Effects of extracorporeal blood purification therapies on organ dysfunction in critically ill patients

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Ph.D. Thesis

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1. Publications

1.1 Publications related to the subject of the thesis

I. **Kanjo A**, Ocskay K, Gede N, Kiss S, Szakács Z, Párniczky A, Mitzner S, Stange J, Hegyi P, Molnár Z.

Efficacy and safety of liver support devices in acute and hyperacute liver failure: a systematic review and network meta-analysis.

Sci Rep. 2021 Feb 18;11(1):4189.

D1, IF: 4.997

II. **Kanjo A**, Molnár Z, Zádori N, Gede N, Erőss B, Szakó L, Kiss T, Márton Z, Malbrain M, Szuldrzynski K, Szrama J, Kusza K, Kogelmann K, Hegyi P.

Dosing of Extracorporeal Cytokine Removal In Septic Shock (DECRISS): protocol of a prospective, randomised, adaptive, multicentre clinical trial.

BMJ Open. 2021 Aug 26;11(8):e050464.

Q1, IF: 3.006

1.2 Publications not closely related to the subject of the thesis

I. Ocskay K, **Kanjo A**, Gede N, Szakács Z, Pár G, Erőss B, Stange J, Mitzner S, Hegyi P, Molnár Z.

Uncertainty in the impact of liver support systems in acute-on-chronic liver failure: a systematic review and network meta-analysis.

Ann Intensive Care. 2021 Jan 18;11(1):10.

D1, IF: 6.925

II. Erőss B, Molnár Z, Szakács Z, Zádori N, Szakó L, Váncsa S, Juhász FM, Ocskay K, Vörhendi N, Márta K, Szentesi A, Párniczky A, Hegyi PJ, Kiss S, Földi M, Dembrovszky F, **Kanjo A**, Pázmány P, Varró A, Csathó Á, Helyes Z, Péterfi Z, Czopf L, Kiss I, Zemplényi A, Czapári D, Hegyi E, Dobszai D, Miklós E, Márta A, Tóth D, Farkas R, Farkas N, Birkás B, Pintér E, Pethő G, Zsigmond B, Sárközi A, Nagy A, Hegyi P.

Personalised health education against health damage of COVID-19 epidemic in the elderly Hungarian population (PROACTIVE-19): protocol of an adaptive randomised controlled clinical trial.

Trials. 2020 Sep 29;21(1):809.

Q1, IF: 2.279

III. Váncsa S, Németh D, Hegyi P, Szakács Z, Farkas Á, Kiss S, Hegyi PJ, **Kanjo A**, Sarlós P, Erőss B, Pár G.

Diabetes Mellitus Increases the Risk of Hepatocellular Carcinoma After Direct-Acting Antiviral Therapy: Systematic Review and Meta-Analysis.

Front Med (Lausanne). 2021 Oct 18;8:744512.

Q1, IF: 5.058

1.3 Scientific metrics

Number of publications **related to the subject of the thesis**: 2 (2 first author)
Cumulative impact factor of publications related to the thesis: 8.003
D1: 1, Q1: 1, Q2: 0, Q3: 0, Q4: 0

Number of **total accepted/published articles**: 5 (2 first author)
Cumulative impact factor of the published articles: 22.265
D1: 2, Q1: 3, Q2: 0, Q3: 0, Q4: 0

Number of total citations by **MTM2**: 28; 23 independent
Hirsch Index: 3

https://m2.mtmt.hu/api/author/10070613/summary, http://jcr.clarivate.com/jcr/home

TABLE OF CONTENTS

2. List of Abbreviations

ABCDE approach	Airway, Breathing, Circulation, Disability, Exposure (ABCDE) approach
AC	Anticoagulant
ACLF	Acute-on-chronic liver failure
AIDS	Acquired Immune Deficiency Syndrome
ALF	Acute liver failure
AO	Acetaminophen overdose
ARDS	Acute respiratory distress syndrome
CrI	Credible interval
CRRT	Continuous renal replacement therapy
DAMP	Damage-associated molecular pattern
Charcoal-HP	Charcoal-hemoperfusion
ECLS	Extracorporeal liver support
eCRF	electronic Case Report Form
ELAD	Extracorporeal Liver Assist Device
ET	Exchange transfusion
EVLW	Extravascular lung water
FHF	Fulminant hepatic failure
GCP	Good Clinical Practice
gr	Grade
GRADE	Grading of Recommendations Assessment, Development, and Evaluation
HD	Hemodialysis
HE	Hepatic encephalopathy
HELLP-syndrome	Haemolysis, elevated liver enzymes, low platelet count
HVPE	High-volume plasma exchange
ICU	Intensive care unit
IDMB	Independent data management board
IL(-6)	Interleukin-(6)
INF-γ	Interferon gamma
IV	Intravenous
max	Maximum

MARS	Molecular Adsorbent Recirculating System
MELD	Model for End-Stage Liver Disease
MOF	Multiple organ failure
NMA	Network meta-analysis
PAMP	Pathogen-associated molecular pattern
PCT	Procalcitonin
PICO	P: patients I: intervention C: comparison O: outcome
PiCCO	Pulse Contour Cardiac Output
PNF	Primary nonfunction following liver transplantation
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
RCT	Randomized controlled trials
RoB2	Cochrane risk-of-bias tool for randomised trials
RR	Risk ratio
SC	Steering Committee
ScvO2	Central venous oxygen saturation
SD	Standard deviation
SHF	Subfulminant hepatic failure
SMT	Standard medical therapy
SOFA	Sequential Organ Failure Assessment
SUCRA	Surface under the cumulative ranking curves
T0	Start of the study period
T6, T12, T24 etc.	In the 6th, 12th, 24th hour of the study period
Te	End of the study period
TLR	Toll-like receptor
UK	United Kingdom
USA	United States of America

3. Introduction

3.1 Critically ill patients in Intensive Care Units

Intensive care is appropriate for critically ill patients with a possibility of recovery who require or likely to require advanced organ support, can benefit from invasive treatment and need more detailed monitoring than it can be applied in a general ward [1].

Adhikari et al. reported that there were 13 to 20 million people requiring mechanical ventilation worldwide in 2004 [2], Rudd et al found that there were 48.9 million cases of sepsis globally in 2017 [3] and with the ageing population, the frequency of comorbidities and the incidence of critical illness syndromes and critical care treatments are increasing [2,4]. Mortality rates are high; in a prospective, multinational cohort study including 16784 patients from 303 Intensive Care Units (ICUs), the average hospital mortality was 28% (17-42%) [5] and in a retrospective cohort analysis conducted in Australia and New Zealand including 223 129 intensive care patients, overall hospital mortality was 16.1% [6].

3.1.1 Pathophysiology

Multiple organ failure (MOF) is the primary cause of late mortality in critically ill patients in ICUs [7]. Significant stimulation of the innate immune system can lead to a dysregulated immune response and subsequently, MOF and death. The possible "insult" can be severe injury, infection, burns or sterile inflammation, however, what determines the outcome and severity of the disease is the host's immune response to the primary injury [8].

The pathogenesis of these changes is still not fully understood, however, it has been revealed that damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs) trigger inflammatory responses through innate immune receptors, such as toll-like receptors (TLRs), causing damage to distant organs far from the primary site of injury [9,10]. Microbial infections leading to robust inflammation are mostly PAMP-mediated, while sterile insults are mainly propagated by DAMPs via the same TLRs, leading to systemic tissue damage and organ disfunction [11]. Within the context of MOF, in addition to lung, circulatory and renal failure, the liver is often damaged as well. Furthermore, acute liver failure (ALF) can also lead to hyperinflammation, eventually evolving into MOF. In ALF caused by viruses, PAMPs are more important, whereas DAMPs take priority in toxic etiologies [12].

Another pathophysiological change that occurs in critically ill patients is the imbalance between oxygen delivery and oxygen consumption. The incapability of fulfilling sufficient oxygen delivery to the tissues, leads to decreased aerobic metabolism, reduced adenosine triphosphate, increased lactate levels, then subsequently, cell dysfunction and cell death [13].

3.2 Treatment options

The amendment of pathophysiological changes – i.e., supporting the vital organ functions of the patient – initially takes priority over the accurate diagnosis [1]. In the resuscitation phase, immediate life-threatening conditions are assessed, generally with the Airway, Breathing, Circulation, Disability, Exposure (ABCDE) approach, while performing the initial treatment at the same time, for instance early adequate fluid management and oxygen therapy [14,15].

In the early phase of the treatment, the affected vital organs are identified – such as respiratory failure, acute coronary syndrome, shock, etc. – and based on this evaluation, organ support is commenced [16].

Causative therapy in case of sepsis involves source control, which can be achieved by antibiotics, operative techniques and interventional radiology, depending on the nature of the infection [8]. In acute liver failure etiology-specific treatment is applied, however, for those who do not recover spontaneously, the definitive therapy is liver transplantation [17].

In adjunction of causative therapy and organ support, additional therapies are applied, such as venous thromboembolism prophylaxis, stress ulcer prophylaxis, which can be supplemented theoretically with adjunctive measures for example intravenous (iv.) immunoglobulins, iv. corticosteroids and blood purification techniques [18].

3.3 Extracorporeal therapies

Extracorporeal life support can be used as bridge to stabilization in critically ill patients until more definitive therapies are applied [19]. In liver failure, extracorporeal liver support systems (ECLS) can be used to aid the liver's detoxification function by removing albumin-bound toxins and water-soluble substances [20]. Furthermore, bioartificial liver support therapies that contain hepatocytes can provide synthetic functions as well [21]. When there is a potential for recovery, liver support systems amend the supportive care until the regeneration of the liver. In other cases, the definitive therapy of liver failure is liver transplantation – which is expensive and restricted by

the number of organs available – however, liver support therapy may keep these patients alive until a suitable organ is found [22].

In septic patients, extracorporeal blood purification techniques were adopted in order to restore the balance of pro- and anti-inflammatory mediators by eliminating them via plasma pheresis or hemofiltration [19]. The results were dubious, therefore new techniques were developed with specific removal of mediators and toxins [8]. In case of high cut-off membranes, high-volume hemofiltration, adsorption alone and coupled plasma filtration adsorption, the goal was to enhance renal replacement therapy and adjust the uncontrolled host immune response [19].

3.4 Aim of the Ph.D. thesis

Critically ill patients represent a very heterogenous patient population with high mortality rates [2]. It has been suggested that extracorporeal blood purification techniques may improve outcomes and enhance recovery [19]. Our aim was to compare the efficacy a few of these therapies in critically ill patients admitted to the ICU.

We selected two critically ill patient populations as our main focus of interest: 1) patients with acute liver failure, and 2) patients in refractory septic shock. Both conditions are associated with high mortality and the role of extracorporeal blood purification remains uncertain. Therefore, we decided to summarize current knowledge and preferably add new findings to it by performing a network meta-analysis (NMA) and by designing a prospective randomized, controlled clinical trial.

Our main questions in the liver failure population were:

1. Which liver support device reduces mortality in acute and hyperacute liver failure most effectively?
2. Which liver support device has the highest probability of reducing the worsening of hepatic encephalopathy (HE)?

Regarding extracorporeal hemoadsorption in septic shock our goal was:

1. To design a prospective, randomized, controlled, multi-centre study for a relatively homogeneous group of septic shock patients.
2. To investigate the efficacy, safety and the appropriate length of CytoSorb therapy.
3. To assess physiologic outcomes as our primary endpoints.

4. Chapter I

4.1 Background

Acute and hyperacute liver failure are potentially life-threatening conditions that can lead to MOF [23,24], affecting 1-6 per million people every year in developed countries [25] with mortality rates of 25-50% [26-28]. The main causes of acute and hyperacute liver failure are drugs – especially paracetamol overdose (46-65%) – and viruses (29-77%), other etiologies are less frequent (11-23%) like mushroom poisoning, Budd-Chiari syndrome, Wilson-disease or HELLP-syndrome [28,29]. Due to the impaired synthetic and detoxification capacities, coagulopathy, jaundice and HE may develop [30]. In hyperacute liver failure considerably elevated transaminase levels and severe coagulopathy can be observed with slightly or not increased bilirubin levels [25]. Patients with hyperacute liver failure have a greater possibility to spontaneously recover without liver transplantation [25].

As it was discussed above *(3.3 Extracorporeal therapies)*, liver support therapies can be applied as a bridging-to-transplantation or bridging-to-recovery; however, considering the effectiveness of these therapies, the results of clinical trials are controversial, thus, currently they are not recommended by thy European Association for the Study of the Liver Clinical Practical Guidelines or the American Association for the Study of Liver Diseases Practice Guidelines outside of clinical trials in acute or hyperacute liver failure [31,32].

In former meta-analyses in this field, the different interventions were considered equivalent and pooled together in comparison with standard medical therapy (SMT) [22,33-35].

4.1.1 The rationale of conducting network meta-analyses

In conventional meta-analyses, two interventions can be compared, however when multiple alternatives exist, NMAs can provide results in a single analysis based on direct and indirect (no head-to-head trials conducted between the interventions before) comparisons as well [36]. Therefore, we decided to perform a NMA, in which we were able to assess the different liver support systems' efficacy and safety in acute and hyperacute liver failure. With the statistical methods of NMA, we (1) compare the interventions to each other and (2) rank them, to choose the best option regarding the outcome.

4.2 Methods

4.2.1. Search strategy and selection criteria

The NMA was reported using the PRISMA Extension Statement for Reporting of Systematic Reviews Incorporating Network Meta-Analyses of Health Care Interventions [37]. We used the classical PICO framework for our clinical question. P: patients with acute or hyperacute liver failure (having regard to the fact that the studies were conducted in a wide range of time (1973-2016) we accepted the articles' definition of hyperacute and acute liver failure); I and C: artificial, bioartificial liver support therapies, SMT; O: overall in-hospital mortality, mortality-by-etiology, HE, number of patients transplanted, laboratory parameters and adverse events. Our network meta-analysis was registered with the PROSPERO registry (CRD42020160133).

For this NMA on the 4th of October 2019, we searched Medline (via PubMed), the Cochrane Central Register of Controlled Trials (CENTRAL), Web of Science, Embase and Scopus for RCTs and conference abstracts of RCTs. No restrictions were imposed on the search.

We used the following search key in all databases (complemented with the MeSH function in MEDLINE): ('hepatic failure' OR 'liver failure' OR 'end stage liver disease' OR cirrhosis OR 'alcoholic hepatitis') AND ('liver support system' OR 'liver support device' OR 'liver assist device' OR 'artificial liver' OR 'bioartificial liver' OR 'extracorporeal liver' OR 'albumin dialysis' OR 'extracorporeal cellular therapy' OR MARS OR Prometheus OR 'fractioned plasma separation and adsorption' OR hemadsorption OR hemoadsorption) AND random*.

Randomized controlled trials studying liver support devices in acute-on-chronic liver failure (ACLF) were excluded. In studies in which patients with ALF and ACLF were both involved and provided individual patient data, we only extracted the data of patients with acute liver failure. Transitivity was assessed clinically, based on the eligibility criteria of the included randomized controlled trials. As acute and hyperacute liver failure have mainly similar symptoms despite etiology, we concluded that, regarding the liver support systems' clinical effect on these symptoms, the conditions of transitivity are satisfied.

Records from each database were downloaded into EndNote X9 citation manager (Clarivate Analytics, Philadelphia, USA) and duplicates were removed by the citation manager based on the title of the article, and then manually. The titles then the abstracts and full texts of the identified studies were screened for inclusion against the eligibility criteria by two independent review authors (KO, AK). A third party (ZM) resolved conflicts. Citing and cited articles were revised

through Google Scholar, where all the additional sources were identified. The PRISMA flowchart shows the process of the article selection *(Fig. 1)* [38].

4.2.2 Data extraction and outcomes

All data according to study type, author and publication information, demographic data, etiology, details of the interventions and comparators, mortality, HE, number of patients transplanted, laboratory parameters, adverse events and notes were collected in the study database (standardized template). The data from intention-to-treat analyses were extracted independently by the first (AK) and second author (KO), when conflicts arose, a third participant resolved any discrepancies (ZM).

The primary outcome of our analysis was in-hospital overall mortality. Secondary outcomes included HE (number of patients improved vs. worsened plus not improved), mortality-by-etiology, liver transplantation, long-term survival, and adverse events. We accepted the articles' definition of adverse events. We planned to analyse changes in laboratory parameters as well but failed to do so because studies reported them in different time instants.

4.2.3 Risk-of-bias assessment and quality of evidence

Risk-of-bias assessment was first performed on individual study-level according to the Revised Cochrane risk-of-bias tool for randomized trials (RoB 2) [39]. From the individual studies' overall RoB assessment, we chose the one which was at the highest risk-of-bias for each intervention's (each arm of the network) overall RoB assessment. Then we summarized the interventions' overall RoB assessment on the comparison level with the same method. The results of the RoB assessment are depicted in league tables.

We used the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) approach to assess the certainty of evidence [40]. Study limitations were evaluated based on RoB 2 tool, as detailed above. Imprecision was judged based on the sample size calculation of the article of Larsen et al [41]. Node splitting could not be performed in any of the networks due to network geometry, consequently inconsistency could not be tested. We compared the individual studies' populations, interventions and outcomes to rate indirectness. Publication bias was judged by the 'comparison-adjusted' funnel plot and Egger's test. The quality of evidence firstly was judged where head-to-head trials exist, then we chose the lowest quality of evidence for the indirect comparisons. In the league tables we marked the quality of evidence for each comparison.

Risk-of-bias and quality of evidence assessment were performed by two independent review authors (KO, AK), a third party (ZM) resolved conflicts.

4.2.4 Statistical analysis

A Bayesian method was used to perform pairwise meta-analyses and network meta-analysis with the random effect model. In case of missing outcome data, we replaced values with the worse outcome, i.e. in case of mortality, death, in case of HE, worsening/not improving. We used risk ratios (RR) for dichotomous data with 95% credible intervals (95% CrI).

We optimized the model and generated posterior samples using the Monte-Carlo methods running in four chains. We set at least 20,000 adaptation iterations to get convergence and 10,000 simulation iterations. Network estimates (pooled direct and indirect data) of each intervention compared to standard medical therapy and to other interventions are presented in forest plots, summarized in a league table (as shown in the results section). In the network geometry the direct comparisons are presented with edges, and the thickness of the edges is proportional to the number of the head-to-head trials, and the size of the nodes is proportional to the number of studies in which the intervention was applied.

We also ranked interventions by their posterior probability via calculating the surface under the cumulative ranking curves (SUCRA) values. 'Comparison-adjusted' funnel plot was created with the frequentist approach, and Egger's tests were performed in the NMA to assess small-study effect of in-hospital mortality. All calculations were performed with R (V. 3.5.2) package gemtc (V. 0.8-2) along with the Markov Chain Monte Carlo engine JAGS (V. 3.4.0) and STATA 17.0 (StataCorp LLC).

4.3 Results

4.3.1 Selection process and study characteristics

Through the initial searches 2774 citations were identified. After reading the titles and abstracts, 99 articles remained for further assessment. 12 articles could be included for qualitative synthesis and 11 for NMA (*Fig. 1*). In the article of Demetriou et al., there were no data reported that we could include in the quantitative synthesis concerning mortality or HE [42].

Figure 1. Study selection process
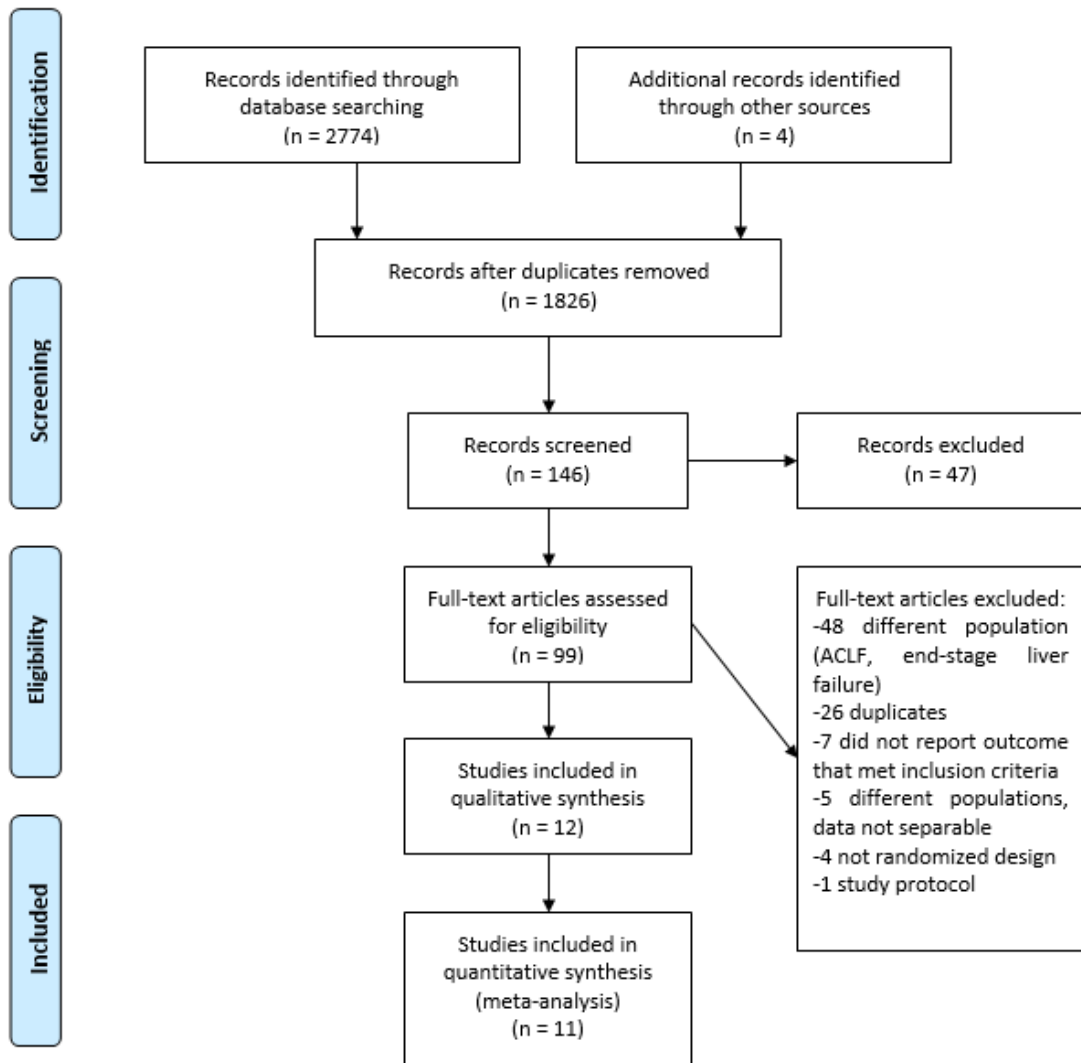
PRISMA flowchart containing results of systematic search and article selection.

