Gastrointestinal victims of alcohol: the liver and the pancreas

PhD Thesis

Doctoral School of Pharmacological and Pharmaceutical Sciences

Head: Erika Pintér, MD, PhD, DSc

Translational Medicine Programme leader: Péter Hegyi, MD, PhD, DSc, MAE

Klementina Ocskay, MD

Supervisors:

Andrea Párniczky, MD, PhD

Institute for Translational Medicine, Medical School, University of Pécs, Pécs, Hungary

Heim Pál National Pediatric Institute, Budapest, Hungary

Péter Hegyi, MD, PhD, DSc, MAE

Institute for Translational Medicine, Medical School, University of Pécs, Pécs, Hungary

Centre for Translational Medicine, Semmelweis University, Budapest, Hungary

Pécs, 2022

Table of contents

List of abbreviations

ACLF: acute-on-chronic liver failure

AE: adverse event

ALD: alcoholic liver disease

AP: acute pancreatitis

APASL: Asian Pacific Association for the Study of the Liver

ATP: adenosine-triphosphate

AUC: area under the curve

AUD: alcohol use disorder

BAMBI: BMP and activin membrane-bound inhibitor homologue

BUN: blood urea nitrogen

DNA: deoxyribonucleic acid

CFTR: cystic fibrosis transmembrane conductance regulator

CI: confidence interval

CP: chronic pancreatitis

CrI: credible interval

CRP: C-reactive protein

DALY: disability-adjusted life years

DAMP: danger-associated molecular patterns

DNA: deoxyribonucleic acid

DSM: Diagnostic and Statistical Manual of Mental Disorders

EASL: European Association for the Study of the Liver

ELAD: Extracorporeal liver assist device

eCRF: electronic Case Report Form

eGFR: estimated glomerular filtration rate

ER: endoplasmic reticulum

EtG: ethyl-glucuronide

FAEE: fatty acid ethyl ester

GGT: gamma glutamyl-transferase

GRADE: Grading of Recommendation, Assessment, Development and Evaluation Working Group

HBALSS: the hybrid bioartificial liver supporting system

HBV: hepatitis B virus

HE: hepatic encephalopathy

HCC: hepatocellular carcinoma

HPSG: Hungarian Pancreatic Study Group

IAP/APA: International Association of Pancreatology/ American Pancreatic Association

ICD: International Classification of Disease

ICU: intensive care unit

IL: interleukin

INR: international normalized ratio

IQR: interquartile range

MARS: Molecular adsorbent and recirculating system

MCV: mean corpuscular volume

MOF: multiple organ failure

NMA: network meta-analysis

OR: odds ratio

OS: overall survival

PAMP: pathogen associated molecular pattern

PE: plasma exchange

PCT: procalcitonin

PIN: personal identification number

RAP: recurrent acute pancreatitis

RCT: randomized controlled trial

ROC: Receiver Operator Characteristic

ROS: reactive oxygen species

RR: risk ratio

SCFA: short-chain fatty acid

SD: standard deviation

SI: sterile inflammation

SMT: standard medical therapy

STROBE: The Strengthening the Reporting of Observational Studies in Epidemiology

SUCRA: surface under cumulative ranking

TFS: transplant-free survival

TNF-α: tumour necrosis factor-α

UPR: unfolded protein response

WBC: white blood cell count

WHO: World Health Organisation

Scientific metrics

Number of publications **related to the subject** of the thesis:	3 (3 first author)
Cumulative impact factor of publications related to the thesis:	13.997
D1: 2, Q1: 1, Q2: -, Q3: -, Q4: -	
Number of **total accepted/published** articles:	23 (6 first author)
Cumulative impact factor of the published articles:	106.091
D1: 6, Q1: 15, Q2: 2, Q3: -, Q4: -	
Number of total citations by **MTM2:**	67 independent
https://m2.mtmt.hu/api/author/10074191	
Hirsch Index: 5	
Number of total citations by Google Scholar	139
https://scholar.google.com/citations?hl=hu&user=1OhNviUAAAAJ&view_op=list_works&sortby=pubdate	
Hirsch Index: 6	

List of publications

2.1 Publications related to the subject of the thesis

Ocskay K, Vinkó Z, Németh D, Szabó L, Bajor J, Gódi S, Sarlós P, Czakó L, Izbéki F, Hamvas J, Papp M, Varga M, Török I, Mickevicius A, Sallinen V, Maldonado ER, Galeev S, Mikó A, Erőss B, Imrei M, Hegyi PJ, Faluhelyi N, Farkas O, Kanizsai P, Miseta A, Nagy T, Hágendorn R, Márton Z, Szakács Z, Szentesi A, Hegyi P, Párniczky A. Hypoalbuminemia affects one third of acute pancreatitis patients and is independently associated with severity and mortality. **Sci Rep.** 2021 Dec 17;11(1):24158. doi: 10.1038/s41598-021-03449-8. MID: 34921151; PMCID: PMC8683470.

IF: 4.380; D1; original publication (1)

Ocskay K, Juhász MF, Farkas N, Zádori N, Szakó L, Szakács Z, Szentesi A, Erőss B, Miklós E, Zemplényi A, Birkás B, Csathó Á, Hartung I, Nagy T, Czopf L, Izbéki F, Gajdán L, Papp M, Czakó L, Illés D, Marino MV, Mirabella A, Małecka-Panas E, Zatorski H, Susak Y, Opalchuk K, Capurso G, Apadula L, Gheorghe C, Saizu IA, Petersen OH, de-Madaria E, Rosendahl J, Párniczky A, Hegyi P; Hungarian Pancreatic Study Group. Recurrent acute pancreatitis prevention by the elimination of alcohol and cigarette smoking (REAPPEAR): protocol of a randomised controlled trial and a cohort study. **BMJ Open**. 2022 Jan 4;12(1):e050821. doi: 10.1136/bmjopen-2021-050821. PMID: 34983758; PMCID: PMC8728419.

IF: 2.692; Q1; original publication (2)

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. doi: 10.1186/s13613-020-00795-0. PMID: 33462764; PMCID: PMC7813174.

IF: 6.925; D1; original publication (3)

2.2 Publications not related to the subject of the thesis

Nagy R, **Ocskay K**, Váradi A, Papp M, Vitális Z, Izbéki F, Boros E, Gajdán L, Szentesi A, Erőss B, Hegyi PJ, Vincze Á, Bajor J, Sarlos P, Mikó A, Márta K, Pécsi D, Párniczky A, Hegyi P. In-Hospital Patient Education Markedly Reduces Alcohol Consumption after Alcohol-Induced Acute Pancreatitis. **Nutrients.** 2022 May 20;14(10):2131. doi: 10.3390/nu14102131.

IF: 5.717; D1; original publication

Virág M, Rottler M, Gede N, **Ocskay K**, Leiner T, Tuba M, Ábrahám S, Farkas N, Hegyi P, Molnár Z. Goal-Directed Fluid Therapy Enhances Gastrointestinal Recovery after Laparoscopic Surgery: A Systematic Review and Meta-Analysis. ***Journal of Personalized Medicine*.** 2022; 12(5):734. https://doi.org/10.3390/jpm12050734

IF: 4.945; Q1; original publication

Leiner T, Nemeth D, Hegyi P, **Ocskay K**, Virag M, Kiss S, Rottler M, Vajda M, Varadi A, Molnar Z. Frailty and Emergency Surgery: Results of a Systematic Review and Meta-Analysis. **Front Med (Lausanne)**. 2022 Mar 31;9:811524. doi: 10.3389/fmed.2022.811524. PMID: 35433739; PMCID: PMC9008569.

IF: 5.093; Q1; original publication

Durst M, Könczöl K, **Ocskay K**, Sípos K, Várnai P, Szilvásy-Szabó A, Fekete C, Tóth ZE. Hypothalamic Nesfatin-1 Resistance May Underlie the Development of Type 2 Diabetes Mellitus in Maternally Undernourished Non-obese Rats. **Front Neurosci**. 2022 Mar 21;16:828571. doi: 10.3389/fnins.2022.828571. PMID: 35386592; PMCID: PMC8978526.

IF: 4.677; Q2; original publication

Nagy R, Gede N, **Ocskay K**, Dobai BM, Abada A, Vereczkei Z, Pázmány P, Kató D, Hegyi P, Párniczky A. Association of Body Mass Index With Clinical Outcomes in Patients With Cystic Fibrosis: A Systematic Review and Meta-analysis. **JAMA Netw Open**. 2022 Mar 1;5(3):e220740. doi: 10.1001/jamanetworkopen.2022.0740. PMID: 35254432; PMCID: PMC8902650.

IF: 8.484; D1; original publication

Rottler M, **Ocskay K**, Sipos Z, Görbe A, Virág M, Hegyi P, Molnár T, Erőss B, Leiner T, Molnár Z. Clinical Frailty Scale (CFS) indicated frailty is associated with increased in-hospital and 30-day mortality in COVID-19 patients: a systematic review and meta-analysis. **Ann Intensive Care**. 2022 Feb 20;12(1):17. doi: 10.1186/s13613-021-00977-4. PMID: 35184215; PMCID: PMC8858439.

IF:6.925; D1; original publication

Juhász MF, Vereczkei Z, **Ocskay K**, Szakó L, Farkas N, Szakács Z, Zádori N, Wilschanski M, Pandol SJ, Joly F, Capurso G, Arcidiacono PG, Izbéki F, Czakó L, Papp M, Czopf L, Hegyi P, Párniczky A. The EFFect of dietary fat content on the recurrence of pancreaTitis (EFFORT): Protocol of a multicenter randomized controlled trial. **Pancreatology**. 2022 Jan;22(1):51-57. doi: 10.1016/j.pan.2021.10.002. Epub 2021 Oct 14. PMID: 34750077.

IF: 3.996; Q1; original publication

Virág M, Rottler M, **Ocskay K**, Leiner T, Horváth B, Blanco DA, Vasquez A, Bucsi L, Sárkány Á, Molnár Z. Extracorporeal Cytokine Removal in Critically Ill COVID-19 Patients: A Case Series. **Front Med (Lausanne).** 2021 Nov 19;8:760435. doi: 10.3389/fmed.2021.760435. PMID: 34869464; PMCID: PMC8639689.

IF: 3.996; Q1; original publication

Ocskay K, Tomescu D, Faltlhauser A, Jacob D, Friesecke S, Malbrain M, Kogelmann K, Bogdanski R, Bach F, Fritz H, Hartjes A, Kortgen A, Soukup J, Utzolino S, van Tellingen M, Träger K, Schumacher U, Brunkhorst FM, Molnar Z. Hemoadsorption in 'Liver Indication'-Analysis of 109 Patients' Data from the CytoSorb International Registry. **J Clin Med**. 2021 Nov 5;10(21):5182. doi: 10.3390/jcm10215182. PMID: 34768702; PMCID: PMC8584981.

IF: 4.242; Q1; original publication

Nagy R, Harangi F, Tárnok A, Vincze Á, **Ocskay K**, Párniczky A, Hegyi P. Revisiting the evidence-based management of paediatric pancreatitis. **Pancreatology.** 2021 Aug;21(5):1011-1013. doi: 10.1016/j.pan.2021.06.008. Epub 2021 Jul 1. PMID: 34244039**.**

IF: 3.996; Q1; original publication

Szakó L, Gede N, Váradi A, Tinusz B, Vörhendi N, Mosztbacher D, Vincze Á, Takács T, Czakó L, Izbéki F, Gajdán L, Dunás-Varga V, Hamvas J, Papp M, Fehér KE, Varga M, Mickevicius A, Török I, **Ocskay K**, Juhász MF, Váncsa S, Faluhelyi N, Farkas O, Miseta A, Vereczkei A, Mikó A, Hegyi PJ, Szentesi A, Párniczky A, Erőss B, Hegyi P. Early occurrence of pseudocysts in acute pancreatitis - A multicenter international cohort analysis of 2275 cases. **Pancreatology**. 2021 May 19:S1424-3903(21)00158-7. doi: 10.1016/j.pan.2021.05.007. Epub ahead of print. PMID: 34059448.

IF: 3.996; Q1; original publication

Hegyi PJ, Váncsa S, **Ocskay K**, Dembrovszky F, Kiss S, Farkas N, Erőss B, Szakács Z, Hegyi P, Pár G. Metabolic Associated Fatty Liver Disease Is Associated With an Increased Risk of Severe COVID-19: A Systematic Review With Meta-Analysis. **Front Med (Lausanne).** 2021 Mar 12;8:626425. doi: 10.3389/fmed.2021.626425. PMID: 33777974; PMCID: PMC7994270.

IF: 5.093; Q1; original publication

Virág M, Leiner T, Rottler M, **Ocskay K**, Molnar Z. Individualized Hemodynamic Management in Sepsis. **J Pers Med**. 2021 Feb 23;11(2):157. doi: 10.3390/jpm11020157. PMID: 33672267; PMCID: PMC7926902.

IF: 4.945; Q1; knowledge publication

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. doi: 10.1038/s41598-021-83292-z. PMID: 33602961; PMCID: PMC7893063.

IF: 4.380; D1; original publication

Hegyi PJ, Soós A, Hegyi P, Szakács Z, Hanák L, Váncsa S, **Ocskay K**, Pétervári E, Balaskó M, Erőss B, Pár G. Pre-transplant Sarcopenic Obesity Worsens the Survival After Liver Transplantation: A Meta-Analysis and a Systematic Review. **Front Med (Lausanne)**. 2020 Dec 16;7:599434. doi: 10.3389/fmed.2020.599434. PMID: 33392221; PMCID: PMC7772841.

IF: 5.093; Q1; original publication

Kiss S, Gede N, Hegyi P, Németh D, Földi M, Dembrovszky F, Nagy B, Juhász MF, **Ocskay K**, Zádori N, Molnár Z, Párniczky A, Hegyi PJ, Szakács Z, Pár G, Erőss B, Alizadeh H. Early changes in laboratory parameters are predictors of mortality and ICU admission in patients with COVID-19: a systematic review and meta-analysis. **Med Microbiol Immunol**. 2020 Nov 21:1–15. doi: 10.1007/s00430-020-00696-w. Epub ahead of print. PMID: 33219397; PMCID: PMC7679241.

IF: 3.402; Q2; original publication

Váncsa S, Hegyi PJ, Zádori N, Szakó L, Vörhendi N, **Ocskay K**, Földi M, Dembrovszky F, Dömötör ZR, Jánosi K, Rakonczay Z Jr, Hartmann P, Horváth T, Erőss B, Kiss S, Szakács Z, Németh D, Hegyi P, Pár G. Pre-existing Liver Diseases and On-Admission Liver-Related Laboratory Tests in COVID-19: A Prognostic Accuracy Meta-Analysis With Systematic Review. **Front Med (Lausanne)**. 2020 Nov 13;7:572115. doi: 10.3389/fmed.2020.572115. PMID: 33282888; PMCID: PMC7691431.

IF: 5.093; Q1; original publication

Juhász MF, **Ocskay K**, Kiss S, Hegyi P, Párniczky A. Insufficient etiological workup of COVID-19-associated acute pancreatitis: A systematic review. **World J Gastroenterol**. 2020 Oct 28;26(40):6270-6278. doi: 10.3748/wjg.v26.i40.6270. PMID: 33177799; PMCID: PMC7596641.

IF: 5.742; Q1; original publication

Demcsák A, Soós A, Kincses L, Capunge I, Minkov G, Kovacheva-Slavova M, Nakov R, Wu D, Huang W, Xia Q, Deng L, Hollenbach M, Schneider A, Hirth M, Ioannidis O, Vincze Á, Bajor J, Sarlós P, Czakó L, Illés D, Izbéki F, Gajdán L, Papp M, Hamvas J, Varga M, Kanizsai P, Bóna E, Mikó A, Váncsa S, Juhász MF, **Ocskay K**…, Papachristou G, Hegyi P. Acid suppression therapy, gastrointestinal bleeding and infection in acute pancreatitis - An international cohort study. **Pancreatology**. 2020 Oct;20(7):1323-1331. doi: 10.1016/j.pan.2020.08.009. Epub 2020 Aug 22. PMID: 32948430.

IF: 3.996; Q1; original publication

Erőss B, Molnár Z, Szakács Z, Zádori N, Szakó L, Váncsa S, Juhász MF, **Ocskay K**, …, 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. doi: 10.1186/s13063-020-04733-0. PMID: 32993779; PMCID: PMC7522906.

IF: 2.279; Q1; original publication

Introduction

3.1 The epidemic of harmful alcohol consumption in the modern world

Alcohol consumption is among the leading causes of death worldwide and is the third among the causes of diseases, the first being smoking, the second hypertension (4). Harmful use of alcohol is a pattern of psychoactive substance use that is causing damage to health, incorporating the pattern and volume of drinking over time, the drinking context and the quality or contamination of the consumed beverages (5). Of note, a protective effect was observed in ischemic stroke and diabetes mellitus, which does not compensate the various detrimental effects alcohol poses on physical and mental health. According to the World Health Organisation's (WHO) report on alcohol consumption from 2018, 43% of the population reports current drinking, which translates as more than 2.3 billion consumers (6). In general, men are more affected by harmful alcohol consumption than women. In 2016, 53.6% percent of men consumed alcohol in the last 12 months, whereas only 32.2% of women drank (6). Interestingly, the prevalence of alcohol use disorders is 5 times higher in men (8.6% compared to 1.7% in women), which may be explained by more excessive consumption and the preference for hard drinks (7).

Among the WHO regions, the highest proportion of current drinkers was measured in Europe (59.9%), with 23.5% lifetime abstainers in 2016. Assessment of the changes in alcohol consumption in the last two decades shows a slight decrease worldwide (5%) and in the European region as well. However, the total alcohol consumption per capita among drinkers increased in almost all regions and was 17.2 litres in Europe. Although the prevalence of heavy episodic drinking (binge drinking) is decreasing worldwide, it is the highest in the European region in general and in adolescents and young adults as well.

Alcohol consumption poses a significant disease burden, measured in disability-adjusted life years (DALY) it takes up 5.1% of the total. Harmful alcohol consumption is related to about 230 diseases and injury-related health conditions and resulted in 3 million deaths in 2016. Furthermore, mostly the 15 to 59 years old population is affected, who are in their active years (8). Losses attributed to alcohol consist of direct costs for the treatment of alcohol-related diseases and indirect costs inflicted by decreased workforce productivity, absenteeism, early retirement and premature mortality (9). The proportion of deaths and DALYs attributed to alcohol were found to be the highest in Europe (10.1% and 10.8%).

Hungary is particularly heavily affected by harmful alcohol use, even compared to other countries in the region. The total alcohol consumption was 11.4 litres of pure alcohol per capita, 17.1 litres among drinkers only in 2016 (compared to 9.8 and 17.2 litres). The majority of the population consumes alcohol (percentage of abstainers in the past 12 months: 33.4), while spirits, wine and beer are consumed in comparable amounts. The prevalence of alcohol use disorders and alcohol dependence is more than 2.4 times higher than the European average (21.2% vs 8.8% and 9.4% vs 3.7%). The number of years of life lost is also particularly high, with an estimate of more than 5200 alcohol-attributable deaths in 2016. The prevalence of alcoholic liver disease (ALD) in Hungary was about 300 per 100.000, while liver-related age-standardized mortality was the second highest after Romania from 35 countries included in

HEPAHEALTH project (10). Still, no national plan or policy is available for the reduction of alcohol use and its consequences in Hungary (6).

Most alcohol-related deaths are due to digestive diseases (21.3%) and they are the second biggest contributors to the alcohol-attributable burden of disease, highlighting the role of gastroenterologists in the fields of prevention and treatment (***Figure 1***).

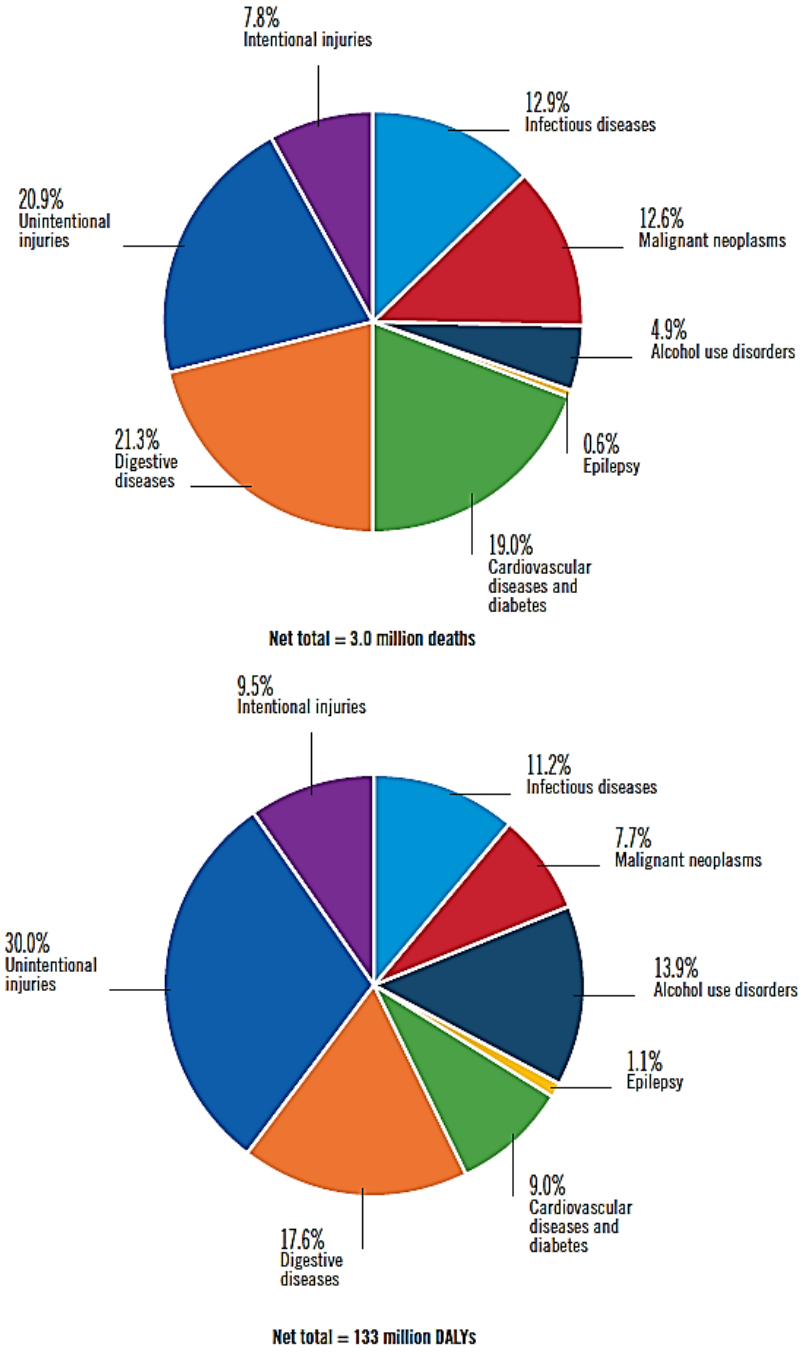


Figure 1 Alcohol-attributable deaths and burden of disease*, shown as a percentage of all alcohol-attributable, as a percentage of all alcohol-attributable disability-adjusted life years (DALYs) by broad disease category, 2016 (WHO, 2018; (6))*

3.2 Definitions of alcohol use disorders

Although alcohol use disorders (AUDs) are commonly diagnosed, terminology varies between diagnostic systems (11). AUDs are defined by the Diagnostic and Statistical Manual of Mental Disorders (DSM) and the International Classification of Disease (ICD). In both systems, the diagnostic criteria for AUDs include tolerance, withdrawal, impaired control, neglect of activities or time spent in alcohol-related activity, continued use despite problems, and compulsion or craving. However, several differences are present, hindering the use of both systems in research context. The DSM-5 combines the previously used dependence and abuse categories. If 2 out of 11 criteria are met within 12 months, AUD is diagnosed, the severity depending on the number of criteria met. In the ICD-11, 2 out of 3 criteria must be met. The ICD system defines harmful pattern of use of alcohol as repetitive alcohol use which resulted in physical or mental damage of health, but impaired control and physiological features or not necessarily present (7). Hazardous alcohol use was introduced in the ICD-11 among the health risk factors. It is defined as "a pattern of alcohol use that appreciably increases the risk of harmful physical or mental health consequences to the user or to others to an extent that warrants attention and advice from health professionals." (6).

3.3 The negative effects of alcohol on the gastrointestinal tract

Digestive diseases are major contributors to the alcohol-attributable burden on society and healthcare (***Figure 1***). Excessive alcohol use – including binge drinking (4 or more standard drinks for men and 5 or more standard drinks for women) and heavy drinking (8 or more drinks per week for men and 15 or more for women)- has been identified as a risk factor for several non-neoplastic and neoplastic gastrointestinal diseases. Its various effects on the gastrointestinal tract have been thoroughly studied and reviewed (12, 13). Summarizing these pathophysiological pathways, a 3-fold categorization seems rational: 1) changes in the microbiome and disruption of the gut barrier, 2) metabolomic changes and 3) effects on absorption and nutritional status (14). Most pathophysiological effects discussed here are results of the disruption in the function of the gut-liver axis. After absorption in the stomach and the proximal small intestine, alcohol passes through the liver via the portal vein. This first-pass mechanism exposes the liver to great amounts of alcohol, as 90% is eliminated by the hepatic tissue. Alcohol damages the mucous barrier function of the gut, therefore causing a disruption in the immune function. Besides its direct effect on the epithelium (cell death and mucosal erosions), one of alcohol's most important negative effects is the disruption of tight junctions. Furthermore, alcohol consumption is proven to induce changes in the microbiota, therefore causing alcohol-induced dysbiosis. Commensal bacteria contribute to barrier integrity by producing short-chain fatty acids (SCFAs) and stimulating Toll-like receptor-mediated immune response. Dysbiosis through the decreased SCFA concentration and immune response contributes to the disruption of the tight junctions in the mucosa, enabling the translocation of endotoxins and pathogen associated molecular patterns (PAMPs) to the portal circulation, therefore generating inflammatory cytokine production and signalling resulting in fibrosis in the hepatic tissue (14, 15). The effects of dysbiosis on the metabolome include alterations in the amount of SCFAs, amino acids and the circulation and conjugation of bile acids. Normally, SCFAs are produced by the gut microbiota by fermentation of indigestible fibres. Alcohol-

induced dysbiosis results in lower amount of SCFAs, which plays a key role in the disruption of tight junctions as previously discussed. Furthermore, the changes induced in the microbiota also result in a disbalance of the amino acid metabolism. Both essential and non-essential amino acids are affected, with lower luminal amino acid levels for some, but higher tyrosine and phenylalanine availability. This also may contribute to the generation of reactive oxygen species (ROS) and toxic intermediates. The entero-hepatic circulation of bile acids is also disturbed by alcohol. They are play a key role in lipid absorption and cholesterol homeostasis and also have regulatory functions including bile acid production, energy expenditure, glucose homeostasis and anti-inflammatory processes (16). Due to the dysbiosis and the consequential changes in amino acid metabolism, the conjugation of bile salts -formed from primary biliary acids- into secondary biliary acids by the microbiota by the removal of the taurine/glycine groups is disrupted. Hence, the concentration of bile acids in total and secondary bile acids is higher as well as the proportion of glycine-conjugated forms. Bile acids directly regulate the gut microbiota by stimulating the production of antimicrobial peptides, inhibiting gut microbial overgrowth.

Chronic alcohol consumption is often accompanied by macro- and micronutrient deficiencies (17). Decreased calory intake is the primary cause of insufficient fat, protein, and micronutrient supply, as alcohol contributes more than 50% to heavy drinkers' daily calories. Malabsorption and maldigestion are also major factors in the development of malnutrition, vitamin- and trace element deficiency (15). Intestinal oedema, delayed gastric emptying, the loss of epithelium at the tips of the villi and mucosal erosions are the major factors causing malabsorption. Disturbed amino acid and bile acid metabolism, altered intestinal permeability and inhibition of transport of several vitamins – including thiamine and zinc - were also described related to alcohol, further hindering the utilisation of micro- and macronutrients (14, 15). Changes in the microbiota and small intestinal bacterial overgrowth are also associated with diarrhoea and vitamin deficiencies. Exocrine pancreatic insufficiency and the reduced amount of biologically active bile acids result in impaired absorption of fat and fat-soluble vitamins (17, 18). Protein malnutrition leads to decreased serum protein levels, including hypoalbuminemia. Nutritional deficit results in decreased adaptive capability to stressors, immune paresis, sarcopenia and frailty, all predisposing to the development of organ failure and complications, associated with lower survival (18).

The most frequent and severe gastroenterological diseases caused by harmful alcohol consumption are cirrhosis, alcohol-induced acute pancreatitis and tumours. Alcohol consumption also facilitates the development of gastro-oesophageal reflux, alcoholic gastritis and gastropathy, peptic ulcer disease, diarrhoea and plays a role in the development of oropharyngeal, oesophageal, gastric, colorectal, pancreatic and liver cancer (13).

3.4 Alcohol and the pancreas

3.4.1 Alcohol-induced pancreas injury

Ductal, acinar and pancreatic stellate cells are all actively participating in the development of acute pancreatitis (AP) and are all affected by alcohol ingestion (***Figure 2***) (19-21). According to our current understanding, noxious stimuli -including alcohol- induce a series of changes in pancreatic cells which leads to the development of sterile inflammation (SI) and cell death. As

a starting block, intracellular calcium signalling, mitochondrial damage, adenosine-triphosphate (ATP) depletion and endoplasmic reticulum (ER) stress are induced in acinar and ductal cells. This results in premature trypsinogen activation and the decrease of fluid and bicarbonate secretion. The trypsinogen activation is further enhanced by the decreasing pH, propagating cell death and inflammation, known as the vicious trypsin cycle (22). The molecules and products released from the dying cells – danger-associated molecular patterns; DAMPs - induce inflammation with cytokine production and inflammatory cell infiltration. This self-reinforcing process may result in extensive necrosis and systemic inflammation with organ failure in severe cases.

SI is dominant in the pancreas as well as in alcohol-induced liver disease (23). Furthermore, several developmental similarities can be found between the two organs, which may facilitate the understanding of the diseases (24). Hepatic and pancreatic stellate cells are very closely related and functionally similar. The same processes are observed in pancreatic stellate cells as described in hepatic stellate cells fuelling inflammation. Pancreatic stellate cells are also activated by alcohol and acetaldehyde and transform to myofibroblast-like cells. Then these cells participate in the generation of ROS, proinflammatory cytokines and mediators (25). This was described by Peter Hegyi as the necroinflammatory amplification loop (21).

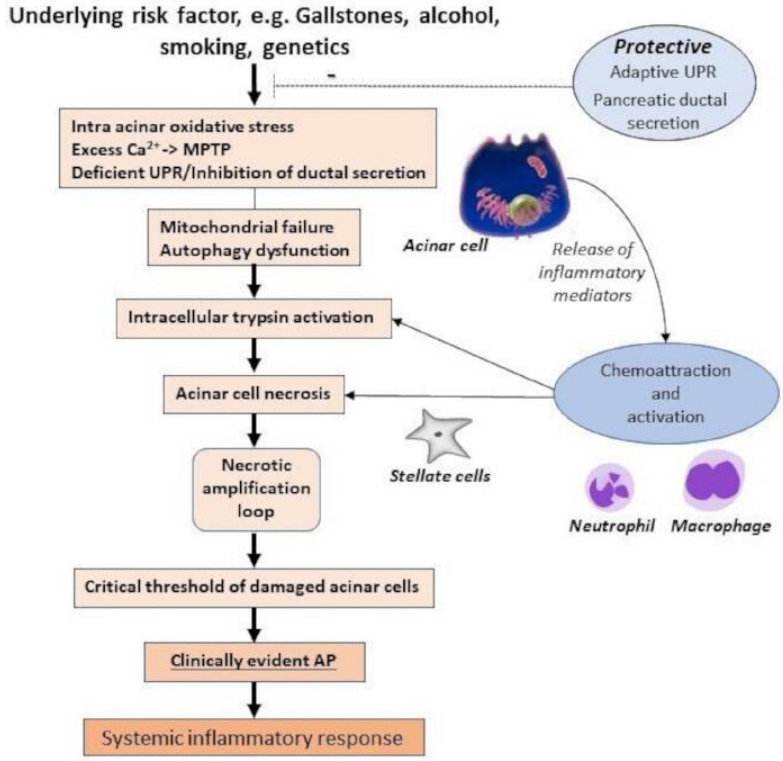


Figure 2 *The multifactorial 'critical threshold' hypothesis in the causation of AP. AP, acute pancreatitis; Ca2+, free cytosolic calcium; MPTP, mitochondrial permeability transition pore; UPR, unfolded protein response. (Barreto et al., 2021; (20))*

Pandol et al. hypothesized that alcohol exerts its deleterious effects on the acinar cells through ER stress by increasing the demand for protein production. Furthermore, in this state, the capacity of acinar cells for processing and recycling unwanted proteins is markedly reduced (26). ER stress and consequential cell death, including both apoptosis and necrosis is also observed in alcohol-induced liver injury. Adaptation to long-term alcohol consumption is mediated through unfolded protein response (UPR), which is a protective factor against AP (***Figure 2***) (27). This mechanism is characteristic of the acinar cells, as they produce the greatest

amount of protein in the body. Alcohol's oxidative effects cause an increase in oxidized glutathione and protein disulphide isomerase, a molecule key to protein maturation. Trough UPR, the transcription of genes regulating ER extension, chaperone proteins and ER degradation is upregulated. It is also known that chronic alcohol consumption affects inflammatory pathways promoting inflammation, decreases pancreatic microperfusion and leads to a block in exocytosis of zymogen granules, enabling premature trypsinogen activation in case the inhibitory mechanisms are compromised by another noxious stimulus. Another key factor regarding alcohol-induced pancreatic damage are the toxic products of alcohol metabolism. In the pancreatic acinar cells, the non-oxidative pathway is dominant which besides acetaldehyde and ROS, leads to the production of fatty acid ethyl esters (FAEEs) (28). FAEE-caused pancreatic damage occurs through destabilisation of lysosomes, direct interaction with cellular membranes; increasing the fragility of stimulation of zymogen granules and lysosomes) and mitochondrial damage (25).

Ductal cells are also affected by alcohol, by blockade of cystic fibrosis transmembrane conductance regulator (CFTR) expression, localization and activity and bicarbonate secretion (29).

However, based on experimental models, alcohol in itself is not capable of inducing AP, but strongly potentiates to inflammation and necrosis, therefore other risk factors are more likely to instigate the disease (25). This explains the clinical observation, that only a minority of patients with alcohol abuse develop AP (28). The 'critical threshold' hypothesis proposed by Barreto et al. (***Figure 2***) puts these observations in context from the clinical point of view (20). The authors describe two thresholds, the first being the development of AP and the second the development of organ failure as a sign of systemic disease. Pancreas damage and amplification loops increase the likelihood of the patient crossing these thresholds. Lankisch at al. proposed that the trigger of alcohol-induced pancreatitis may be increased gut permeability, as a consequence of chronic alcohol ingestion, similarly to alcoholic liver disease (25). In an animal model, alcohol-feeding alone did not induce AP, but a single endotoxin stimulus resulted in acinar cell vacuolization and necrosis, oedema, inflammatory infiltration, and haemorrhage in the pancreas (30). Although the role of smoking is hard to deduce, because often smoking and drinking go hand-in-hand, evidence prompts that smoking plays a prominent role in the development and progression of alcohol-induced AP (25, 31). Similarly to alcohol, smoking also induces oxidative stress, decreases fluid and bicarbonate secretion, promotes fibrosis and inhibits CFTR. The nature of the relationship between alcohol and smoking regarding pancreatic damage is still not entirely clear (32). Lugea et al. described a possible synergistic effect through UPR, as smoking creates a maladaptive response thus hindering the adaptive one to alcohol (33). A prospective cohort analysis also confirmed existence of a dose-dependent and synergistic effect between alcohol and smoking (34).

3.4.2 Alcohol-induced acute pancreatitis

3.4.2.1 Epidemiology

Acute pancreatitis is the third most frequent cause of hospitalization and fourth most frequent reason for 30-day readmission in the United States. It is associated with immense costs,

significant morbidity and mortality (35, 36). Its incidence varies between 2.8 and 60.3 per 100,000, with an increasing tendency of 3% per year (37, 38).

Although alcohol is reportedly the first or second most frequent cause of AP worldwide, only a very small proportion (2-5%) of high-risk drinkers develop the disease (25, 39). Alcoholic AP is almost eight times more frequent in men (10.3 vs 1.3 per 100,000 and year), mirroring the difference in heavy drinking between men and women. However, the incidence of alcoholic AP is similar in the two groups in the heavy drinker population (40). Alcoholic AP is often seen together with smoking and hypertriglyceridemia, further worsening prognosis (41). Yadav et al. found a linear increase in the prevalence and amount of smoking with alcohol consumption in AP patients (42).

3.4.2.2 Diagnosis and severity

The diagnosis of AP is established by the Revised Atlanta Classification (43). Two out of the following three criteria must be met: (a) abdominal pain suggestive of pancreatitis, (b) serum amylase or lipase level greater than three times the upper normal value, or (c) characteristic imaging findings.

Aetiological workup is crucial in AP, as the aetiology strongly influences treatment and prognosis. It is crucial to differentiate biliary and alcohol-induced AP as early as possible during the disease course. In biliary cases, the diagnosis is established based on imaging after which endoscopic retrograde cholangiopancreatography or cholecystectomy is performed, resolving pancreatic duct obstruction, and preventing recurrence. Although alcohol is the other most frequent aetiological factor, neither clinical practice nor evidence-based guidelines provide detailed information on prerequisites to diagnose alcohol-induced AP (44, 45). Therefore, it remains the responsibility of the attending physician to determine the aetiological role of alcohol in a certain episode of AP.

Early adequate treatment is necessary to prevent the development of necrosis and organ failures. Three grades of severity are distinguished in the classification: mild, moderate and severe AP. Local or systemic complications without persistent organ failure are characteristic of moderately severe cases, while persistent organ failure is requisite in severe AP. Prognostic factors are also widely researched, to help identify high-risk patients likely in need of intensive care. Alcoholic aetiology is associated with more severe disease course and complications compared to non-alcoholic cases (34, 46, 47).

3.4.2.3 Development and progression

The threshold of alcohol consumption is not clear in AP (48), but a dose-dependent relationship was observed in a meta-analysis between the amount of alcohol and the risk of AP (49). However, in women a threshold of 40 g/day was found, whereas in men no safe amount of alcohol consumption could be established. Results regarding the risk associated with different types of alcohol and the role of binge drinking are controversial (25). The American College of Gastroenterology Guideline advises to only consider the diagnosis of alcohol-induced AP if more than five years of heavy alcohol consumption (>50 g per day) is present in the patient's medical history (50).

Additional genetic and environmental factors increase the risk of AP in drinkers. A dose-response association was found between smoking and AP (51, 52), and combined with heavy drinking, smoking can further increase the risk of AP up to four times compared to non-smokers (53, 54).

If the toxic and environmental effects persist after the first episode, progression to recurrent AP (RAP) and chronic pancreatitis (CP) may take place. This process is explained by the two-hit hypothesis and reinforced by clinical experience (55). Although recurrence rates differ greatly among cohort studies and patients with alcohol-induced AP are significantly younger, it is established by multiple studies that recurrence rates are significantly higher in alcohol-induced AP compared to other aetiologies (41). Chronic harmful alcohol consumption is the primary cause of CP in most countries. Progression to CP is also faster in these patients, meaning that the consequences of CP are a long-term burden for the patients and the health-care system.

3.4.2.4 Prevention

Alcohol cessation is the only preventive measure to reduce the risk of recurrence and progression to CP in alcohol-induced AP. Therefore, all patients with alcohol-induced AP must be offered and provided help to reduce their alcohol consumption, with a long-term goal of abstinence. Unfortunately, as stated in the American Gastroenterological Association Institute Technical review (56), there is a knowledge gap concerning alcohol interventions in AP patients. So far, only one randomized controlled trial (RCT) was conducted in AP on alcohol counselling by Nordback et al., with remarkable efficacy (57). Based on their results, the American Gastroenterological Association Institute Guideline on Initial Management of Acute Pancreatitis recommends brief alcohol intervention during admission (45). Despite the lack of information, a strong recommendation was articulated by the panel, underlining the importance of the issue. Moreover, based on the same study, the International Association of Pancreatology/ American Pancreatic Association (IAP/APA) evidence-based guidelines recommend dedicated follow-up visits after alcoholic pancreatitis to prevent recurrence (58). Taking into consideration the data available on alcohol and smoking regarding the development of CP, Yadav et al. proposed that counselling for not solely alcohol but also smoking cessation should be routine following the first episode of AP (41). A survey from the Dutch Pancreatic Study Group from 2021 highlighted the need for a uniform and validated approach in alcohol counselling and the missed opportunities regarding the prevention of RAP and CP in alcohol-induced pancreatitis (59).

3.5 Alcohol and the liver

3.5.1 Alcoholic liver disease

As previously mentioned, the liver and the gut are functionally forming one unit, the gut-liver axis. The majority of alcohol absorbed into the portal blood is metabolised in the liver to acetaldehyde, mainly by the alcohol dehydrogenase and then to acetate by aldehyde dehydrogenase. Then, in the periphery acetate is further converted to water and carbon dioxide (14). Further pathways include the cytochrome P450 enzyme (CYP2E1) as part of the mitochondrial enzyme oxidation system and catalase. Acetaldehyde is highly reactive, binding to lipids, proteins and forming deoxyribonucleic acid (DNA) adducts it is a major contributor

to liver damage. These adducts induce adaptive immune response-mediated inflammation through the release of inflammatory cytokines and chemokines. Furthermore, mitochondrial damage and alterations result in decreased ATP generation, ROS production and decreased alcohol dehydrogenase activity. Acetaldehyde also promotes collagen synthesis, advancing liver fibrosis. In chronic alcohol exposure, a switch to the CYP2E1 pathway occurs, which results in further ROS production, inducing lipid peroxidation and oxidative stress. Moreover, acetaldehyde-induced glutathione depletion impairs the antioxidant capacity, worsening the damage induced by ROS and oxidative stress. The upregulation of UPR genes was described in alcohol-fed mice and pigs (60). The contribution of UPR to hepatocellular damage in alcoholic liver disease is beyond dispute (61).

Besides alcohol's and its toxic products' role in hepatocellular damage, the crosstalk between the gut and the liver is of significant importance from the pathophysiological point of view (16). Bacterial translocation and the recognition of PAMPs in the liver by Kupffer and stellate cells, as a consequence of the 'leaky gut', leads to the activation of a pro-inflammatory cascade and signalling promoting fibrosis, which can progress to HCC. PAMPs as well as DAMPs are recognized by the Kupffer and stellate cells via pattern-recognition receptors and endotoxins activate Toll-like receptors 4, 9 and 2 resulting in a pro-inflammatory cascade, leading to the activation of nuclear factor-κB. Fibrosis is promoted through the downregulation of BMP and activin membrane-bound inhibitor homologue (BAMBI) in stellate cells. As a result, transforming growth factor-β activity increases, which enhances extracellular matrix production (62).

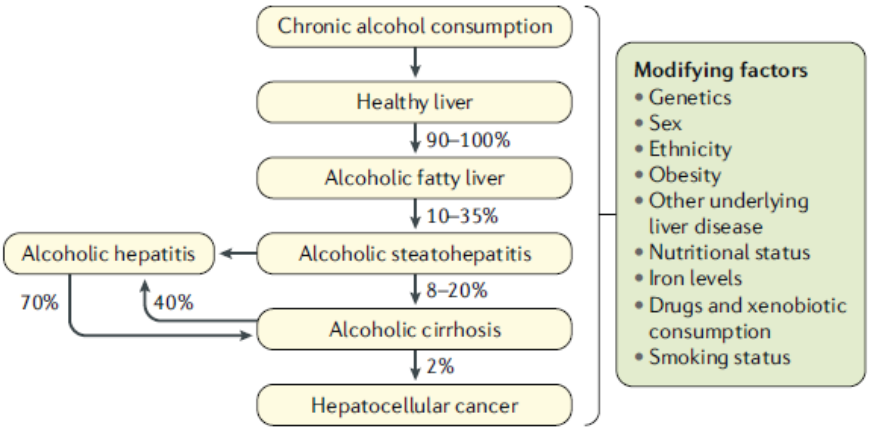


Figure 3 The natural disease course of alcoholic liver disease. *The percentage of patients progressing from each alcoholic liver disease is shown. (Seitz et al., 2018; (63))*

Alcoholic liver disease (ALD) ranges from steatosis, through alcoholic steatohepatitis to cirrhosis, liver failure and hepatocellular carcinoma (HCC) (**Figure 3**) (14). The development of ALD is multifactorial, genetic factors, the immune system, the gut microbiome and environmental factors, such as diet all influence the disease course. Alcohol-related steatosis is the universal response of hepatic tissue to chronic alcohol use, diagnosed based on fat content (>5-10%). The mechanism behind the accumulation of lipids within hepatocytes is complex, but it is fully reversible. The annual progression to cirrhosis was found to be 3% and liver-related mortality 1% by a recent meta-analysis (64). In alcoholic steatohepatitis, local inflammation develops which is the starting block for cirrhosis and HCC. Alcoholic

steatohepatitis is characterized by specific histological features, namely ballooning and neutrophil infiltration. Apart from the slow progression to cirrhosis and eventually HCC, acute worsening of alcoholic steatohepatitis with the development of jaundice and clinical decompensation is called alcoholic hepatitis. This condition often necessitates hospital admission and has a 20-50% mortality rate (63). With further progression of ALD the accumulation of collagen and other extracellular matrix proteins (liver fibrosis) mainly by activated hepatic stellate cells leads to the formation of regenerative nodules surrounded by fibrous bands, called cirrhosis. Cirrhosis is characterized by the severe disruption of hepatic venous flow, portal hypertension and a decline in both synthetic and detoxifying liver function (65). At first, compensated cirrhosis is associated with no or minor symptoms and slow but continuous progression of fibrotic changes and rising portal pressure. The development of complications, namely ascites, hepatic encephalopathy, or variceal bleeding marks the turning point to decompensated cirrhosis. Although cirrhosis was previously considered an end-stage disease, with the elimination of the cause, regression to compensated cirrhosis or even pre-cirrhotic stages may be achieved (66). The dysfunction of five main organ systems is incorporated in most of prognostic scoring systems (hepatic, renal, respiratory, cardiovascular and central nervous system). However, a wide range of organ dysfunctions can be observed in cirrhotic patients, including the immune system, adrenal glands, thyroid glands and muscles. In most patients, acute deterioration occurs with the development of organ failures, which often leads to death. 30% of patients hospitalized with decompensated cirrhosis develop acute-on-chronic liver failure, which will be discussed later.

3.5.2 Acute-on-chronic liver failure

3.5.2.1 Definitions and diagnostic criteria

Acute-on-chronic liver failure (ACLF) is a clinical entity, observed in patients with chronic liver disease, characterized by the failure of one or more organ systems and high short-term mortality (65). International consensus regarding the definition of ACLF is lacking. The most widely used definition is the one introduced by the European Association for the Study of the Liver – Chronic Liver Failure Consortium (EASL-CLIF), based on the CANONIC prospective, multicentre observational study. ACLF, by the EASL-CLIF definition has three major characteristics: acute decompensation of cirrhosis, the presence of simple or multiple organ failure (MOF) and the high probability of mortality within 28 days. It is often preceded by a precipitating event (**Figure 4**). The CANONIC study was designed to assess the prevalence and clinical course of the disease, while improving the performance of prognostic scores, comprising of 1343 patients' data (67). The EASL-CLIF definition applies to patients with cirrhosis -without taking previous decompensation into account-, includes extrahepatic organ failures and does not exclude extrahepatic precipitating events (68). Definitions of organ failures and cut-off points for laboratory parameters (total bilirubin and International Normalized Ratio) are also non-identical. Organ dysfunction, the less severe form of impaired functioning of the six organ systems included in the ACLF definition (liver, brain, kidneys, lungs, coagulation and circulation) is defined as dysfunction and is a trait of decompensated cirrhosis. In the CANONIC study, the CLIF-SOFA score was used to define organ failure, which was later modified to improve prognostic accuracy. ACLF severity is graded based on the number of failing organs. ACLF grade 1 includes single kidney failure, single liver,

coagulation, circulatory or lung failure associated with 1.5-1.9 mg/dl serum creatinine levels or grade 1 or 2 hepatic encephalopathy and single brain failure with 1.5-1.9 mg/dl serum creatinine levels. Two and three organ failures are defined as grade 2 and grade 3 ACLF.

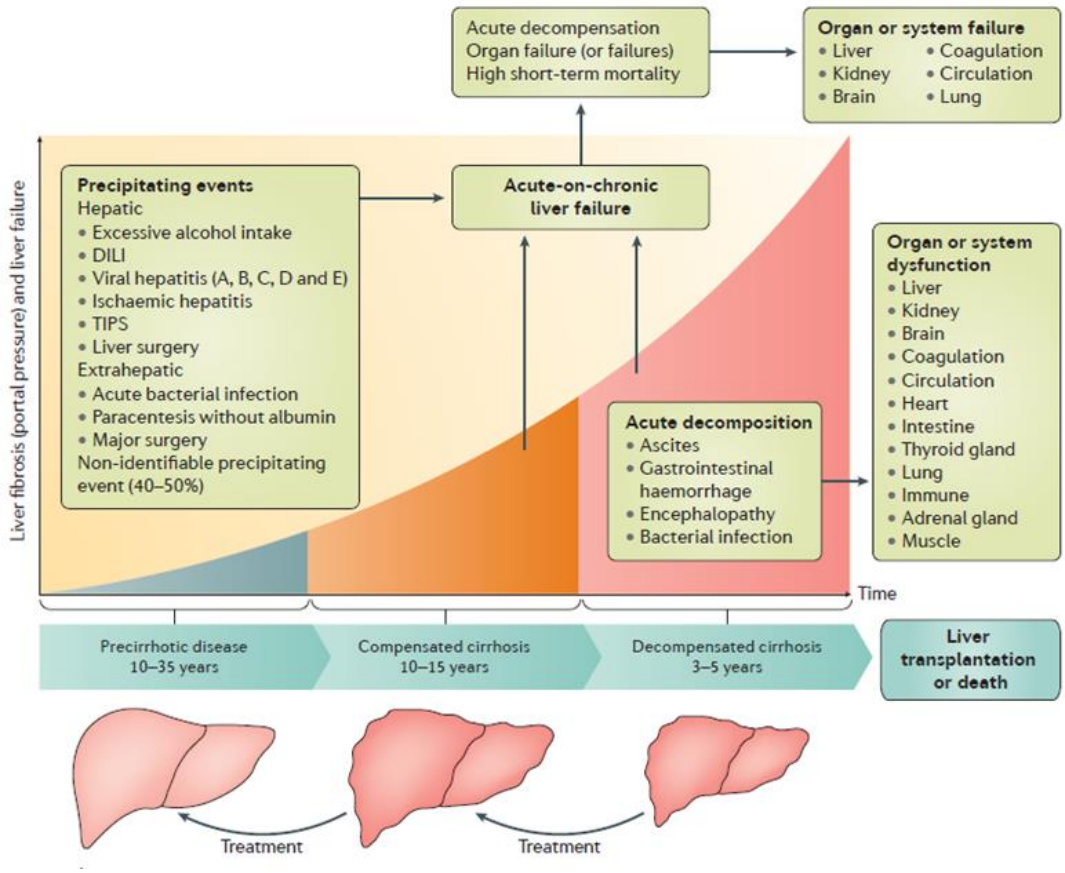


Figure 4 The clinical course of cirrhosis. *Acute-on-chronic liver failure (ACLF) can develop at any stage from compensated to decompensated cirrhosis and can involve hepatic or extrahepatic precipitating events. A considerable proportion of patients have no identifiable triggering event. DILI, drug-induced liver injury; TIPS, transjugular intrahepatic portosystemic shunt (Moreau, 2013; (67))*

The consensus definition used by the Asian Pacific Association for the Study of the Liver (APASL) is fundamentally different: it applies only to patients without prior decompensation but does not necessitate the presence of cirrhosis. The APASL defines ACLF as the development of liver failure due to a hepatic insult. Liver failure is described as the development of hepatic encephalopathy (HE) within 4 weeks of jaundice. The acute occurrence of decompensating events in patients with chronic liver disease, including patients with prior decompensation is categorized as acute decompensation. These two patient groups are distinguished by the APASL experts, to create a more homogenous ACLF population compared to the European definition (69). Sepsis and infections are viewed as consequences of liver failure rather than precipitants, which is contradictory to the European definition.

