ANESTHESIOLOGY

Blood Purification and Mortality in Sepsis and Septic Shock

A Systematic Review and Meta-analysis of Randomized Trials

Alessandro Putzu, M.D., Raoul Schorer, M.D., Juan Carlos Lopez-Delgado, M.D., Ph.D., Tiziano Cassina, M.D., Giovanni Landoni, M.D.

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EDITOR'S PERSPECTIVE

What We Already Know about This Topic:

- Among patients with sepsis or septic shock, a variety of extracorporeal blood purification techniques are available
- Individual existing trials evaluating these options are underpowered to provide clear evidence

What This Paper Tells Us That Is New

- Meta-analysis of very low-quality randomized controlled trial evidence demonstrates a potential benefit of hemoperfusion, hemofiltration, or plasmapheresis
- Additional high-quality trials demonstrating benefit in modern clinical practice are needed before recommending these therapies

ABSTRACT

Background: Sepsis and septic shock are severe inflammatory conditions related to high morbidity and mortality. We performed a systematic review with meta-analysis of randomized trials to assess whether extracorporeal blood purification reduces mortality in this setting.

Methods: Electronic databases were searched for pertinent studies up to January 2019. We included randomized controlled trials on the use of hemoperfusion, hemofiltration without a renal replacement purpose, and plasmapheresis as a blood purification technique in comparison to conventional therapy in adult patients with sepsis and septic shock. The primary outcome was mortality at the longest follow-up available. We calculated relative risks and 95% CIs. The grading of recommendations assessment, development and evaluation methodology for the certainty of evidence was used.

Results: Thirty-seven trials with 2,499 patients were included in the meta-analysis. Hemoperfusion was associated with lower mortality compared to conventional therapy (relative risk = 0.88 [95% CI, 0.78 to 0.98], $P = 0.02$, very low certainty evidence). Low risk of bias trials on polymyxin B immobilized filter hemoperfusion showed no mortality difference *versus* control (relative risk = 1.14 [95% CI, 0.96 to 1.36], $P = 0.12$, moderate certainty evidence), while recent trials found an increased mortality (relative risk = 1.22 [95% CI, 1.03 to 1.45], $P = 0.02$, low certainty evidence); trials performed in the United States and Europe had no significant difference in mortality (relative risk = 1.13 [95% CI, 0.96 to 1.34], $P = 0.15$), while trials performed in Asia had a positive treatment effect (relative risk = 0.57 [95% CI, 0.47 to 0.69], $P < 0.001$). Hemofiltration (relative risk = 0.79 [95% CI, 0.63 to 1.00], $P = 0.05$, very low certainty evidence) and plasmapheresis (relative risk = 0.63 [95% CI, 0.42 to 0.96], $P = 0.03$, very low certainty evidence) were associated with a lower mortality.

Conclusions: Very low-quality randomized evidence demonstrates that the use of hemoperfusion, hemofiltration, or plasmapheresis may reduce mortality in sepsis or septic shock. Existing evidence of moderate quality and certainty does not provide any support for a difference in mortality using polymyxin B hemoperfusion. Further high-quality randomized trials are needed before systematic implementation of these therapies in clinical practice.

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Today, sepsis remains one of the main causes of morbidity and mortality in the intensive care unit. Despite recent advancement in intensive care unit and sepsis management, mortality still remains high.[1–4]

The pathogenesis of sepsis involves many complex cellular and biochemical interactions between leukocytes, platelets, endothelial cells, and the complement system that trigger an inflammatory response.[5] Inflammation is caused by the production of pro- and antiinflammatory mediators, such as cytokines, in the presence of infection and/or bacterial toxins, and the imbalance between these mediators or their excessive production may lead to multiorgan failure due to a prolonged or inadequate systemic inflammatory response syndrome.[5,6]

Extracorporeal blood purification techniques have been proposed as adjunctive therapy in sepsis. These techniques are based on the principle that removal and modulation of blood pro- and antiinflammatory mediators or bacterial toxins (or both) could attenuate the sepsis-related massive

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systemic inflammatory response, reducing morbidity and mortality.[7,8] Several different extracorporeal techniques have been studied for this purpose.

Hemoperfusion involves the placement of a sorbent cartridge in direct contact with blood *via* an extracorporeal circuit. The removal characteristics of hemoperfusion are dependent on the different types of sorbent used and could also target high-molecular-weight molecules, usually not captured by conventional hemofilters. The most studied therapy is polymyxin B immobilized fiber column hemoperfusion with Toraymyxin (Toray Industries Ltd., Japan), that could capture circulating bacterial endotoxin[9] and modulate the inflammatory response.[10] Another device is the CytoSorb (CytoSorbents Corporation, USA), a novel filter potentially able to remove both pro-inflammatory and antiinflammatory cytokines.[11]

Renal replacement devices such as hemofiltration or hemodiafiltration could be used to remove part of the inflammatory mediators and toxins in septic patients without renal indication for kidney replacement therapy, by employing standard or special filters with adsorptive properties.[12] Limited data are available on plasmapheresis, a technique based on plasma replacement with fresh frozen plasma or albumin,[12] that has the potential to remove inflammatory cytokines and restore deficient plasma proteins.

Despite the large number of available techniques, actual evidence is scarce, and these therapies have not entered into daily clinical practice around the world yet. Several small trials were published on various devices, and the most comprehensive meta-analysis summarizing the evidence on blood purification is outdated.[13] Some more recent meta-analyses focusing on polymyxin B immobilized fiber column hemoperfusion[14,15] or hemofiltration[16] did not include some relevant trials nor the final results of the largest randomized study performed on the topic so far.[17] Therefore, we performed a meta-analysis of randomized control trials in order to determine whether extracorporeal blood purification decreased mortality in patients with sepsis and septic shock.

Materials and Methods

The current systematic review was conducted in compliance with the PRISMA (Preferred Reporting Items Systematic Reviews and Meta-Analysis) guidelines[18] (Supplemental Digital Content, table S1, http://links.lww.com/ALN/B977) and Cochrane methodology[19] and according to a prepublished protocol (PROSPERO database, CRD42018104643).

Search Strategy

Two investigators (A.P. and R.S.) independently searched PubMed, the Cochrane Central Register of clinical trials, and Embase up to January 1, 2019, for relevant articles (Supplemental Digital Content, table S2, http://links.lww.com/ALN/B977). The search strategy aimed to include any randomized study performed with any type of extracorporeal blood purification technique compared to conventional therapy in adult critically ill patients with sepsis and septic shock. Abstracts from recent international conferences were searched for additional studies. In addition, we hand-scanned references of retrieved articles and pertinent reviews to identify other eligible trials (backward snowballing).

Study Selection

References obtained from searches were first independently examined at the abstract level by two authors (A.P. and R.S.) and then collected as full-text articles if potentially relevant. Eligible studies met the following PICOS criteria: (1) Population: adult critically ill patients with sepsis with or without septic shock; (2) Intervention: any extracorporeal blood purification technique (hemoperfusion, renal replacement therapy techniques, plasmapheresis); (3) Comparison intervention: conventional therapy; (4) Outcome: mortality at longest follow-up available; and (5) Study design: randomized controlled trial. The exclusion criteria were blood purification for renal failure indication at randomization, trials with overlapping populations with a previously included article (*e.g.*, manuscripts with different follow-up or subanalyses of a previously published trial), and pediatric studies. Two authors (A.P. and R.S.) independently assessed selected studies for the final analysis, with disagreements resolved by consensus with a third author (G.L.). If the article did not include data on mortality or was not full-text, the corresponding author was contacted for further data. No language restrictions were imposed.

Data Abstraction

One author (A.P.) extracted relevant information from each selected study. These data were checked by another author (R.S.). Disagreement was resolved by consensus with a third author (G.L.). We specifically extracted potential sources of significant clinical heterogeneity (*e.g.*, study design, clinical setting, inclusion and exclusion criteria, blood purification regimen).

The primary endpoint of this review was mortality at the longest follow-up available, and the secondary endpoint was mortality at 28 to 30 days.

Quality Assessment

Two authors (A.P. and R.S.) independently assessed the internal validity of each included trial according to the Cochrane Collaboration methods.[19,20] We assessed the risk of bias associated with the random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, completeness of outcome data, selective reporting, and other bias. The other bias domain included the classic items reported by the "Cochrane Handbook for Systematic Reviews of

Interventions"[19] but also the presence of an intention-to-treat analysis, sample size calculation, and ethical approval of the trial. If one or more of the domains were judged as having a high or unclear risk of bias, we classified the trial as having a high risk of bias. Due to the nature of the intervention, blinding of participants and personnel seemed difficult and was therefore not judged as crucial for bias assessment. We evaluated the potential risk of bias by applying a rating of "Low," "High," or "Unclear" to each study.

Two authors (A.P. and R.S.) independently reviewed the presence of authors' possible conflict of interest and the funding source for each study, then rated each trial as of "Low," "High," or "Unclear" risk regarding those specific points.

The certainty of the body of evidence was assessed using the grading of recommendations assessment, development, and evaluation framework.[21,22] The grading of recommendations assessment, development, and evaluation framework characterizes the certainty of a body of evidence on the basis of study limitations, imprecision, inconsistency, indirectness, and other considerations.

Statistical Analysis

Individual trial and summary results were reported as relative risk with 95% CI. We used a random-effects model except in cases where few trials dominated the available evidence or where significant publication bias was present, as random-effects meta-analysis applied in these contexts may give inappropriately high weight to smaller studies. Statistical heterogeneity was explored by the Cochran Q statistic and characterized using the I^2 metric. Publication bias was assessed by visually inspecting the funnel plot for the primary outcome. Statistical significance was set at $P = 0.05$. The meta-analysis was performed using Review Manager (RevMan, version 5.3; The Nordic Cochrane Center, The Cochrane Collaboration, Copenhagen, Denmark, 2014).