All studies included in the quantitative synthesis are parallel randomized controlled trials comparing liver support systems to SMT, published between 1973 and 2016, including 479 patients. Overall, 243 patients were assigned to a liver support therapy and 236 to SMT. In four of the studies BioLogic-DT [43-46] (BioLogic-DT has been redesigned and now called Liver Dialysis

Device. [35]), in three of them the Molecular Adsorbent Recirculating System (MARS) was applied [47-49]. Through the systematic search we found one study from each modalities analysing high-volume plasma exchange [41], exchange transfusion [50], Extracorporeal Liver Assist Device (ELAD) [51] and charcoal hemoperfusion [52]. Bioartificial modalities are ELAD therapy (Vital Therapies Inc., San Diego, CA, USA) and HepatAssist device (Circe Biomedical Inc., Lexington, MA, USA). HepatAssist device was included only in the systematic review.

Seven studies reported detailed demographic characteristics. The mean age was 38.8 years, two studies included adolescents as well. About half of the sample population were female (55.8% - 226 of 405). The majority of the studies included patients with different etiologies, however, the distribution of the different etiologic factors was similar to the general population. Seven RCTs recruited patients across Europe (58%), three in the USA (25%) and 2 multicentric trials recruited patients at the study sites across continents (17%) (*Table 1*).

Table 1. Randomized controlled trials included in the systematic review and network metaanalysis

Study	Country	Population	Etiology	Intervention (Nº of patients)	Nº of sessions	Ancillary hemodialysis (HD) and use of anticoagulant (AC) therapy	Comparator (Nº of patients)	Age range (mean)	Women (%)
Redeker (1973)	USA	ALF with gr. IV HE	acute viral hepatitis (100%)	Exchange transfusion (n= 15)	mean, SD: 1,1+/- 0.35, median: 1, range: 1–2, max: 2	AC: received	Standard medical therapy (n= 13)	16–67 (25.1)	39
O'Grady (1988)	UK	FHF with gr. IV HE	acetaminophen overdose (AO) (52%), viral hepatitis (40%) drug reaction (8%)	Charcoal hemoperfusion (n= 29)	median: 2, max: 4	HD: at the physician's discretion AC: received	Standard medical therapy (n= 33)		
Hughes (1994)	UK	FHF with gr. IV HE	AO (60%), viral hepatitis (40%)	BioLogic-DT (n= 5)	mean: 3.6, median: 4,	HD:	Standard medical	19–64 (37.3)	30

Study	Country	Condition	Etiology	Device	Duration	AC/HD	Control	Age	
					range: 2–5, max: 5	in case of renal failure, patients were excluded AC: not applied (producer's suggestion)	therapy (n= 5)		
Ellis (1996)	UK	ALF	AO (71%), viral hepatitis (21%), drug induced (8%)	ELAD (n= 12)	continuous	HD: at the physician's discretion	Standard medical therapy (n= 12)	14–65	50
Mazariegos (1997)	USA	ALF with coma		BioLogic-DT (n= 5)	max. 5		Standard medical therapy (n= 1)	35–65 (48.3)	67
Wilkinson (1998)	USA	ALF with gr. III-IV HE	viral hepatitis (66%) heat stroke (33%)	BioLogic-DT (n= 1)	mean: 3.6, max: 5	HD: in case of renal failure, patients were excluded	Standard medical therapy (n= 2)	27–58 (42.7)	33

						AC: not applied (producer's suggestion)			
Ellis (1999)	UK	ALF with gr. II or greater HE	acute alcoholic hepatitis (100%)	BioLogic-DT (n= 5)	mean: 2.6, median: 3, range: 1–3, max: 3	HD: at the physician's discretion AC: received	Standard medical therapy (n= 5)	36–64	30
Demetriou (2004)	USA and Europe	FHF/SHF with gr. III-IV HE, PNF	viral hepatitis+AO+other drug induced (49%) indeterminate (37%), PNF (14%)	HepatAssist (n= 85)	mean: 2.9, range: 1–9		Standard medical therapy (n= 86)	10–69 (37)	70
Pollock (2004)	UK	FHF	AO (100%)	MARS (n= 6)	max. 14		Standard medical therapy (n= 6)		

| Study	Country	Type	Etiology	Intervention		HD/AC	Control		
El Banayosi (2007)	Germany	ALF	cardiogenic shock after cardiac surgery (100%)	MARS (n= 20)	range: 1–54		Standard medical therapy (n= 20)		28
Saliba (2013)	France	ALF	AO (38%), viral hepatitis 14%) autoimmune hepatitis (12%), mushroom induced (8%), unknown (8%), drug reaction (6%), toxic agents (6%), other (9%)	MARS (n= 53)	median: 1, range: 0-7	HD: at the physician's discretion	Standard medical therapy (n= 49)	(40.4)	57
Larsen (2016)	Denmark, UK, Finland	ALF with gr. II or greater HE	AO (59%), unknown (21%), toxic agents (9%), viral hepatitis 6%), Budd-Chiari syndrome (1%), other (3%)	High-volume plasma exchange (n= 92)	mean, SD: 2.4+/-0,8, max: 3	HD: at the physician's discretion AC: received based on local guidelines	Standard medical therapy (n= 90)	33–56	68

Table contains study characteristics of the included trials. Blank cells indicate that the data were not reported in the article. Abbreviations: ALF: acute liver failure, HE: hepatic encephalopathy, HD: hemodialysis, AC: anticoagulant, SD: standard deviation, max: maximum, USA: United States of America, FHF: fulminant hepatic failure, gr.: grade, UK: United Kingdom, AO: acetaminophen overdose, SHF: subfulminant hepatic failure, PNF: primary nonfunction following liver transplantation

4.3.2 In-hospital mortality

The network (*Fig. 2*) includes eleven studies. All liver support systems were compared to standard medical therapy.

Figure 2. The network interventions regarding in-hospital mortality
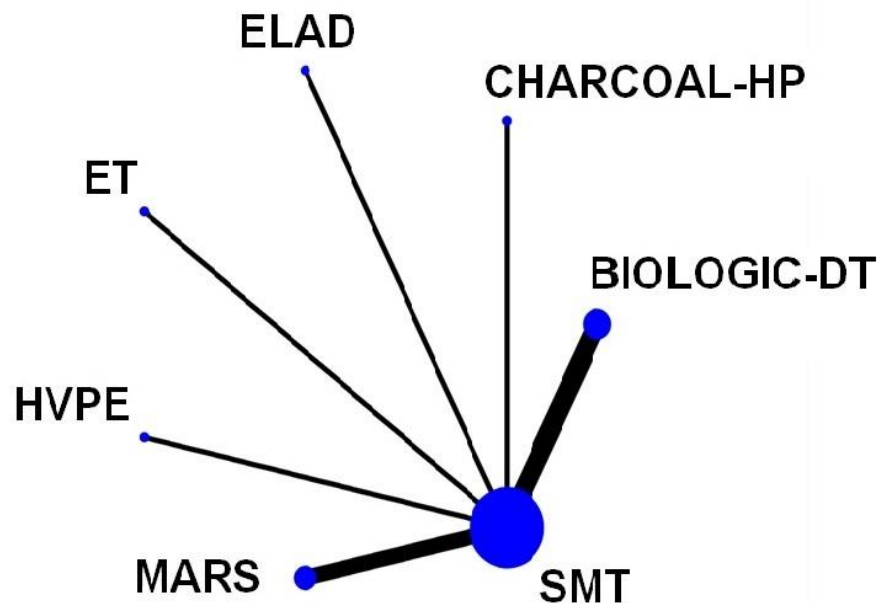
The thickness of the edges is proportional to the number of the head-to-head trials, and the size of the nodes is proportional to the number of studies in which the intervention was applied.

The SUCRA values (*Fig. 3*) indicate that BioLogic-DT and MARS are most likely to result in the lowest mortality. However, the results of the analysis (*Supplementary Fig. 1-7)* presented in the league table (*Table 2*) show that there were no statistically significant differences between the interventions.

Figure 3. Surface under the cumulative ranking curves (SUCRA%) values of in-hospital mortality
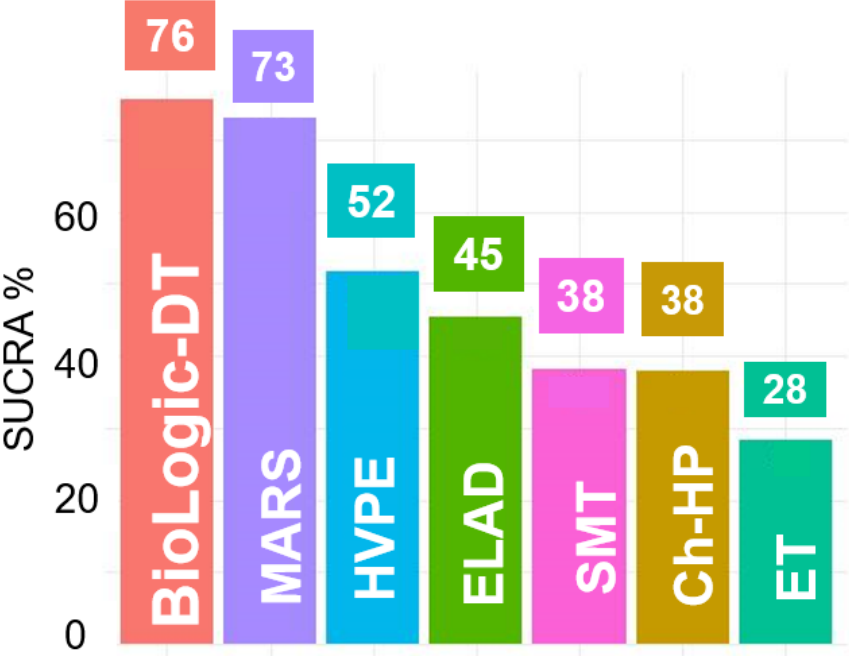
The higher the SUCRA value, the higher the probability for the interventions to be the best option.

**Table 2. League table of pairwise comparisons regarding in-hospital mortality**

BioLogic-DT						
0.91 (0.12, 4.7) ⊕○○○	MARS					
0.60 (0.05, 4.5) ⊕○○○	0.67 (0.07, 5.2) ⊕○○○	HVPE				
0.50 (0.03, 4.9) ⊕○○○	0.56 (0.05, 5.2) ⊕○○○	0.86 (0.058, 13) ⊕○○○	ELAD			
0.47 (0.09, 1.6) ⊕○○○	0.53 (0.15, 1.5) ⊕○○○	0.80 (0.13, 4.9) ⊕⊕⊕⊕	0.93 (0.13, 7.2) ⊕○○○	SMT		
0.44 (0.03, 3.4) ⊕○○○	0.49 (0.05, 3.9) ⊕○○○	0.74 (0.054, 9.3) ⊕○○○	0.85 (0.05, 13) ⊕○○○	0.91 (0.14, 5.7) ⊕○○○	Charcoal-HP	
0.34 (0.03, 2.6) ⊕○○○	0.38 (0.04, 3.1) ⊕○○○	0.58 (0.044, 7.2) ⊕○○○	0.67 (0.05, 11) ⊕○○○	0.72 (0.12, 4.5) ⊕○○○	0.79 (0.06, 9.9) ⊕○○○	ET

Values are given as relative risk (95% credible interval). The colour of the boxes indicates the comparisons' overall risk-of-bias assessment (green: low risk-of-bias, yellow: some concerns, red: high risk-of-bias). The number of ⊕ symbols refer to the quality of evidence according to the GRADE approach (⊕⊕⊕⊕ high quality, ⊕⊕⊕○ moderate quality, ⊕⊕○○ low quality, ⊕○○○ very low quality)

4.3.3 Secondary outcomes

The network of in-hospital mortality among nonparacetamol-poisoned patients is depicted in *Fig. 4.*

Figure 4. The network geometry of the eligible comparisons of in-hospital mortality in nonparacetamol-poisoned patients.
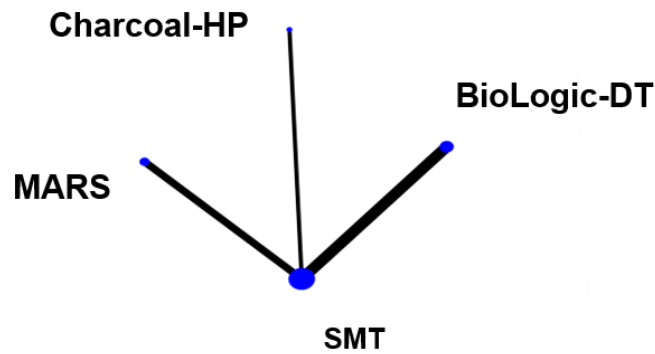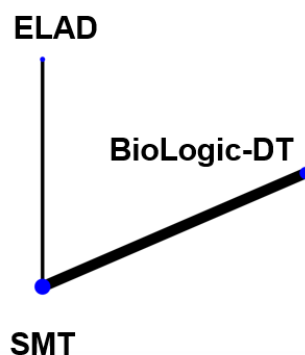

Figure 5. Surface under the cumulative ranking curves (SUCRA%) values of in-hospital mortality in nonparacetamol-poisoned patients.
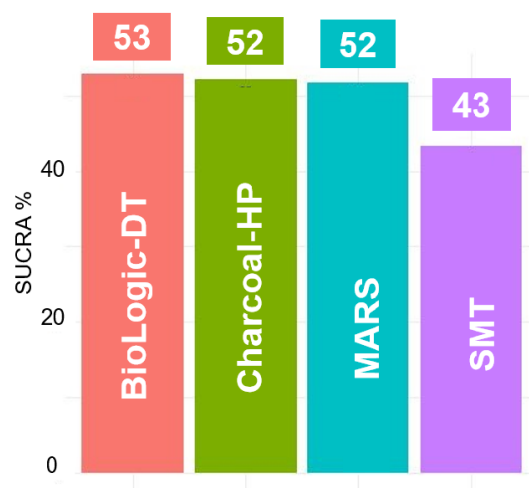

The SUCRA values show that BioLogic-DT, charcoal hemoperfusion and MARS may be equally efficient to decrease mortality (53%, 52% and 52%, respectively) while SMT seems less effective (43%) in the nonparacetamol-poisoned patient population (*Fig. 5*). Considering HE *(Fig. 6.)*, the SUCRA rankings indicate (*Fig. 7*) that ELAD therapy has the highest probability to reduce the worsening of HE while BioLogic-DT seems noticeably less appealing than SMT or ELAD (78%, 44% and 28%).

Figure 6. The network geometry of the eligible comparisons of HE

Figure 7. Surface under the cumulative ranking curves (SUCRA%) values of HE
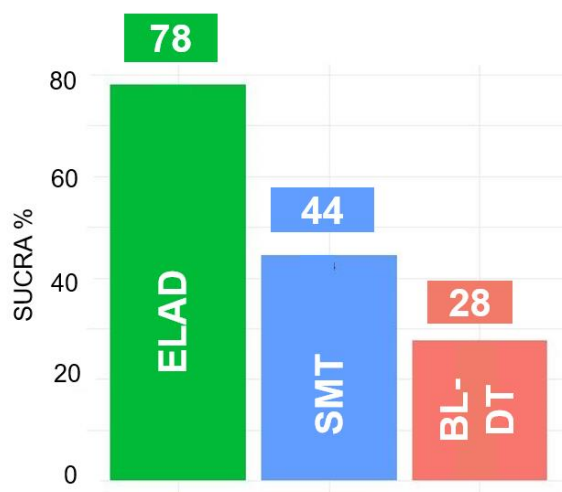
On the other hand, the results from the league table (*Table 3 and 4*) based on the forest plots (*Supplementary Fig. 10-13, Supplementary Fig. 15-17*) for both outcomes confirm that no statistically significant differences can be found between the interventions.

Table 3. League table of in-hospital mortality of non-paracetamol poisoned patients

BioLogic-DT			
1.0 (0.17, 4.4)	Charcoal-HP		
0.99 (0.20, 3.7)	0.99 (0.16, 5.5)	MARS	
0. 93 (0.32, 2.1)	0. 94 (0.23, 3.6)	0.94 (0.32, 2.9)	SMT

The league table contains the risk ratios /RR/ (credible intervals /CrI/) for every possible comparison of the interventions. All the comparisons' overall risk-of-bias assessments were judged to raise some concern and according to the GRADE approach all comparisons were judged as very low quality ⊕○○○.

Table 4. League table of HE

ELAD		
0.65 (0.17-2.1)	**SMT**	
0.56 (0.12-2.3)	0.85 (0.37-2)	**BioLogic-DT**

The league table contains the risk ratios /RR/ (credible intervals /CrI/) for every possible comparison of the interventions. The event was the number of patients whose HE worsened/not improved. The colour of the boxes indicates the comparisons' overall risk-of-bias assessment (green: low risk-of-bias, yellow: some concerns, red: high risk-of-bias). According to the GRADE approach all comparisons were judged as very low quality ⊕○○○.

4.3.4 Long-term survival

We assessed articles in which the follow-up period was at least 30 days. In the trial of Demetriou et al. 30-day survival was 71% in the bioartificial liver-treated group and 62% in the control group (p=0.26, generated with Whitehead Triangular Test) [42]. Saliba et al. reported that 6-month overall survival was not significantly different in the MARS and control groups (82.1 and 75.5%, respectively, p=0.50) [49]. Considering HVPE, Larsen et al. reported that 3-month overall survival was not improved significantly in the plasma exchange group compared to the control group, however transplant-free survival was significantly better in the HVPE-treated group after 3 months (p=0.0058)[41].

4.3.5 Transplantation

Six trials reported on liver transplantation. Three large RCTs did not find significant differences between the control and treatment groups in the number of patients transplanted and survival rates analysing HepatAssist device, HVPE and MARS [41,42,49]. Ellis et al. examining ELAD therapy reported that 2 patients underwent transplantation and 1 survived in each group [51]. In the trial published by Wilkinson et al. 2 fulminant hepatic failure patients

had liver transplantation, 1 survived and 1 underwent transplantation before the start of the trial period [44]. In the study from Mazariegos et al. 3 patients from the treatment group had liver transplantation and survived, and no patients were transplanted from control group [46].

4.3.6 Adverse events

Nine studies reported adverse events. In three trials no adverse events were observed during BioLogic-DT treatment [43-45]. With ELAD therapy tachypnoea, tachycardia, fever and bleeding occurred in two patients [51]. In a trial examining HepatAssist device thrombocytopenia was the most frequent adverse event with similar incidences between groups (33.7% vs 38.8% for controls vs interventions, respectively) [42]. During charcoal hemoperfusion renal failure, cerebral oedema and uncompensated metabolic acidosis were detected [52]. Examining HVPE, cardiac arrhythmia, acute respiratory distress syndrome (ARDS), pancreatitis, deteriorating in gas exchange, transfusion-related acute lung injury, infections confirmed by blood culture and bleeding could be observed. The rate of adverse events were not statistically different in the treatment and control group [41].

In a multi-center RCT MARS was tested, bleeding, death or sepsis did not occur related to MARS therapy, the majority of adverse events were related to liver transplantation and were more frequent in the not paracetamol-poisoned population [49].

In patients with ALF due to cardiogenic shock after cardiac surgery treated with MARS no bleeding was detected due to thrombocytopenia, other adverse events were not reported [48].

4.3.7 Risk-of-bias and quality of evidence of NMA assessing liver support systems in ALF

Two trials were published in abstract form [46,47]. Three of the trials were adjudicated as overall low risk-of-bias (33%) [41,42,49], and nine studies were judged to raise some concerns (67%) (*Supplementary Fig. 18-20*) [43-45,48,50-54] considering mortality outcomes. Regarding HE three studies were judged to raise some concerns [43-45] and one article was considered to be at high risk-of-bias [51].

Certainty of evidence for the outcomes was rated as very low for most comparisons (*Supplementary Table 1-3*). Except for the study of Larsen et al. [41], none of the articles had the appropriate number of patients, thus we downgraded the quality of evidence in each comparison in every outcome by two.

The study populations were heterogenous in most of the studies, with different etiologies and disease onset. Methodological differences were found among the studies according to renal replacement and AC therapy detailed in *Table 1, Ancillary hemodialysis and use of*

anticoagulation therapy. Differences in outcome measures were found concerning HE, according to the different scores applied. Indirectness could not be measured where there was only one head-to-head trial between two interventions.

'Comparison-adjusted' funnel plot was created with the frequentist approach, and Egger's test were performed in a NMA to assess small-study effect of in-hospital mortality (*Supplementary Fig. 21*). Asymmetry was not significant thus downgrading was not necessary. Considering in-hospital mortality in nonparacetamol-poisoned patients and HE due to the low number of articles funnel plot and Egger's test could not be performed.

4.4 Discussion

The role of liver support therapies in acute liver failure is still controversial, and to the best of our knowledge, no NMA has been published in this field before. Eleven RCTs were included in the current study with mortality and HE being the patient-important outcomes. BioLogic-DT was ranked as the best treatment for in-hospital mortality and worse for HE, however this modality is not applied in clinical practice anymore. MARS therapy was the best option from the available treatments in reducing in-hospital mortality. However, with no statistically significant results, there is no solid evidence that the differences that we can see from the SUCRA values are due to chance or the interventions truly differ in their effects.