The overlap between the EASL-CLIF and APASL definitions was examined in the CANONIC study (67) as well as in a Korean cohort (70). It is clear, that the definitions capture distinct patient populations, with a small overlap (67). Regarding survival, the EASL-CLIF definition predicts prognosis more accurately, therefore may be more useful in everyday patient care.

3.5.2.2 Epidemiology

While the prevalence, aetiology and survival are vastly different using the EASL and APASL definitions, I will mostly focus on ACLF defined by the European definition.

Studies using the EASL-CLIF definition reported a worldwide prevalence of 24-34% in at-risk population (mostly patients hospitalized with decompensated cirrhosis) (65). 28-day transplant-free mortality in patients with decompensated cirrhosis without ACLF was 1.9% in the CANONIC study (67) and 2.6% in a Chinese analysis (71). Mortality in ACLF correlates with the number of organ failures, ranging from 23% to 23.6% in grade 1, from 31% to 40.8% in grade 2 and from 60.2 to 74% in grade 3 ACLF. 90-day mortality rates ranged from 2.1% in patients without ACLF to 84.7% in grade 3 ACLF.

The leading cause of chronic liver disease in ACLF patients in Europe and North America is alcohol. Alcoholic cirrhosis was identified in more than 60% of all cases in the CANONIC study, while active alcoholism was determined as the precipitating event in 16.8% of the cases (67). Hepatitis C virus infection is the second most frequent etiological factor, while mostly bacterial infections, alcohol and gastrointestinal bleeding trigger the development of organ failure.

3.5.2.3 Pathophysiology and clinical features

SI is the key to understand the development of ACLF. As previously discussed, SI may be provoked by PAMPs and DAMPs recognized by pattern recognition receptors (***Figure 5***) (72). SI results in tissue hypoperfusion, mitochondrial dysfunction and immune-mediated tissue damage. The latter creates a vicious cycle, creating an excess of DAMPs, further worsening the patient's state.

Per the EASL-CLIF definition, both hepatic and extrahepatic events may provoke the development of ACLF (***Figure 4***), both in compensated and decompensated cirrhosis. In about one fourth of the patients (23%), no prior decompensation was registered in the CANONIC study. With the progression of cirrhosis, not only the liver architecture suffers a distortion, but several organs' and systems' dysfunction have been proven in decompensated cirrhosis, such as the brain, kidneys, circulation, coagulation, lungs, heart, intestines, adrenal and immune system (65). When this fragile, altered system is confronted with a precipitating factor, it can result in organ failure. Regardless of the endogenous or exogenous trigger, in ACLF an even more severe systemic inflammatory process takes place than in cirrhosis. This results in cytokine storm, causing additional tissue damage.

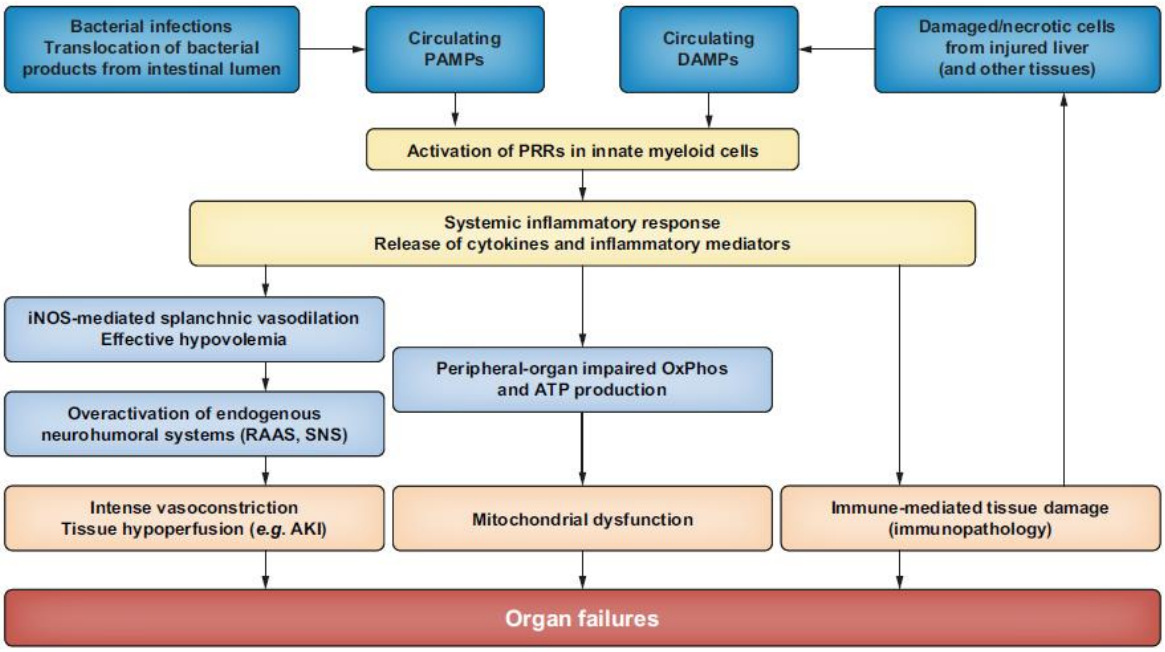


Figure 5 Pathophysiology of acute-on-chronic liver failure *Schematic of induction of systemic inflammation and its role in the development of organ failures. ACLF, acute-on-chronic liver failure; AKI, acute kidney injury; DAMPs, damage-associated molecular patterns; PAMPs, pathogen-associated molecular patterns; PRRs, pattern-recognition receptors; iNOS, inducible nitric oxide synthase; OxPhos, oxidative phosphorylation; RAAS, renin-angiotensin-aldosterone system; SNS, sympathetic nervous system.*

Bacterial infections and alcohol consumption are the most common precipitating events in Europe, seen in about 33% and 25% of the cases (67). On the other hand, the leading cause of ACLF in Asia, HBV reactivation is extremely rare in Europe, as well as hepatitis A and E infections. Liver surgery, transjugular intrahepatic portosystemic shunt placement, ischemic hepatitis mostly in sepsis and drug-induced liver injury can also trigger the development of ACLF. In about 40% of patients, the precipitating factor cannot be identified. This prompts toward the investigation of bacterial translocation's role in the pathophysiology of the disease. It is hypothesised that consequential splanchnic vasodilation results in organ hypoperfusion and the systemic inflammation is capable of inflicting organ damage and cell death throughout the body (73).

Based on the findings of the CANONIC study, the most frequently affected organs are the kidneys (55.8%) and the liver (43.6%), followed by coagulation, brain, circulation and the lungs. The high threshold of bilirubin (≥12 mg/dl) partly explains this phenomenon (only less than half of the patients having liver failure), and it must be noted that almost all patients have liver dysfunction of some degree. However, the concept of ACLF includes the failure of other organ systems as a consequence of liver dysfunction and cirrhosis, when stimulated by a precipitating factor (73).

The first week is crucial regarding the course of the disease. 50% of patients admitted with ACLF grade 1 improve in the first 7 days of hospitalization, 25% is stable and 25% shows decline. These patient groups are vastly different regarding 28-day survival (67).

3.5.2.4 The role of alcohol in acute-on-chronic liver failure

The prevalence of ACLF was found to be much higher in patients with alcoholic cirrhosis with (42.9%) or without (32.5%) active alcohol consumption compared to patients with non-alcoholic cirrhosis (24.3). A more severe disease course can be observed in the alcoholic population, the proportion of patients with grade 3 ACLF being 22% (from ACLF cases in the group) in patients with active alcohol consumption and alcoholic cirrhosis, 10% in patients without active alcohol consumption and alcoholic cirrhosis and only 7% in non-alcoholic cirrhosis in the CANONIC study. One fifth of ACLF cases was caused by severe alcoholic hepatitis. A Chinese study found comparable short-term mortality, but significantly higher 90-day and 1-year mortality in patients with extrahepatic precipitating events (74).

3.5.2.5 Treatment of ACLF

As the first 7 days are decisive to prognosis and survival of ACLF, early intervention is critical (65). The two pillars of medical management of ACLF are the identification and treatment of the precipitating factor and supportive care (72). The prevalence of infection at admission and during the first four weeks of ACLF is very high (reaching 80%) (75), therefore immediate empirical antibacterial therapy is recommended at diagnosis, considering the suspected site of infection and local resistance patterns. Corticosteroid therapy is the gold standard in alcoholic hepatitis; however, the risk of infection necessitates careful consideration. The Lille score is used to identify responders at day seven of steroid therapy. Human albumin solution is indicated in three scenarios: after high-volume paracentesis, acute kidney injury stage 2 and 3 and spontaneous bacterial peritonitis. Adequate organ support needs a multidisciplinary approach and is often provided in the intensive care unit (ICU) (76). However, ICU admission may not be beneficial for all patients with ACLF – in some cases a palliative approach should be chosen. Intensive therapy also may serve as bridge to transplantation.

Liver transplantation is the definitive therapy in ACLF. The 1-year survival rate post-transplantation in ACLF grade 1 and 2 is comparable to patients without ACLF (77). Despite the promising results, allocation and prioritization remain problematic on the transplant waiting list (72). The stimulation or replacement of hepatic functions came into focus as new therapeutic prospects emerged. The development of extracorporeal liver support systems dates back to the seventies with the aim to stabilize patients at the time of acute decompensation when transplant is not available or bridge patients to transplant (78). At first, these devices were designed to replace only excretory functions and were based on hemoperfusion and adsorption (79). The newer technologies combined these methods with bioreactors containing hepatocytes creating bioartificial liver support systems with the potential of synthetic activity, known as bioartificial liver support systems. Liver support systems are being tested in animal models and humans as well, but data is insufficient to draw firm conclusions so far (80, 81). The administration of granulocyte colony-stimulating factor and stem cell transplant are also under consideration (82).

Objectives

In the work conducted for this thesis we used a triad of clinical research methodologies – a cohort analysis, a randomized controlled trial, and a meta-analysis – to assess prognostic factors, preventive and therapeutic interventions in the two gastrointestinal diseases most frequently seen in patients with harmful alcohol consumption: acute pancreatitis and acute-on-chronic liver failure.

In each project, the following objectives were set:

1) We aimed to assess the risk for severity and mortality associated with hypoalbuminemia and its predictive value in acute pancreatitis.

2) We aimed to create a cessation program utilizing brief intervention methods for the prevention of recurrent alcohol-induced acute pancreatitis.

3) We aimed to assess the efficacy and safety of different liver support devices in acute-on-chronic liver failure.

The studies

5.1 Hypoalbuminemia in acute pancreatitis: a prospective cohort analysis

5.1.1 Introduction

AP is a common gastroenterological disorder, with rising incidence and high medical costs. The commonly used revised Atlanta Classification distinguishes between mild, moderate and severe disease based on the development and duration of organ failure (see chapter 1.4.2.2) (43). As the mortality rate can reach 30% in severe cases, identifying risk factors and potential therapeutic targets is of utmost importance.

Human serum albumin is the most abundant protein in human serum with a very diverse role. Although this hypothesis has been contradicted by recent data, declining albumin levels during inflammation have long prompted physicians to underestimate its contribution to maintaining homeostasis during inflammation. However, albumin plays a pivotal role in maintaining the plasma redox state (83), and its scavenging activity is likely to influence vascular resistance through the regulation of nitric oxide levels (84). Furthermore, low albumin levels result in dilution and increased drug clearance, ultimately causing sub-optimal treatment (85).

Small retrospective cohort studies have shown that hypoalbuminemia is an independent risk factor for severe AP and in-hospital mortality in adults and children (86, 87). Low serum albumin has been reported to be associated with persistent organ failure and prolonged hospital stay (88). However, whether albumin is only a marker or there is a cause-effect relationship between hypoalbuminemia and disease severity and mortality should be further evaluated.

While comprehensive analyses are missing on AP patients with hypoalbuminemia and albumin loss in AP, we aimed to evaluate (1) on-admission and in-hospital hypoalbuminemia as a risk factor in AP, (2) the prognostic potential of human serum albumin, (3) whether there is a dose-dependent relationship between albumin level and disease outcomes and (4) the relation of albumin loss to severity and mortality.

5.1.2 Methods

5.1.2.1 Study design and definitions

This analysis of an international, prospective, multicentre cohort was conducted using data from the Acute Pancreatitis Registry operated by the Hungarian Pancreatic Study Group (HPSG) (89). Patient data were collected from establishment of the registry to 31st December 2019 on electronic case report forms and validated using a four-tiered data validation protocol. Contributing centres are shown in the online supplementary material (see *chapter 5.1.2.5.*). The registry was approved by the Scientific and Research Ethics Committee of the Medical Research Council of Hungary (222254-1/2012/EKU) in 2012. It conforms to the Declaration of Helsinki, as revised in 2013. All participants provided written informed consent. Data collection and validation are detailed by Párniczky et al. (90). The HPSG published analyses from the registry, the population of which may overlap with our analysed cohort (90-100).

Diagnosis of AP was established using the IAP/APA guidelines (58), while severity and complications were defined using the Revised Atlanta Classification (43).

5.1.2.2 Participants

Analyses were performed on patients' data with albumin measurement anytime during hospitalization (lowest measured albumin cohort, n=1272) and in the first 48 hours of hospitalization (on-admission albumin cohort, n=1149) to answer a post-hoc clinical research question. The cut-off value between the low and normal albumin group was 35 g/L in both cases, based on the commonly used lower normal value. Subjects were further divided into seven subgroups (Groups 1 to 7) using the lowest (n=1272) or first measured (n=1149) albumin values.

The analyses of albumin change involved selected patients (n=335) with at least two albumin measurements. Delta albumin was calculated as the difference between the first and lowest measured albumin levels.

Data quality for the analysed variables is presented in the online supplementary material (see *chapter 2.1.2.1*).

5.1.2.3 Statistical analysis

Descriptive statistics are presented as the median with 25% and 75% percentiles (IQR) or mean with standard deviation (SD) for continuous variables and as numbers and proportions for categorical variables. The Chi-squared test or Fisher's exact test was used to assess the relationship between categorical variables. The Mann–Whitney U test or Kruskal–Wallis test followed by Dunnett's post hoc test was used to evaluate differences between groups in the case of continuous variables. Multivariate binary logistic regression analysis was performed to identify the risk factors independently associated with severe disease and mortality. Odds ratios (OR) with 95% confidence intervals (CI) were calculated. The Receiver Operator Characteristic (ROC) curve and Area Under the Curve (AUC) with 95% CI were used to identify the ability of albumin levels to predict the mortality or severity of AP (The various AUC values were classified as follows: between 0.5 and 0.6 – fail; between 0.6 and 0.7 – poor; between 0.7 and 0.8 – fair; between 0.8 and 0.9 – good; and over 0.9 – excellent.) Best cut-offs were calculated using the Youden index (101). $P<0.05$ was considered statistically significant, except for the Kruskal–Wallis test followed by Dunnett's post hoc test, where $p<0.025$ was considered statistically significant. All analyses were carried out in R statistical software, version 4.0.2 (R Core Team, 2020, Vienna, Austria), packages: pROC (v. 1.17.0.1) and PMCMRplus (v. 1.9.0.) (102, 103).

5.1.2.4 Representativity

The main characteristics of the analysed cohorts are consistent with the literature data. However, they differed significantly from the entire cohort (n=2461) in terms of severity, length of stay and mortality (online supplementary material; see *chapter 2.1.2.5*).

Cohorts were compared to the original (n=2461). The analysed cohorts differed significantly in severity ($p=0.025$ for on-admission and $p=0.005$ for lowest albumin) and length of stay ($p<0.001$ for both cohorts). The lowest albumin cohort also differed in mortality ($p=0.026$).

5.1.2.5 Supplementary material

The online supplementary material can be found at (https://www.nature.com/articles/s41598-021-03449-8).

5.1.2.6 Reporting

We report our results following The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement, using the checklist provided (104).

5.1.3 Results

5.1.3.1 Incidence of hypoalbuminemia on admission

Nineteen per cent of patients (n=218/1149) presented with hypoalbuminemia (<35g/L). 12.4% of patients were admitted with 30–34.99 g/L albumin levels (Group 5), whereas 4.4% and 2.2% of patients had 25–29.99 g/L (Group 6) and <25 g/L (Group 7) on-admission albumin levels (online supplementary material; see *chapter 2.1.2.5*).

5.1.3.2 Variables associated with on-admission hypoalbuminemia

Hypoalbuminemia was associated with older age (average 59.7 ± 18.0 and 56.0 ± 16.1 years; p=0.005, (online supplementary material; see *chapter 2.1.2.5*).). Males were overrepresented in the analysed cohort (57%) and all subgroups (online supplementary material; see *chapter 2.1.2.5*). Although biliary aetiology was the most frequent in all subgroups, significantly fewer patients had biliary aetiology (34.4% vs 42.2%; p=0.042) in the low albumin group, and a tendency of more alcoholic episodes (24.3% and 19%; p=0.096) was seen (online supplementary material; see *chapter 2.1.2.5*).

Significantly lower body mass index (average 28.23 and 27.23; p=0.012) was found in the low albumin group compared to the normal albumin group (online supplementary material; see *chapter 2.1.2.5*). Diabetes mellitus (22.6% vs. 19.3%; p=0.318) and CP (7.3% vs. 6.1%, p=0.507) were overrepresented in patients with hypoalbuminemia; however, fewer patients with hypoalbuminemia had recurrent AP (17.4% vs. 21.9%, p=0.144) (online supplementary material; see *chapter 2.1.2.5*).

As regards the signs and symptoms, fewer hypoalbuminemia patients presented with abdominal pain (94.9% and 99.2%; p<0.001) and more with abdominal guarding (27.2% and 19.9%; p=0.023). General signs, such as duration and intensity of abdominal pain, abdominal tenderness, nausea and vomiting, did not differ significantly. Hypoalbuminemia was associated with a dose-dependent increase in heart rate and a decrease in systolic and diastolic blood pressure on admission (online supplementary material; see *chapter 2.1.2.5*).

The fulfilment of diagnostic criteria differed significantly (p<0.001) among the low and normal albumin groups on admission. Low albumin patients were less likely to present with pancreatic enzyme elevation, abdominal pain and characteristic imaging findings at the same time (42.7% versus 58.4%) (online supplementary material; see *chapter 2.1.2.5*).

5.1.3.3 Dose-dependent association of CRP, PCT and on-admission hypoalbuminemia

The low albumin group had significantly lower serum amylase (p<0.001) and lipase (p=0.002) levels on admission. An increase in dose-dependent C-reactive protein (CRP) (p<0.001) and procalcitonin (PCT) (p<0.001) was observed in the lower albumin groups. White blood cell count (WBC) (p=0.017) levels were also significantly elevated in the low albumin group (online supplementary material; see *chapter 2.1.2.5*). As regards laboratory markers of renal function, hypoalbuminemia patients had significantly higher blood urea nitrogen (BUN) (p=0.002) and creatinine (p=0.002) levels and a lower estimated glomerular filtration rate (eGFR) (p<0.001) (online supplementary material; see *chapter 2.1.2.5*). Liver enzymes and total bilirubin levels

did not differ between the low and normal albumin groups, but hypoalbuminemia was associated with higher direct bilirubin levels (p=0.005) and a higher international normalized ratio (INR) (p<0.001). Haematological parameters, lipids, ions and glucose levels are shown in (online supplementary material; see *chapter 2.1.2.5*).

5.1.3.4 Dose-dependent association of hypoalbuminemia with complications, severity and mortality

Significantly more patients developed local complications and organ failure in the low albumin group (p=0.016 and p<0.001, respectively) (***Figures 6-7***). Lower albumin levels correlated with a higher rate of peripancreatic fluid collection and respiratory failure (p<0.001 and p=0.051). The rate of pancreatic necrosis, pseudocyst or heart failure did not differ significantly between the groups.

Most importantly, hypoalbuminemia was associated with increased mortality (p=0.020), disease severity (p=0.015) and hospital stay (p=0.025) (***Figure 8***). Groups 6 and 7 had significantly higher mortality (p=0.005 and p=0.007, respectively) and severity (p=0.028 and p<0.001, respectively) compared to the normal group. Maximum CRP levels during the course of AP significantly and dose-dependently increased with the degree of serum albumin (p<0.001) (***Figure 8***).

5.1.3.5 On-admission hypoalbuminemia is an independent risk factor for severity and mortality

Age, hypertriglyceridemia-induced (with or without concomitant alcoholic aetiology) and idiopathic AP were independently associated with mortality. Severe on-admission hypoalbuminemia proved to be an independent risk factor for mortality with an OR of 3.782 (CI: 1.313–9.462) in Group 6 (<30 g/L) and an OR of 5.256 (CI: 1.389–16.112) in Group 7 (<25 g/L) (***Table 1***). Albumin levels were examined with a 35 g/L cut-off in a separate analysis, which found an independent relation between hypoalbuminemia and mortality (OR: 2.070; CI: 1.021–4.033; online supplementary material; see *chapter 2.1.2.5*). Age, hypertriglyceridemia-induced AP, and, among the multifactorial aetiologies, a combination of hypertriglyceridemia and alcohol were independent risk factors for disease severity. On-admission albumin levels <25 g/L were independently associated with severe AP (OR: 3.620; CI: 1.128–9.978; ***Table 1***).

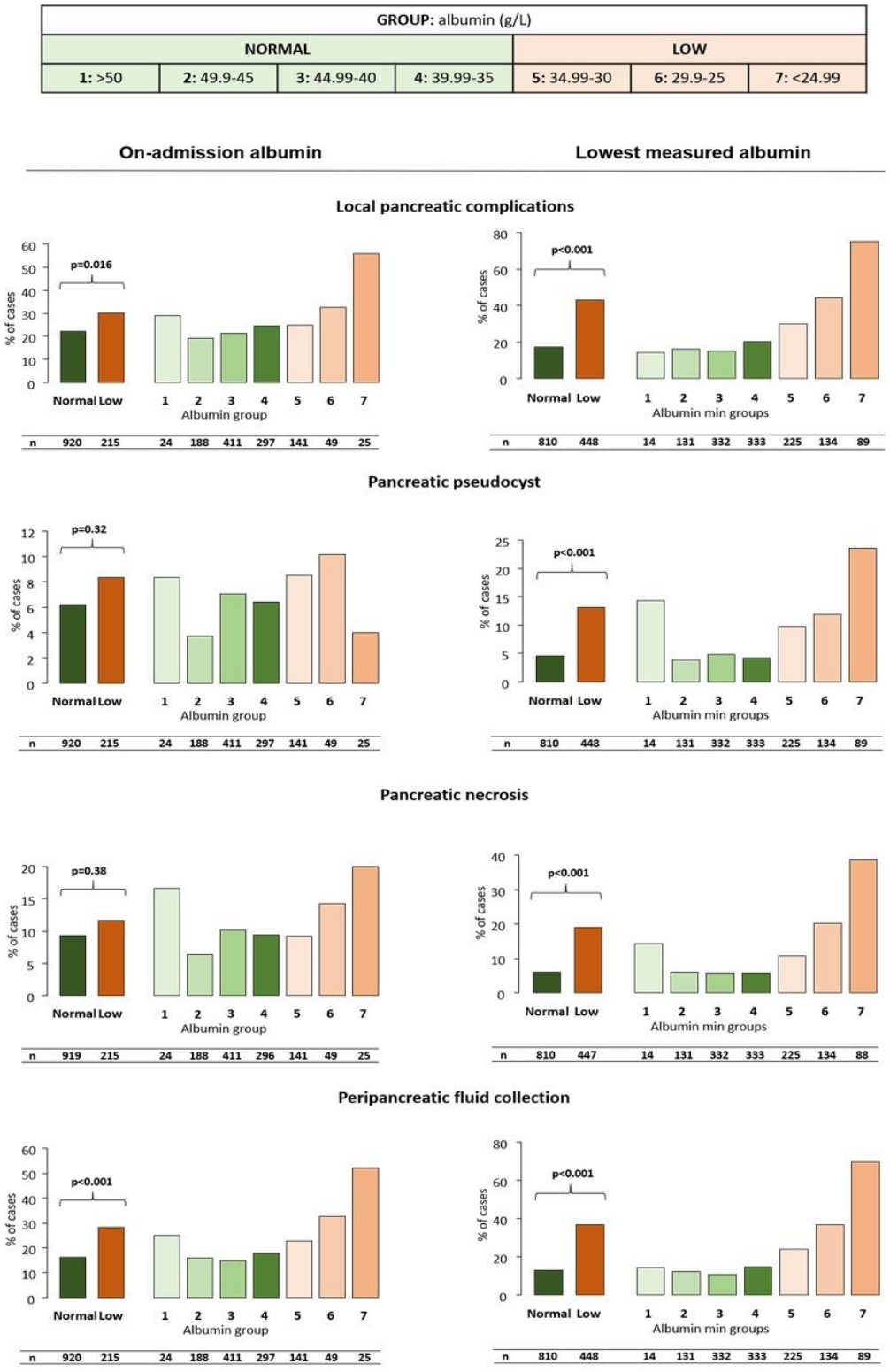


Figure 6 Relation between albumin level and local complications, as defined by the Revised Atlanta Criteria in acute pancreatitis *All types of local complications were significantly more frequent in the low albumin group. A dose-dependent increase was seen in the rate of local complications and peripancreatic fluid collection in both cohorts and in pancreatic necrosis and pseudocyst in the lowest measured albumin cohort. P<0.05 is considered significant. Patients with albumin levels <35 g/L were included in the low albumin group (Groups 5–7).*

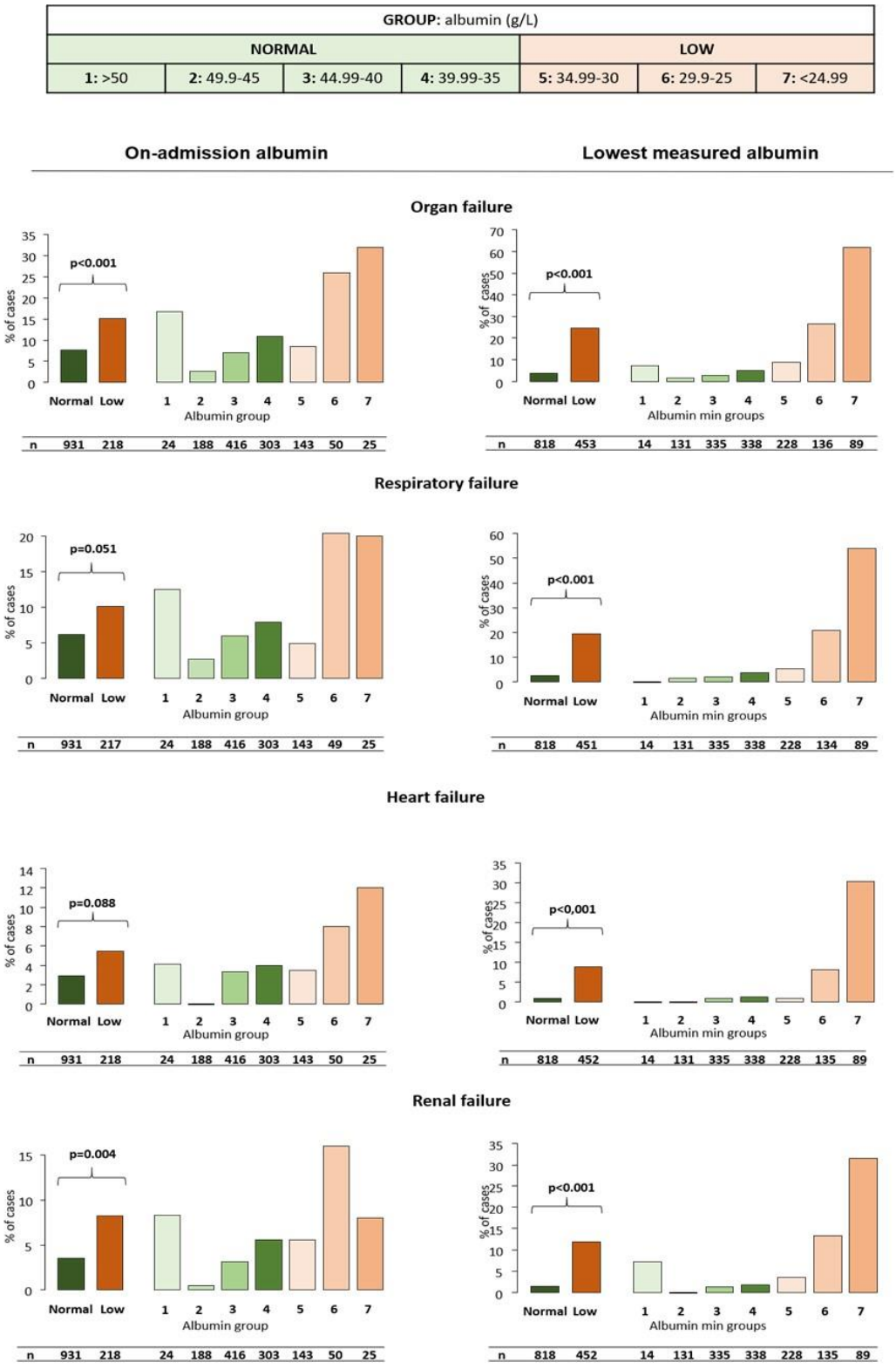
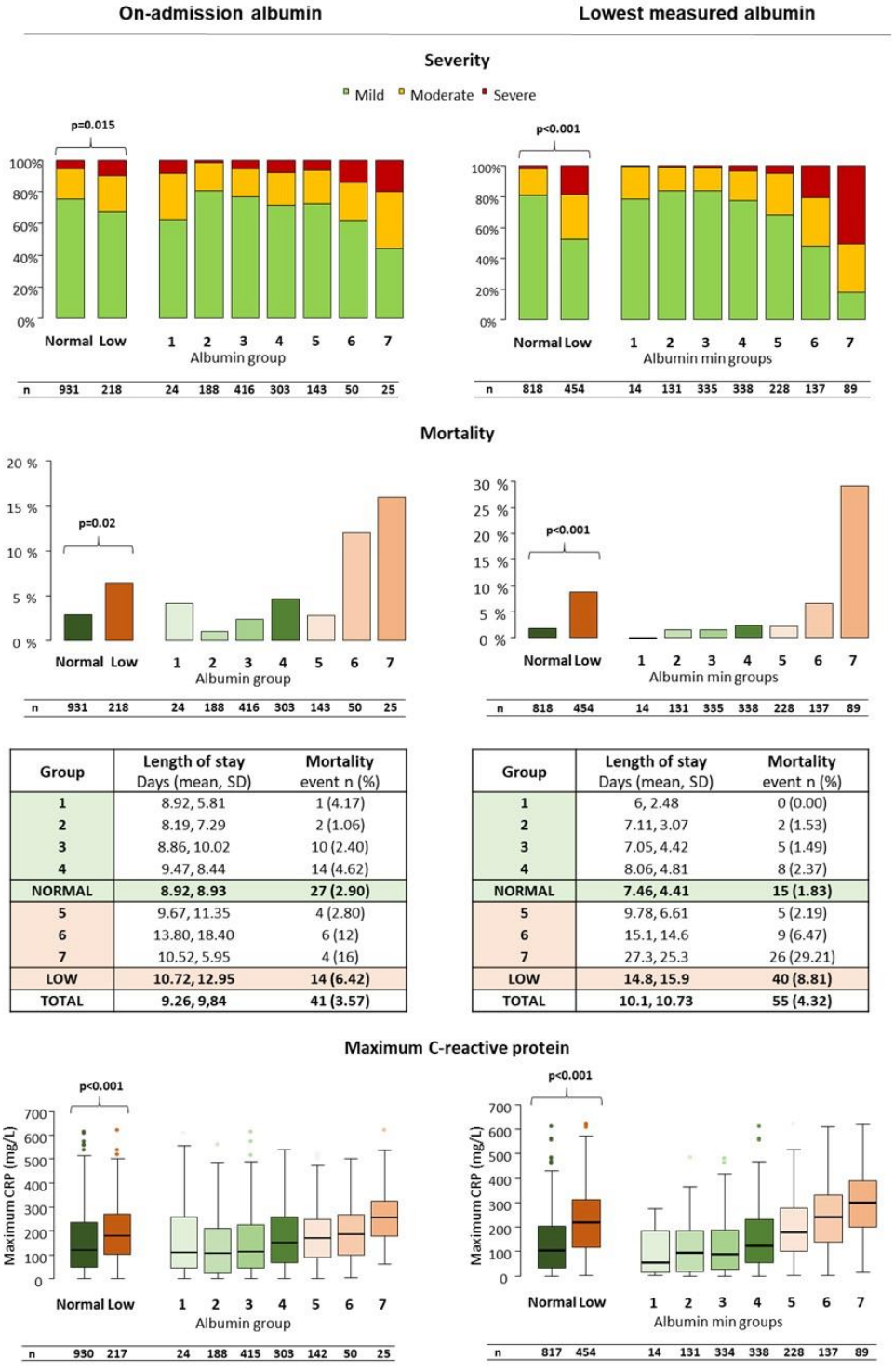


Figure 7 Relation between albumin level and organ failure, as defined by the Revised Atlanta Criteria in acute pancreatitis *Significantly more patients developed organ failure in the low albumin group in both cohorts. A dose-dependent increase was seen in the case of all analyses in the lowest measured albumin cohort. Heart failure was dose-dependently increased in the on-admission cohort as well. P<0.005 is considered significant.*

GROUP: albumin (g/L)						
NORMAL				LOW		
1: >50	2: 49.9-45	3: 44.99-40	4: 39.99-35	5: 34.99-30	6: 29.9-25	7: <24.99



Group	Length of stay Days (mean, SD)	Mortality event n (%)
1	8.92, 5.81	1 (4.17)
2	8.19, 7.29	2 (1.06)
3	8.86, 10.02	10 (2.40)
4	9.47, 8.44	14 (4.62)
NORMAL	**8.92, 8.93**	**27 (2.90)**
5	9.67, 11.35	4 (2.80)
6	13.80, 18.40	6 (12)
7	10.52, 5.95	4 (16)
LOW	**10.72, 12.95**	**14 (6.42)**
TOTAL	**9.26, 9,84**	**41 (3.57)**

Group	Length of stay Days (mean, SD)	Mortality event n (%)
1	6, 2.48	0 (0.00)
2	7.11, 3.07	2 (1.53)
3	7.05, 4.42	5 (1.49)
4	8.06, 4.81	8 (2.37)
NORMAL	**7.46, 4.41**	**15 (1.83)**
5	9.78, 6.61	5 (2.19)
6	15.1, 14.6	9 (6.47)
7	27.3, 25.3	26 (29.21)
LOW	**14.8, 15.9**	**40 (8.81)**
TOTAL	**10.1, 10.73**	**55 (4.32)**

Figure 8 Relation between albumin level and disease severity, mortality, length of stay and maximum C-reactive protein level in acute pancreatitis *Severity, mortality, length of stay and maximum C-reactive protein levels were significantly and dose-dependently associated with hypoalbuminemia in both cohorts. P<0.05 is considered significant.*

Predictor	β	SE	OR	95% CI	p	β	SE	OR	95% CI	p
Mortality	**On-admission albumin (n = 1149)**					**Lowest measured albumin (n = 1272)**				
On-admission albumin level (vs. ≥35 g/L)						**On-admission albumin level** (vs. ≥35 g/L)				
30–34.99 g/L	-0.108	0.553	0.898	0.259–2.390	0.845	-0.016	0.531	0.984	0.313–2.621	0.976
25–29.99 g/L	1.330	0.496	3.782	1.313–9.462	**0.007**	1.069	0.448	2.912	1.166–6.893	**0.017**
<25 g/L	1.659	0.611	5.256	1.389–16.112	**0.007**	2.823	0.365	16.828	8.323–35.129	**<0.001**
Age						**Age**				
per years	0.037	0.012	1.037	0.014–1.063	**0.003**	0.043	0.012	1.044	1.021–1.070	**<0.001**
Gender (vs. male)						**Gender** (vs. male)				
female	-0.222	0.370	0.801	0.383–1.648	0.548	-0.352	0.347	0.703	0.352–1.380	0.309
Aetiology (vs. biliary)						**Aetiology** (vs. biliary)				
alcohol	0.669	0.554	1.952	0.636–5.725	0.227	0.909	0.523	2.481	0.880–6.960	0.083
HTG	1.669	0.747	5.304	1.037–21.022	**0.025**	1.569	0.766	4.803	0.914–19.900	**0.041**
biliary + alcohol	1.234	1.100	3.436	0.178–20.816	0.262	1.651	0.793	5.215	0.949–22.798	**0.037**
biliary + HTG	-12.903	783.282	-	-	0.987	-12.335	786.272	-	-	0.987
alcohol + HTG	1.781	0.768	5.938	1.123–24.693	**0.020**	1.356	0.793	3.880	0.709–17.009	0.087
idiopathic	1.119	0.427	3.061	1.330–7.223	**0.009**	1.402	0.402	4.063	1.878–9.181	**<0.001**
other	0.010	0.790	1.010	0.152–3.964	0.990	0.213	0.807	1.237	0.182–5.045	0.792
Severity	**On-admission albumin (n = 1149)**					**Lowest measured albumin (n = 1272)**				
On-admission albumin level (vs. ≥35 g/L)						**On-admission albumin level** (vs. ≥35 g/L)				
30–34.99 g/L	0.029	0.383	1.030	0.457–2.086	0.939	0.858	0.410	2.359	1.030–5.240	**0.036**
25–29.99 g/L	0.829	0.449	2.292	0.882–5.238	0.065	2.460	0.345	11.709	6.038–23.515	**<0.001**
<25 g/L	1.286	0.548	3.620	1.118–9.968	**0.019**	3.887	0.346	48.761	25.276–98.908	**<0.001**
Age						**Age**				
per years	0.040	0.010	1.041	1.022–1.061	**<0.001**	0.032	0.009	1.032	1.015–1.051	**<0.001**
Gender (vs. male)						**Gender** (vs. male)				
female	-0.183	0.281	0.830	0.478–1.442	0.515	-0.332	0.274	0.718	0.417–1.225	0.226
Aetiology (vs. biliary)						**Aetiology** (vs. biliary)				
alcohol	0.522	0.420	1.685	0.751–3.673	0.195	0.093	0.403	1.097	0.492–2.403	0.818
HTG	1.712	0.546	5.543	1.776–15.536	**0.002**	1.060	0.565	2.885	0.910–8.476	0.061
biliary + alcohol	1.056	0.802	2.874	0.426–11.572	0.188	0.172	0.778	1.188	0.222–5.006	0.825
biliary + HTG	-13.792	785.525	-	-	0.986	-13.429	753.256	-	-	0.986
alcohol + HTG	1.316	0.632	3.727	0.952–11.941	**0.037**	0.497	0.657	1.643	0.422–5.688	0.450
idiopathic	0.536	0.330	1.709	0.884–3.247	0.104	0.541	0.320	1.718	0.915–3.218	0.091
other	-0.475	0.629	0.622	0.145–1.852	0.450	0.008	0.547	1.008	0.310–2.744	0.988

Table 1 Multivariate logistic regression analysis for severity and mortality using the on-admission and the lowest measured albumin cohort HTG*: hypertriglyceridemia; β: β coefficient; SE: standard error; OR: odds ratio; CI: confidence interval*

5.1.3.6 Poor predictive value on-admission

On-admission albumin levels have an AUC of 0.615 (sensitivity: 57.6%; specificity: 61.1%) for severity with a cut-off at 39.3 g/L (***Figure 9***). The AUC for mortality was 0.660 (sensitivity: 72.1%; specificity: 53.7%) with a cut-off at 37.0 g/L. These data suggest that albumin plays a crucial role in the pathophysiology and clinical outcome of AP; however, it cannot be used as a single biomarker for predicting severity and mortality. Next, we wanted to understand whether albumin loss during the course of AP is related in any way to outcome of the disease; therefore, we regrouped our patients based on the lowest measured albumin levels.

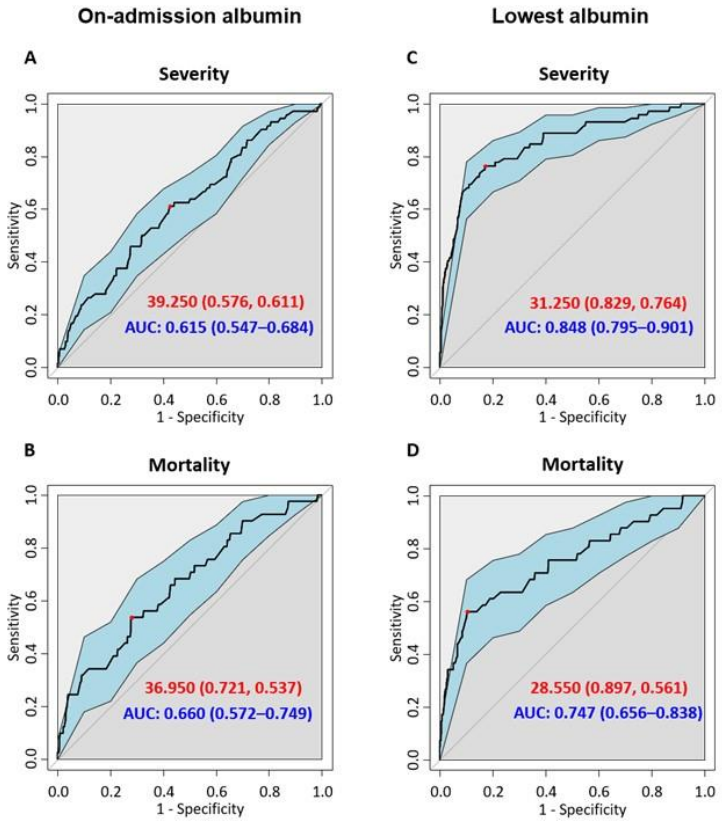


Figure 9 Receiver operating curves for mortality and severity *On-admission albumin levels have poor predictive value, while lowest albumin values have good classification accuracy for severity and fair classification accuracy for mortality. AUC: area under the curve; best cut-offs are shown in red*

5.1.3.7 Dose-dependent association of hypoalbuminemia during hospitalization with severity and mortality

The proportion of patients with hypoalbuminemia anytime during hospitalization was 35.7% (454 patients). A significant, dose-dependent increase was seen in the low albumin groups (Group 5–7) compared to the normal albumin group as regards the rate of all examined systemic and local complications (***Figures 6-7***). The lowest measured albumin levels throughout hospitalization (n=1272) were significantly and dose-dependently associated with severity ($p<0.001$), mortality ($p<0.001$), length of stay ($p<0.001$) and maximum CRP values ($p<0.001$) (***Figure 8***).

5.1.3.8 *Lower albumin levels and greater albumin loss is associated with severity and mortality*

Albumin loss was analysed using data from patients with at least two albumin measurements (n=335; ***Figure 10***). Compared to mild cases, patients with moderate and severe AP showed a greater decrease in albumin levels (medians 5.4 vs. 9 and 15.25 g/L; p<0.001 for both comparisons). The comparison of delta albumin between the moderate and severe groups also yielded significant results (p=0.003). Patients who died also lost significantly more albumin during hospitalization (medians 6.7 vs. 15.75 g/L; p=0.002). The median time to the lowest albumin levels from admission was 4 days (IQR: 3–7 days).

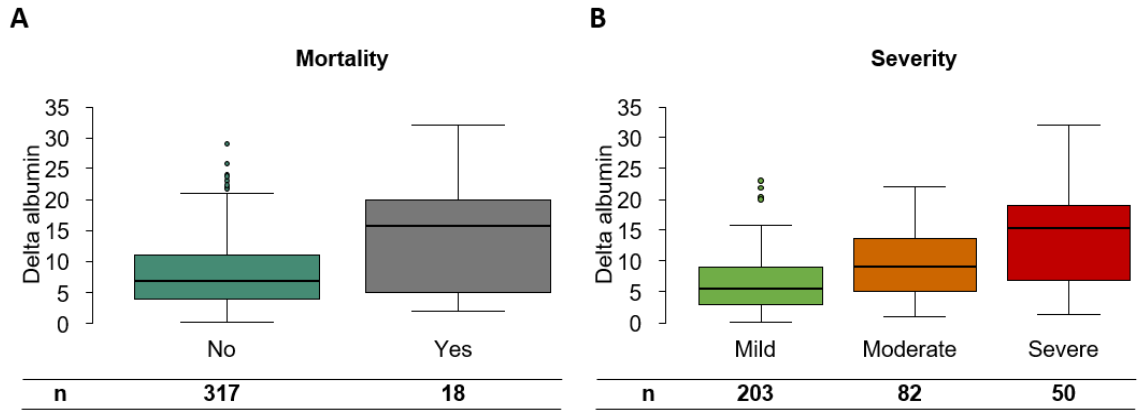


Figure 10 Albumin loss *Delta albumin values (g/L) are shown on boxplots for patients with at least two albumin measurements (n=335) grouped by severity and mortality.*

5.1.3.9 *Extreme hypoalbuminemia increases the risk of severe AP and death*

Age is an independent risk factor for severe AP and mortality, whereas hypertriglyceridemia-induced and idiopathic AP and a combination of alcoholic and biliary causes are independently associated with mortality (***Table 1*** and online supplementary material; see *chapter 2.1.2.5*). Hypoalbuminemia below 25–29.99 g/L (OR: 2.912; CI: 1.176–6.893) and below 25 g/L (OR: 16.828; CI: 8.323–35.129) were associated with an increased risk of mortality (***Table 1***). In a separate analysis, hypoalbuminemia (<35 g/L) was also an independent risk factor for mortality (OR: 4.185; CI: 2.286–8.039) (online supplementary material; see *chapter 2.1.2.5*). Furthermore, hypoalbuminemia anytime during hospitalization was associated with a higher risk for severe AP (OR: 10.664; CI: 6.188–19.614), and a gradual increase of odds ratios can be observed in the low albumin groups (OR: 2.359; CI: 1.030–5.240 for Group 5; OR: 11.709; CI: 6.038–23.515 for Group 6; and OR: 48.761; CI: 25.276–98.908 for Group 7).

5.1.3.10 *The predictive value of lowest albumin values*

The lowest measured albumin levels have higher AUC values: 0.848 for severity and 0.747 for mortality (***Figure 9***). The best cut-off values were 31.3 g/L for severity (sensitivity: 82.9%; specificity: 76.4%) and 28.6 g/L for mortality (sensitivity: 89.9%; specificity: 56.1%). The day of the lowest albumin measurement - including patients with a single measurement- ranged from 1 to 56 days, with a median of 2 days. Most patients only had a single measurement around the time of admission.

5.1.4 Discussion

To date, this is the most comprehensive evaluation of AP patients with hypoalbuminemia, using the largest, prospectively collected, high-quality dataset. We found that almost one-fifth of patients had hypoalbuminemia on admission (19%), and a further 25% developed hypoalbuminemia during hospitalization, meaning that every third patient was affected.

In our analysis, hypoalbuminemia under 25 g/L anytime during hospitalization was independently associated with a more than 47-fold higher chance for severe AP and a more than 16-fold higher chance for mortality. Our findings are consistent with results for hypoalbuminemia in other diseases. Hypoalbuminemia was a prominent risk factor in community-acquired bloodstream infection with severe sepsis and septic shock (105). A retrospective analysis of data from more than 20,000 emergency medical patients in Ireland found that hypoalbuminemia is independently associated with 30-day in-hospital mortality, with a non-linear relationship between mortality and on-admission albumin levels (106). Moreover, in a secondary analysis of a prospective cohort, AP patients with MOF (n=18) demonstrated a sharper decline in serum albumin ($P<0.001$) compared to non-MOF patients (n=39) (107).

We have not only proved that hypoalbuminemia is a risk factor but have also shown the dose-dependent relation between low albumin levels and severity, mortality, number of patients with any local complications, number of patients developing organ failure and maximum CRP levels in both analyses (on-admission and lowest measured albumin levels).

These relations can be explained by the numerous physiological functions of human serum albumin. Albumin was long considered a negative acute-phase protein, with decreasing production giving way to inflammatory cytokines in inflammation (108). Serum albumin levels undoubtedly decrease in inflammatory states, which may be due to a shorter half-life and a larger interstitial pool, which causes the dilution of albumin (109-111). Capillary leak resulting from inflammatory processes plays a role in the decline of serum albumin, but it is argued that the escape of albumin to the tissues may be beneficial because of its antioxidant and scavenging activity (112). Although a more than twofold higher production rate was observed in critically ill ICU patients, this increased production is still not able to balance the higher demand. This can be considered as a relative synthetic insufficiency of hepatic function (113).

Albumin loss was significantly associated with severity and mortality in our analysis. However, only 51.7% of patients in the HPSG database had albumin measurements at least once during their hospitalization, and 13.6% had them at least twice during that time. This highlights how neglected albumin measurements are in AP.

On admission albumin levels were found to have poor predictive values for mortality and severity. Previous studies were mainly retrospective and had a much smaller sample size (86, 114, 115). They only assessed the predictive value of serum albumin for persistent organ failure and peripancreatic infection, or were limited to severe AP.

From the clinician's point of view, the decline of serum albumin levels – regardless of on-admission albumin levels – signals clinical worsening and may aid in identifying high-risk AP patients. However, clinicians mostly miss the opportunity to pre-emptively and frequently measure serum albumin, thus delaying timely intervention.

To date, no clinical trial examined therapeutic albumin administration in AP. As we know, albumin is similarly associated with outcomes in sepsis and septic shock; randomized controlled trials in this field could be a start (112, 116). The controversial results of studies and meta-analyses in this field may be explained by heterogeneous patient populations and the time sensitivity of this treatment (117).

To further exploit the potential in therapeutic albumin administration in AP, more detailed clinical studies are needed to identify the patient subpopulations benefiting the most from this therapeutic option.

5.1.4.1 Strengths and limitations

We conducted the most extensive, most comprehensive cohort study on the role of hypoalbuminemia in acute pancreatitis to date. We analysed high-quality data from a prospective, international, multicentric registry. We identified hypoalbuminemia as an independent risk factor in AP, present in at least every third patient. We also found a dose-dependent relationship between albumin levels and main outcomes, which was previously not described.

Among the limitations, we must mention the arbitrary classification of albumin levels (except for the low-normal cut-off), the missing data on albumin levels and albumin administration during hospital stay, and the limited number of albumin measurements during the hospital stay, which could introduce bias. The limited number of albumin measurements did not enable more detailed analyses of serum albumin at different time points. Our analysed cohorts differed from the total cohort in some aspects, thus potentially signalling performance bias, as albumin measurements are more frequently ordered for patients with expected hypoalbuminemia.

5.1.5 Conclusion

Hypoalbuminemia is remarkably common in AP (seen in 19% of patients on admission and 35.7% during hospitalization) and represents an independent risk factor for severity and mortality. Importantly, albumin loss during hospitalization was also associated with severity and mortality, suggesting that routine monitoring of serum albumin is recommended, and that albumin administration should be examined as a therapeutic intervention in AP.

5.1.6 Implications for research

Clinical trials are needed to assess the potential benefit of albumin replacement in AP.

5.1.7 Implications for practice

(1) Albumin levels should be measured for all AP patients, (2) albumin levels should be controlled at least in those patients whose condition is worsening during AP, and (3) albumin administration should at least be considered in patients with severe hypoalbuminemia (<25 g/L).

5.2 Recurrence prevention in alcohol-induced acute pancreatitis: protocol of a randomized controlled trial

5.2.1 Introduction

AP is an often-unheeded issue by clinicians and healthcare professionals, with significant medical charges (36, 118). Alcohol and biliary obstruction are the two main causes of AP in adulthood, alcohol being the diagnosed inducing factor in 25-35% of the cases (119).

Cohort studies have found that 10 to 30% of patients have recurrent attacks based on medical history, and a recent meta-analysis has shown that 10% of the patients after a single episode of AP and 26% of those with RAP later progress to CP (120). It Is known, that RAP (more than one episodes of AP) significantly lowers physical and mental quality of life (QoL) (121) and alcoholic etiology has been identified in 19% of RAP patients (41). Despite the importance and potentially preventable nature of alcoholic RAP, preventive efforts are still scarce (122, 123).

A pivotal study from Nikkola et al. found that abstinent patients experienced no RAPs during a 9-year follow-up period. On the other hand, 34% of patients who did not stop drinking developed a recurrent attack (124). The median time between the index AP and the first alcoholic RAP ranges from 8.5 months to 2.2 years, but around 80% of the registered first recurrent attacks occur in the first 4 years of follow-up (125, 126). With 6-monthly interventions, Nordback et al. achieved a significant reduction in the recurrence rate of AP in Finland (57, 127).