The primary analysis was stratified by blood purification technique: hemoperfusion, hemofiltration, hemoperfusion combined with hemofiltration, or plasmapheresis. Hemoperfusion subgroup analyses including trials on polymyxin B immobilized fiber column hemoperfusion or hemoperfusion with other devices were carried out. To explore the sources of heterogeneity, we performed some subgroup analyses: (1) low risk of bias *versus* unclear/high risk of bias trials; (2) trials conducted in Asia *versus* Europe and America; (3) trials from the Nakamura group *versus* other trials; and (4) trials published after 2010 *versus* older trials.

To explore the relationship between treatment effect and disease severity, we performed various analyses: (1) a random-effects meta-regression on the APACHE II (Acute Physiology, Age, Chronic Health Evaluation II) score,[23] sepsis-related organ failure assessment score,[24] and control group mortality;[14] (2) subgroup analyses according to conventional therapy group mortality: low-risk group (mortality rate less than 30%), intermediate-risk group (30 to 60%), and high-risk group (greater than 60%).[14] We also performed a meta-regression for age to investigate a possible influence on outcome estimates. Finally, sensitivity analyses were performed by analyzing the data with a fixed or random effects model and using other summary statistics.

We performed a predefined random-effects trial sequential analysis,[25–27] with the intent of maintaining an overall 5% risk of type I error and a 10% risk of type II error. We assumed a relative risk reduction of 15% and derived the control event proportion from the actual dataset. The resulting required information size was further diversity (D^2)-adjusted. In case of $D^2 = 0$ we performed a sensitivity analysis assuming a $D^2 = 25\%$. We used the trial sequential analysis software (TSAViewer [Computer program], version 0.9.5.5 Beta, Copenhagen Trial Unit, Center for Clinical Intervention Research, Rigshospitalet, Copenhagen, Denmark, 2016). Deviations from the initial protocol are reported in the supplement (Supplemental Digital Content, eMethods 1, http://links.lww.com/ALN/B977).

Results

Search Results and Study Characteristics

The search strategy identified 6,933 citations and, after exclusion of inadequate reports (Supplemental Digital Content, table S3, http://links.lww.com/ALN/B977), 37 trials with 2,499 patients were included in the meta-analysis (fig. 1).[17,28–63]

The characteristics of the included studies are shown in table 1 and in the supplement (Supplemental Digital Content, tables S4-S6, http://links.lww.com/ALN/B977). Two trials had four treatment arms.[43,58] Twenty trials used a hemoperfusion technique, 13 used hemofiltration or hemodiafiltration, 4 trials combined hemofiltration with hemoperfusion, and 2 trials used plasma exchange. In three cases we received further information from corresponding authors.[17,59,62]

Three trials were judged to be at low risk of bias,[17,30,46] 20 at unclear risk, and 14 at high risk (Supplemental Digital Content, figs. S1 and S2, http://links.lww.com/ALN/B977). The grading of recommendations assessment, development, and evaluation assessment is reported in table S7 in the Supplemental Digital Content (http://links.lww.com/ALN/B977).

Hemoperfusion Techniques

Hemoperfusion (20 trials and 1,548 patients), which comprises various techniques differing among other things on the presence or absence of polymyxin B in the treatment regimen, was associated with a lower mortality compared to the control group (relative risk = 0.87 [95% CI, 0.78 to 0.98], $P = 0.02$, trial sequential analysis inconclusive, very low-certainty evidence) the analysis was limited by publication bias, small trial effects, and high heterogeneity (Supplemental Digital Content, figs S3-S5 and table S8, http://links.lww.com/ALN/B977). Subanalyses are reported in the

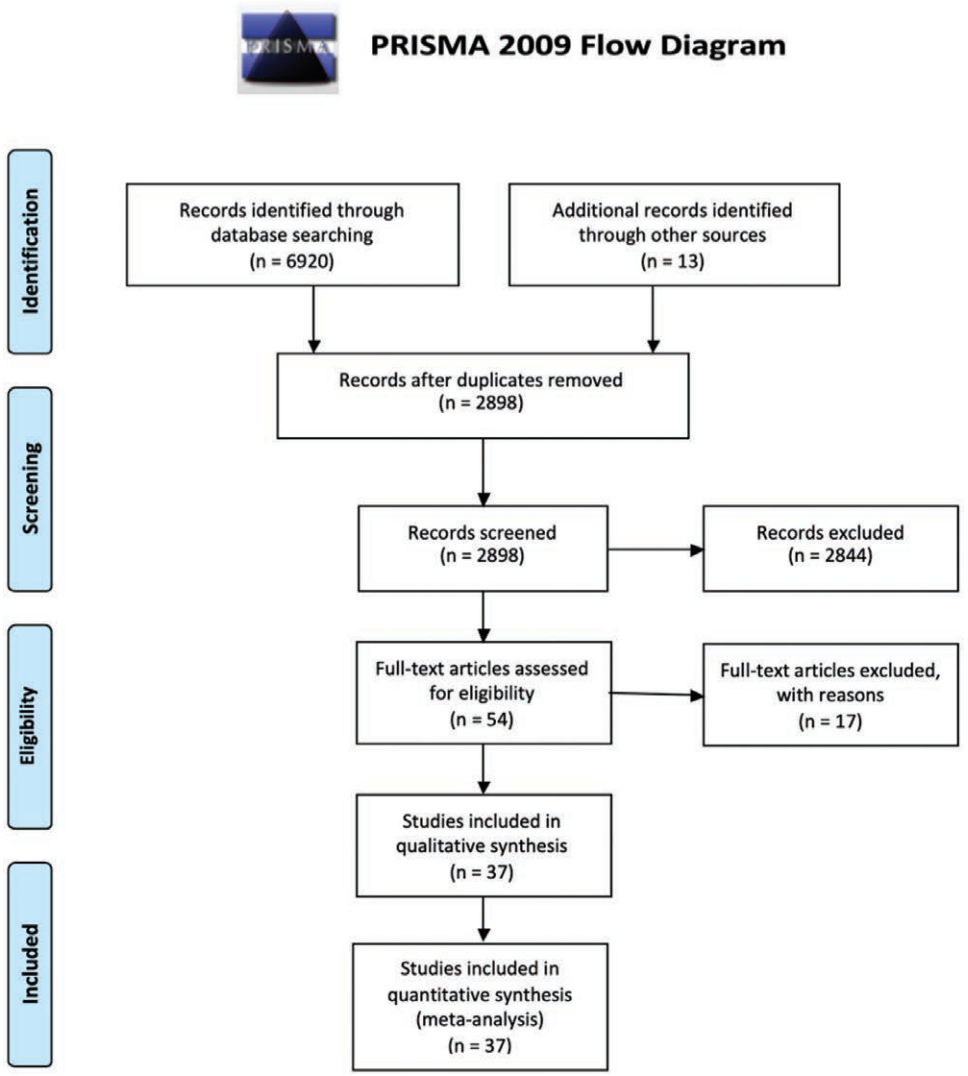

Fig. 1. Flow diagram for the selection of studies. PRISMA, Preferred Reporting Items Systematic Reviews and Meta-Analysis.

supplement (Supplemental Digital Content, figs. S6—S9 and eResults 1, http://links.lww.com/ALN/B977).

Polymyxin B Immobilized Fiber Column Hemoperfusion. Polymyxin B immobilized fiber column hemoperfusion (13 trials and 1,163 patients) was associated with a lower mortality at longest follow-up available compared to control (relative risk = 0.87 [95% CI, 0.77 to 0.98], $P = 0.03$, very low-certainty evidence), although the analysis was limited by very high heterogeneity ($I^2 = 74\%$, $P_{heterogeneity} < 0.001$) (fig. 2). No significant difference in 30-day mortality was found (Supplemental Digital Content, fig. S9, http://links.lww.com/ALN/B977).

Low risk of bias trials (three trials and 745 patients) found no difference in mortality with polymyxin B immobilized fiber column hemoperfusion *versus* control (relative risk = 1.14 [95% CI, 0.96 to 1.36], $P = 0.12$, moderate-certainty evidence; fig. 2). Recent trials published after 2010 (three trials and 740 patients) showed that polymyxin B immobilized fiber column hemoperfusion was associated with higher mortality than conventional therapy (relative risk = 1.22 [95% CI, 1.03 to 1.45], $P = 0.02$, $I^2 = 0\%$, low-certainty evidence), while trials published before 2011 were associated with a mortality benefit (relative risk = 0.58 [95% CI, 0.49 to 0.69], $P < 0.001$, $I^2 = 8\%$; $P_{groups} < 0.001$). Studies conducted in Asia (seven trials in Japan and one in Thailand, with a total of 367 patients) showed that polymyxin B immobilized fiber column hemoperfusion decreased mortality (relative risk = 0.62 [95% CI, 0.52 to 0.75], $P < 0.001$, $I^2 = 57\%$, $P_{heterogeneity} = 0.02$), while aggregate data from trials conducted in the United States and Europe (five trials and 796