Former meta-analyses reported conflicting results considering liver support devices' effect on mortality in acute liver failure. Zheng et al. found that bioartificial devices reduced mortality in ALF (RR: 0.69, 95% CI=0.50-0.94, P=0.018), although from the three studies analysed two represented the same patient population [55]. Stutchfield et al. reported that based on three RCTs, liver assist devices reduced mortality (RR: 0.7, 95% CI=0.49, 1.00, P=0.05), although the significance is not robust given the confidence interval [35]. Other previous meta-analyses did not find any significant difference between SMT and liver support techniques in the ALF population by subgroup analysis [22,33,34,56-58].

Acetaminophen overdose is the leading cause of ALF in the USA, Australia and Europe [59-61]. Spontaneous recovery is more frequent in this patient population compared to other drug-induced, autoimmune or idiopathic ALF [59]. Therefore, emergency transplantation as a routine intervention in paracetamol poisoning has been questioned [62]. We did not have enough data in this patient population for a quantitative synthesis, however in the nonparacetamol-poisoned population no significant difference could be observed between SMT and extracorporeal liver assist devices, and the different liver support therapies applied.

HE is an important symptom of ALF [30]. However, because of the disease's complexity there are several different measurement scales [63] and the result is greatly affected by the assessor [64]. Furthermore, the patients are usually sedated and mechanically ventilated, which makes the evaluation more difficult. In former meta-analyses in populations from both ACLF and ALF patients, significant improvement was found in HE with ECLS systems [22,33,34,57].

The greatest strength of this study is that the different interventions were compared to each other and were not assessed together in comparison with standard medical therapy. However, this study has certain limitations. The most important limitations are the small sample sizes, the heterogeneity of the patient populations, outcomes, and study design and the inconsistency in definitions of liver failure. We were unable to use the node-splitting analysis to examine consistency assumption because there was not enough information from the comparisons in the network. Long-term survival could not be quantitatively analysed, although it is a particularly important factor to assess the efficacy of the interventions. Finally, our NMA covers a period of more than 40 years, during which SMT has improved remarkably (that is, chronological bias).

5. Chapter II

5.1 Background

Sepsis and septic shock are devastating conditions with mortality rates between 20-50% [65-67]. Sepsis has an outstandingly complex pathophysiology, therefore the clinical presentation of sepsis is often diverse and unpredictable [68,69]. The process begins with the host's immune response triggered by various insults [70]. This response becomes uncontrolled, and an imbalance occurs between pro- and anti-inflammatory mediators. This condition is also referred to as the 'cytokine storm' [71]. During the cascade-like inflammatory response, cytokines are released, which are a heterogeneous group of proteins, mostly in the mass range of 40 kDa [72]. The theory that cytokine storm may be responsible for the observed deleterious sequence of events in sepsis, raises the pathophysiological rationale of extensive removal of circulating cytokines [73]. A disturbance in vascular tone regulation also develops in sepsis: vasoplegia is thought to be a key factor responsible for the death of patients with septic shock, due to persistent hypotension [74].

When standard therapeutic measures, such as adequate early resuscitation, source control and organ support fail to improve the patients' condition, additional therapeutic alternatives,

called 'adjuvant therapies' are applied to reduce morbidity and mortality by providing some extra help [8]. Several adjuvant therapies have been tested over the decades with non-conclusive results [75-77]. One of the most recent alternatives is extracorporeal cytokine adsorption with a device called CytoSorb (CytoSorbents® Corporation, New Jersey, USA) that has become available in clinical practice in 2011. It is a high-flow, low-resistance cytokine adsorbent, containing specially developed polymer beads with a large adsorption surface and a spectrum of adsorption between 5 and 60 kDa [78].

Over 100 case studies describing the use of CytoSorb in many clinical scenarios and in general, the effects are promising, and the treatment is well tolerated [79-81]. Concerning the treatment of sepsis, clinical trials are lacking at present, and we have mainly small case series [82-85]. There is also an international CytoSorb Registry, and recent data analysis on 198 patients indicated, that observed mortality (65%) was substantially better as compared to the predicted (80-20%) and the treatment also proved to be safe [86]. Furthermore, recent case series and case-control studies reported profound benefit on the outcome in patients with septic shock and treated with CytoSorb [87,88]. Recently, the ACESS-trial (Adsorption of Cytokines Early in Septic Shock) was published, which is the first randomized clinical trial (RCT) on CytoSorb as a stand-alone hemoperfusion treatment (i.e., without continuous renal replacement therapy -CRRT) in patients with septic shock [89]. It was a proof-of-concept pilot study on 20 medical patients randomized into a CytoSorb and a standard treatment group, with cytokine adsorption initiated within the first 24 hours after the onset of septic shock. The treatment proved to be safe and resulted in a significant reduction in norepinephrine requirement and serum procalcitonin (PCT) levels in the CytoSorb group as compared to controls. In a more recent propensity-score-weighted retrospective study on more than 100 patients with septic shock requiring CRRT, when patients were weighted by stabilized inverse probability of treatment weights the results suggested that CytoSorb therapy may be associated with decreased all-cause mortality at 28 days compared to CRRT alone [90].

Despite the promising case series and preliminary results, several questions need to be clarified before recommendations can be made, including the right target population, the timing and the length of a single treatment and the overall duration of the therapy. Some preliminary data are suggesting that PCT is removed by the adsorber in a time-dependent manner [91] being most efficient during the first 12 hours, after which removal is negligible.

5.2 Aim of the study

This study aims to compare the efficacy of standard medical therapy (SMT, Group A) and continuous extracorporeal cytokine removal with CytoSorb therapy in patients with early refractory septic shock. Furthermore, we compare the dosing of CytoSorb adsorber device changed every 12 (Group B) or 24 hours (Group C).

5.3 Methods and analysis

5.3.1 Study design

It is a prospective, randomized, controlled, three-arm, open-label, international, multi-centre, phase III study with adaptive "sample size re-estimation" design.
The study protocol was constructed in accordance with the SPIRIT 2013 statement [92].

5.3.2 Randomization

A computer-generated random number sequence will be conducted with randomly varied multiple block sizes stratified according to the participating centres with an equal (1:1:1) allocation ratio. The medical personnel in each study centre will have credentials to access the randomization site. On this site, the medical staff has to check all inclusion criteria and the absence of all the exclusion criteria. Patients will be recruited consecutively. After the participant was registered, the allocation appears but the following allocations and the block sizes are concealed.

5.3.3 Blinding

It is not possible for the staff who are providing patient care to be unaware of the group assignments after randomization. Sham procedures for the control group would be unethical. Statisticians are blinded to treatment assignments.

5.3.4 Duration

Duration per patient: The study starts after randomization. In the CytoSorb groups, measurements, blood sampling and other recordings are performed immediately after the start of CytoSorb therapy (indicated as T_0). In the SMT group, T_0 is defined as the first recordings after randomization. The study period ends (T_e) 12 hours after shock reversal or on day 5 after randomization, at the time of death within this period or in case of deterioration of the patient after a minimum of a 24-hours treatment, whichever happens first. The patients will be followed up on day 28±7 and day 90±7 after randomization. Duration of the entire study: the planned starting date of the study is June 2023, and the planned completion date is June 2027.

5.3.5 Study groups

Patients eligible for the study in terms of the inclusion and exclusion criteria (defined below), will be randomly assigned to one of the three study groups after informed consent. In case the patient is unable to give consent, informed consent will be obtained from the next of kin or his/her legal guardian, information on the study and the treatment will be provided by the attending physician. Patients in Group A will be treated with standard medical therapy (SMT). Patients in Group B will be treated with continuous CytoSorb therapy in addition to standard medical therapy; CytoSorb device will be changed every 12 hours. Patients in Group C will also be treated with continuous CytoSorb therapy in addition to standard treatment, however CytoSorb device will be changed every 24 hours. *(Figure 8)*.

Figure 8: Flowchart of the therapy according to the SPIRIT 2013 statement [92]
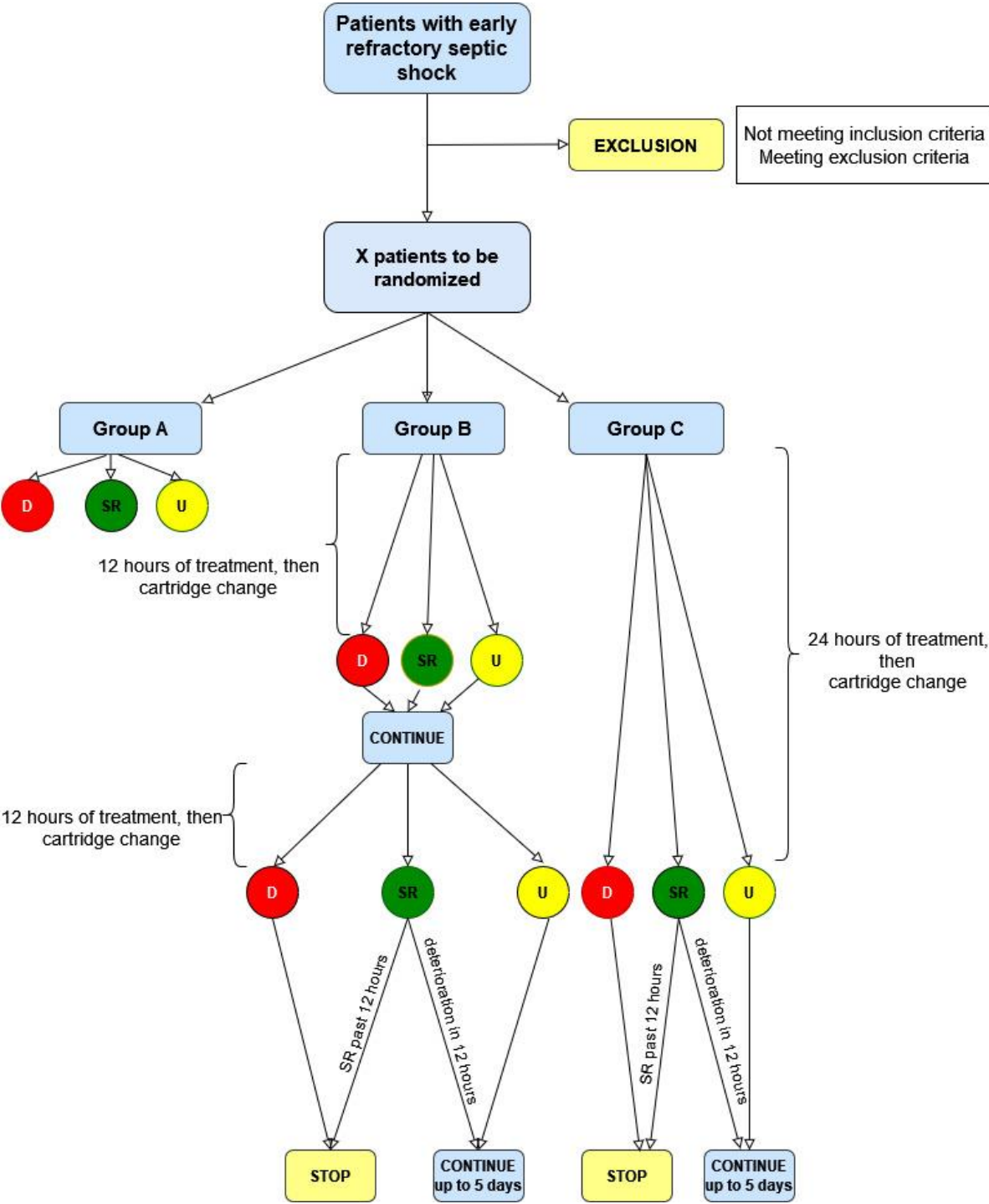
The figure presents the first 24 hours of the treatment period. D: deterioration, U: unchanged state, SR: shock reversal

5.3.6 Patient enrolment

The inclusion and exclusion criteria are based on the results of previous case series [87,88], on the ACESS trial [89] and modified accordingly:

5.3.6.1. Inclusion criteria
- Septic shock as defined by the Sepsis-3 criteria [93]
- Septic shock of both medical and surgical etiology (except for re-operation)
- APACHE II > 25 [87-89] (APACHE II score will be assessed at T_0)
- Mechanical ventilation
- Norepinephrine requirement ≥0.4 µg/kg/min for at least 30 minutes, when hypovolemia is highly unlikely as indicated by invasive hemodynamic measurements [87-89] assessed by the attending physician
- Invasive hemodynamic monitoring to determine cardiac output and derived variables
- Procalcitonin level ≥ 10 ng/ml [87-89]
- Inclusion within 6-24 hours after the onset of vasopressor need and after all standard therapeutic measures (including steroid therapy and/or second vasopressor) have been implemented without clinical improvement (i.e.: the shock is considered refractory)
- Written informed consent

5.3.6.2. Exclusion criteria
- Patients under 18 years of age and over 80
- Lack of health insurance
- Pregnancy
- Criteria of standard guideline-based medical treatment not exhausted *(detailed below at 3.7) standard medical therapy)*
- End-stage organ failure [94]
   - New York Heart Association Class IV.
   - Chronic renal failure with an estimated glomerular filtration rate< 15 ml/min/1,73 m^2
   - End-stage liver disease (MELD score >30, Child-Pugh score Class C)
- Unlikely survival for 24 hours according to the attending physician
- Acute onset of haemato-oncological illness
- Post cardiopulmonary resuscitation care
- Re-operation in the context of a septic insult
- Immunosuppression

- systemic steroid therapy (>10 mg prednisolone/day)

- immunosuppressive agents (i.e.: methotrexate, azathioprine, cyclosporin, tacrolimus, cyclophosphamide)

- Human immunodeficiency virus infection (active AIDS): HIV viral load > 50 copies/mL [95]

- Patients with transplanted vital organs

- Thrombocytopenia (<20.000/ml)

- More than 10%-of body surface area with a third-degree burn

- Acute coronary syndrome

- In case of the need for a transfer of the patient to radiology or surgery, and if the device has to be disconnected, then the adsorber should be kept in a recirculation mode. In case of the need for changing the adsorber (i.e.: clotting) or if the disconnection lasted more than 2 hours, the patient should be excluded from the study

5.3.7. Standard medical therapy

Patients will receive standard monitoring and care according to the centers' local standard protocols based on international guidelines [96]. It includes 5-lead ECG, pulse oximetry, continuous invasive blood pressure monitoring, central venous cannulation and advanced hemodynamic monitoring with the Pulse Contour Cardiac Output (PiCCO) technology. Advanced haemodynamic monitoring will be undertaken to optimize haemodynamics. Study teams will be encouraged to wean catecholamine support as soon as possible (mean arterial pressure between 65-70 mmHg in general) [97], but this should remain at the physician's discretion and should be tailored to each patient's individual need, based on other indices of global hemodynamic parameters and tissue perfusion such as urine output, serum lactate levels, central venous O_2 saturation (ScvO2), etc. The first choice of vasopressor is norepinephrine. For the second line, vasopressin is the recommended vasopressor -, also including steroid support decided by the attending physician. In case of the need for an inotrope, dobutamine is suggested as first-line treatment. Standard medical therapy will be performed according to the 'Surviving Sepsis Campaign' Guidelines [96].

Patients in both Group B and C will receive a haemodialysis catheter inserted into a central vein (femoral, subclavian or internal jugular, as appropriate). Treatment will be performed as instructed by the manufacturer's user guide.

5.3.8. CytoSorb therapy

In short, CytoSorb will be placed in a blood pump circuit in pre-haemofilter position (hemoperfusion) using a renal replacement device – of the choice of the given site - as a stand-alone treatment or in combination with renal replacement therapy. The device will be run in continuous veno-venous hemofiltration, continuous veno-venous hemodialysis or continuous veno-venous hemodiafiltration mode. Intravenous anticoagulation will be performed - according to the current standards recommended by the manufacturers - with heparin, low molecular weight heparin or citrate as required, and a pump flow rate of 100-400 mL/min will be aimed and flow rate recorded.

Physicians are strongly advised to start CytoSorb therapy as soon as possible after randomization, but not later than 2 hours. In case of further delay, the patient should be removed from the study.

In Group B and C, special attention will be paid to coagulation, therefore, in addition to standard laboratory tests (prothrombin time, activated thromboplastin time, international normalized ratio), rotational thromboelastometry (ROTEM) will be performed whenever necessary and available.

Antibiotic serum concentrations are recommended to be monitored – in centres where it is available - according to international standards and doses should be altered as recommended if necessary.

Shock reversal will be assessed by the attending physician and the treatment will be immediately continued or terminated with a new adsorber. Criteria for termination are as follows:

1. *Discontinuation:* shock reversal (see below) has been achieved and remains so after finishing 12 hours of SMT [89].

2. *Restarting:* treatment can be restarted within 12 hours if vasopressor requirement increases despite normovolaemia confirmed with hemodynamic monitoring and in case of worsening organ function such as deterioration in gas exchange, increased extravascular lung water extravascular lung water (EVLW), etc., which is considered by the attending physician as a result of a new onset of hyperinflammatory response.

3. *Defining non-responders:* It is expected that there will be patients who do not respond to CytoSorb treatment. Therefore, patients whose clinical condition deteriorates during and within the first 24 hours of CytoSorb therapy will be considered as non-responders

and CytoSorb will not be continued after 24 hours. Non-responsiveness will be defined as:

a) increasing vasopressor requirement not related to hypovolemia or bleeding

b) increasing lactate not associated with acute liver failure

c) when the worsening clinical picture is accompanied by increasing PCT/IL-6 levels despite the likely presence of adequate source control.

Patients' data will be recorded on the electronic case report form (eCRF) at T_0, T_6, T_{12}, T_{24} and then daily until the end of the study period (T_e) that is until 12 hours after shock reversal or up to a maximum of 5 days or until the patient's death, whichever occurs first. Follow-up visits/calls are scheduled on day 28±7 and day 90±7 after randomization.

Primary endpoints:

1. Time to shock reversal: the hours elapsed from T_0 to shock reversal

2. Shock reversal: In previous studies, shock reversal occurred in 65% [87], 38,5% [88] and 65% [89] of patients, within a 24-hour CytoSorb treatment, which has been considered as the most important clinical effect of the therapy. Based on the results "shock reversal" will be defined as:

   a. No further need or reduced (≤10% of the maximum dose) vasopressor requirement (including norepinephrine and/or vasopressin) for 3 hours [88,98] *(In case of multiple vasopressor agents are required, the reduction of one of them (≤10% of the maximum dose) is sufficient if the other agent(s)' dosage does not need to be increased)*

   b. Low doses of vasopressor (≤10% of the maximum dose) may be required to compensate for sedation or to maintain adequate organ perfusion

   c. In case of 2.a) invasive hemodynamic measurements will be performed to confirm hemodynamic stability

   d. In case of 2.a), arterial and central venous blood gas analysis will be performed, to determine arterial lactate levels (the target is ≤2 mmol/l), venous to arterial pCO_2-gap (normal value is: ≤ 7 mmHg) and $ScvO_2$ (increase above 70% at T_e if it was lower than 70% at T_0 or returning into 70-75% by T_e in case it was greater than 75-80% at T_0).

Secondary endpoints:

1. Blood samples will be collected at T_0, T_6, T_{12}, T_{24} and then daily, and the change from T_0 to T_e of the following parameters will be assessed:

    a. inflammatory parameters: 1. PCT, 2. IL-6, 3. CRP, 4. IL-1, 5. IL-1ra, 6. IL-8, 7. IL-10, 8. tumour necrosis factor alpha, 9. syndecan-1, 10. heparan sulphate

    b. arterial lactate levels

2. Change in SOFA score from T_0 to T_e (SOFA score will be assessed at T_0, T_{24} and then daily)

3. Change in EVLW from T_0 to T_e

4. Duration of mechanical ventilation in days (every 24 hours when the patient required the organ support therapy counts as one)

5. Duration of catecholamine requirement in days

6. Duration of renal replacement therapy in days

7. Need for dialysis on day 28±7

8. Need for dialysis on day 90±7

9. Length of stay at the ICU

10. Length of stay at the hospital

11. Survival: ICU

12. Survival: hospital

13. Survival at day 28

14. Survival at day 90

15. Survival: number of days (every finished 24 hours counts one)

16. Adverse events

5.3.9. Adverse and Serious Adverse Events: Definition and Recording

Adverse events will be collected from the start of the intervention period until follow-up. All adverse events (AEs) and device deficiencies including all serious adverse events (SAEs) are collected and documented in the source document and the Adverse event report form during the entire study period, i.e. from the patient's informed consent until the last follow-up visit/call. Dates of the event, the seriousness of the event and the relationship to the study device need to be documented. The Adverse event report form has to be forwarded to the steering committee (SC) and the independent data management board (IDMB). Provided that the adverse event is confirmed by the SC, the national ethics committee needs to be notified (http://www. ett. hu/ tukeb. htm).

5.3.10 Follow-up

A follow-up assessment will be conducted 28±7 days and 90±7 days after randomization using a follow-up letter/e-mail or a phone call. In case the patient or the next-of-kin cannot be reached, medical records will be used to obtain the needed information. At day 28 and 90 survival, need for dialysis and adverse events will be assessed.

5.3.11. Statistical analysis

5.3.11.1. Sample size calculation

Based on the previous case series and the ACESS pilot data the most apparent clinical benefit is expected to be the reduction in norepinephrine requirement; therefore, we chose shock reversal as the most important outcome [87-89]. In the ACESS trial it was found that one single 24-hour treatment resulted in an almost 70% reduction in the required norepinephrine dose. A similar observation was made in a recent case series [87], in which a 50% reduction was found after a 24-hour treatment. Furthermore, in our pilot study, the most profound effect occurred within the first 12 hours of treatment, as far as norepinephrine requirement and PCT level reduction are concerned [91]. Based on these results it is postulated that cytokine removal may be most effective in the first hours of treatment, therefore shock reversal could occur faster in Group-B as compared to Group-C and faster in both groups as in Group-A (controls).