Smoking is a long-established independent risk factor of AP and CP (see chapter 1.4.2.3). Findings are controversial regarding the effects of smoking cessation. A study published by Sadr-Azodi found that the risk of AP is statistically comparable to never-smokers' after 20 non-smoking years (54). In contrast, a meta-analysis showed an elevated risk of AP in former smokers compared to never-smokers (53).

Limiting alcohol use and smoking apart from their positive effects on the pancreas generally improve health (128) and up to a certain extent, organ damage caused by these substances is reversible (63, 129-131). Smoking cessation alone can prolong life with 1.4-8.5 years (132).

In a Hungarian cohort study of 600 patients, alcohol consumption was 4 times more frequent in males, alcoholic aetiology represented 26.5% of all cases and was often associated with smoking. Alcoholic RAP accounted for 21.2% of all cases in the cohort (133). In a CP cohort, daily alcohol consumption, as an etiological factor, was present in 56% of the cases, and 56% of the participants smoked more than 10 cigarettes/day (134).

It is known that more than half of patients suffering from AUD are also dependent on tobacco, and that continued tobacco use represents a more than two-fold risk for relapse (135, 136). To this day there are no adjusted protocols for the treatment and follow-up of heavy-drinking smokers (137, 138). It is proven that, in contrast with previous assumptions, smoking cessation programs for patients at risk or living with AUD improve alcohol-related outcomes (135, 139) and a brief alcohol intervention improves the rate of successful smoking cessation (140).

However, to date, no study has examined the effects of a combined intervention for the reduction of nicotine and alcohol consumption in RAP and guidance is very limited on this topic (141-144). Based on the above-mentioned reasons, while all patients with alcoholic AP

should receive counselling, a one-time brief intervention will be provided to all participants, without further counselling in the control group.

5.2.2 Objectives

The study encompasses a randomized controlled trial (REAPPEAR-T) and a concomitant cohort study (REAPPEAR-C). The REAPPEAR-T's objective is to investigate the effect of an alcohol and smoking cessation program combined with patient education on the recurrence rate of alcohol-induced AP, CP and QoL. Additionally, the REAPPEAR-C's objective is to investigate the effect of alcohol and smoking cessation (irrespective of intervention) on the recurrence rate of alcohol-induced AP, CP and QoL.

5.2.3 Methods

5.2.3.1 Design

The REAPPEAR study, designed in accordance with the SPIRIT statement (145), utilizes a combined design to answer two questions in one particular patient population. The REAPPEAR-T will be an international, single-blind, 2-arm, parallel group, superiority randomized controlled trial, testing the efficacy of a cessation program for alcohol and smoking, using brief interventions. The REAPPEAR-C is a prospective 4-arm cohort study, which includes all patients participating in REAPPEAR-T with further enrolment after the termination of enrolment to the trial. In the cohort, patients will be grouped by smoking status and alcohol consumption at the end of the study, irrespective of intervention. The same eligibility criteria and outcomes will be used in both sub-studies and differences will be described in the appropriate sections in detail.

For the list of centres please see the online supplementary material (see *chapter 5.2.3.19*). The enhance the visibility of this project and centre recruitment, the protocol is being presented on national and international conferences.

5.2.3.2 Population

Inclusion criteria:

- patient hospitalized with alcohol-induced AP (defined by the revised Atlanta criteria(43))
- regular consumption of at least 40g (women)/ 50g (men) alcohol daily or 280g (women)/ 350g (men) alcohol during the preceding week of onset of abdominal pain
- every day smoker (defined as an adult patient who smoked at least 100 cigarettes in his or her lifetime, and now smokes on a daily basis; as per the CDC definition), with at least 1-year history of smoking
- aged 18-65 years (146)
- completed the standard intervention (see below)
- provided written informed consent (online supplementary material; see *chapter 5.2.3.19*).

Exclusion criteria:

- possible aetiologies for AP other than alcohol (eg. gallstone-related, hypertriglyceridemia above 11.5 mM (99, 147, 148), hypercalcemia, viral infection) and cases with combined etiological factors will be excluded

- major psychiatric illnesses (e.g., schizophrenia, bipolar disorder, dementia)
- currently receiving therapy for AUD or taking part in a smoking cessation program
- three or more documented lifetime episodes of AP (149) or CP criteria are met (150)
- undergoing active or palliative treatment for malignancy
- pregnancy
- life expectancy is less than two years

Medical personnel not involved in the treatment of the patient will perform formal screening and obtain informed consent.

5.2.3.3 Standard intervention

The standard intervention will be incorporated into standard medical therapy in all centres, and will be provided to all patients hospitalized for alcohol-induced AP. Standard intervention will be delivered by a specially trained nurse because interventions delivered by nurses have been found to be the most effective in reducing the quantity of alcohol consumed (151). The intervention will be based on the WHO initiative 'Assist-linked brief intervention', using psychoeducational and motivational interviewing techniques (152). For the standard intervention, we calculated with an average length of 30 minutes, based on a recent Cochrane review including 69 RCT-s, according to which longer interventions on alcohol had no benefit, the median duration being 25 minutes (153). The standard intervention will also provide educational information about the nature of alcoholic AP and the risk of recurrence to the patients. Feasibility and cost-effectiveness were also considered.

5.2.3.4 Intervention in REAPPEAR-T

The repeated intervention will be provided by the same specially trained personnel and structured similarly to the standard intervention. Each session will have the same structure but can be tailored to the patient's needs to strengthen motivation. Sessions will consist of 3 parts. First, the negative effects of alcohol and smoking on the pancreas will be highlighted. Second, the patient's motivation for abstinence and smoking cessation will be discussed. Third, the individual's responsibility in achieving the goals set after motivation assessment, with personalized advice (57). We wish to enhance the efficacy of the repeated intervention by providing feedback for the patient based on the mean corpuscular volume (MCV) and gamma glutamyl-transferase (GGT) values measured at the day of the interview (154). The trained personnel providing the interventions will not take part in patient care in any form.

5.2.3.5 Concomitant care

Patients participating in cessation programs or psychotherapy at the time of enrolment will not be eligible. Patients using self-help programs and nicotine replacement therapy with commonly available products will not be excluded. The provided interventions encourage patients to seek help and try different strategies for alcohol and smoking cessation.

5.2.3.6 Outcomes

The primary endpoint of the REAPPEAR Study will be the composite of 2-year recurrence rate of AP irrespective of aetiology and 2-year all-cause mortality.

Secondary endpoints:

1) RAP irrespective of aetiology (given as cumulative incidence and as rate of event) within 6, 12, 18 and 24 months.
2) Recurrence of alcohol-induced AP (rate of event) within 2 years.
3) The condition of 'likely pancreatitis' (fulfilling the diagnostic criteria of epigastric pain, a serum amylase or lipase level at least two times the upper normal level, and elevated leukocyte count or CRP levels, defined by Pelli et al (155))
4) Length of hospital stay given in days (specifically due to recurrent pancreatitis and cumulative during follow-up)
5) Presentation to the emergency unit with and without hospital re-admission (cumulative incidence)
6) Change of alcohol consumption and tobacco use (compared to baseline), estimated separately form biomarker levels and patient-reported consumption
7) Chronic pancreatitis (incidence within 2 years (150))
8) Changes in body mass index and blood pressure (compared to baseline)
9) Healthcare cost from the perspective of the health insurance fund within 2 years and quality adjusted life years

5.2.3.7 Recruitment

Consecutively, all patients under treatment for alcohol-induced AP who received the standard intervention according to standard protocol will be screened for eligibility, all eligible patients will be offered to participate in the REAPPEAR study. The potential benefits of participation will be highlighted to facilitate patient recruitment.

5.2.3.8 Biologic sample collection and biomarker measurements

At enrolment and every visit, basic laboratory tests from blood will be carried out and participants will provide blood, hair and urine samples for storage in the biobank.

Laboratory parameters measured are shown in the online supplementary material (see *chapter 5.2.3.19*). Laboratory results will be evaluated by a physician, who will decide whether further medical attention is necessary. All patients will receive the results of their laboratory tests in written form.

The samples in the biobank will be stored at −80°C and identified by the personal identification number (PIN) given at study entry. Planned alcohol and smoking biomarker measurements include urine and serum ethyl-glucuronide (or ethyl-sulphate) and hair nicotine measurements (156, 157). All samples will be collected and sent together to the laboratory when the patient number reached the pre-set goal for analysis. The results of the biomarker measurements will not be made accessible for patients. These measurements are only available in specialized laboratories, therefore can be changed later due to feasibility issues.

5.2.3.9 Trial organization, committees and boards

The corresponding center of the REAPPEAR study is the Centre for Translational Medicine, Medical School, University of Pécs (www.tm-centre.org), whereas the coordinator and designer research team is the HPSG (https://tm-centre.org/en/study-groups/hungarian-pancreatic-study-group/). HPSG has been running high-quality international, multicenter clinical trials since 2014 (148, 158-160) and has published relevant guidelines for pancreatic diseases to improve patient care in pancreatology (161, 162).

The Steering Committee will be led by PH (principal investigator, specialist in internal medicine, gastroenterology and clinical pharmacology). Members will be KO (study coordinator), a patient representative, NF (biostatistician), IH (psychologist) and the center leaders. All data gathered for research purposes will be handled confidentially and anonymously, which will be ensured by the Data Monitoring Committee. 6-monthly audits are planned in each center with continuous monitoring of the electronic case report forms (eCRFs) (online supplementary material; see *chapter 5.2.3.19*). For each participant, a PIN will be generated, and it will be present on all forms and documents of each individual. The International Advisory Board will include Ole Petersen, Enrique de-Madaria and Jonas Rosendahl, providing independent external advice and guidance on strategic matters. The study was designed by the Steering Committee and was supported by the University of Pécs, Medical School. The sponsor had no role in the design of the trial and will have no access to the randomization codes or the data. The sponsor will not participate in data monitoring, analysis and publication of results. The independent Safety Monitor will be LC, who will ensure the safety of the patients and revise all reported harms possibly related to the intervention.

5.2.3.10 Data handling

Investigators will be responsible for the accuracy, reliability and quality of the collected data. Detailed data flow will be described in a Data Management Plan.

The Data Monitoring Committee will perform an independent assessment of trial-related documents and activities to ensure respect for subjects' rights, safety and well-being and to guarantee the plausibility of clinical data. After written consent of the subjects, data will be recorded by the investigators. Personal data will not be made accessible to third parties. We will fully comply with the GDPR regulations.

5.2.3.11 Safety

Based on the nature of the combined brief intervention in REAPPEAR-T, we do not expect serious adverse events. However, minor or moderate adverse events may occur. Participants will be provided with information on alcohol and nicotine withdrawal alongside with the available options of professional help for addiction treatment. In case a potentially serious health problem is detected by the investigators related to the intervention, the Safety Monitoring Board will be notified. The REAPPEAR-C is an observational study, hence adverse events are not applicable.

5.2.3.12 Randomization, allocation concealment and blinding in REAPPEAR-T

Central randomization will be performed with randomly permuted block size (2 to 6) and allocation ratio of 1:1 using a computer-generated random sequence. Inclusion and exclusion criteria will be re-checked prior to computer-aided randomization via an online platform. The platform generates the PIN and a follow-up plan (with appointment dates). The randomization procedure will be performed by the same person who screened and consented the patient. This person must be a doctor not actively participating in the treatment of the participant.

Outcome assessors will be blinded to allocation. The medical personnel involved in the check-ups and treatment during a potential hospital re-admission will not be aware of the allocation. Since the nature of the intervention, the patient and the study nurse cannot be properly blinded.

5.2.3.13 Statistical analysis

Sample size calculation for REAPPEAR-T was based on the only published interventional randomized study assessing the effects of repeated brief interventions in alcohol-induced RAP, counting with a 2-year recurrence rate of 21.3% and an absolute reduction to 8.5% (51, 54, 57, 133). Considering one interim analysis on efficacy (with the Pocock correction), 80% power, 5% alpha (superiority design, two-sided) and a drop-out rate of 30%, the estimated sample size is 182 subject per study arm. This sample size calculation is expected to overestimate the minimum number of participants for 3 reasons: (i) the use of a combined intervention on alcohol and smoking and more frequent visits are expected to result in greater reduction of recurrence, (ii) the use of a composite primary endpoint may result in higher event numbers, (iii) the recurrence rate of AP is expected to be higher in the heavy- drinking smoker population, than in a mixed sample. The calculation was performed by Stata (version 15, Philadelphia, USA).

Safety analysis will be carried out upon reaching 10% of the target patient enrolment, and a single interim analysis for efficacy and sample size re-estimation taking into consideration the observed drop-out rate at 50% of the expected total events of the primary outcome, which is 21. Early stopping will be executed (1) if safety concerns arise during the interim analysis or anytime later (stopping for safety concerns), (2) if the statistical power reaches at least 80% and $p<0.05$ for the primary outcome at the interim analysis (stopping for benefit), (3) if the results of the interim analysis show equal effects in both groups (stopping for futility), (4) if power does not reach 80%, sample size will be re-estimated using the observed event and drop-out rate. In case the newly calculated sample size is unfeasible for the trial, both groups will continue follow-up according to the schedule of REAPPEAR-C (stopping for feasibility).

In the final analysis, intention-to-treat will be favoured over per-protocol (or "as-treated") analysis. Information on mortality and hospitalizations will be obtained from the organization responsible for handling data. The "last observation carried forward" strategy will be followed to impute missing data for other outcomes measured during the study.

Sample size calculation for the REAPPEAR-C will be carried out at the final analysis of the REAPPEAR-T, using available data from participants. Further enrolment will be performed according to the estimated sample size. These additional participants will receive the more effective or in case of equality the less costly intervention for alcohol and smoking cessation as determined by the results of the REAPPEAR-T. Participants of the cohort will be categorized into four groups primarily, according to smoking and drinking status (quit smoking; quit drinking; quit both; still smokes and drinks). Time of smoking and alcohol cessation will be taken into consideration. Participants who started smoking or drinking again after an abstinent period will be excluded from analysis in the REAPPEAR-C.

In descriptive statistics, the count and percentage will be provided for each treatment arm for binary outcomes. For continuous outcomes, number (n), mean, median, IQR, SD, minimum, and maximum values will be provided for each treatment arm. In the univariate comparative analysis, we will calculate relative risk with 95% CI when comparing the primary endpoint between two groups (alpha=5%) with a reference arm using the control group complemented with chi-square or Fisher's exact test (the same strategy will be followed for binary secondary outcomes). For continuous variables, we will use t-test assuming unequal variances or the

Mann-Whitney test. We will perform univariate (Kaplan-Meier and Cox-regression) and multivariate (Cox-regression) survival analyses for binary outcomes. An adjustment will be carried out at least for age, sex, socioeconomic status, the number of prior RAPs, comorbidities, history of alcohol consumption (cumulative) and smoking (package year), severity and complications of index AP, body mass index, cholecystectomy and enrolling centre. Mixed effect logistic regression will be conducted to estimate the effect of the multicomponent intervention on the outcomes, where the subject PINs will be used as a random subject. The model will be adjusted for changes in smoking habits, alcohol consumption, body mass index, socioeconomic status, blood pressure and Maddrey score (163). All analyses will be carried out with SPSS version 26 and Stata version 15.

5.2.3.14 Drop-outs

Information on the primary outcome will be obtained either from the patient's documentation or from the National Health Insurance Fund or similar organization managing data on healthcare costs and mortality, therefore information on the primary outcome will be available for most patients regardless of attendance of the study visits. Only withdrawal of consent will result in missing data.

Considering per protocol analysis, in the REAPPEAR-T trial, missing more than one consecutive interventions after the initial assessment or withdrawal of consent during follow-up will result in the drop-out of the patient. In the REAPPEAR-C investigation, patients who withdraw consent during follow-up or miss the 2-year visit will be considered drop-outs, since data on current alcohol consumption and smoking can only be obtained from the patient.

5.2.3.15 Flow and timing

Patients who met the inclusion criteria and none of the exclusion criteria will be offered to participate in the REAPPEAR-T trial. The enrolment period lasts from 48 hours before, until one week after hospital discharge. After informed consent and randomization, participants will be assigned to the cessation program or the control group (***Figure 11***). All patients will appear at the clinic according to the study schedule (***Figure 12***), within ±14 days from the pre-scheduled date.

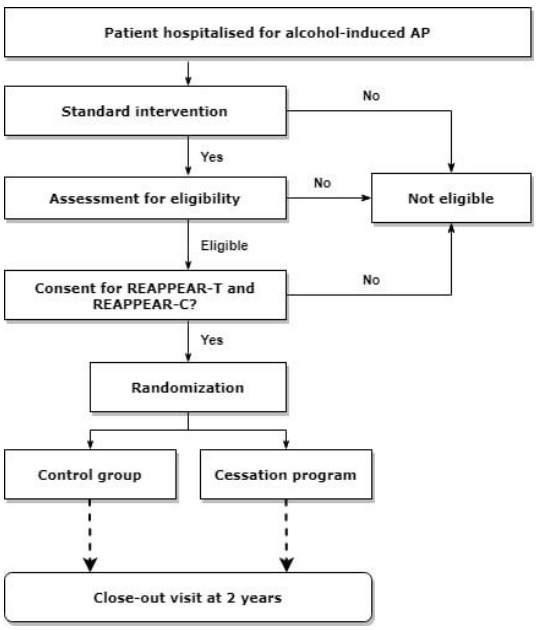


Figure 11 SPIRIT flowchart *Standard intervention will be provided for every patient as part of standard therapy. All randomized participants will be included in the REAPPEAR-T (trial) and REAPPEAR-C (cohort) as well. After reaching the required patient numbers for the REAPPEAR-T, further patients will be enrolled to the REAPPEAR-C in accordance with the estimated sample size.*

We chose 3-monthly visits in the cessation program based on a Swedish cohort study, in which 3-monthly brief interventions for selected patients with increased GGT levels were introduced and GGT levels were used for feedback. These interventions were found to reduce mortality, hospitalization and sick leave significantly (154). Hopefully, frequent visits will help in upholding motivation and improve adherence. Patients in the control group will have 2 pre-scheduled appointments, at 12 and 24 months.

	STUDY PERIOD	Screening enrollment period	Allocation day 0	Visit 1 3 months		Visit 2 6 months		Visit 3 9 months		Visit 4 12 months		Visit 5 15 months		Visit 6 18 months		Visit 7 21 months		Close-out 24 months
	GROUP	Both	Both	CG	CP	CG	CP	CG	CP	CG	CP	CG	CP	CG	CP	CG	CP	Both
ENROLLMENT	Eligibility screen	X																
	Standard intervention	X																
	Informed consent	X																
	Allocation		X															
INTERVENTION					X		X		X		X		X		X		X	
ASSESSMENT	BP, HR, BMI		X		X		X		X	X	X		X		X		X	X
	Laboratory testing		X		X		X		X	X	X		X		X		X	X
	Questionnaires		X		X		X		X	X	X		X		X		X	X
	Sample collection		X		X		X		X	X	X		X		X		X	X

Figure 12 SPIRIT timetable *Abbreviations: control group (CG), cessation program (CP), blood pressure (BP), heart rate (HR), body mass index (BMI)*

5.2.3.16 Assessment

For the assessment of addiction and motivation to quit will be assessed by internationally recognized and validated questionnaires (online supplementary material; see *chapter 5.2.3.19*) (164-170). This will enable the person who provides the intervention to individualize it and motivate the subject. Data on coffee consumption will be collected as well, as caffeine might counter the effects of alcohol in AP (171). For the assessment of quality of life, the EQ-5D-5L questionnaire will be used at baseline and every visit (172, 173). Socioeconomic status will be assessed at baseline and at 12 and 24 months with the questionnaire used in the LIFESPAN study (174). The aetiology of each recurrent episode will be determined following current international guidelines, but all episodes will be included in the primary endpoint (119, 142). Blood pressure, heart rate and body weight will be measured by an independent nurse blind to the allocation at every visit. Body mass index will be calculated.

5.2.3.17 Cost-effectiveness

Cost-effectiveness analysis will be performed to examine the impact of the cessation program on QoL, survival and health expenditure compared to the controls. We calculate the incremental cost-effectiveness ratio, which is defined by the difference in cost between the compared interventions (cessation program with 3-monthly visits versus usual care), divided by the difference in their effect (quality adjusted life years). The incremental cost-effectiveness ratio will be evaluated based on the Hungarian cost-effectiveness threshold. The total cost of treatment per each individual will be obtained from the national database at the completion of the study.

5.2.3.18 Patient and public involvement

Five randomly selected patients from the HPSG database were invited. All of them had previous AP and would have been eligible for the study. Three patients attended the joint consultation.

The original aims, hypotheses and protocol of the study were fully introduced to them. Patients insights were as follows: (i) they welcomed the study with great pleasure and felt it is highly important, (ii) they found the primary endpoint fundamental, (iii) they found the questionnaires and information sheets understandable, (iv) they highlighted the importance of frequent visits to the clinic, and found the duration of the visits feasible (v) they pointed out the necessity of high quality training of personnel providing the interventions, (vi) they had absolutely no disapproval or negative feelings regarding regular blood tests, (vii) they had no ethical objection concerning the control group, (viii) they expressed high difficulties considering smoking cessation and favoured a step-down approach rather than immediate quitting. We have revised and modified the original protocol accordingly.

5.2.3.19 Supplementary material

The online supplementary material can be found at https://bmjopen.bmj.com/content/12/1/e050821.

5.2.4 Discussion

Although alcohol and smoking are individual risk factors for AP, RAP and CP, they can synergize each other's effects (31). In addition, there is a lack of evidence as to the means of preventive measures that could be used in everyday clinical practice concerning alcohol and tobacco use for AP patients. Also, the effect of smoking cessation on recurrence in drinkers and non-drinkers is not yet clear.

The REAPPEAR study aims to fill these gaps and provide specialists and primary care physicians with valuable information on the importance of alcohol and smoking cessation in AP and RAP. Furthermore, the feasibility, efficacy and cost-effectiveness of an intervention program will be tested in this population to provide basis for large-scale intervention in alcohol-induced pancreatitis.

5.2.5 Ethics and dissemination

The REAPPEAR study is open for participation. Results of the planned analyses will be presented at national and international conferences and in peer-reviewed journals. Additional long-term follow-up of the participants is planned within the confines of the REAPPEAR+ study.

The trial has been registered at the clinicaltrials.gov (NCT04647097). Amendments will be published under this registration number. The Scientific and Research Ethics Committee of the Hungarian Medical Research Council approved the study (40394-10/2020/EÜIG). All local ethical approvals are in place. The study will be performed in accordance with the declaration of Helsinki, the principles of ICH-GCP guidelines and local legal and regulatory requirements.

5.3 Liver support therapy in acute-on-chronic liver failure: a network meta-analysis and systematic review

5.3.1 Introduction

ACLF is a clinical syndrome defined by the acute deterioration of chronic liver disease and the rapid development of organ failures, associated with high short-term mortality.

Most patients developing ACLF have pre-existing cirrhosis, which is a hyperinflammatory state (175, 176). Another aggravating factor is the immune paralysis described by several studies (177-181), which prevents effective countermeasures against infection and makes patients prone to serious infective complications.

Several therapies have been tested for the replacement of hepatic functions. So far, liver transplantation is the only curative therapy available. Survival rates are good, but availability and eligibility for transplant in ACLF differs by country (182). In the CANONIC study, only 4.5% of ACLF patients received transplant. Reportedly, low transplant rates are due to the high prevalence of infection and organ failure. Waiting-list mortality exceeds 50% in this population (182).

The APASL consensus guideline from 2019 states that “plasma exchange appears to be a promising and effective bridging therapy in patients with ACLF to liver transplant or spontaneous regeneration [1, C]” (183). The EASL Clinical Practice Guidelines do not recommend liver support therapies for the treatment of ACLF, but underlines the importance of further studies, because in specific subgroups ACLF seems beneficial (184).

Numerous pairwise meta-analyses of RCTs have been published assessing short-, middle- and long-term survival benefit of liver support therapies with controversial results (185-192). These meta-analyses faced serious limitations, as they pooled together data from studies testing different devices, in some cases with different follow-up lengths. A network meta-analysis (NMA), on the other hand, can handle multiple interventions and rank them, if the assumption of transitivity is met (193).

To facilitate international discussion and consensus, we decided to perform the first NMA comparing all available and tested liver support systems to each other and standard medical therapy (SMT) in patients with ACLF and ranking these treatments by survival benefit.

5.3.2 Methods

The protocol for this review was registered in the PROSPERO database under registration number CRD42020155850. There were no protocol deviations. This meta-analysis was reported according to The PRISMA Extension Statement for Reporting of Systematic Reviews Incorporating Network Meta-analyses of Health Care Interventions (PRISMA-NMA) (194).

5.3.2.1 Eligibility criteria

Parallel randomized controlled trials assessing the safety and efficacy of artificial and bioartificial liver support therapies in adult patients with ACLF were eligible for inclusion, regardless of the current availability of the tested therapy and length of follow-up. Conference abstracts were included to reduce publication bias. Crossover studies were excluded from the analyses of survival due to concerns about the carryover effect but were included in the systematic review. ACLF definitions used in the included RCTs were accepted, as there is a

lack of international consensus regarding this matter. For the studies published before ACLF was introduced as a clinical entity, the review authors decided eligibility based on the eligibility criteria used in the study. In case data for the same outcome was reported with substantially different time frames or different definitions were used among studies, outcomes were reported in the systematic review. Studies with shorter or longer follow-up periods than the assessed outcomes were also included in the systematic review.

5.3.2.2 Search strategy and selection

The systematic search was conducted up to the 15th December 2019 in the following databases: MEDLINE (via PubMed), Embase, CENTRAL, Web of Science and Scopus, with the search key designed based on the PICO format: ('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*. No filters or restrictions were applied. References of included studies, citing articles, and authors' accessible publications in a search engine (Google Scholar) and ResearchGate were hand searched for further eligible publications.

5.3.2.3 Data extraction

Data extraction was performed by two independent investigators (KO, AK) in duplicate using Endnote X9, Clarivate Analytics and Windows Excel 2016, Microsoft. In the case of discrepancies, agreement was reached by two experts (ZM or ZS). As a measure of inter-rater reliability, Cohen's kappa coefficients (κ) for the selection of abstracts and full-texts were counted. Information collected from each study and additional information used are detailed in the supplementary material.

5.3.2.4 Risk of bias assessment and quality of evidence

The risk of bias assessment was conducted in duplicate using Version 2 of the Cochrane risk-of-bias tool for randomized trials (RoB 2) for overall (OS) and transplant-free survival (TFS) separately (195).

For the four outcomes assessed in the NMA, quality of evidence was assessed in duplicate according to the Grading of Recommendation, Assessment, Development and Evaluation Working Group's recommendations, using a modified GRADE approach (196).

5.3.2.5 Statistical analysis

A Bayesian method was used to perform pairwise meta-analyses and NMAs with the random effect model for OS and TFS. For the analysis of transplant-free survival, transplant counted as an event similar to death. In case no patient received liver transplantation, OS and TFS were identical. If available, data for the intention-to-treat population was used.

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 other interventions are presented in forest plots, summarized in a league table. We were unable to use the node-splitting analysis to examine the

consistency assumption because of the star-shaped configuration of the networks (197). We ranked the interventions by their posterior probability by calculating the surface under cumulative ranking (SUCRA) curve values ranging from 0 to 100%. The higher the SUCRA value, and the closer to 100%, the higher the likelihood that a therapy is in the top rank or one of the top ranks; the closer to 0 the SUCRA value, the more likely that a therapy is in the bottom rank, or one of the bottom ranks (198). We also provided rankograms, showing the probability of achieving certain ranks. Frequentist comparison-adjusted funnel plots were created for 1- and 3-month OS, and Egger's tests were performed to assess small-study effect. The low number of studies in the TFS analyses did not enable this method. In an additional analysis, methodology-based evaluation was performed. 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 16.0 (StataCorp LLC).

5.3.2.6 Supplementary material

The online supplementary material can be found at https://annalsofintensivecare.springeropen.com/articles/10.1186/s13613-020-00795-0.

5.3.3 Results

5.3.3.1 Search and selection

The systematic search yielded 2,797 records. Four additional articles were identified through manual search and from previous meta-analyses. κ for abstracts and full-texts was 0.87 and 0.90 respectively, marking almost perfect agreement in both cases. One hundred three full texts were assessed for eligibility. Twenty-three articles proved to meet the eligibility criteria for the systematic review and 16 were included in the data synthesis (***Figure 13***).

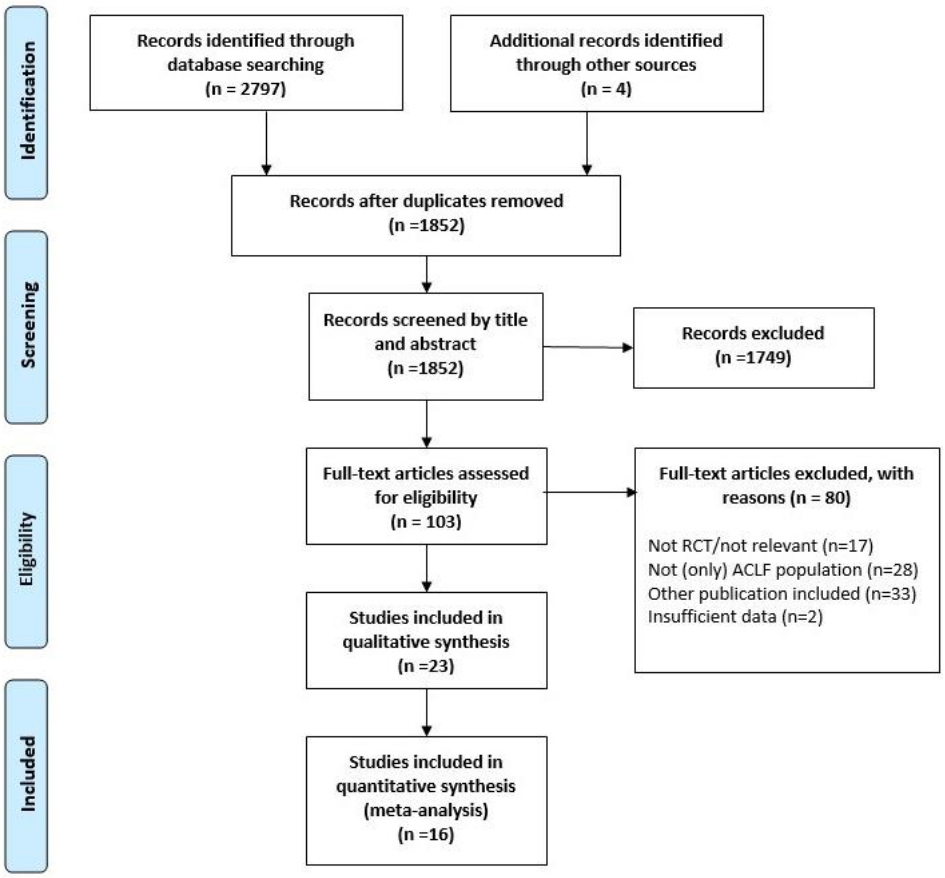


Figure 13 Flowchart of study selection *according to the PRISMA Statement*

5.3.3.2 Characteristics of the included studies

The main characteristics of the 23 eligible studies included in qualitative synthesis are shown in ***Table 2***. Of the 16 studies, enrolling 1670 patients included in the meta-analysis 15 compared a type of artificial (199-208) or bioartificial (209-213) liver support system to standard medical therapy and one study compared Molecular adsorbent and recirculating system (MARS) versus MARS plus plasma exchange (PE) (214). The most common aetiologies of underlying diseases were viral infection and alcohol. From the 1526 participants with available information on gender, 1064 were males (69.8%). ACLF definitions, eligibility criteria, baseline characteristics and outcomes of the individual studies are reported in ***Table 2***.

5.3.3.3 Survival

Survival was reported in most of the included studies, with greatly varying follow-up lengths. Data synthesis was feasible in four cases: 1-month (28-31 days) and 3-month (84-91 days) data were pooled for overall and transplant-free survival. The summary of the findings for these four outcomes is presented in ***Table 3.***

Plasma exchange demonstrated a statistically significant survival benefit compared to SMT in the analysis for 3-month OS (RR 0.74; CrI 0.60 to 0.94), with 86% SUCRA, 46% probability of being the best and 41% probability of being the second-best option from the six listed treatments (***Figures14-15***). PE also ranked first on the cumulative curves in three out of four analyses: both 1- and 3-month OS and 1-month TFS (***Figure 14*** and online supplementary material; see *chapter 2.3.2.6.*). In the analysis for 1-month TFS PE rank second after extracorporeal liver assist device (ELAD), with 76% versus 79% SUCRA values, but had a slightly higher cumulative probability of being in the first two places than ELAD (90% versus 88%) (online supplementary material; see *chapter 2.3.2.6.*).

MARS ranked second in both OS outcomes ***(Figures 14-15*** and online supplementary material*; see chapter 2.3.2.6.)* with 73% SUCRA at 1 month and 71% at 3 months. Concerning TFS, MARS ranked second last and last with SUCRA values of 27% at 1 month and 33% at 3 months (online supplementary material; see *chapter 2.3.2.6.*). Prometheus was included in both OS analyses and in 3-month TFS. Only MARS, PE and their combination performed better than this device in the OS outcomes and it ranked second after PE for 3-month TFS. However, the SUCRA values and the probabilities for the first ranks are much lower than for PE (SUCRA: 40% for both OS and 51% for 3-month TFS, first rank probabilities 5% for 1-month OS, 4% for 3-month OS and 13% for 3-month TFS, shown on ***(Figures 14-15*** and online supplementary material*; see chapter 2.3.2.6.)*. Although ELAD therapy, the only biological device ranked first for 1-month TFS, in the analysis for 3-month TFS it had a SUCRA of 38%, even lower than SMT (41%). BioLogicDT was included in the OS analyses and ranked second last in both cases. SMT had the lowest probability of being the best or second-best option in all four analyses.

First author, publication year	Eligibility criteria	Aetiology and baseline characteristics	Intervention(s) /comparison	Outcomes
Banares (2013)	Inclusion: presumptive diagnosis of cirrhosis with an identifiable triggering event; an increase of TBIL >5 mg/dL and at least one of the following: HRS, HE≥ grade II, rapidly progressive hyperbilirubinemia (>50% increase from TBIL levels at admission) >20 mg/dL at admission Exclusion: progressive jaundice as a consequence of the natural course of cirrhosis; extrahepatic cholestasis; PLT<50,000/mm3; INR>2.3; suspected or evident DIC; need for RRT; intrinsic renal disease; uncontrolled infection; active bleeding; HCC >4 cm in diameter; portal vein thrombosis; severe cardiopulmonary disease; MAP<60 mmHg despite vasopressor therapy); major surgical procedure within the last 4 weeks; HIV infection	Mostly alcoholic, viral, autoimmune, drug-induced, NASH etc. Age (years)[a]: 51.8/50.0 Males (%): 66.7/70.8 MELD[a]:25.6/24.1	MARS/SMT	Survival, HE, laboratory parameters, AEs
Duan (2018)	Inclusion: 15–65 years ; clinical diagnosis of ACLF; obvious gastrointestinal and/or systemic toxic symptoms; TBIL >5 times upper limit of normal or daily increase >1 mg/dl; prothrombin activity of 10%–50%; INR 1.6–4.0, or prothrombin time >5s longer than the control but <20s, HE absent or grade I-II; no or mild ascites/pleural effusion Exclusion: primary or metastatic liver cancer; uncontrolled severe infection; shock; active bleeding within 3 days; grade III-IV HE; PLT <40×109/l; creatinine >1.5 mg/ml; severe oesophageal varices	Mostly alcoholic and viral; drug-induced, autoimmune, unknown, 'acute/subacute' Age[a] (years): 39.5/39.2 Males (%): 96.9/88.2 MELD[a]: 28.0/30.8	ELAD/SMT	Survival, AEs
Ellis (1999)	Inclusion: acute alcoholic hepatitis, HE ≥ grade II Exlusion: pregnancy; MAP <50 mmHg despite adequate volume loading and appropriate use of inotropes;respiratory failure; cerebrovascular event within the previous 12 months, a recent upper gastrointestinal haemorrhage; poorly controlled epilepsy; recent myocardial infarction/ischaemia	Alcoholic Age[b] (years): 46/43 Males (%): 60/80 MELD/CTP: NR	BioLogicDT/SMT	Survival, HE, physical and laboratory parameters, AEs
Hassanein (2007)	Inclusion: ≥18 years, manifestations of cirrhosis and HE grade III-IV Exclusion: active haemorrhage; hemodynamic instability; acute cardiopulmonary complications (pulmonary oedema, massive aspiration pneumonia, heart failure); pregnancy; active RRT; drug intoxication or irreversible brain damage or nonhepatic causes of altered mental status; acute liver failure; HCC; received transplant	Mostly alcoholic or viral; autoimmune, drug induced, unknown Age[b] (years): 49/56 Males (%): 61.5/48.4 MELD[b]: 33/38	MARS/SMT	HE, AEs, laboratory parameters (survival was additional)
Heemann (2002)	Inclusion: 18-65 years; cirrhosis (CTP≥7) and a superimposed acute liver injury leading to decompensation and severe hyperbilirubinemia (TBIL≥20 mg/dL) Exclusion: hepatobiliary obstruction; active bleeding or sepsis causing hemodynamic instability; comorbid conditions associated with a poor outcome; coma of nonhepatic origin; extensive surgery 30 days preceding admission; HRS; pregnancy	Mostly alcoholic; viral, drug induced Age[b] (years): 48/57 Males (%): 50/63.6 CTP[b]: 11.5/12	MARS/SMT	Survival, HE, AEs, laboratory parameters

First author, publication year	Eligibility criteria	Aetiology and baseline characteristics	Intervention(s) /comparison	Outcomes
Hillebrand (2010)	Inclusion: acute decompensation of cirrhosis; SOFA score ≥9; and either a MELD score of ≥32, or MELD ≥24 and at least one of HE grade III-IV or type I HRS Exclusion: NR	Aetiology, age, sex NR MELD[a]: 34.3/40.8	ELAD/SMT	Survival, AEs
Huang (2012)	Inclusion: chronic severe hepatitis B with HE ≥grade II Exclusion: late stage disease; previous irreversible respiratory failure; severe brain odema with hernia; severe systemic circulation disorder accompanied by DIC; serious active bleeding	HBV Age[b] (years): 43/42 Males (%): 78.3/75 MELD/CTP: NR	MARS±PE	Survival, HE, AEs, laboratory parameters, cost of treatment
Kramer (2001)	Inclusion: documented cirrhosis and encephalopathy grades II or III had not improved with conventional treatment Exclusion: renal failure; hypotension (MAP <55 mmHg); respiratory or multiorgan failure; fever of >38,5 °C; bleeding requiring transfusion of >2 units within the preceding 24 hours; insulin dependent diabetes mellitus; administration of sedatives within the preceding 2 days	Alcoholic, viral, autoimmune, unknown Age[b] (years): 55/56 Males[c] (%): 65% CTP[b]: 14/14.5	BioLogicDT/SMT	HE, laboratory and physical parameters, AEs (survival is additional)
Kribben (2012)	Inclusion: 18-70 years; severe deterioration of chronic liver disease; CTP ≥10 (over 72 hours); TBIL ≥5 mg/dL (over 72 hours) Exclusion: Pregnancy/lactation; HIV infection, intracranial bleeding; cerebrovascular disease; ARDS; circulatory shock with vasopressor therapy; persistent bleeding needing perfusion; chronic renal failure stage V; acute necrotizing pancreatitis; HCC, malignancy; INR >3.0 or PLT<30,000/L; extrahepatic cholestasis; liver resections or major hepatobiliary surgery in the previous 6 months except laparoscopic cholecystectomy; LT within 2 years, ALSS therapy within 7 days; participation in another clinical trial or this study priorly	Mostly alcoholic and viral; others not specified Age[a] (years): 50/51 Males (%): 62/65 MELD[b]: 28/27	Prometheus/SMT	Survival, laboratory parameters; AEs
Mitzner (2000)	Inclusion: 18-60 years; HRS (serum creatinine >1.5mg/dL, oliguria <500mL/d, urine sodium < 20mmol/L, central venous pressure >8 cmH2O); need of haemodialysis/filtration treatment; chronic liver failure (3 of 4 criteria): ultrasonic signs of chronic damage or impaired synthesis function (hypoalbuminemia ,30g/L, prolonged prothrombin time (quick value <70%), AT III <70%, serum cholinesterase <40 umol/s/L or hyperbilirubinemia (>15mg/dL) or grade III-IV HE Exclusion: fulminant hepatic failure; sepsis unresponsive to antibiotics; severe acute haemorrhages; malignancies; obstructive/chronic renal failure; pregnancy; severe cardiopulmonary disease	Mostly alcoholic; HBV, primary and secondary biliary cirrhosis Age[a] (years): 49.6/43.8 Males (%): 37.5/40 CTP[a]: 12.5/12.2	MARS/SMT	Survival

First author, publication year	Eligibility criteria	Aetiology and baseline characteristics	Intervention(s) /comparison	Outcomes
Pyrsopoulos (2019)	Inclusion: sAH, age 18-50 years, total bilirubin ≥16 mg/dL, Maddrey score ≥32, not eligible for transplant Exclusion: PLT<40000/mm3; INR >2.5; serum creatinine ≥1.3 mg/dL; MELD score ≥30; AST >500 IU/L; infection unresponsive to antibiotics; reduction in TBIL ≥20% in the previous 72 hours; hemodynamic instability; active bleeding; major haemorrhage; liver size reduction due to cirrhosis; occlusive portal vein thrombosis; bile duct obstruction; life expectancy of less than 3 months due to concomitant diseases; subject on haemodialysis; Wilson's disease; NAFLD; Budd-Chiari Syndrome; active viral hepatitis; pregnancy; received liver transplant	Alcoholic hepatitis Age[a] (years): 39.1/39.5 Males (%): 60.3/60.3 MELD[a]: 24.8/25.6	ELAD/SMT	Survival, AEs
Qin (2014)	Inclusion: 18-70 years; presumptive diagnosis of CHB, HBV-associated cirrhosis, or hepatitis B surface antigen (HBsAg) carrier; rapidly progressive hyperbilirubinemia with TBIL >10 mg/dL, within 28 days from symptom onset; INR>1.5 or plasma prothrombin activity <40% Exclusion: acute HBV infection; hepatitis E, A, D, or or HIV superinfection; alcohol- or drug-induced liver injury; severe gastrointestinal bleeding; HCC; pregnancy	HBV Age[a] (years): 44.1/48.7 Males (%): 82.7/72.3 MELD[a]: 28.6/29.5	PE/SMT	Survival, AEs
Sen (2004)	Inclusion: 18-75 years old; alcoholic liver disease; acute deterioration in liver function over 2–4 weeks leading to severe progressive clinical deterioration despite supportive care (over 48 hours); jaundice (TBIL >100 mol/L) and either HE Grade 2 or HRS; cirrhosis Exclusion: prior enrolment in another study; known hepatic / extrahepatic malignancy; uncontrolled infection or upper gastrointestinal bleeding over the previous 48 hours; pregnancy; prior treatment with terlipressin for HRS; coexisting HIV infection; severe cardiorespiratory disease	Alcoholic Age[b] (years): 45/44 Males (%): 78/67 MELD[b]: 16.5/19.4	MARS/SMT	Survival, HE, laboratory and physical parameters
Teperman (2012)	Inclusion: acute alcoholic hepatitis or acute decompensation of cirrhosis, MELD 18-35 Exclusion: NR	Alcoholic and not specified (baseline only given for PP subjects)	ELAD/SMT	Survival, time to progression, AEs
Thompson (2018)	Inclusion: ≥18 years, history of heavy alcohol abuse, maximum of 6 weeks between the last consumption, rapid onset of jaundice (TBIL≥ 8 mg/dL) and coagulopathy (Maddrey's DF ≥ 32), stratum A: liver biopsy confirmed sAH/ 2 of the following: AST>ALT, leucocytosis, ascites stratum B: sAH+underlying chronic liver disease confirmed by biopsy, laboratory findings and/or medical history Exclusion: end-stage cirrhosis; portal vein thrombosis; MELD>35,	Alcoholic hepatitis (superimposed or primary); Age[a] (years): 46.5/44.8 Males%: 57.3/60.7 MELD[a]: 27.6/27.1	ELAD/SMT	Survival, laboratory parameters, AEs

First author, publication year	Eligibility criteria	Aetiology and baseline characteristics	Intervention(s) /comparison	Outcomes
	PLT<40000/mm3; severe concomitant disease; uncontrolled bleeding; infection unresponsive to antibiotics; hemodynamic instability; chronic dialysis			
Yu (2008)	Inclusion: acute-on-chronic hepatitis B liver failure (HBV-DNA ≥10000 copies/mL); defined as severe jaundice (TBIL> 171 mmol/L), coagulopathy, and /or HE > grade II; previous lamivudine treatment; MELD>30 Exclusion: obstructive and haemolytic jaundice; prolonged PTT due to hematologic diseases; drug-induced hepatitis; Wilson's disease; alcoholic liver disease; autoimmune hepatitis; hepatitis C or D or HIV infection	HBV Age[a] (years): 45.2/46.4 Males (%): 80/78.6 MELD[a,d]: 41.4	PE/SMT	Survival, laboratory parameters
He (2000)*	Inclusion: severe viral hepatitis according to the criteria of the 1995 national symposium Exclusion: NR	Mostly viral, alcoholic Age,sex, MELD/CTP: NR	PE, PP, DHP/SMT	Survival, laboratory parameters, HE, AEs
Hu (2005)*	Inclusion: chronic severe hepatitis complicated with multiorgan failure Exclusion: NR	NR	MARS/SMT	Survival, HE, laboratory parameters
Krisper (2005)*	Inclusion: ACLF Exclusion: NR	Mostly alcoholic, HCV Age[c] (years): 57 Males[c] (%): 67% MELD[b]: 35.4	MARS and Prometheus, crossover	Laboratory parameters, AEs
Laleman (2006)*	Inclusion: 18-75 years; histologically proven alcoholic cirrhosis with superposed alcoholic hepatitis; portal hypertension with associated hyperdynamic circulation and ACLF (persistent deterioration in liver function despite treatment of the precipitating event and elevated bilirubin>12 mg%) Exclusion: extrahepatic cholestasis; coma of non-hepatic origin; active gastrointestinal bleeding in the past five days; comorbidities associated with poor outcome (acute necrotizing pancreatitis, neoplasia, severe cardiopulmonary disease, oxygen dependent or steroid-dependent COPD); ongoing infection; HRS type I	Alcoholic hepatitis Age[a] (years): 54.5/43.2/55.8 Males (%): 83.3/66.7/50 MELD[a]: 22.7/29.7/24.3	MARS/Prometheus /SMT	Laboratory parameters, AEs

First author, publication year	Eligibility criteria	Aetiology and baseline characteristics	Intervention(s) /comparison	Outcomes
Meijers (2012)*	Inclusion: ≥18 years, compensated chronic liver disease; developed intrahepatic cholestasis (TBIL > 5 mg/dl); at least one of the following complications within 4-8 weeks after a potential identifiable acute superposed hepatic insult: a) a progressive hyperbilirubinemia ≥ 50% increase of TBIL > 20 mg/dl, b) HE ≥ II, c) de novo development of ascites, and/or d) HRS Exclusion: extrahepatic cholestasis; severe hypocalcaemia ($Ca2+ < 0.9$ $mmol \cdot l^{-1}$); acidosis (pH < 7.25)	Mostly alcoholic, HCV, NASH and others Age[c] (years): 54.6 Males (%): NR MELD[a,c]: 32.1	MARS ± citrate, crossover	Laboratory parameters, AEs
Wilkinson (1998)*	Inclusion: decompensated chronic liver disease[x] and grade III-IV encephalopathy Exclusion: NR	Alcoholic, HCV, HBV, autoimmune, unknown Age[a] (years): 58.3/42.7 Males (%): 60/100 MELD/CTP: NR	BioLogicDT/SMT	Physiologic and neurologic improvement, AEs
You (2011)*	Inclusion: ACLF defined by the Chinese Medical Association definition (2006) Exclusion: NR	Viral (?) Age[a] (years): 42.7/43.5 Males (%): 100/83 MELD[a]: 23/24.1	HBALSS/PE	Survival, AEs, laboratory parameters

Table 2 Characteristics of included studies *Outcomes included in the network meta-analysis and systematic review (in grey) are listed here. [a]mean values; [b]median values; [c]all patients; [d]only reported in the intervention group; TBIL: Total bilirubin; HRS: Hepatorenal syndrome; HE: Hepatic encephalopathy, PLT: platelet, INR: International normalized ratio, DIC: Disseminated intravascular coagulation, RRT: Renal replacement therapy, HCC: Hepatocellular carcinoma, MAP: Mean arterial pressure, HIV: Human immunodeficiency virus, NASH: Non-alcoholic steatohepatitis, MELD: Model for end-stage liver disease, MARS: Molecular adsorbent and recirculating system, SMT: Standard medical therapy, AE: Adverse events, ACLF: Acute-on-chronic liver failure, ELAD: Extracorporeal liver assist device, CTP: Child-Turcotte-Pugh, NR: Not reported, HRS: Hepatorenal syndrome, SOFA: Sequential organ failure assessment, HBV: Hepatitis B virus, PE: Plasma exchange, ARDS: Adult respiratory distress syndrome, sAH: Severe alcoholic hepatitis, AST: Aspartate-aminotransferase, NAFLD: Non-alcoholic fatty liver disease, PP: Plasma perfusion, DHP: Direct hemoperfusion, HCV: Hepatitis C virus, COPD: chronic obstructive pulmonary disease*

Intervention (Studies)	Rank	Study event rates (%)		Relative effect (95% CI)	Anticipated absolute effects		Overall certainty of evidence
		SMT	Liver support		Risk with SMT	Risk difference with liver support	
1-month overall survival							
PE (1 RCT)	1	122/359 (34.0%)	19/104 (18.3%)	**RR 0.51** (0.12 to 2.40)	34 per 100	**17 fewer per 100** (from 30 fewer to 48 more)	⊕⊕⊕◯ MODERATE
MARS (3 RCTs)	2		109/113 (96.5%)	**RR 0.60** (0.15 to 1.30)		**14 fewer per 100** (from 29 fewer to 10 more)	⊕◯◯◯ VERY LOW
MARS+PE (indirect)	3		7/60 (11.7%)	**RR 0.60** (0.07 to 3.20)		**14 fewer per 100** (from 32 fewer to 75 more)	⊕◯◯◯ VERY LOW
Prometheus (1 RCT)	4		29/77 (37.7%)	**RR 1.00** (0.25 to 4.30)		**0 fewer per 100** (from 25 fewer to 100 more)	⊕◯◯◯ VERY LOW
BioLogicDT (1 RCT)	6		6/10 (60.0%)	**RR 1.10** (0.24 to 5.40)		**3 more per 100** (from 26 fewer to 100 more)	⊕◯◯◯ VERY LOW
ELAD (3 RCTs)	7		26/117 (22.2%)	**RR 1.40** (0.56 to 3.60)		**14 more per 100** (from 15 fewer to 88 more)	⊕◯◯◯ VERY LOW
1-month transplant-free survival							
ELAD (2 RCTs)	1	109/264 (41.3%)	14/43 (32.6%)	**RR 0.47** (0.13 to 1.20)	41 per 100	**22 fewer per 100** (from 36 fewer to 8 more)	⊕◯◯◯ VERY LOW
PE (1 RCT)	2		47/104 (45.2%)	**RR 0.52** (0.21 to 1.20)		**20 fewer per 100** (from 33 fewer to 8 more)	⊕⊕⊕◯ MODERATE
MARS (3 RCTs)	3		60/122 (49.2%)	**RR 0.96** (0.50 to 1.50)		**2 fewer per 100** (from 21 fewer to 21 more)	⊕◯◯◯ VERY LOW
3-month overall survival							
PE (2 RCTs)	1	334/569 (58.7%)	136/244 (55.7%)	**RR 0.74** (0.60 to 0.94)	59 per 100	**15 fewer per 100** (from 23 fewer to 4 fewer)	⊕◯◯◯ VERY LOW
MARS (2 RCTs)	2		12/17 (70.6%)	**RR 0.78** (0.38 to 1.40)		**13 fewer per 100** (from 36 fewer to 23 more)	⊕◯◯◯ VERY LOW
Prometheus (1 RCT)	3		46/77 (59.7%)	**RR 0.97** (0.68 to 1.40)		**2 fewer per 100** (from 19 fewer to 23 more)	⊕◯◯◯ VERY LOW
ELAD (4 RCTs)	4		78/213 (36.6%)	**RR 0.99** (0.76 to 1.30)		**1 fewer per 100** (from 14 fewer to 18 more)	⊕◯◯◯ VERY LOW
BioLogicDT (1 RCT)	5		5/5 (100.0%)	**RR 1.00** (0.55 to 2.10)		**0 fewer per 100** (from 26 fewer to 65 more)	⊕◯◯◯ VERY LOW
3-month transplant-free survival							
PE (1 RCT)	1	189/396 (47.7%)	42/104 (40.4%)	**RR 0.77** (0.51 to 1.10)	41 per 100	**11 fewer per 100** (from 23 fewer to 5 more)	⊕⊕⊕◯ MODERATE
Prometheus (1 RCT)	2		52/77 (67.5%)	**RR 0.96** (0.67 to 1.40)		**2 fewer per 100** (from 16 fewer to 19 more)	⊕◯◯◯ VERY LOW
ELAD (4 RCTs)	4		76/217 (35.0%)	**RR 1.00** (0.78 to 1.40)		**0 fewer per 100** (from 11 fewer to 19 more)	⊕◯◯◯ VERY LOW
MARS (1 RCT)	5		7/8 (87.5%)	**RR 1.10** (0.61 to 2.10)		**5 more per 100** (from 19 fewer to 53 more)	⊕◯◯◯ VERY LOW

Table 3 Summary of findings *Significant results are highlighted in bold. Interventions are compared to SMT as reference comparator SMT: standard medical therapy; CrI: Credible interval; PE: Plasma exchange; RCT: Randomized controlled trial; RR: Risk ratio: MARS: Molecular adsorbent and recirculating system; ELAD: Extracorporeal liver assist device*

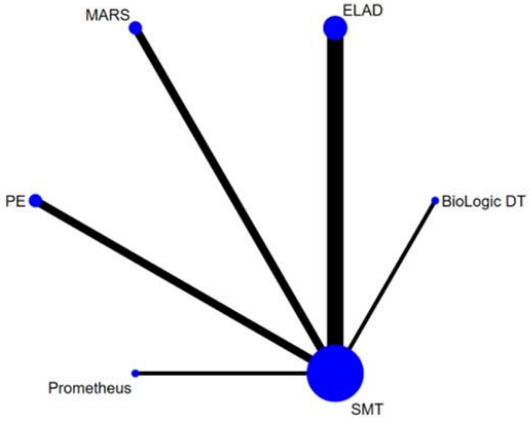
A.