Table 1. Trials Characteristics

Trial	Country	Sample Size	Major Inclusion Criteria	Blood Purification Technique	Treatment Duration	Control Group Mortality	Risk of Bias
Hemoperfusion							
Polymyxin B–immobilized filter column hemoperfusion							
Cantaluppi 2008	Italy	16	Sepsis with positive culture for Gram-negative bacteria	PMX-HP	2 sessions of 2 h at 24-h interval	38%	Unclear
Cruz 2009	Italy	64	Severe sepsis or septic shock from an abdominal source	PMX-HP	2 sessions of 2 h at 24-h interval	67%	Low
Dellinger 2018	USA and Canada	449	Septic shock and an high endotoxin activity assay level	PMX-HP	2 sessions of 2 h at 24-h interval	42%	Low
Nakamura 1999	Japan	50	Septic shock	PMX-HP	2 sessions of 2 h at 24-h distance	70%	Unclear
Nakamura 2002(a)	Japan	18	Sepsis and trauma	PMX-HP	2 sessions of 2 h at 24-h interval	78%	Unclear
Nakamura 2002(b)	Japan	14	Sepsis	PMX-HP	2 sessions of 2 h at 24-h interval	86%	Unclear
Nakamura 2003(a)	Japan	20	Sepsis and MRSA-associated glomerulonephritis	PMX-HP	2 sessions of 2 h at 24-h interval	80%	Unclear
Nakamura 2003(b)	Japan	60	MRSA sepsis	PMX-HP	2 sessions of 2 h at 24-h interval	64%	Unclear
Nemoto 2001	Japan	98	Sepsis, severe sepsis, or septic shock	PMX-HP	1 or 2 sessions of 4 h	89%	Unclear
Payen 2015	France	232	Septic shock from and abdominal source	PMX-HP	2 sessions of 1.5 h at 22–24-h interval	24%	Low
Srisawat 2018	Thailand	59	Severe sepsis or septic shock, high endotoxin activity assay level, mostly under renal replacement therapy	PMX-HP	2 sessions of 2 h at 24-h interval	50%	High
Suzuki 2002	Japan	48	Septic shock	PMX-HP	1 HP session of 4 h, then 1 CVVHDF session until 24 h	75%	Unclear
Vincent 2005	Europe	35	Severe sepsis or septic shock from an intraabdominal source	PMX-HP	1 session of 2 h	28%	Unclear
Other hemoperfusion devices							
Hawchar 2019	Hungary	20	Septic shock of medical origin	HP with CytoSorb	1 session of 24 h	20%	High
Huang 2010	China	44	Severe sepsis or septic shock	HP with HA330 resin cartridge (Lizhu Industries, China)	3 sessions of 2 h at 24-h interval	55%	Unclear
Huang 2013	China	46	Severe sepsis or septic shock with acute lung injury from extrapulmonary source	HP with HA330 resin cartridge	3 sessions of 2 h at 24-h interval	67%	High
Reinhart 2004	Europe	143	Severe sepsis or septic shock	HP with Matisse EN500 endotoxin adsorber (Fresenius HemoCare Adsorber Technology GmbH, Germany).	A daily session for the first 4 d	25%	High
Schädler 2017	Germany	97	Severe sepsis or septic shock and ALI/ARDS	HP with CytoSorb	1 daily session of 6 h up to 7 d	26%	High
Shum 2014	China	15	Septic shock from an intra-abdominal source	HP with Alteco endotoxin hemoadsorber (Alteco Medical AB, Sweden)	2 sessions of 2 h at 24-h interval	25%	High
Zheng 2017	China	20	Sepsis, severe sepsis, or septic shock	HP with Adsorba 300 filter (manufacturer not reported)	1 HP session of 2.5 h	80%	Unclear
Hemofiltration							
Chung 2017	USA	37	Septic shock and burn	CVVH	nr	57%	Unclear
Cole 2002	nr	24	Severe sepsis with end-organ dysfunction or septic shock	CVVH with AN69 Filtral 12 filter (Hospal, France)	1 session of 2 d	33%	Unclear
Guo 2017	China	22	Severe sepsis or septic shock	CVVH with AN69 filter	1 session of 2 d	45%	Unclear
Han 2011	China	45	Severe sepsis	CVVH with AN69 filter	1 session of 3 d	41%	Unclear
Jing 2015	China	97	Severe sepsis or septic shock	CVVH	1 session of at least 3 d	37%	High

(*Continued*)

Table 1. (Continued)

Trial	Country	Sample Size	Major Inclusion Criteria	Blood Purification Technique	Treatment Duration	Control Group Mortality	Risk of Bias
Meng 2016	China	56	Septic shock and ARDS	CVVH with AN69 filter (Gambro Industries, France)	1 session of 3 d	32%	High
Payen 2009	France	76	Severe sepsis or septic shock	CVVH with Duraflo II filter (Edwards Lifesciences, USA)	1 session of at least 4 d	44%	Unclear
Peng 2010	China	22	Severe sepsis	CVVH with AN69 filter	1 session of 3 d	18%	Unclear
Quenot 2015	France	60	Septic shock	CVVH	1 session of 2 d	48%	High
Sander 1997	Germany	26	Severe sepsis or septic shock	CVVH with AN69 Multiflow 60 filter (Hospal, France)	1 session of at least 2 d	92%	High
Wang 2009	China	89	Septic shock	CVVH	1 session of 7 d	17	Unclear
Xu 2014	China	22	Sepsis and burn	CVVHDF	1 session of 12 h	18%	Unclear
Zheng 2017	China	20	Sepsis, severe sepsis, or septic shock	CVVH with M100 set (manufacturer not reported)	1 session of 24 h	80%	Unclear
Combined hemofiltration and hemoperfusion							
Hassan 2013	Malaysia	23	Severe sepsis or septic shock	CPFA with DF-140 (Infomed, Switzerland)	1 session of 24 h or until clinical improvement	83%	High
Livigni 2014	Italy	184	Septic shock	CPFA with Lynda (Bellco, Italy)	5 session of at least 10 h, in 5 consecutive days	49%	Unclear
Peng 2005	China	20	Sepsis and burn	CVVH with AN69 Multiflow-60 filter (manufacturer not reported) + PMX-HP	nr	20%	Unclear
Zheng 2017	China	20	Sepsis, severe sepsis, or septic shock	CVVH with M100 filter + HP with Adsorba-300 (manufacturers not reported)	1 CVVH-session of 24 h and 1 HP session of 2.5 h	80%	Unclear
Plasmapheresis							
Busund 2002	Russia	106	Severe sepsis or septic shock	na	2 sessions of about 2 h in 24 h	54%	High
Reeves 1999	Australia	22	Severe sepsis	na	1 session of 36 h	46%	Unclear

ALI, acute lung injury; ARDS, acute respiratory distress syndrome; CVVH, continuous veno-venous hemofiltration; CVVHDF, continuous veno-venous hemodiafiltration; CPFA, coupled plasma filtration adsorption; HP, hemoperfusion; MRSA, methicillin-resistant *Staphylococcus aureus*; PMX, polymyxin B–immobilized fiber column; na, not applicable; nr, not reported.

patients) found no difference (relative risk = 1.11 [95% CI, 0.94 to 1.32], $P = 0.21$, $I^2 = 50\%$, $P_{heterogeneity} = 0.09$), ($P_{groups} < 0.001$). Similarly, when excluding trials performed in Japan by the Nakamura group (five trials and 162 overall patients), polymyxin B immobilized fiber column hemoperfusion was associated with no difference in mortality compared to conventional therapy (relative risk = 0.98 [95% CI, 0.86 to 1.12], $P = 0.80$; Supplemental Digital Content, figs. S10-S13, http://links.lww.com/ALN/B977).

Hemoperfusion with Other Devices. Hemoperfusion with devices other than polymyxin B–immobilized filter column (seven trials and 385 patients) was not associated with a difference in mortality compared to conventional therapy (relative risk = 0.81 [95% CI, 0.53 to 1.21], $P = 0.30$, very low-certainty evidence). The hemoperfusion devices included were Adsorba-300 filter (one trial, relative risk = 0.50 [95% CI, 0.22 to 1.14], $P = 0.10$); Alteco endotoxin hemoadsorber (one trial, relative risk = 0.57 [95% CI, 0.06 to 5.03], $P = 0.61$); CytoSorb (two trials, relative risk = 0.94 [95% CI, 0.14 to 6.49], $P = 0.95$); HA330 resin cartridge (two trials, relative risk = 0.61 [95% CI, 0.31 to 1.19], $P = 0.15$); and Matisse EN 500 endotoxin adsorber (one trial, relative risk = 1.13 [95% CI, 0.66 to 1.96], $P = 0.65$; Supplemental Digital Content, fig. S3, http://links.lww.com/ALN/B977).

Hemofiltration Techniques

The use of hemofiltration with a blood purification aim was associated with lower mortality compared to control

