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A CRITICAL EVALUATION OF THE MARS TREATMENT IN FINLAND

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ACADEMIC DISSERTATION

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To my lovely little flower girl, Lilja Alexandra

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications I-VI which are referred to by their Roman numerals in the text. The original publications are presented with the permission from the publishers and copyright holders.

I Anna-Maria Koivusalo, Taru Teikari, Krister Höckerstedt, Helena Isoniemi "Albumin dialysis has a favorable effect on amino acid profile in hepatic encephalopathy" *Metabolic Brain Disease* 2008 Dec;23(4):387-98.

II Taru Kantola, Anna-Maria Koivusalo, Krister Höckerstedt, Helena Isoniemi "The effect of molecular adsorbent recirculating system treatment on survival, native liver recovery and need for liver transplantation in acute liver failure patients" *Transplant International* 2008 Sep;21(9):857-66.

III Taru Kantola, Teemu Kantola, Anna-Maria Koivusalo, Krister Höckerstedt, Helena Isoniemi "Early molecular adsorbents recirculating system treatment of *Amanita* mushroom poisoning" *Therapeutic Apheresis and Dialysis* 2009 Oct;13(5):399-403.

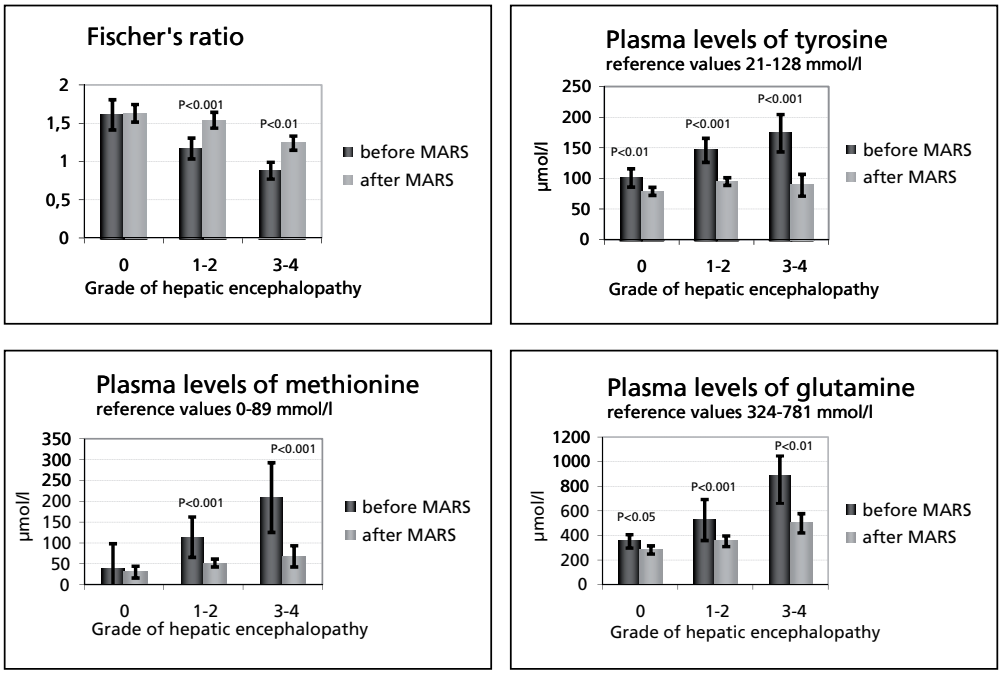
IV Anna-Maria Koivusalo, Taru Kantola, Johanna Arola, Krister Höckerstedt, Pekka Kairaluoma, Helena Isoniemi "Is it possible to gain extra waiting time to liver transplantation in acute liver failure patients using albumin dialysis?" *Therapeutic Apheresis and Dialysis* 2009 Oct;13(5):413-418.

V Taru Kantola, Anna-Maria Koivusalo, Satu Parmanen, Krister Höckerstedt, Helena Isoniemi "Survival predictors in patients treated with a molecular adsorbent recirculating system" *World Journal of Gastroenterology* 2009 Jun 28;15(24):3015-24.

VI Taru Kantola, Suvi Mäklin, Anna-Maria Koivusalo, Pirjo Räsänen, Anne Rissanen, Risto Roine, Harri Sintonen, Krister Höckerstedt, Helena Isoniemi "The cost-utility of molecular adsorbent recirculating system (MARS) treatment in acute liver failure". *Submitted.*

ERRATUM

Study I, Figure 1.



Study II, Table 1.