The sample size calculation was based on patient data from the study of Kogelmann et al. [88]. The time of shock reversal was separately calculated for those in whom the first adsorber was changed after 12 hours (n=3), and for those who received therapy for 24-hours each time (n=17) (48 ± 30 hours vs. 68 ± 21, respectively). In a recent prospective RCT on patients with sepsis and septic shock, vasopressors were weaned in 96±40 hours in the control group (n=50) [99].

We considered these differences as clinically relevant and not to be overlooked between the 3 groups. Sample size calculation suggests that 135 patients (1:1:1) will need to be enrolled (45 in each study arm) to confirm or reject the hypothesis for the primary endpoint with a 20% dropout, 80% power and 95% significance level. Non-responders will be handled as dropouts and will continue to receive standard medical therapy.

V.3.11.2. Analysis plan and statistics

Descriptive statistics – mean, median, standard deviation, quartiles and relative frequency – weighted generalized linear model with contrasts (continuous variable) for the primary endpoint, and mixed models (continuous variable), a weighted generalized linear model with

contrasts (continuous variable), relative risk (dichotomous variables) for secondary endpoints. Affiliated statistical analyses will be performed with an error probability of 0.0294 (type-I error probability) for Per Protocol (PP) and Intention-To-Treat (ITT) population. All statistical analyses are performed with R (V. 3.5.2).

V.3.11.3. Interim analysis

Appropriate sample size calculation was not possible due to the lack of available high-quality clinical data [88]. Therefore, it is highly likely that the event rate of shock reversal will occur in substantially less than 100%. In order to adapt the required sample size to maintain statistical power, we decided to allow sample size re-estimation after an interim analysis at the 50% recruitment rate. If no more subjects are needed, early termination will be applied. For this reason, the p-value should be adjusted to diminish the probability of type I error; therefore, the corrected level of significance (p-value) will be 0.0294.

The following rules will be applied:

1) If the treatment in any of the groups proves to be significantly (p<0.0294) less effective than the others and it is already obvious that there is no hope for ascertaining a significant difference between the other two groups, the study will be stopped.

2) If the treatment in any of the groups are significantly (p<0.0294) less effective than the others and it is already visible that there is hope of ascertaining a significant difference between the other two groups, the inferior treatment will be dropped, and the study will be continued with the remaining two arms.

3) If any of the groups proves to be significantly (p<0.0294) more effective than the others, the study will be discontinued.

V.3.11.4. Study populations

Safety Analysis Set (SAS, all patients enrolled in the study), Per Protocol Set (PPS, all enrolled patients who finished the study conforming to the requirements of the study protocol) and Intention to Treat (ITT, all randomized participants who start on a treatment, excluding consent withdrawals) will be performed.

V.3.11.5. Withdrawal of a subject from PPS

Patients will not be included in the per-protocol analysis if: (1) during the trial any exclusion criteria is met; (2) a serious adverse effect occurs; (3) data required for the primary endpoints are missing; or (4) serious medical conditions not related to septic shock occur (eg, myocardial infarction, stroke, etc),(5) commencement of CytoSorb more than 2 hours after randomization

(6) the duration of CytoSorb therapy did not reach 24 hours or the patient died within 24 hours from enrolment in Groups B and C.

5.4 Ethics and dissemination

5.4.1 Ethical and legal considerations

This clinical study will be conducted following the Declaration of Helsinki. It will be conducted in compliance with the protocol, good clinical practice (GCP) (2001/20/EEC, CPMP/ICH/135/95), designated standard operating procedures, and local laws and regulations relevant to the country of conduct. This protocol was approved by the Scientific and Research Ethics Committee of the Hungarian Medical Research Council (OGYÉI/65049/2020). The study was registered in the ClinicalTrials.gov Protocol Registration and Results System NCT04742764.

5.4.2. Data management

IDMB will handle data, eCRF will be applied. The Investigator will guarantee that the data in the eCRF are accurate, complete and clear. Data Management Plan (DMP) will detail the data handling during and after the trial. Data from completed eCRFs will be assessed under the direction of the Data Manager at IDMB according to a Data Cleaning Plan (DCP). In case of missing, improbable or inconsistent data in the eCRFs will be referred back to the Investigator using a data query form (DQF).

5.4.3. Publication policy

Centres recruiting more than 10 patients can nominate two authors to the authorship list. Every additional 10 patients will give the opportunity to nominate an additional author. According to a new translational medicine cycle model [100,101], we plan to summarise and communicate our findings to all the members of the cycle and to use them in everyday practice.

5.5 Trial organisation, committees and boards

DECRISS was designed by the Centre for Translational Medicine at the Medical School of University of Pécs and will be coordinated by the Centre for Translational Medicine at University of Pécs.

5.5.1. Steering committee

The SC will be led by ZM (intensive care specialist). The members will be AK (medical doctor), MM (intensive care specialist), KK (intensive care specialist), LB (intensive care

specialist), BE (clinical research specialist) and PH (clinical pharmacologist). SC will discuss all important questions including adverse events and the dropouts during the study.

5.5.2. Participating centers

The trial will start in 3 centres (University of Pécs, Pécs, Hungary; Hospital Emden, Emden, Germany; Poznan University of Medical Sciences, Poznan, Poland), then the trial is open for other centres. The center will be assessed by the IDMB and will be presented to the SC. The SC has the right to decide whether the center meets the required quality to join the study. Compulsory requirements for a centre are: (1) it needs to treat at least 50 patients with septic shock a year; (2) it needs to have all the equipment required for the study; (3) besides the regular medical team, the centre has to have human resources (doctors, nurse/administrator) available for the trial; (4) before study commencement a meeting will be held; at least one person/center needs to attend who completed a GCP course. All the details of the study protocol will be discussed thoroughly. A letter of intent needs to be sent to the corresponding author by email in case of a center wishing to participate in the study.

5.6 Discussion

To our best knowledge, this is the first multi-centre clinical trial, assessing the dosing of CytoSorb treatment alone as well as in combination with standard CRRT and compared to standard treatment in patients with refractory septic shock.

5.6.1. Strengths and limitations of the study

Study design intends to aim a relatively homogeneous group of patients in order to overcome the drawbacks of previous large sepsis trials, that resulted in non-significant findings [102,103]. Therefore, in addition to the broad term Sepsis-3 definition of septic shock [93], other prerequisites will be incorporated into the inclusion criteria such as the minimum APACHE II score, norepinephrine dose, PCT levels, mechanical ventilation, etc.

Most sepsis randomized trials applied hard endpoints to evaluate the effects of a single treatment, such as mortality, length of hospital stay or ventilator and vasopressor-free days [104,105]. However, this approach has been criticised by several internationally acknowledged experts for numerous reasons (i.e., the heterogeneity of the patients based on the severity, the onset of the disease, the endpoints) [106,107]. One of the possible solutions is to design trials with physiologic primary endpoints [106]. CytoSorb therapy has been shown to reduce the need of vasopressor support in several case series and studies [87,88,103]. Therefore, we decided to choose "shock reversal" as our primary outcome measure. Furthermore, it is not only the

occurrence of shock reversal but the "time to shock reversal" from the start of treatment that is of particular interest in the current study.

The current practice of applying one adsorber for 24 hours is an arbitrary one, based on the company's recommendation and theoretical considerations. Nevertheless, several centres change the cartridge earlier (most often after 12 hours), based simply on their experience, but no study investigated this issue yet. Therefore, the current study should have important results to determine if there is any difference in the effects when the adsorber is "fresh" as compared to its later performance. For this purpose, we designed a 3-arm trial comparing standard therapy to 12 and 24 hours CytoSorb adsorber changing strategies to assess, which leads to faster shock reversal.

Another strength of our study is that in addition to well-acknowledged parameters indicating organ dysfunction a specific issue in the current trial will be the investigation of the evolution of EVLW during the treatment. Extravascular lung water is an indicator of increased pulmonary capillary permeability, often due to systemic inflammation [108]. There is one case report indicating that CytoSorb therapy may have protective effects on vascular barrier function [109]. As mechanical ventilation is also an inclusion criterium, our study may provide further insight into the relationship between cytokine removal and pulmonary function.

Although it has been shown in several experimental models that CytoSorb removes cytokines but clinical data, especially from prospective randomised trials are missing. An array of inflammatory markers and mediators are planned to be determined during the study, which can provide a further understanding of the removal properties of the device.

One of the limitations of the study is that shock reversal per se has not been used as a primary outcome, therefore sample size calculation was based on data from a limited number of patients and a heterogeneous population of septic patients. Another potential limitation is the heterogeneity of the study population. Patients with septic shock both due to medical and surgical origin will be included, while the inflammatory response might be different in the 2 groups [110]. However, currently available clinical data indicate that both patient populations can benefit similarly from the therapy [88]. Another concern regarding heterogeneity could be that CytoSorb treatment will be applied on its own as hemoperfusion and in combination with CRRT. However, we have no data yet, neither pro nor con that these two therapies interact in any way. For safety measures, we decided to treat patients in both CytoSorb-treated groups for at least 24 hours – as pre-current practice –, therefore, we will not be able to assess sustained shock reversal after 12 hours during the first 24 hours.

6. Conclusions and new discoveries

Extracorporeal therapies may improve patients' outcome, however, based on previous studies their role is still controversial in our examined patient populations. To the best of our knowledge, no network meta-analysis – which studies liver support therapies in acute and hyperacute liver failure patients – had been published before. With this method we were able to compare liver support therapies to each other as well as to standard medical therapy.

The concept of conducting randomized controlled trials in critically ill patients in intensive care units was criticized by various experts. However, these studies carry the highest level of evidence, therefore, we attempted to correct the mentioned issues in our study. We designed the first prospective, randomized, controlled, multi-centre trial with a relatively homogeneous group of septic shock patients, applying physiologic parameters as our primary endpoints, to investigate the efficacy, safety and the appropriate length of CytoSorb therapy.

6.1 Liver support therapies in hyperacute and acute liver failure

Based on our results, the following new statements can be made:

1. BioLogic-DT was ranked as the best treatment for in-hospital mortality and worse for HE, however this modality is not applied in clinical practice anymore, therefore – from the available treatments – MARS therapy was the best option in reducing in-hospital mortality.
2. Considering HE, the SUCRA rankings indicate that the ELAD therapy has the highest probability to reduce the worsening of HE.
3. However, with no statistically significant results, there is no solid evidence that the differences that we can see from the SUCRA values are due to chance or the interventions truly differ in their effects, therefore, good-quality randomized trials are needed on currently available and new blood purification modalities to define the role of extracorporeal liver support in patients with acute liver failure.

6.2 Extracorporeal cytokine removal in patients with septic shock

New statements cannot be drawn, but the novelty of the trial design is the following:

1. We designed a 3-arm trial comparing standard therapy to 12 and 24 hours CytoSorb adsorber changing strategies to assess, which causes faster shock reversal - which has not been investigated before.

2. Instead of the internationally criticised hard endpoints in sepsis trials, physiologic outcomes were chosen as our primary endpoints.

3. A specific issue in our trial will be the investigation of the evolution of EVLW during the treatment, therefore this study may provide further insight in the relationship between cytokine removal and pulmonary function.

7. Financial support

8. Author's own contribution

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The author performed the database search and read the articles for eligibility, collected the data from the articles to the study database, performed the bias analysis and quality assessment, completed the PRISMA checklist. The author drafted the majority of the manuscript and edited the tables and figures.

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The author studied the available literature, played a key role in the study design, wrote the majority of the manuscript and edited the study figure.

9. Acknowledgements

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109. David, S., Thamm, K., Schmidt, B.M.W., Falk, C.S. & Kielstein, J.T. Effect of extracorporeal cytokine removal on vascular barrier function in a septic shock patient. *Journal of intensive care* **5**, 12 (2017).

110. Trasy, D.*, et al.* Early procalcitonin kinetics and appropriateness of empirical antimicrobial therapy in critically ill patients: A prospective observational study. *Journal of critical care* **34**, 50-55 (2016).

11. Appendix

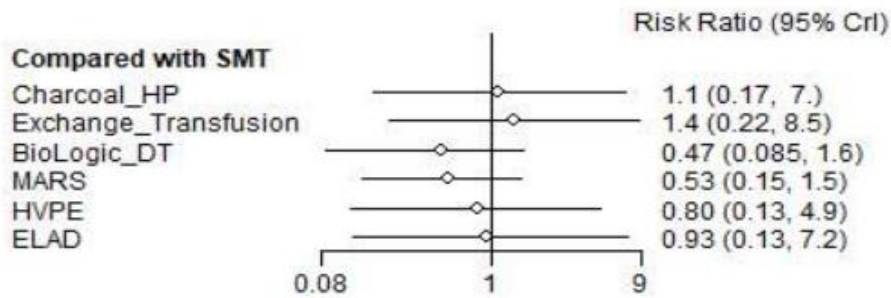Figure S1. Forest plot for in-hospital mortality, interventions compared to SMT

Figure S2. Forest plot for in-hospital mortality, interventions compared to HVPE
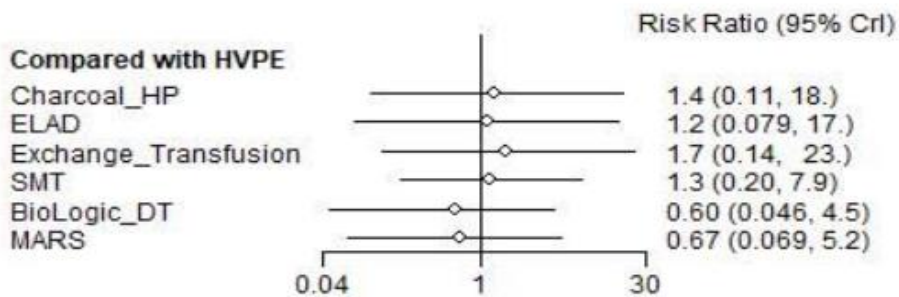

Figure S3. Forest plot for in-hospital mortality, interventions compared to ELAD
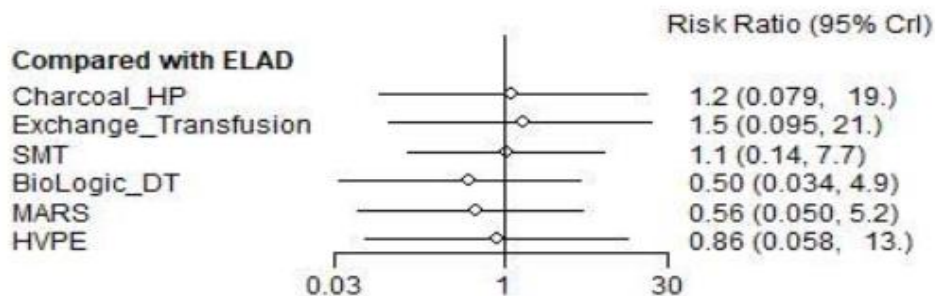

Figure S4. Forest plot for in-hospital mortality, interventions compared to charcoal-hemoperfusion
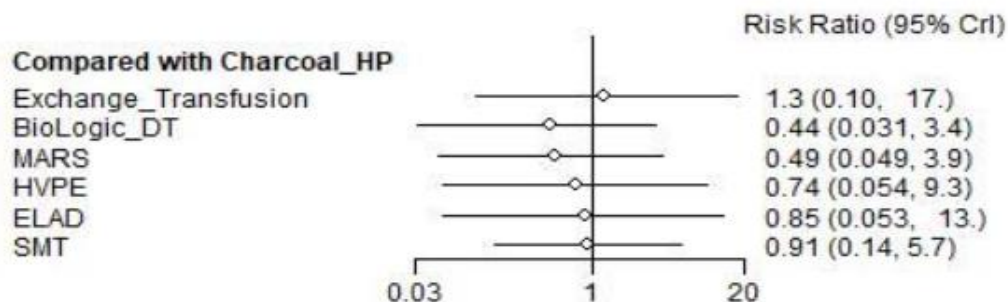
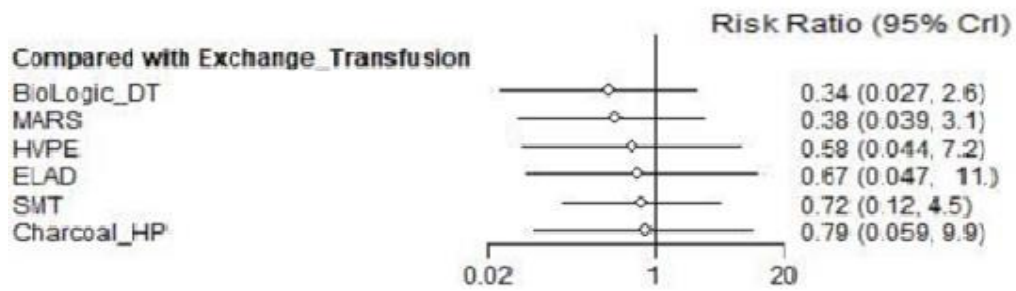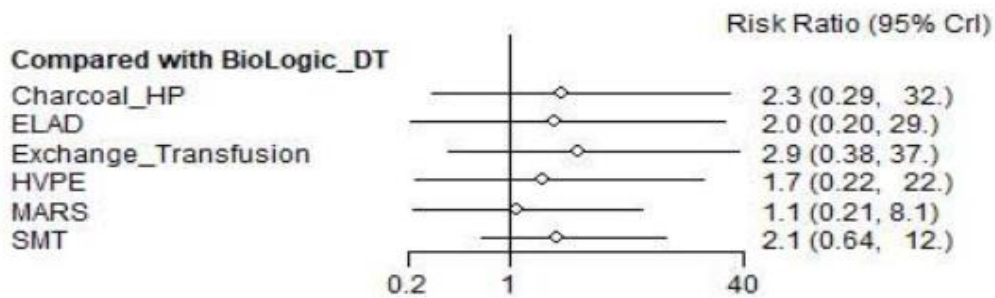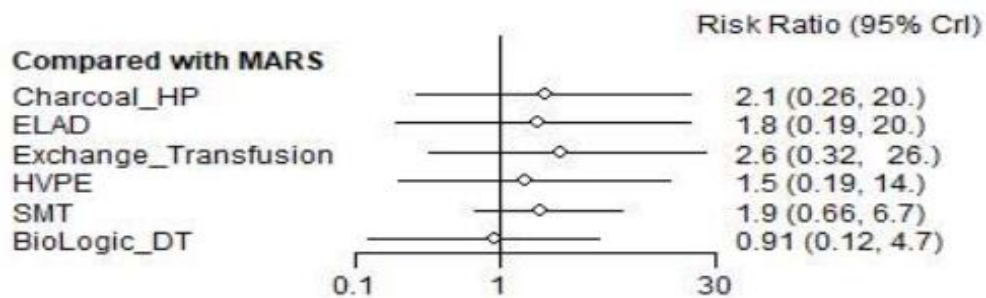Figure S5. Forest plot for in-hospital mortality, interventions compared to exchange-transfusion

Risk Ratio (95% CrI)

Compared with Exchange_Transfusion

BioLogic_DT	0.34 (0.027, 2.6)
MARS	0.38 (0.039, 3.1)
HVPE	0.58 (0.044, 7.2)
ELAD	0.67 (0.047, 11.)
SMT	0.72 (0.12, 4.5)
Charcoal_HP	0.79 (0.059, 9.9)

0.02   1   20

Figure S6. Forest plot for in-hospital mortality, interventions compared to BioLogic-DT

Risk Ratio (95% CrI)

Compared with BioLogic_DT

Charcoal_HP	2.3 (0.29, 32.)
ELAD	2.0 (0.20, 29.)
Exchange_Transfusion	2.9 (0.38, 37.)
HVPE	1.7 (0.22, 22.)
MARS	1.1 (0.21, 8.1)
SMT	2.1 (0.64, 12.)