Treatment 1 / RR (95% CrI)					Treatment 2
PE					
0.95 (0.53, 2.0)	MARS				
0.76 (0.50, 1.2)	0.81 (0.36, 1.5)	Prometheus			
0.75 (0.53, 1.1)	0.79 (0.37, 1.5)	0.98 (0.63, 1.6)	ELAD		
0.72 (0.35, 1.4)	0.75 (0.28, 1.7)	0.93 (0.43, 1.9)	0.95 (0.46, 1.9)	BioLogicDT	
0.74 (0.60, 0.94)	0.78 (0.38, 1.4)	0.97 (0.68, 1.4)	0.99 (0.76, 1.3)	1.0 (0.55, 2.1)	SMT

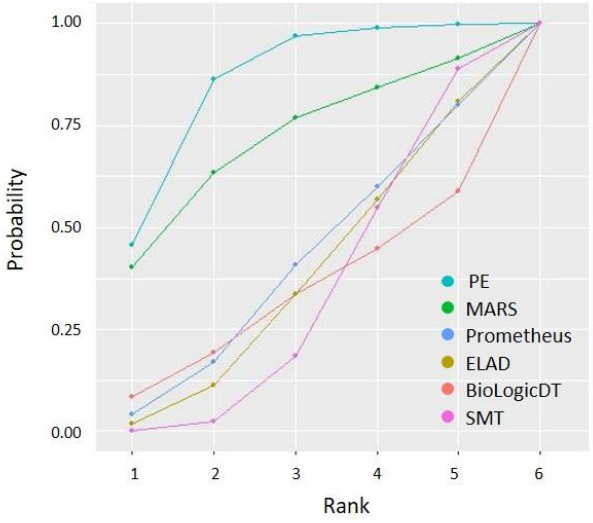
B.

Study ID	Intervention(s)	Patients/group
Thompson (2018)	ELAD/SMT	96/107
Hillebrand (2010)	ELAD/SMT	14/4
Teperman (2012)	ELAD/SMT	25/28
Pyrsopoulos (2019)	ELAD/SMT	78/73
Qin (2014)	PE/SMT	104/130
Yu (2008)	PE/SMT	140/140
Ellis (1999)	BioLogicDT/SMT	5/5
Sen (2004)	MARS/SMT	9/9
Mitzner (2000)	MARS/SMT	8/5
Kribben (2012)	Prometheus/SMT	77/68

C.



D.



E.

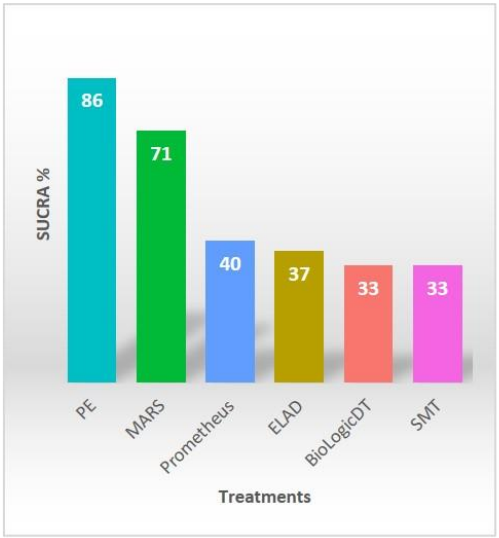


Figure 14 3-month overall survival *League table containing risk ratios and credible intervals for all comparisons (**A**), studies included in the analysis for (**B**), geometry of the network (**C**), cumulative ranking curves (**D**) and surface under the cumulative ranking curves (SUCRA) (**E**) RR: risk ratio; CrI: credible interval; PE: plasma exchange; MARS: Molecular adsorbent and recirculating system; ELAD: Extracorporeal liver assist device; SMT: standard medical therapy*

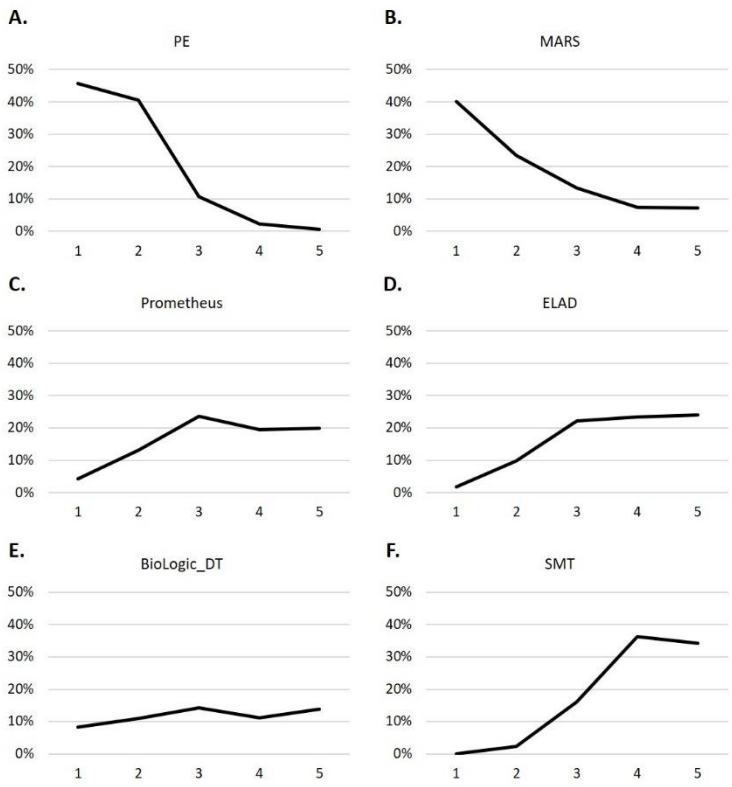


Figure 15 Rankograms for 3-month overall survival *showing the probability (x axis) of the respective treatment (**A-E**) and standard medical therapy (**F**) achieving certain ranks (y axis). PE: plasma exchange; MARS: Molecular adsorbent and recirculating system; ELAD: Extracorporeal liver assist device; SMT: standard medical therapy*

Methodology-based analyses were also performed grouping the albumin-based (MARS and Prometheus) techniques, with very similar results (only the PE-SMT comparison for 3-month OS reaching statistical significance, online supplementary material*; see chapter 2.3.2.6.)*.

Wilkinson et al. (215) provided data only for 5-day survival comparing BioLogicDT with SMT in a small number of patients. The device seemed to be effective in bridging to transplant. Hu et al. (216) has found that MARS improved the survival of patients with chronic severe hepatitis with MOF. You et al. (217) tested the hybrid bioartificial liver supporting system (HBALSS) in 6 patients with similar mortality rate to controls. He et al. (218) tested the effects of plasma perfusion, PE and direct hemoperfusion compared with SMT and the results were reported in Chinese. A higher survival rate was reported in the intervention group (68.75% vs 46.67%) for the whole study population. Extracted data for mortality in the ACLF subgroup by Alshamsi et al. did not show a significant difference (RR 0.59, 95% CI 0.33-1.04) (189).

Long-term survival was assessed in six studies. Six-month survival was reported to be identical in both groups by Hassanein, Heemann and Pyrsopoulos (additionally presented at a conference, together with 1-year survival) (201, 208, 212). Duan et al. reported higher transplant-free survival in the ELAD group, maintained until the end of the 5-year follow-up (210). On the contrary, Thompson et al. found comparable mortality in the two groups at five years (209). Interestingly, Qin et al. showed that in the PE group the 5-year cumulative survival probability was significantly higher (43% vs 31% survived) and have found that treatment added about 6 months to the life expectancy of patients with HBV-associated ACLF.

5.3.3.4 Hepatic encephalopathy and ammonia

Altogether ten studies reported the changes in mental status, but hepatic encephalopathy (HE) different scales and definitions were used (online supplementary material*; see chapter 2.3.2.6.)*.

All studies reported improvement, which was statistically significant only in five cases, all using MARS therapy. Ten studies reported changes in blood ammonia levels (online supplementary material*; see chapter 2.3.2.6.)*. Findings are controversial for MARS. Prometheus and BioLogicDT does not remove ammonia effectively.

5.3.3.5 Bilirubin and bile acids

Changes in total bilirubin were reported in twenty studies (online supplementary material*; see chapter 2.3.2.6.)*. Results were not pooled on account of different treatment doses, measurement time-points and definitions for bilirubin reduction. Hassanein et al. rightly pointed out that the time between the last treatment session and post-treatment measurements could greatly influence this outcome (208). They showed that a single session of MARS reduced total bilirubin levels significantly, but this difference decreased by the end of the 5-day treatment period. MARS, PE, MARS combined with PE, Prometheus, ELAD and HBALSS treatment significantly reduced bilirubin levels. Krisper et al. compared MARS and Prometheus in a crossover design and reported Prometheus to be more effective in the removal of conjugated and unconjugated bilirubin. BioLogicDT does not remove bilirubin effectively.

Hassanein, Heeman and Laleman found that both MARS and Prometheus reduced bile acid levels significantly (P<0.001 and P<0.001, respectively) (201, 208, 219). Krisper et al. reported that MARS and Prometheus remove individual bile acids with different clearance rates (220). On the other hand, Meijers et al. observed no significant reduction in bile acid levels after MARS sessions.

5.3.3.6 Creatinine and blood urea nitrogen

Changes in creatinine levels were reported in 12 cases (online supplementary material*; see chapter 2.3.2.6.)*. Findings for MARS and BioLogic-DT are controversial regarding creatinine removal from blood and Prometheus and plasma exchange therapy do not influence creatinine levels. MARS, Prometheus and BioLogic-DT were found to decrease blood urea nitrogen levels effectively.

5.3.3.7 Cytokines

Tumour necrosis factor-α (TNF-α) levels were reduced after 6 hours of BioLogic-DT treatment (p=0.04) as reported by Kramer et al. (202) but only small changes were observed by Ellis et al. (207). MARS and Prometheus treatment did not reduce TNF-α levels (204, 221). He et al. reported significant TNF-α reduction after treatment (218). MARS did not change IL-6, IL-8 and IL-10 levels, similarly to TNF-receptor 1 and 2 (204, 221). Higher IL-8 levels were measured in the BioLogic-DT group (207). Levels of anti-inflammatory protein IL-1 receptor antagonist were significantly elevated for days in ELAD treated subjects (209).

5.3.3.8 Harms

In the numbers of adverse events (AEs) and reporting protocols an immense heterogeneity was shown; therefore, quantitative data synthesis was not carried out. All devices were evaluated to be safe, and the number of AEs was comparable to the control groups. Hassanein et al. described nine possibly treatment-related adverse events in the MARS group, however, the nature of these was not detailed (208). Acute hemolysis developed in one patient in the ELAD group (210) and

treatment was discontinued in several cases due to adverse events not specified (209, 211, 213). Heemann et al. compared AEs in the MARS group to patients who received dialysis and found no significant difference. Two out of the twelve patients treated with MARS had fever/sepsis possibly related to the catheter (201).

Adverse events were reported in all but four papers in general. The most frequent complications were bleeding at the site of the catheter, clotting in the apparatus, and thrombocytopenia. Hypotension was reported in patients treated with PE and Prometheus (203, 219).

5.3.3.9 Risk of bias assessment and quality of evidence

The quality of evidence is shown in ***Table 3*** (for more information see online supplementary material*; see chapter 2.3.2.6.)*. Quality of evidence was moderate for PE in the analysis of OS at 1 month and both TFS outcomes. All other results were of very low certainty. Results of the risk of bias assessment conducted separately for OS and TFS are shown the online supplementary material *(see chapter 2.3.2.6.)*. Overall risk of bias was low in 50% of the studies included in the OS analyses. 33% carried moderate and 22% high risk of bias. For TFS, 22% of studies carried low, 22% moderate and 46% high risk of bias.

5.3.4 Discussion

Extracorporeal liver support therapies have been and will remain of fundamental interest in the management of ACLF (222). However, their benefits have been debated for long. Therefore, we conducted the first network meta-analysis focusing on patients with ACLF, assessing OS and TFS at one and three months. The analyses for OS yielded similar results, with PE ranking first and MARS second on the cumulative ranking curves in both cases. From all comparisons, only PE was associated with a statistically significant improvement, when compared to SMT in the analysis of 3-month OS, but with very low certainty of evidence. Other comparisons did not reach statistical significance, but SMT had very low probabilities of being the best option in all analyses.

Until then, evidence on the efficacy of PE in ACLF mostly originated from cohort studies. The APASL consensus guideline recommended the use of PE in ACLF for bridging to transplantation or recovery. The EASL did not find the available evidence to be sufficient for recommending the use of any liver support therapy for the treatment of ACLF. High volume PE was found to reduce mortality and effectively remove DAMPs, TNF-α and IL-6 in ALF patients in an RCT (223, 224).

The role of immune dysfunction and dysregulated immune response in ACLF has recently come into focus. Both hyper-inflammation and immunosuppression plays a role in acute decompensation (179, 225). Inflammation represented by elevated inflammatory markers was previously thought to be a consequence of ongoing infection, but lately endogenous inducers were identified as underlying causes (226). Bioartificial devices have the potential of synthetic functions and contribution to the immune response (227). So far, only ELAD was tested in RCTs, always compared to SMT. Although ELAD did not perform well on the cumulative ranking curves, significantly higher IL-1 receptor antagonist levels were measured during ELAD therapy than in controls (209). Based on this finding, the immunomodulatory functions of bioartificial devices should be further assessed.

Several new devices are being tested in animal models of liver failure including both artificial and bioartificial devices (228, 229) and ongoing clinical trials are enrolling ACLF patients ((230), NCT03882346, NCT04051437). Other blood purification methods, such as CytoSorb ™ therapy, also seem promising (231, 232), but they have not yet been evaluated in a randomized setting. Nevertheless, according to a recent *in vitro* experimental model, CytoSorb hemoperfusion lead to an initially faster removal of cytokines like TNF-α and IL-6, as well as more effective reduction of albumin-bound toxins such as indirect bilirubin and bile acids compared to MARS (233).

There are some strengths and several limitations to our study. This is the first NMA in this field using the latest recommendations from the Cochrane Collaboration for statistical analysis, risk of bias and QE assessment. We evaluated OS and TFS separately, at one and three months. We did not pool in-hospital, short-term and long-term survival data. Studies enrolling patients with hepatorenal syndrome were not excluded with the aim of including cases with poorer prognosis. This new methodology enabled the comparison and ranking of different devices and highlighted the need for international consensus on the definition of ACLF and further trials testing already existing and new devices.

The absence of loops in all of the created networks limits statistical analysis in Bayesian networks and results in wider credible intervals. Transitivity could not be directly tested, but we think that the differences between the study populations do not violate the assumption of transitivity. The analyses included relatively few studies, some of them only enrolling less than 10 subjects per group, raising concerns about the beta-type error. Most importantly, due to the different definitions of ACLF used, patient characteristics can differ significantly among studies, resulting in a highly heterogenous population in our study. Eligibility criteria and the ratio of viral and alcoholic aetiology differs in the included studies, but all patients were diagnosed with ACLF. Differences in the study populations may explain some of the controversial results of RCTs included in this meta-analysis. Also, in some of the included studies mortality was not a primary endpoint and was reported additionally, therefore bias arise. The recruitment period for the included trials ranges from March 1997 until February 2015, which could impose chronological bias. Variance in SMT and treatment dose also could have influenced outcomes (234). Due to the differences in treatment dose, cut-offs and reporting protocols, data on HE, laboratory parameters and AEs could not be analysed quantitatively.

5.3.5 Implication for research

International consensus is needed to standardize the definition of ACLF. Further RCTs targeting carefully selected subgroups of the ACLF population, using already existing and new therapeutic methods are needed to produce high quality evidence for guideline development.

5.3.6 Implication for practice

PE seems to have the most beneficial effect at present, but liver support devices in general had higher probabilities for the first two ranks than SMT. Choosing the best option remains in the hands of the attending physician.

Discussion

6.1 Common features and differences of AP and ACLF

AP and ACLF show resemblance in their core pathophysiologic features, which may facilitate the understanding of the diseases and synthesis of scientific results for the benefit of patients. Chronic damage by noxious stimuli – in many cases by harmful alcohol consumption – preconditions pancreatic and hepatic tissue to the development of SI, which is set off by an often-unidentified trigger. More profound understanding of the development of the diseases resulted in the concept of the sentinel acute pancreatitis model (55) and the clinical distinction of ACLF from acute decompensation (65).

Although the similarities between ACLF and AP are numerous, certain fundamental differences must be highlighted. Organ failure is a diagnostic criterion for ACLF and the prevalence of MOF is much higher compared to AP. It represents an advanced stage of liver disease, while AP is usually self-limiting and represents the first phase of a potentially chronic disease. It is often only an acute inflammatory episode without the development of organ failure and recurrence.

6.2 Stressors and amplification loops – current evidence on alcohol, smoking and hypoalbuminemia

There is general consensus that both in AP and ACLF, cell death and organ failure are a result of an imbalance of protective factors and stressors. In both cases, environmental and genetic factors result in a state of decreased capacity to mediate inflammation and susceptibility to cell damage and death. After the insult reaches the clinical threshold and SI is induced, it is amplified by vicious cycles seen both in AP and ACLF (21, 60). However, individual sensitivity to noxious stimuli and harmful substances creates a more nuanced picture, further complicating the utilization of experimental and clinical data. Interpersonal differences in response to harmful substances are key to understanding the pathophysiology of these diseases, targeting at-risk individuals, and offering effective treatment options.

The scientific community is not yet able to answer the controversy of why only a small proportion of individuals with harmful alcohol consumption develop cirrhosis and alcohol-induced acute pancreatitis. Nevertheless, the relationship of commonly occurring chronic stressors, such as alcohol and smoking is being explored in depths. Alcohol and smoking are closely associated, and smoking is known to play a role in disease progression, inflammation and carcinogenesis both in ALD and AP (235). It has been shown that alcohol and smoking produce an additive or multiplicative effect in AP (32), and smoking is also associated with higher risk of alcoholic cirrhosis (235). The REAPPEAR study provides a specifically designed intervention program for patients with concurrent harmful alcohol consumption and nicotine dependency. By the utilization of information gathered from this gap-filling RCT, the follow-up and cessation program of these patients can be organized more effectively in the future. These programs may prevent or slow the progression of both AP and ALD, preventing CP and ACLF.

Albumin is a biologically active protein with multiple functions. It is not only a transport protein, but has anti-inflammatory, anti-oxidant and anti-thrombotic effects and is involved in

endothelial stabilization (236). As a negative acute phase protein, the complex mechanisms behind the deterioration of serum albumin levels during inflammation were mainly attributed to extravasation. Hypoalbuminemia is a generally accepted sign of clinical deterioration, is associated with disease severity and negatively correlates with disease prognosis both in AP and ALD. Our analysis on the role of hypoalbuminemia in AP showed that severe hypoalbuminemia is independently associated with both severity and mortality, and that there is a dose-dependent association between albumin levels and systemic and local complications in AP. As expected, we have found that on-admission levels have poor predictive value for AP severity and mortality. However, the prospect of efficient albumin concentration looks promising to improve the prognostic value of albumin (236).

6.3 Therapeutic prospects

Treatment of the precipitating event, the limitation of the inflammatory process and the prevention of organ failure are the main therapeutic goals in AP and ACLF.

The anti-inflammatory properties of albumin, the rapid development of hypoalbuminemia in inflammatory conditions and the successful use of human serum albumin solution for the prevention of hepatorenal syndrome in ACLF highlight the potential therapeutic effects of albumin resuscitation both in AP and liver diseases (65, 72). Jalan et al. postulated that albumin function rather than quantity influences prognosis. They measured albumin the transport and detoxification efficiency of albumin in patients with alcoholic cirrhosis, hospitalized due to acute deterioration. They have found that albumin function was significantly reduced in cirrhotic patients compared to healthy controls, with further reduction in patients with ACLF (237). We have shown that one in five AP patients is affected by hypoalbuminemia on-admission, and the prevalence of hypoalbuminemia during hospitalization reaches 35%, warranting further attention. However, information is lacking regarding efficient albumin concentration in AP. These findings underpin the need for trials assessing albumin's immunomodulatory effects and the efficacy of albumin resuscitation in these conditions.

Liver support therapies theoretically offer replacement for numerous liver functions, such as the removal of several toxic product form the blood and synthesis of albumin, coagulation factors, cytokines, and other biologically active substances. Nevertheless, most clinical trials are underpowered and fail to present reliable evidence on the efficacy of liver support systems in ACLF or lack thereof. Network meta-analyses enable the inclusion of multiple types of interventions – in this case liver support systems – and the ranking of the above. We have found that PE may be the most beneficial of the liver support systems tested in human RCTs. Results from clinical trials of the newly developed bioartificial systems are much anticipated.

6.4 Clinical significance and implementation of the findings presented in the thesis

The immense burden of harmful alcohol consumption in the form of digestive system diseases – including AP and ACLF – necessitates the close collaboration of pancreatologists and hepatologists, to reduce alcohol-related gastrointestinal morbidity and mortality. The various similarities in the pathophysiology and therapeutic goals in AP and ACLF offer a starting block for scientific and clinical collaboration between these two fields. Due to the social and cultural context of alcohol use, effective treatment and the prevention of progression are of utmost

importance and must be in the focus of gastroenterologists. Index hospitalisations with alcohol-induced AP or ALD provide an opportunity for the assessment of both organs and the initiation of preventive measures and cessation programs. As a result of early intervention, the damage caused by harmful alcohol consumption can be reversed, and several organs – including the liver and the pancreas – spared.

Summary of novel findings and perspectives

Hypoalbuminemia in acute pancreatitis: a prospective cohort analysis

- We found that hypoalbuminemia is remarkably common in AP (seen in 19% of patients on admission and 35.7% during hospitalization).
- We saw not only that hypoalbuminemia represents an independent risk factor for severe disease course but there is a dose-dependent association with severity and mortality in AP (<25 g/L serum albumin anytime during hospitalization have a 16.8-fold higher risk of death and 48.8-fold higher risk of severe AP than patients with normal albumin levels).
- We also observed that albumin loss during AP is associated with severity and mortality.
- Based on these findings, we formulated the following implications for practice which immediately change the treatment of AP patients:
    - Albumin levels recommended to be measured for all AP patients
    - Albumin levels suggested to be followed up at least in those patients whose condition is worsening during AP
    - Albumin administration should at least be considered in patients with severe hypoalbuminemia (<25 g/L)
- Our results point towards RCTs focusing on albumin replacement in AP.

Recurrence prevention in alcohol-induced acute pancreatitis: protocol of a randomized controlled trial

- We provide a gap-filling international, multicentre, randomized controlled trial to examine the effects of a combined intervention for the reduction of nicotine and alcohol consumption in alcohol-induced AP.
- We created a tailored alcohol and smoking cessation program for all patients with alcoholic AP in order to reduce the recurrence rate and to prevent the development of CP.
- Incorporating the REAPPEAR cessation program to the everyday practice will revolutionize the care of patients with AUD, furthermore, it could result in the decrease of medical expenses in the future.

Liver support therapy in acute-on-chronic liver failure: a network meta-analysis and systematic review

- We performed the first network meta-analysis comparing all available and tested liver support systems to each other and standard medical therapy in patients with ACLF, assessing safety and efficacy, ranking these treatments by survival benefit.
- We found that all liver support systems are safe with low occurrence of complications.
- Plasma exchange therapy showed significant survival benefit at 3 months and seems to be the most effective treatment option currently available.
- Our results facilitate international discussion and a consensus expected in the near future on the clinical utility of liver support systems.

Author's contribution

In all three articles used in the thesis, the author played a key role in conceptualization, planning, performing the analyses and writing the manuscript.

Additional contributions:

Ocskay et al. Scientific Reports, 2021

The author took part in patient management at centres participating in the Acute Pancreatitis Registry of the HPSG, participated in patient enrolment, curated the data, designed the analyses, edited the figures and tables, interpreted the results, and wrote the original draft.

Ocskay et al. BMJ Open, 2021

The author was responsible for the design of the intervention program and coordination of the contributors as well as conducting the test patient interviews. The author wrote the original draft of the manuscript.

Ocskay et al. Annals of Intensive Care, 2021

The author conducted data extraction, risk of bias and certainty of evidence assessment, designed the analyses and wrote the original draft of the manuscript.

Acknowledgements

I would like to express my gratitude to my mentors, Professor Péter Hegyi and Andrea Párniczky. Working with them has given me much more than I could hope for within the confines of a PhD program. Thanks to them, I have not only grown as a researcher and a medical doctor, but as a critical thinker and a person as well.

I would also like to thank Professor Zsolt Molnár for his advice and the glimpse at intensive therapy, which I hope will stay with me shaping my mentality as a physician.

I am fortunate to have been part of the Hungarian Pancreatic Study Group. I would like to thank all collaborators who uploaded clinical data, data managers and clinical administrators taking part in the quality control, and last but not least, all patients involved in the research related to the thesis, making this work possible. I am also indebted to the funders for providing the financial means to my projects.

Many thanks to my fellow PhD students for sharing their knowledge with me and for the experience of effective and interesting teamwork. I would like to acknowledge all colleagues at the Institute for Translational Medicine, Medical School, University of Pécs who contributed to my work in any way.

I am eternally grateful for the support of my husband Félix – your calmness helps me overtake every hurdle that comes my way. I would also like to thank my parents and my family for their support throughout the years and their trust that gives me confidence at the hardest times. I am also thankful to my son, who although being a new-born generously let me finish this thesis.

References

1. Ocskay K, Vinkó Z, Németh D, Szabó L, Bajor J, Gódi S, et al. Hypoalbuminemia affects one third of acute pancreatitis patients and is independently associated with severity and mortality. Sci Rep. 2021;11(1):24158.
2. Ocskay K, Juhász MF, Farkas N, Zádori N, Szakó L, Szakács Z, et al. Recurrent acute pancreatitis prevention by the elimination of alcohol and cigarette smoking (REAPPEAR): protocol of a randomised controlled trial and a cohort study. BMJ Open. 2022;12(1):e050821.
3. Ocskay K, Kanjo A, Gede N, Szakács Z, Pár G, Erőss B, et al. 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;11(1):10.
4. Asrani SK, Devarbhavi H, Eaton J, Kamath PS. Burden of liver diseases in the world. J Hepatol. 2019;70(1):151-71.
5. WHO. ICD-10 classification of mental and behavioural disorders: clinical descriptions and diagnostic guidelines. Geneva, Switzerland: World Health Orgnization; 1992.
6. WHO. Global status report on alcohol and health 2018.: World Health Organization; 2018.
7. Saunders JB, Degenhardt L, Reed GM, Poznyak V. Alcohol Use Disorders in ICD-11: Past, Present, and Future. Alcohol Clin Exp Res. 2019;43(8):1617-31.
8. Axley PD, Richardson CT, Singal AK. Epidemiology of Alcohol Consumption and Societal Burden of Alcoholism and Alcoholic Liver Disease. Clin Liver Dis. 2019;23(1):39-50.
9. Laramée P, Kusel J, Leonard S, Aubin HJ, François C, Daeppen JB. The economic burden of alcohol dependence in Europe. Alcohol Alcohol. 2013;48(3):259-69.
10. Pimpin L, Cortez-Pinto H, Negro F, Corbould E, Lazarus JV, Webber L, et al. Burden of liver disease in Europe: Epidemiology and analysis of risk factors to identify prevention policies. Journal of Hepatology. 2018;69(3):718-35.
11. Carvalho AF, Heilig M, Perez A, Probst C, Rehm J. Alcohol use disorders. Lancet. 2019;394(10200):781-92.
12. Rocco A, Compare D, Angrisani D, Sanduzzi Zamparelli M, Nardone G. Alcoholic disease: liver and beyond. World J Gastroenterol. 2014;20(40):14652-9.
13. Haber PS, Kortt NC. Alcohol use disorder and the gut. Addiction. 2021;116(3):658-67.
14. Pohl K, Moodley P, Dhanda AD. Alcohol's Impact on the Gut and Liver. Nutrients. 2021;13(9).
15. Bujanda L. The effects of alcohol consumption upon the gastrointestinal tract. Am J Gastroenterol. 2000;95(12):3374-82.
16. Tripathi A, Debelius J, Brenner DA, Karin M, Loomba R, Schnabl B, et al. The gut–liver axis and the intersection with the microbiome. Nature Reviews Gastroenterology & Hepatology. 2018;15(7):397-411.
17. Bhavsar-Burke I, Jansson-Knodell CL, Gilmore AC, Crabb DW. Review article: the role of nutrition in alcohol-associated liver disease. Aliment Pharmacol Ther. 2021;53(12):1268-76.
18. Kamran U, Towey J, Khanna A, Chauhan A, Rajoriya N, Holt A. Nutrition in alcohol-related liver disease: Physiopathology and management. World J Gastroenterol. 2020;26(22):2916-30.
19. Clemens DL, Schneider KJ, Arkfeld CK, Grode JR, Wells MA, Singh S. Alcoholic pancreatitis: New insights into the pathogenesis and treatment. World J Gastrointest Pathophysiol. 2016;7(1):48-58.
20. Barreto SG, Habtezion A, Gukovskaya A, Lugea A, Jeon C, Yadav D, et al. Critical thresholds: key to unlocking the door to the prevention and specific treatments for acute pancreatitis. Gut. 2021;70(1):194-203.

21. Hegyi P. Necrotic amplification loop in acute pancreatitis: pancreatic stellate cells and nitric oxide are important players in the development of the disease. J Physiol. 2018;596(14):2679-80.
22. Pallagi P, Venglovecz V, Rakonczay Z, Jr., Borka K, Korompay A, Ozsvári B, et al. Trypsin reduces pancreatic ductal bicarbonate secretion by inhibiting CFTR Cl^- channels and luminal anion exchangers. Gastroenterology. 2011;141(6):2228-39.e6.
23. Hoque R, Farooq A, Mehal WZ. Sterile inflammation in the liver and pancreas. J Gastroenterol Hepatol. 2013;28 Suppl 1(0 1):61-7.
24. Clemens DL, Mahan KJ. Alcoholic pancreatitis: lessons from the liver. World J Gastroenterol. 2010;16(11):1314-20.
25. Lankisch PG, Apte M, Banks PA. Acute pancreatitis. The Lancet. 2015;386(9988):85-96.
26. Borrello MT, Martin MB, Pin CL. The unfolded protein response: An emerging therapeutic target for pancreatitis and pancreatic ductal adenocarcinoma. Pancreatology. 2022;22(1):148-59.
27. Pandol S, Gorelick F, Lugea A. Environmental and Genetic Stressors and the Unfolded Protein Response in Exocrine Pancreatic Function – A Hypothesis. Frontiers in Physiology. 2011;2.
28. Pandol SJ, Saluja AK, Imrie CW, Banks PA. Acute pancreatitis: bench to the bedside. Gastroenterology. 2007;132(3):1127-51.
29. Maléth J, Balázs A, Pallagi P, Balla Z, Kui B, Katona M, et al. Alcohol disrupts levels and function of the cystic fibrosis transmembrane conductance regulator to promote development of pancreatitis. Gastroenterology. 2015;148(2):427-39.e16.
30. Vonlaufen A, Xu Z, Daniel B, Kumar RK, Pirola R, Wilson J, et al. Bacterial endotoxin: a trigger factor for alcoholic pancreatitis? Evidence from a novel, physiologically relevant animal model. Gastroenterology. 2007;133(4):1293-303.
31. Sahin-Tóth M, Hegyi P. Smoking and Drinking Synergize in Pancreatitis: Multiple Hits on Multiple Targets. Gastroenterology. 2017;153(6):1479-81.
32. Yadav D, Whitcomb DC. The role of alcohol and smoking in pancreatitis. Nature Reviews Gastroenterology & Hepatology. 2010;7(3):131-45.
33. Lugea A, Gerloff A, Su HY, Xu Z, Go A, Hu C, et al. The Combination of Alcohol and Cigarette Smoke Induces Endoplasmic Reticulum Stress and Cell Death in Pancreatic Acinar Cells. Gastroenterology. 2017;153(6):1674-86.
34. Szentesi A, Farkas N, Sipos Z, Mátrai P, Vincze Á, Izbéki F, et al. Alcohol consumption and smoking dose-dependently and synergistically worsen local pancreas damage. Gut. 2022.
35. Peery AF, Crockett SD, Murphy CC, Lund JL, Dellon ES, Williams JL, et al. Burden and Cost of Gastrointestinal, Liver, and Pancreatic Diseases in the United States: Update 2018. Gastroenterology. 2019;156(1):254-72.e11.
36. Wadhwa V, Patwardhan S, Garg SK, Jobanputra Y, Lopez R, Sanaka MR. Health Care Utilization and Costs Associated With Acute Pancreatitis. Pancreas. 2017;46(3):410-5.
37. Iannuzzi JP, King JA, Leong JH, Quan J, Windsor JW, Tanyingoh D, et al. Global Incidence of Acute Pancreatitis Is Increasing Over Time: A Systematic Review and Meta-Analysis. Gastroenterology. 2022;162(1):122-34.
38. Ouyang G, Pan G, Liu Q, Wu Y, Liu Z, Lu W, et al. The global, regional, and national burden of pancreatitis in 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. BMC Medicine. 2020;18(1):388.
39. Yadav D, Eigenbrodt ML, Briggs MJ, Williams DK, Wiseman EJ. Pancreatitis: prevalence and risk factors among male veterans in a detoxification program. Pancreas. 2007;34(4):390-8.

40. Lankisch PG, Lowenfels AB, Maisonneuve P. What is the risk of alcoholic pancreatitis in heavy drinkers? Pancreas. 2002;25(4):411-2.
41. Yadav D, O'Connell M, Papachristou GI. Natural history following the first attack of acute pancreatitis. Am J Gastroenterol. 2012;107(7):1096-103.
42. Yadav D, Hawes RH, Brand RE, Anderson MA, Money ME, Banks PA, et al. Alcohol consumption, cigarette smoking, and the risk of recurrent acute and chronic pancreatitis. Arch Intern Med. 2009;169(11):1035-45.
43. Banks PA, Bollen TL, Dervenis C, Gooszen HG, Johnson CD, Sarr MG, et al. Classification of acute pancreatitis—2012: revision of the Atlanta classification and definitions by international consensus. Gut. 2013;62(1):102.
44. Koizumi M, Takada T, Kawarada Y, Hirata K, Mayumi T, Yoshida M, et al. JPN Guidelines for the management of acute pancreatitis: diagnostic criteria for acute pancreatitis. J Hepatobiliary Pancreat Surg. 2006;13(1):25-32.
45. Crockett SD, Wani S, Gardner TB, Falck-Ytter Y, Barkun AN, Crockett S, et al. American Gastroenterological Association Institute Guideline on Initial Management of Acute Pancreatitis. Gastroenterology. 2018;154(4):1096-101.
46. Bálint ER, Fűr G, Kiss L, Németh DI, Soós A, Hegyi P, et al. Assessment of the course of acute pancreatitis in the light of aetiology: a systematic review and meta-analysis. Sci Rep. 2020;10(1):17936.
47. Zhu Y, Pan X, Zeng H, He W, Xia L, Liu P, et al. A Study on the Etiology, Severity, and Mortality of 3260 Patients With Acute Pancreatitis According to the Revised Atlanta Classification in Jiangxi, China Over an 8-Year Period. Pancreas. 2017;46(4):504-9.
48. Yadav D. Recent advances in the epidemiology of alcoholic pancreatitis. Curr Gastroenterol Rep. 2011;13(2):157-65.
49. Samokhvalov AV, Rehm J, Roerecke M. Alcohol Consumption as a Risk Factor for Acute and Chronic Pancreatitis: A Systematic Review and a Series of Meta-analyses. EBioMedicine. 2015;2(12):1996-2002.
50. Tenner S, Baillie J, DeWitt J, Vege SS. American College of Gastroenterology Guideline: Management of Acute Pancreatitis. Official journal of the American College of Gastroenterology | ACG. 2013;108(9).
51. Tolstrup JS, Kristiansen L, Becker U, Grønbæk M. Smoking and Risk of Acute and Chronic Pancreatitis Among Women and Men: A Population-Based Cohort Study. Archives of Internal Medicine. 2009;169(6):603-9.
52. Lindkvist B, Appelros S, Regner S, Manjer J. A prospective cohort study on risk of acute pancreatitis related to serum triglycerides, cholesterol and fasting glucose. Pancreatology. 2012;12(4):317-24.
53. Majumder S, Gierisch JM, Bastian LA. The association of smoking and acute pancreatitis: a systematic review and meta-analysis. Pancreas. 2015;44(4):540-6.
54. Sadr-Azodi O, Andrén-Sandberg Å, Orsini N, Wolk A. Cigarette smoking, smoking cessation and acute pancreatitis: a prospective population-based study. Gut. 2012;61(2):262-7.
55. Whitcomb DC. Hereditary pancreatitis: new insights into acute and chronic pancreatitis. Gut. 1999;45(3):317.
56. Vege SS, DiMagno MJ, Forsmark CE, Martel M, Barkun AN. Initial Medical Treatment of Acute Pancreatitis: American Gastroenterological Association Institute Technical Review. Gastroenterology. 2018;154(4):1103-39.
57. Nordback I, Pelli H, Lappalainen-Lehto R, Järvinen S, Räty S, Sand J. The Recurrence of Acute Alcohol-Associated Pancreatitis Can Be Reduced: A Randomized Controlled Trial. Gastroenterology. 2009;136(3):848-55.
58. IAP/APA evidence-based guidelines for the management of acute pancreatitis. Pancreatology. 2013;13(4, Supplement 2):e1-e15.

59. Sissingh NJ, Umans DS, Goudriaan AE, Sijbom M, Verdonk RC, van Hooft JE. Alcohol Reduction to Reduce Relapse in Acute Alcoholic Pancreatitis-Missed Opportunities. Alcohol Alcohol. 2021;56(6):678-82.
60. Dara L, Ji C, Kaplowitz N. The contribution of endoplasmic reticulum stress to liver diseases. Hepatology. 2011;53(5):1752-63.
61. Xia SW, Wang ZM, Sun SM, Su Y, Li ZH, Shao JJ, et al. Endoplasmic reticulum stress and protein degradation in chronic liver disease. Pharmacol Res. 2020;161:105218.
62. Friedman SL. A deer in the headlights: BAMBI meets liver fibrosis. Nature Medicine. 2007;13(11):1281-2.
63. Seitz HK, Bataller R, Cortez-Pinto H, Gao B, Gual A, Lackner C, et al. Alcoholic liver disease. Nature Reviews Disease Primers. 2018;4(1):16.
64. Parker R, Aithal GP, Becker U, Gleeson D, Masson S, Wyatt JI, et al. Natural history of histologically proven alcohol-related liver disease: A systematic review. J Hepatol. 2019;71(3):586-93.
65. Arroyo V, Moreau R, Kamath PS, Jalan R, Ginès P, Nevens F, et al. Acute-on-chronic liver failure in cirrhosis. Nat Rev Dis Primers. 2016;2:16041.
66. Ramachandran P, Iredale JP, Fallowfield JA. Resolution of liver fibrosis: basic mechanisms and clinical relevance. Semin Liver Dis. 2015;35(2):119-31.
67. Moreau R, Jalan R, Gines P, Pavesi M, Angeli P, Cordoba J, et al. Acute-on-Chronic Liver Failure Is a Distinct Syndrome That Develops in Patients With Acute Decompensation of Cirrhosis. Gastroenterology. 2013;144(7):1426-37.e9.
68. Arroyo V, Moreau R, Jalan R. Acute-on-Chronic Liver Failure. New England Journal of Medicine. 2020;382(22):2137-45.
69. Sarin SK, Choudhury A, Sharma MK, Maiwall R, Al Mahtab M, Rahman S, et al. Acute-on-chronic liver failure: consensus recommendations of the Asian Pacific association for the study of the liver (APASL): an update. Hepatology International. 2019;13(4):353-90.
70. Kim TY, Song DS, Kim HY, Sinn DH, Yoon EL, Kim CW, et al. Characteristics and Discrepancies in Acute-on-Chronic Liver Failure: Need for a Unified Definition. PLoS One. 2016;11(1):e0146745.
71. Li H, Chen LY, Zhang NN, Li ST, Zeng B, Pavesi M, et al. Characteristics, Diagnosis and Prognosis of Acute-on-Chronic Liver Failure in Cirrhosis Associated to Hepatitis B. Sci Rep. 2016;6:25487.
72. Zaccherini G, Weiss E, Moreau R. Acute-on-chronic liver failure: Definitions, pathophysiology and principles of treatment. JHEP Reports. 2021;3(1).
73. Bernardi M, Moreau R, Angeli P, Schnabl B, Arroyo V. Mechanisms of decompensation and organ failure in cirrhosis: From peripheral arterial vasodilation to systemic inflammation hypothesis. J Hepatol. 2015;63(5):1272-84.
74. Shi Y, Yang Y, Hu Y, Wu W, Yang Q, Zheng M, et al. Acute-on-chronic liver failure precipitated by hepatic injury is distinct from that precipitated by extrahepatic insults. Hepatology. 2015;62(1):232-42.
75. Fernández J, Acevedo J, Wiest R, Gustot T, Amoros A, Deulofeu C, et al. Bacterial and fungal infections in acute-on-chronic liver failure: prevalence, characteristics and impact on prognosis. Gut. 2018;67(10):1870-80.
76. Dong V, Karvellas CJ. Acute-on-chronic liver failure: Objective admission and support criteria in the intensive care unit. JHEP Rep. 2019;1(1):44-52.
77. Artru F, Louvet A, Ruiz I, Levesque E, Labreuche J, Ursic-Bedoya J, et al. Liver transplantation in the most severely ill cirrhotic patients: A multicenter study in acute-on-chronic liver failure grade 3. J Hepatol. 2017;67(4):708-15.
78. García Martínez JJ, Bendjelid K. Artificial liver support systems: what is new over the last decade? Ann Intensive Care. 2018;8(1):109-.

79. Dunlop EH, Weston MJ, Gazzard BG, Langley PG, Mellon PJ, Williams R. Artificial liver support based on haemoperfusion of adsorbents. Biomedical engineering. 1975;10(6):213-8.
80. Larsen FS. Artificial liver support in acute and acute-on-chronic liver failure. Curr Opin Crit Care. 2019;25(2):187-91.
81. MacDonald AJ, Karvellas CJ. Emerging Role of Extracorporeal Support in Acute and Acute-on-Chronic Liver Failure: Recent Developments. Semin Respir Crit Care Med. 2018;39(5):625-34.
82. Hernaez R, Solà E, Moreau R, Ginès P. Acute-on-chronic liver failure: an update. Gut. 2017;66(3):541-53.
83. Oettl K, Stauber RE. Physiological and pathological changes in the redox state of human serum albumin critically influence its binding properties. British journal of pharmacology. 2007;151(5):580-90.
84. Stamler JS, Jaraki O, Osborne J, Simon DI, Keaney J, Vita J, et al. Nitric oxide circulates in mammalian plasma primarily as an S-nitroso adduct of serum albumin. Proceedings of the National Academy of Sciences of the United States of America. 1992;89(16):7674-7.
85. Ulldemolins M, Roberts JA, Rello J, Paterson DL, Lipman J. The effects of hypoalbuminaemia on optimizing antibacterial dosing in critically ill patients. Clinical pharmacokinetics. 2011;50(2):99-110.
86. Zhang W, Hu J, Yao B, Yang X, Song L, Yin T, et al. Evaluation of Early Prognostic Factors of Mortality in Patients with Acute Pancreatitis: A Retrospective Study. Gastroenterology research and practice. 2017;2017:8363561.
87. Wang X, Cui Z, Li H, Saleen AF, Zhang D, Miao B, et al. Nosocomial mortality and early prediction of patients with severe acute pancreatitis. Journal of gastroenterology and hepatology. 2010;25(8):1386-93.
88. Hong W, Lin S, Zippi M, Geng W, Stock S, Basharat Z, et al. Serum Albumin Is Independently Associated with Persistent Organ Failure in Acute Pancreatitis. Canadian journal of gastroenterology & hepatology. 2017;2017:5297143.
89. Acute Pancreatitis Registry https://tm-centre.org/en/registries/acute-pancreatitis-registry/ [
90. Párniczky A, Lantos T, Tóth EM, Szakács Z, Gódi S, Hágendorn R, et al. Antibiotic therapy in acute pancreatitis: From global overuse to evidence based recommendations. Pancreatology : official journal of the International Association of Pancreatology (IAP) [et al]. 2019;19(4):488-99.
91. Párniczky A, Kui B, Szentesi A, Balázs A, Szűcs Á, Mosztbacher D, et al. Prospective, Multicentre, Nationwide Clinical Data from 600 Cases of Acute Pancreatitis. PloS one. 2016;11(10):e0165309.
92. Mosztbacher D, Hanák L, Farkas N, Szentesi A, Mikó A, Bajor J, et al. Hypertriglyceridemia-induced acute pancreatitis: A prospective, multicenter, international cohort analysis of 716 acute pancreatitis cases. Pancreatology : official journal of the International Association of Pancreatology (IAP) [et al]. 2020;20(4):608-16.
93. Farkas N, Hanák L, Mikó A, Bajor J, Sarlós P, Czimmer J, et al. A Multicenter, International Cohort Analysis of 1435 Cases to Support Clinical Trial Design in Acute Pancreatitis. Frontiers in physiology. 2019;10:1092.
94. Hágendorn R, Vincze Á, Izbéki F, Gajdán L, Gódi S, Illés A, et al. Development of disturbance of consciousness is associated with increased severity in acute pancreatitis. Pancreatology : official journal of the International Association of Pancreatology (IAP) [et al]. 2020;20(5):806-12.
95. Demcsák A, Soós A, Kincses L, Capunge I, Minkov G, Kovacheva-Slavova M, et al. Acid suppression therapy, gastrointestinal bleeding and infection in acute pancreatitis - An

international cohort study. Pancreatology : official journal of the International Association of Pancreatology (IAP) [et al]. 2020;20(7):1323-31.
96. Gódi S, Erőss B, Gyömbér Z, Szentesi A, Farkas N, Párniczky A, et al. Centralized care for acute pancreatitis significantly improves outcomes. Journal of gastrointestinal and liver diseases : JGLD. 2018;27(2):151-7.
97. Szakács Z, Gede N, Pécsi D, Izbéki F, Papp M, Kovács G, et al. Aging and Comorbidities in Acute Pancreatitis II.: A Cohort-Analysis of 1203 Prospectively Collected Cases. Frontiers in physiology. 2018;9:1776.
98. Halász A, Pécsi D, Farkas N, Izbéki F, Gajdán L, Fejes R, et al. Outcomes and timing of endoscopic retrograde cholangiopancreatography for acute biliary pancreatitis. Digestive and liver disease : official journal of the Italian Society of Gastroenterology and the Italian Association for the Study of the Liver. 2019;51(9):1281-6.
99. Szentesi A, Párniczky A, Vincze Á, Bajor J, Gódi S, Sarlós P, et al. Multiple Hits in Acute Pancreatitis: Components of Metabolic Syndrome Synergize Each Other's Deteriorating Effects. Frontiers in physiology. 2019;10:1202.
100. Hegyi PJ, Soós A, Tóth E, Ébert A, Venglovecz V, Márta K, et al. Evidence for diagnosis of early chronic pancreatitis after three episodes of acute pancreatitis: a cross-sectional multicentre international study with experimental animal model. Scientific Reports. 2021;11(1):1367.
101. Fluss R, Faraggi D, Reiser B. Estimation of the Youden Index and its associated cutoff point. Biom J. 2005;47(4):458-72.
102. Robin X, Turck N, Hainard A, Tiberti N, Lisacek F, Sanchez J-C, et al. pROC: an open-source package for R and S+ to analyze and compare ROC curves. BMC Bioinformatics. 2011;12(1):77.
103. PMCMRplus: Calculate Pairwise Multiple Comparisons of Mean Rank Sums Extended https://CRAN.R-project.org/package=PMCMRplus [
104. von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. Lancet (London, England). 2007;370(9596):1453-7.
105. Artero A, Zaragoza R, Camarena JJ, Sancho S, González R, Nogueira JM. Prognostic factors of mortality in patients with community-acquired bloodstream infection with severe sepsis and septic shock. Journal of critical care. 2010;25(2):276-81.
106. Lyons O, Whelan B, Bennett K, O'Riordan D, Silke B. Serum albumin as an outcome predictor in hospital emergency medical admissions. European journal of internal medicine. 2010;21(1):17-20.
107. Komara NL, Paragomi P, Greer PJ, Wilson AS, Breze C, Papachristou GI, et al. Severe acute pancreatitis: capillary permeability model linking systemic inflammation to multiorgan failure. American journal of physiology Gastrointestinal and liver physiology. 2020;319(5):G573-g83.
108. Charlie-Silva I, Klein A, Gomes JMM, Prado EJR, Moraes AC, Eto SF, et al. Acute-phase proteins during inflammatory reaction by bacterial infection: Fish-model. Scientific Reports. 2019;9(1):4776.
109. Barle H, Gamrin L, Essén P, McNurlan MA, Garlick PJ, Wernerman J. Growth hormone does not affect albumin synthesis in the critically ill. Intensive Care Medicine. 2001;27(5):836-43.
110. Barle H, Hammarqvist F, Westman B, Klaude M, Rooyackers O, Garlick Peter J, et al. Synthesis rates of total liver protein and albumin are both increased in patients with an acute inflammatory response. Clinical Science. 2005;110(1):93-9.

