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

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

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2. List of Abbreviations

AIDS	Acquired Immune Deficiency Syndrome
ALF	Acute liver failure
CrI	Credible interval
DAMP	Damage-associated molecular pattern
Charcoal-HP	Charcoal-hemoperfusion
ECLS	Extracorporeal liver support
ELAD	Extracorporeal Liver Assist Device
ET	Exchange transfusion
EVLW	Extravascular lung water
GRADE	Grading of Recommendations Assessment, Development, and Evaluation
HE	Hepatic encephalopathy
HVPE	High-volume plasma exchange
ICU	Intensive care unit
IL(-6)	Interleukin-(6)
INF-γ	Interferon gamma
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
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
RCT	Randomized controlled trials
RoB2	Cochrane risk-of-bias tool for randomised trials
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

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.

Rudd et al found that there were 48.9 million cases of sepsis globally in 2017 and with the ageing population, the frequency of comorbidities and the incidence of critical illness syndromes and critical care, treatments are increasing. 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%).

3.1.1 Pathophysiology

Multiple organ failure (MOF) is the primary cause of late mortality in critically ill patients in ICUs. Significant stimulation of the innate immune system through the damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs) 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. 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 hyperinflammation, eventually evolving into MOF. Another pathophysiological change that occurs in critically ill patients is the imbalance between oxygen delivery and oxygen consumption.

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. In the resuscitation phase, immediate life-threatening conditions are assessed, while initial treatment is commenced. Causative therapy in case of sepsis involves source control, which can be achieved by antibiotics, operative techniques or interventional radiology. In acute liver failure etiology-specific treatment is applied, however, for those who do not recover spontaneously, the definitive therapy is liver transplantation.

In adjunction of causative therapy and organ support, additional therapies, such as blood purification techniques may also be applied.

3.3 Extracorporeal therapies

Extracorporeal life support can be used as bridge to stabilization in critically ill patients until more definitive therapies are applied. In liver failure, extracorporeal liver support systems (ECLS) can be used to aid the liver's detoxification function, furthermore, bioartificial liver support therapies can provide synthetic functions as well.

In septic patients, extracorporeal blood purification techniques were adopted in order to restore the balance of pro- and anti-inflammatory mediators by eliminating or deactivating them.

3.4 Aim of the Ph.D. thesis

Critically ill patients represent a very heterogenous patient population with high mortality rates. It has been suggested that extracorporeal blood purification techniques may improve outcomes and enhance recovery. 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, affecting 1-6 per million people every year in developed countries with mortality rates of 25-50%. The main causes of acute and hyperacute liver failure are drugs – especially paracetamol overdose (46-65%) – and viruses (29-77%). Regarding liver support therapies, results of clinical trials are controversial.

4.1.1 The rationale of conducting network meta-analyses

In conventional meta-analyses, two interventions can be compared, however when multiple alternatives exist, network meta-analyses can provide results in a single analysis, therefore, we can (1) compare the interventions to each other and (2) rank them, to choose the best option regarding the outcome.

4.2 Methods

After systematic search, randomized controlled trials (RCTs) comparing liver support devices in adults with acute or hyperacute liver failure were included. In-hospital mortality was the primary outcome, the secondary outcomes were HE and mortality-by-aetiology. Our network meta-analysis was registered with the PROSPERO registry (CRD42020160133).

Risk-of-bias assessment was first performed according to the Revised Cochrane risk-of-bias tool for randomized trials (RoB 2). We used the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) approach to assess the certainty of evidence.

A Bayesian method was used to perform network meta-analysis and to calculate surface under the cumulative ranking curve (SUCRA) values to rank interventions. We used risk ratios for dichotomous data with 95% credible intervals (95% CrI). The details of the methodology can be found in the main thesis document.

4.3 Results

12 articles could be included for qualitative synthesis and 11 RCTs for network meta-analysis. The studied liver support devices were BioLogic-DT (that has been since redesigned), Molecular Adsorbent Recirculating System (MARS), high-volume plasma exchange, exchange transfusion, Extracorporeal Liver Assist Device (ELAD) and charcoal hemoperfusion. Bioartificial modalities are ELAD therapy and HepatAssist device.