0.2   1   40

Figure S7. Forest plot for in-hospital mortality, interventions compared to MARS

Risk Ratio (95% CrI)

Compared with MARS

Charcoal_HP	2.1 (0.26, 20.)
ELAD	1.8 (0.19, 20.)
Exchange_Transfusion	2.6 (0.32, 26.)
HVPE	1.5 (0.19, 14.)
SMT	1.9 (0.66, 6.7)
BioLogic_DT	0.91 (0.12, 4.7)

0.1   1   30

54

Figure S8. Cumulative ranking curves of in-hospital mortality
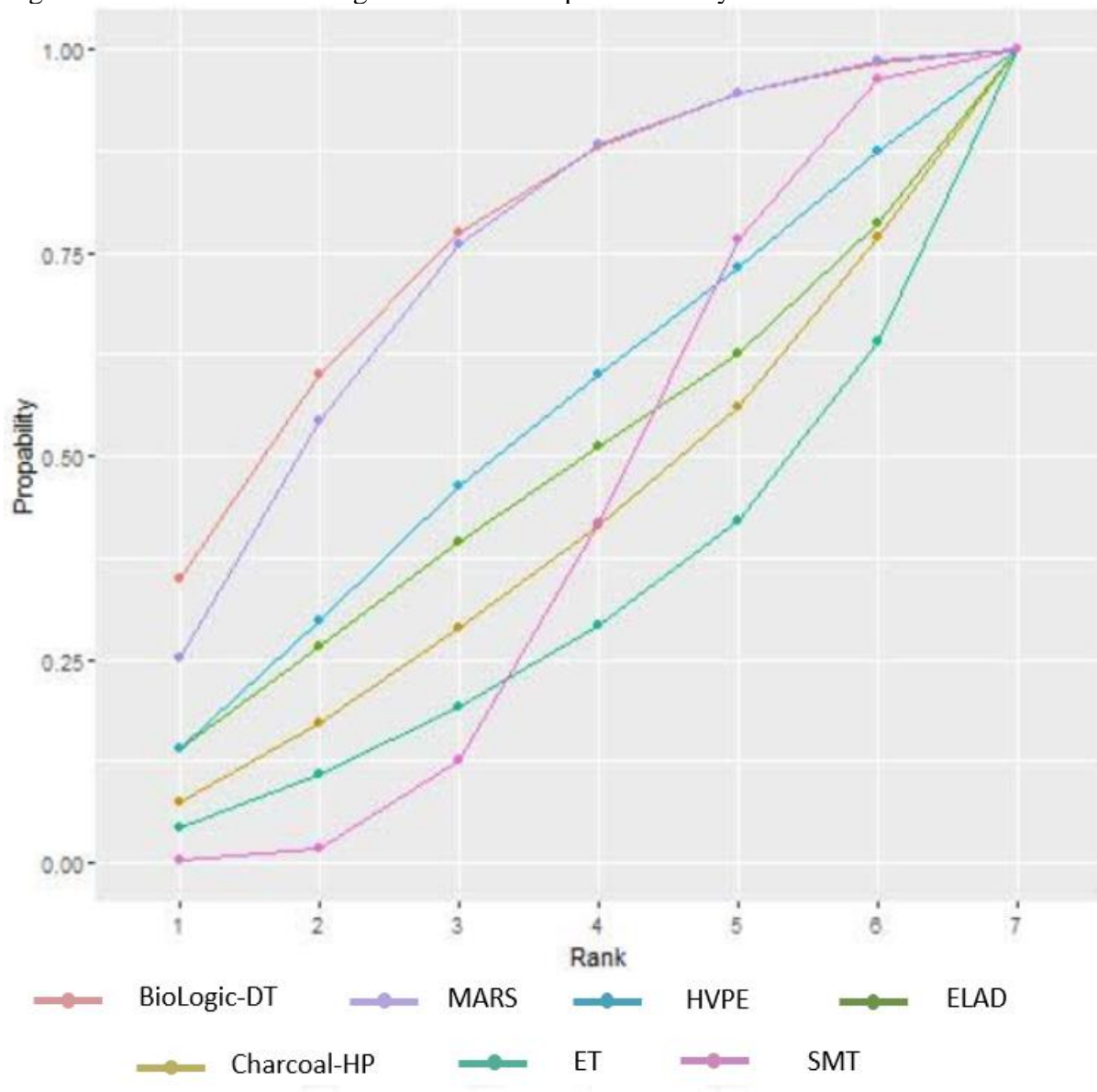
Figure S9. Cumulative ranking curves of in-hospital mortality in nonparacetamol-poisoned
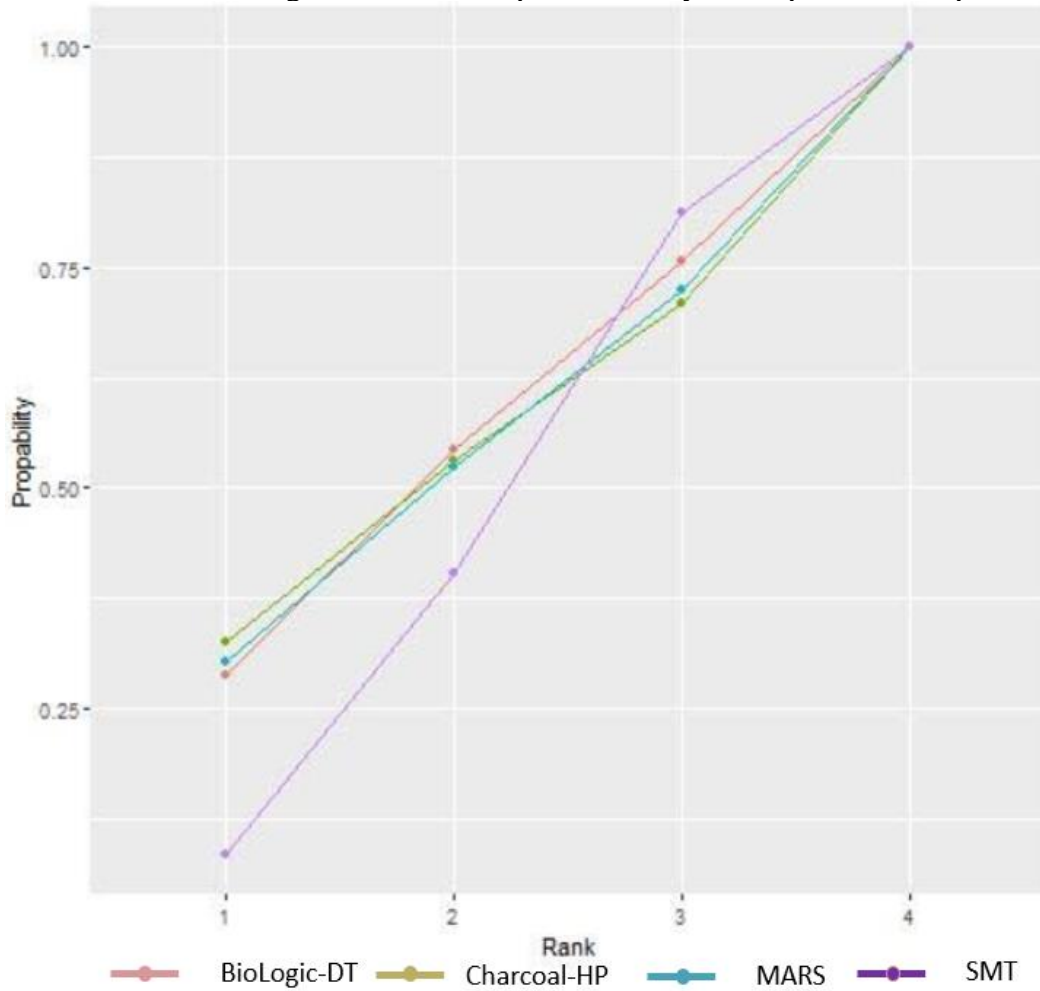
patients

Figure S10. Forest plot for in-hospital mortality in nonparacetamol-poisoned patients, interventions compared to charcoal-hemoperfusion
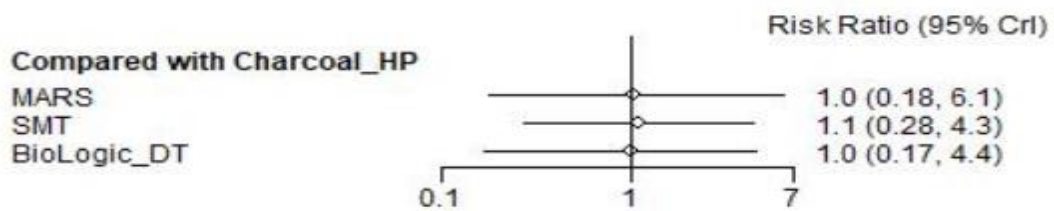
Figure S11. Forest plot for in-hospital mortality in nonparacetamol-poisoned patients, interventions compared to MARS
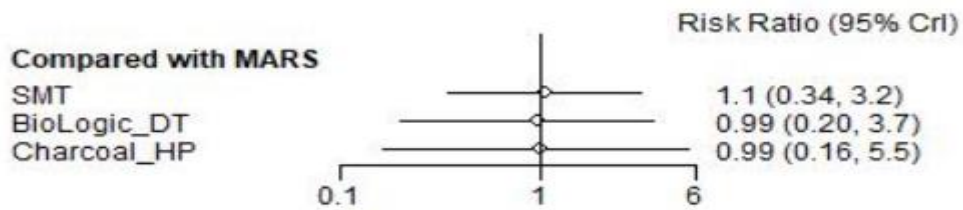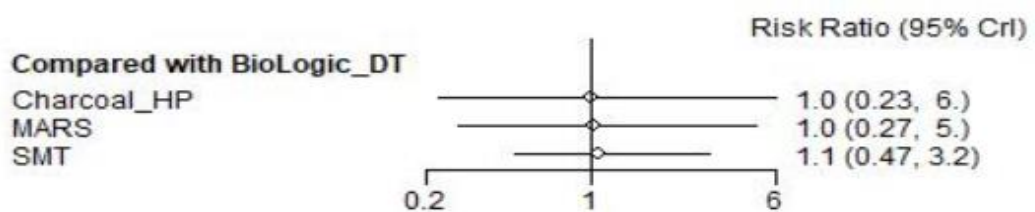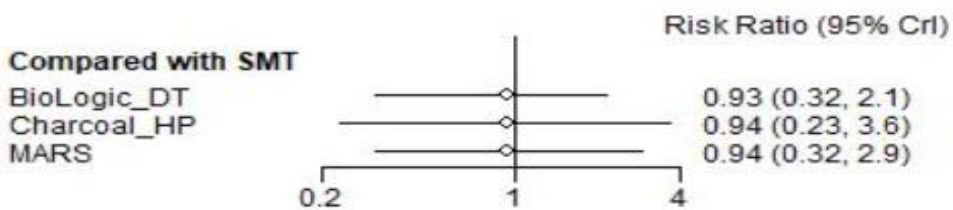
Risk Ratio (95% Crl)

Compared with MARS

SMT		1.1 (0.34, 3.2)
BioLogic_DT		0.99 (0.20, 3.7)
Charcoal_HP		0.99 (0.16, 5.5)

0.1   1   6

Figure S12. Forest plot for in-hospital mortality in nonparacetamol-poisoned patients, interventions compared to BioLogic-DT

Risk Ratio (95% Crl)

Compared with BioLogic_DT

Charcoal_HP		1.0 (0.23, 6.)
MARS		1.0 (0.27, 5.)
SMT		1.1 (0.47, 3.2)

0.2   1   6

Figure S13. Forest plot for in-hospital mortality in nonparacetamol-poisoned patients, interventions compared to standard medical therapy

Risk Ratio (95% Crl)

Compared with SMT

BioLogic_DT		0.93 (0.32, 2.1)
Charcoal_HP		0.94 (0.23, 3.6)
MARS		0.94 (0.32, 2.9)

0.2   1   4

Figure S14. Cumulative ranking curves of hepatic encephalopathy
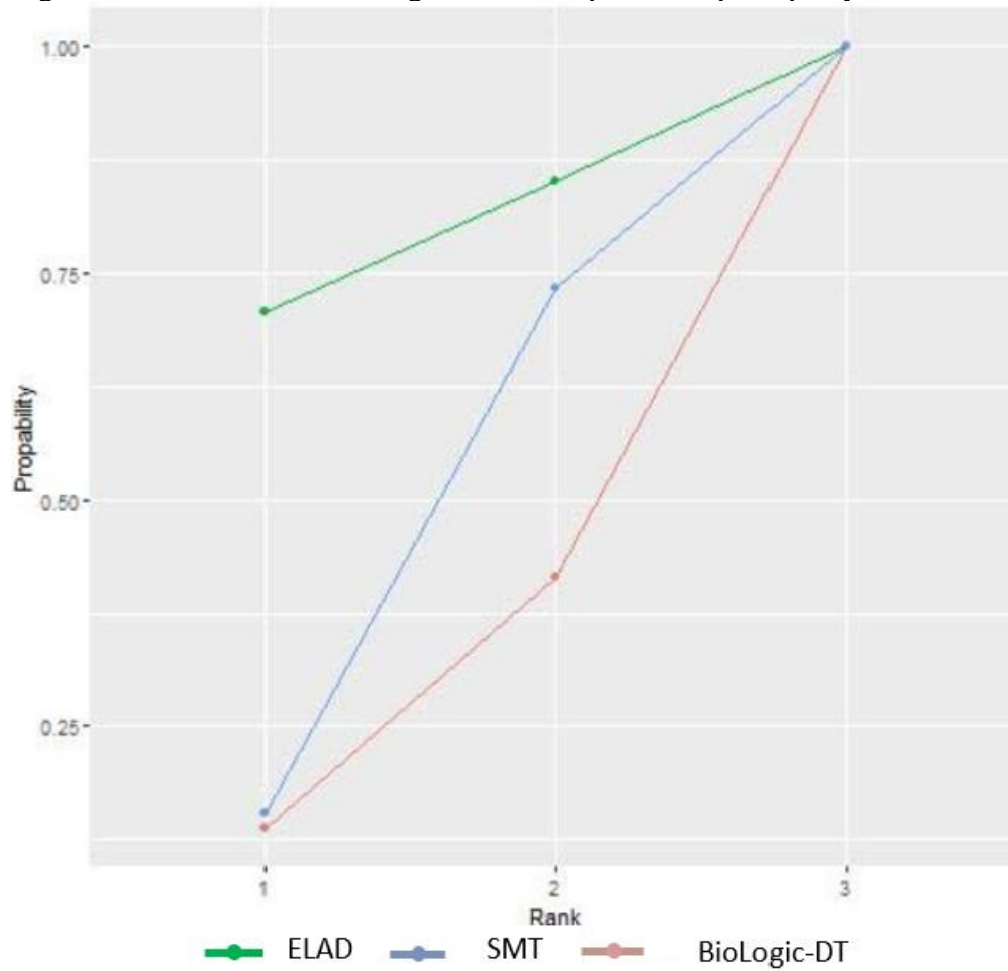
Figure S15. Forest plot hepatic encephalopathy, interventions compared to standard medical therapy
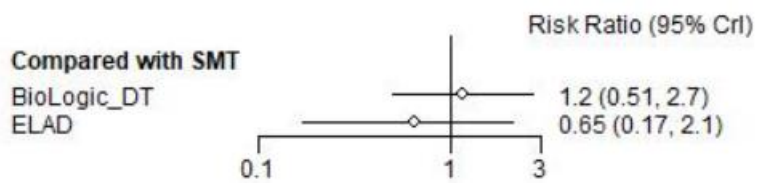
Figure S16. Forest plot hepatic encephalopathy, interventions compared to BioLogic-DT
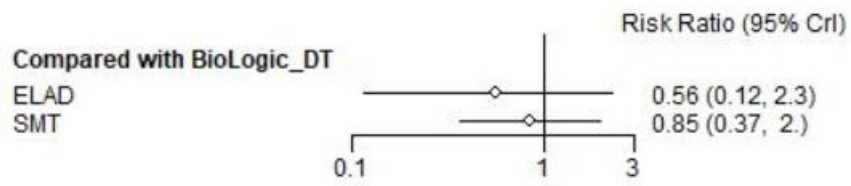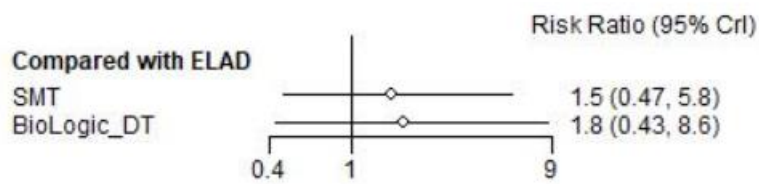
Risk Ratio (95% CrI)

Compared with BioLogic_DT

ELAD	0.56 (0.12, 2.3)
SMT	0.85 (0.37, 2.)

0.1   1   3

Figure S17. Forest plot hepatic encephalopathy, interventions compared to ELAD

Risk Ratio (95% CrI)

Compared with ELAD

SMT	1.5 (0.47, 5.8)
BioLogic_DT	1.8 (0.43, 8.6)

0.4   1   9

Figure S18. Risk-of-bias assessment

Figure S19. Risk-of-bias assessment of mortality outcomes, broken down to tools, shown in percentage
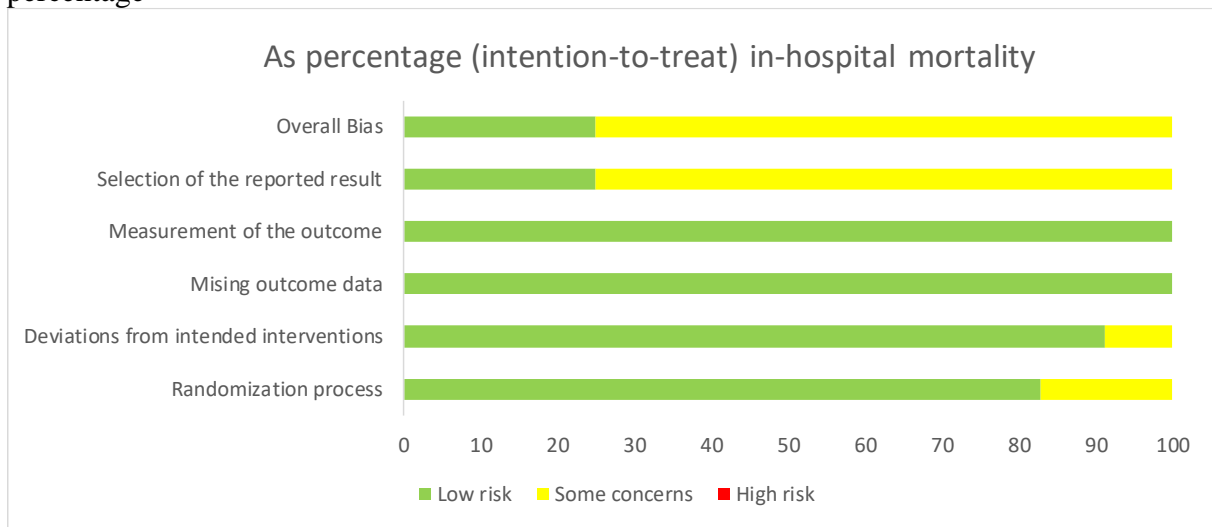

As percentage (intention-to-treat) in-hospital mortality

Figure S20. Risk-of-bias assessment of hepatic encephalopathy, broken down to tools, shown in percentage

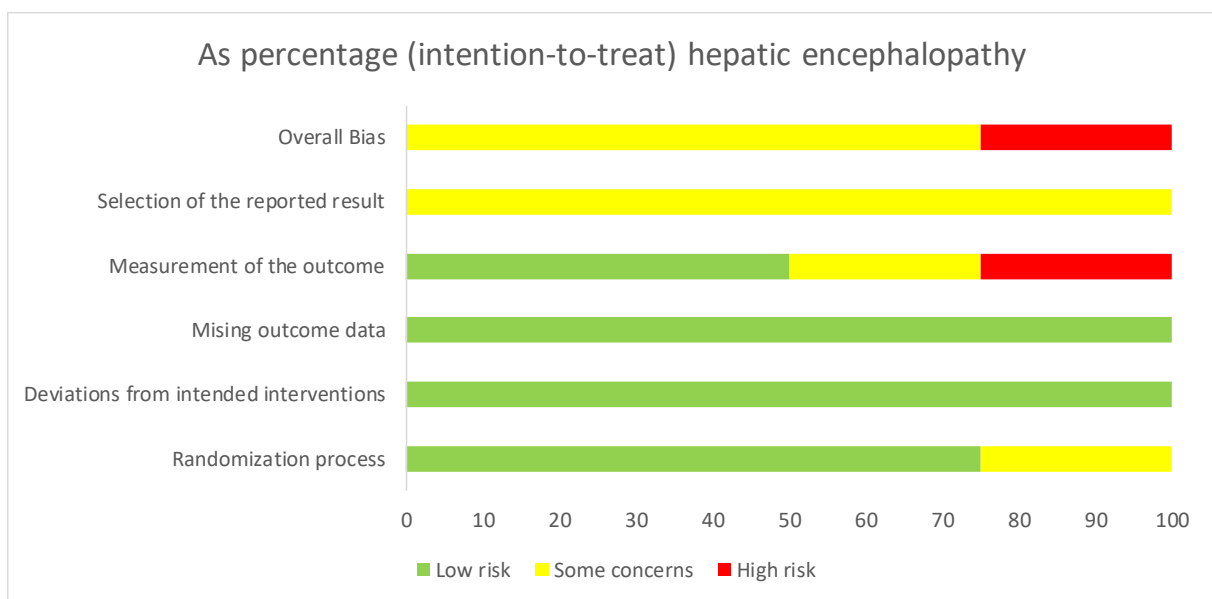As percentage (intention-to-treat) hepatic encephalopathy

Table S1 Summary of findings table of in-hospital mortality

	BioLogic-DT vs SMT	MARS vs SMT	HVPE vs SMT	ELAD vs SMT	Charcoal-HP vs SMT	ET vs SMT
Study limitations[1]	↓	↓	-	↓	↓	↓
Comments	some concerns	some concerns	low risk of bias	some concerns	some concerns	some concerns
Imprecision[2]	↓↓	↓↓	-	↓↓	↓↓	↓↓
Inconsistency[3]	-	-	-	-	-	-
Indirectness[4]	↓	↓	-	-	-	-
Comments	different study populations, HD was performed at the physician's discretion (Ellis, 1999) or was not allowed (Wilkinson, 1998; Hughes 1994)	different study populations, HD was performed at the physician's discretion				
Publication bias[5]	-	-	-	-	-	-
GRADE	very low quality ⊕○○○	very low quality ⊕○○○	high quality ⊕⊕⊕⊕	very low quality ⊕○○○	very low quality ⊕○○○	very low quality ⊕○○○

The table includes information from 11 studies and 479 patients

[1] Detailed information on study limitations can be found in *Figure S18-20*

[2] Imprecision was judged based on the sample size calculation of the article of Larsen, 2016.

[3] Node splitting could not be performed due to network geometry, inconsistency could not be tested.

[4] Indirectness could not be judged where there was only one head-to-head trial between two interventions

[5] Publication bias was judged by the 'comparison-adjusted' funnel plot and Egger's test (*Figure S21*), asymmetry is not significant thus downgrading was not necessary

Table S2 Summary of findings table of in-hospital mortality in nonparacetamol-poisoned patients

	BioLogic-DT vs SMT	MARS vs SMT	Charcoal-HP vs SMT
Study limitations[1]	↓	↓	↓
Comments	some concerns	some concerns	some concerns
Imprecision[2]	↓↓	↓↓	↓↓
Inconsistency[3]	-	-	-
Indirectness[4]	↓	↓	-
Comments	different study populations, HD was performed at the physician's discretion (Ellis, 1999) or was not allowed (Wilkinson, 1998; Hughes 1994)	different study populations, HD was performed at the physician's discretion	
Publication bias[5]	-	-	-
GRADE	very low quality ⊕○○○	very low quality ⊕○○○	very low quality ⊕○○○

The table includes information from 6 studies and 150 patients

[1] Detailed information on study limitations can be found in Figure S18-20

[2] Imprecision was judged based on the sample size calculation of the article of Larsen, 2016.

[3] Node splitting could not be performed due to network geometry, inconsistency could not be tested.