111. Dahn MS, Mitchell RA, Lange MP, Smith S, Jacobs LA. Hepatic metabolic response to injury and sepsis. Surgery. 1995;117(5):520-30.
112. Soeters PB, Wolfe RR, Shenkin A. Hypoalbuminemia: Pathogenesis and Clinical Significance. 2019;43(2):181-93.
113. Levitt DG, Levitt MD. Human serum albumin homeostasis: a new look at the roles of synthesis, catabolism, renal and gastrointestinal excretion, and the clinical value of serum albumin measurements. Int J Gen Med. 2016;9:229-55.
114. Li S, Zhang Y, Li M, Xie C, Wu H. Serum albumin, a good indicator of persistent organ failure in acute pancreatitis. BMC Gastroenterology. 2017;17(1):59.
115. Brand M, Götz A, Zeman F, Behrens G, Leitzmann M, Brünnler T, et al. Acute necrotizing pancreatitis: laboratory, clinical, and imaging findings as predictors of patient outcome. AJR Am J Roentgenol. 2014;202(6):1215-31.
116. Vincent J-L, Russell JA, Jacob M, Martin G, Guidet B, Wernerman J, et al. Albumin administration in the acutely ill: what is new and where next? Critical Care. 2014;18(4):231.
117. Flannery AH, Owen GD, Coz A, Thompson Bastin ML, Patel K. Impact of Hyperoncotic Albumin on Duration of Vasopressor Support inSeptic Shock: A PropensityScore-Matched Analysis. The Annals of pharmacotherapy. 2020:1060028020963645.
118. Szentesi A, Tóth E, Bálint E, Fanczal J, Madácsy T, Laczkó D, et al. Analysis of Research Activity in Gastroenterology: Pancreatitis Is in Real Danger. PLoS One [Internet]. 2016 2016; 11(10):[e0165244 p.].
119. Tenner S, Baillie J, DeWitt J, Vege SS. American College of Gastroenterology guideline: management of acute pancreatitis. The American journal of gastroenterology. 2013;108(9):1400-15; 16.
120. Guda NM, Muddana V, Whitcomb DC, Levy P, Garg P, Cote G, et al. Recurrent Acute Pancreatitis: International State-of-the-Science Conference With Recommendations. Pancreas. 2018;47(6):653-66.
121. Cote GA, Yadav D, Abberbock JA, Whitcomb DC, Sherman S, Sandhu BS, et al. Recurrent Acute Pancreatitis Significantly Reduces Quality of Life Even in the Absence of Overt Chronic Pancreatitis. The American journal of gastroenterology. 2018;113(6):906-12.
122. Mandalia A, Wamsteker EJ, DiMagno MJ. Recent advances in understanding and managing acute pancreatitis. F1000Res. 2018;7.
123. Krishna SG, Kamboj AK, Hart PA, Hinton A, Conwell DL. The Changing Epidemiology of Acute Pancreatitis Hospitalizations: A Decade of Trends and the Impact of Chronic Pancreatitis. Pancreas. 2017;46(4):482-8.
124. Nikkola J, Raty S, Laukkarinen J, Seppanen H, Lappalainen-Lehto R, Jarvinen S, et al. Abstinence after first acute alcohol-associated pancreatitis protects against recurrent pancreatitis and minimizes the risk of pancreatic dysfunction. Alcohol and alcoholism (Oxford, Oxfordshire). 2013;48(4):483-6.
125. Magnusdottir BA, Baldursdottir MB, Kalaitzakis E, Bjornsson ES. Risk factors for chronic and recurrent pancreatitis after first attack of acute pancreatitis. Scandinavian journal of gastroenterology. 2019;54(1):87-94.
126. Nikkola J, Laukkarinen J, Lahtela J, Seppanen H, Jarvinen S, Nordback I, et al. The Long-term Prospective Follow-up of Pancreatic Function After the First Episode of Acute Alcoholic Pancreatitis: Recurrence Predisposes One to Pancreatic Dysfunction and Pancreatogenic Diabetes. Journal of clinical gastroenterology. 2017;51(2):183-90.
127. Lappalainen-Lehto R, Koistinen N, Aalto M, Huhtala H, Sand J, Nordback I, et al. Goal-related outcome after acute alcohol-pancreatitis -- a two-year follow-up study. Addictive behaviors. 2013;38(12):2805-9.
128. Schuckit MA. Alcohol-use disorders. The Lancet. 2009;373(9662):492-501.

129. Peris P, Parés A, Guañabens N, Del Río L, Pons F, De Osaba MJM, et al. Bone mass improves in alcoholics after 2 years of abstinence. Journal of Bone and Mineral Research. 1994;9(10):1607-12.
130. Aguilera MT, de la Sierra A, Coca A, Estruch R, Fernandez-Sola J, Urbano-Marquez A. Effect of alcohol abstinence on blood pressure: assessment by 24-hour ambulatory blood pressure monitoring. Hypertension (Dallas, Tex : 1979). 1999;33(2):653-7.
131. Jandikova H, Duskova M, Starka L. The influence of smoking and cessation on the human reproductive hormonal balance. Physiological research. 2017;66(Supplementum 3):S323-s31.
132. Taylor DH, Jr., Hasselblad V, Henley SJ, Thun MJ, Sloan FA. Benefits of smoking cessation for longevity. American journal of public health. 2002;92(6):990-6.
133. Párniczky A, Kui B, Szentesi A, Balázs A, Szűcs Á, Mosztbacher D, et al. Prospective, Multicentre, Nationwide Clinical Data from 600 Cases of Acute Pancreatitis. PLoS One. 2016;11(10):e0165309-e.
134. Szucs A, Marjai T, Szentesi A, Farkas N, Parniczky A, Nagy G, et al. Chronic pancreatitis: Multicentre prospective data collection and analysis by the Hungarian Pancreatic Study Group. PLoS One. 2017;12(2):e0171420.
135. Weinberger AH, Platt J, Jiang B, Goodwin RD. Cigarette Smoking and Risk of Alcohol Use Relapse Among Adults in Recovery from Alcohol Use Disorders. Alcoholism, clinical and experimental research. 2015;39(10):1989-96.
136. Yardley MM, Mirbaba MM, Ray LA. Pharmacological Options for Smoking Cessation in Heavy-Drinking Smokers. CNS drugs. 2015;29(10):833-45.
137. Mirbaba M. Heavy-Drinking Smokers: Pathophysiology and Pharmacologic Treatment Options. American Journal of Psychiatry Residents' Journal. 2016;11(6):8-11.
138. Fucito LM, Hanrahan TH. Heavy-Drinking Smokers' Treatment Needs and Preferences: A Mixed-Methods Study. Journal of substance abuse treatment. 2015;59:38-44.
139. McKelvey K, Thrul J, Ramo D. Impact of quitting smoking and smoking cessation treatment on substance use outcomes: An updated and narrative review. Addictive behaviors. 2017;65:161-70.
140. Toll BA, Martino S, O'Malley SS, Fucito LM, McKee SA, Kahler CW, et al. A randomized trial for hazardous drinking and smoking cessation for callers to a quitline. Journal of consulting and clinical psychology. 2015;83(3):445-54.
141. Crockett SD, Wani S, Gardner TB, Falck-Ytter Y, Barkun AN. American Gastroenterological Association Institute Guideline on Initial Management of Acute Pancreatitis. Gastroenterology. 2018;154(4):1096-101.
142. IAP/APA evidence-based guidelines for the management of acute pancreatitis. Pancreatology. 2013;13(4 Suppl 2):e1-15.
143. Samarasekera E, Mahammed S, Carlisle S, Charnley R. Pancreatitis: summary of NICE guidance. BMJ (Clinical research ed). 2018;362:k3443.
144. Isaji S, Takada T, Mayumi T, Yoshida M, Wada K, Yokoe M, et al. Revised Japanese guidelines for the management of acute pancreatitis 2015: revised concepts and updated points. Journal of hepato-biliary-pancreatic sciences. 2015;22(6):433-45.
145. Chan AW, Tetzlaff JM, Gotzsche PC, Altman DG, Mann H, Berlin JA, et al. SPIRIT 2013 explanation and elaboration: guidance for protocols of clinical trials. BMJ (Clinical research ed). 2013;346:e7586.
146. Yadav D. Reassessing the Risk of Pancreatitis With Alcohol. Pancreas. 2016;45(6):781-2.
147. Mosztbacher D, Hanák L, Farkas N, Szentesi A, Mikó A, Bajor J, et al. Hypertriglyceridemia-induced acute pancreatitis: A prospective, multicenter, international cohort analysis of 716 acute pancreatitis cases. Pancreatology. 2020.

148. Zádori N, Gede N, Antal J, Szentesi A, Alizadeh H, Vincze Á, et al. EarLy Elimination of Fatty Acids iN hypertriglyceridemia-induced acuTe pancreatitis (ELEFANT trial): Protocol of an open-label, multicenter, adaptive randomized clinical trial. Pancreatology. 2020;20(3):369-76.
149. Hegyi P, Erőss B, Mátrai P, Hamvas J, Pécsi D, Németh B, et al. The first definition for early chronic pancreatitis. Pancreatology. 2018;18(4, Supplement):S172.
150. Lohr JM, Dominguez-Munoz E, Rosendahl J, Besselink M, Mayerle J, Lerch MM, et al. United European Gastroenterology evidence-based guidelines for the diagnosis and therapy of chronic pancreatitis (HaPanEU). United European gastroenterology journal. 2017;5(2):153-99.
151. Platt L, Melendez-Torres GJ, O'Donnell A, Bradley J, Newbury-Birch D, Kaner E, et al. How effective are brief interventions in reducing alcohol consumption: do the setting, practitioner group and content matter? Findings from a systematic review and metaregression analysis. BMJ open. 2016;6(8):e011473.
152. The Alcohol, Smoking and Substance Involvement Screening Test (ASSIST): development, reliability and feasibility. Addiction (Abingdon, England). 2002;97(9):1183-94.
153. Kaner EFS, Beyer FR, Muirhead C, Campbell F, Pienaar ED, Bertholet N, et al. Effectiveness of brief alcohol interventions in primary care populations. Cochrane Database of Systematic Reviews. 2018(2).
154. Kristenson H, Öhlin H, Hultén-Nosslin M-B, Trell E, Hood B. Identification and Intervention of Heavy Drinking in Middle-Aged Men: Results and Follow-up of 24–60 Months of Long-Term Study with Randomized Controls. Alcoholism: Clinical and Experimental Research. 1983;7(2):203-9.
155. Pelli H, Lappalainen-Lehto R, Piironen A, Sand J, Nordback I. Risk factors for recurrent acute alcohol-associated pancreatitis: a prospective analysis. Scandinavian journal of gastroenterology. 2008;43(5):614-21.
156. Kim S, Apelberg BJ, Avila-Tang E, Hepp L, Yun D, Samet JM, et al. Utility and cutoff value of hair nicotine as a biomarker of long-term tobacco smoke exposure, compared to salivary cotinine. Int J Environ Res Public Health. 2014;11(8):8368-82.
157. Andresen-Streichert H, Müller A, Glahn A, Skopp G, Sterneck M. Alcohol Biomarkers in Clinical and Forensic Contexts. Dtsch Arztebl Int. 2018;115(18):309-15.
158. Mikó A, Erőss B, Sarlós P, Hegyi P, Jr., Márta K, Pécsi D, et al. Observational longitudinal multicentre investigation of acute pancreatitis (GOULASH PLUS): follow-up of the GOULASH study, protocol. BMJ open. 2019;9(8):e025500.
159. Márta K, Szabó AN, Pécsi D, Varjú P, Bajor J, Gódi S, et al. High versus low energy administration in the early phase of acute pancreatitis (GOULASH trial): protocol of a multicentre randomised double-blind clinical trial. BMJ open. 2017;7(9):e015874.
160. Párniczky A, Mosztbacher D, Zsoldos F, Tóth A, Lásztity N, Hegyi P. Analysis of Pediatric Pancreatitis (APPLE Trial): Pre-Study Protocol of a Multinational Prospective Clinical Trial. Digestion. 2016;93(2):105-10.
161. Párniczky A, Abu-El-Haija M, Husain S, Lowe M, Oracz G, Sahin-Tóth M, et al. EPC/HPSG evidence-based guidelines for the management of pediatric pancreatitis. Pancreatology. 2018;18(2):146-60.
162. Hritz I, Czakó L, Dubravcsik Z, Farkas G, Kelemen D, Lásztity N, et al. [Acute pancreatitis. Evidence-based practice guidelines, prepared by the Hungarian Pancreatic Study Group]. Orvosi hetilap. 2015;156(7):244-61.
163. Gholam PM. Prognosis and Prognostic Scoring Models for Alcoholic Liver Disease and Acute Alcoholic Hepatitis. Clinics in liver disease. 2016;20(3):491-7.
164. Hedman* L, Katsaounou* PA, Filippidis FT, Ravara SB, Lindberg A, Janson C, et al. Receiving support to quit smoking and quit attempts among smokers with and without smoking

related diseases: Findings from the EUREST-PLUS ITC Europe Surveys. Tob Induc Dis. 2018;16(2).
165. Saunders JB, Aasland OG, Babor TF, de la Fuente JR, Grant M. Development of the Alcohol Use Disorders Identification Test (AUDIT): WHO Collaborative Project on Early Detection of Persons with Harmful Alcohol Consumption--II. Addiction (Abingdon, England). 1993;88(6):791-804.
166. Németh ZK, Urbán, Róbert, Farkas, Judit, Kuntsche, Emmanuel, Demetrovics, Zsolt. Hungarian adaptation of the long and the short form of the drinking motives questionnaire (DMQ-R). Magyar Pszichológiai Szemle, 67 (4) pp 673-694. 2012.
167. Kuntsche E, Kuntsche S. Development and validation of the Drinking Motive Questionnaire Revised Short Form (DMQ-R SF). Journal of clinical child and adolescent psychology : the official journal for the Society of Clinical Child and Adolescent Psychology, American Psychological Association, Division 53. 2009;38(6):899-908.
168. Heatherton TF, Kozlowski LT, Frecker RC, Fagerstrom KO. The Fagerstrom Test for Nicotine Dependence: a revision of the Fagerstrom Tolerance Questionnaire. British journal of addiction. 1991;86(9):1119-27.
169. Capacities HMoH. Az Emberi Erőforrások Minisztériuma egészségügyi szakmai irányelve a dohányzásról való leszokás támogatásáról. https://tudogyogyasz.hu2019.
170. Varga L. Segítségnyújtás kockázatos ivók számára National Addiction Institute; 2006.
171. Petersen OH, Sutton R. Ca2+ signalling and pancreatitis: effects of alcohol, bile and coffee. Trends in pharmacological sciences. 2006;27(2):113-20.
172. Rencz F, Gulacsi L, Drummond M, Golicki D, Prevolnik Rupel V, Simon J, et al. EQ-5D in Central and Eastern Europe: 2000-2015. Quality of life research : an international journal of quality of life aspects of treatment, care and rehabilitation. 2016;25(11):2693-710.
173. Lloyd A, Pickard AS. The EQ-5D and the EuroQol Group. Value in health : the journal of the International Society for Pharmacoeconomics and Outcomes Research. 2019;22(1):21-2.
174. Koncz B, Darvasi E, Erdosi D, Szentesi A, Marta K, Eross B, et al. LIFEStyle, Prevention and Risk of Acute PaNcreatitis (LIFESPAN): protocol of a multicentre and multinational observational case-control study. BMJ open. 2020;10(1):e029660.
175. Wu W, Yan H, Zhao H, Sun W, Yang Q, Sheng J, et al. Characteristics of systemic inflammation in hepatitis B-precipitated ACLF: Differentiate it from No-ACLF. Liver International. 2018;38(2):248-57.
176. Clària J, Stauber RE, Coenraad MJ, Moreau R, Jalan R, Pavesi M, et al. Systemic inflammation in decompensated cirrhosis: Characterization and role in acute-on-chronic liver failure. Hepatology. 2016;64(4):1249-64.
177. Markwick LJ, Riva A, Ryan JM, Cooksley H, Palma E, Tranah TH, et al. Blockade of PD1 and TIM3 restores innate and adaptive immunity in patients with acute alcoholic hepatitis. Gastroenterology. 2015;148(3):590-602.e10.
178. Bernsmeier C, Pop OT, Singanayagam A, Triantafyllou E, Patel VC, Weston CJ, et al. Patients with acute-on-chronic liver failure have increased numbers of regulatory immune cells expressing the receptor tyrosine kinase MERTK. Gastroenterology. 2015;148(3):603-15.e14.
179. Wasmuth HE, Kunz D, Yagmur E, Timmer-Stranghöner A, Vidacek D, Siewert E, et al. Patients with acute on chronic liver failure display ‘sepsis-like’ immune paralysis. Journal of Hepatology. 2005;42(2):195-201.
180. Berres ML, Schnyder B, Yagmur E, Inglis B, Stanzel S, Tischendorf JJ, et al. Longitudinal monocyte human leukocyte antigen-DR expression is a prognostic marker in critically ill patients with decompensated liver cirrhosis. Liver international : official journal of the International Association for the Study of the Liver. 2009;29(4):536-43.

181. O'Brien AJ, Fullerton JN, Massey KA, Auld G, Sewell G, James S, et al. Immunosuppression in acutely decompensated cirrhosis is mediated by prostaglandin E2. Nature medicine. 2014;20(5):518-23.
182. Finkenstedt A, Nachbaur K, Zoller H, Joannidis M, Pratschke J, Graziadei IW, et al. Acute-on-chronic liver failure: Excellent outcomes after liver transplantation but high mortality on the wait list. Liver Transplantation. 2013;19(8):879-86.
183. Sarin SK, Choudhury A, Sharma MK, Maiwall R, Al Mahtab M, Rahman S, et al. Acute-on-chronic liver failure: consensus recommendations of the Asian Pacific association for the study of the liver (APASL): an update. Hepatology international. 2019;13(4):353-90.
184. EASL Clinical Practice Guidelines for the management of patients with decompensated cirrhosis. J Hepatol. 2018;69(2):406-60.
185. Kjaergard LL, Liu J, Als-Nielsen B, Gluud C. Artificial and bioartificial support systems for acute and acute-on-chronic liver failure: a systematic review. Jama. 2003;289(2):217-22.
186. Liu JP, Gluud LL, Als-Nielsen B, Gluud C. Artificial and bioartificial support systems for liver failure. Cochrane Database Syst Rev. 2004(1):Cd003628.
187. Zheng Z, Li X, Li Z, Ma X. Artificial and bioartificial liver support systems for acute and acute-on-chronic hepatic failure: A meta-analysis and meta-regression. Experimental and therapeutic medicine. 2013;6(4):929-36.
188. Shen Y, Wang XL, Wang B, Shao JG, Liu YM, Qin Y, et al. Survival Benefits With Artificial Liver Support System for Acute-on-Chronic Liver Failure: A Time Series-Based Meta-Analysis. Medicine. 2016;95(3):e2506.
189. Alshamsi F, Alshammari K, Belley-Cote E, Dionne J, Albrahim T, Albudoor B, et al. Extracorporeal liver support in patients with liver failure: a systematic review and meta-analysis of randomized trials. Intensive care medicine. 2020;46(1):1-16.
190. Stutchfield BM, Simpson K, Wigmore SJ. Systematic review and meta-analysis of survival following extracorporeal liver support. Br J Surg. 2011;98(5):623-31.
191. Khuroo MS, Khuroo MS, Farahat KL. Molecular adsorbent recirculating system for acute and acute-on-chronic liver failure: a meta-analysis. Liver Transpl. 2004;10(9):1099-106.
192. He GL, Feng L, Duan CY, Hu X, Zhou CJ, Cheng Y, et al. Meta-analysis of survival with the molecular adsorbent recirculating system for liver failure. International journal of clinical and experimental medicine. 2015;8(10):17046-54.
193. Rouse B, Chaimani A, Li T. Network meta-analysis: an introduction for clinicians. Intern Emerg Med. 2017;12(1):103-11.
194. Hutton B, Salanti G, Caldwell DM, Chaimani A, Schmid CH, Cameron C, et al. The PRISMA Extension Statement for Reporting of Systematic Reviews Incorporating Network Meta-analyses of Health Care Interventions: Checklist and Explanations. Annals of Internal Medicine. 2015;162(11):777-84.
195. Sterne JAC, Savović J, Page MJ, Elbers RG, Blencowe NS, Boutron I, et al. RoB 2: a revised tool for assessing risk of bias in randomised trials. BMJ. 2019;366:l4898.
196. Juan José Yepes-Nunes HJS. 'Summary of findings' tables in network meta-analysis (NMA) 2018 [Available from: https://training.cochrane.org/resource/SoF-tables-nma.
197. Dias S, Welton NJ, Caldwell DM, Ades AE. Checking consistency in mixed treatment comparison meta-analysis. Statistics in medicine. 2010;29(7-8):932-44.
198. Mbuagbaw L, Rochwerg B, Jaeschke R, Heels-Andsell D, Alhazzani W, Thabane L, et al. Approaches to interpreting and choosing the best treatments in network meta-analyses. Systematic Reviews. 2017;6(1):79.
199. Mitzner SR, Stange J, Klammt S, Risler T, Erley CM, Bader BD, et al. Improvement of hepatorenal syndrome with extracorporeal albumin dialysis MARS: results of a prospective, randomized, controlled clinical trial. Liver Transpl. 2000;6(3):277-86.

200. Bañares R, Nevens F, Larsen FS, Jalan R, Albillos A, Dollinger M, et al. Extracorporeal albumin dialysis with the molecular adsorbent recirculating system in acute-on-chronic liver failure: The RELIEF trial. Hepatology. 2013;57(3):1153-62.
201. Heemann U, Treichel U, Loock J, Philipp T, Gerken G, Malago M, et al. Albumin dialysis in cirrhosis with superimposed acute liver injury: a prospective, controlled study. Hepatology. 2002;36(4 Pt 1):949-58.
202. Kramer L, Gendo A, Madl C, Mullen KD, Kaminski-Russ K, Sunder-Plassmann G, et al. A controlled study of sorbent suspension dialysis in chronic liver disease and hepatic encephalopathy. International Journal of Artificial Organs. 2001;24(7):434-42.
203. Qin G, Shao JG, Wang B, Shen Y, Zheng J, Liu XJ, et al. Artificial liver support system improves short- and long-term outcomes of patients with HBV-associated acute-on-chronic liver failure: A single-center experience. Medicine (United States). 2014;93(28):e338.
204. Sen S, Davies NA, Mookerjee RP, Cheshire LM, Hodges SJ, Williams R, et al. Pathophysiological effects of albumin dialysis in acute-on-chronic liver failure: A randomized controlled study. Liver Transplantation. 2004;10(9):1109-19.
205. Kribben A, Gerken G, Haag S, Hergetrosenthal S, Treichel U, Betz C, et al. Effects of fractionated plasma separation and adsorption on survival in patients with acute-on-chronic liver failure. Gastroenterology. 2012;142(4):782-9.e3.
206. Yu JW, Sun LJ, Zhao YH, Li SC. Prediction value of model for end-stage liver disease scoring system on prognosis in patients with acute-on-chronic hepatitis B liver failure after plasma exchange and lamivudine treatment. J Gastroenterol Hepatol. 2008;23(8 Pt 1):1242-9.
207. Ellis AJ, Hughes RD, Nicholl D, Langley PG, Wendon JA, O'Grady JG, et al. Temporary extracorporeal liver support for severe acute alcoholic hepatitis using the BioLogic-DT. The International journal of artificial organs. 1999;22(1):27-34.
208. Hassanein TI, Tofteng F, Brown Jr RS, McGuire B, Lynch P, Mehta R, et al. Randomized controlled study of extracorporeal albumin dialysis for hepatic encephalopathy in advanced cirrhosis. Hepatology. 2007;46(6):1853-62.
209. Thompson J, Jones N, Al-Khafaji A, Malik S, Reich D, Munoz S, et al. Extracorporeal cellular therapy (ELAD) in severe alcoholic hepatitis: A multinational, prospective, controlled, randomized trial. Liver Transplantation. 2018;24(3):380-93.
210. Duan Z, Xin S, Zhang J, You S, Chen Y, Liu H, et al. Comparison of extracorporeal cellular therapy (ELAD®) vs standard of care in a randomized controlled clinical trial in treating Chinese subjects with acute-on-chronic liver failure. Hepatic Medicine: Evidence and Research. 2018;10:139-52.
211. Teperman L. A phase 2b study of safety & efficacy in subjects with acute-on-chronic hepatitis (AOCH) due either to acute alcoholic hepatitis or acute decompensation of cirrhosis & the use of a support system (elad®) a human cell-based biological liver. American Journal of Transplantation. 2012;12:64.
212. Pyrsopoulos NT, Hassanein T, Subramanian R, Thompson J, Caldwell S, Al-Khafaji A, et al. A study investigating the effect of extracorporeal cellular therapy with C3A cells on the survival of alcoholic hepatitis designed along the guidelines of the NIAAA. Journal of Hepatology. 2019;70(1):e282.
213. Hillebrand DJ, Frederick RT, Williams WW, Brown RS, Napotilano LM, Te HS, et al. Safety and efficacy of the Extracorporeal Liver Assist Device (ELAD®) in patients with acute on chronic liver failure. Journal of Hepatology. 2010;52:S323-S4.
214. Huang YK, Tan DM, Xie YT, Fan XG, Huang Y, Liu ZB, et al. Randomized controlled study of plasma exchange combined with molecular adsorbent re-circulating system for the treatment of liver failure complicated with hepatic encephalopathy. Hepato-Gastroenterology. 2012;59(117):1323-6.

215. Wilkinson AH, Ash SR, Nissenson AR. Hemodiabsorption in treatment of hepatic failure. Journal of transplant coordination : official publication of the North American Transplant Coordinators Organization (NATCO). 1998;8(1):43-50.
216. Hu XB, Zhou Y, Zhao YQ, Liao ME, He MR, Wang MM. Therapeutic application of molecular adsorbents recirculating system (MARS) in chronic severe hepatitis patients complicated with multi organ failure. Journal of hepatology. 2005;42(Suppl 2):179.
217. You SL, Liu HL, Rong YH, Zhu B, Liu WS, Mao PY, et al. [Clinical study on hybrid bioartificial liver supporting system for acute on chronic liver failure patients]. Zhonghua shi yan he lin chuang bing du xue za zhi = Zhonghua shiyan he linchuang bingduxue zazhi = Chinese journal of experimental and clinical virology. 2011;25(5):387-9.
218. He JQ CC, Deng JT, Qi HX, Zhang XQ, Chen ZQ Clinical study on the treatment of fatal hepatitis with artificial liver support system. Chin Crit Care Med. 2000(12):105–8.
219. Laleman W, Wilmer A, Evenepoel P, Elst IV, Zeegers M, Zaman Z, et al. Effect of the molecular adsorbent recirculating system and Prometheus devices on systemic haemodynamics and vasoactive agents in patients with acute-on-chronic alcoholic liver failure. Critical Care. 2006;10(4).
220. Stadlbauer V, Krisper P, Beuers U, Haditsch B, Schneditz D, Jung A, et al. Removal of bile acids by two different extracorporeal liver support systems in acute-on-chronic liver failure. ASAIO Journal. 2007;53(2):187-93.
221. Stadlbauer V, Krisper P, Aigner R, Haditsch B, Jung A, Lackner C, et al. Effect of extracorporeal liver support by MARS and Prometheus on serum cytokines in acute-on-chronic liver failure. Critical Care. 2006;10.
222. Fuhrmann V. Extracorporeal therapies in liver failure. Therapeutic Apheresis and Dialysis. 2018;22(4):427.
223. Karvellas CJ, Stravitz RT. High volume plasma exchange in acute liver failure: Dampening the inflammatory cascade? J Hepatol. 2016;64(1):10-2.
224. Larsen FS, Schmidt LE, Bernsmeier C, Rasmussen A, Isoniemi H, Patel VC, et al. High-volume plasma exchange in patients with acute liver failure: An open randomised controlled trial. J Hepatol. 2016;64(1):69-78.
225. Clària J, Arroyo V, Moreau R. The Acute-on-Chronic Liver Failure Syndrome, or When the Innate Immune System Goes Astray. The Journal of Immunology. 2016;197(10):3755.
226. Chen P, Wang Y-Y, Chen C, Guan J, Zhu H-H, Chen Z. The immunological roles in acute-on-chronic liver failure: An update. Hepatobiliary & Pancreatic Diseases International. 2019;18(5):403-11.
227. Carpentier B, Gautier A, Legallais C. Artificial and bioartificial liver devices: present and future. Gut. 2009;58(12):1690.
228. He Y-T, Qi Y-N, Zhang B-Q, Li J-B, Bao J. Bioartificial liver support systems for acute liver failure: A systematic review and meta-analysis of the clinical and preclinical literature. World J Gastroenterol. 2019;25(27):3634-48.
229. Defterevos G, Nastos C, Papalois A, Kalimeris K, Margelos V, Fragulidis G, et al. Peritoneal Albumin Dialysis as a Novel Approach for Liver Support: Study in a Porcine Model of Acute Hepatic Failure. Artificial Organs. 2016;40(8):755-64.
230. Stange J, Klammt S, Mitzner S, Koball S, Hinz M, Gloger M, et al. Extracorporeal albumin dialysis using microparticle charcoal for albumin recycling is superior to using mars macroparticle adsorbents in removing albumin bound toxins (ABT). Hepatology. 2017;66:672A.
231. Acar U, Gokkaya Z, Akbulut A, Ferah O, Yenidunya O, Acik ME, et al. Impact of Cytokine Adsorption Treatment in Liver Failure. Transplantation proceedings. 2019;51(7):2420-4.

232. Dhokia VD, Madhavan D, Austin A, Morris CG. Novel use of Cytosorb haemadsorption to provide biochemical control in liver impairment. Journal of the Intensive Care Society. 2019;20(2):174-81.
233. Dominik A, Stange J. Similarities, Differences, and Potential Synergies in the Mechanism of Action of Albumin Dialysis Using the MARS Albumin Dialysis Device and the CytoSorb Hemoperfusion Device in the Treatment of Liver Failure. Blood purification. 2020:1-10.
234. Banares R, Ibanez-Samaniego L, Torner JM, Pavesi M, Olmedo C, Catalina MV, et al. Meta-analysis of individual patient data of albumin dialysis in acute-on-chronic liver failure: focus on treatment intensity. Therapeutic advances in gastroenterology. 2019;12:1756284819879565.
235. Premkumar M, Anand AC. Tobacco, Cigarettes, and the Liver: The Smoking Gun. J Clin Exp Hepatol. 2021;11(6):700-12.
236. Sun L, Yin H, Liu M, Xu G, Zhou X, Ge P, et al. Impaired albumin function: a novel potential indicator for liver function damage? Ann Med. 2019;51(7-8):333-44.
237. Jalan R, Schnurr K, Mookerjee RP, Sen S, Cheshire L, Hodges S, et al. Alterations in the functional capacity of albumin in patients with decompensated cirrhosis is associated with increased mortality. Hepatology. 2009;50(2):555-64.

scientific reports

Check for updates

OPEN

Hypoalbuminemia affects one third of acute pancreatitis patients and is independently associated with severity and mortality

Klementina Ocskay[1,2], Zsófia Vinkó[1], Dávid Németh[1], László Szabó[1,3], Judit Bajor[4], Szilárd Gódi[4], Patrícia Sarlós[4], László Czakó[5], Ferenc Izbéki[6], József Hamvas[7], Mária Papp[8], Márta Varga[9], Imola Török[10], Artautas Mickevicius[11,12], Ville Sallinen[13], Elena Ramirez Maldonado[14], Shamil Galeev[15], Alexandra Mikó[1], Bálint Erőss[1,2,16], Marcell Imrei[1,2], Péter Jenő Hegyi[1,2,16], Nándor Faluhelyi[17], Orsolya Farkas[17], Péter Kanizsai[18], Attila Miseta[19], Tamás Nagy[19], Roland Hágendorn[20], Zsolt Márton[20], Zsolt Szakács[1,20], Andrea Szentesi[1,2,3], Péter Hegyi[1,2,16,22] & Andrea Párniczky[1,2,21,22]✉

The incidence and medical costs of acute pancreatitis (AP) are on the rise, and severe cases still have a 30% mortality rate. We aimed to evaluate hypoalbuminemia as a risk factor and the prognostic value of human serum albumin in AP. Data from 2461 patients were extracted from the international, prospective, multicentre AP registry operated by the Hungarian Pancreatic Study Group. Data from patients with albumin measurement in the first 48 h (n = 1149) and anytime during hospitalization (n = 1272) were analysed. Multivariate binary logistic regression and Receiver Operator Characteristic curve analysis were used. The prevalence of hypoalbuminemia (< 35 g/L) was 19% on admission and 35.7% during hospitalization. Hypoalbuminemia dose-dependently increased the risk of severity, mortality, local complications and organ failure and is associated with longer hospital stay. The predictive value of hypoalbuminemia on admission was poor for severity and mortality. Severe hypoalbuminemia (< 25 g/L) represented an independent risk factor for severity (OR 48.761; CI 25.276–98.908) and mortality (OR 16.83; CI 8.32–35.13). Albumin loss during AP was strongly associated with severity (p < 0.001) and mortality (p = 0.002). Hypoalbuminemia represents an independent risk factor for severity and mortality in AP, and it shows a dose-dependent relationship with local complications, organ failure and length of stay.

[1]Institute for Translational Medicine, Szentágothai Research Centre, Medical School, University of Pécs, Pécs, Hungary. [2]Centre for Translational Medicine, Semmelweis University, Budapest, Hungary. [3]Department of Medicine, Centre for Translational Medicine, University of Szeged, Szeged, Hungary. [4]Division of Gastroenterology, First Department of Medicine, Medical School, University of Pécs, Pécs, Hungary. [5]Department of Medicine, University of Szeged, Szeged, Hungary. [6]Szent György University Teaching Hospital of Fejér County, Székesfehérvár, Hungary. [7]Péterfy Hospital, Budapest, Hungary. [8]Department of Internal Medicine, Division of Gastroenterology, University of Debrecen, Debrecen, Hungary. [9]Dr. Réthy Pál Hospital, Békéscsaba, Hungary. [10]County Emergency Clinical Hospital – Gastroenterology and University of Medicine, Pharmacy, Sciences and Technology, Targu Mures, Romania. [11]Vilnius University Hospital Santaros Clinics, Vilnius, Lithuania. [12]Clinics of Abdominal Surgery, Nephrourology and Gastroenterology, Faculty of Medicine, Vilnius University, Vilnius, Lithuania. [13]Department of Transplantation and Liver Surgery, Helsinki University Hospital and University of Helsinki, Helsinki, Finland. [14]General Surgery, Consorci Sanitari del Garraf, Sant Pere de Ribes, Barcelona, Spain. [15]North-Western State Medical University, Saint-Petersburg, Russia. [16]Division of Pancreatic Diseases, Heart and Vascular Center, Semmelweis University, Budapest, Hungary. [17]Department of Medical Imaging, Medical School, University of Pécs, Pécs, Hungary. [18]Department of Emergency Medicine, Medical School, University of Pécs, Pécs, Hungary. [19]Department of Laboratory Medicine, Medical School, University of Pécs, Pécs, Hungary. [20]First Department of Medicine, Medical School, University of Pécs, Pécs, Hungary. [21]Heim Pál National Paediatric Institute, Budapest, Hungary. [22]These authors jointly supervised this work: Péter Hegyi and Andrea Párniczky. ✉email: andrea.parniczky@gmail.com

Acute pancreatitis is a common gastroenterological disorder, with rising incidence and high medical costs. The commonly used revised Atlanta Classification distinguishes between mild, moderate and severe disease based on the development and duration of organ failure[1]. As the mortality rate can reach 30% in severe cases, identifying risk factors and potential therapeutic targets is of utmost importance.

Human serum albumin is the most abundant protein in human serum with a very diverse role. Although this hypothesis has been contradicted by recent data, declining albumin levels during inflammation have long prompted physicians to underestimate its contribution to maintaining homeostasis during inflammation. However, albumin plays a pivotal role in maintaining the plasma redox state[2], and its scavenging activity is likely to influence vascular resistance through the regulation of nitric oxide levels[3]. Furthermore, low albumin levels result in dilution and increased drug clearance, ultimately causing sub-optimal treatment[4].

Small retrospective cohort studies have shown that hypoalbuminemia is an independent risk factor for severe AP and in-hospital mortality in adults and children[5,6]. Serum albumin has been reported to be associated with persistent organ failure and prolonged hospital stay[7]. However, whether albumin is only a marker or there is a cause-effect relationship between hypoalbuminemia and disease severity and mortality should be further evaluated.

While comprehensive analyses are missing on AP patients with hypoalbuminemia and albumin loss in AP, we aimed to evaluate (1) on-admission and in-hospital hypoalbuminemia as a risk factor in AP, (2) the prognostic potential of human serum albumin, (3) whether there is a dose-dependent relationship between albumin level and disease outcomes and (4) the relation of albumin loss to severity and mortality.

We found evidence that AP patients with < 25 g/L serum albumin anytime during hospitalization have a 16.8-fold higher risk of death and 48.8-fold higher risk of severe AP than patients with normal albumin levels. We also observed that albumin loss during AP is associated with severity and mortality. These data highlight the unmet need for randomized controlled trials focusing on albumin replacement.

Results

One in every five patients suffering from acute pancreatitis has hypoalbuminemia on admission. Nineteen percent of patients (n = 218/1149) presented with hypoalbuminemia (< 35 g/L). 12.4% of patients were admitted with 30–34.99 g/L albumin levels (Group 5), whereas 4.4% and 2.2% of patients had 25–29.99 g/L (Group 6) and < 25 g/L (Group 7) on-admission albumin levels (Sup. Fig. S3).

Older age, lower body mass index, abdominal guarding on physical examination and non-biliary aetiology are associated with on-admission hypoalbuminemia. Hypoalbuminemia was associated with older age (average 59.7 ± 18.0 and 56.0 ± 16.1 years; p = 0.005, Sup. Fig. S3). Males were overrepresented in the analysed cohort (57%) and all subgroups (Sup. Fig. S3). Although biliary aetiology was the most frequent in all subgroups, significantly fewer patients had biliary aetiology (34.4% vs 42.2%; p = 0.042) in the low albumin group, and a tendency of more alcoholic episodes (24.3% and 19%; p = 0.096) was seen (Sup. Fig. S3).

Significantly lower body mass index (average 28.23 and 27.23; p = 0.012) was found in the low albumin group compared to the normal albumin group (Sup. Fig. S4). Diabetes mellitus (22.6% vs. 19.3%; p = 0.318) and chronic pancreatitis (7.3% vs. 6.1%, p = 0.507) were overrepresented in patients with hypoalbuminemia; however, fewer patients with hypoalbuminemia had recurrent AP (17.4% vs. 21.9%, p = 0.144) (Sup. Fig. S4).

As regards the signs and symptoms, fewer hypoalbuminemia patients presented with abdominal pain (94.9% and 99.2%; p < 0.001) and more with abdominal guarding (27.2% and 19.9%; p = 0.023) (Fig. S5). General signs, such as duration and intensity of abdominal pain, abdominal tenderness, nausea and vomiting, did not differ significantly. Hypoalbuminemia was associated with a dose-dependent increase in heart rate and a decrease in systolic and diastolic blood pressure on admission (Sup. Fig. S5).

The fulfilment of diagnostic criteria differed significantly (p < 0.001) among the low and normal albumin groups on admission. Low albumin patients were less likely to present with pancreatic enzyme elevation, abdominal pain and characteristic imaging findings at the same time (42.7% versus 58.4%) (Sup. Table S2).

On-admission hypoalbuminemia is dose-dependently associated with elevated CRP and PCT levels in AP. The low albumin group had significantly lower serum amylase (p < 0.001) and lipase (p = 0.002) levels on admission. An increase in dose-dependent C-reactive protein (CRP) (p < 0.001) and procalcitonin (PCT) (p < 0.001) was observed in the lower albumin groups. White blood cell count (WBC) (p = 0.017) levels were also significantly elevated in the low albumin group (Figs. S6–S7). As regards laboratory markers of renal function, hypoalbuminemia patients had significantly higher blood urea nitrogen (BUN) (p = 0.002) and creatinine (p = 0.002) levels and a lower estimated glomerular filtration rate (eGFR) (p < 0.001) (Sup. Figs. S8–S9). Liver enzymes and total bilirubin levels did not differ between the low and normal albumin groups, but hypoalbuminemia was associated with higher direct bilirubin levels (p = 0.005) and a higher international normalized ratio (INR) (p < 0.001) (Sup. Figs. S10–S13). Haematological parameters, lipids, ions and glucose levels are shown in Supplementary Figs. S14–S17.

On-admission hypoalbuminemia is dose-dependently associated with complications, severity and mortality in AP. Significantly more patients developed local complications and organ failure in the low albumin group (p = 0.016 and p < 0.001, respectively) (Figs. 1, 2). Lower albumin levels correlated with a higher rate of peripancreatic fluid collection and respiratory failure (p < 0.001 and p = 0.051). The rate of pancreatic necrosis, pseudocyst or heart failure did not differ significantly between the groups.

Most importantly, hypoalbuminemia was associated with increased mortality (p = 0.020), disease severity (p = 0.015) and hospital stay (p = 0.025) (Fig. 3). Groups 6 and 7 had significantly higher mortality (p = 0.005

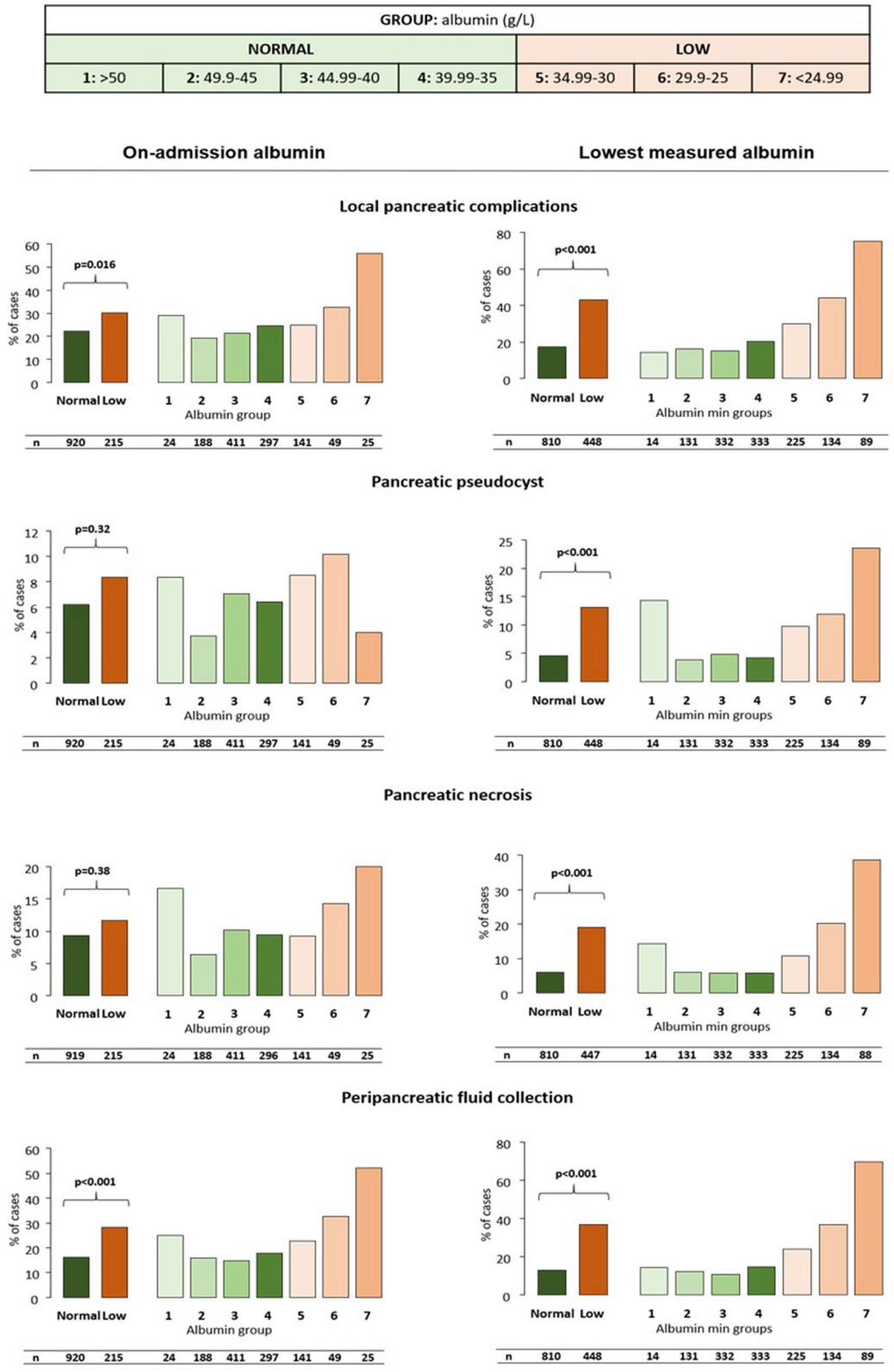


Figure 1. Relation between albumin level and local complications, as defined by the Revised Atlanta Criteria in acute pancreatitis. All types of local complications were significantly more frequent in the low albumin group. A dose-dependent increase was seen in the rate of local complications and peripancreatic fluid collection in both cohorts and in pancreatic necrosis and pseudocyst in the lowest measured albumin cohort. $P < 0.05$ is considered significant. Patients with albumin levels < 35 g/L were included in the low albumin group (Groups 5–7).

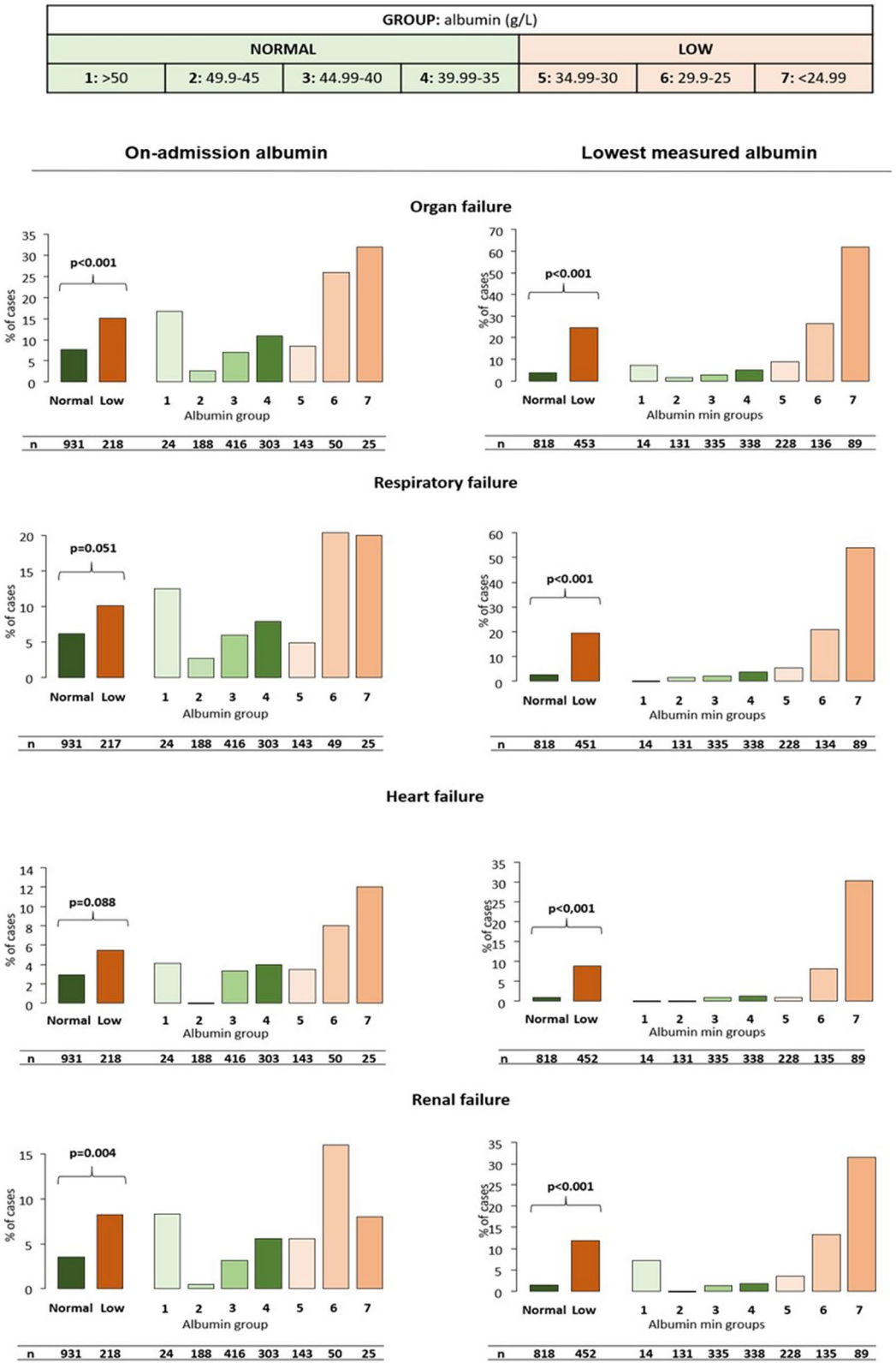
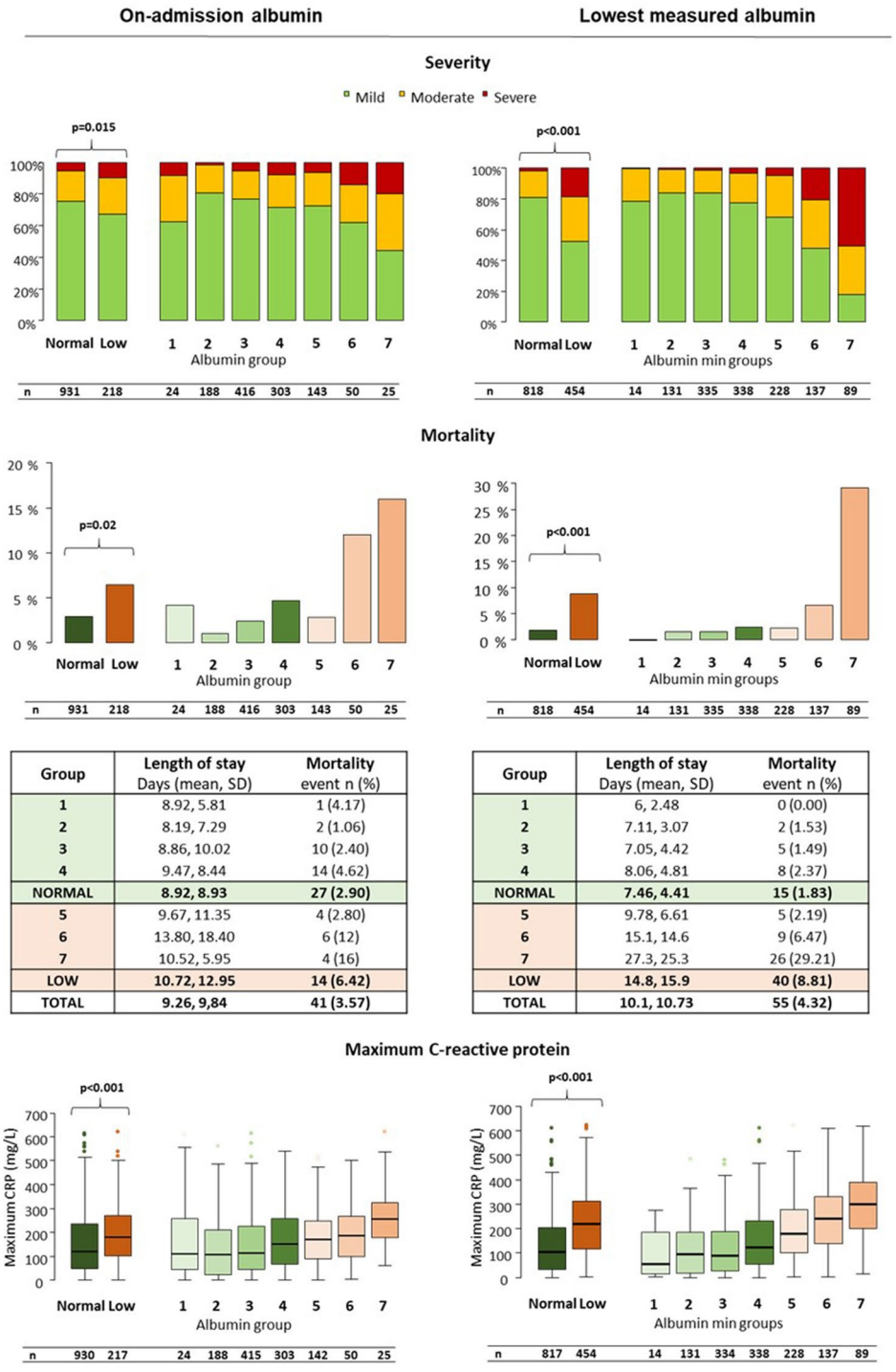


Figure 2. Relation between albumin level and organ failure, as defined by the Revised Atlanta Criteria in acute pancreatitis. Significantly more patients developed organ failure in the low albumin group in both cohorts. A dose-dependent increase was seen in the case of all analyses in the lowest measured albumin cohort. Heart failure was dose-dependently increased in the on-admission cohort as well. $P < 0.005$ is considered significant.