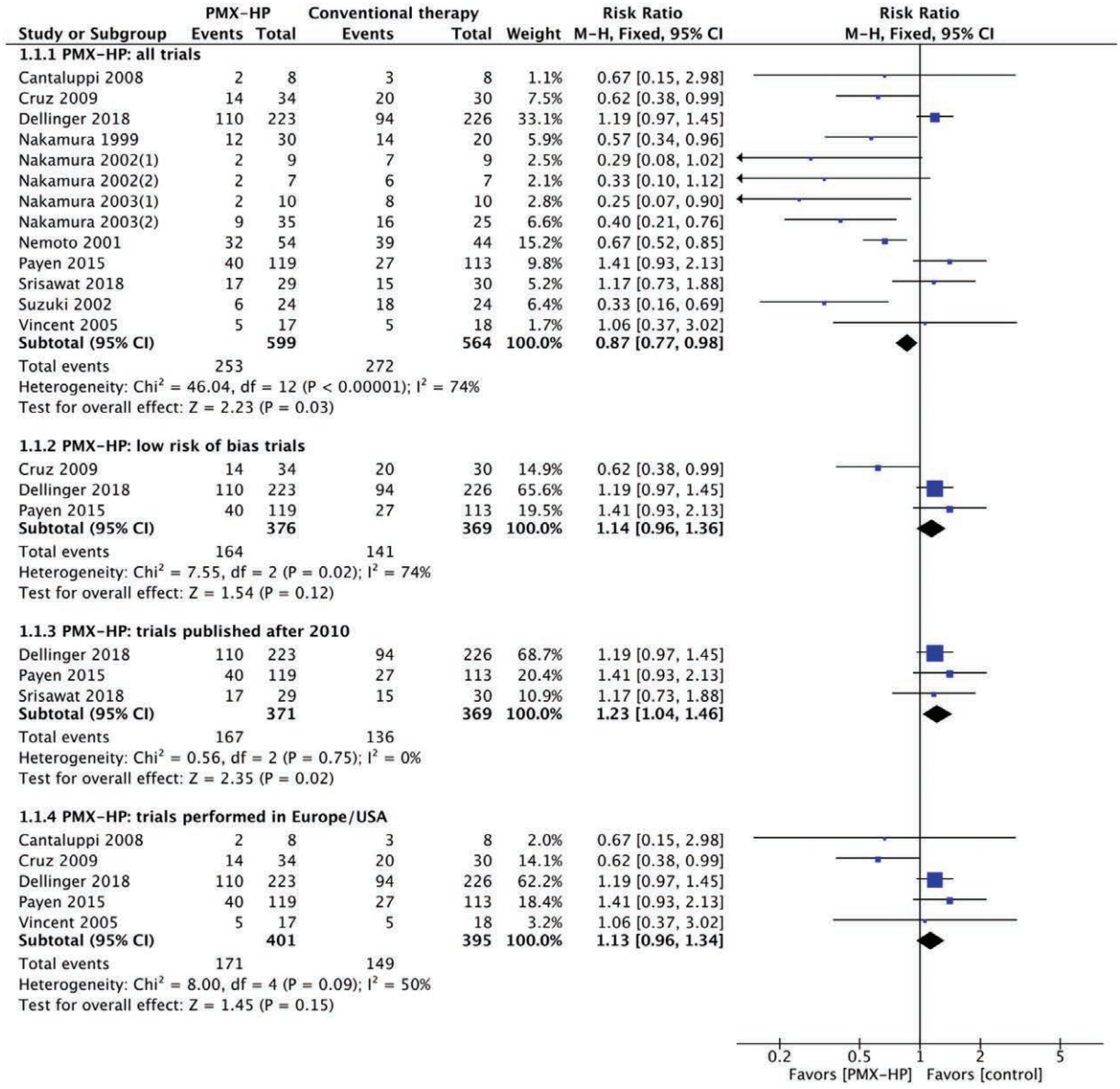

Fig. 2. Forest plot of the relative risk of mortality at longest follow up available with polymyxin B-immobilized fiber column hemoperfusion. Various subanalyses are also reported. M-H, Mantel-Haenszel; PMX-HP, polymyxin B immobilized fiber column hemoperfusion.

(relative risk = 0.79 [95% CI, 0.63, 1.00], $P = 0.05$, trial sequential analysis inconclusive, very low-certainty evidence) in 13 trials and 596 patients without acute kidney injury requiring renal replacement therapy (fig. 3 and Supplemental Digital Content, fig. S14, http://links.lww.com/ALN/B977). On subgroup analysis, hemofiltration was not associated with a difference in mortality in trials conducted in Europe and the United States (relative risk = 0.94 [95% CI, 0.74 to 1.19], $P = 0.61$, $I^2 = 0\%$, five trials and 223 patients) but was associated with a decrease in mortality in trials conducted in Asia (relative risk = 0.58 [95% CI, 0.40 to 0.82], $P = 0.002$, $I^2 = 0\%$, eight trials and 373 patients; $P_{groups} = 0.02$); other analyses are reported in the supplement (Supplemental Digital Content, figs. S15-S18, table S9 and eResults 2, http://links.lww.com/ALN/B977).

Combined Hemofiltration and Hemoperfusion Techniques

The association of hemoperfusion and hemofiltration was not associated with a significant difference in mortality compared to control (relative risk = 0.63 [95% CI, 0.36 to 1.13], $P = 0.12$, trial sequential analysis inconclusive, very

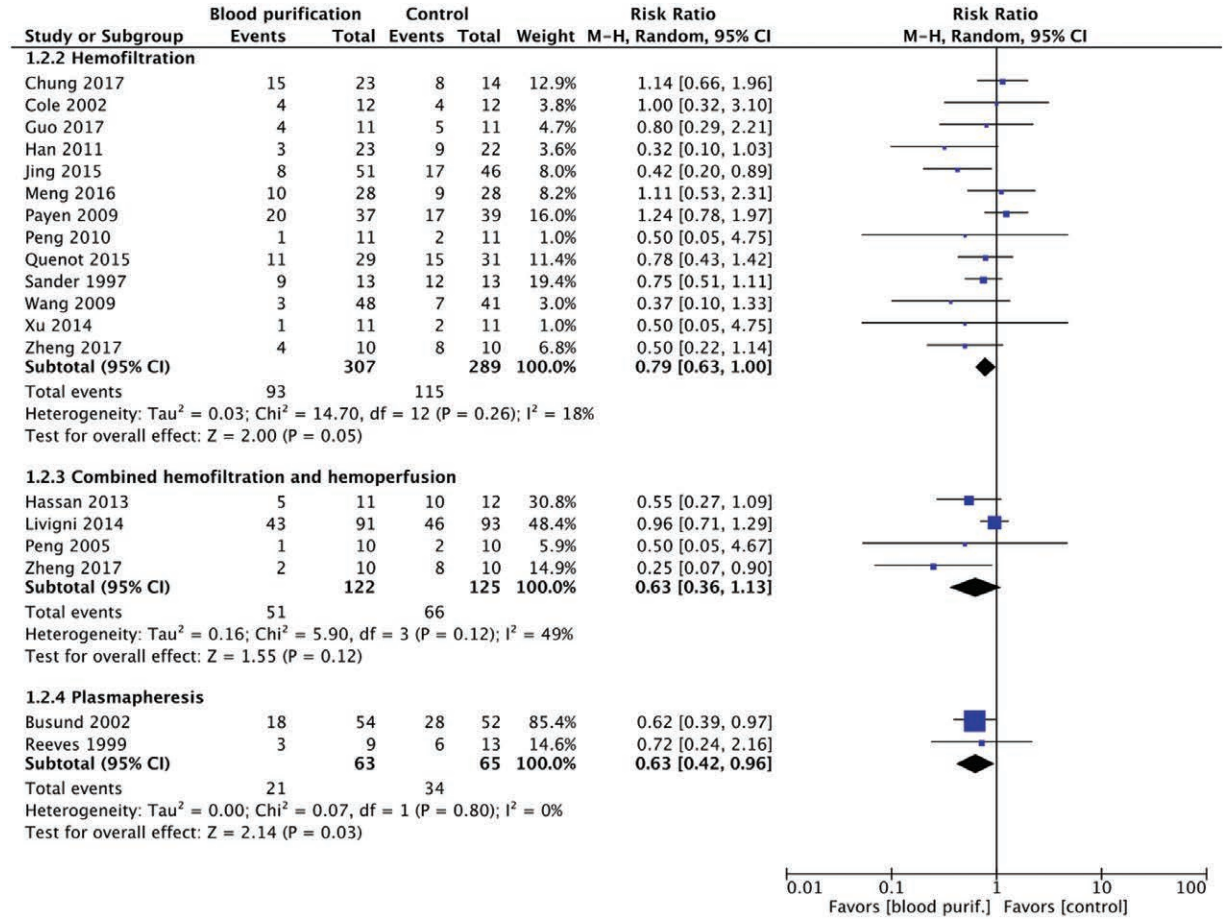

Fig. 3. Forest plot of the relative risk of mortality at the longest follow-up available with hemofiltration, combined hemofiltration and hemoperfusion, or plasmapheresis. Blood purif., blood purification; M-H, Mantel-Haenszel.

low-certainty evidence) in four trials including a total of 247 patients without acute kidney injury requiring renal replacement therapy (fig. 3).

Plasmapheresis Techniques

Plasmapheresis was associated with a lower mortality compared to standard treatment (relative risk = 0.63 [95% CI, 0.42 to 0.96], $P = 0.03$, trial sequential analysis inconclusive, very low-certainty evidence) with two trials and 128 patients included (fig. 3).

Discussion

We performed a comprehensive systematic review and meta-analysis on the mortality effects of blood purification with extracorporeal techniques in sepsis. The certainty of evidence underlying the use of blood purification therapies in sepsis is very low, and does not support their systematic use in patients with sepsis with or without septic shock.

Hemoperfusion

A variety of hemoperfusion techniques exists. Only a few randomized clinical trials were published on hemoperfusion techniques other than polymyxin B–immobilized filter column (*e.g.*, CytoSorb, Alteco endotoxin hemoadsorber), suggesting the need for further clinical trials. However, polymyxin B immobilized fiber column hemoperfusion emerged as a promising therapy in septic shock with elevated endotoxin levels, and several studies were published on the topic in the past 20 yr. This technique consists of using a sorbent cartridge containing fibers coated with polymyxin B, an antibiotic with high affinity for lipopolysaccharide.[9] Lipopolysaccharide is a cell wall component in Gram-negative bacteria that acts as an endotoxin by stimulating the production of inflammatory mediators by macrophages in a dose-dependent way and enhancing the inflammatory response.[9,64] Endotoxemia seems to be more pronounced when tissue hypoperfusion is present and lipopolysaccharide blood levels seem to correlate with sepsis severity.[9,65] Promising results in

pilot studies showed improvement in inflammatory mediators,[10] cardiac and renal dysfunction,[56] hemodynamics, organ dysfunction, and 28-day mortality[30] in patients with abdominal septic shock. All these promising findings, together with the significant increase in arterial pressure after therapy initiation,[15,17,30] made polymyxin B immobilized fiber column hemoperfusion an attractive therapy for clinicians. Conversely, recent large high-quality trials such as the EUPHRATES (Evaluating the Use of Polymyxin B Hemoperfusion in a Randomized Controlled trial of Adults Treated for Endotoxemia and Septic Shock)[17] and the ABDO-MIX (Effects of Hemoperfusion With a Polymyxin B Membrane in Peritonitis With Septic Shock) group[46] trials yielded inconclusive results and reported a nonsignificant increase in mortality with polymyxin B immobilized fiber column hemoperfusion at the longest follow-up assessed.

