6, article R181, 2009.
- [45] C. M. Pastor and P. M. Suter, "Hepatic hemodynamics and cell functions in human and experimental sepsis," *Anesthesia and Analgesia*, vol. 89, no. 2, pp. 344–352, 1999.
- [46] R. Moreno, J.-L. Vincent, R. Matos et al., "The use of maximum SOFA score to quantify organ dysfunction/failure in intensive care. Results of a prospective, multicentre study," *Intensive Care Medicine*, vol. 25, no. 7, pp. 686–696, 1999.
- [47] S. Kimura, T. Yoshioka, M. Shibuya, T. Sakano, R. Tanaka, and S. Matsuyama, "Indocyanine green elimination rate detects hepatocellular dysfunction early in septic shock and correlates with survival," *Critical Care Medicine*, vol. 29, no. 6, pp. 1159–1163, 2001.
- [48] S. G. Sakka, K. Reinhart, and A. Meier-Hellmann, "Prognostic value of the indocyanine green plasma disappearance rate in critically ill patients," *Chest*, vol. 122, no. 5, pp. 1715–1720, 2002.
- [49] F. Weis, E. Kilger, A. Beiras-Fernandez et al., "Indocyanine green clearance as an outcome prediction tool in cardiac surgery: a prospective study," *Journal of Critical Care*, vol. 29, no. 2, pp. 224–229, 2014.
- [50] B. Zoller, K. Spanaus, R. Gerster et al., "ICG-liver test versus new biomarkers as prognostic markers for prolonged length of stay in critically ill patients—a prospective study of accuracy for prediction of length of stay in the ICU," *Annals of Intensive Care*, vol. 4, article 19, 2014.
- [51] A. Stehr, F. Ploner, K. Traeger et al., "Plasma disappearance of indocyanine green: a marker for excretory liver function?" *Intensive Care Medicine*, vol. 31, no. 12, pp. 1719–1722, 2005.
- [52] B. M. Halle, T. D. Poulsen, and H. P. Pedersen, "Indocyanine green plasma disappearance rate as dynamic liver function test in critically ill patients," *Acta Anaesthesiologica Scandinavica*, vol. 58, no. 10, pp. 1214–1219, 2014.
- [53] T. Regueira, P. M. Lepper, S. Brandt et al., "Hypoxia inducible factor-1 α induction by tumour necrosis factor- α , but not by toll-like receptor agonists, modulates cellular respiration in cultured human hepatocytes," *Liver International*, vol. 29, no. 10, pp. 1582–1592, 2009.
- [54] M. A. El-Saadany, H. M. Rawel, J. Raila, M. S. El-Dashloty, and F. J. Schweigert, "Antioxidants modulate the IL-6 induced inhibition of negative acute-phase protein secretion in HepG2 cells," *Cell Biochemistry and Function*, vol. 26, no. 1, pp. 95–101, 2008.
- [55] K. Nakai, H. Tanaka, K. Hanada et al., "Decreased expression of cytochromes P450 1A2, 2E1, and 3A4 and drug transporters Na⁺-taurocholate-cotransporting polypeptide, organic cation transporter 1, and organic anion-transporting peptide-C correlates with the progression of liver fibrosis in chronic hepatitis C patients," *Drug Metabolism & Disposition*, vol. 36, no. 9, pp. 1786–1793, 2008.
- [56] T. Li, A. Jahan, and J. Y. L. Chiang, "Bile acids and cytokines inhibit the human cholesterol 7 α -hydroxylase gene via the JNK/c-Jun pathway in human liver cells," *Hepatology*, vol. 43, no. 6, pp. 1202–1210, 2006.