GROUP: albumin (g/L)						
NORMAL				LOW		
1: >50	2: 49.9-45	3: 44.99-40	4: 39.99-35	5: 34.99-30	6: 29.9-25	7: <24.99



Group	Length of stay Days (mean, SD)	Mortality event n (%)
1	8.92, 5.81	1 (4.17)
2	8.19, 7.29	2 (1.06)
3	8.86, 10.02	10 (2.40)
4	9.47, 8.44	14 (4.62)
NORMAL	**8.92, 8.93**	**27 (2.90)**
5	9.67, 11.35	4 (2.80)
6	13.80, 18.40	6 (12)
7	10.52, 5.95	4 (16)
LOW	**10.72, 12.95**	**14 (6.42)**
TOTAL	**9.26, 9,84**	**41 (3.57)**

Group	Length of stay Days (mean, SD)	Mortality event n (%)
1	6, 2.48	0 (0.00)
2	7.11, 3.07	2 (1.53)
3	7.05, 4.42	5 (1.49)
4	8.06, 4.81	8 (2.37)
NORMAL	**7.46, 4.41**	**15 (1.83)**
5	9.78, 6.61	5 (2.19)
6	15.1, 14.6	9 (6.47)
7	27.3, 25.3	26 (29.21)
LOW	**14.8, 15.9**	**40 (8.81)**
TOTAL	**10.1, 10.73**	**55 (4.32)**

Figure 3. Relation between albumin level and disease severity, mortality, length of stay and maximum C-reactive protein level in acute pancreatitis. Severity, mortality, length of stay and maximum C-reactive protein levels were significantly and dose-dependently associated with hypoalbuminemia in both cohorts. $P < 0.05$ is considered significant.

Predictor	β	SE	OR	95% CI	p
On-admission albumin (n = 1149)—mortality					
On-admission albumin level					
30–34.99 g/L (vs. ≥ 35 g/L)	− 0.108	0.553	0.898	0.259–2.390	0.845
25–29.99 g/L (vs. ≥ 35 g/L)	1.330	0.496	3.782	1.313–9.462	**0.007**
< 25 g/L (vs. ≥ 35 g/L)	1.659	0.611	5.256	1.389–16.112	**0.007**
Age					
Per years	0.037	0.012	1.037	0.014–1.063	**0.003**
Gender					
Female (vs. male)	− 0.222	0.370	0.801	0.383–1.648	0.548
Aetiology					
Alcohol (vs. biliary)	0.669	0.554	1.952	0.636–5.725	0.227
HTG (vs. biliary)	1.669	0.747	5.304	1.037–21.022	**0.025**
Biliary + alcohol (vs. biliary)	1.234	1.100	3.436	0.178–20.816	0.262
Biliary + HTG (vs. biliary)	− 12.903	783.282	–	–	0.987
Alcohol + HTG (vs. biliary)	1.781	0.768	5.938	1.123–24.693	**0.020**
Idiopathic (vs. biliary)	1.119	0.427	3.061	1.330–7.223	**0.009**
Other (vs. biliary)	0.010	0.790	1.010	0.152–3.964	0.990
On-admission albumin (n = 1149)—severity					
On-admission albumin					
30–34.99 g/L (v. ≥ 35 g/L)	0.029	0.383	1.030	0.457–2.086	0.939
25–29.99 g/L (v. ≥ 35 g/L)	0.829	0.449	2.292	0.882–5.238	0.065
< 25 g/L (v. ≥ 35 g/L)	1.286	0.548	3.620	1.118–9.968	**0.019**
Age					
Per years	0.040	0.010	1.041	1.022–1.061	**< 0.001**
Gender					
Female (vs. male)	− 0.183	0.281	0.830	0.478–1.442	0.515
Aetiology					
Alcohol (vs. biliary)	0.522	0.420	1.685	0.751–3.673	0.195
HTG (vs. biliary)	1.712	0.546	5.543	1.776–15.536	**0.002**
Biliary + alcohol (vs. biliary)	1.056	0.802	2.874	0.426–11.572	0.188
Biliary + HTG (vs. biliary)	− 13.792	785.525	–	–	0.986
Alcohol + HTG (vs. biliary)	1.316	0.632	3.727	0.952–11.941	**0.037**
Idiopathic (vs. biliary)	0.536	0.330	1.709	0.884–3.247	0.104
Other (vs. biliary)	− 0.475	0.629	0.622	0.145–1.852	0.450

Table 1. Multivariate logistic regression analysis on the prognostic role of on-admission hypoalbuminemia in acute pancreatitis. *HTG* hypertriglyceridemia, *β* β coefficient, *SE* standard error *OR* odds ratio, *CI* confidence interval.

and p = 0.007, respectively) and severity (p = 0.028 and p < 0.001, respectively) compared to the normal group. Maximum CRP levels during the course of AP significantly and dose-dependently increased with the degree of serum albumin (p < 0.001, Fig. 3).

On-admission hypoalbuminemia is an independent risk factor for severity and mortality, with an odds ratio of up to 5.3 for mortality in acute pancreatitis. Age, hypertriglyceridemia-induced (with or without concomitant alcoholic aetiology) and idiopathic AP were independently associated with mortality. Severe on-admission hypoalbuminemia proved to be an independent risk factor for mortality with an OR of 3.782 (CI 1.313–9.462) in Group 6 (< 30 g/L) and an OR of 5.256 (CI 1.389–16.112) in Group 7 (< 25 g/L) (Table 1). Albumin levels were examined with a 35 g/L cut-off in a separate analysis, which found an independent relation between hypoalbuminemia and mortality (OR 2.070; CI 1.021–4.033; Supplementary Table S3). Age, hypertriglyceridemia-induced AP, and, among the multifactorial aetiologies, a combination of hypertriglyceridemia and alcohol were independent risk factors for disease severity. On-admission albumin levels < 25 g/L were independently associated with severe AP (OR 3.620; CI 1.128–9.978; Table 1).

On-admission albumin levels alone have poor predictive value in AP. On-admission albumin levels have an AUC of 0.615 (sensitivity: 57.6%; specificity: 61.1%) for severity with a cut-off at 39.3 g/L (Fig. 4). The AUC for mortality was 0.660 (sensitivity: 72.1%; specificity: 53.7%) with a cut-off at 37.0 g/L.

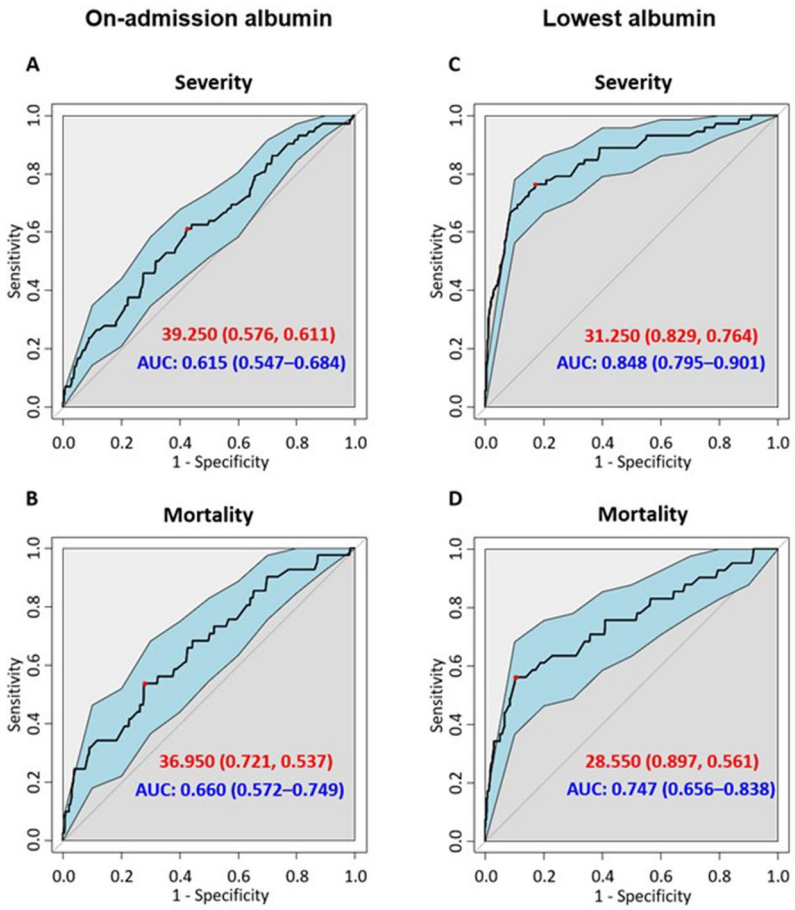


Figure 4. Receiver operating curves for mortality and severity. *AUC* area under the curve; best cut-offs are shown in red.

These data suggest that albumin plays a crucial role in the pathophysiology and clinical outcome of AP; however, it cannot be used as a single biomarker for predicting severity and mortality. Next, we wanted to understand whether albumin loss during the course of AP is related in any way to outcome of the disease; therefore, we regrouped our patients based on the lowest measured albumin levels.

One out of three patients suffer from hypoalbuminemia in AP during hospitalization, which dose-dependently correlates with disease severity and mortality in AP. The proportion of patients with hypoalbuminemia anytime during hospitalization was 35.7% (454 patients). A significant, dose-dependent increase was seen in the low albumin groups (Group 5–7) compared to the normal albumin group as regards the rate of all examined systemic and local complications (Figs. 1, 2). The lowest measured albumin levels throughout hospitalization ($n = 1272$) were significantly and dose-dependently associated with severity ($p < 0.001$), mortality ($p < 0.001$), length of stay ($p < 0.001$) and maximum CRP values ($p < 0.001$) (Fig. 3).

Moderate and severe AP and mortality are associated with significantly lower albumin levels and greater albumin loss. Albumin loss was analysed using data from patients with at least two albumin measurements ($n = 335$; Sup. Fig. S18). Compared to mild cases, patients with moderate and severe AP showed a greater decrease in albumin levels (medians 5.4 vs. 9 and 15.25 g/L; $p < 0.001$ for both comparisons). The comparison of delta albumin between the moderate and severe groups also yielded significant results ($p = 0.003$). Patients who died also lost significantly more albumin during hospitalization (medians 6.7 vs. 15.75 g/L; $p = 0.002$). The median time to the lowest albumin levels from admission was 4 days (IQR: 3–7 days).

AP patients with less than 25 g/L serum albumin have a 16.8-fold higher risk of death and a 48.8-fold higher risk of severe AP compared to patients with normal albumin levels. Age is an independent risk factor for severe AP and mortality, whereas hypertriglyceridemia-induced and idiopathic AP and a combination of alcoholic and biliary causes are independently associated with mortality (Table 2 and Supplementary Table S4). Hypoalbuminemia below 25–29.99 g/L (OR 2.912; CI 1.176–6.893) and below 25 g/L (OR 16.828; CI 8.323–35.129) were associated with an increased risk of mortality (Table 2). In a separate analysis, hypoalbuminemia (< 35 g/L) was also an independent risk factor for mortality (OR 4.185; CI 2.286–8.039)

Predictor	β	SE	OR	95% CI	p
Lowest measured albumin (n = 1272)—mortality					
On-admission albumin level					
30–34.99 g/L (vs. ≥ 35 g/L)	− 0.016	0.531	0.984	0.313–2.621	0.976
25–29.99 g/L (vs. ≥ 35 g/L)	1.069	0.448	2.912	1.166–6.893	**0.017**
< 25 g/L (vs. ≥ 35 g/L)	2.823	0.365	16.828	8.323–35.129	< **0.001**
Age					
Per years	0.043	0.012	1.044	1.021–1.070	< **0.001**
Gender					
Female (vs. male)	− 0.352	0.347	0.703	0.352–1.380	0.309
Aetiology					
Alcohol (vs. biliary)	0.909	0.523	2.481	0.880–6.960	0.083
HTG (vs. biliary)	1.569	0.766	4.803	0.914–19.900	**0.041**
Biliary + alcohol (vs. biliary)	1.651	0.793	5.215	0.949–22.798	**0.037**
Biliary + HTG (vs. biliary)	− 12.335	786.272	–	–	0.987
Alcohol + HTG (vs. biliary)	1.356	0.793	3.880	0.709–17.009	0.087
Idiopathic (vs. biliary)	1.402	0.402	4.063	1.878–9.181	< **0.001**
Other (vs. biliary)	0.213	0.807	1.237	0.182–5.045	0.792
Lowest measured albumin (n = 1272)—severity					
On-admission albumin					
30–34.99 g/L (v. ≥ 35 g/L)	0.858	0.410	2.359	1.030–5.240	**0.036**
25–29.99 g/L (v. ≥ 35 g/L)	2.460	0.345	11.709	6.038–23.515	< **0.001**
< 25 g/L (v. ≥ 35 g/L)	3.887	0.346	48.761	25.276–98.908	< **0.001**
Age					
Per years	0.032	0.009	1.032	1.015–1.051	< **0.001**
Gender					
Female (vs. male)	− 0.332	0.274	0.718	0.417–1.225	0.226
Aetiology					
Alcohol (vs. biliary)	0.093	0.403	1.097	0.492–2.403	0.818
HTG (vs. biliary)	1.060	0.565	2.885	0.910–8.476	0.061
Biliary + alcohol (vs. biliary)	0.172	0.778	1.188	0.222–5.006	0.825
Biliary + HTG (vs. biliary)	− 13.429	753.256	–	–	0.986
Alcohol + HTG (vs. biliary)	0.497	0.657	1.643	0.422–5.688	0.450
Idiopathic (vs. biliary)	0.541	0.320	1.718	0.915–3.218	0.091
Other (vs. biliary)	0.008	0.547	1.008	0.310–2.744	0.988

Table 2. Logistic regression for severity and mortality using the lowest measured albumin cohort. *HTG* hypertriglyceridemia, *β* β coefficient, *SE* standard error, *OR* odds ratio, *CI* confidence interval.

(Table S4). Furthermore, hypoalbuminemia anytime during hospitalization was associated with a higher risk for severe AP (OR 10.664; CI 6.188–19.614), and a gradual increase of odds ratios can be observed in the low albumin groups (OR 2.359; CI 1.030–5.240 for Group 5; OR 11.709; CI 6.038–23.515 for Group 6; and OR 48.761; CI 25.276–98.908 for Group 7).

The lowest albumin values have good and fair predictive value for severity and mortality in acute pancreatitis. The lowest measured albumin levels have higher AUC values: 0.848 for severity and 0.747 for mortality (Fig. 3). The best cut-off values were 31.3 g/L for severity (sensitivity: 82.9%; specificity: 76.4%) and 28.6 g/L for mortality (sensitivity: 89.9%; specificity: 56.1%). The day of the lowest albumin measurement ranged from 1 to 56 days, with a median of 2 days. Most patients only had a single measurement around the time of admission.

Discussion

To date, this is the most comprehensive evaluation of AP patients with hypoalbuminemia, using the largest, prospectively collected, high-quality dataset[8,9].

We found that almost one-fifth of patients had hypoalbuminemia on admission (19%), and a further 25% developed hypoalbuminemia during hospitalization, meaning that every third patient was affected.

In our analysis, hypoalbuminemia under 25 g/L anytime during hospitalization was independently associated with a more than 47-fold higher chance for severe AP and a more than 16-fold higher chance for mortality.

Our findings are consistent with results for hypoalbuminemia in other diseases. Hypoalbuminemia was a prominent risk factor in community-acquired bloodstream infection with severe sepsis and septic shock[10]. A

retrospective analysis of data from more than 20,000 emergency medical patients in Ireland found that hypoalbuminemia is independently associated with 30-day in-hospital mortality, with a non-linear relationship between mortality and on-admission albumin levels[11]. Moreover, in a secondary analysis of a prospective cohort, AP patients with multiorgan failure (MOF; n = 18) demonstrated a sharper decline in serum albumin ($P < 0.001$) compared to non-MOF patients (n = 39)[12].

We have not only proved that hypoalbuminemia is a risk factor, but have also shown the dose-dependent relation between low albumin levels and severity, mortality, number of patients with any local complications, number of patients developing organ failure and maximum CRP levels in both analyses (on-admission and lowest measured albumin levels).

These relations can be explained by the numerous physiological functions of human serum albumin. Albumin was long considered a negative acute-phase protein, with decreasing production giving way to inflammatory cytokines in inflammation[13]. Serum albumin levels undoubtedly decrease in inflammatory states, which may be due to a shorter half-life and a larger interstitial pool, which causes the dilution of albumin[14–16]. Capillary leak resulting from inflammatory processes plays a role in the decline of serum albumin, but it is argued that the escape of albumin to the tissues may be beneficial because of its antioxidant and scavenging activity[17]. Although a more than twofold higher production rate was observed in critically ill ICU patients, this increased production is still not able to balance the higher demand. This can be considered as a relative synthetic insufficiency of hepatic function[18].

Albumin loss was significantly associated with severity and mortality in our analysis. However, only 51.7% of patients in the HPSG database had albumin measurements at least once during their hospitalization, and 13.6% had them at least twice during that time. This highlights how neglected albumin measurements are in AP.

On admission albumin levels were found to have poor predictive values for mortality and severity. Previous studies were mainly retrospective and had a much smaller sample size[5,19,20]. They only assessed the predictive value of serum albumin for persistent organ failure and peripancreatic infection, or were limited to severe AP.

From the clinician's point of view, the decline of serum albumin levels—regardless of on-admission albumin levels—signals clinical worsening and may aid in identifying high-risk AP patients. However, clinicians mostly miss the opportunity to pre-emptively and frequently measure serum albumin, thus delaying timely intervention.

To date, no clinical trial examined therapeutic albumin administration in AP. As we know, albumin is similarly associated with outcomes in sepsis and septic shock; randomized controlled trials in this field could be a start[17,21]. The controversial results of studies and meta-analyses in this field may be explained by heterogeneous patient populations and the time sensitivity of this treatment[22].

To further exploit the potential in therapeutic albumin administration in AP, more detailed clinical studies are needed to identify the patient subpopulations benefiting the most from this therapeutic option.

Strengths and limitations. We conducted the most extensive, most comprehensive cohort study on the role of hypoalbuminemia in acute pancreatitis to date. We analysed high-quality data from a prospective, international, multicentric registry. We identified hypoalbuminemia as an independent risk factor in AP, present in at least every third patient. We also found a dose-dependent relationship between albumin levels and main outcomes, which was previously not described.

Among the limitations, we must mention the arbitrary classification of albumin levels (except for the low-normal cut-off), the missing data on albumin levels and albumin administration during hospital stay, and the limited number of albumin measurements during the hospital stay, which could introduce bias. The limited number of albumin measurements did not enable more detailed analyses of serum albumin at different time points. Our analysed cohorts differed from the total cohort in some aspects, thus potentially signalling performance bias, as albumin measurements are more frequently ordered for patients with expected hypoalbuminemia.

Conclusion

Hypoalbuminemia is remarkably common in AP (seen in 19% of patients on admission and 35.7% during hospitalization) and represents an independent risk factor for severity and mortality. Importantly, albumin loss during hospitalization was also associated with severity and mortality, suggesting that routine monitoring of serum albumin is recommended and that albumin administration should be examined as a therapeutic intervention in AP.

Implications for research. Clinical trials are needed to assess the potential benefit of albumin replacement in AP.

Implications for practice. (1) Albumin levels should be measured for all AP patients, (2) albumin levels should be controlled at least in those patients whose condition is worsening during AP, and (3) albumin administration should at least be considered in patients with severe hypoalbuminemia (< 25 g/L).

Methods

Study design and definitions. This analysis of an international, prospective, multicentre cohort was conducted using data from the Acute Pancreatitis Registry operated by the Hungarian Pancreatic Study Group (HPSG)[23]. Patient data were collected from establishment of the registry to 31 December 2019 on electronic case report forms and validated using a four-tiered data validation protocol. Contributing centres are shown in the supplementary material (Table S1 and Fig. S1). The registry was approved by the Scientific and Research Ethics Committee of the Medical Research Council of Hungary (222254-1/2012/EKU) in 2012. It conforms to the Declaration of Helsinki, as revised in 2013. All participants provided written informed consent. Data collection

and validation are detailed by Párniczky et al.[24]. The Hungarian Pancreatic Study Group published analyses from the registry, the population of which may overlap with our analysed cohort[24–34].

Diagnosis of AP was established using the IAP/APA guidelines[35], while severity and complications were defined using the Revised Atlanta Classification[1].

Participants. Analyses were performed on patients' data with albumin measurement anytime during hospitalization (lowest measured albumin cohort, n = 1272) and in the first 48 h of hospitalization (on-admission albumin cohort, n = 1149) to answer a post-hoc clinical research question. The cut-off value between the low and normal albumin group was 35 g/L in both cases, based on the commonly used lower normal value. Subjects were further divided into seven subgroups (Groups 1 to 7) using the lowest (n = 1272) or first measured (n = 1149) albumin values.

The analyses of albumin change involved selected patients (n = 335) with at least two albumin measurements. Delta albumin was calculated as the difference between the first and lowest measured albumin levels.

Statistical analysis. Descriptive statistics are presented as the median with 25% and 75% percentiles (IQR) or mean with standard deviation (SD) for continuous variables and as numbers and proportions for categorical variables.

The Chi-squared test or Fisher's exact test was used to assess the relationship between categorical variables. The Mann–Whitney U test or Kruskal–Wallis test followed by Dunnett's post hoc test was used to evaluate differences between groups in the case of continuous variables.

Multivariate binary logistic regression analysis was performed to identify the risk factors independently associated with severe disease and mortality. Odds ratios (OR) with 95% confidence intervals (CI) were calculated.

The Receiver Operator Characteristic (ROC) curve and Area Under the Curve (AUC) with 95% CI were used to identify the ability of albumin levels to predict the mortality or severity of AP (The various AUC values were classified as follows: between 0.5 and 0.6—fail; between 0.6 and 0.7—poor; between 0.7 and 0.8—fair; between 0.8 and 0.9—good; and over 0.9—excellent.) Best cut-offs were calculated using the Youden index[36].

$P < 0.05$ was considered statistically significant, except for the Kruskal–Wallis test followed by Dunnett's post hoc test, where $p < 0.025$ was considered statistically significant.

All analyses were carried out in R statistical software, version 4.0.2 (R Core Team, 2020, Vienna, Austria), packages: pROC (v. 1.17.0.1) and PMCMRplus (v. 1.9.0.)[37,38].

Representativity. The main characteristics of the analysed cohorts are consistent with the literature data. However, they differed significantly from the entire cohort (n = 2461) in terms of severity, length of stay and mortality (Fig. S2).

Reporting. We report our results following The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement, using the checklist provided[39].

Data availability

The full dataset is available upon reasonable request.

Received: 11 June 2021; Accepted: 23 November 2021
Published online: 17 December 2021

References

1. Sarr, M. G. 2012 revision of the Atlanta classification of acute pancreatitis. *Pol. Arch. Med. Wewn.* **123**, 118–124. https://doi.org/10.20452/pamw.1627 (2013).
2. Oettl, K. & Stauber, R. E. Physiological and pathological changes in the redox state of human serum albumin critically influence its binding properties. *Br. J. Pharmacol.* **151**, 580–590. https://doi.org/10.1038/sj.bjp.0707251 (2007).
3. Stamler, J. S. *et al.* Nitric oxide circulates in mammalian plasma primarily as an S-nitroso adduct of serum albumin. *Proc. Natl. Acad. Sci. U.S.A.* **89**, 7674–7677. https://doi.org/10.1073/pnas.89.16.7674 (1992).
4. Ulldemolins, M., Roberts, J. A., Rello, J., Paterson, D. L. & Lipman, J. The effects of hypoalbuminaemia on optimizing antibacterial dosing in critically ill patients. *Clin. Pharmacokinet.* **50**, 99–110. https://doi.org/10.2165/11539220-000000000-00000 (2011).
5. Zhang, W. *et al.* Evaluation of early prognostic factors of mortality in patients with acute pancreatitis: A retrospective study. *Gastroenterol. Res. Pract.* **2017**, 8363561. https://doi.org/10.1155/2017/8363561 (2017).
6. Wang, X. *et al.* Nosocomial mortality and early prediction of patients with severe acute pancreatitis. *J. Gastroenterol. Hepatol.* **25**, 1386–1393. https://doi.org/10.1111/j.1440-1746.2010.06376.x (2010).
7. Hong, W. *et al.* Serum albumin is independently associated with persistent organ failure in acute pancreatitis. *Can. J. Gastroenterol. Hepatol.* **2017**, 5297143. https://doi.org/10.1155/2017/5297143 (2017).
8. Hegyi, P. *et al.* Accelerating the translational medicine cycle: The Academia Europaea pilot. *Nat. Med.* **27**(8), 1317–1319. https://doi.org/10.1038/s41591-021-01458-8 (2021).
9. Hegyi, P. *et al.* Academia Europaea position paper on translational medicine: The cycle model for translating scientific results into community benefits. *J. Clin. Med.* **9**(5), 1532. https://doi.org/10.3390/jcm9051532 (2020).
10. Artero, A. *et al.* Prognostic factors of mortality in patients with community-acquired bloodstream infection with severe sepsis and septic shock. *J. Crit. Care* **25**, 276–281. https://doi.org/10.1016/j.jcrc.2009.12.004 (2010).
11. Lyons, O., Whelan, B., Bennett, K., O'Riordan, D. & Silke, B. Serum albumin as an outcome predictor in hospital emergency medical admissions. *Eur. J. Intern. Med.* **21**, 17–20. https://doi.org/10.1016/j.ejim.2009.10.010 (2010).
12. Komara, N. L. *et al.* Severe acute pancreatitis: Capillary permeability model linking systemic inflammation to multiorgan failure. *Am. J. Physiol.* **319**, G573–G583. https://doi.org/10.1152/ajpgi.00285.2020 (2020).
13. Charlie-Silva, I. *et al.* Acute-phase proteins during inflammatory reaction by bacterial infection: Fish-model. *Sci. Rep.* **9**, 4776. https://doi.org/10.1038/s41598-019-41312-z (2019).

14. Barle, H. *et al.* Growth hormone does not affect albumin synthesis in the critically ill. *Intensive Care Med.* **27**, 836–843. https://doi.org/10.1007/s001340100914 (2001).
15. Barle, H. *et al.* Synthesis rates of total liver protein and albumin are both increased in patients with an acute inflammatory response. *Clin. Sci.* **110**, 93–99. https://doi.org/10.1042/CS20050222 (2005).
16. Dahn, M. S., Mitchell, R. A., Lange, M. P., Smith, S. & Jacobs, L. A. Hepatic metabolic response to injury and sepsis. *Surgery* **117**, 520–530. https://doi.org/10.1016/s0039-6060(05)80251-x (1995).
17. Soeters, P. B., Wolfe, R. R. & Shenkin, A. Hypoalbuminemia: Pathogenesis and clinical significance. *J. Parenteral Enteral Nutr.* **43**, 181–193. https://doi.org/10.1002/jpen.1451 (2019).
18. Levitt, D. G. & Levitt, M. D. Human serum albumin homeostasis: A new look at the roles of synthesis, catabolism, renal and gastrointestinal excretion, and the clinical value of serum albumin measurements. *Int. J. Gen. Med.* **9**, 229–255. https://doi.org/10.2147/ijgm.S102819 (2016).
19. Li, S., Zhang, Y., Li, M., Xie, C. & Wu, H. Serum albumin, a good indicator of persistent organ failure in acute pancreatitis. *BMC Gastroenterol.* **17**, 59. https://doi.org/10.1186/s12876-017-0615-8 (2017).
20. Brand, M. *et al.* Acute necrotizing pancreatitis: Laboratory, clinical, and imaging findings as predictors of patient outcome. *AJR Am. J. Roentgenol.* **202**, 1215–1231. https://doi.org/10.2214/ajr.13.10936 (2014).
21. Vincent, J.-L. *et al.* Albumin administration in the acutely ill: What is new and where next?. *Crit. Care* **18**, 231. https://doi.org/10.1186/cc13991 (2014).
22. Flannery, A. H., Owen, G. D., Coz, A., Thompson Bastin, M. L. & Patel, K. Impact of hyperoncotic albumin on duration of vasopressor support in septic shock: A propensityscore-matched analysis. *Ann. Pharmacother.* https://doi.org/10.1177/1060028020963645 (2020).
23. *Acute Pancreatitis Registry*.
24. Párniczky, A. *et al.* Antibiotic therapy in acute pancreatitis: From global overuse to evidence based recommendations. *Pancreatology* **19**, 488–499. https://doi.org/10.1016/j.pan.2019.04.003 (2019).
25. Párniczky, A. *et al.* Prospective, multicentre, nationwide clinical data from 600 cases of acute pancreatitis. *PLoS ONE* **11**, e0165309. https://doi.org/10.1371/journal.pone.0165309 (2016).
26. Mosztbacher, D. *et al.* Hypertriglyceridemia-induced acute pancreatitis: A prospective, multicenter, international cohort analysis of 716 acute pancreatitis cases. *Pancreatology* **20**, 608–616. https://doi.org/10.1016/j.pan.2020.03.018 (2020).
27. Farkas, N. *et al.* A multicenter, international cohort analysis of 1435 cases to support clinical trial design in acute pancreatitis. *Front. Physiol.* **10**, 1092. https://doi.org/10.3389/fphys.2019.01092 (2019).
28. Hágendorn, R. *et al.* Development of disturbance of consciousness is associated with increased severity in acute pancreatitis. *Pancreatology* **20**, 806–812. https://doi.org/10.1016/j.pan.2020.05.009 (2020).
29. Demcsák, A. *et al.* Acid suppression therapy, gastrointestinal bleeding and infection in acute pancreatitis: An international cohort study. *Pancreatology* **20**, 1323–1331. https://doi.org/10.1016/j.pan.2020.08.009 (2020).
30. Gódi, S. *et al.* Centralized care for acute pancreatitis significantly improves outcomes. *J. Gastrointest. Liver Dis.* **27**, 151–157. https://doi.org/10.15403/jgld.2014.1121.272.pan (2018).
31. Szakács, Z. *et al.* Aging and comorbidities in acute pancreatitis II: A cohort-analysis of 1203 prospectively collected cases. *Front. Physiol.* **9**, 1776. https://doi.org/10.3389/fphys.2018.01776 (2018).
32. Halász, A. *et al.* Outcomes and timing of endoscopic retrograde cholangiopancreatography for acute biliary pancreatitis. *Dig. Liver Dis.* **51**, 1281–1286. https://doi.org/10.1016/j.dld.2019.03.018 (2019).
33. Szentesi, A. *et al.* Multiple hits in acute pancreatitis: Components of metabolic syndrome synergize each other's deteriorating effects. *Front. Physiol.* **10**, 1202. https://doi.org/10.3389/fphys.2019.01202 (2019).
34. Hegyi, P. J. *et al.* Evidence for diagnosis of early chronic pancreatitis after three episodes of acute pancreatitis: A cross-sectional multicentre international study with experimental animal model. *Sci. Rep.* **11**, 1367. https://doi.org/10.1038/s41598-020-80532-6 (2021).
35. Besselink, M. *et al.* IAP/APA evidence-based guidelines for the management of acute pancreatitis. *Pancreatology* **13**, e1–e15. https://doi.org/10.1016/j.pan.2013.07.063 (2013).
36. Fluss, R., Faraggi, D. & Reiser, B. Estimation of the Youden Index and its associated cutoff point. *Biom J* **47**, 458–472. https://doi.org/10.1002/bimj.200410135 (2005).
37. Robin, X. *et al.* pROC: An open-source package for R and S+ to analyze and compare ROC curves. *BMC Bioinform.* **12**, 77. https://doi.org/10.1186/1471-2105-12-77 (2011).
38. *PMCMRplus: Calculate Pairwise Multiple Comparisons of Mean Rank Sums Extended*.
39. von Elm, E. *et al.* The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: Guidelines for reporting observational studies. *Lancet (Lond., Engl.)* **370**, 1453–1457. https://doi.org/10.1016/s0140-6736(07)61602-x (2007).

Acknowledgements

We would like to thank all the patients and personnel who contributed to the registry and this analysis. The authors are also very thankful to those Hungarian Pancreatic Study Group Members who uploaded clinical data but did not achieve the contribution necessary to be listed among the authors: Áron Vincze, József Czimmer, Imre Szabó, Anita Illés, Patrícia Sarlós, Hágendorn Roland, Gabriella Pár, Balázs Kui, Balázs Csaba Németh, Zoltán Szepes, Tamás Takács, Judit Gervain, Adrienn Halász, László Gajdán, Roland Fejes, Veronika Dunás-Varga, Zsuzsanna Vitális, György Kovács, Eszter Fehér, Ildikó Földi, Klára Csefkó, Hunor Farkas, Melania Macarie. János Novák, Stefan Crai, Barnabás Bod, Attila Szepes, Zsolt Dubravcsik, István Hritz, Goran Poropat, Davor Stimac, Csaba Góg, Árpád Patai, Miklós Ihász, Imanta Ozola-Zalite, Aldis Pukitis, Kristina Zadorozhna, Zsolt Szentkereszty, András Gelley, Masayasu Horibe and Georgiana Robu.

Author contributions

K.O., P.H. and A.P. drafted the concept and interpreted the data. K.O. wrote the majority of the manuscript. D.N. and L.S. performed the statistical analyses. V.Z. prepared the figures. J.B., S.G., P.S., L.C., F.I., J.H., M.P., M.V., I.T., A.M., V.S., E.R.M., S.G., P.K., R.H., B.E. and Z.M. all provided a substantial number of enrolled participants. A.M., Z.S., M.I. and P.J.H. dealt with patient enrolment and supervised data quality. N.F. and O.F. acted as radiological supervisors, ensuring data quality. A.M. and T.N. provided insight into laboratory markers and contributed to patient enrolment. A.S., P.H. and A.P. provided methodological and medical guidance and supervised the writing of the article. All the co-authors have read and approved the final version of the manuscript. A.P. and P.H. contributed equally as last authors.

Funding
Our study was supported by the "EFOP-3.6.2-16-2017-00006—LIVE LONGER" Human Resources Development Operational Programme Grant (PH) and the "GINOP-2.3.2-15-2016-00015—I-KOM" Economic Development and Innovation Operative Programme Grant (PH) co-financed by the European Union (European Regional Development Fund) within the framework of the Széchenyi 2020 Programme. Further grants: a János Bolyai Research Scholarship from the Hungarian Academy of Sciences and the ÚNKP-20-5 New National Excellence Programme, Ministry for Innovation and Technology, from the National Research, Development and Innovation Fund (AP and AM), and National Research, Development and Innovation Office Project Grants (K116634 (PH); FK131864 (AM); FK138929 (AP)). The funders have played no role in planning the concept, data analysis, interpretation and writing of the manuscript or choosing to publish.

Competing interests
The authors declare no competing interests.

Additional information
Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-021-03449-8.

Correspondence and requests for materials should be addressed to A.P.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

BMJ Open

Recurrent acute pancreatitis prevention by the elimination of alcohol and cigarette smoking (REAPPEAR): protocol of a randomised controlled trial and a cohort study

Klementina Ocskay ,[1,2] Márk Félix Juhász,[3] Nelli Farkas,[3,4] Noémi Zádori,[3] Lajos Szakó,[3] Zsolt Szakács,[1] Andrea Szentesi,[1] Bálint Erőss,[1,2] Emőke Miklós,[3] Antal Zemplényi,[5,6] Béla Birkás,[7] Árpád Csathó,[7] István Hartung,[7] Tamás Nagy,[8] László Czopf,[9] Ferenc Izbéki,[10] László Gajdán,[10] Mária Papp,[11] László Czakó ,[12] Dóra Illés ,[12] Marco V Marino,[13] Antonello Mirabella,[13] Ewa Małecka-Panas,[14] Hubert Zatorski,[14] Yaroslav Susak,[15] Kristina Opalchuk,[15] Gabriele Capurso,[16] Laura Apadula,[16] Cristian Gheorghe,[17,18] Ionut Adrian Saizu,[17,18] Ole H Petersen,[19] Enrique de-Madaria,[20] Jonas Rosendahl,[21] Andrea Párniczky,[3,22] Péter Hegyi ,[1,2] On behalf of the Hungarian Pancreatic Study Group

To cite: Ocskay K, Juhász MF, Farkas N, *et al*. **Re**current acute pancreatitis prevention by the elimination of alcohol and cigarette smoking (REAPPEAR): protocol of a randomised controlled trial and a cohort study. *BMJ Open* 2022;**12**:e050821. doi:10.1136/bmjopen-2021-050821

► Prepublication history and additional supplemental material for this paper are available online. To view these files, please visit the journal online (http://dx.doi.org/10.1136/bmjopen-2021-050821).

AP and PH contributed equally.

Received 01 March 2021
Accepted 12 November 2021

For numbered affiliations see end of article.

Correspondence to
Professor Péter Hegyi;
hegyi2009@gmail.com

ABSTRACT

Background/objectives Acute recurrent pancreatitis (ARP) due to alcohol and/or tobacco abuse is a preventable disease which lowers quality of life and can lead to chronic pancreatitis. The REAPPEAR study aims to investigate whether a combined patient education and cessation programme for smoking and alcohol prevents ARP.

Methods and analysis The REAPPEAR study consists of an international multicentre randomised controlled trial (REAPPEAR-T) testing the efficacy of a cessation programme on alcohol and smoking and a prospective cohort study (REAPPEAR-C) assessing the effects of change in alcohol consumption and smoking (irrespective of intervention). Daily smoker patients hospitalised with alcohol-induced acute pancreatitis (AP) will be enrolled. All patients will receive a standard intervention priorly to encourage alcohol and smoking cessation. Participants will be subjected to laboratory testing, measurement of blood pressure and body mass index and will provide blood, hair and urine samples for later biomarker analysis. Addiction, motivation to change, socioeconomic status and quality of life will be evaluated with questionnaires. In the trial, patients will be randomised either to the cessation programme with 3-monthly visits or to the control group with annual visits. Participants of the cessation programme will receive a brief intervention at every visit with direct feedback on their alcohol consumption based on laboratory results. The primary endpoint will be the composite of 2-year all-cause recurrence rate of AP and/or 2-year all-cause mortality. The cost-effectiveness of the cessation programme will be evaluated. An estimated 182 participants will be enrolled per group to the REAPPEAR-T with further enrolment to the cohort.

Ethics and dissemination The study was approved by the Scientific and Research Ethics Committee of the Hungarian Medical Research Council (40394-10/2020/EÜIG), all local ethical approvals are in place. Results will be disseminated at conferences and in peer-reviewed journals.

Trial registration number NCT04647097

Strengths and limitations of this study

- This is the first study assessing a combined brief intervention programme for recurrence prevention in acute pancreatitis.
- The study could provide a cost-effective and easy-to-use preventive method, reducing the recurrence rate of alcoholic acute pancreatitis.
- The lack of a conventional control group could result in underestimating the efficacy of the cessation programme.
- The results will be specific to the enrolled patient population, which does not cover all patients with recurrent acute pancreatitis.

INTRODUCTION

Acute pancreatitis (AP) is an often-unheeded issue by clinicians and healthcare professionals, with significant medical charges.[1 2] The incidence rate of the first attack of AP ranges from 15 to 45 per 100 000 per year.[3] Alcohol and biliary obstruction are the two main causes of AP in adulthood, alcohol being the diagnosed inducing factor in 25%–35% of the cases.[4]

Cohort studies have found that 10%–30% of patients have recurrent attacks based on medical history, and a recent meta-analysis has shown that 10% of the patients after a single episode of AP and 26% of those with acute recurrent pancreatitis (ARP) later progress to chronic pancreatitis (CP).[5] It Is known, that ARP (more than one episodes of AP) significantly lowers physical and mental quality of life (QoL)[6] and alcoholic aetiology has been identified in 19% of ARP patients.[7] Despite the importance and potentially preventable nature of alcoholic ARP, preventive efforts are still scarce.[8 9]

A pivotal study from Nikkola *et al* found that abstinent patients experienced no ARPs during a 9-year follow-up period. On the other hand, 34% of patients who did not stop drinking developed a recurrent attack.[10] The median time between the index AP and the first alcoholic ARP ranges from 8.5 months to 2.2 years, but around 80% of the registered first recurrent attacks occur in the first 4 years of follow-up.[11 12] With 6-monthly interventions, Nordback *et al* achieved a significant reduction in the recurrence rate of AP in Finland.[13 14]

Smoking is a long-established independent risk factor of AP and CP. A dose–response association was found between smoking and AP,[15 16] and combined with heavy drinking, smoking can further increase the risk of AP up to four times compared with non-smokers.[17 18] Findings are controversial regarding the effects of smoking cessation. A study published by Sadr-Azodi found that the risk of AP is statistically comparable to never-smokers' after 20 non-smoking years.[17] In contrast, a meta-analysis showed an elevated risk of AP in former smokers compared with never-smokers.[18]

Limiting alcohol use and smoking apart from their positive effects on the pancreas generally improve health[19] and up to a certain extent, organ damage caused by these substances is reversible.[20–23] Smoking cessation alone can prolong life with 1.4–8.5 years.[24]

In a Hungarian cohort study of 600 patients, alcohol consumption was four times more frequent in males, alcoholic aetiology represented 26.5% of all cases and was often associated with smoking. Alcoholic ARP accounted for 21.2% of all cases in the cohort.[25] In a CP cohort, daily alcohol consumption, as an etiological factor, was present in 56% of the cases, and 56% of the participants smoked more than 10 cigarettes/day.[26]

It is known that more than half of patients suffering from alcohol use disorder (AUD) are also dependent on tobacco, and that continued tobacco use represents a more than two-fold risk for relapse.[27 28] To this day, there are no adjusted protocols for the treatment and follow-up of heavy-drinking smokers.[29 30] It is proven that, in contrast with previous assumptions, smoking cessation programmes for patients at risk or living with AUD improve alcohol-related outcomes[27 31] and a brief alcohol intervention improves the rate of successful smoking cessation.[32]

However, to date, no study has examined the effects of a combined intervention for the reduction of nicotine and alcohol consumption in ARP and guidance is very limited on this topic,[33–36] (online supplemental table S1). Based on the above-mentioned reasons, while all patients with alcoholic AP should receive counselling, a one-time brief intervention will be provided to all participants, without further counselling in the control group.

Objectives

The study encompasses a randomised controlled trial (RCT) (REAPPEAR-T: Recurrent acute pancreatitis prevention by the elimination of alcohol and cigarette smoking; trial) and a concomitant cohort study (REAPPEAR-C: Recurrent acute pancreatitis prevention by the elimination of alcohol and cigarette smoking; cohort). The REAPPEAR-T's objective is to investigate the effect of an alcohol and smoking cessation programme combined with patient education on the recurrence rate of alcohol-induced AP, CP and QoL. Additionally, the REAPPEAR-C's objective is to investigate the effect of alcohol and smoking cessation (irrespective of intervention) on the recurrence rate of alcohol-induced AP, CP and QoL.

METHODS

Design

The REAPPEAR study, designed in accordance with the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) statement,[37] uses a combined design to answer two questions in one particular patient population. The REAPPEAR-T will be an international, single-blind, two-arm, parallel group, superiority RCT, testing the efficacy of a cessation programme for alcohol and smoking, using brief interventions. The REAPPEAR-C is a prospective four-arm cohort study, which includes all patients participating in REAPPEAR-T with further enrolment after the termination of enrolment to the trial. In the cohort, patients will be grouped by smoking status and alcohol consumption at the end of the study, irrespective of intervention. The same eligibility criteria and outcomes will be used in both substudies and differences will be described in the appropriate sections in detail.

The study will be conducted in Hungary, Ukraine, Italy and Romania (list of centres in online supplemental file 1). Centres are welcome to join. The enhance the visibility of this project and centre recruitment, the protocol is being presented on national and international conferences. Patients will be enrolled during their hospitalisation for AP and will be followed during ambulatory visits to the same hospital.

Population

Inclusion criteria

- Patient hospitalised with alcohol-induced AP (defined by the revised Atlanta criteria.[38]
- Regular consumption of at least 40 g (women)/ 50 g (men) alcohol daily or 280 g (women)/ 350 g (men) alcohol during the preceding week of onset of abdominal pain

- Every day smoker (defined as an adult patient who smoked at least 100 cigarettes in his or her lifetime, and now smokes on a daily basis; as per the CDC definition), with at least 1-year history of smoking.
- Aged 18–65 years.[39]
- Completed the standard intervention (SI) (see below).
- Provided written informed consent (online supplemental file 2).

Exclusion criteria

- Possible aetiologies for AP other than alcohol (eg, gallstone-related, hypertriglyceridaemia above 11.5 mM,[40–42] hypercalcaemia, viral infection) and cases with combined etiological factors will be excluded.
- Major psychiatric illnesses (eg, schizophrenia, bipolar disorder, dementia).
- Currently receiving therapy for AUD.
- Currently taking part in a smoking cessation programme.
- Three or more documented lifetime episodes of AP.[43]
- CP.[44]
- Undergoing active or palliative treatment for malignancy.
- Pregnancy.
- Life expectancy is less than 2 years.

Medical personnel not involved in the treatment of the patient will perform formal screening and obtain informed consent.

Standard intervention

The SI will be incorporated into standard medical therapy in all centres, and will be provided to all patients hospitalised for alcohol-induced AP. SI will be delivered by a specially trained nurse because interventions delivered by nurses have been found to be the most effective in reducing the quantity of alcohol consumed.[45] The intervention will be based on the WHO initiative 'Assist-linked brief intervention', using psychoeducational and motivational interviewing techniques.[46] For SI, we calculated with an average length of 30 min, based on a recent Cochrane review including 69 RCTs, according to which longer interventions on alcohol had no benefit, the median duration being 25 min.[47] SI will also provide educational information about the nature of alcoholic AP and the risk of recurrence to the patients. Feasibility and cost-effectiveness were also considered.

Intervention in REAPPEAR-T

The repeated intervention will be provided by the same specially trained personnel and structured similarly to the SI. Each session will have the same structure but can be tailored to the patient's needs to strengthen motivation. Sessions will consist of three parts. First, the negative effects of alcohol and smoking on the pancreas will be highlighted. Second, the patient's motivation for abstinence and smoking cessation will be discussed. Third, the individual's responsibility in achieving the goals set after motivation assessment, with personalised advice.[13] We wish to enhance the efficacy of the repeated intervention by providing feedback for the patient based on the mean corpuscular volume and gamma glutamyl-transferase (GGT) values measured at the day of the interview.[48] The trained personnel providing the interventions will not take part in patient care in any form. A detailed protocol will be provided on request.

Concomitant care

Patients participating in cessation programmes or psychotherapy at the time of enrolment will not be eligible. Patients using self-help programmes and nicotine replacement therapy with commonly available products will not be excluded. The provided interventions encourage patients to seek help and try different strategies for alcohol and smoking cessation.

Outcomes

Primary endpoint

The primary endpoint of the REAPPEAR Study will be the composite of 2-year recurrence rate of AP irrespective of aetiology and 2-year all-cause mortality.

Secondary endpoints

1. ARP irrespective of aetiology (given as cumulative incidence and as rate of event) within 6, 12, 18 and 24 months.
2. Recurrence of alcohol-induced AP (rate of event) within 2 years.
3. The condition of 'likely pancreatitis' (fulfilling the diagnostic criteria of epigastric pain, a serum amylase or lipase level at least two times the upper normal level, and elevated leucocyte count or CRP levels, defined by Pelli *et al*.[49]
4. Length of hospital stay given in days (specifically due to recurrent pancreatitis and cumulative during follow-up).
5. Presentation to the emergency unit with and without hospital readmission (cumulative incidence).
6. Change of alcohol consumption and tobacco use (compared with baseline), estimated separately form biomarker levels and patient-reported consumption
7. CP (incidence within 2 years).[44]
8. Changes in body mass index (BMI) and blood pressure (compared with baseline).
9. Healthcare cost from the perspective of the health insurance fund within 2 years and quality-adjusted life-years (QALY).

Recruitment

Consecutively, all patients under treatment for alcohol-induced AP who received the SI according to standard protocol will be screened for eligibility, all eligible patients will be offered to participate in the REAPPEAR study. The potential benefits of participation will be highlighted to facilitate patient recruitment. The planned start and end dates of patient recruitment are 1 March 2021 and 1 December 2024.

Biologic sample collection and biomarker measurements

At enrolment and every visit, basic laboratory tests from blood will be carried out and participants will provide blood, hair and urine samples for storage in the biobank.

Laboratory parameters measured are shown in online supplemental file 3. Laboratory results will be evaluated by a physician, who will decide whether further medical attention is necessary. All patients will receive the results of their laboratory tests in written form.

The samples in the biobank will be stored at −80°C and identified by the personal identification number (PIN) given at study entry. Planned alcohol and smoking biomarker measurements include urine and serum ethyl-glucuronide (or ethyl-sulfate) and hair nicotine measurements.[50 51] All samples will be collected and sent together to the laboratory when the patient number reached the preset goal for analysis. The results of the biomarker measurements will not be made accessible for patients. These measurements are only available in specialised laboratories, therefore, can be changed later due to feasibility issues.

Trial organisation, committees and boards

The corresponding centre of the REAPPEAR study is the Centre for Translational Medicine, Medical School, University of Pécs (www.tm-centre.org), whereas the coordinator and designer research team is the Hungarian Pancreatic Study Group (HPSG, https://tm-centre.org/en/study-groups/hungarian-pancreatic-study-group/). HPSG has been running high-quality international, multicentre clinical trials since 2014[41 52–54] and has published relevant guidelines for pancreatic diseases to improve patient care in pancreatology.[55 56]

The steering committee (SC) will be led by PH (principal investigator, specialist in internal medicine, gastroenterology and clinical pharmacology). SC members will be KO (study coordinator), a patient representative, NF (biostatistician), IH (psychologist) and the centre leaders. The SC will supervise the trial primarily and will make decisions regarding all critical questions overseeing patient safety, the progress of the trial, adherence to protocol, considering new information relevant to the trial and ensuring dissemination and implementation of the results.

All data gathered for research purposes will be handled confidentially and anonymously, which will be ensured by the data monitoring committee (DMC). Six-monthly audits are planned in each centre with continuous monitoring of the electronic case report forms (eCRFs) (online supplemental file 3) For each participant, a PIN will be generated and it will be present on all forms and documents of each individual.

The International Advisory Board will include Ole Petersen, Enrique de-Madaria and Jonas Rosendahl, providing independent external advice and guidance on strategic matters.

The study was designed by the SC and was supported by the University of Pécs Medical School. The sponsor had no role in the design of the trial and will have no access to the randomisation codes or the data. The sponsor will not participate in data monitoring, analysis and publication of results.

The independent safety monitor will be LC, who will ensure the safety of the patients and revise all reported harms possibly related to the intervention.

Data handling

Investigators will be responsible for the accuracy, reliability and quality of the collected data. Detailed data flow will be described in a data management plan. Data from completed eCRFs will be validated under the direction of the data manager on the DMC according to the data cleaning plan. Any missing, implausible or inconsistent recordings in the eCRFs will be referred back to the Investigator using a data query form and will be documented for each subject before clean file status is declared. All changes to eCRFs will be recorded.

The DMC will perform an independent assessment of trial-related documents and activities to ensure respect for subjects' rights, safety and well-being and to guarantee the plausibility of clinical data. The similarity of groups at baseline will also be checked.

After written consent of the subjects, data will be recorded by the investigators. Clinical research data will be processed separately from participants' personal data. Data may only be accessed by a person acting under the authority of the controller and in accordance with the authorisation system established within the controller's organisational structure, only to the extent and in the manner necessary for the performance of tasks. Personal data will not be made accessible to third parties. We will fully comply with the General Data Protection Regulation (GDPR).

Safety

Based on the nature of the combined brief intervention in REAPPEAR-T, we do not expect serious adverse events. However, minor or moderate adverse events may occur. Participants will be provided with information on alcohol and nicotine withdrawal alongside with the available options of professional help for addiction treatment. In case a potentially serious health problem is detected by the investigators related to the intervention, the safety monitoring board will be notified. The REAPPEAR-C is an observational study, hence adverse events are not applicable.

Randomisation and allocation concealment in REAPPEAR-T

Central randomisation will be performed with randomly permuted block size (2–6) and allocation ratio of 1:1 using a computer-generated random sequence. Inclusion and exclusion criteria will be rechecked prior to computer-aided randomisation via an online platform. The platform generates the PIN and a follow-up plan (with appointment dates). The randomisation procedure will be performed by the same person who screened and

consented the patient. This person must be a doctor not actively participating in the treatment of the participant.

Blinding in REAPPEAR-T

Outcome assessors will be blinded to allocation. The medical personnel involved in the check-ups and treatment during a potential hospital re-admission will not be aware of the allocation. Since the nature of the intervention, the patient and the study nurse cannot be properly blinded.