[4] Indirectness could not be judged where there was only one head-to-head trial between two interventions

[5] Due to the low number of articles funnel plot and Egger's test could not be performed

Table S3 Summary of findings table of hepatic encephalopathy

	BioLogic-DT vs SMT	ELAD vs SMT
Study limitations[1]	↓	↓↓
Comments	some concerns	high risk of bias
Imprecision[2]	↓↓	↓↓
Inconsistency[3]	-	-
Indirectness[4]	↓	-
Comments	different applied neurological tests/scales, no detailed information on the implementation, the result is greatly affected by the assessor	
Publication bias	-	-
GRADE	very low quality ⊕◯◯◯	very low quality ⊕◯◯◯

The table includes information from 4 studies and 47 patients

[1] Detailed information on study limitations can be found in Figure S18-20

[2] Imprecision was judged based on the sample size calculation of the article of Larsen, 2016.

[3] Node splitting could not be performed due to network geometry, inconsistency could not be tested.

[4] Indirectness could not be judged where there was only one head-to-head trial between two interventions

[5] Due to the low number of articles funnel plot and Egger's test could not be performed

Figure S21 'Comparison-adjusted' Funnel plot and Egger's test of in-hospital mortality

I.

scientific reports
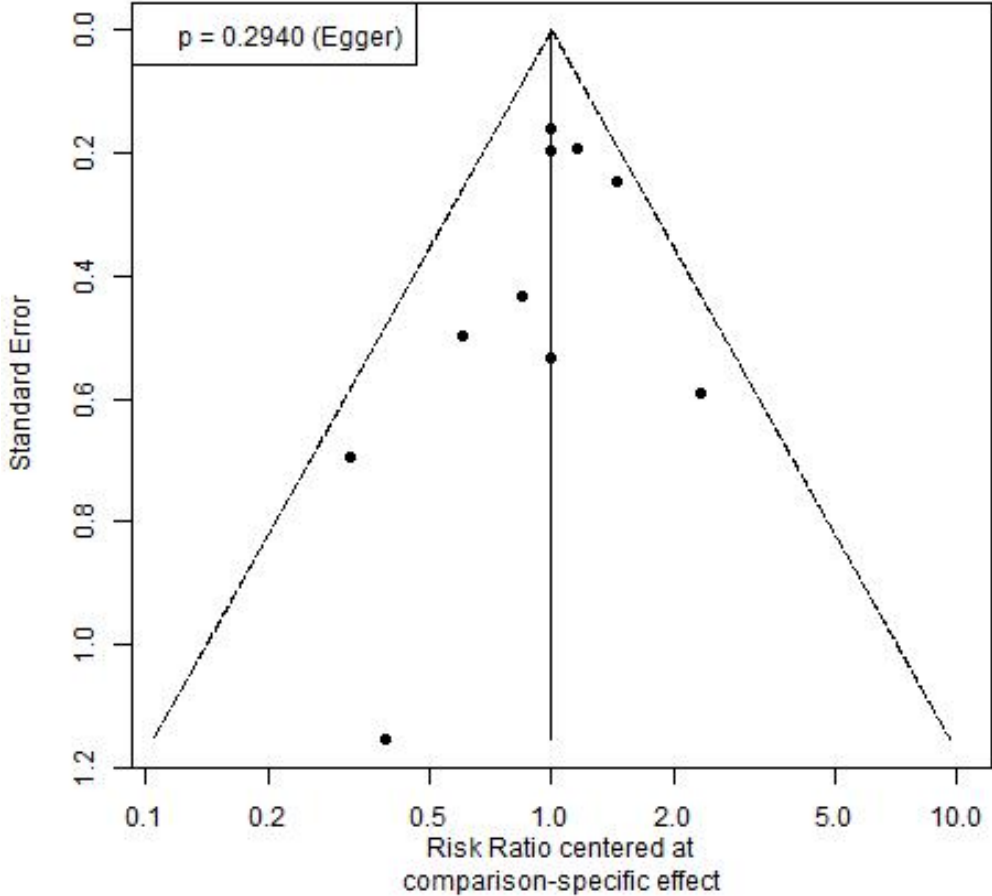
OPEN

Efficacy and safety of liver support devices in acute and hyperacute liver failure: a systematic review and network meta-analysis

Anna Kanjo[1,2,3], Klementina Ocskay[1], Noémi Gede[1], Szabolcs Kiss[1,3], Zsolt Szakács[1,4], Andrea Párniczky[1,2,3], Steffen Mitzner[5], Jan Stange[5], Péter Hegyi[1,4] & Zsolt Molnár[1,3,6]✉

Acute liver failure (ALF) is a potentially life-threatening condition. Liver support therapies can be applied as a bridging-to-transplantation or bridging-to-recovery; however, results of clinical trials are controversial. Our aim was to compare liver support systems in acute and hyperacute liver failure with network meta-analysis. After systematic search, randomized controlled trials (RCT) comparing liver support therapies in adults with acute or hyperacute liver failure were included. In-hospital mortality was the primary outcome, the secondary outcomes were hepatic encephalopathy and mortality-by-aetiology. A Bayesian-method was used to perform network meta-analysis and calculate surface under the cumulative ranking curve (SUCRA) values to rank interventions. Eleven RCTs were included. BioLogic-DT and molecular adsorbent recirculating system (MARS) resulted in the lowest mortality (SUCRAs: 76% and 73%, respectively). In non-paracetamol-poisoned patients, BioLogic-DT, charcoal hemoperfusion and MARS may be equally efficient regarding mortality (SUCRAs: 53%, 52% and 52%, respectively). Considering hepatic encephalopathy, extracorporeal liver assist device (ELAD) may be the most effective option (SUCRA: 78%). However, in pairwise meta-analysis, there were no statistically significant differences between the interventions in the outcomes. In conclusion, MARS therapy seems to be the best available option in reducing mortality. Further research is needed on currently available and new therapeutic modalities. (CRD42020160133).

Abbreviations

AASLD	American Association for the Study of Liver Diseases
AC	Anticoagulant
ACLF	Acute-on-chronic liver failure
ALF	Acute liver failure
AO	Acetaminophen overdose
ARDS	Acute respiratory distress syndrome
BAL	Bioartificial liver
CrI	Credible interval
Charcoal-HP	Charcoal-hemoperfusion
EASL	European Association for the Study of the Liver
ECLS	Extracorporeal liver support
ELAD	Extracorporeal Liver Assist Device
ET	Exchange transfusion
FHF	Fulminant hepatic failure
gr	Grade
GRADE	Grading of Recommendations Assessment, Development, and Evaluation

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HD	Hemodialysis
HE	Hepatic encephalopathy
HELLP-syndrome	Haemolysis, elevated liver enzymes, low platelet count
HVPE	High-volume plasma exchange
IL-6	Interleukin 6
max	Maximum
MARS	Molecular adsorbent recirculating system
PICO	P: patients I: intervention C: comparison O: outcome
PNF	Primary nonfunction following liver transplantation
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
RCT	Randomized controlled trials
RoB2	Cochrane risk-of-bias tool for randomised trials
RR	Risk ratio
SD	Standard deviation
SHF	Subfulminant hepatic failure
SMT	Standard medical therapy
SUCRA	Surface under the cumulative ranking curves
TNF α	Tumor necrosis factor alpha
TRALI	Transfusion-related acute lung injury
UK	United Kingdom
USA	United States of America

Acute and hyperacute liver failure are potentially life-threatening conditions that can lead to multiorgan failure[1,2], affecting one one and six per million people every year in developed countries[3] with mortality rates of 25–50%[4–6]. The main causes of acute and hyperacute liver failure are drugs—especially paracetamol overdose (46–65%)—and viruses (29–77%), other etiologies are less frequent (11–23%) like mushroom poisoning, Budd-Chiari syndrome, Wilson-disease or HELLP-syndrome[6,7]. Due to the impaired synthetic and detoxification capacities, coagulopathy, jaundice and hepatic encephalopathy may develop[8]. In hyperacute liver failure considerably elevated transaminase levels and severe coagulopathy can be observed with slightly or not increased bilirubin levels[3]. Patients with hyperacute liver failure have a greater possibility to spontaneously recover without liver transplantation[3].

Extracorporeal liver support systems (ECLS) can be used to aid the liver's detoxification function by removing albumin-bound toxins and water-soluble substances[9]. Furthermore, bioartificial liver support therapies that contain hepatocytes can provide synthetic functions as well[10]. In liver failure when there is a potential for recovery, liver support systems amend the supportive care until the regeneration of the liver. In other cases, the definitive therapy of liver failure is liver transplantation—which is expensive and restricted by the number of organs available—however, liver support therapy can keep these patients alive until a suitable organ is found[11]. Considering the effectiveness of these therapies the results of clinical trials are controversial, thus, currently they are not recommended by thy European Association for the Study of the Liver (EASL) Clinical Practical Guidelines or the American Association for the Study of Liver Diseases (AASLD) Practice Guidelines outside of clinical trials in acute or hyperacute liver failure[12,13].

In former meta-analyses in this field, the different interventions were considered equivalent and pooled together in comparison with standard medical therapy (SMT)[11,14–16].
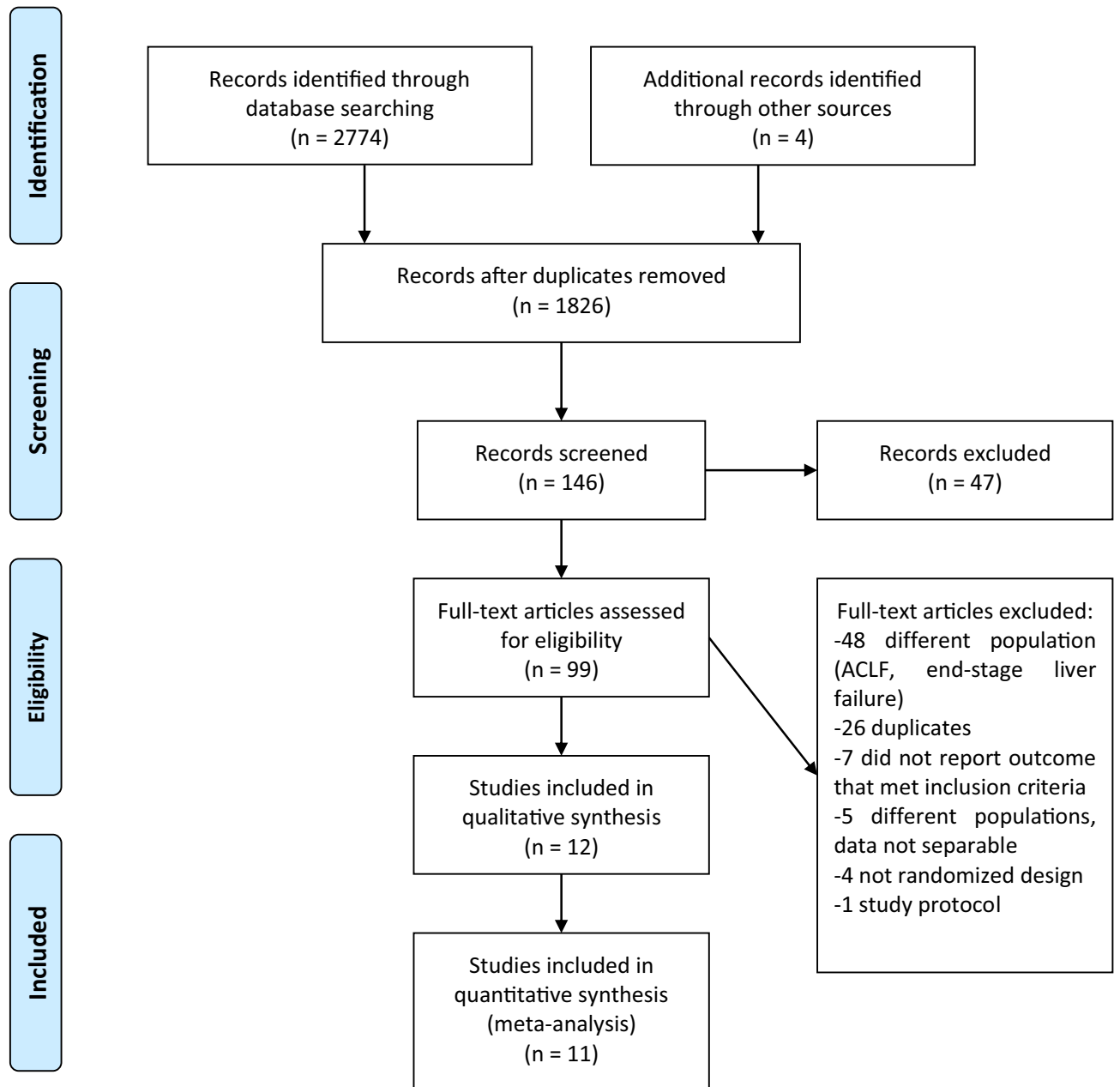
In conventional meta-analyses two interventions can be compared, however when multiple alternatives exist, network meta-analyses can provide results in a single analysis based on direct and indirect (no head-to-head trials conducted between the interventions before) comparisons as well[17]. Therefore, we decided to perform a network meta-analysis, in which we are able to assess the different liver support systems' efficacy and safety in acute and hyperacute liver failure. With the statistical methods of network meta-analysis, we (1) compare the interventions to each other and (2) rank them, to choose the best option regarding the outcome.

Results

Selection process and study characteristics. Through the initial searches 2774 citations were identified. After reading the titles and abstracts, 99 articles remained for further assessment. 12 articles could be included for qualitative synthesis and 11 for network meta-analysis (Fig. 1). In the article of Demetriou et al., there were no data reported that we could include in the quantitative synthesis concerning mortality or hepatic encephalopathy[18].

All studies included in the quantitative synthesis are parallel randomized controlled trials comparing liver support systems to SMT, published between 1973 and 2016, including 479 patients. Overall, 243 patients were assigned to a liver support therapy and 236 to SMT. In four of the studies BioLogic-DT[19–22] (BioLogic-DT has been redesigned and now called Liver Dialysis Device[16].), in three of them the Molecular Adsorbent Recirculating System (MARS) was applied[23–25]. Through the systematic search we found one study from each modalities analysing high-volume plasma exchange[26], exchange transfusion[27], Extracorporeal Liver Assist Device (ELAD)[28] and charcoal hemoperfusion[29]. Bioartificial modalities are ELAD therapy (Vital Therapies Inc., San Diego, CA, USA) and HepatAssist device (Circe Biomedical Inc., Lexington, MA, USA). HepatAssist device was included only in the systematic review.

Seven studies reported detailed demographic characteristics. The mean age was 38.8 years, two studies included adolescents as well. About half of the sample population were female (55.8%—226 of 405). The majority of the studies included patients with different etiologies, however, the distribution of the different etiologic

Figure 1. Study selection process. PRISMA flowchart containing results of systematic search and article selection. ACLF, acute-on-chronic liver failure.

factors was similar to the general population. Seven RCTs recruited patients across Europe (58%), three in the USA (25%) and 2 multicentric trials recruited patients at the study sites across continents (17%) (Table 1).

In-hospital mortality. The network (Fig. 2) includes eleven studies. All liver support systems were compared to standard medical therapy.

The SUCRA values (Fig. 3) indicate that BioLogic-DT and MARS are most likely to result in the lowest mortality. However, the results of the analysis presented in the league table (Table 2) show that there were no statistically significant differences between the interventions.

Secondary outcomes. The networks of in-hospital mortality among nonparacetamol-poisoned patients and hepatic encephalopathy are depicted in Supplementary Fig. S9 and S16.

The SUCRA values show that BioLogic-DT, charcoal hemoperfusion and MARS may be equally efficient to decrease mortality (53%, 52% and 52%, respectively) while SMT seems less effective (43%) in the nonparacetamol-poisoned patient population (Supplementary Fig. S11). Considering hepatic encephalopathy, the SUCRA rankings indicate (Supplementary Fig. S18) the ELAD therapy has the highest probability to reduce the worsening of hepatic encephalopathy while BioLogic-DT seems noticeably less appealing than SMT or ELAD (78%, 44%

Study	Country	Population	Aetiology	Intervention (Nº of patients)	Nº of sessions	Ancillary hemodialysis (HD) and use of anticoagulant (AC) therapy	Comparator (Nº of patients)	Age range (mean)	Women (%)
Redeker (1973)	USA	ALF with gr. IV HE	Acute viral hepatitis (100%)	Exchange transfusion (n = 15)	Mean, SD: 1,1 ± 0.35, median: 1, range: 1–2, max: 2	AC: received	Standard medical therapy (n = 13)	16–67 (25.1)	39
O'Grady (1988)	UK	FHF with gr. IV HE	Acetaminophen overdose (AO) (52%), viral hepatitis (40%) drug reaction (8%)	Charcoal hemoperfusion (n = 29)	Median: 2, max: 4	HD: at the physician's discretion AC: received	Standard medical therapy (n = 33)		
Hughes (1994)	UK	FHF with gr. IV HE	AO (60%), viral hepatitis (40%)	BioLogic-DT (n = 5)	Mean: 3.6, median: 4, range: 2–5, max: 5	HD: in case of renal failure, patients were excluded AC: not applied (producer's suggestion)	Standard medical therapy (n = 5)	19–64 (37.3)	30
Ellis (1996)	UK	ALF	AO (71%), viral hepatitis (21%), drug induced (8%)	ELAD (n = 12)	Continuous	HD: at the physician's discretion	Standard medical therapy (n = 12)	14–65	50
Mazariegos (1997)	USA	ALF with coma		BioLogic-DT (n = 5)	Max. 5		Standard medical therapy (n = 1)	35–65 (48.3)	67
Wilkinson (1998)	USA	ALF with gr. III-IV HE	Viral hepatitis (66%) heat stroke (33%)	BioLogic-DT (n = 1)	Mean: 3.6, max: 5	HD: in case of renal failure, patients were excluded AC: not applied (producer's suggestion)	Standard medical therapy (n = 2)	27–58 (42.7)	33
Ellis (1999)	UK	ALF with gr. II or greater HE	Acute alcoholic hepatitis (100%)	BioLogic-DT (n = 5)	Mean: 2.6, median: 3, range: 1–3, max: 3	HD: at the physician's discretion AC: received	Standard medical therapy (n = 5)	36–64	30
Demetriou (2004)	USA and Europe	FHF/SHF with gr. III-IV HE, PNF	Viral hepatitis + AO + other drug induced (49%) indeterminate (37%), PNF (14%)	HepatAssist (n = 85)	Mean: 2.9, range: 1–9		Standard medical therapy (n = 86)	10–69 (37)	70
Pollock (2004)	UK	FHF	AO (100%)	MARS (n = 6)	Max. 14		Standard medical therapy (n = 6)		
El Banayosi (2007)	Germany	ALF	Cardiogenic shock after cardiac surgery (100%)	MARS (n = 20)	Range: 1–54		Standard medical therapy (n = 20)		28
Saliba (2013)	France	ALF	AO (38%), viral hepatitis 14%) autoimmune hepatitis (12%), mushroom induced (8%), unknown (8%), drug reaction (6%), toxic agents (6%), other (9%)	MARS (n = 53)	Median: 1, range: 0–7	HD: at the physician's discretion	Standard medical therapy (n = 49)	(40.4)	57
Larsen (2016)	Denmark, UK, Finland	ALF with gr. II or greater HE	AO (59%), unknown (21%), toxic agents (9%), viral hepatitis 6%), Budd-Chiari syndrome (1%), other (3%)	High-volume plasma exchange (n = 92)	Mean, SD: 2.4 ± 0,8, max: 3	HD: at the physician's discretion AC: received based on local guidelines	Standard medical therapy (n = 90)	33–56	68

Table 1. Randomized controlled trials included in the systematic review and network metaanalysis. Table contains study characteristics of the included trials. Blank cells indicate that the data were not reported in the article. Abbreviations: ALF: acute liver failure, HE: hepatic encephalopathy, HD: hemodialysis, AC: anticoagulant, SD: standard deviation, max: maximum, USA: United States of America, FHF: fulminant hepatic failure, gr.: grade, UK: United Kingdom, AO: acetaminophen overdose, SHF: subfulminant hepatic failure, PNF: primary nonfunction following liver transplantation.
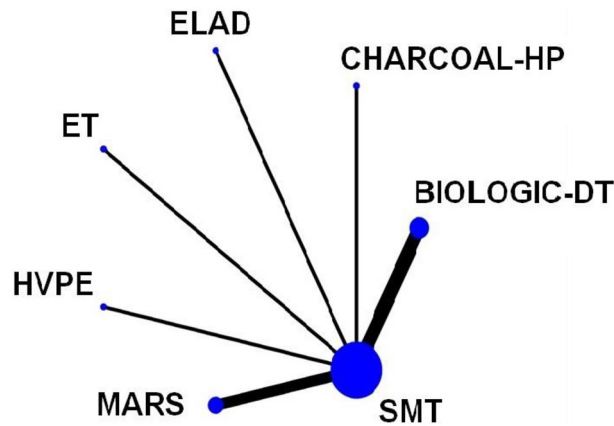
Figure 2. The network geometry of the eligible comparisons of in-hospital mortality. The thickness of the edges is proportional to the number of the head-to-head trials, and the size of the nodes is proportional to the number of studies in which the intervention was applied. SMT, standard medical therapy; HVPE, high-volume plasma exchange; ET, exchange transfusion; Charcoal-HP, charcoal-hemoperfusion.
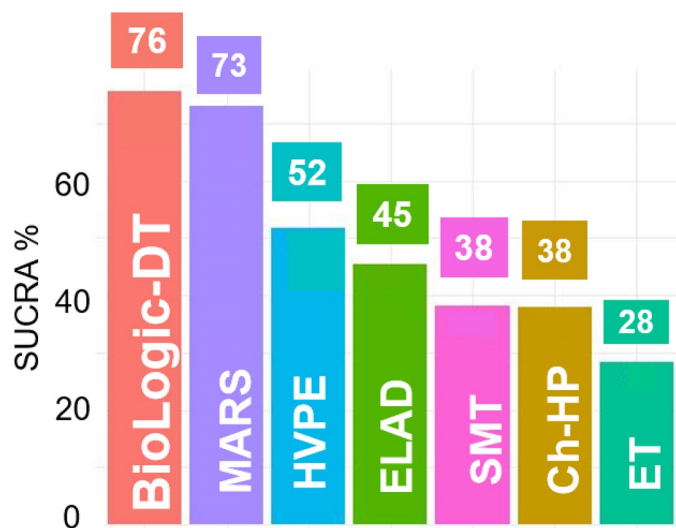
Figure 3. Surface under the cumulative ranking curves (SUCRA%) values of in-hospital mortality. Interventions were ranked by their posterior probability via calculating the surface under cumulative ranking (SUCRA) curve values. The higher the SUCRA value, the higher the probability for the interventions to be the best option. HVPE, high-volume plasma exchange; SMT, standard medical therapy; Ch-HP, Charcoal hemoperfusion; ET, exchange transfusion.

and 28%). On the other hand, the results from the league table (Table S1 and S2) for both outcomes confirm that no statistically significant differences can be found between the interventions.