The EUPHRATES trial, which is the largest and highest-quality randomized clinical trial performed so far, randomized 450 patients with septic shock and a high endotoxin activity assay level to two sessions of polymyxin B immobilized fiber column hemoperfusion of 90 to 120 min at a 24 h distance or to a sham treatment aiming at reducing 28-day mortality. The trial found no significant difference in the primary endpoint in the overall population or in the higher disease severity subgroup.[17]

Our meta-analysis including 13 trials on polymyxin B immobilized fiber column hemoperfusion is the largest and most comprehensive to date. Recently, some meta-analyses[14,15,66] on polymyxin B immobilized fiber column hemoperfusion have appeared but failed to include some old and new randomized clinical trials. A meta-analysis concluded that this therapy may reduce mortality in patients with severe sepsis and septic shock in high disease severity subgroups based upon the aggregate analysis of 12 nonrandomized trials and 5 small randomized clinical trials representing a very low-quality evidence.[14] Two other meta-analyses respectively including only six and seven randomized clinical trials concluded that only low-quality evidence supported polymyxin B immobilized fiber column hemoperfusion for mortality reduction in sepsis.[15,66] Since the release of EUPHRATES and other trials, a more comprehensive analysis was made possible. The positive results previously reported regarding polymyxin B immobilized fiber column hemoperfusion were driven by small randomized clinical trials conducted in Asia of low methodologic quality. Interestingly, when limiting the analysis to trials published after 2010 and including the two largest randomized clinical trials performed on the topic,[17,46] polymyxin B immobilized fiber column hemoperfusion is associated with a higher mortality rate at the longest follow-up available. This together with inconclusive results on trial sequential analysis suggests that the current aggregate randomized evidence cannot consistently refute potential positive or detrimental effects on mortality. These findings do not support the use of polymyxin B immobilized fiber column hemoperfusion in sepsis and septic shock.

Hemofiltration

The use of hemofiltration techniques as a blood purification treatment in patients without renal failure has also been suggested, with controversial results and insufficient evidence to recommend its use outside of experimental clinical settings.[16,67] High-volume hemofiltration, further increasing plasma exchanges, was also investigated with limited results in patients with or without renal failure.[29,68] We found a positive survival trend associated to hemofiltration, although those results are driven by small, low-quality randomized trials, and further investigation is therefore warranted.

Plasmapheresis

The first randomized clinical trial to ever address plasmapheresis as a blood purification technique reported a decrease in the intensity of acute-phase response.[50] A second randomized clinical trial with a larger sample population found an absolute mortality risk reduction of 20.5%.[28] Despite those promising results, the evidence is still too weak to recommend the use of plasmapheresis for blood purification in sepsis.[69]

Disease Severity

Previous meta-analyses found that hemoperfusion was associated with a large positive effect in trials with a control group mortality rate greater than 60%, suggesting that hemoperfusion could be useful in the setting of higher disease severity.[14,66] Our study yielded similar findings and also found a trend toward increased mortality in the lower disease severity subgroup (mortality less than 30%). Meta-regressions on APACHE II and sepsis-related organ failure assessment scores, both predictors of sepsis mortality, did not find any significant trend supporting those findings. Furthermore, most trials with a greater than 60% control group mortality are at unclear/high risk of bias, are small in size, and were conducted in Asia. In the EUPHRATES trial, the per-protocol subgroup analysis with high disease severity, including patients with a Multiple Organ Dysfunction Score greater than 9 at randomization and a control group 1-year mortality rate of 50%, was inconclusive and did not suggest any trend favoring polymyxin B immobilized fiber column hemoperfusion.[17]

Those inconsistencies make a beneficial effect of hemoperfusion or polymyxin B immobilized fiber column hemoperfusion in high-disease-severity patients unlikely. This specific question merits further investigation.

Future Directions

Current randomized evidence cannot support the use of extracorporeal blood purification techniques; further

trials are warranted before systemic implementation of these techniques. Furthermore, an increase in mortality related to extracorporeal therapies should not be excluded. Some randomized clinical trials describe a trend toward higher mortality with polymyxin B immobilized fiber column hemoperfusion[46] or CytoSorb-HP[54], for example. Numerically higher adverse events with polymyxin B immobilized fiber column hemoperfusion[17,46] and greater disease severity with hemofiltration[45] were also reported. Our meta-analysis found an increased mortality at the longest follow-up available with polymyxin B immobilized fiber column hemoperfusion in a *post hoc* subgroup analysis including only the trials published after 2010. The unspecific removal of cytokines may remove mediators necessary to the function of the immune system, eventually provoking a deleterious outcome. Furthermore, the complex interaction between extracorporeal devices and inflammatory systems, micronutrients,[70] trace elements, electrolytes, and antibiotics levels and activity remain uninvestigated. Only few studies assessed the impact of those therapies on antibiotics, the only proven therapy in sepsis. A recent study on *in vitro* removal of antiinfective agents by CytoSorb-HP showed that all tested antibiotics were adsorbed by the cartridge in substantial amounts.[71] The authors speculated that an additional dose within the first hours of treatment and therapeutic drug monitoring should be considered in this population.[71] Similarly, an *in vitro* study assessing the effects of polymyxin B immobilized fiber column hemoperfusion on nine antibiotics reported adsorption of linezolid, suggesting a necessity for the monitoring of blood antimicrobial concentrations during polymyxin B immobilized fiber column hemoperfusion.[72,73] A larger literature is present on hemofiltration, suggesting an increased antibiotic clearance with these devices.[74–76]

Strengths and Limitations

We performed a comprehensive meta-analysis on blood purification techniques in sepsis and septic shock, which represents an important update to the literature in comparison to previous meta-analyses.[13–16] Limitations of this study may appear similar to those of previous meta-analyses. Most included studies are small in size and at unclear or high risk of bias. Some studies assessed technical feasibility, but side effects were rarely reported, and a systematic assessment of adverse events is warranted in future trials. Heterogeneity in sepsis management, blood purification regimens (*e.g.*, modality, hemofiltration volume, duration of the session, filter and cartridge change, and so forth), and populations across different centers is evident, but we made an attempt at an exploration through several subanalyses in order to further assess the clinical potential of blood purification modalities in sepsis. The positive treatment effect found in trials conducted in Asia was also reported elsewhere[13] and could be explained by publication bias, small studies effect, low methodologic quality, or a higher burden of disease as suggested by the high control group mortality. Furthermore, seven polymyxin B immobilized fiber column hemoperfusion trials from Japan were performed before 2005, and the progress in conventional therapy management and outcome in the past years could have diluted or cancelled the beneficial effects of this treatment.

Conclusions

Very low-quality randomized evidence demonstrates that the use of hemoperfusion, hemofiltration, or plasmapheresis may reduce mortality in sepsis or septic shock. Moderate-certainty evidence supports that polymyxin B immobilized fiber column hemoperfusion is not associated with any significant difference in mortality in comparison to conventional treatment regimen. Detrimental effects on survival could not be excluded by aggregate randomized evidence. Further high-quality randomized controlled trials adequately powered for mortality are needed to assess the real impact of blood purification techniques before such therapies can be systematically implemented in clinical practice.

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Competing Interests

The authors declare no competing interests.

Correspondence

Address correspondence to Dr. Landoni: Department of Cardiothoracic Anaesthesia and Intensive Care, Ospedale San Raffaele, Via Olgettina 60, Milano, 20132 Italy. landoni.giovanni@hsr.it. Information on purchasing reprints may be found at www.anesthesiology.org or on the masthead page at the beginning of this issue. Anesthesiology's articles are made freely accessible to all readers, for personal use only, 6 months from the cover date of the issue.

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Christina König[1,2], Anka C Röhr[3], Otto R Frey[3], Alexander Brinkmann[4], Jason A Roberts[5,6,7], Dominic Wichmann[1], Stephan Braune[1], Stefan Kluge[1] and Axel Nierhaus[1]

Abstract

Objectives: The aim of this study is to describe the in vitro adsorption of anti-infective drugs onto an extracorporeal cytokine adsorber.

Methods: Various anti-infective drugs (β-lactams, quinolones, aminoglycosides, glycopeptides, azole antimycotics) were prepared in normal saline 0.9% and human albumin 5%, and pumped through a cytokine cartridge (CytoSorb®; CytoSorbents Corporation, Monmouth Junction, NJ, USA) at a flow rate of 1.2 L/h for 1.5 h. In addition, meropenem and ciprofloxacin were dissolved in reconstituted blood and run through a CytoSorb cartridge, which was integrated into a continuous renal replacement therapy circuit with a flow rate of 2 L/h for 18 h. Samples from the solution, pre- and post-filter, were quantified by high-performance liquid chromatography with ultraviolet detection and fluorescence polarisation immunoassay.

Results: Observed mean clearance of the drugs in normal saline was 1.22 ± 0.07 L/h. In human albumin, clearance was 1.29 ± 0.08 L/h. In reconstituted blood, clearance of meropenem decreased from 5.4 to 1.4 L/h and for ciprofloxacin from 6.3 to 4.3 L/h within the first 1.5 h because of early drug adsorption. Continuous renal replacement therapy clearance measured without CytoSorb was stable at 2 and 1.7 L/h, respectively. Approximately 400 mg of meropenem and 300 mg of ciprofloxacin had been adsorbed by CytoSorb, suggesting that these amounts are the maximum adsorptive capacity for these drugs.