Statistical analysis

Sample size calculation for REAPPEAR-T was based on the only published interventional randomised study assessing the effects of repeated brief interventions in alcohol-induced ARP, counting with a 2-year recurrence rate of 21.3% and an absolute reduction to 8.5%.[13 15 17 25] Considering one interim analysis on efficacy (with the Pocock correction), 80% power, 5% alpha (superiority design, two-sided) and a drop-out rate of 30%, the estimated sample size is 182 subject per study arm. This sample size calculation is expected to overestimate the minimum number of participants for three reasons: (1) the use of a combined intervention on alcohol and smoking and more frequent visits are expected to result in greater reduction of recurrence, (2) the use of a composite primary endpoint may result in higher event numbers and (3) the recurrence rate of AP is expected to be higher in the heavy-drinking smoker population, than in a mixed sample. The calculation was performed by Stata (V.15).

Safety analysis will be carried out on reaching 10% of the target patient enrolment, and a single interim analysis for efficacy and sample size re-estimation taking into consideration the observed drop-out rate at 50% of the expected total events of the primary outcome, which is 21. Early stopping will be executed (1) if safety concerns arise during the interim analysis or anytime later (stopping for safety concerns), (2) if the statistical power reaches at least 80% and $p<0.05$ for the primary outcome at the interim analysis (stopping for benefit), (3) if the results of the interim analysis show equal effects in both groups (stopping for futility) and (4) if power does not reach 80%, sample size will be re-estimated using the observed event and drop-out rate. In case the newly calculated sample size is unfeasible for the trial, both groups will continue follow-up according to the schedule of REAPPEAR-C (stopping for feasibility).

In the final analysis, intention-to-treat will be favoured over per-protocol (or 'as-treated') analysis. Information on mortality and hospitalisations will be obtained from the organisation responsible for handling data.

The 'last observation carried forward' strategy will be followed to impute missing data for other outcomes measured during the study.

Sample size calculation for the REAPPEAR-C will be carried out at the final analysis of the REAPPEAR-T, using available data from participants. Further enrolment will be performed according to the estimated sample size. These additional participants will receive the more effective or in case of equality the less costly intervention for alcohol and smoking cessation as determined by the results of the REAPPEAR-T. Participants of the cohort will be categorised into four groups primarily, according to smoking and drinking status (quit smoking; quit drinking; quit both; still smokes and drinks). Time of smoking and alcohol cessation will be taken into consideration. Participants who started smoking or drinking again after an abstinent period will be excluded from analysis in the REAPPEAR-C.

In descriptive statistics, the count and percentage will be provided for each treatment arm for binary outcomes. For continuous outcomes, number (n), mean, median, IQR (Q3–Q1), SD, minimum and maximum values will be provided for each treatment arm. In the univariate comparative analysis, we will calculate relative risk with 95% CI when comparing the primary endpoint between two groups (alpha=5%) with a reference arm using the control group complemented with χ^2 or Fisher's exact test (the same strategy will be followed for binary secondary outcomes). For continuous variables, we will use t-test assuming unequal variances or the Mann-Whitney test. We will perform univariate (Kaplan-Meier and Cox regression) and multivariate (Cox regression) survival analyses for binary outcomes. An adjustment will be carried out at least for age, sex, socioeconomic status, the number of prior ARPs, comorbidities, history of alcohol consumption (cumulative) and smoking (package year), severity and complications of index AP, BMI, cholecystectomy and enrolling centre. Mixed effect logistic regression will be conducted to estimate the effect of the multicomponent intervention on the outcomes, where the subject PINs will be used as a random subject. The model will be adjusted for changes in smoking habits, alcohol consumption, BMI, socioeconomic status, blood pressure and Maddrey score.[57]

All analyses will be carried out with SPSS V.26 and Stata V.15.

Drop-outs

Information on the primary outcome will be obtained either from the patient's documentation or from the National Health Insurance Fund or similar organisation managing data on healthcare costs and mortality, therefore information on the primary outcome will be available for most patients regardless of attendance of the study visits. Only withdrawal of consent will result in missing data.

Considering per-protocol analysis, in the REAPPEAR-T trial, missing more than one consecutive interventions after the initial assessment or withdrawal of consent during follow-up will result in the drop-out of the patient. In the REAPPEAR-C investigation, patients who withdraw consent during follow-up or miss the 2-year visit will be considered drop-outs, since data on current alcohol

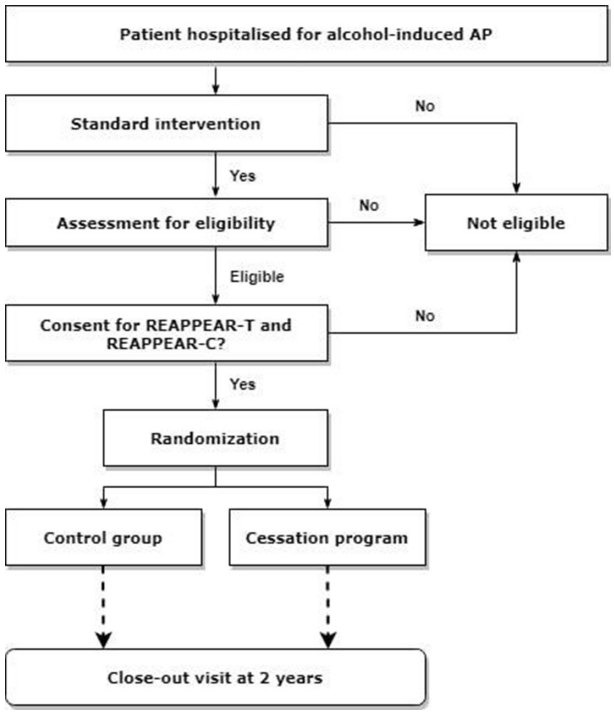


Figure 1 SPIRIT flow chart. Standard intervention will be provided for every patient as part of standard therapy. All randomised participants will be included in the REAPPEAR-T (trial) and REAPPEAR-C (cohort) as well. After reaching the required patient numbers for the REAPPEAR-T, further patients will be enrolled to the REAPPEAR-C in accordance with the estimated sample size. AP, acute pancreatitis. SPIRIT, Standard Protocol Items: Recommendations for Interventional Trials, REAPPEAR, Recurrent acute pancreatitis prevention by the elimination of alcohol and cigarette smoking.

consumption and smoking can only be obtained from the patient.

Flow and timing

Patients who met the inclusion criteria and none of the exclusion criteria will be offered to participate in the REAPPER-T trial. The enrolment period lasts from 48 hours before, until 1 week after hospital discharge. After informed consent and randomisation, participants will be assigned to the cessation programme or the control group (see at figure 1). All patients will appear at the clinic according to the study schedule (figure 2), within ±14 days from the prescheduled date.

We chose 3-monthly visits in the cessation programme based on a Swedish cohort study, in which 3-monthly brief interventions for selected patients with increased GGT levels were introduced and GGT levels were used for feedback. These interventions were found to reduce mortality, hospitalisation and sick leave significantly.[48] Hopefully, frequent visits will help in upholding motivation and improve adherence. Patients in the control group will have two prescheduled appointments, at 12 and 24 months.

	STUDY PERIOD	Screening enrollment period	Allocation	Visit 1		Visit 2		Visit 3		Visit 4		Visit 5		Visit 6		Visit 7		Close-out
			day 0	3 months		6 months		9 months		12 months		15 months		18 months		21 months		24 months
	GROUP	Both	Both	CG	CP	CG	CP	CG	CP	CG	CP	CG	CP	CG	CP	CG	CP	Both
ENROLLMENT	Eligibility screen	X																
	Standard intervention	X																
	Informed consent	X																
	Allocation		X															
	INTERVENTION				X		X		X		X		X		X		X	
ASSESSMENT	BP, HR, BMI		X		X		X		X	X	X		X		X		X	X
	Laboratory testing		X		X		X		X	X	X		X		X		X	X
	Questionnaires		X		X		X		X	X	X		X		X		X	X
	Sample collection		X		X		X		X	X	X		X		X		X	X

Figure 2 SPIRIT time table. SPIRIT, Standard Protocol Items: Recommendations for Interventional Trials; BMI, body mass index; BP, blood pressure; CG; control group, CP; cessation programme, HR; heart rate.

Assessment

For the assessment of addiction and motivation to quit will be assessed by internationally recognised and validated questionnaires (online supplemental file 3).[58–64] This will enable the person who provides the intervention to individualise it and motivate the subject. Data on coffee consumption will be collected as well, as caffeine might counter the effects of alcohol in AP.[65] For the assessment of QoL, the EQ-5D-5L questionnaire will be used at baseline and every visit.[66 67] Socioeconomic status will be assessed at baseline and at 12 and 24 months with the questionnaire used in the LIFESPAN study.[68]

The aetiology of each recurrent episode will be determined following current international guidelines, but all episodes will be included in the primary endpoint.[4 34]

Blood pressure, heart rate and body weight will be measured by an independent nurse blind to the allocation at every visit. BMI will be calculated.

Cost-effectiveness

Cost-effectiveness analysis will be performed to examine the impact of the cessation programme on QoL, survival and health expenditure compared with the controls. We calculate the incremental cost-effectiveness ratio (ICER), which is defined by the difference in cost between the compared interventions (cessation programme with 3-monthly visits vs usual care), divided by the difference in their effect (QALY). The ICER will be evaluated based on the Hungarian cost-effectiveness threshold. The total cost of treatment per each individual will be obtained from the national database at the completion of the study.

Patient and public involvement

Five randomly selected patients from the HPSG database were invited. All of them had previous AP and would have been eligible for the study. Three patients attended the joint consultation. The original aims, hypotheses and protocol of the study were fully introduced to them. Patients insights were as follows: (1) they welcomed the study with great pleasure and felt it is highly important, (2) they found the primary endpoint fundamental, (3) they found the questionnaires and information sheets understandable, (4) they highlighted the importance of frequent visits to the clinic, and found the duration of the visits feasible, (5) they pointed out the necessity of

high quality training of personnel providing the interventions, (6) they had absolutely no disapproval or negative feelings regarding regular blood tests, (7) they had no ethical objection concerning the control group and (8) they expressed high difficulties considering smoking cessation and favoured a step-down approach rather than immediate quitting.

We have revised and modified the original protocol accordingly.

DISCUSSION

Although alcohol and smoking are individual risk factors for AP, ARP and CP, they can synergise each other's effects.[69] In addition, there is a lack of evidence as to the means of preventive measures that could be used in everyday clinical practice concerning alcohol and tobacco use for AP patients. Also, the effect of smoking cessation on recurrence in drinkers and non-drinkers is not yet clear.

The REAPPEAR study aims to fill these gaps and provide specialists and primary care physicians with valuable information on the importance of alcohol and smoking cessation in AP and ARP. Furthermore, the feasibility, efficacy and cost-effectiveness of an intervention programme will be tested in this population to provide basis for large-scale intervention in alcohol-induced pancreatitis.

ETHICS AND DISSEMINATION

The REAPPEAR study is open for participation. Results of the planned analyses will be presented at national and international conferences and in peer-reviewed journals. Additional long-term follow-up of the participants is planned within the confines of the REAPPEAR+study.

The trial has been registered at the clinicaltrials.gov (NCT04647097). Amendments will be published under this registration number.

The Scientific and Research Ethics Committee of the Hungarian Medical Research Council approved the study (40394-10/2020/EÜIG). All local ethical approvals are in place. The study will be performed in accordance with the declaration of Helsinki, the principles of International Conference on Harmonisation-Good Clinical Practice (ICH-GCP) guidelines and local legal and regulatory requirements.

Author affiliations
[1]Institute for Translational Medicine, Szentágothai Research Centre, Medical School, University of Pécs, Pécs, Hungary
[2]Centre for Translational Medicine, Semmelweis University, Budapest, Hungary
[3]Institute for Translational Medicine, Medical School, University of Pécs, Pécs, Hungary
[4]Medical School, Institute of Bioanalysis, University of Pécs, Pécs, Hungary
[5]Faculty of Pharmacy, Division of Pharmacoeconomics, University of Pécs, Pécs, Hungary
[6]Center for Health Technology Assessment, University of Pécs, Pécs, Hungary
[7]Medical School, Institute of Behavioural Sciences, University of Pécs, Pécs, Hungary
[8]Medical School, Department of Laboratory Medicine, University of Pécs, Pécs, Hungary
[9]Medical School, First Department of Medicine, Division of Cardiology and Angiology, University of Pécs, Pécs, Hungary
[10]First Department of Internal Medicine, Szent György University Teaching Hospital of Fejér County, Székesfehérvár, Hungary
[11]Department of Internal Medicine, Division of Gastroenterology, University of Debrecen, Debrecen, Hungary
[12]Faculty of Medicine, First Department of Medicine, University of Szeged, Szeged, Hungary
[13]General and Emergency Surgery Department, Azienda Ospedaliera Ospedali Riuniti Villa Sofia-Cervello, Palermo, Italy
[14]Department of Digestive Tract Diseases, Medical University of Lodz, Lodz, Poland
[15]Department of Surgery, Bogomolets National Medical University, Kyiv, Ukraine
[16]Pancreas Translational and Clinical Research Center, San Raffaele Scientific Institute, Vita Salute San Raffaele University, Milan, Italy
[17]Clinical Institute Fundeni, Bucuresti, Romania
[18]Carol Davila University of Medicine and Pharmacy, Bucuresti, Romania
[19]Cardiff University, Cardiff, UK
[20]Gastroenterology Department, Alicante University General Hospital, Alicante Institute for Health and Biomedical Research (ISABIAL), Alicante, Spain
[21]Department of Internal Medicine I, Martin Luther University, Halle, Germany
[22]Heim Pál National Pediatric Institute, Budapest, Hungary

Twitter Ionut Adrian Saizu @saizuadrian and Enrique de-Madaria @DeMadaria

Acknowledgements We would like to thank the interdisciplinary team of the Institute for Translational Medicine and the staff from all centres.

Contributors All authors were involved in the study design and edited the manuscript. KOc conducted the literature search, conceptualised and wrote the manuscript and prepared the figures. FJ participated in manuscript writing, critical revision, abstract writing and figure preparation. NF performed the sample size calculation and planned the statistical analyses. NZ and LS facilitated the ethical approval and trial registration. ZS provided methodological guidance, critically reviewed and revised the manuscript, EM facilitated patient invovlement and will coordinate data management, BB, AC and IH designed the intervention and chose the questionnaires, AZ will carry out the healthcare cost calculations. LC provided expert opinion and will perform safety monitoring. TN provided expert opinion on laboratory measurements and biological sample collection. FI, LG, MP, LC, DI, MVM, AM, EM-P, HZ, YS, KOp, GC, LA, CG and IAS provided insight on feasibility and will coordinate local enrolment and follow-up. OHP, Ed-M and JR provided expert opinion. PH, AP, AS, BE critically reviewed and revised the manuscript. PH and AP have given significant intellectual input and supervised the work. All authors have read and approved the final version of the manuscript. AP and PH co-supervised this work as joint last authors.

Funding Center costs (IT, biostatistics, trial organisation, etc) are covered by the University of Pécs Medical School (grant number: not applicable). This work was supported by 'GINOP-2.3.2-15-2016-00048 - STAY ALIVE' (PH) cofinanced by the European Union (European Regional Development Fund) within the framework of Programme Széchenyi 2020, and by EFOP 3.6.2-16-2017-00006 – LIVE LONGER (PH) Human Resources Development Operational Programme Grant which is cofinanced by the European Union (European Regional Development Fund) within the framework of Programme Széchenyi 2020 as well as the Translational Medicine Foundation. Further grants: a János Bolyai Research Scholarship from the Hungarian Academy of Sciences (AP) and National Research, Development and Innovation Office Project Grants (K116634, (PH);, FK138929 (AP)).

Disclaimer The sponsor was not involved in the design of the study and will have no access to the database or the randomisation code.

Competing interests None declared.

Patient consent for publication Not applicable.

Provenance and peer review Not commissioned; externally peer reviewed.

terminology, drug names and drug dosages), and is not responsible for any error and/or omissions arising from translation and adaptation or otherwise.

ORCID iDs
Klementina Ocskay http://orcid.org/0000-0001-5848-2506
László Czakó http://orcid.org/0000-0002-6331-0802
Dóra Illés http://orcid.org/0000-0003-3138-8039
Péter Hegyi http://orcid.org/0000-0003-0399-7259

REFERENCES

1 Wadhwa V, Patwardhan S, Garg SK, *et al*. Health care utilization and costs associated with acute pancreatitis. *Pancreas* 2017;46:410–5.
2 Szentesi A, Tóth E, Bálint E, *et al*. Analysis of research activity in gastroenterology: pancreatitis is in real danger. *PLoS One*;11:e0165244.
3 Machicado JD, Yadav D. Epidemiology of recurrent acute and chronic pancreatitis: similarities and differences. *Dig Dis Sci* 2017;62:1683–91.
4 Tenner S, Baillie J, DeWitt J, *et al*. American College of gastroenterology guideline: management of acute pancreatitis. *Am J Gastroenterol* 2013;108:1400–15.
5 Guda NM, Muddana V, Whitcomb DC, *et al*. Recurrent acute pancreatitis: international state-of-the-science conference with recommendations. *Pancreas* 2018;47:653–66.
6 Coté GA, Yadav D, Abberbock JA, *et al*. Recurrent acute pancreatitis significantly reduces quality of life even in the absence of overt chronic pancreatitis. *Am J Gastroenterol* 2018;113:906–12.
7 Yadav D, O'Connell M, Papachristou GI. Natural history following the first attack of acute pancreatitis. *Am J Gastroenterol* 2012;107:1096–103.
8 Mandalia A, Wamsteker E-J, DiMagno MJ. Recent advances in understanding and managing acute pancreatitis. *F1000Res* 2018;7. doi:10.12688/f1000research.14244.1. [Epub ahead of print: 28 06 2018].
9 Krishna SG, Kamboj AK, Hart PA, *et al*. The changing epidemiology of acute pancreatitis hospitalizations: a decade of trends and the impact of chronic pancreatitis. *Pancreas* 2017;46:482–8.
10 Nikkola J, Räty S, Laukkarinen J, *et al*. Abstinence after first acute alcohol-associated pancreatitis protects against recurrent pancreatitis and minimizes the risk of pancreatic dysfunction. *Alcohol Alcohol* 2013;48:483–6.
11 Magnusdottir BA, Baldursdottir MB, Kalaitzakis E, *et al*. Risk factors for chronic and recurrent pancreatitis after first attack of acute pancreatitis. *Scand J Gastroenterol* 2019;54:87–94.
12 Nikkola J, Laukkarinen J, Lahtela J, *et al*. The long-term prospective follow-up of pancreatic function after the first episode of acute alcoholic pancreatitis: recurrence predisposes one to pancreatic dysfunction and pancreatogenic diabetes. *J Clin Gastroenterol* 2017;51:183–90.
13 Nordback I, Pelli H, Lappalainen-Lehto R, *et al*. The recurrence of acute alcohol-associated pancreatitis can be reduced: a randomized controlled trial. *Gastroenterology* 2009;136:848–55.
14 Lappalainen-Lehto R, Koistinen N, Aalto M, *et al*. Goal-related outcome after acute alcohol-pancreatitis -- a two-year follow-up study. *Addict Behav* 2013;38:2805–9.
15 Tolstrup JS, Kristiansen L, Becker U, *et al*. Smoking and risk of acute and chronic pancreatitis among women and men: a population-based cohort study. *Arch Intern Med* 2009;169:603–9.
16 Lindkvist B, Appelros S, Regnér S, *et al*. A prospective cohort study on risk of acute pancreatitis related to serum triglycerides, cholesterol and fasting glucose. *Pancreatology* 2012;12:317–24.
17 Sadr-Azodi O, Andrén-Sandberg Å, Orsini N, *et al*. Cigarette smoking, smoking cessation and acute pancreatitis: a prospective population-based study. *Gut* 2012;61:262–7.
18 Majumder S, Gierisch JM, Bastian LA. The association of smoking and acute pancreatitis: a systematic review and meta-analysis. *Pancreas* 2015;44:540–6.
19 Schuckit MA. Alcohol-use disorders. *The Lancet* 2009;373:492–501. doi:10.1016/S0140-6736(09)60009-X
20 Peris P, Parés A, Guañabens N, *et al*. Bone mass improves in alcoholics after 2 years of abstinence. *J Bone Miner Res* 1994;9:1607–12.
21 Aguilera MT, de la Sierra A, Coca A, *et al*. Effect of alcohol abstinence on blood pressure: assessment by 24-hour ambulatory blood pressure monitoring. *Hypertension* 1999;33:653–7.
22 Seitz HK, Bataller R, Cortez-Pinto H, *et al*. Alcoholic liver disease. *Nat Rev Dis Primers* 2018;4:16.
23 Jandíková H, Dušková M, Stárka L. The influence of smoking and cessation on the human reproductive hormonal balance. *Physiol Res* 2017;66:S323–31.
24 Taylor DH, Hasselblad V, Henley SJ, *et al*. Benefits of smoking cessation for longevity. *Am J Public Health* 2002;92:990–6.
25 Párniczky A, Kui B, Szentesi A, *et al*. Prospective, multicentre, nationwide clinical data from 600 cases of acute pancreatitis. *PLoS One* 2016;11:e0165309.
26 Szücs Ákos, Marjai T, Szentesi A, *et al*. Chronic pancreatitis: multicentre prospective data collection and analysis by the Hungarian pancreatic study group. *PLoS One* 2017;12:e0171420.
27 Weinberger AH, Platt J, Jiang B, *et al*. Cigarette smoking and risk of alcohol use relapse among adults in recovery from alcohol use disorders. *Alcohol Clin Exp Res* 2015;39:1989–96.
28 Yardley MM, Mirbaba MM, Ray LA. Pharmacological options for smoking cessation in heavy-drinking smokers. *CNS Drugs* 2015;29:833–45.
29 Mirbaba M. Heavy-Drinking smokers: pathophysiology and pharmacologic treatment options. *Am J Psychiatry Resid J* 2016;11:8–11.
30 Fucito LM, Hanrahan TH. Heavy-Drinking smokers' treatment needs and preferences: a mixed-methods study. *J Subst Abuse Treat* 2015;59:38–44.
31 McKelvey K, Thrul J, Ramo D. Impact of quitting smoking and smoking cessation treatment on substance use outcomes: an updated and narrative review. *Addict Behav* 2017;65:161–70.
32 Toll BA, Martino S, O'Malley SS, *et al*. A randomized trial for hazardous drinking and smoking cessation for callers to a quitline. *J Consult Clin Psychol* 2015;83:445–54.
33 Crockett SD, Wani S, Gardner TB, *et al*. American Gastroenterological Association Institute Guideline on Initial Management of Acute Pancreatitis. *Gastroenterology* 2018;154:1096–101.
34 Working Group IAP/APA Acute Pancreatitis Guidelines. IAP/APA evidence-based guidelines for the management of acute pancreatitis. *Pancreatology* 2013;13:e1–15.
35 Samarasekera E, Mahammed S, Carlisle S, *et al*. Pancreatitis: summary of NICE guidance. *BMJ* 2018;362:k3443.
36 Isaji S, Takada T, Mayumi T, *et al*. Revised Japanese guidelines for the management of acute pancreatitis 2015: revised concepts and updated points. *J Hepatobiliary Pancreat Sci* 2015;22:433–45.
37 Chan A-W, Tetzlaff JM, Gøtzsche PC, *et al*. Spirit 2013 explanation and elaboration: guidance for protocols of clinical trials. *BMJ* 2013;346:e7586.
38 Banks PA, Bollen TL, Dervenis C, *et al*. Classification of acute pancreatitis--2012: revision of the Atlanta classification and definitions by international consensus. *Gut* 2013;62:102–11.
39 Yadav D. Reassessing the risk of pancreatitis with alcohol. *Pancreas* 2016;45:781–2.
40 Mosztbacher D, Hanák L, Farkas N, *et al*. Hypertriglyceridemia-induced acute pancreatitis: a prospective, multicenter, international cohort analysis of 716 acute pancreatitis cases. *Pancreatology* 2020;20:608–16.
41 Zádori N, Gede N, Antal J, *et al*. Early elimination of fatty acids in hypertriglyceridemia-induced acute pancreatitis (ELEFANT trial): protocol of an open-label, multicenter, adaptive randomized clinical trial. *Pancreatology* 2020;20:369–76.
42 Szentesi A, Párniczky A, Vincze Áron, *et al*. Multiple hits in acute pancreatitis: components of metabolic syndrome synergize each other's deteriorating effects. *Front Physiol* 2019;10:1202.
43 Hegyi P, Erőss B, Mátrai P, *et al*. The first definition for early chronic pancreatitis. *Pancreatology* 2018;18:S172.
44 Löhr JM, Dominguez-Munoz E, Rosendahl J, *et al*. United European gastroenterology evidence-based guidelines for the diagnosis and therapy of chronic pancreatitis (HaPanEU). *United European Gastroenterol J* 2017;5:153–99.
45 Platt L, Melendez-Torres GJ, O'Donnell A, *et al*. How effective are brief interventions in reducing alcohol consumption: do the setting, practitioner group and content matter? Findings from a systematic review and metaregression analysis. *BMJ Open* 2016;6:e011473.
46 WHO ASSIST Working Group. The alcohol, smoking and substance involvement screening test (assist): development, reliability and feasibility. *Addiction* 2002;97:1183–94.
47 Kaner EF, Beyer FR, Muirhead C, *et al*. Effectiveness of brief alcohol interventions in primary care populations. *Cochrane Database Syst Rev* 2018;2:CD004148.

48 Kristenson H, Öhlin H, Hultén-Nosslin MB, *et al*. Identification and intervention of heavy drinking in middle-aged men: results and follow-up of 24-60 months of long-term study with randomized controls. *Alcohol Clin Exp Res* 1983;7:203–9.
49 Pelli H, Lappalainen-Lehto R, Piironen A, *et al*. Risk factors for recurrent acute alcohol-associated pancreatitis: a prospective analysis. *Scand J Gastroenterol* 2008;43:614–21.
50 Kim S, Apelberg BJ, Avila-Tang E, *et al*. Utility and cutoff value of hair nicotine as a biomarker of long-term tobacco smoke exposure, compared to salivary cotinine. *Int J Environ Res Public Health* 2014;11:8368–82.
51 Andresen-Streichert H, Müller A, Glahn A, *et al*. Alcohol biomarkers in clinical and forensic contexts. *Dtsch Arztebl Int* 2018;115:309–15.
52 Mikó A, Erőss B, Sarlós P, *et al*. Observational longitudinal multicentre investigation of acute pancreatitis (GOULASH plus): follow-up of the GOULASH study, protocol. *BMJ Open* 2019;9:e025500.
53 Márta K, Szabó AN, Pécsi D, *et al*. High versus low energy administration in the early phase of acute pancreatitis (GOULASH trial): protocol of a multicentre randomised double-blind clinical trial. *BMJ Open* 2017;7:e015874.
54 Párniczky A, Mosztbacher D, Zsoldos F, *et al*. Analysis of pediatric pancreatitis (apple trial): Pre-Study protocol of a multinational prospective clinical trial. *Digestion* 2016;93:105–10.
55 Párniczky A, Abu-El-Haija M, Husain S, *et al*. EPC/HPSG evidence-based guidelines for the management of pediatric pancreatitis. *Pancreatology* 2018;18:146–60.
56 Hritz I, Czakó L, Dubravcsik Z, *et al*. [Acute pancreatitis. Evidence-based practice guidelines, prepared by the Hungarian Pancreatic Study Group]. *Orv Hetil* 2015;156:244–61.
57 Gholam PM. Prognosis and prognostic scoring models for alcoholic liver disease and acute alcoholic hepatitis. *Clin Liver Dis* 2016;20:491–7.
58 Hedman L, Katsaounou PA, Filippidis FT, *et al*. Receiving support to quit smoking and quit attempts among smokers with and without smoking related diseases: findings from the EUREST-PLUS ITC Europe surveys. *Tob Induc Dis* 2018;16:A14.
59 Saunders JB, Aasland OG, Babor TF, *et al*. Development of the Alcohol Use Disorders Identification Test (AUDIT): WHO Collaborative Project on early detection of persons with harmful alcohol consumption--II. *Addiction* 1993;88:791–804.
60 Németh Z, Urbán R, Farkas J, *et al*. Hungarian adaptation of the long and the short form of the drinking motives questionnaire (DMQ-R). *Magyar Pszichológiai Szemle* 2012;67:673–94.
61 Kuntsche E, Kuntsche S. Development and validation of the drinking motive questionnaire revised short form (DMQ-R SF). *J Clin Child Adolesc Psychol* 2009;38:899–908.
62 Heatherton TF, Kozlowski LT, Frecker RC, *et al*. The Fagerström test for nicotine dependence: a revision of the Fagerström tolerance questionnaire. *Br J Addict* 1991;86:1119–27.
63 Capacities HMoH Az emberi erőforrások minisztériuma egészségügyi szakmai irányelve a dohányzásról való leszokás támogatásáról. Available: https://tudogyogyasz.hu2019
64 Varga L. Segítségnyújtás kockázatos ivók számára, 2006. National addiction Institute
65 Petersen OH, Sutton R. Ca2+ signalling and pancreatitis: effects of alcohol, bile and coffee. *Trends Pharmacol Sci* 2006;27:113–20.
66 Rencz F, Gulácsi L, Drummond M, *et al*. EQ-5D in central and eastern Europe: 2000-2015. *Qual Life Res* 2016;25:2693–710.
67 Lloyd A, Pickard AS. The EQ-5D and the EuroQol group. *Value Health* 2019;22:21–2.
68 Koncz B, Darvasi E, Erdősi D, *et al*. Lifestyle, prevention and risk of acute pancreatitis (lifespan): protocol of a multicentre and multinational observational case-control study. *BMJ Open* 2020;10:e029660.
69 Sahin-Tóth M, Hegyi P. Smoking and drinking synergize in pancreatitis: multiple hits on multiple targets. *Gastroenterology* 2017;153:1479–81.

Annals of Intensive Care

REVIEW

Open Access

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

Klementina Ocskay[1], Anna Kanjo[1,2], Noémi Gede[1,3], Zsolt Szakács[1], Gabriella Pár[4], Bálint Erőss[1], Jan Stange[5], Steffen Mitzner[5], Péter Hegyi[1,6,7] and Zsolt Molnár[1,8*]

Abstract

Background: The role of artificial and bioartificial liver support systems in acute-on-chronic liver failure (ACLF) is still controversial. We aimed to perform the first network meta-analysis comparing and ranking different liver support systems and standard medical therapy (SMT) in patients with ACLF.

Methods: The study protocol was registered with PROSPERO (CRD42020155850). A systematic search was conducted in five databases. We conducted a Bayesian network meta-analysis of randomized controlled trials assessing the effect of artificial or bioartificial liver support systems on survival in patients with ACLF. Ranking was performed by calculating the surface under cumulative ranking (SUCRA) curve values. The RoB2 tool and a modified GRADE approach were used for the assessment of the risk of bias and quality of evidence (QE).

Results: In the quantitative synthesis 16 trials were included, using MARS®, Prometheus®, ELAD®, plasma exchange (PE) and BioLogic-DT®. Overall (OS) and transplant-free (TFS) survival were assessed at 1 and 3 months. PE significantly improved 3-month OS compared to SMT (RR 0.74, CrI: 0.6–0.94) and ranked first on the cumulative ranking curves for both OS outcomes (SUCRA: 86% at 3 months; 77% at 1 month) and 3-month TFS (SUCRA: 87%) and second after ELAD for 1-month TFS (SUCRA: 76%). Other comparisons did not reach statistical significance. QE was moderate for PE concerning 1-month OS and both TFS outcomes. Other results were of very low certainty.

Conclusion: PE seems to be the best currently available liver support therapy in ACLF regarding 3-month OS. Based on the low QE, randomized trials are needed to confirm our findings for already existing options and to introduce new devices.

Keywords: Network meta-analysis, Liver support therapy, Overall survival, Transplant-free survival, SUCRA, Plasma exchange, ELAD, MARS, Prometheus, BioLogic-DT

Introduction

Acute-on-chronic liver failure (ACLF) is a clinical syndrome defined by the acute deterioration of chronic liver disease and the rapid development of organ failures, associated with high short-term mortality.

ACLF is due to exogenous and endogenous precipitating factors called pathogen- and damage-associated molecular patterns (PAMPs and DAMPs) [1, 2]. The release of these molecules by necrosis or infection triggers an excessive inflammatory response, resulting in organ failures. Most patients developing ACLF have pre-existing cirrhosis, which is in itself a hyperinflammatory state [3, 4]. Another aggravating factor is the immune paralysis described by several studies [5–9], which

*Correspondence: zsoltmolna@gmail.com
[1] Institute for Translational Medicine, Medical School, University of Pécs, Szigeti út 12. 2nd floor, Pécs 7624, Hungary
Full list of author information is available at the end of the article

prevents effective countermeasures against infection and makes patients prone to serious infective complications.

Several therapies have been tested for the replacement of hepatic functions. So far, liver transplantation is the only curative therapy available. Survival rates are good, but availability and eligibility for transplant in ACLF differs by country [10]. In the CANONIC study, only 4.5% of ACLF patients received transplant. Reportedly, low transplant rates are due to the high prevalence of infection and organ failure. Waiting-list mortality exceeds 50% in this population [10].

The development of extracorporeal liver support systems dates back to the seventies with the aim to stabilize patients at the time of acute decompensation when transplant is not available or bridge patients to transplant [11]. At first, these devices were designed to replace only excretory functions and were based on hemoperfusion and adsorption [12]. The newer technologies combined these methods with bioreactors containing hepatocytes creating bioartificial liver support systems with the potential of synthetic activity.

The Asian Pacific Association for the Study of the Liver (APASL) consensus guideline from 2019 states that "plasma exchange appears to be a promising and effective bridging therapy in patients with ACLF to liver transplant or spontaneous regeneration [1, C]" [13]. The European Association for the Study of the Liver (EASL) Clinical Practice Guidelines do not recommend liver support therapies for the treatment of ACLF, but underline the importance of further studies, because in specific subgroups ACLF seems beneficial [14].

Numerous pairwise meta-analyses of randomized controlled trials (RCTs) have been published assessing short-, middle-, and long-term survival benefit of liver support therapies with controversial results [15–22]. These meta-analyses faced serious limitations, as they pooled together data from studies testing different devices, in some cases with different follow-up lengths. A network meta-analysis (NMA), on the other hand, can handle multiple interventions and rank them, if the assumption of transitivity is met [23].

To facilitate international discussion and consensus, we decided to perform the first NMA comparing all available and tested liver support systems to each other and standard medical therapy (SMT) in patients with ACLF and ranking these treatments by survival benefit.

Methods and materials

The protocol for this review was registered in the PROSPERO database under registration number CRD42020155850. There were no protocol deviations. This meta-analysis was reported according to The PRISMA Extension Statement for Reporting of Systematic Reviews Incorporating Network Meta-analyses of Health Care Interventions (PRISMA-NMA) [24].

Eligibility criteria

Parallel randomized controlled trials assessing the safety and efficacy of artificial and bioartificial liver support therapies in adult patients with acute-on-chronic liver failure (ACLF) were eligible for inclusion, regardless of the current availability of the tested therapy and length of follow-up. Conference abstracts were included to reduce publication bias. Crossover studies were excluded from the analyses of survival due to concerns about the carryover effect, but were included in the systematic review. ACLF definitions used in the included RCTs were accepted, as there is a lack of international consensus regarding this matter. For the studies published before ACLF was introduced as a clinical entity, the review authors decided eligibility based on the eligibility criteria used in the study. Due to substantial heterogeneity regarding the definitions or the timing of measurements, some outcomes were included only in the qualitative synthesis. Studies with shorter or longer follow-up periods than the assessed outcomes were also included in the systematic review.

Search strategy and selection

The systematic search was conducted up to the 15th December 2019 in the following databases: MEDLINE (via PubMed), Embase, CENTRAL, Web of Science, and Scopus, with the search key designed based on the PICO format––("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 "hemoadsorption") AND random*. No filters or restrictions were applied. References of included studies, citing articles, and authors' accessible publications in a search engine (Google Scholar) and ResearchGate were hand searched for further eligible publications.

Data extraction

Data extraction was performed by two independent investigators (KO and AK) in duplicate using Endnote X9, Clarivate Analytics and Windows Excel 2016, Microsoft. In the case of discrepancies, agreement was reached by two experts (ZM or ZS). As a measure of inter-rater reliability, Cohen's kappa coefficients (κ) for the selection of abstracts and full texts were counted. Information

collected from each study and additional information used are detailed in Additional file 1.

Risk of bias assessment and quality of evidence

The risk of bias assessment was conducted in duplicate using Version 2 of the Cochrane risk-of-bias tool for randomized trials (RoB 2) for overall and transplant-free survival separately [25].

For the four outcomes assessed in the NMA, quality of evidence was assessed in duplicate according to the Grades of Recommendation, Assessment, Development and Evaluation Working Group's recommendations, using a modified GRADE approach [26].

Statistical analysis

A Bayesian method was used to perform pairwise meta-analyses and NMAs with the random effect model for overall survival (OS) and transplant-free survival (TFS). For the analysis of transplant-free survival, transplant counted as an event similar to death. In case no patient received liver transplantation, OS and TFS were identical. If available, data for the intention-to-treat population were used.

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 other interventions are presented in forest plots, summarized in a league table (as shown in the results section). We were unable to use the node-splitting analysis to examine the consistency assumption because of the star-shaped configuration of the networks [27]. We ranked the interventions by their posterior probability by calculating the surface under cumulative ranking (SUCRA) curve values ranging from 0 to 100%. The higher the SUCRA value, and the closer to 100%, the higher the likelihood that a therapy is in the top rank or one of the top ranks; the closer to 0 the SUCRA value, the more likely that a therapy is in the bottom rank, or one of the bottom ranks [28]. We also provided rankograms, showing the probability of achieving certain ranks. Frequentist comparison-adjusted funnel plots were created for 1- and 3-month OS, and Egger's tests were performed to assess small-study effect. The low number of studies in the TFS analyses did not enable this method. In an additional analysis, methodology-based evaluation was performed. 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 16.0 (StataCorp LLC).

Results

Search and selection

The systematic search yielded 2797 records. Four additional articles were identified through manual search and from previous meta-analyses. κ for abstracts and full texts was 0.87 and 0.90, respectively, marking almost perfect agreement in both cases. One hundred three full texts were assessed for eligibility. Twenty-three articles proved to meet the eligibility criteria for the systematic review and 16 were included in the data synthesis (Fig. 1).

Characteristics of the included studies

The main characteristics of the 23 eligible studies included in qualitative synthesis are shown in Table 1. Of the 16 studies, enrolling 1670 patients included in the meta-analysis, 15 compared a type of artificial [29–38] or bioartificial [39–43] liver support system to standard medical therapy and one study compared MARS versus MARS plus plasma exchange [44]. The most common etiologies of underlying diseases were viral infection and alcohol. From the 1526 participants with available information on gender, 1064 were males (69.8%). ACLF definitions, eligibility criteria, baseline characteristics, and outcomes of the individual studies are reported in Table 1.

Synthesis

Survival

Survival was reported in most of the included studies, with greatly varying follow-up lengths. Data synthesis was feasible in four cases: 1-month (28–31 days) and 3-month (84–91 days) data were pooled for overall and transplant-free survival. The summary of the findings for these four outcomes is presented in Table 2.

Plasma exchange demonstrated a statistically significant survival benefit compared to SMT in the analysis for 3-month OS (RR 0.74; CrI 0.60 to 0.94), with 86% SUCRA, 46% probability of being the best, and 41% probability of being the second-best option from the six listed treatments (Figs. 2 and 3). PE also ranked first on the cumulative curves in three out of four analyses: both 1- and 3-month OS and 1-month TFS (Fig. 2, Additional file 1: Figure S3, S7). In the analysis for 1-month TFS PE ranked second after ELAD, with 76% versus 79% SUCRA values, but had a slightly higher cumulative probability of being in the first two places than ELAD (90% versus 88%) (Additional file 1: Figure S11).

MARS ranked second in both OS outcomes (Fig. 2, Additional file 1: Figure S3) with 73% SUCRA at 1 month and 71% at 3 months. Concerning TFS, MARS ranked second last and last with SUCRA values of 27% at 1 month and 33% at 3 months (Additional file 1: Figures S7, S11). Prometheus was included in both OS

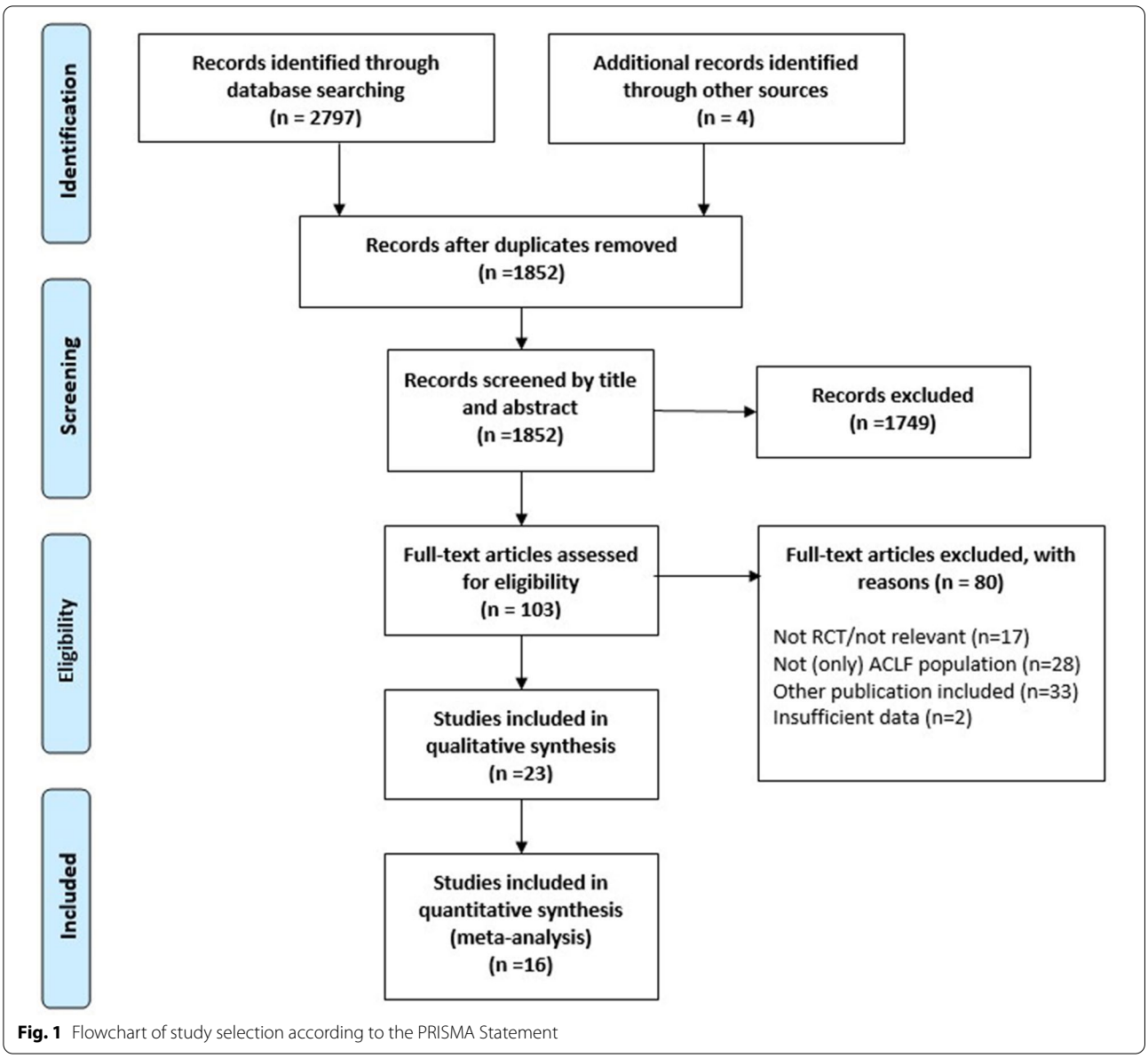


Fig. 1 Flowchart of study selection according to the PRISMA Statement

analyses and in 3-month TFS. Only MARS, PE, and their combination performed better than this device in the OS outcomes and it ranked second after PE for 3-month TFS. However, the SUCRA values and the probabilities for the first ranks are much lower than for PE (SUCRA: 40% for both OS and 51% for 3-month TFS, first rank probabilities 5% for 1-month OS, 4% for 3-month OS, and 13% for 3-month TFS, shown in Figs. 2, 3, Additional file 1: Figures S3, S4, S7, S8). Despite ELAD therapy, the only biological device ranked first for 1-month TFS, in the analysis for 3-month TFS, it had a SUCRA of 38%, even lower than SMT (41%). BioLogic-DT was included in the OS analyses and ranked second last in both cases. SMT had the lowest probability of being the best or second-best option in all four analyses.

Methodology-based analyses were also performed grouping the albumin-based (MARS and Prometheus) techniques, with very similar results (only the PE-SMT comparison for 3-month OS reaching statistical significance, Additional file 1: Figures S21 and S22).