Long-term survival. We assessed articles in which the follow-up period was at least 30 days. In the trial of Demetriou et al. 30-day survival was 71% in the bioartificial liver-treated group (BAL) and 62% in the control group (p = 0.26, generated with Whitehead Triangular Test)[18]. Saliba et al. reported that 6-month overall survival was not significantly different in the MARS and control groups (82.1 and 75.5%, respectively, p = 0.50)[25]. Considering HVPE, Larsen et al. reported that 3-month overall survival was not improved significantly in the plasma exchange group compared to the control group, however transplant-free survival was significantly better in the HVPE-treated group after 3 months (p = 0.0058)[26].

Transplantation. Six trials reported on liver transplantation. Three large RCTs did not find significant differences between the control and treatment groups in the number of patients transplanted and survival rates analysing HepatAssist device, HVPE and MARS[18,25,26]. Ellis et al. examining ELAD therapy reported that 2 patients underwent transplantation and 1 survived in each group[28]. In the trial published by Wilkinson et al. 2 fulminant hepatic failure patients had liver transplantation, 1 survived and 1 underwent transplantation before

BioLogic-DT						
0.91 (0.12, 4.7) ⊕○○○	MARS					
0.60 (0.05, 4.5) ⊕○○○	0.67 (0.07, 5.2) ⊕○○○	HVPE				
0.50 (0.03, 4.9) ⊕○○○	0.56 (0.05, 5.2) ⊕○○○	0.86 (0.058, 13) ⊕○○○	ELAD			
0.47 (0.09, 1.6) ⊕○○○	0.53 (0.15, 1.5) ⊕○○○	0.80 (0.13, 4.9) ⊕⊕⊕⊕	0.93 (0.13, 7.2) ⊕○○○	SMT		
0.44 (0.03, 3.4) ⊕○○○	0.49 (0.05, 3.9) ⊕○○○	0.74 (0.054, 9.3) ⊕○○○	0.85 (0.05, 13) ⊕○○○	0.91 (0.14, 5.7) ⊕○○○	Charcoal-HP	
0.34 (0.03, 2.6) ⊕○○○	0.38 (0.04, 3.1) ⊕○○○	0.58 (0.044, 7.2) ⊕○○○	0.67 (0.05, 11) ⊕○○○	0.72 (0.12, 4.5) ⊕○○○	0.79 (0.06, 9.9) ⊕○○○	ET

Table 2. League table of pairwise comparisons regarding in-hospital mortality. Values are given as relative risk (95% credible interval). The colour of the boxes indicates the comparisons' overall risk of bias assessment (green: low risk of bias, yellow: some concerns, red: high risk of bias). The number of ⊕ symbols refer to the quality of evidence according to the GRADE approach (⊕⊕⊕⊕ high quality, ⊕⊕⊕○ moderate quality, ⊕⊕○○ low quality, ⊕○○○ very low quality).

the start of the trial period[20]. In the study from Mazariegos et al. 3 patients from the treatment group had liver transplantation and survived, and no patients were transplanted from control group[22].

Adverse events. Nine studies reported adverse events. In three trials no adverse events were observed during BioLogic-DT treatment[19–21]. With ELAD therapy tachypnoea, tachycardia, fever and bleeding occurred in two patients[28]. In a trial examining HepatAssist device thrombocytopenia was the most frequent adverse event with similar incidences between groups (33.7% vs 38.8% for controls vs interventions, respectively)[18]. During charcoal hemoperfusion renal failure, cerebral oedema and uncompensated metabolic acidosis were detected[29]. Examining HVPE, cardiac arrhythmia, acute respiratory distress syndrome (ARDS), pancreatitis, deteriorating in gas exchange, transfusion-related acute lung injury (TRALI), infections confirmed by blood culture and bleeding could be observed. The rate of adverse events were not statistically different in the treatment and control group[26].

In a multi-center RCT MARS was tested, bleeding, death or sepsis did not occur related to MARS therapy, the majority of adverse events were related to liver transplantation and were more frequent in the not paracetamol-poisoned population[25].

In patients with ALF due to cardiogenic shock after cardiac surgery treated with MARS no bleeding was detected due to thrombocytopenia, other adverse events were not reported[24].

Risk of bias and quality of evidence. Two trials were published in abstract form[22,23]. Three of the trials were adjudicated as overall low risk of bias (33%)[18,25,26], and nine studies were judged to raise some concerns (67%) (Supplementary Fig. S22)[19–21,24,27–31] considering mortality outcomes. Regarding hepatic encephalopathy three studies were judged to raise some concerns[19–21] and one article was considered to be at high risk of bias[28]. Certainty of evidence for the outcomes was rated as very low for most comparisons (Supplementary, Table S3-S5).

Discussion

The role of liver support therapies in acute liver failure is still controversial, and to the best of our knowledge, no network meta-analysis has been published in this field before. Eleven RCTs were included in the current study with mortality and hepatic encephalopathy being the patient-important outcomes. BioLogic-DT was ranked as the best treatment for in-hospital mortality and worse for hepatic encephalopathy, however this modality is not applied in clinical practice anymore. MARS therapy was the best option from the available treatments in reducing in-hospital mortality. However, with no statistically significant results, there is no solid evidence that

the differences that we can see from the SUCRA values are due to chance or the interventions truly differ in their effects.

Former meta-analyses reported conflicting results considering liver support devices' effect on mortality in acute liver failure. Zheng et al. found that bioartificial devices reduced mortality in ALF (RR: 0.69, 95% CI 0.50–0.94, P = 0.018), although from the three studies analysed two represented the same patient population[32]. Stutchfield et al. reported that based on three RCTs, liver assist devices reduced mortality (RR: 0.7, 95% CI 0.49, 1.00, P = 0.05), although the significance is not robust given the confidence interval[16]. Other previous meta-analyses did not find any significant difference between SMT and liver support techniques in the ALF population by subgroup analysis[11,14,15,33–35].

Acetaminophen overdose is the leading cause of ALF in the USA, Australia and Europe[36–38]. Spontaneous recovery is more frequent in this patient population compared to other drug-induced, autoimmune or idiopathic ALF[36]. Therefore, emergency transplantation as a routine intervention in paracetamol poisoning has been questioned[39]. We did not have enough data in this patient population for a quantitative synthesis, however in the nonparacetamol-poisoned population no significant difference could be observed between SMT and extracorporeal liver assist devices, and the different liver support therapies applied.

Hepatic encephalopathy is an important symptom of ALF[8]. However, because of the disease's complexity there are several different measurement scales[40] and the result is greatly affected by the assessor[41]. Furthermore, the patients are usually sedated and mechanically ventilated, which makes the evaluation more difficult. In former meta-analyses in populations from both ACLF and ALF patients significant improvement was found in hepatic encephalopathy with ECLS systems[11,14,15,34].

The greatest strength of this study is that the different interventions were compared to each other and were not assessed together in comparison with standard medical therapy. However, this study has certain limitations. The most important limitations are the small sample sizes, the heterogeneity of the patient populations, outcomes, and study design and the inconsistency in definitions of liver failure. We were unable to use the node-splitting analysis to examine consistency assumption because there was not enough information from the comparisons in the network. Long-term survival could not be quantitatively analysed, although it is a particularly important factor to assess the efficacy of the interventions. Finally, our network meta-analysis covers a period of more than 40 years, during which SMT has improved remarkably (that is, chronological bias).

Conclusion

This network meta-analysis demonstrated that—as BioLogic-DT is not applied in clinical practice anymore—MARS therapy seems to be the best available option in reducing in-hospital mortality, however, no statistically significant differences could be observed among the treatments of acute liver failure considering in-hospital mortality and hepatic encephalopathy. Good-quality randomized trials are needed on currently available and new blood purification modalities to define the role of extracorporeal liver support in patients with acute liver failure.

Methods

Search strategy and selection criteria.   The network meta-analysis was reported using the PRISMA Extension Statement for Reporting of Systematic Reviews Incorporating Network Meta-Analyses of Health Care Interventions[42]. We used the classical PICO framework for our clinical question. P: patients with acute or hyperacute liver failure (having regard to the fact that the studies were conducted in a wide range of time (1973–2016) we accepted the articles' definition of hyperacute and acute liver failure); I and C: artificial, bioartificial liver support therapies, SMT; O: overall in-hospital mortality, mortality-by-aetiology, hepatic encephalopathy, number of patients transplanted, laboratory parameters and adverse events. Our network meta-analysis was registered with the PROSPERO registry (CRD42020160133).

For this network meta-analysis on the 4th of October 2019 we searched Medline (via PubMed), the Cochrane Central Register of Controlled Trials (CENTRAL), Web of Science, Embase and Scopus for RCTs and conference abstracts of RCTs. No restrictions were imposed on the search.

We used the following search key in all databases (complemented with the MeSH function in MEDLINE): ('hepatic failure' OR 'liver failure' OR 'end stage liver disease' OR cirrhosis OR 'alcoholic hepatitis') AND ('liver support system' OR 'liver support device' OR 'liver assist device' OR 'artificial liver' OR 'bioartificial liver' OR 'extracorporeal liver' OR 'albumin dialysis' OR 'extracorporeal cellular therapy' OR MARS OR Prometheus OR 'fractioned plasma separation and adsorption' OR hemadsorption OR hemoadsorption) AND random*.

Randomized controlled trials studying liver support devices in acute-on-chronic liver failure were excluded. In studies in which patients with ALF and ACLF were both involved and provided individual patient data, we only extracted the data of patients with acute liver failure. Transitivity was assessed clinically, based on the eligibility criteria of the included randomized controlled trials. As acute and hyperacute liver failure have mainly similar symptoms despite etiology, we concluded that, regarding the liver support systems' clinical effect on these symptoms, the conditions of transitivity are satisfied.

Records from each database were downloaded into EndNote X9 citation manager (Clarivate Analytics, Philadelphia, USA) and duplicates were removed by the citation manager based on the title of the article, and then manually. The titles then the abstracts and full texts of the identified studies were screened for inclusion against the eligibility criteria by two independent review authors (KO, AK). A third party (ZM) resolved conflicts. Citing and cited articles were revised through Google Scholar, where all the additional sources were identified. The PRISMA flowchart shows the process of the article selection (Fig. 1)[43].

Data extraction and outcomes.   All data according to study type, author and publication information, demographic data, aetiology, details of the interventions and comparators, mortality, hepatic encephalopathy,

number of patients transplanted, laboratory parameters, adverse events and notes were collected in the study database (standardized template). The data from intention-to-treat analyses were extracted independently by the first (AK) and second author (KO), when conflicts arose, a third participant resolved any discrepancies (ZM).

The primary outcome of our analysis was in-hospital overall mortality. Secondary outcomes included hepatic encephalopathy (number of patients improved versus worsened plus not improved), mortality-by-aetiology, liver transplantation, long-term survival, and adverse events. We accepted the articles' definition of adverse events. We planned to analyse changes in laboratory parameters as well but failed to do so because studies reported them in different time instants.

Risk of bias assessment and quality of evidence. Risk of bias assessment was first performed on individual study-level according to the Revised Cochrane risk-of-bias tool for randomized trials (RoB 2)[44]. From the individual studies' overall RoB assessment, we chose the one which was at the highest risk of bias for each intervention's (each arm of the network) overall RoB assessment. Then we summarized the interventions' overall RoB-assessment on the comparison level with the same method. The results of the RoB assessment are depicted in league tables. The colour of the boxes indicates the comparisons' overall risk of bias assessment (green: low risk of bias, yellow: some concerns, red: high risk of bias). We used the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) approach to assess the certainty of evidence[45]. Study limitations were evaluated based on RoB 2 tool, as detailed above. Imprecision was judged based on the sample size calculation of the article of Larsen et al.[26]. Node splitting could not be performed in any of the networks due to network geometry, consequently inconsistency could not be tested. We compared the individual studies' populations, interventions and outcomes to rate indirectness. Publication bias was judged by the 'comparison-adjusted' funnel plot and Egger's test. In the league tables we marked the quality of evidence for each comparison. Risk of bias and quality of evidence assessment were performed by two independent review authors (KO, AK), a third party (ZM) resolved conflicts.

Statistical analysis. A Bayesian-method was used to perform pairwise meta-analyses and network meta-analysis with the random effect model. In case of missing outcome data, we replaced values with the worse outcome, i.e. in case of mortality, death, in case of hepatic encephalopathy, worsening/not improving. We used risk ratios (RR) for dichotomous data with 95% credible intervals (95% CrI). We optimized the model and generated posterior samples using the Monte-Carlo methods running in four chains. We set at least 20,000 adaptation iterations to get convergence and 10,000 simulation iterations. Network estimates (pooled direct and indirect data) of each intervention compared to standard medical therapy and to other interventions are presented in forest plots, summarized in a league table (as shown in the results section). In the network geometry the direct comparisons are presented with edges, and the thickness of the edges is proportional to the number of the head-to-head trials, and the size of the nodes is proportional to the number of studies in which the intervention was applied. We also ranked interventions by their posterior probability via calculating the SUCRA values. 'Comparison-adjusted' funnel plot was created with the frequentist approach, and Egger's tests were performed in the network meta-analysis to assess small-study effect of in-hospital mortality. All calculations were performed with R (V. 3.5.2) package gemtc (V. 0.8-2) along with the Markov Chain Monte Carlo engine JAGS (V. 3.4.0) and STATA 17.0 (StataCorp LLC).

Data availability
All data generated or analysed during the current study are available from the corresponding author on reasonable request.

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Author contributions

A.K. and K.O. performed the database search and read the articles for eligibility; when a conflict arose, a third participant, Z.M. made the decision. A.K. and K.O. collected the data from the articles to the study database. Statistical analysis was conducted by N.G., Z.S. and S.K. helped interpreting the analysis. S.M. and J.S. provided useful information on the practical use of liver support therapies. A.K. and K.O. performed the bias analysis and quality assessment. A.K. and Z.M. drafted the manuscript. A.K., K.O., N.G., Z.S., A.P., S.K., S.M., J.S., P.H. and Z.M. edited the manuscript. A.K. edited the tables and figures. A.K. completed the PRISMA checklist. Z.M. made the critical revision on the finalized manuscript. All authors reviewed and approved the final manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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Dosing of Extracorporeal Cytokine Removal In Septic Shock (DECRISS): protocol of a prospective, randomised, adaptive, multicentre clinical trial

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ABSTRACT

Introduction Sepsis and septic shock have mortality rates between 20% and 50%. In sepsis, the immune response becomes dysregulated, which leads to an imbalance between proinflammatory and anti-inflammatory mediators. When standard therapeutic measures fail to improve patients' condition, additional therapeutic alternatives are applied to reduce morbidity and mortality. One of the most recent alternatives is extracorporeal cytokine adsorption with a device called CytoSorb. This study aims to compare the efficacy of standard medical therapy and continuous extracorporeal cytokine removal with CytoSorb therapy in patients with early refractory septic shock. Furthermore, we compare the dosing of CytoSorb adsorber device changed every 12 or 24 hours.

Methods and analysis It is a prospective, randomised, controlled, open-label, international, multicentre, phase III study. Patients fulfilling the inclusion criteria will be randomly assigned to receive standard medical therapy (group A) or—in addition to standard treatment—CytoSorb therapy. CytoSorb treatment will be continuous and last for at least 24 hours, CytoSorb adsorber device will be changed every 12 (group B) or 24 hours (group C). Our primary outcome is shock reversal (no further need or a reduced (≤10% of the maximum dose) vasopressor requirement for 3 hours) and time to shock reversal (number of hours elapsed from the start of the treatment to shock reversal).

Based on sample size calculation, 135 patients (1:1:1) will need to be enrolled in the study. A predefined interim analysis will be performed after reaching 50% of the planned sample size, therefore, the corrected level of significance (p value) will be 0.0294.

Ethics and dissemination Ethics approval was obtained from the Scientific and Research Ethics Committee of the Hungarian Medical Research Council (OGYÉI/65049/2020). Results will be submitted for publication in a peer-reviewed journal.

Trial registration number NCT04742764; Pre-results.

BACKGROUND

Sepsis and septic shock are devastating conditions with mortality rates between

Strengths and limitations of this study

► It is a prospective, randomised, controlled, multi-centre study with a relatively homogeneous group of patients.

► Instead of the internationally criticised hard end-points in sepsis trials, physiological outcomes were chosen as our primary endpoints.

► Shock reversal has not been used as a primary outcome in randomised trials before, therefore, sample size calculation was based on a heterogeneous population of patients with sepsis from a limited number of studies.

► For safety measures we decided to treat patients in both CytoSorb-treated groups for at least 24 hours—according to current practice—therefore, we will not able to assess sustained shock reversal without haemadsorption therapy during the first 24 hours.

20% and 50%.[1–3] Sepsis has an outstandingly complex pathophysiology, therefore, the clinical presentation of sepsis is often diverse and unpredictable.[4 5] The process begins with the host's immune response triggered by various insults.[6] This response becomes uncontrolled and an imbalance occurs between proinflammatory and anti-inflammatory mediators. This condition is also referred to as the 'cytokine storm'.[7] During the cascade-like inflammatory response, cytokines are released, which are a heterogeneous group of proteins, mostly in the mass range of 40 kDa.[8] The theory that cytokine storm may be responsible for the observed deleterious sequence of events in sepsis, raises the pathophysiological rationale of extensive removal of circulating cytokines.[9] A disturbance in vascular tone regulation also develops in sepsis:

vasoplegia is thought to be a key factor responsible for the death of patients with septic shock, due to persistent hypotension.[10]

When standard therapeutic measures, such as adequate early resuscitation, source control and organ support fail to improve the patients' condition, additional therapeutic alternatives, called 'adjuvant therapies' are applied to reduce morbidity and mortality by providing some extra help.[11] Several adjuvant therapies have been tested over the decades with non-conclusive results.[12–14] One of the most recent alternatives is extracorporeal cytokine adsorption with a device called CytoSorb (CytoSorbents, New Jersey, USA) that has become available in clinical practice in 2011. It is a high-flow, low-resistance cytokine adsorbent, containing specially developed polymer beads with a large adsorption surface and a spectrum of adsorption between 5 and 60 kDa.[15]

Over 100 case studies describing the use of CytoSorb in many clinical scenarios and in general, the effects are promising, and the treatment is well tolerated.[16–18] Concerning the treatment of sepsis, clinical trials are lacking at present, and we have mainly small case series.[19–22] There is also an international CytoSorb Registry, and recent data analysis on 198 patients indicated, that observed mortality (65%) was substantially better as compared with the predicted (80%–20%) and the treatment also proved to be safe.[23] Furthermore, recent case series and case–control studies reported profound benefit on the outcome in patients with septic shock and treated with CytoSorb.[24 25] Recently, the Adsorption of Cytokines Early in Septic Shock (ACESS trial) was published, which is the first randomised clinical trial (RCT) on CytoSorb as a stand-alone haemoperfusion treatment (ie, without continuous renal replacement therapy (CRRT)) in patients with septic shock.[26] It was a proof-of-concept pilot study on 20 medical patients randomised into a CytoSorb and a standard treatment group, with cytokine adsorption initiated within the first 24 hours after the onset of septic shock. The treatment proved to be safe and resulted in a significant reduction in norepinephrine requirement and serum procalcitonin (PCT) levels in the CytoSorb group as compared with controls. In a more recent propensity-score-weighted retrospective study on more than 100 patients with septic shock requiring CRRT, when patients were weighted by stabilised inverse probability of treatment weights the results suggested that CytoSorb therapy may be associated with decreased all-cause mortality at 28 days compared with CRRT alone.[27]

Despite the promising case series and preliminary results, several questions need to be clarified before recommendations can be made, including the right target population, the timing and the length of a single treatment and the overall duration of the therapy. Some preliminary data are suggesting that PCT is removed by the adsorber in a time-dependent manner[28] being most efficient during the first 12 hours, after which removal is negligible.

AIM OF THE STUDY

This study aims to compare the efficacy of standard medical therapy (SMT, group A) and continuous extracorporeal cytokine removal with CytoSorb therapy in patients with early refractory septic shock. Furthermore, we compare the dosing of CytoSorb adsorber device changed every 12 (group B) or 24 hours (group C).

METHODS AND ANALYSIS
Study design

It is a prospective, randomised, controlled, three-arm, open-label, international, multicentre, phase III study with adaptive 'sample size re-estimation' design.

The study protocol was constructed in accordance with the Standard Protocol Items: Recommendations for Interventional Trials 2013 statement.[29]

Randomisation

A computer-generated random number sequence will be conducted with randomly varied multiple block sizes stratified according to the participating centres with an equal (1:1:1) allocation ratio. The medical personnel in each study centre will have credentials to access the randomisation site. On this site, the medical staff has to check all inclusion criteria and the absence of all the exclusion criteria. Patients will be recruited consecutively. After the participant was registered, the allocation appears but the following allocations and the block sizes are concealed.

Blinding

It is not possible for the staff who are providing patient care to be unaware of the group assignments after randomisation. Sham procedures for the control group would be unethical. Statisticians are blinded to treatment assignments.

Duration

Duration per patient: The study starts after randomisation. In the CytoSorb groups, measurements, blood sampling and other recordings are performed immediately after the start of CytoSorb therapy (indicated as T_0). In the SMT group, T_0 is defined as the first recordings after randomisation. The study period ends (T_e) 12 hours after shock reversal or on day 5 after randomisation or at the time of death within this period, whichever happens first. The patients will be followed up on day 28±7 and day 90±7 after randomisation. Duration of the entire study: the planned starting date of the study is June 2021, and the planned completion date is June 2024.