Conclusion: In these settings, all tested drugs were adsorbed by the cartridge in relevant amounts. The identified maximum adsorptive capacity and the rapid decline in concentration during the first 1.5 h of CytoSorb use suggest that the administration of an additional dose within the first hours of CytoSorb treatment may be reasonable. In addition, early therapeutic drug monitoring should be considered.

Keywords

Antibiotic, pharmacokinetic, cytokine adsorber, extracorporeal elimination

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[1]Department of Intensive Care Medicine, University Medical Centre Hamburg-Eppendorf, Hamburg, Germany
[2]Hospital Pharmacy, University Medical Centre Hamburg-Eppendorf, Hamburg, Germany
[3]Hospital Pharmacy, Heidenheim General Hospital, Heidenheim, Germany
[4]Department of Anaesthesia and Critical Care Medicine, Heidenheim General Hospital, Heidenheim, Germany
[5]Centre for Translational Anti-Infective Pharmacodynamics, School of Pharmacy, The University of Queensland, Brisbane, QLD, Australia
[6]Centre for Clinical Research, Faculty of Medicine, The University of Queensland, Brisbane, QLD, Australia
[7]Royal Brisbane and Women's Hospital, Brisbane, QLD, Australia

Corresponding author:
Christina König, Department of Intensive Care Medicine, University Medical Centre Hamburg-Eppendorf, Martinistraße 52, Hamburg 20251, Germany.
Email: ch.koenig@uke.de

Introduction

Recently, novel cytokine adsorber systems have been developed as a treatment for the 'cytokine storm' that occurs during sepsis and septic shock.[1,2] These systems are designed for the elimination of middle molecular weight substances like cytokines and mediators. The CytoSorb® adsorber cartridge (CytoSorbents Corporation, Monmouth Junction, NJ, USA) is filled with a porous adsorbent made of polymer beads, which are designed to adsorb molecules from 10,000 to 50,000 Da. Adsorption is achieved through hydrophobic interactions of molecules and the lipophilic surface of the inner channels and pores of the beads.[3] The filter can be used as a stand-alone device or in combination with continuous renal replacement therapy (CRRT). Due to its non-specific adsorptive mechanism, elimination of anti-infective drugs through the cytokine adsorber is possible, even though they usually have a molecular size <2000 Da. After regulatory approval for clinical use, cytokine adsorbers are now increasingly used in critically ill patients with sepsis and septic shock, resulting in various case reports.[4–7] In addition, the international CytoSorb register with over 170 centres participating was implemented to record the use and efficacy of cytokine adsorber systems under real-life conditions.[8] However, to date, there is limited data showing the efficacy and effectiveness for clinical outcome parameters. Little is known about pharmacokinetics of anti-infective agents with concomitant use of cytokine adsorptive systems such as CytoSorb. Therefore, the aim of this in vitro study was to evaluate the adsorption capacity of CytoSorb in aqueous solutions and reconstituted blood for commonly used anti-infectives such as vancomycin, gentamicin, meropenem, flucloxacillin, piperacillin, ciprofloxacin, rifampicin, fluconazole and voriconazole.

Materials and methods

The adsorptive capacity of the cytokine adsorbent system CytoSorb was tested using three different experimental settings. Adsorptive capacity for all anti-infective drugs was investigated in an aqueous medium using normal saline (NaCl 0.9%) solution. Moreover, human albumin (HA) 5% was used as a carrier to investigate the effect of plasma-protein binding (PB) on drug clearance (CL). During the experiments, the cytokine adsorber was macroscopically inspected for colour changes. Finally, for meropenem and ciprofloxacin, reconstituted blood was used to determine the potential influence of erythrocytes and potential saturation effects on the CL of these two drugs.

Normal saline and human albumin solutions

Standardised concentrations of vancomycin (40 mg/L), gentamicin (20 mg/L), meropenem (20 mg/L), ciprofloxacin (15 mg/L), piperacillin (80 mg/L), flucloxacillin (80 mg/L), voriconazole (10 mg/L), rifampicin (10 mg/L) and fluconazole (40 mg/L) were used. All anti-infective drugs were dissolved in a total of 1000 mL NaCl 0.9% and 500 mL HA 5%. These solutions were pumped through the cytokine filter at a flow rate of 1.2 L/h with an infusion pump (Infusomat Space®; B. Braun Melsungen, Germany). To ensure drug mixing, the solutions were stirred (190 r/min) continuously throughout the experiments. To avoid recirculation, inlet and outlet of the circulation were fixed to opposing sides of the reservoir (Figure 1). During a circulation time of 1.5 h, samples of the solutions as well as post- and pre-filter samples were obtained. Samples were taken at 0, 5, 20, 35, 55, 75 and 95 min and then stored at −80°C until analysis.

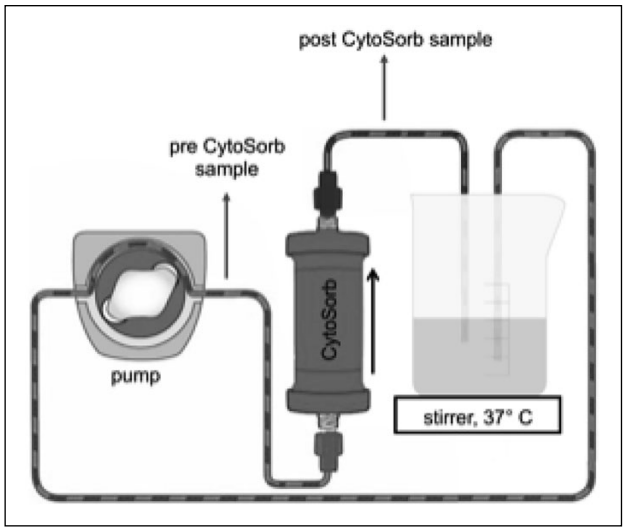

Figure 1. Experimental setup for normal saline and human albumin solutions (adapted from CytoSorbents Europe GmbH).

Reconstituted blood

Reconstituted blood (2.9 L) was obtained by mixing matched plasma and erythrocyte concentrates to achieve a haematocrit of 23%. To avoid clotting, heparin was added to the circuit via continuous infusion of 10,000 I.U./50 mL NaCl 0.9% with a flow rate of 5 mL/h. The Erlenmeyer flask was primed with heparin (10,000 I.U. heparin/50 mL NaCl 0.9%) prior to the start of the experiment. Meropenem and ciprofloxacin were added to the blood solution to achieve a concentration of 16 and 2.5 mg/L, respectively, and infused continuously (meropenem, 192 mg/h; ciprofloxacin, 30 mg/h) during the experiment. Both solutions were run through the CytoSorb cartridge built into a CRRT system (multiFiltrate®; haemofilter AV 600S, Fresenius Medical Care, Bad Homburg, Germany) in continous veno-venous haemodialysis (CVVHD) mode with a blood flow of 12 L/h and a dialysate flow of 2 L/h (Figure 2). Samples were taken from pre- and post-cytokine adsorber and

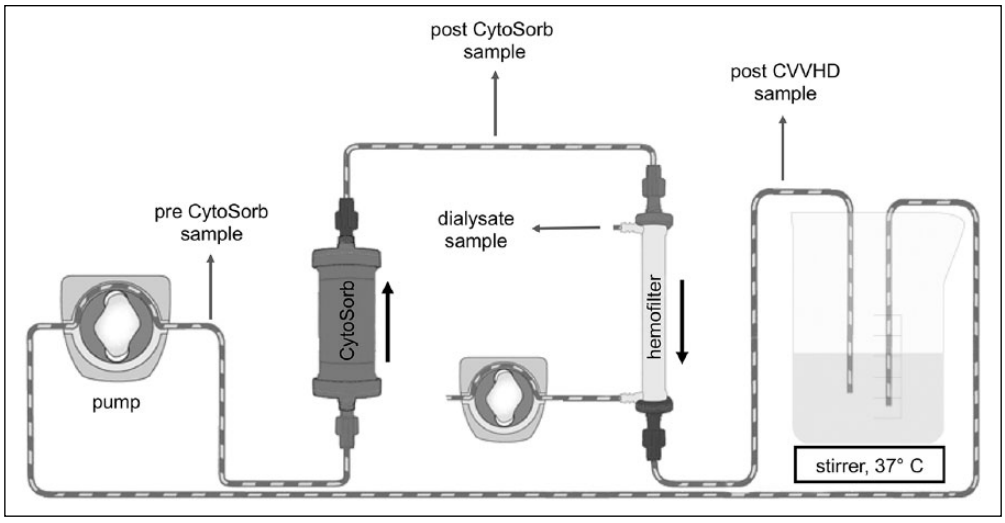

Figure 2. Experimental setup for reconstituted blood using CytoSorb and continous veno-venous hemodialysis (CVVHD) (adapted from CytoSorbents Europe GmbH).

dialysis filter, as well as from the dialysate over a sampling time of 18 h. Samples were taken at 0.5, 1.5, 2.6, 3.6, 4.6, 5.6, 6.6, 8.6, 10.6, 14.5 und 18.3 h after starting the circuit.

To ensure drug mixing and to avoid clotting, the solutions were kept at 38°C and stirred (190 r/min) continuously throughout the experiments. All samples were immediately centrifuged (3000 r/min, 10 min, 25°C); plasma was separated and then stored at −80°C until analysis.