Wilkinson et al. [45] provided data only for 5-day survival comparing BioLogic-DT with SMT in a small number of patients. The device seemed to be effective in bridging to transplant. Hu et al. [46] has found that MARS improved the survival of patients with chronic severe hepatitis with multiorgan failure. You

Table 1 Characteristics of included studies

First author, publication year	Eligibility criteria	Etiology of the underlying disease and baseline characteristics	Intervention(s) and/or comparison	Outcomes
Banares (2013)	Inclusion: presumptive diagnosis of cirrhosis with an identifiable triggering event; an increase of TBIL > 5 mg/dl and at least one of the following: HRS, HE ≥ grade II, rapidly progressive hyperbilirubinemia (> 50% increase from TBIL levels at admission) > 20 mg/dl at admission Exclusion: progressive jaundice as a consequence of the natural course of cirrhosis; extrahepatic cholestasis; PLT < 50,000/mm^3; INR > 2.3; suspected or evident DIC; need for RRT; intrinsic renal disease; uncontrolled infection; active bleeding; HCC > 4 cm in diameter; portal vein thrombosis; severe cardiopulmonary disease; MAP < 60 mmHg despite vasopressor therapy; major surgical procedure within the last 4 weeks; HIV infection	Mostly alcoholic; viral, autoimmune, drug-induced, NASH, etc. Age (years)[a]: 51.8/50.0 Males (%): 66.7/70.8 MELD[a]:25.6/24.1	MARS/SMT	Survival, HE, laboratory parameters, AEs
Duan (2018)	Inclusion: 15–65 years; clinical diagnosis of ACLF; obvious gastrointestinal and/or systemic toxic symptoms; TBIL > 5 times upper limit of normal or daily increase > 1 mg/dl; prothrombin activity of 10–50%; INR 1.6–4.0, or prothrombin time > 5 s longer than the control but < 20 s, HE absent or grade I–II; no or mild ascites/pleural effusion Exclusion: primary or metastatic liver cancer; uncontrolled severe infection; shock; active bleeding within 3 days; grade III–IV HE; PLT < 40 × 10^9/l; creatinine > 1.5 mg/ml; severe esophageal varices	Mostly alcoholic and viral; drug-induced, autoimmune, unknown, "acute/subacute" Age[a] (years): 39.5/39.2 Males (%): 96.9/88.2 MELD[a]: 28.0/30.8	ELAD/SMT	Survival, AEs
Ellis (1999)	Inclusion: acute alcoholic hepatitis, HE ≥ grade II Exclusion: pregnancy; MAP < 50 mmHg despite adequate volume loading and appropriate use of inotropes; respiratory failure; cerebrovascular event within the previous 12 months, a recent upper gastrointestinal hemorrhage; poorly controlled epilepsy; recent myocardial infarction/ischemia	Alcoholic Age[b] (years): 46/43 Males (%): 60/80 MELD/CTP: NR	BioLogic-DT/SMT	Survival, HE, physical and laboratory parameters, AEs
Hassanein (2007)	Inclusion: ≥ 18 years, manifestations of cirrhosis and HE grade III–IV Exclusion: active hemorrhage; hemodynamic instability; acute cardiopulmonary complications (pulmonary edema, massive aspiration pneumonia, heart failure); pregnancy; active RRT; drug intoxication or irreversible brain damage or nonhepatic causes of altered mental status; acute liver failure; HCC; received transplant	Mostly alcoholic or viral; autoimmune, drug induced, unknown Age[b] (years): 49/56 Males (%): 61.5/48.4 MELD[b]: 33/38	MARS/SMT	HE, AEs, laboratory parameters (survival is additional)

Table 1 (continued)

First author, publication year	Eligibility criteria	Etiology of the underlying disease and baseline characteristics	Intervention(s) and/or comparison	Outcomes
Heemann (2002)	Inclusion: 18–65 years; cirrhosis (CTP ≥ 7) and a superimposed acute liver injury leading to decompensation and severe hyperbilirubinemia (TBIL ≥ 20 mg/dl) Exclusion: hepatobiliary obstruction; active bleeding or sepsis causing hemodynamic instability; comorbid conditions associated with a poor outcome; coma of nonhepatic origin; extensive surgery 30 days preceding admission; HRS; pregnancy	Mostly alcoholic; viral, drug induced Age[b] (years): 48/57 Males (%): 50/63.6 CTP[b]: 11.5/12	MARS/SMT	Survival, HE, AEs, laboratory parameters
Hillebrand (2010)	Inclusion: acute decompensation of cirrhosis; SOFA score ≥ 9; and either a MELD score of ≥ 32, or MELD ≥ 24 and at least one of HE grade III–IV or type I HRS Exclusion: NR	Etiology, age, sex NR MELD[a]: 34.3/40.8	ELAD/SMT	Survival, AEs
Huang (2012)	Inclusion: chronic severe hepatitis B with HE ≥ grade II Exclusion: late stage disease; previous irreversible respiratory failure; severe brain edema with hernia; severe systemic circulation disorder accompanied by DIC; serious active bleeding	HBV Age[b] (years): 43/42 Males (%): 78.3/75 MELD/CTP: NR	MARS ± PE	Survival, HE, AEs, laboratory parameters, cost of treatment
Kramer (2001)	Inclusion: documented cirrhosis and encephalopathy grade II or III had not improved with conventional treatment Exclusion: renal failure; hypotension (MAP < 55 mmHg); respiratory or multiorgan failure; fever of > 38.5 °C; bleeding requiring transfusion of > 2 units within the preceding 24 h; insulin-dependent diabetes mellitus; administration of sedatives within the preceding 2 days	Alcoholic, viral, autoimmune, unknown Age[b] (years): 55/56 Males[c] (%): 65% CTP[b]: 14/14.5	BioLogic-DT/SMT	HE, laboratory and physical parameters, AEs (survival is additional)
Kribben (2012)	Inclusion: 18–70 years; severe deterioration of chronic liver disease; CTP ≥ 10 (over 72 h); TBIL ≥ 5 mg/dl (over 72 h) Exclusion: pregnancy/lactation; HIV infection, intracranial bleeding; cerebrovascular disease; ARDS; circulatory shock with vasopressor therapy; persistent bleeding needing perfusion; chronic renal failure stage V; acute necrotizing pancreatitis; HCC, malignancy; INR > 3.0 or PLT < 30,000/l; extrahepatic cholestasis; liver resections or major hepatobiliary surgery in the previous 6 months except laparoscopic cholecystectomy; LT within 2 years, ALSS therapy within 7 days; participation in another clinical trial or this study priorly	Mostly alcoholic and viral; others not specified Age[a] (years): 50/51 Males (%): 62/65 MELD[b]: 28/27	Prometheus/SMT	Survival, laboratory parameters; AEs

Table 1 (continued)

First author, publication year	Eligibility criteria	Etiology of the underlying disease and baseline characteristics	Intervention(s) and/or comparison	Outcomes
Mitzner (2000)	Inclusion: 18–60 years; HRS (serum creatinine > 1.5 mg/dl, oliguria < 500 ml/d, urine sodium < 20 mmol/l, central venous pressure > 8 cmH_2O); need of hemodialysis/filtration treatment; chronic liver failure (3 of 4 criteria): ultrasonic signs of chronic damage or impaired synthesis function (hypoalbuminemia, 30 g/l, prolonged prothrombin time (quick value < 70%), AT III < 70%, serum cholinesterase < 40 umol/s/l) or hyperbilirubinemia (> 15 mg/dl) or grade III–IV HE Exclusion: fulminant hepatic failure; sepsis unresponsive to antibiotics; severe acute hemorrhages; malignancies; obstructive/chronic renal failure; pregnancy; severe cardiopulmonary disease	Mostly alcoholic; HBV, primary and secondary biliary cirrhosis Age[a] (years): 49.6/43.8 Males (%): 37.5/40 CTP[a]: 12.5/12.2	MARS/SMT	Survival
Pyrsopoulos (2019)	Inclusion: SAH, age 18–50 years, total bilirubin ≥ 16 mg/dl, Maddrey score ≥ 32, not eligible for transplant Exclusion: PLT < 40,000/mm3; INR > 2.5; serum creatinine ≥ 1.3 mg/dl; MELD score ≥ 30; AST > 500 IU/l; infection unresponsive to antibiotics; reduction in TBIL ≥ 20% in the previous 72 h; hemodynamic instability; active bleeding; major hemorrhage; liver size reduction due to cirrhosis; occlusive portal vein thrombosis; bile duct obstruction; life expectancy of less than 3 months due to concomitant diseases; subject on hemodialysis; Wilson's disease; NAFLD; Budd-Chiari syndrome; active viral hepatitis; pregnancy; received liver transplant	Alcoholic hepatitis Age[a] (years): 39.1/39.5 Males (%): 60.3/60.3 MELD[a]: 24.8/25.6	ELAD/SMT	Survival, AEs
Qin (2014)	Inclusion: 18–70 years; presumptive diagnosis of chronic hepatitis B infection, HBV-associated cirrhosis, or hepatitis B surface antigen (HBsAg) carrier; rapidly progressive hyperbilirubinemia with TBIL > 10 mg/dl, within 28 days from symptom onset; INR > 1.5 or plasma prothrombin activity < 40% Exclusion: acute HBV infection; hepatitis E, A, D, or HIV superinfection; alcohol- or drug-induced liver injury; severe gastrointestinal bleeding; HCC; pregnancy	HBV Age[a] (years): 44.1/48.7 Males (%): 82.7/72.3 MELD[a]: 28.6/29.5	PE/SMT	Survival, AEs

Table 1 (continued)

First author, publication year	Eligibility criteria	Etiology of the underlying disease and baseline characteristics	Intervention(s) and/or comparison	Outcomes
Sen (2004)	Inclusion: 18–75 years old; alcoholic liver disease; acute deterioration in liver function over 2–4 weeks leading to severe progressive clinical deterioration despite supportive care (over 48 h); jaundice (TBIL > 100 mol/l) and either HE Grade II or HRS; cirrhosis Exclusion: prior enrollment in another study; known hepatic/extrahepatic malignancy; uncontrolled infection or upper gastrointestinal bleeding over the previous 48 h; pregnancy; prior treatment with terlipressin for HRS; coexisting HIV infection; severe cardiorespiratory disease	Alcoholic Age[b] (years): 45/44 Males (%): 78/67 MELD[b]: 16.5/19.4	MARS/SMT	Survival, HE, laboratory and physical parameters
Teperman (2012)	Inclusion: acute alcoholic hepatitis or acute decompensation of cirrhosis, MELD 18–35 Exclusion: NR	Alcoholic and not specified (baseline only given for PP subjects)	ELAD/SMT	Survival, time to progression, AEs
Thompson (2018)	Inclusion: ≥ 18 years, history of heavy alcohol abuse, maximum of 6 weeks between the last consumption, rapid onset of jaundice (TBIL ≥ 8 mg/dl), and coagulopathy (Maddrey's DF ≥ 32), stratum A: liver biopsy confirmed SAH/ 2 of the following: AST > ALT, leukocytosis, ascites stratum B: SAH + underlying chronic liver disease confirmed by biopsy, laboratory findings, and/or medical history Exclusion: end-stage cirrhosis; portal vein thrombosis; MELD > 35, PLT < 40,000/mm^3; severe concomitant disease; uncontrolled bleeding; infection unresponsive to antibiotics; hemodynamic instability; chronic dialysis	Alcoholic hepatitis (superimposed or primary) Age[a] (years): 46.5/44.8 Males%: 57.3/60.7 MELD[a]: 27.6/27.1	ELAD/SMT	Survival, laboratory parameters, AEs
Yu (2008)	Inclusion: acute-on-chronic hepatitis B liver failure (HBV-DNA ≥ 10,000 copies/mL); defined as severe jaundice (TBIL > 171 mmol/l), coagulopathy, and/or HE > grade II; previous lamivudine treatment; MELD > 30 Exclusion: obstructive and hemolytic jaundice; prolonged prothrombin time due to hematologic diseases; drug-induced hepatitis; Wilson's disease; alcoholic liver disease; autoimmune hepatitis; hepatitis C or D or HIV infection	HBV Age[a] (years): 45.2/46.4 Males (%): 80/78.6 MELD[a,d]: 41.4	PE/SMT	Survival, laboratory parameters
He (2000)*	Inclusion: severe viral hepatitis according to the criteria of the 1995 national symposium Exclusion: NR	Mostly viral; alcoholic Age, sex, MELD/CTP: NR	PE, PP, DHP/SMT	Survival, laboratory parameters, HE, AEs
Hu (2005)*	Inclusion: chronic severe hepatitis complicated with multiorgan failure Exclusion: NR	NR	MARS/SMT	Survival, HE, laboratory parameters

Table 1 (continued)

First author, publication year	Eligibility criteria	Etiology of the underlying disease and baseline characteristics	Intervention(s) and/or comparison	Outcomes
Krisper (2005)*	Inclusion: ACLF Exclusion: NR	Mostly alcoholic; HCV Age[c] (years): 57 Males[c] (%): 67% MELD[b]: 35.4	MARS and Prometheus, crossover	Laboratory parameters, AEs
Laleman (2006)*	Inclusion: 18–75 years; histologically proven alcoholic cirrhosis with superposed alcoholic hepatitis; portal hypertension with associated hyperdynamic circulation and ACLF (persistent deterioration in liver function despite treatment of the precipitating event and elevated bilirubin > 12 mg%) Exclusion: extrahepatic cholestasis; coma of nonhepatic origin; active gastrointestinal bleeding in the past 5 days; comorbidities associated with poor outcome (acute necrotizing pancreatitis, neoplasia, severe cardiopulmonary disease, oxygen-dependent or steroid-dependent COPD); ongoing infection; HRS type I	Alcoholic Age[a] (years): 54.5/43.2/55.8 Males (%): 83.3/66.7/50 MELD[a]: 22.7/29.7/24.3	MARS/Prometheus /SMT	Laboratory parameters, AEs
Meijers (2012)*	Inclusion: ≥ 18 years, compensated chronic liver disease; developed intrahepatic cholestasis (TBIL > 5 mg/dl); at least one of the following complications within 4–8 weeks after a potential identifiable acute superposed hepatic insult: (a) a progressive hyperbilirubinemia ≥ 50% increase of TBIL > 20 mg/dl, (b) HE grade ≥ II, (c) de novo development of ascites, and/or (d) HRS Exclusion: extrahepatic cholestasis; severe hypocalcemia ($Ca2+ < 0.9$ mmol·l^{-1}); acidosis (pH < 7.25)	Mostly alcoholic; HCV, NASH, and others Age[c] (years): 54.6 Males (%): NR MELD[a,c]: 32.1	MARS ± citrate, crossover	Laboratory parameters, AEs
Wilkinson (1998)*	Inclusion: decompensated chronic liver disease and grade III–IV encephalopathy Exclusion: NR	Alcoholic, HCV, HBV, autoimmune, unknown Age[a] (years): 58.3/42.7 Males (%): 60/100 MELD/CTP: NR	BioLogic-DT/SMT	Physiologic and neurologic improvement, AEs
You (2011)*	Inclusion: ACLF defined by the Chinese Medical Association's definition (2006) Exclusion: NR	Viral (?) Age[a] (years): 42.7/43.5 Males (%): 100/83 MELD[a]: 23/24.1	HBALSS/PE	Survival, AEs, laboratory parameters

Articles included in the quantitative and qualitative synthesis (indicated by *) are listed here

TBIL total bilirubin, *HRS* hepatorenal syndrome, *HE* hepatic encephalopathy, *PLT* platelet, *INR* international normalized ratio, *DIC* disseminated intravascular coagulation, *RRT* renal replacement therapy, *HCC* hepatocellular carcinoma, *MAP* mean arterial pressure, *HIV* human immunodeficiency virus, *NASH* non-alcoholic steatohepatitis, *MELD* Model for end-stage liver disease, *MARS* molecular adsorbent and recirculating system, *SMT* standard medical therapy, *AEs* adverse events, *ACLF* acute-on-chronic liver failure, *ELAD* extracorporeal liver assist device, *CTP* Child–Turcotte–Pugh, *NR* not reported, *SOFA* sequential organ failure assessment, *HBV* hepatitis B virus, *PE* plasma exchange, *ARDS* adult respiratory distress syndrome, *SAH* severe alcoholic hepatitis, *AST* aspartate aminotransferase, *ALT* alanine aminotrasferase, *NAFLD* nonalcoholic fatty liver disease, *PP* plasma perfusion, *DHP* direct hemoperfusion, *HCV* hepatitis C virus, *COPD* chronic obstructive pulmonary disease

[a] Mean values

[b] Median values

[c] All patients

[d] Only reported in the intervention group

et al. [47] tested the hybrid bioartificial liver supporting system (HBALSS) in 6 patients with similar mortality rate to controls. He et al. [48] tested the effects of plasma perfusion (PP), plasma exchange (PE), and direct hemoperfusion (DHP) compared with SMT and the results were reported in Chinese. A higher survival rate was reported in the intervention group (68.75% vs 46.67%) for the whole study population. Extracted data for mortality in the ACLF subgroup by Alshamsi et al. did not show a significant difference (RR 0.59, 95% CI 0.33–1.04) [19].

Long-term survival was assessed in six studies. Six-month survival was reported to be identical in both groups by Hassanein, Heemann, and Pyrsopoulos (additionally presented at a conference, together with 1-year survival) [31, 38, 42]. Duan et al. reported higher transplant-free survival in the ELAD group, maintained until the end of the 5-year follow-up [40]. On the contrary, Thompson et al. found comparable mortality in the two groups at 5 years [39]. Interestingly, Qin et al. showed that in the PE group the 5-year cumulative survival probability was significantly higher (43% vs 31% survived) and have found that treatment added about 6 months to the life expectancy of patients with HBV-associated ACLF.

Hepatic encephalopathy and ammonia

Altogether ten studies reported the changes in mental status, but for hepatic encephalopathy (HE) different scales and definitions were used (Additional file 1: Table S2). All studies reported improvement, which was statistically significant only in five cases, all using MARS therapy.

Ten studies reported changes in blood ammonia levels (Additional file 1: Table S4). Findings are controversial for MARS. Prometheus and BioLogic-DT do not remove ammonia effectively.

Bilirubin

Changes in total bilirubin (TBIL) were reported in twenty studies (Additional file 1: Table S3). The results were not pooled on account of different treatment doses, measurement time points, and definitions for bilirubin reduction. Hassanein et al. rightly pointed out that the time between the last treatment session and post-treatment measurements could greatly influence this outcome [38]. They showed that a single session of MARS reduced TBIL levels significantly, but this difference decreased by the end of the 5-day treatment period. MARS, PE, MARS combined with PE, Prometheus, ELAD, and HBALSS treatments significantly reduced bilirubin levels. Krisper et al. compared MARS and Prometheus in a crossover design and reported Prometheus to be more effective in the removal of conjugated and unconjugated bilirubin. BioLogic-DT does not remove bilirubin effectively.

Bile acids

Hassanein, Heemann, and Laleman found that both MARS and Prometheus reduced bile acid levels significantly ($P<0.001$ and $P<0.001$, respectively) [31, 38, 49]. Krisper et al. reported that MARS and Prometheus remove individual bile acids with different clearance rates [50]. On the other hand, Meijers et al. observed no significant reduction in bile acid levels after MARS sessions.

Creatinine and blood urea nitrogen

Changes in creatinine levels were reported in 12 cases (Additional file 1: Table S5). Findings for MARS and BioLogic-DT are controversial regarding creatinine removal from the blood, and Prometheus and plasma exchange therapy do not influence creatinine levels.

MARS, Prometheus, and BioLogic-DT were found to decrease blood urea nitrogen levels effectively.

Cytokines

TNF-α levels were reduced after 6 hours of BioLogic-DT treatment ($P=0.04$) as reported by Kramer et al. [32], but only small changes were observed by Ellis et al. [37]. MARS and Prometheus treatment did not reduce TNF-α levels [34, 51]. He et al. reported significant TNF-α reduction after treatment [48]. MARS did not change IL-6, IL-8, and IL-10 levels, similarly to TNF receptors 1 and 2 [34, 51]. Higher IL-8 levels were measured in the BioLogic-DT group [37]. Levels of anti-inflammatory protein IL-1 receptor antagonist were significantly elevated for days in ELAD-treated subjects [39].

Harms

In the numbers of adverse events (AEs) and reporting protocols, an immense heterogeneity was shown; therefore, quantitative data synthesis was not carried out. All devices were evaluated to be safe, and the number of AEs was comparable to the control groups. Hassanein et al. described nine possibly treatment-related adverse events in the MARS group; however, the nature of these was not detailed [38]. Acute hemolysis developed in one patient in the ELAD group [40] and treatment was discontinued in several subjects due to adverse events not specified [39, 41, 43]. Heemann et al. compared AEs in the MARS group to patients who received dialysis and found no significant difference. Two out of the twelve patients treated with MARS had fever/sepsis possibly related to the catheter [31].

Table 2 Summary of findings

Summary of findings							Quality of evidence
Intervention[1] (Studies[2])	Rank	Study event rates (%)		Risk ratio (95% CrI)	Anticipated absolute effects		Overall certainty of evidence
		With standard medical therapy[3]	With extracorporeal liver support devices[4]		Risk with standard medical therapy	Risk difference with extracorporeal liver support devices	
3-month overall survival (follow-up: range 84 days to 91 days)							
PE (2 RCTs)	1	334/569 (58.7%)	136/244 (55.7%)	RR 0.74 (0.60 to 0.94)	59 per 100	15 fewer per 100 (from 23 to 4 fewer)	⊕◯◯◯ Very low
MARS (2 RCTs)	2		12/17 (70.6%)	RR 0.78 (0.38 to 1.40)		13 fewer per 100 (from 36 fewer to 23 more)	⊕◯◯◯ Very low
Prometheus (1 RCT)	3		46/77 (59.7%)	RR 0.97 (0.68 to 1.40)		2 fewer per 100 (from 19 fewer to 23 more)	⊕◯◯◯ Very low
ELAD (4 RCTs)	4		78/213 (36.6%)	RR 0.99 (0.76 to 1.30)		1 fewer per 100 (from 14 fewer to 18 more)	⊕◯◯◯ Very low
BioLogic-DT (1 RCT)	5		5/5 (100.0%)	RR 1.00 (0.55 to 2.10)		0 fewer per 100 (from 26 fewer to 65 more)	⊕◯◯◯ Very low
1 month overall survival (follow-up: range 28 days to 31 days)							
PE (1 RCT)	1	122/359 (34.0%)	19/104 (18.3%)	RR 0.51 (0.12 to 2.40)	34 per 100	17 fewer per 100 (from 30 fewer to 48 more)	⊕⊕⊕◯ Moderate
MARS (3 RCTs)	2		109/113 (96.5%)	RR 0.60 (0.15 to 1.30)		14 fewer per 100 (from 29 fewer to 10 more)	⊕◯◯◯ Very low
MARS + PE (indirect)	3		7/60 (11.7%)	RR 0.60 (0.07 to 3.20)		14 fewer per 100 (from 32 fewer to 75 more)	⊕◯◯◯ Very low
Prometheus (1 RCT)	4		29/77 (37.7%)	RR 1.00 (0.25 to 4.30)		0 fewer per 100 (from 25 fewer to 100 more)	⊕◯◯◯ Very low
BioLogic-DT (1 RCT)	6		6/10 (60.0%)	RR 1.10 (0.24 to 5.40)		3 more per 100 (from 26 fewer to 100 more)	⊕◯◯◯ Very low
ELAD (3 RCTs)	7		26/117 (22.2%)	RR 1.40 (0.56 to 3.60)		14 more per 100 (from 15 fewer to 88 more)	⊕◯◯◯ Very low
3-month transplant-free survival (follow-up: range 84 days to 91 days)							
PE (1 RCT)	1	189/396 (47.7%)	42/104 (40.4%)	RR 0.77 (0.51 to 1.10)	41 per 100	11 fewer per 100 (from 23 fewer to 5 more)	⊕⊕⊕◯ Moderate
Prometheus (1 RCT)	2		52/77 (67.5%)	RR 0.96 (0.67 to 1.40)		2 fewer per 100 (from 16 fewer to 19 more)	⊕◯◯◯ Very low
ELAD (4 RCTs)	4		76/217 (35.0%)	RR 1.00 (0.78 to 1.40)		0 fewer per 100 (from 11 fewer to 19 more)	⊕◯◯◯ VERY LOW
MARS (1 RCT)	5		7/8 (87.5%)	RR 1.10 (0.61 to 2.10)		5 more per 100 (from 19 fewer to 53 more)	⊕◯◯◯ Very low

Table 2 (continued)

Summary of findings							Quality of evidence
Intervention[1] (Studies[2])	Rank	Study event rates (%)		Risk ratio (95% CrI)	Anticipated absolute effects		Overall certainty of evidence
		With standard medical therapy[3]	With extracorporeal liver support devices[4]		Risk with standard medical therapy	Risk difference with extracorporeal liver support devices	
1-month transplant-free survival (follow-up: range 28 days to 31 days)							
ELAD (2 RCTs)	1	109/264 (41.3%)	14/43 (32.6%)	RR 0.47 (0.13 to 1.20)	41 per 100	22 fewer per 100 (from 36 fewer to 8 more)	⊕◯◯◯ Very low
PE (1 RCT)	2		47/104 (45.2%)	RR 0.52 (0.21 to 1.20)		20 fewer per 100 (from 33 fewer to 8 more)	⊕⊕⊕◯ Moderate
MARS (3 RCTs)	3		60/122 (49.2%)	RR 0.96 (0.50 to 1.50)		2 fewer per 100 (from 21 fewer to 21 more)	⊕◯◯◯ Very low

Significant results are highlighted in italic

CrI credible interval, *PE* plasma exchange, *RCT* randomized controlled trial, *RR* risk ratio, *MARS* molecular adsorbent and recirculating system, *ELAD* extracorporeal liver assist device

[1] Intervention compared to SMT as reference comparator

[2] Number of studies included in the direct comparison

[3] Data from all studies

[4] Data from studies included in the direct comparison

Adverse events were reported in all but four papers in general. The most frequent complications were bleeding at the site of the catheter, clotting in the apparatus, and thrombocytopenia. Hypotension was reported in patients treated with PE and Prometheus [33, 49].

Risk of bias assessment and quality of evidence

The quality of evidence is shown in Table 2 (see Additional file 1: Table S1 for more detail). Quality of evidence was moderate for PE in the analysis of OS at 1 month and both TFS outcomes. All other results were of very low certainty. The results of the risk of bias assessment conducted separately for OS and TFS are shown in Additional file 1: Figures S13 and S14. Overall risk of bias was low in 50% of the studies included in the OS analyses. 33% carried moderate and 22% high risk of bias. For TFS, 22% of studies carried low, 22% moderate, and 46% high risk of bias.

Discussion

Extracorporeal liver support therapies have been and will remain of fundamental interest in the management of ACLF [52]. However, their benefits have been debated for long. Therefore, we conducted the first network meta-analysis focusing on patients with ACLF, assessing overall and transplant-free survival at 1 and 3 months. The analyses for OS yielded similar results, with PE ranking first and MARS second on the cumulative ranking curves in both cases. From all comparisons, only plasma exchange was associated with a statistically significant improvement, when compared to SMT in the analysis of 3-month overall survival, but with very low certainty of evidence. Other comparisons did not reach statistical significance, but SMT had very low probabilities of being the best option in all analyses.

Until then, evidence on the efficacy of PE in ACLF mostly originated from cohort studies. The APASL consensus guideline recommended the use of PE in ACLF

(See figure on next page.)

Fig. 2 **b** Studies included in the analysis for 3-month overall survival (OS). **c** Geometry of the network: the nodes represent the number of studies and the thickness of the lines corresponds to the number of direct comparisons. **a** League table: The league table contains the risk ratios (RR) and credible intervals (CrI) for every possible comparison of the interventions. Events were defined as death during the follow-up period (84–91 days). Significant results are highlighted in bold. **d** Cumulative ranking curves: On the x axis the cumulative probability of the treatment being in the first n rank is shown, while the y axis shows the ranks. **e** Surface under the cumulative ranking curves: The surface under the cumulative ranking curve (SUCRA) is a numeric presentation of the overall ranking and presents a single number associated with each treatment. SUCRA values range from 0 to 100%. The higher the SUCRA value, and the closer to 100%, the higher the likelihood that a therapy is in the top rank or one of the top ranks. The height of each bar corresponds to the SUCRA value of the respective treatment

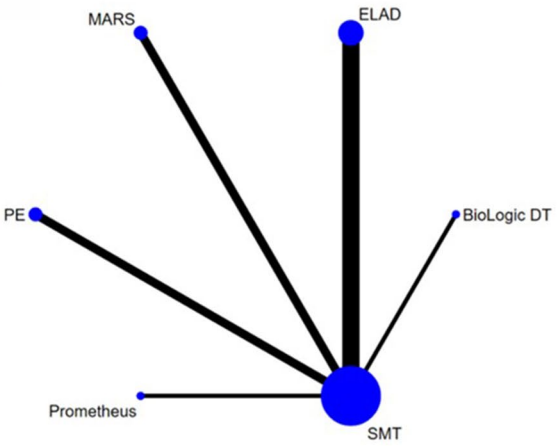
a

Treatment 1 / RR (95% CrI)	MARS	Prometheus	ELAD	BioLogicDT	SMT
PE	0.81 (0.36, 1.5)	0.98 (0.63, 1.6)	0.95 (0.46, 1.9)	1.0 (0.55, 2.1)	**0.74 (0.60, 0.94)**
0.95 (0.53, 2.0)	0.76 (0.50, 1.2)	0.75 (0.53, 1.1)	0.72 (0.35, 1.4)	0.78 (0.38, 1.4)	0.97 (0.68, 1.4)
MARS	Prometheus	ELAD	BioLogicDT	SMT	0.99 (0.76, 1.3)
0.79 (0.37, 1.5)	0.93 (0.43, 1.9)	1.0 (0.55, 2.1)	Treatment 2		

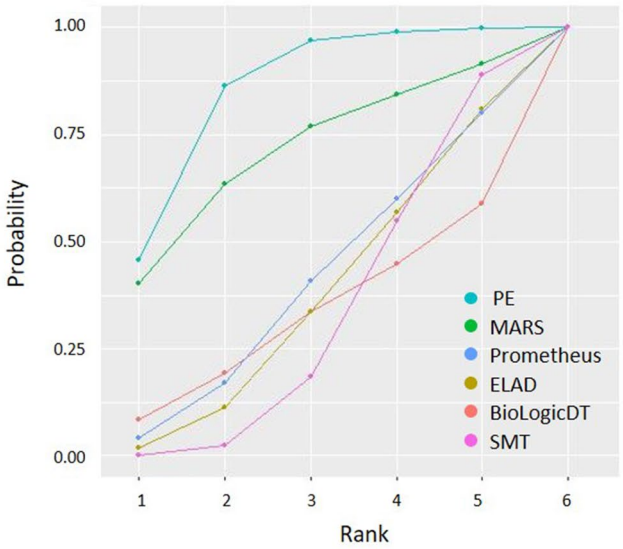
b

Study ID	Intervention(s)	Patients/group
Thompson (2018)	ELAD/SMT	96/107
Hillebrand (2010)	ELAD/SMT	14/4
Teperman (2012)	ELAD/SMT	25/28
Pyrsopoulos (2019)	ELAD/SMT	78/73
Qin (2014)	PE/SMT	104/130
Yu (2008)	PE/SMT	140/140
Ellis (1999)	BioLogicDT/SMT	5/5
Sen (2004)	MARS/SMT	9/9
Mitzner (2000)	MARS/SMT	8/5
Kribben (2012)	Prometheus/SMT	77/68

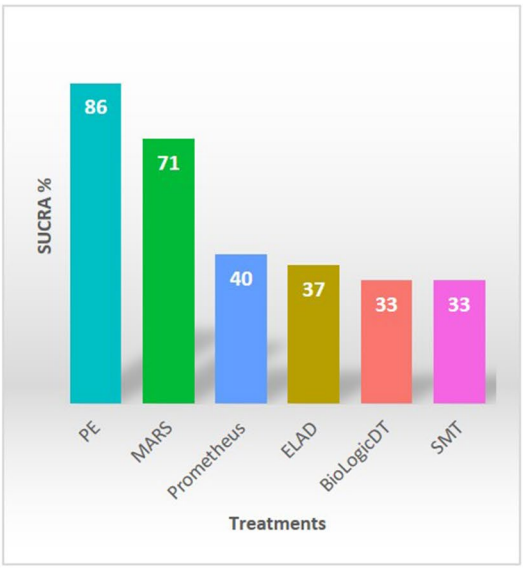
c



d



e



for bridging to transplantation or recovery. The EASL did not find the available evidence to be sufficient for recommending the use of any liver support therapy for the treatment of ACLF. High-volume PE was found to reduce mortality and effectively remove DAMPs, TNF-α, and IL-6 in ALF patients in an RCT [53, 54].

The role of immune dysfunction and dysregulated immune response in ACLF has recently come into focus.

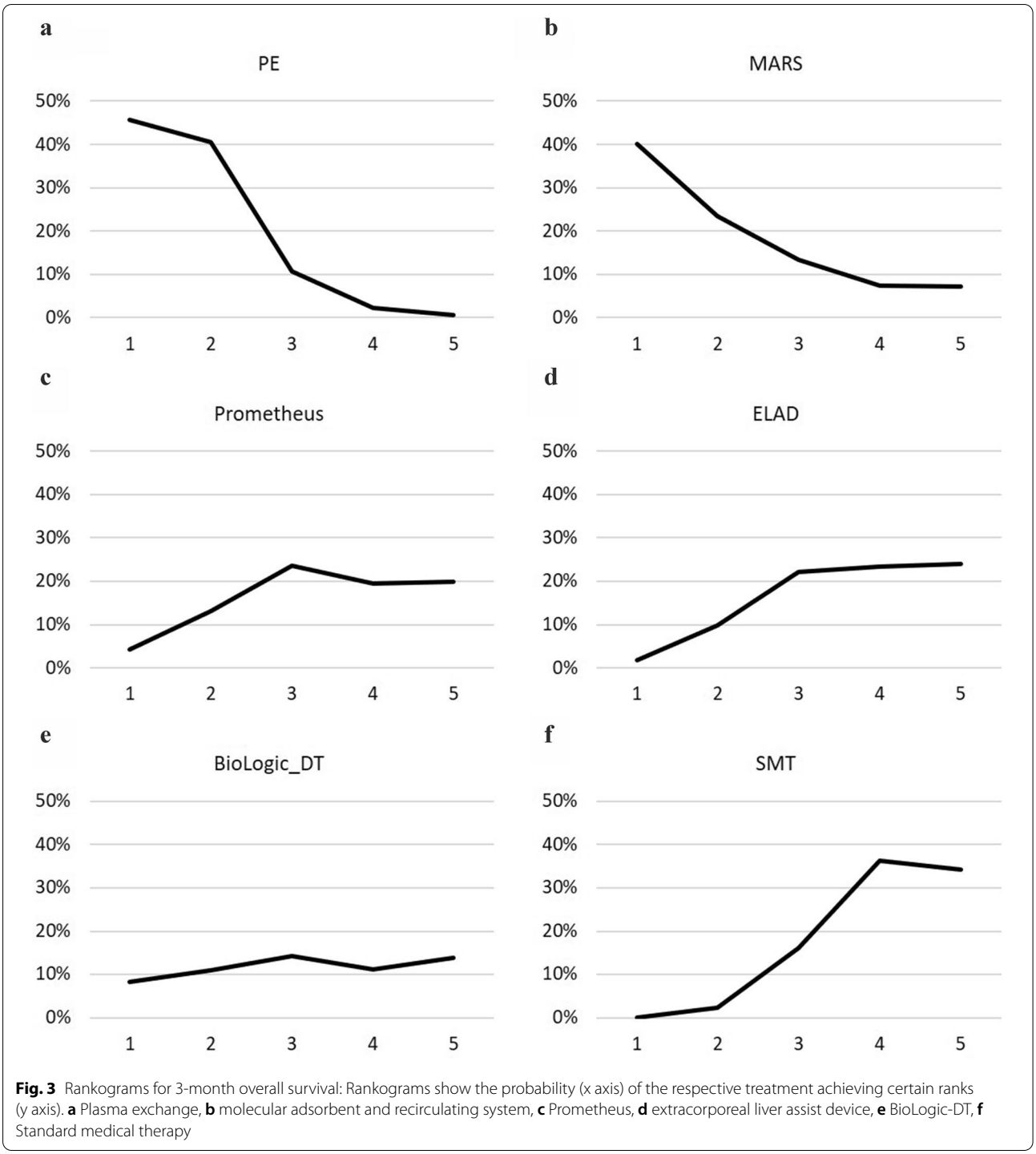


Fig. 3 Rankograms for 3-month overall survival: Rankograms show the probability (x axis) of the respective treatment achieving certain ranks (y axis). **a** Plasma exchange, **b** molecular adsorbent and recirculating system, **c** Prometheus, **d** extracorporeal liver assist device, **e** BioLogic-DT, **f** Standard medical therapy

Both hyper-inflammation and immunosuppression play a role in acute decompensation [1, 7]. Inflammation represented by elevated inflammatory markers was previously thought to be a consequence of ongoing infection, but lately endogenous inducers were identified as underlying causes [2]. Bioartificial devices have the potential of synthetic functions and contribution to the immune response [55]. So far, only ELAD was tested in RCTs, always compared to SMT. Although ELAD did not perform well on the cumulative ranking curves, significantly higher IL-1 receptor antagonist levels were measured during ELAD therapy than in controls [39]. Based on this

finding, the immunomodulatory functions of bioartificial devices should be further assessed.

Several new devices are being tested in animal models of liver failure, including both artificial and bioartificial ones [56, 57], and ongoing clinical trials are enrolling ACLF patients ([58], NCT03882346, NCT04051437). Other blood purification methods, such as CytoSorb ™ therapy, also seem promising [59, 60], but they have not yet been evaluated in a randomized setting. Nevertheless, according to a recent in vitro experimental model, CytoSorb hemoperfusion leads to an initially faster removal of cytokines, like TNF-α and IL-6, as well as more effective reduction of albumin-bound toxins, such as indirect bilirubin and bile acids, compared to MARS [61].

There are some strengths and several limitations to our study. This is the first NMA in this field using the latest recommendations from the Cochrane Collaboration for statistical analysis, risk of bias, and QE assessment. We evaluated OS and TFS separately, at 1 and 3 months. We did not pool in-hospital, short-term, and long-term survival data. Studies enrolling patients with hepatorenal syndrome were not excluded with the aim of including cases with poorer prognosis. This new methodology enabled the comparison and ranking of different devices and highlighted the need for international consensus on the definition of ACLF and further trials testing already existing and new devices.

The absence of loops in all of the created networks limits statistical analysis in Bayesian networks and results in wider credible intervals. Transitivity could not be directly tested, but we think that the differences between the study populations do not violate the assumption of transitivity. The analyses included relatively few studies, some of them only enrolling less than 10 subjects per group, raising concerns about the beta-type error. Most importantly, due to the different definitions of ACLF used (Table 1), patient characteristics can differ significantly among studies, resulting in a highly heterogeneous population in our study. Eligibility criteria and the ratio of viral and alcoholic etiology differs in the included studies, but all patients were diagnosed with ACLF. Differences in the study populations may explain some of the controversial results of RCTs included in this meta-analysis. Also, in some of the included studies mortality was not a primary endpoint and was reported additionally; therefore, bias arises. The recruitment period for the included trials ranges from March 1997 until February 2015, which could impose chronological bias. Variance in SMT and treatment dose also could have influenced outcomes [62]. Due to the differences in treatment dose, cut-offs and reporting protocols, data on HE, laboratory parameters, and AEs could not be analyzed quantitatively.

Conclusion

Implication for practice

Plasma exchange seems to have the most beneficial effect at present, but liver support devices in general had higher probabilities for the first two ranks than SMT. Choosing the best option remains in the hands of the attending physician.

Implication for research

International consensus is needed to standardize the definition of ACLF. Further RCTs targeting carefully selected subgroups of the ACLF population, using already existing and new therapeutic methods are needed to produce high-quality evidence for guideline development.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13613-020-00795-0.

Additional file 1. Information collected form each study, additional information used. **Figure S1.** Geometry of the network and included studies for the analysis of 1-month overall survival. **Figure S2.** League table of 1-month overall survival. **Figure S3.** Cumulative ranking curves and SUCRA values for 1-month overall survival. **Figure S4.** Rankograms for 1-month overall survival. **Figure S5.** Geometry of the network and included studies for the analysis of 3-month transplant-free survival. **Figure S6.** League table of 3-month transplant-free survival. **Figure S7.** Ranking of treatments for 3-month transplant-free survival. **Figure S8.** Rankograms for 3-month transplant-free survival. **Figure S9.** Geometry of the network and included studies for the analysis of 1-month transplant-free survival. **Figure S10.** League table of 1-month transplant-free survival. **Figure S11.** Ranking of treatments for 1-month transplant-free survival. **Figure S12.** Rankograms for 1-month transplant-free survival. **Figure S13.** Risk of bias assessment for overall survival. **Figure S14.** Risk of bias assessment for transplant-free survival. **Table S1.** Quality of evidence. **Table S2.** Assessment of hepatic encephalopathy in the included studies. **Table S3.** Assessment of bilirubin reduction in the included studies. **Table S4.** Assessment of ammonia reduction in the included studies. **Table S5.** Assessment of creatinine reduction in the included studies. **Figure S15.** Forrest plots for 3-month overall survival. **Figure S16.** Forrest plots for 1-month overall survival. **Figure S17.** Forrest plots for 3-month transplant-free survival. **Figure S18.** Forrest plots for 1-month transplant-free survival. **Figure S19.** Funnel plot and Egger's test for 3-month overall survival. **Figure S20.** Funnel plot and Egger's test for 1-month overall survival. **Figure S21.** Cummulative ranking curves and SUCRA for methodology-based evaluation. **Figure S22.** Methodology-based evaluation league tables.

Abbreviations

ACLF: Acute-on-chronic liver failure; APASL: Asian Pacific Association for the Study of the Liver; AE: Adverse event; CrI: Credible interval; DAMP: Damage-associated molecular pattern; DHP: Direct hemoperfusion; EASL: European Association for the Study of the Liver; ELAD: Extracorporeal liver assist device; HBALSS: Hybrid bioartificial liver support system; HBV: Hepatitis B virus; IL: Interleukin; MARS: Molecular adsorbent recirculating system; NMA: Network meta-analysis; OS: Overall survival; PAMP: Pathogen-associated molecular patterns; PE: Plasma exchange; PP: Plasma perfusion; RCT: Randomized controlled trial; RR: Risk ratio; SMT: Standard medical therapy; SUCRA: Surface under the cumulative ranking curve; QE: Quality of evidence; TBIL: Total bilirubin; TNF-α: Tumor necrosis factor alpha; TFS: Transplant-free survival.

Acknowledgements

The authors would like to thank Szabolcs Kiss for the methodological advice and education on network meta-analyses.

Authors' contributions
KO registered the study, performed the selection, data collection, risk of bias, and quality of evidence assessment; contributed to the graphical presentation of the results; and wrote the draft of the manuscript. AK performed the selection, data collection, risk of bias, and quality of evidence assessment. NG performed the statistical analysis and contributed to the interpretation of findings. ZS provided methodological guidance and was a major contributor in writing the manuscript. GP, BE, JS, and SM provided insight from the clinical perspective and contributed to the interpretation of findings. They also corrected and shaped the manuscript. PH provided the funding and infrastructure in carrying out the study along with counsel on the interpretation and presentation of the results. ZM coordinated the work group and substantially revised the manuscript. All the authors read and approved the final manuscript.

Funding
Any costs were funded by GINOP-2.3.2-15-2016-00048—STAY ALIVE co-financed by the European Union (European Regional Development Fund) within the framework of the Széchenyi 2020 program and by a Human Resources Development Operational Program Grant, Grant Number EFOP 3.6.2-16-2017-00006—LIVE LONGER, co-financed by the European Union (European Regional Development Fund) within the framework of the Széchenyi 2020 program as well as the Translational Medicine Foundation. The sponsor was not involved in the design of the study.

Availability of data and materials
The dataset(s) supporting the conclusions of this article is(are) included within the article (and its additional file(s)).

Ethics approval and consent to participate
Given the nature of this study, ethic approval and consent was not required. Already published data were used.

Consent for publication
This study does not contain individual data.

Competing interests
ZM is one of the Medical directors at CytoSorbents Europe.

Author details
[1] Institute for Translational Medicine, Medical School, University of Pécs, Szigeti út 12. 2nd floor, Pécs 7624, Hungary. [2] Heim Pál National Paediatric Institute, Budapest, Hungary. [3] Institute of Bioanalysis, Medical School, University of Pécs, Pécs, Hungary. [4] Division of Gastroenterology, First Department of Medicine, Medical School, University of Pécs, Pécs, Hungary. [5] Division of Nephrology, Department of Medicine, University of Rostock, Rostock, Germany. [6] First Department of Medicine, University of Szeged, Szeged, Hungary. [7] Translational Medicine Foundation, Szeged, Hungary. [8] Department of Anaesthesiology and Intensive Therapy, Faculty of Medicine, Poznan University of Medical Sciences, Poznan, Poland.

Received: 15 July 2020 Accepted: 18 December 2020
Published online: 18 January 2021

References

1. Clària J, Arroyo V, Moreau R. The acute-on-chronic liver failure syndrome, or when the innate immune system goes astray. J Immunol. 2016;197(10):3755.
2. Chen P, Wang Y-Y, Chen C, Guan J, Zhu H-H, Chen Z. The immunological roles in acute-on-chronic liver failure: an update. Hepatobiliary Pancreat Dis Int. 2019;18(5):403–11.
3. Wu W, Yan H, Zhao H, Sun W, Yang Q, Sheng J, et al. Characteristics of systemic inflammation in hepatitis B-precipitated ACLF: Differentiate it from No-ACLF. Liver Int. 2018;38(2):248–57.
4. Clària J, Stauber RE, Coenraad MJ, Moreau R, Jalan R, Pavesi M, et al. Systemic inflammation in decompensated cirrhosis: Characterization and role in acute-on-chronic liver failure. Hepatology. 2016;64(4):1249–64.
5. Markwick LJ, Riva A, Ryan JM, Cooksley H, Palma E, Tranah TH, et al. Blockade of PD1 and TIM3 restores innate and adaptive immunity in patients with acute alcoholic hepatitis. Gastroenterology. 2015;148(3):590-602.e10.
6. Bernsmeier C, Pop OT, Singanayagam A, Triantafyllou E, Patel VC, Weston CJ, et al. Patients with acute-on-chronic liver failure have increased numbers of regulatory immune cells expressing the receptor tyrosine kinase MERTK. Gastroenterology. 2015;148(3):603-15.e14.
7. Wasmuth HE, Kunz D, Yagmur E, Timmer-Stranghöner A, Vidacek D, Siewert E, et al. Patients with acute on chronic liver failure display & #x2018;sepsis-like' immune paralysis. J Hepatol. 2005;42(2):195–201.
8. Berres ML, Schnyder B, Yagmur E, Inglis B, Stanzel S, Tischendorf JJ, et al. Longitudinal monocyte human leukocyte antigen-DR expression is a prognostic marker in critically ill patients with decompensated liver cirrhosis. Liver Int. 2009;29(4):536–43.
9. O'Brien AJ, Fullerton JN, Massey KA, Auld G, Sewell G, James S, et al. Immunosuppression in acutely decompensated cirrhosis is mediated by prostaglandin E2. Nat Med. 2014;20(5):518–23.
10. Finkenstedt A, Nachbaur K, Zoller H, Joannidis M, Pratschke J, Graziadei IW, et al. Acute-on-chronic liver failure: Excellent outcomes after liver transplantation but high mortality on the wait list. Liver Transpl. 2013;19(8):879–86.
11. García Martínez JJ, Bendjelid K. Artificial liver support systems: what is new over the last decade? Ann Intensive Care. 2018;8(1):109.
12. Dunlop EH, Weston MJ, Gazzard BG, Langley PG, Mellon PJ, Williams R. Artificial liver support based on haemoperfusion of adsorbents. Biomed Eng. 1975;10(6):213–8.
13. Sarin SK, Choudhury A, Sharma MK, Maiwall R, Al Mahtab M, Rahman S, et al. Acute-on-chronic liver failure: consensus recommendations of the Asian Pacific association for the study of the liver (APASL): an update. Hep Int. 2019;13(4):353–90.
14. European Association for the Study of the Liver. EASL Clinical Practice Guidelines for the management of patients with decompensated cirrhosis. J Hepatol. 2018;69(2):406–60.
15. Kjaergard LL, Liu J, Als-Nielsen B, Gluud C. Artificial and bioartificial support systems for acute and acute-on-chronic liver failure: a systematic review. JAMA. 2003;289(2):217–22.
16. Liu JP, Gluud LL, Als-Nielsen B, Gluud C. Artificial and bioartificial support systems for liver failure. Cochrane Database Syst Rev. 2004;2004(1):CD003628.
17. Zheng Z, Li X, Li Z, Ma X. Artificial and bioartificial liver support systems for acute and acute-on-chronic hepatic failure: a meta-analysis and meta-regression. Exp Ther Med. 2013;6(4):929–36.
18. Shen Y, Wang XL, Wang B, Shao JG, Liu YM, Qin Y, et al. Survival benefits with artificial liver support system for acute-on-chronic liver failure: a time series-based meta-analysis. Medicine. 2016;95(3):e2506.
19. Alshamsi F, Alshammari K, Belley-Cote E, Dionne J, Albrahim T, Albudoor B, et al. Extracorporeal liver support in patients with liver failure: a systematic review and meta-analysis of randomized trials. Intensive Care Med. 2020;46(1):1–16.
20. Stutchfield BM, Simpson K, Wigmore SJ. Systematic review and meta-analysis of survival following extracorporeal liver support. Br J Surg. 2011;98(5):623–31.
21. Khuroo MS, Khuroo MS, Farahat KL. Molecular adsorbent recirculating system for acute and acute-on-chronic liver failure: a meta-analysis. Liver Transpl. 2004;10(9):1099–106.
22. He GL, Feng L, Duan CY, Hu X, Zhou CJ, Cheng Y, et al. Meta-analysis of survival with the molecular adsorbent recirculating system for liver failure. Int J Clin Exp Med. 2015;8(10):17046–54.
23. Rouse B, Chaimani A, Li T. Network meta-analysis: an introduction for clinicians. Intern Emerg Med. 2017;12(1):103–11.
24. Hutton B, Salanti G, Caldwell DM, Chaimani A, Schmid CH, Cameron C, et al. The PRISMA extension statement for reporting of systematic reviews incorporating network meta-analyses of health care interventions: checklist and explanations. Ann Intern Med. 2015;162(11):777–84.
25. Sterne JAC, Savović J, Page MJ, Elbers RG, Blencowe NS, Boutron I, et al. RoB 2: a revised tool for assessing risk of bias in randomised trials. BMJ. 2019;366:l4898.
26. Juan José Yepes-Nunes HJS. 'Summary of findings' tables in network meta-analysis (NMA) 2018. https://training.cochrane.org/resource/SoF-tables-nma.

27. Dias S, Welton NJ, Caldwell DM, Ades AE. Checking consistency in mixed treatment comparison meta-analysis. Stat Med. 2010;29(7–8):932–44.
28. Mbuagbaw L, Rochwerg B, Jaeschke R, Heels-Andsell D, Alhazzani W, Thabane L, et al. Approaches to interpreting and choosing the best treatments in network meta-analyses. Syst Rev. 2017;6(1):79.
29. Mitzner SR, Stange J, Klammt S, Risler T, Erley CM, Bader BD, et al. Improvement of hepatorenal syndrome with extracorporeal albumin dialysis MARS: results of a prospective, randomized, controlled clinical trial. Liver Transpl. 2000;6(3):277–86.
30. Bañares R, Nevens F, Larsen FS, Jalan R, Albillos A, Dollinger M, et al. Extracorporeal albumin dialysis with the molecular adsorbent recirculating system in acute-on-chronic liver failure: the RELIEF trial. Hepatology. 2013;57(3):1153–62.
31. Heemann U, Treichel U, Loock J, Philipp T, Gerken G, Malago M, et al. Albumin dialysis in cirrhosis with superimposed acute liver injury: a prospective, controlled study. Hepatology. 2002;36(4 Pt 1):949–58.
32. Kramer L, Gendo A, Madl C, Mullen KD, Kaminski-Russ K, Sunder-Plassmann G, et al. A controlled study of sorbent suspension dialysis in chronic liver disease and hepatic encephalopathy. Int J Artif Organs. 2001;24(7):434–42.
33. Qin G, Shao JG, Wang B, Shen Y, Zheng J, Liu XJ, et al. Artificial liver support system improves short- and long-term outcomes of patients with HBV-associated acute-on-chronic liver failure: a single-center experience. Medicine (United States). 2014;93(28):e338.
34. Sen S, Davies NA, Mookerjee RP, Cheshire LM, Hodges SJ, Williams R, et al. Pathophysiological effects of albumin dialysis in acute-on-chronic liver failure: a randomized controlled study. Liver Transpl. 2004;10(9):1109–19.
35. Kribben A, Gerken G, Haag S, Hergetrosenthal S, Treichel U, Betz C, et al. Effects of fractionated plasma separation and adsorption on survival in patients with acute-on-chronic liver failure. Gastroenterology. 2012;142(4):782-9.e3.
36. Yu JW, Sun LJ, Zhao YH, Li SC. Prediction value of model for end-stage liver disease scoring system on prognosis in patients with acute-on-chronic hepatitis B liver failure after plasma exchange and lamivudine treatment. J Gastroenterol Hepatol. 2008;23(8 Pt 1):1242–9.
37. Ellis AJ, Hughes RD, Nicholl D, Langley PG, Wendon JA, O'Grady JG, et al. Temporary extracorporeal liver support for severe acute alcoholic hepatitis using the BioLogic-DT. Int J Artif Organs. 1999;22(1):27–34.
38. Hassanein TI, Tofteng F, Brown RS Jr, McGuire B, Lynch P, Mehta R, et al. Randomized controlled study of extracorporeal albumin dialysis for hepatic encephalopathy in advanced cirrhosis. Hepatology. 2007;46(6):1853–62.
39. Thompson J, Jones N, Al-Khafaji A, Malik S, Reich D, Munoz S, et al. Extracorporeal cellular therapy (ELAD) in severe alcoholic hepatitis: a multinational, prospective, controlled, randomized trial. Liver Transpl. 2018;24(3):380–93.
40. Duan Z, Xin S, Zhang J, You S, Chen Y, Liu H, et al. Comparison of extracorporeal cellular therapy (ELAD®) vs standard of care in a randomized controlled clinical trial in treating Chinese subjects with acute-on-chronic liver failure. Hepat Med Evid Res. 2018;10:139–52.
41. Teperman L. A phase 2b study of safety & efficacy in subjects with acute-on-chronic hepatitis (AOCH) due either to acute alcoholic hepatitis or acute decompensation of cirrhosis & the use of a support system (elad®) a human cell-based biological liver. Am J Transplant. 2012;12:64.
42. Pyrsopoulos NT, Hassanein T, Subramanian R, Thompson J, Caldwell S, Al-Khafaji A, et al. A study investigating the effect of extracorporeal cellular therapy with C3A cells on the survival of alcoholic hepatitis designed along the guidelines of the NIAAA. J Hepatol. 2019;70(1):e282.
43. Hillebrand DJ, Frederick RT, Williams WW, Brown RS, Napotilano LM, Te HS, et al. Safety and efficacy of the Extracorporeal Liver Assist Device (ELAD®) in patients with acute on chronic liver failure. J Hepatol. 2010;52:S323–4.
44. Huang YK, Tan DM, Xie YT, Fan XG, Huang Y, Liu ZB, et al. Randomized controlled study of plasma exchange combined with molecular adsorbent re-circulating system for the treatment of liver failure complicated with hepatic encephalopathy. Hepatogastroenterology. 2012;59(117):1323–6.
45. Wilkinson AH, Ash SR, Nissenson AR. Hemodiabsorption in treatment of hepatic failure. J Transplant Coord. 1998;8(1):43–50.
46. Hu XB, Zhou Y, Zhao YQ, Liao ME, He MR, Wang MM. Therapeutic application of molecular adsorbents recirculating system (MARS) in chronic severe hepatitis patients complicated with multi organ failure. J Hepatol. 2005;42(Suppl 2):179.
47. You SL, Liu HL, Rong YH, Zhu B, Liu WS, Mao PY, et al. Clinical study on hybrid bioartificial liver supporting system for acute on chronic liver failure patients. Zhonghua shi yan he lin chuang bing du xue za zhi Zhonghua shiyan he linchuang bingduxue zazhi Chin J Exp Clin Virol. 2011;25(5):387–9.
48. He JQCC, Deng JT, Qi HX, Zhang XQ. Chen ZQ Clinical study on the treatment of fatal hepatitis with artificial liver support system. Chin Crit Care Med. 2000;12:105–8.
49. Laleman W, Wilmer A, Evenepoel P, Elst IV, Zeegers M, Zaman Z, et al. Effect of the molecular adsorbent recirculating system and Prometheus devices on systemic haemodynamics and vasoactive agents in patients with acute-on-chronic alcoholic liver failure. Crit Care. 2006;10(4):R108.
50. Stadlbauer V, Krisper P, Beuers U, Haditsch B, Schneditz D, Jung A, et al. Removal of bile acids by two different extracorporeal liver support systems in acute-on-chronic liver failure. ASAIO J. 2007;53(2):187–93.
51. Stadlbauer V, Krisper P, Aigner R, Haditsch B, Jung A, Lackner C, et al. Effect of extracorporeal liver support by MARS and Prometheus on serum cytokines in acute-on-chronic liver failure. Crit Care. 2006;10:R169.
52. Fuhrmann V. Extracorporeal therapies in liver failure. Ther Apheresis Dialysis. 2018;22(4):427.
53. Karvellas CJ, Stravitz RT. High volume plasma exchange in acute liver failure: dampening the inflammatory cascade? J Hepatol. 2016;64(1):10–2.
54. Larsen FS, Schmidt LE, Bernsmeier C, Rasmussen A, Isoniemi H, Patel VC, et al. High-volume plasma exchange in patients with acute liver failure: an open randomised controlled trial. J Hepatol. 2016;64(1):69–78.
55. Carpentier B, Gautier A, Legallais C. Artificial and bioartificial liver devices: present and future. Gut. 2009;58(12):1690.
56. He Y-T, Qi Y-N, Zhang B-Q, Li J-B, Bao J. Bioartificial liver support systems for acute liver failure: a systematic review and meta-analysis of the clinical and preclinical literature. World J Gastroenterol. 2019;25(27):3634–48.
57. Defterevos G, Nastos C, Papalois A, Kalimeris K, Margelos V, Fragulidis G, et al. Peritoneal albumin dialysis as a novel approach for liver support: study in a porcine model of acute hepatic failure. Artif Organs. 2016;40(8):755–64.
58. Stange J, Klammt S, Mitzner S, Koball S, Hinz M, Gloger M, et al. Extracorporeal albumin dialysis using microparticle charcoal for albumin recycling is superior to using mars macroparticle adsorbents in removing albumin bound toxins (ABT). Hepatology. 2017;66:672A.
59. Acar U, Gokkaya Z, Akbulut A, Ferah O, Yenidunya O, Acik ME, et al. Impact of cytokine adsorption treatment in liver failure. Transpl Proc. 2019;51(7):2420–4.
60. Dhokia VD, Madhavan D, Austin A, Morris CG. Novel use of Cytosorb haemadsorption to provide biochemical control in liver impairment. J Intensive Care Soc. 2019;20(2):174–81.
61. Dominik A, Stange J. Similarities, differences, and potential synergies in the mechanism of action of albumin dialysis using the MARS albumin dialysis device and the cytosorb hemoperfusion device in the treatment of liver failure. Blood Purif. 2020. https://doi.org/10.1159/000508810.
62. Banares R, Ibanez-Samaniego L, Torner JM, Pavesi M, Olmedo C, Catalina MV, et al. Meta-analysis of individual patient data of albumin dialysis in acute-on-chronic liver failure: focus on treatment intensity. Ther Adv Gastroenterol. 2019;12:1756284819879565.

Publisher's Note