4.3.1 In-hospital mortality

The network includes eleven studies. All liver support systems (BioLogic-DT, Charcoal-HP, ELAD, ET, HVPE, MARS) were compared directly to standard medical therapy (SMT). The SUCRA values indicate that BioLogic-DT (76%) and MARS (73%) are most likely to result in the lowest mortality. However, there were no statistically significant differences between the interventions.

4.3.2 Secondary outcomes

The SUCRA values show that BioLogic-DT, charcoal hemoperfusion and MARS may be equally efficient to decrease in-hospital mortality (53%, 52% and 52%, respectively) while SMT seems less effective (43%) in the nonparacetamol-poisoned patient population. Considering HE, SUCRA rankings indicate 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%).

Although, the results from the league table for both outcomes confirm that no statistically significant differences can be found between the interventions.

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

Three of the trials were adjudicated as overall low risk-of-bias (33%), and nine studies were judged to raise some concerns (67%) considering mortality outcomes. Regarding HE three studies were judged to raise some concerns and one article was considered to be at high risk-of- bias. Certainty of evidence for the outcomes was rated as very low for most comparisons.

4.4 Discussion

The role of liver support therapies in acute liver failure is still controversial. 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. Therefore, good-quality randomized trials of currently available and new blood purification modalities are needed to define the role of extracorporeal liver support in patients with acute liver failure.

5. Chapter II

5.1 Background and aims

Sepsis and septic shock have mortality rates between 20-50%. In sepsis, the immune response becomes dysregulated which leads to an imbalance between pro-, and anti-inflammatory mediators. The theory that the cytokine storm may be responsible for the observed deleterious sequence of events in sepsis, raises the pathophysiological rationale of extensive removal of circulating cytokines. 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.

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 SMT and continuous extracorporeal cytokine removal with CytoSorb therapy in patients with early refractory septic shock. Furthermore, we compare the dosing of the CytoSorb adsorber device changed every 12 or 24 hours.

5.2 Methods and analysis

It is a prospective, randomized, controlled, open-label, international, multi-centre, phase III study. Patients fulfilling the inclusion criteria will be randomly assigned to receive SMT (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).

5.2.1 Patient enrolment

5.2.1.1. Inclusion criteria

- Septic shock as defined by the Sepsis-3 criteria
- Septic shock of both medical and surgical etiology (except for re-operation)
- APACHE II > 25 (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 assessed by the attending physician
- Invasive hemodynamic monitoring to determine cardiac output and derived variables

- Procalcitonin level ≥ 10 ng/ml
- 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

5.2.1.2. 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
- End-stage organ failure
    - New York Heart Association Class IV.
    - Chronic renal failure with 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
- 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

5.2.2 Outcomes

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).