Analytical quantification

Vancomycin and gentamicin concentrations were analysed with fluorescence polarisation immunoassay (Axsym; Abbott Diagnostics, Lake Forest, IL, USA). All other drug concentrations were analysed with high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection. Assay validation was performed according to the FDA Guidance for Industry for biochemical method validation using calibration standards and quality controls.[9] Assay performance was previously reported by Roehr et al.[10] Reconstituted blood samples were centrifuged. HA and serum samples were treated with acetonitrile and methanol (1:1) for protein precipitation and centrifuged at 3000 r/min for 10 min prior to analysis. The supernatant was transferred and used for drug quantification.

Pharmacokinetic calculations

Pharmacokinetic parameters of half-life ($t_{1/2}$) and CL of each drug were calculated using descriptive methods and assuming first-order kinetics and a one-compartment model. The elimination constant (k_e) was determined by the slope of the regression line of concentrations and was used for the estimation of drug $t_{1/2}$ via $t_{1/2}=\ln(2)/k_e$. CL was calculated as follows: $CL=\ln(2)\times Vd/t_{1/2}$, with Vd being the volume of the solution in litre (L). Adsorption in the reconstituted blood setting was calculated as follows: *Adsorption* = *Dose added* (mg) – (*Concentration* (mg/L) × *Total fluid volume* (L)).

To determine the amount eliminated via CRRT and CytoSorb, calculation was as follows

$$\begin{aligned} &\textit{Amount in CytoSorb cartridge}\,(\text{mg}) \\ &\quad = \textit{total amount infused}\,(\text{mg}) \\ &\quad - \big(\textit{amount in dialysate}\,(\text{mg}) \\ &\quad + \textit{amount in reconstituted blood}\,(\text{mg})\big) \end{aligned}$$

For comparison, similar equations were used to investigate and determine the kinetic properties of renal replacement therapies in clinical data.[11]

Results

All experiments were performed under stable conditions without technical problems. The cytokine cartridge was perfused without interruptions. Haematocrit (23%) and pH (7.4) of reconstituted blood remained stable throughout the experiment. There were no signs of blood stasis and no clotting occurred.

Normal saline solutions

There was a steep decrease of all drug concentrations during the first 20 min of the experiment. After this time, no drug was detectable in post-filter samples of the saline solutions except for gentamicin, which was detectable in

Table 1. Pharmacokinetic parameters determined in HA 5% and NaCl 0.9% and general chemical properties.

Drug	$t_{1/2}$ NaCl (min)	CL NaCl (L/h)	$t_{1/2}$ HA (min)	CL HA (L/h)	Log P	PB (%)	Da
Vancomycin	24.49	1.19	22.80	1.28	−3.1	55	1449
Gentamicin	25.67	1.13	23.34	1.25	−3.1	10	477
Meropenem	22.00	1.32	22.65	1.29	−0.6	5	383
Ciprofloxacin	22.07	1.32	23.42	1.24	0.28	30	331
Piperacillin	22.58	1.29	20.27	1.44	0.3	20	517
Flucloxacillin	24.93	1.17	24.07	1.20	2.58	97	453
Voriconazole	24.58	1.18	20.69	1.41	1	60	349
Fluconazole	25.96	1.12	23.82	1.22	0.4	10	306
Mean	24.40	1.22	21.67	1.29	–	–	–
SD	1.39	0.07	2.35	0.08	–	–	–

HA: human albumin; NaCl: normal saline; CL: clearance in L/h; $t_{1/2}$: half-life in minutes (min); PB: protein binding; SD: standard deviation; Da: Dalton.

Table 2. Pre- and post-CytoSorb drug concentrations in NaCl 0.9%.

Time (min)	Meropenem (mg/L)		Ciprofloxacin (mg/L)		Piperacillin (mg/L)		Flucloxacillin (mg/L)		Gentamicin (mg/L)		Vancomycin (mg/L)		Voriconazole (mg/L)		Fluconazole (mg/L)	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
0	20.00		15.00		80.00		80.00		23.48		35.54		10.00		40.00	
5	17.47	15.1	12.87	14.0	66.11	4.0	61.02	n.a.	21.5	5.9	30.12	0.0	7.06	0.4	43.91	2.4
20	11.27	0.0	8.34	0.0	39.67	0.2	37.45	3.7	19.23	4.4	25.65	0.2	4.56	0.0	19.39	0.8
35	7.15	0.0	5.22	0.0	24.97	0.4	22.73	0.0	15.62	3.2	22.97	0.4	2.52	0.0	13.28	0.7
55	3.91	0.0	2.82	0.0	13.73	0.3	14.39	0.0	9.58	1.5	15.61	0.7	1.65	0.0	7.73	0.9
75	1.89	0.0	1.44	0.0	7.65	0.3	9.86	0.0	8.68	1.1	12.43	0.9	1.18	0.0	4.80	1.1
95	1.02	0.0	0.76	0.0	4.23	0.3	0.00	0.0	7.65	0.9	10.38	1.4	0.00	0.0	3.00	0.7
Drug removed (%)	95		94		94		100		67		70		100		92	

the post-filter samples for 35 min after the start of the experiment (Table 1).

Observed $t_{1/2}$ and apparent CL in normal saline were similar across the observed drugs. For example, meropenem, ciprofloxacin and fluconazole showed a $t_{1/2}$ of 21.07, 22.29 and 22.73 min, and apparent CL values of 1.29, 1.32 and 1.28 L/h, respectively. However, rifampicin showed a lower $t_{1/2}$ of only 15 min resulting in a higher CL of 2.05 L/h. The mean observed drug CL and $t_{1/2}$ in normal saline was 1.22 ± 0.07 L/h and 24.40 ± 1.39 min, respectively (Table 1). The decrease in drug concentration in pre-filter samples followed first-order kinetics. After 1.5 h perfusion of the cytokine adsorber, concentrations of the antibiotics meropenem, ciprofloxacin, vancomycin, piperacillin and flucloxacillin were very low or not further detectable (Table 2). The antimycotics voriconazole and fluconazole also showed low concentrations with 0 and 3 mg/L, respectively.

Rifampicin was completely adsorbed within the first 15 min of the experiment. This was accompanied with a visible colour change from white to red of the adsorber cartridge.

Human albumin solutions

Within the first 60 min of the investigation, the decrease in drug concentration followed first-order kinetics. Observed $t_{1/2}$ and CL were homogeneous among the anti-infective drugs. For example, meropenem, ciprofloxacin and fluconazole showed a $t_{1/2}$ of 22.65, 23.42 and 23.82 min, respectively. The corresponding CL values were 1.29, 1.24 and 1.22 L/h, respectively. The mean CL and $t_{1/2}$ in HA were 1.29 ± 0.08 L/h and 21.67 ± 2.35 min, respectively (Table 1). Antibiotic and antimycotic concentrations fell rapidly within the first 20 min. After approximately 1 h, there was no further drug removal for flucloxacillin, voriconazole, meropenem and ciprofloxacin. In contrast, vancomycin, gentamicin, fluconazole and piperacillin were steadily removed throughout the experimental period (Table 3).

Since no rising in concentrations was measured at the end of both experiments, no redistribution could be detected for all of the investigated drugs.

Reconstituted blood

Within the first 1.5 h, the cytokine adsorber CL (L/h) for ciprofloxacin decreased from 6.3 to 4.9 L/h with an estimated

Table 3. Pre- and post-CytoSorb drug concentrations in HA 5%.

Time (min)	Meropenem (mg/L)		Ciprofloxacin (mg/L)		Piperacillin (mg/L)		Flucloxacillin (mg/L)		Gentamicin (mg/L)		Vancomycin (mg/L)		Voriconazole (mg/L)		Fluconazole (mg/L)	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
0	20.0		15.00		80.00		80.00		10.52		38.33		10.00		20.00	
5	11.6	1.2	8.48	0.5	88.41	4.7	n.a.	0.0	9.07	4.2	30.66	0.9	9.84	0.0	22.22	3.8
20	7.3	1.5	5.04	0.2	80.86	5.4	n.a.	0.0	6.95	1.3	20.22	0.7	n.a.	0.0	16.28	3.4
35	4.3	1.9	2.58	0.2	37.24	4.5	18.12	0.0	4.28	0.7	12.02	0.5	7.72	0.0	10.05	2.4
55	3.4	2.2	1.60	0.2	23.86	0.0	18.92	0.0	2.26	0.3	6.40	1.0	1.24	0.0	6.14	2.2
75	3.3	3.2	0.90	0.2	12.11	0.0	0.00	0.0	0.9	0.3	3.46	1.0	0.00	0.0	3.35	0.8
95	3.1	3.3	0.90	0.2	7.25	0.0	0.00	0.0	0.77	0.23	2.22	1.0	0.00	0.0	1.59	0.7
Drug removed (%)	85		99		90		100		99		95		100		92	

HA: human albumin.

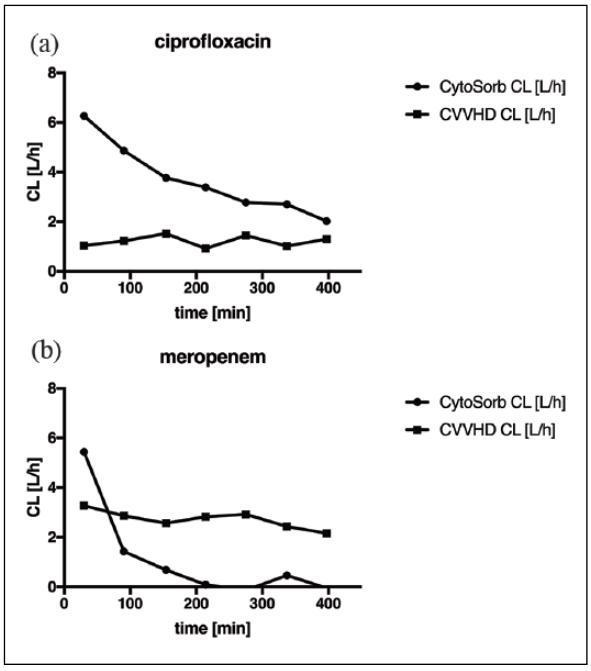

Figure 3. Ciprofloxacin (a) and meropenem (b) clearance (CL) under CytoSorb and continous veno-venous haemodialysis (CVVHD).