Nonparacetamol-related toxic ALF:
MARS group: % male patients: 56% (17), % of contra-indications 16% (5)
Control group: % of male patients: 33% (6), % of contra-indications 16% (5).
In unknown etiology ALF: contra-indications to Ltx in MARS group 10% (4) and Control group 12% (3).

Study IV, Table 1.

Etiology	All patients N=37 patients	Group I 100% necrosis N=9 patients	Group II 80%-99% necrosis N=10 patients	Group III < 80% necrosis N=18 patients
Unknown	26	6	8	12
Toxic	9	3 (3 disulfiram)	2 (1 idiosyncratic drug reaction, 1 amanita	4 (1 paracetamol, 1 iron, 2 idiosyncratic drug reactions)
Other	2			2 (Hepatitis A, Budd-Chiari)

Study V, text (p.3019)

1-year survival rate in non-transplanted other AOCLF patients: 19% (8/43).

ABBREVIATIONS

AAAs	Aromatic amino acids
ALF	Acute liver failure
AMC-BAL	Amsterdam medical center - Bioartificial liver
AOCLF	Acute-on-chronic liver failure
APACHE II	Acute physiology and chronic health evaluation II
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BAL	Bioartificial liver-assist device
BCAAs	Branched chain amino acids
BLSS	Bioartificial liver support system
C.I.	Confidence interval
ELAD	Extracorporeal liver-assist device
FHF	Fulminant hepatic failure
FLTR	Finnish liver transplant registry
FV	Coagulation Factor V
Gc-protein	Group specific protein
GF	Graft failure
GI	Gastrointestinal
HE	Hepatic encephalopathy
HRQoL	Health-related quality of life
HRS	Hepatorenal syndrome
HUCH	Helsinki university central hospital
HVPF	High-volume plasmapheresis
ICP	Intracranial pressure
ICU	Intensive care unit
INR	International normalized ratio
kD	KiloDaltons (atomic mass unit)
Ltx	Liver transplantation
MAP	Mean arterial blood pressure
MARS	Molecular adsorbent recirculating system
MELD	Model for end-stage liver disease-score
MELS	Modular extracorporeal liver support
NAC	N-acetylcystein
OR	Odds ratio
PDG	Primary dysfunctioning graft
PNF	Primary non-functioning graft
QALY	Quality-adjusted life year
RCT	Randomized controlled trial
SMT	Standard medical therapy
SBP	Spontaneous bacterial peritonitis
SFHF	Subfulminant hepatic failure
SPAD	Single-pass albumin dialysis

ABSTRACT

Background: The molecular adsorbent recirculating system (MARS) is an extracorporeal albumin dialysis device that has been used in the treatment of liver failure patients to enable native liver regeneration or as a bridging treatment to liver transplantation (Ltx). MARS treatment was first used in Finland in 2001, and since then, over 200 patients have been treated.

So far, small randomized controlled trials and numerous case series have shown the favorable effects of the MARS treatment on surrogate markers. However, adequate data are still lacking on the possible survival benefit offered by the MARS treatment when compared to the standard conservative medical therapy.

Aims: The aim of this thesis is to evaluate the impact of the MARS treatment on the patient outcome, clinical and biochemical variables as well as the psychological and economic aspects of the treatment for the different types of liver failure patients in Finland.

Patients and methods: This thesis encompasses 195 MARS-treated patients including patients who had acute liver failure (ALF), acute-on-chronic liver failure (AOCLF) and graft failure, and a historical control group of 46 ALF patients who did not undergo the MARS treatment. All patients received similar standard medical therapy at the same liver-disease specialized intensive care unit in Helsinki. The baseline demographics, biochemical laboratory parameters, and clinical variables as well as the MARS treatment-related and health-related quality of life data were recorded before and after the MARS treatment. The direct medical costs, which incurred during a time period of 3.5 years, were determined. The outcome was determined for patients including their survival, native liver recovery rate and their need for Ltx. Additionally, survival predicting factors were investigated in each liver failure etiological subgroup.

Results: In the outcome analysis, the 6-month survival was higher for the MARS-treated ALF patients as compared to the historical control group (75% vs. 61%, P=0.07), and rate of native liver recovery was higher (49% vs. 17%, P<0.001) and the need for Ltx was lower (29% vs. 57%, P= 0.001), respectively. However, the etiological distribution of the ALF patients referred to our unit has changed considerably over the past decade and the percentage of patients with a more favorable prognosis (e.g. toxic etiology) has increased. The etiology of liver failure was the most important predictor

of the outcome (P<0.0001). Other survival predicting factors included the grade of the hepatic encephalopathy, the plasma concentration of coagulation factors and the liver enzyme levels prior to MARS treatment in ALF patients. In terms of prognosis, the MARS treatment of the cirrhotic AOCLF patients does not seem meaningful unless the patient is eligible for Ltx.