Study groups

Patients eligible for the study in terms of the inclusion and exclusion criteria (defined below) will be randomly assigned to one of the three study groups after informed consent. In case the patient is unable to give consent, informed consent will be obtained from the next of kin or his/her legal guardian, information on the study and the treatment will be provided by the attending
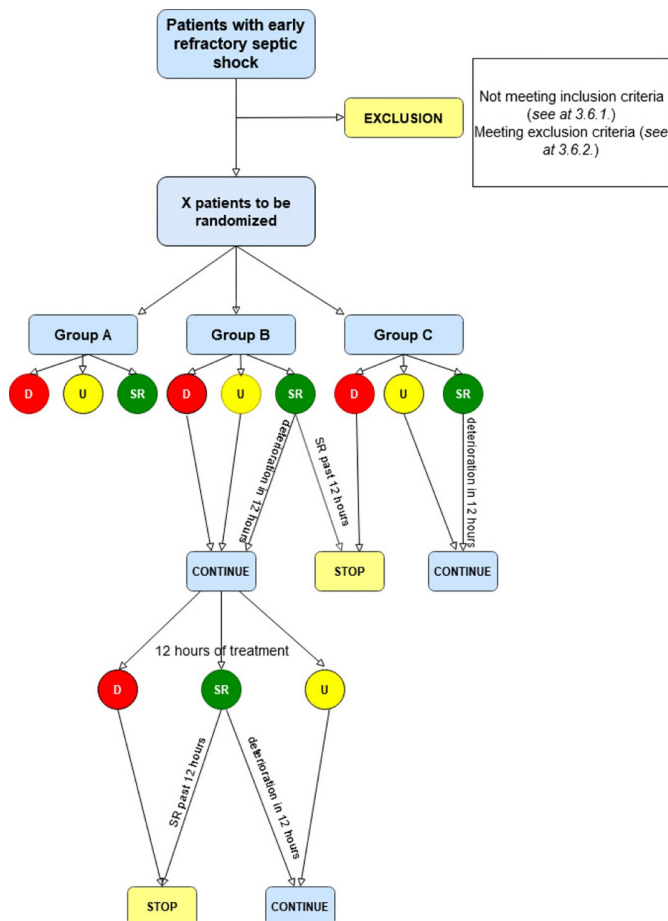
Figure 1 Flow chart of the therapy according to the SPIRIT 2013 statement.[29] The figure presents 24 hours of the treatment period. D, deterioration, SPIRIT, Standard Protocol Items: Recommendations for Interventional Trials; SR: shock reversal; U: unchanged state.

physician. Patients in group A will be treated with SMT. Patients in group B will be treated with continuous Cyto-Sorb therapy in addition to SMT; CytoSorb device will be changed every 12 hours. Patients in group C will also be treated with continuous CytoSorb therapy in addition to standard treatment, however, CytoSorb device will be changed every 24 hours. In each group, the treatment will be continued for a minimum of 24 hours, after that until shock reversal occurs, for a maximum of 5 days or until the patient's death (figure 1).

Patient enrolment
The inclusion and exclusion criteria are based on the results of previous case series,[24 25] on the ACESS trial[26] and modified accordingly:

Inclusion criteria
► Septic shock as defined by the Sepsis-3 criteria.[30]
► Septic shock of both medical and surgical aetiology (except for reoperation).
► Acute Physiology and Chronic Health Evaluation II (APACHE II) score >25[24–26] (APACHE II score will be assessed at T_0).

► Mechanical ventilation.
► Norepinephrine requirement ≥0.4 µg/kg/min for at least 30 min, when hypovolaemia is highly unlikely as indicated by invasive haemodynamic measurements[24–26] assessed by the attending physician.
► Invasive haemodynamic monitoring to determine cardiac output and derived variables.
► PCT level ≥10 ng/mL.[24–26]
► Inclusion within 6–24 hours after the onset of vasopressor need and after all standard therapeutic measures (including steroid therapy and/or second vasopressor) have been implemented without clinical improvement (ie, the shock is considered refractory).
► Written informed consent.

Exclusion criteria
► Patients under 18 years of age and over 80.
► Lack of health insurance.
► Pregnancy.
► Criteria of standard guideline-based medical treatment not exhausted (detailed below at 3.7) SMT).
► End-stage organ failure.[31]
► New York Heart Association class IV.
► Chronic renal failure with estimated glomerular filtration rate (eGFR) <15 mL/min/1.73 m[2.]
► Model for End-Stage Liver Disease Score (>30, Child-Pugh score class C.
► Unlikely survival for 24 hours according to the attending physician.
► Acute onset of haemato-oncological illness.
► Postcardiopulmonary resuscitation care.
► Reoperation in the context of a septic insult.
► Immunosuppression.
► Systemic steroid therapy (>10 mg prednisolone/day).
► Immunosuppressive agents (ie, methotrexate, azathioprine, ciclosporin, tacrolimus, cyclophosphamide).
► HIV infection (active AIDS): HIV-VL >50 copies/mL.[32]
► Patients with transplanted vital organs.
► Thrombocytopaenia (<20.000/mL).
► More than 10%-of body surface area with a third-degree burn.
► Acute coronary syndrome.
► In case of the need for a transfer of the patient to radiology or surgery, and if the device has to be disconnected, then the adsorber should be kept in a recirculation mode. In case of the need for changing the adsorber (ie, clotting) or if the disconnection lasted more than 2 hours, the patient should be excluded from the study.

Standard medical therapy
Patients will receive standard monitoring and care according to the centres' local standard protocols based on international guidelines.[33] It includes 5-lead ECG, pulse oximetry, continuous invasive blood pressure monitoring, central venous cannulation and advanced haemodynamic monitoring with the PiCCO-technology.

Advanced haemodynamic monitoring will be undertaken to optimise haemodynamics. Study teams will be encouraged to wean catecholamine support as soon as possible (mean arterial pressure (MAP) between 65 and 70 mm Hg in general),[34] but this should remain at the physician's discretion and should be tailored to each patient's individual need, based on other indices of global haemodynamic parameters and tissue perfusion such as urine output, serum lactate levels, ScvO2, etc. The first choice of vasopressor is norepinephrine. For the second line, vasopressin is the recommended vasopressor— also including steroid support decided by the attending physician. In case of the need for an inotrope, dobutamine is suggested as first-line treatment. SMT will be performed according to the 'Surviving Sepsis Campaign' Guidelines.[33]

Patients in both group B and C will receive a haemodialysis catheter inserted into a central vein (femoral, subclavian or internal jugular, as appropriate). Treatment will be performed as instructed by the manufacturer's user guide.

CytoSorb therapy

In short, CytoSorb will be placed in a blood pump circuit in prehaemofilter position (haemoperfusion) using a renal replacement device—of the choice of the given site—as a stand-alone treatment or in combination with renal replacement therapy. The device will be run in continuous venovenous hemofiltration (CVVH), continuous venovenous hemodialysis (CVVHD) or continuous venovenous hemodiafiltration (CVVHDF) mode. Intravenous anticoagulation will be performed—according to the current standards recommended by the manufacturers—with heparin, low-molecular-weight heparin or citrate as required, and a pump flow rate of 100–400 mL/min will be aimed and flow rate recorded.

Physicians are strongly advised to start CytoSorb therapy as soon as possible after randomisation, but not later than 2 hours. In case of further delay, the patient should be removed from the study.

In groups B and C, special attention will be paid to coagulation, therefore, in addition to standard laboratory tests (prothrombin time, activated thromboplastin time, international normalised ratio), rotational thromboelastometry will be performed whenever necessary and available.

Antibiotic serum concentrations are recommended to be monitored—in centres where it is available—according to international standards and doses should be altered as recommended if necessary.

Shock reversal will be assessed by the attending physician and the treatment will be immediately continued or terminated with a new adsorber. Criteria for termination are as follows:

1. Discontinuation: shock reversal (see below) has been achieved and remains so after finishing 12 hours of SMT.[26]

2. Restarting: treatment can be restarted within 12 hours if vasopressor requirement increases despite normovolaemia confirmed with haemodynamic monitoring and in case of worsening organ function such as deterioration in gas exchange, increased extravascular lung water (EVLW), etc, which is considered by the attending physician as a result of a new onset of hyperinflammatory response.

3. Defining non-responders: It is expected that there will be patients who do not respond to CytoSorb treatment. Therefore, patients whose clinical condition deteriorates during and within the first 24 hours of CytoSorb therapy will be considered as non-responders and CytoSorb will not be continued. Non-responsiveness will be defined as: (A) increasing vasopressor requirement not related to hypovolaemia or bleeding, (B) increasing lactate not associated with acute liver failure and (C) when the worsening clinical picture is accompanied by increasing PCT/Interleukin-6 (IL-6) levels despite the likely presence of adequate source control.

Patients' data will be recorded on the electronic case report form (eCRF) at T_0, T_6, T_{12}, T_{24} and then daily until the end of the study period (T_e) that is until 12 hours after shock reversal or up to a maximum of 5 days or until the patient's death, whichever occurs first. Follow-up visits/calls are scheduled on day 28±7 and day 90±7 after randomisation.

Primary endpoints

1. Time to shock reversal: the hours elapsed from T_0 to shock reversal.

2. Shock reversal: In previous studies, shock reversal occurred in 65%,[24] 38.5%[25] and 65%[26] of patients, within a 24-hour CytoSorb treatment, which has been considered as the most important clinical effect of the therapy. Based on the results 'shock reversal' will be defined as:

    i. No further need or reduced (≤10% of the maximum dose) in the vasopressor requirement (including norepinephrine and/or vasopressin) for 3 hours[25 35]
    (In case of multiple vasopressor agents are required, the reduction of one of them (≤10% of the maximum dose) is sufficient if the other agent(s)' dosage does not need to be increased).

    ii. Low doses of vasopressor (≤10% of the maximum dose) may be required to compensate for sedation or to maintain adequate organ perfusion.

    iii. In case of (2.a) invasive haemodynamic measurements will be performed to confirm haemodynamic stability.

    iv. In case of (2.a), arterial and central venous blood gas analysis will be performed, to determine arterial lactate levels (the target is ≤2 mmol/L), venous to arterial partial pressure of carbon dioxide gap (normal value is: ≤7 mm Hg) and central venous O_2 saturation (ScvO$_2$) (increase above 70%

at T_e if it was lower than 70% at T_0 or returning into 70%–75% by T_e in case it was greater than 75%–80% at T_0).

Secondary endpoints

1. Blood samples will be collected at T_0, T_6, T_{12}, T_{24} and then daily, and the change from T_0 to T_e of the following parameters will be assessed:
   i. Inflammatory parameters: 1. PCT, 2. IL-6, 3. C-Reactive Protein (CRP), 4. IL-1, 5. IL-1ra, 6. IL-8, 7. IL-10, 8. Tumour necrosis factor-alpha (TNF-α), 9. syndecan-1, 10. heparan sulfate.
   ii. Arterial lactate levels.
2. Change in Sequential Organ Failure Assessment (SOFA) score from T_0 to T_e (SOFA score will be assessed at T_0, T_{24} and then daily).
3. Change in EVLW from T_0 to T_e.
4. Duration of mechanical ventilation in days (every 24 hours when the patient required the organ support therapy counts as one).
5. Duration of catecholamine requirement in days.
6. Duration of renal replacement therapy in days.
7. Need for dialysis on day 28±7.
8. Need for dialysis on day 90±7.
9. Length of stay at the Intensive Care Unit (ICU).
10. Length of stay at the hospital.
11. Survival: ICU.
12. Survival: hospital.
13. Survival at day 28
14. Survival at day 90.
15. Survival: number of days (every finished 24 hours counts one).
16. Adverse events (AEs).

AEs and serious AEs: definition and recording

AEss will be collected from the start of the intervention period until follow-up.

All AEs and device deficiencies including all serious AEs (SAEs) are collected and documented in the source document and the AE report form (see at online supplemental file, AEs) during the entire study period, that is, from the patient's informed consent until the last follow-up visit/call. Dates of the event, the seriousness of the event and the relationship to the study device need to be documented. The AE report form has to be forwarded to the SC and the independent data management board (IDMB). Provided that the AE is confirmed by the SC, the national ethics committee needs to be notified (http://www.ett.hu/tukeb. htm).

Follow-up

A follow-up assessment will be conducted 28±7 days and 90±7 days after randomisation using a follow-up letter/email or a phone call. In case the patient or the next-of-kin cannot be reached, medical records will be used to obtain the needed information. At day 28 and 90 survival, need for dialysis and AEs will be assessed.

Statistical analysis

Sample size calculation

Based on the previous case series and the ACESS pilot data, the most apparent clinical benefit is expected to be the reduction in norepinephrine requirement; therefore, we chose shock reversal as the most important outcome.[24–26] In the ACESS trial, it was found that one single 24-hour treatment resulted in an almost 70% reduction in the required norepinephrine dose. A similar observation was made in a recent case series,[24] in which a 50% reduction was found after a 24-hour treatment. Furthermore, in our pilot study, the most profound effect occurred within the first 12 hours of treatment, as far as norepinephrine requirement and PCT-level reduction are concerned.[28] Based on these results, it is postulated that cytokine removal may be most effective in the first hours of treatment, therefore, shock reversal could occur faster in group B as compared with group C and faster in both groups as in group A (controls).

The sample size calculation was based on patient data from the study of Kogelmann et al.[25] The time of shock reversal was separately calculated for those in whom the first adsorber was changed after 12 hours (n=3), and for those who received therapy for 24-hours each time (n=17) (48±30 hours vs 68±21, respectively). In a recent prospective RCT on patients with sepsis and septic shock, vasopressors were weaned in 96±40 hours in the control group (n=50).[36]

We considered these differences as clinically relevant and not to be overlooked between the three groups. Sample size calculation suggests that 135 patients (1:1:1) will need to be enrolled (45 in each study arm) to confirm or reject the hypothesis for the primary endpoint with a 20% drop-out, 80% power and 95% significance level. Non-responders will be handled as dropouts and will continue to receive SMT.

Analysis plan and statistics

Descriptive statistics—mean, median, SD, quartiles and relative frequency—weighted generalised linear model with contrasts (continuous variable) for the primary endpoint and mixed models (continuous variable), a weighted generalised linear model with contrasts (continuous variable), relative risk (dichotomous variables) for secondary endpoints. Affiliated statistical analyses will be performed with an error probability of 0.0294 (type I error probability) for per-protocol (PP) and intention-to-treat population. All statistical analyses are performed with R (V.3.5.2).

Interim analysis

Appropriate sample size calculation was not possible due to the lack of available high-quality clinical data.[25] Therefore, it is highly likely that the event rate of shock reversal will occur in substantially less than 100%. In order to adapt the required sample size to maintain statistical power, we decided to allow sample size re-estimation after an interim analysis at the 50% recruitment rate. If

no more subjects are needed, early termination will be applied. For this reason, the p value should be adjusted to diminish the probability of type I error; therefore, the corrected level of significance (p value) will be 0.0294.

The following rules will be applied:

1. If the treatment in any of the groups proves to be significantly ($p<0.0294$) less effective than the others and it is already obvious that there is no hope for ascertaining a significant difference between the other two groups, the study will be stopped.
2. If the treatment in any of the groups are significantly ($p<0.0294$) less effective than the others and it is already visible that there is hope of ascertaining a significant difference between the other two groups, the inferior treatment will be dropped, and the study will be continued with the remaining two arms.
3. If any of the groups proves to be significantly ($p<0.0294$) more effective than the others, the study will be discontinued.

Study populations

Safety analysis set (all patients enrolled in the study), PP Set (PPS, all enrolled patients who finished the study conforming to the requirements of the study protocol) and ITT (all randomised participants who start on a treatment, excluding consent withdrawals) will be performed.

Withdrawal of a subject from PPS

Patients will not be included in the per-protocol analysis if: (1) during the trial any exclusion criteria is met; (2) a serious adverse effect occurs; (3) data required for the primary endpoints are missing; or (4) serious medical conditions not related to septic shock occur (eg, myocardial infarction, stroke); (5) commencement of CytoSorb more than 2 hours after randomisation and (6) the duration of CytoSorb therapy did not reach 24 hours or the patient died within 24 hours from enrolment in groups B and C.

Patient and public involvement

Patients or the public were not involved in the design, conduct, reporting or dissemination plans of our research.

ETHICS AND DISSEMINATION
Ethical and legal considerations

This clinical study will be conducted following the Declaration of Helsinki. It will be conducted in compliance with the protocol, Good Clinical Practice (GCP) (2001/20/EEC, CPMP/ICH/135/95), designated standard operating procedures, and local laws and regulations relevant to the country of conduct. This protocol in its current version was approved by the Scientific and Research Ethics Committee of the Hungarian Medical Research Council (OGYÉI/65049/2020).

Data management

IDMB will handle data, eCRF will be applied. The investigator will guarantee that the data in the eCRF are accurate, complete and clear. Data management plan will detail the data handling during and after the trial. Data from completed eCRFs will be assessed under the direction of the data manager at IDMB according to a data cleaning plan. In case of missing, improbable or inconsistent data in the eCRFs will be referred back to the Investigator using a data query form.

Publication policy

Centres recruiting more than 10 patients can nominate two authors to the authorship list. Every additional 10 patients will give the opportunity to nominate an additional author.

Trial organisation, committees and boards

DECRISS is designed and coordinated by the Centre for Translational Medicine at the Medical School of University of Pécs.

Steering committee

The steering committee (SC) will be led by ZM (intensive care specialist). The members will be AK (medical doctor, full-time employee on the project), MM (intensive care specialist), KKo (intensive care specialist), LS (intensive care specialist), BE (clinical research specialist) and PH (clinical pharmacologist). SC will discuss all important questions including AEs and the drop-outs during the study.

Participating centres

The trial will start in two centres (University of Pécs, Pécs, Hungary; Poznan University of Medical Sciences, Poznan, Poland), then the trial is open for other centres. The centre will be assessed by the IDMB and will be presented to the SC. The SC has the right to decide whether the centre meets the required quality to join the study. Compulsory requirements for a centre are: (1) it needs to treat at least 50 patients with septic shock a year; (2) it needs to have all the equipment required for the study; (3) besides the regular medical team, the centre has to have human resources (doctors, nurse/administrator) available for the trial; and (4) before study commencement a meeting will be held; at least one person/centre needs to attend who completed a GCP course. All the details of the study protocol will be discussed thoroughly. A letter of intent needs to be sent to the corresponding author by email in case of a centre wishing to participate in the study.

DISCUSSION

To our best knowledge, this is the first multicentre clinical trial, assessing the dosing of CytoSorb treatment alone as well as in combination with standard CRRT and compared with standard treatment in patients with refractory septic shock.

Strengths and limitations of the study

Study design intends to aim a relatively homogeneous group of patients in order to overcome the drawbacks of

previous large sepsis trials, that resulted in non-significant findings.[37][38] Therefore, in addition to the broad term Sepsis-3 definition of septic shock,[30] other prerequisites will be incorporated into the inclusion criteria such as the minimum APACHE II score, norepinephrine dose, PCT levels, mechanical ventilation, etc.

Most sepsis randomised trials applied hard endpoints to evaluate the effects of a single treatment, such as mortality, length of hospital stay or ventilator and vasopressor-free days.[39][40] However, this approach has been criticised by several internationally acknowledged experts for numerous reasons.[41][42] One of the possible solutions is to design trials with physiologic primary endpoints.[41] CytoSorb therapy has been shown to reduce the need of vasopressor support in several case series and studies.[24][25][38] Therefore, we decided to choose 'shock reversal' as our primary outcome measure. Furthermore, it is not only the occurrence of shock reversal but the 'time to shock reversal' from the start of treatment that is of particular interest in the current study.

The current practice of applying one adsorber for 24 hours is an arbitrary one, based on the company's recommendation and theoretical considerations. Nevertheless, several centres change the cartridge earlier (most often after 12 hours), based simply on their experience, but no study investigated this issue yet. Therefore, the current study should have important results to determine if there is any difference in the effects when the adsorber is 'fresh' as compared with its later performance. For this purpose, we designed a three-arm trial comparing standard therapy to 12 and 24 hours CytoSorb adsorber changing strategies to assess, which leads to faster shock reversal.

Another strength of our study is that in addition to well-acknowledged parameters indicating organ dysfunction a specific issue in the current trial will be the investigation of the evolution of EVLW during the treatment. EVLW is an indicator of increased pulmonary capillary permeability, often due to systemic inflammation.[43] There is one case report indicating that CytoSorb therapy may have protective effects on vascular barrier function.[44] As mechanical ventilation is also an inclusion criterium, our study may provide further insight into the relationship between cytokine removal and pulmonary function.

Although it has been shown in several experimental models that CytoSorb removes cytokines but clinical data, especially from prospective randomised trials are missing. An array of inflammatory markers and mediators are planned to be determined during the study, which can provide a further understanding of the removal properties of the device.

One of the limitations of the study is that shock reversal per se has not been used as a primary outcome, therefore, sample size calculation was based on data from a limited number of patients and a heterogeneous population of patients with sepsis. Another potential limitation is the heterogeneity of the study population. Patients with septic shock both due to medical and surgical origin will be included, while the inflammatory response might be

different in the two groups.[45] However, currently available clinical data indicate that both patient populations can benefit similarly from the therapy.[25] Another concern regarding heterogeneity could be that CytoSorb treatment will be applied on its own as haemoperfusion and in combination with CRRT. However, we have no data yet, neither pro nor con that these two therapies interact in any way. For safety measures, we decided to treat patients in both CytoSorb-treated groups for at least 24 hours—as precurrent practice—therefore, we will not be able to assess sustained shock reversal after 12 hours during the first 24 hours.

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of the translations (including but not limited to local regulations, clinical guidelines, terminology, drug names and drug dosages), and is not responsible for any error and/or omissions arising from translation and adaptation or otherwise.

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