$t_{1/2}$ of 28 min (Figure 3(a)). For meropenem cytokine adsorber, CL decreased from 5.4 to 1.4 L/h with a $t_{1/2}$ of 34 min (Figure 3(b)). Significant removal of meropenem and ciprofloxacin occurred within the first 1.5 h of cytokine adsorber use. Within the first 0.5 h, a decrease in meropenem concentration of approximately 45% and for ciprofloxacin of approximately 52% occurred, measured by pre- and post-cytokine cartridge levels.

After 4 h, there were no significant changes in meropenem concentrations across pre- and post-cytokine adsorber samples, whereas significant changes in concentration of ciprofloxacin could be measured for up to 10 h while using the cytokine cartridge (Table 4). During the total observation period of 18 h, 394 mg of meropenem and 284 mg of ciprofloxacin were adsorbed by the cytokine cartridge. In contrast to that, 2870 mg of meropenem and 235 mg of ciprofloxacin were removed by continous veno-venous haemodialysis (CVVHD).

The mean dialysis CLs (L/h) of meropenem and ciprofloxacin were 2.0 and 1.7, respectively (Figure 3(a) and (b)). They remained stable throughout the experiment and no saturation effects were observed.

Discussion

During the experiment, commonly used antibiotics and antimycotic drugs for the treatment of bacterial and fungal infections were investigated. The substances showed a wide range in PB (0%–97%),[12] lipophilicity (log P -3.1- 2.7)[13] as well as in molecular weight (306–1449 Da).[12] In contrast to the previous investigation by Reiter et al.,[14] where initial drug concentrations were very high, for example, vancomycin 1048 mg/L or gentamicin 153 mg/L, we chose initial drug concentrations according to those achieved therapeutically, for example, gentamicin 20 mg/L and vancomycin 40 mg/L, representing observed serum concentrations in humans. We observed a considerable high apparent adsorptive CL of anti-infective compounds during the first 20 min of the use of the cytokine adsorber in HA and normal saline. This is in accordance to the findings of Reiter et al.[14] who also found a high adsorption of vancomycin, gentamicin and teicoplanin during the first 15 min of treatment with a cytokine adsorber. Comparing the adsorption results in HA and normal saline showed no significant deviation within the CL values. Even though several drugs such as flucloxacillin show a high PB of up to 97%,[15] no major effect of PB could be identified. In critically ill patients, lower serum albumin concentrations with less

Table 4. Pre- and post-CytoSorb concentrations of meropenem (MER) and ciprofloxacin (CIP) in the reconstituted blood setting.

Time (h)	MER (mg/L) Pre-CytoSorb	MER (mg/L) Post-CytoSorb	CIP (mg/L) Pre-CytoSorb	CIP (mg/L) Post-CytoSorb
0	19.89	–	2.29	–
0.5	28.22	15.43	3.14	1.50
1.5	47.09	41.48	4.26	2.53
2.6	70.50	66.50	5.06	3.47
3.6	82.56	81.97	5.63	4.04
4.6	87.53	88.55	6.34	4.87
5.6	91.88	88.38	7.43	5.76
6.6	97.06	97.43	8.42	7.00
8.6	94.05	95.14	9.71	8.87
10.6	99.59	94.93	10.76	9.58
14.5	96.75	91.18	10.01	11.82
18.3	95.66	105.43	15.10	16.25

than 35 g/L are frequently observed.[16] However, even when using higher albumin concentrations of 50 g/L (5%), representing more potential binding sites for anti-infective agents, no significant differences in CL during the first hour of the experiment could be identified. Taking a closer look to the concentrations, it becomes obvious that some drug concentrations in normal saline fall more rapidly than in albumin and vice versa. Therefore, further studies are needed to confirm which physicochemical characteristics of drugs are relevant for the adsorption on cytokine cartridges. But this suggests that the adsorber is potentially able to eliminate even substances with a high PB. This might be due to a potentially higher binding affinity between the anti-infective drugs and the cytokine adsorber than between drug and albumin.

In the aqueous solution setting rather low flow rates (1.2 L/h) were used, this was due to the use of an infusion pump and its maximum flow rate. This flow rate results in a long contact time between drug and sorbent surface, potentially leading to a higher adsorption ratio. In clinical practice, much higher blood flow rates (9–12 L/h) are used, especially in combination with CRRT resulting in a shorter contact time. Therefore, our results might have overestimated the adsorbent capacity for anti-infective drugs in aqueous solutions. This also indicates that only experiments using blood would be relevant for clinical practice.

Furthermore, high drug elimination rates were also observed in the reconstituted blood experiment. The initial drug CL by CytoSorb was 5.4 L/h for meropenem, which was higher than reported for CRRT in clinical data (approximately 3 L/h).[17] The mean CRRT CL for meropenem and ciprofloxacin of 2.0 and 1.7 L/h, respectively, corresponds to the selected dialysate flow of 2 L/h, which is in accordance with the findings of Roehr et al.[10] who found CLs of 1.9 for meropenem and 1.7 L/h for ciprofloxacin during CRRT.

During the whole experiment using reconstituted blood, approximately 300 mg of ciprofloxacin and 400 mg of meropenem were eliminated over 18 h via CytoSorb. However, since the CL was significantly lower after 1.5 h, the largest share of meropenem and ciprofloxacin removal occurred early after initiation of CytoSorb. Therefore, higher doses of anti-infective drugs for the first hours of therapy with CytoSorb might be advisable to saturate drug binding to the adsorber and achieve adequate plasma concentrations according to their pharmacokinetic/pharmacodynamic targets and break points for susceptible bacteria.[18] Timely and optimal drug exposure could be maintained effectively through routine therapeutic drug monitoring.[19]

For all in vitro experiments, maximum adsorption occurs within the first 60 min after the installation of the CytoSorb System. Irrespective of the carrier solution, a very high initial drug loss could be observed in all experiments. Since a combination of several anti-infective drugs was tested in one solution, this might not represent the clinical setting where less substances are used simultaneously. The presence of many drugs potentially binding to the cytokine adsorber might have influenced its binding capacity. Therefore, a potential underestimation of the adsorptive capacity is possible but might not be relevant because of the high overall CL. In septic shock and especially in recurrent sepsis episodes, often multi-drug resistant gram-negative pathogens are present (e.g. *Pseudomonas* spp.). Therefore, our unit commonly uses empirical combination therapy containing double Gram-negative coverage with meropenem and ciprofloxacin until culture results are available. Thus, we focussed on meropenem and ciprofloxacin in our investigation. Therefore, further studies are needed to confirm similar behaviour under CytoSorb therapy.

Limitations

Due to the experimental setting, there are several limitations of these in vitro results. The results from the aqueous and HA solutions cannot be extrapolated to critically ill

patients. Furthermore, the solutions do not contain cellular components such as erythrocytes, platelets and human proteins, which may influence the adsorptive capacity of the CytoSorb cartridge and apparent drug CL. To partially compensate this effect, reconstituted blood composed of plasma and erythrocyte concentrates was used at physiologically relevant concentrations. However, platelets and other proteins such as alpha-1 globulins, alpha-2 globulins and immunoglobulins were not present. The haematocrit of 23% was relatively low, but this is not uncommon in critically ill patients.[20] A higher proportion of cellular components in vitro and in vivo may decrease the adsorptive capacity by blocking the surface of the polymer beads in the cartridge. Moreover, we have not studied a possible release of anti-infectives from a fully saturated system. With using a continuous infusion method, we created a fully saturated system, shown by the accumulation of meropenem and ciprofloxacin levels. But the accumulation ratio of anti-infectives was nearly equivalent to the infusion ratio, and therefore desorption of meropenem and ciprofloxacin seems less likely. Furthermore, we used small volumes (2.9 L reconstituted blood) and drug amounts to achieve pharmacokinetically relevant concentrations which are different drug amounts which are present in a clinical scenario. Therefore, the time course for decreasing concentrations of drug as it binds to the adsorber may be slower in clinical practice. This was confirmed by the experiment in reconstituted blood, where we quantified the amount of drug able to be adsorbed by the cartridge.

Conclusion

The results of our in vitro experiments show that all investigated drugs are adsorbed to the surface of the CytoSorb adsorber and are removed by the combined CRRT system to varying but clinically relevant degrees. Anti-infective drugs are removed from the circulation by CytoSorb in a non-linear fashion as opposed to CRRT. In contrast to CRRT where drugs are removed in a continuous fashion, the use of CytoSorb appeared to saturate the adsorbent surface leading to a reduction in CL over time. For meropenem and ciprofloxacin, we found that the initial adsorptive capacity corresponded to a single dose of approximately 400 and 300 mg, respectively, meaning that this represents the maximum amount of drug that could be adsorbed. This suggests that the administration of an additional dose within the first hours of initiation of the cytokine adsorber may be reasonable. Moreover, with each routine change of the cytokine adsorber system after 8, 12 or 24 h, an extra dose of meropenem and ciprofloxacin should be considered to maintain optimal drug exposure. To optimise anti-infective drug dosing in septic patients treated with CytoSorb and CRRT, further studies are needed to investigate the in vivo effect of extracorporeal blood purification systems on the pharmacokinetics of these drugs. Most importantly, our results emphasise the importance of early therapeutic drug monitoring for patients not only on renal replacement therapy but also on cytokine adsorber therapy to avoid underdosing of anti-infective drugs.

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