From the clinical and biochemical viewpoint, the MARS treatment appears to reduce or halt the progression of encephalopathy in all the liver failure etiologies. Moreover, the MARS treatment reduced significantly the plasma concentration of most neuroactive amino acids and other albumin-bound (e.g. bilirubin) and water-soluble toxins (e.g. creatinine and urea). The MARS treatment effects seem to stabilize the patients, thus allowing additional time either for the native liver to recover and to regenerate, or for the patient to endure the prolonged waiting for an Ltx.

From the perspective of the economics and the health-related quality of life, in the cost utility analysis, the MARS treatment appeared to be less costly and more cost-efficient than the standard medical therapy alone in ALF patients because it reduced the need for Ltx.

Conclusions: The MARS treatment appears to have a beneficial impact on the outcome of ALF patients and those AOCLF patients who can be bridged to Ltx. On the other hand, the AOCLF patients with end-stage cirrhosis who are not eligible for Ltx have an extremely poor prognosis and do not seem to benefit from MARS treatment.

INTRODUCTION

In liver failure, the impairment of the normal metabolic, synthetic and detoxification function of the liver cells leads to the accumulation of various toxins. Currently, this toxin overload is considered to be the main cause of the patophysiological signs and of the end-organ damage in liver failure patients[319, 339]. Some of these toxins are water-soluble (e.g. ammonia, lactate and urea) and can be removed by conventional hemodialysis or hemofiltration. However, a substantial part of the endogenous toxins (such as bilirubin, bile acids and aromatic amino acids) are albumin-bound in the blood and cannot be removed with dialysis.

The molecular adsorbent recirculating system (MARS) machine was first introduced in the 1990s as a novel extracorporeal treatment modality for liver failure patients[336-339]. The main goal of the MARS albumin dialysis is to remove both endo- and exogenous water-soluble and albumin-bound toxins from the patient's blood, and thereby compensate for the loss of the detoxification function of the liver. In liver failure patients, the MARS treatment has been used to sustain vital organ functions either to facilitate native liver recovery or as a bridging treatment to liver transplantation (Ltx) until a suitable organ is found.

The effect of the MARS treatment on the clinical variables and on the outcome of patients has been investigated in a wide range of liver failure etiologies with promising results[54, 69, 95, 145, 248, 354, 366]. Only a few small case series have been published that have concentrated solely on acute liver failure (ALF) patients[69, 190, 200, 393]. Mostly ALF patients have been reported in studies as a small subgroup within other indications[54, 245, 340, 344, 387]. The impact of the MARS treatment on surrogate markers such as laboratory variables, toxin removal capacity and the effect on clinical variables (encephalopathy, hemodynamic profile, etc.) have also been the focus of extensive research[54, 191, 231, 232, 313, 344, 388].

Despite numerous studies of the MARS treatment in liver failure patients, conclusive evidence is still lacking on its beneficial effect on patient outcome[176]. In fact, only eight small randomized controlled trials (RCTs) have been published on the MARS treatment containing mainly chronic liver failure patients[95, 138, 141, 191, 231, 320, 333, 335].

In Finland, the MARS treatments were initiated in May of 2001 at the liver-disease specialized intensive care unit (ICU) and Ltx center at the Helsinki University Central Hospital (HUCH) and currently over 200 patients have been treated. Nevertheless, few uncontrolled case series have been published on the outcome of the very first MARS-treated patients in

Finland[180, 181, 189]. These studies concluded that the MARS treatment seems to be a promising new therapy especially for ALF patients. However, usefulness of this treatment was questioned for chronic liver failure patients who were not eligible for Ltx[181]. In light of these encouraging preliminary results, further studies were planned.

There are a number of commonly recognized problems in designing studies to evaluate the efficiency of liver assist devices[33]. The first challenge is that there is a great variation in etiology and severity of liver failure from country to country and this has a substantial impact on the prognosis of the patients[202, 252, 258]. The second problem is that due to the absence of the well-recognized guidelines for the initiation and duration of the MARS treatment, the different centers have their own inclusion criterion and treatment schedules. Thirdly, the availability and waiting time for Ltx differ substantially worldwide, and in some countries, cadaveric donor organs are virtually non-existent. All of the abovementioned factors make it difficult to compare results from different centers and studies. In addition, the low incidence and high mortality in ALF and graft failure (GF) make it challenging to enroll large number of patients for any study[204].