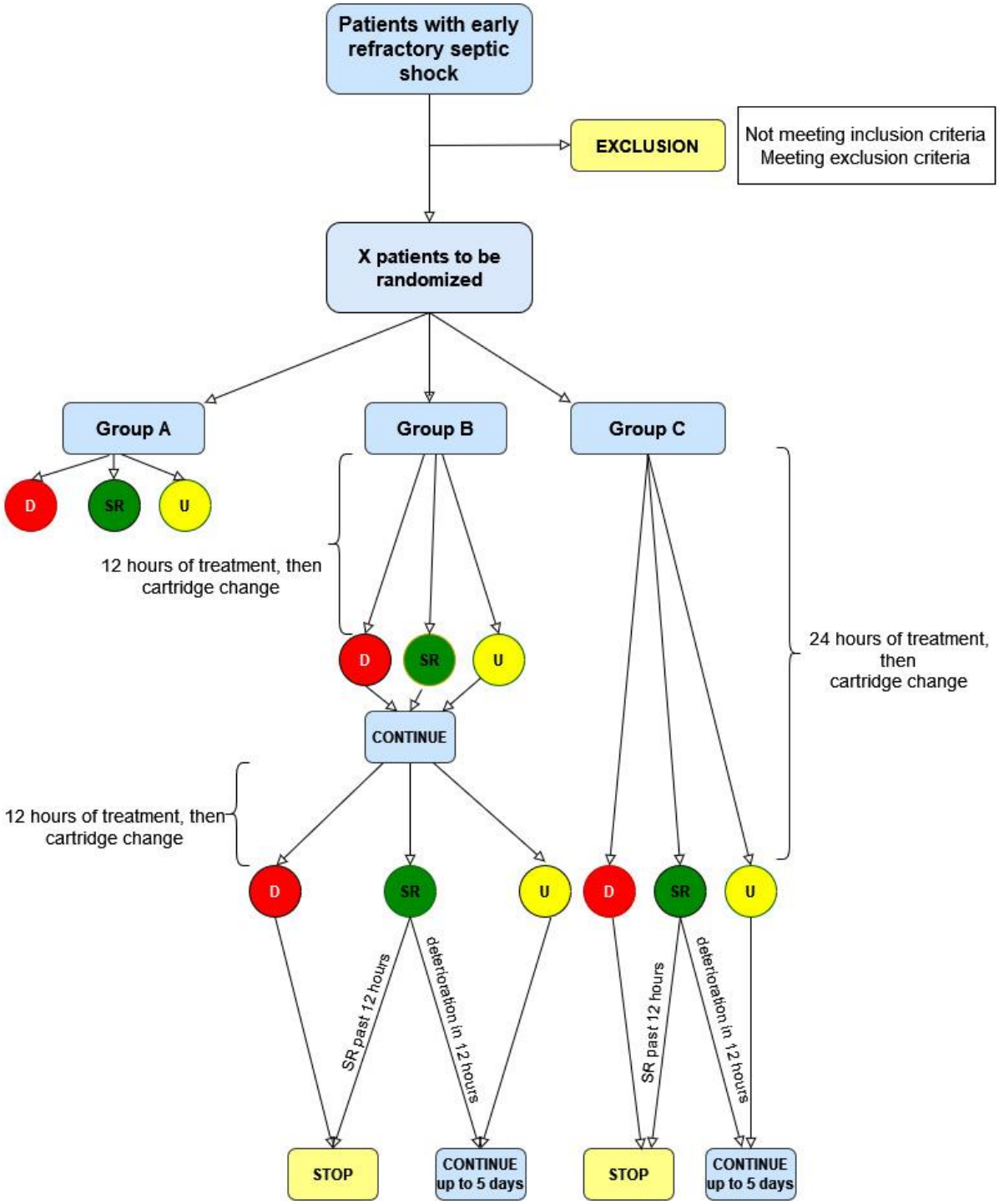
Secondary endpoints:

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. Procalcitonin, 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 extravascular lung water (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

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. The patients will be followed up on day 28±7 and day 90±7 after randomization.

Figure 1. Flowchart of the therapy according to the SPIRIT 2013 statement



The figure presents the first 24 hours of the treatment period. D: deterioration, U: unchanged state, SR: shock reversal

Based on sample size calculation, 135 patients (1:1:1) will need to be enrolled in the study. A pre-defined 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.

5.3 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.

The trial protocol was registered at ClinicalTrials.gov Protocol Registration and Results System: NCT04742764.

5.4 Trial organisation, committees and boards

5.4.1. Steering committee

The Steering committee 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.

5.4.2. Participating centres

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 will be open for other centres.

5.5 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 continuous renal replacement therapy and compared to standard treatment in patients with refractory septic shock.

5.5.1. Strengths and limitations of the study

It is a prospective, randomized, controlled, multi-centre study with a relatively homogeneous group of patients. Instead of the internationally criticised hard endpoints in sepsis trials, physiologic outcomes were chosen as our primary endpoints. One of the study limitations

is that shock reversal has not been used as a primary outcome in RCTs before, therefore sample size calculation was based on a heterogeneous population of septic patients 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 hemadsorption therapy during the first 24 hours.

6. Conclusions – 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, physiological 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

Our network meta-analysis was funded by the Economic Development and Innovation Operational Programme Grant (GINOP-2.3.2-15-2016-00048 - STAY ALIVE and GINOP-2.3.4-15-2020-00010 Competence Center for Health Data Analysis, Data Utilisation and Smart Device and Technology Development at the University of Pécs).

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8. Author's own contribution

8.1 Kanjo at al. Scientific Reports, 2021

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.

8.2 Kanjo et al, BMJ Open, 2021

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.

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