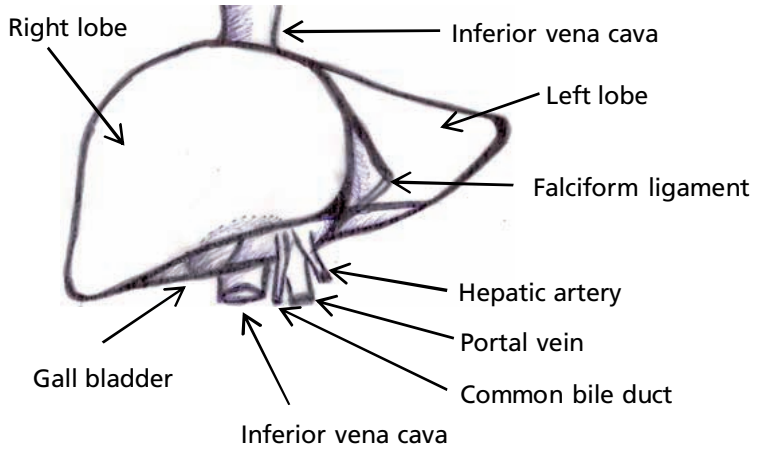
The aim of this thesis was to investigate the multiple aspects of the MARS treatment with long follow-up periods to better understand the mechanisms of treatment effects and to evaluate which patients most benefit from the MARS therapy.

REVIEW OF THE LITERATURE

FUNCTIONS OF THE HEALTHY LIVER

The liver is the largest organ in the human body weighing approximately 1.5 kg in a healthy adult. The liver receives its blood flow through two systems, the portal vein and the hepatic artery. Together these vessels carry approximately one-third of the cardiac output. The portal vein brings venous blood from the small and large intestines and pancreas to the liver so that the nutrients and other substances adsorbed from the gastrointestinal (GI) tract come into contact with hepatocytes through large fenestrations in the liver endothelium. The hepatic artery, carrying oxygenated blood, provides only one-fifth of the entire blood flow to liver (Figure 1.).

Figure 1. Anatomy of the liver



The liver has a wide range of complex functions, which are listed in Table 1. A complete loss of hepatic function leads to irreversible multi-organ failure and inevitable death, usually within 48 hours. However, unlike most other organs in the human body, the liver has a remarkable capacity to regenerate after an insult, as has already been noted in the 1960s[206]. This unique quality of the liver was even recognized by the ancient Greeks and depicted in the legend of Prometheus.

Table 1. Various functions of the healthy liver

Metabolism & Synthesis
Proteins
Amino acid and nitrogen metabolism (ammonia production through nucleic acid and protein metabolism)
Production of plasma proteins (50g/day) (e.g, transferrin, ceruloplasmin, albumin, C-reactive protein, lipoproteins)
Production of coagulation factors (fibrinogen, prothrombin, factors V, VII, IX, X,XI and XII, protein S and C and antithrombin III)
Production of hormones (e.g. Insulin-like growth factor, Angiotensinogen)
Carbohydrates
Glucogenesis (synthesis of glucose from amino acids alanine and glutamine, lactate and glycerol)
Glycogenesis (production of glycogen from glucose)
Glycogenolysis (conversion of storage glycogen to glucose)
Metabolism of galactose and fructose
Lipids
Synthesis and degradation of cholestrol, fatty acids and lipoproteins
Lipogenesis (synthesis of triglyserides)
Ketogenesis (production of ketone bodies from fatty acids)
Bile formation and excretion (~600ml/day)
Urea synthesis from ammonia
Breakdown and Detoxification
Breakdown of many hormones and hemoglobin
Metabolism and detoxification of endo- and exogenous toxins and drugs
Storage
Glycogen, various vitamins (e.g. A, D, B_{12})**, copper, iron, blood reservoir**
Immunology
Via the active reticuloendothelial system
Production of the complement system factors

LIVER FAILURE

In a clinical setting, liver failure can be divided into three main categories, depending on the mechanism by which the damage occurs: acute liver failure (ALF), acute exacerbation of chronic liver disease i.e. acute-on-chronic liver failure (AOCLF) and liver graft failure (GF) after an Ltx[25]. The distinctive features of all the main liver failure categories will be discussed separately in the following chapters.

ACUTE LIVER FAILURE

Definitions

The first definition of ALF in the 1970s was based on the presence of coagulopathy and the development of an altered mental status (e.g. hepatic encephalopathy (HE)) within 8 weeks of the onset of the first symptoms in a person with no previous history of liver disease[359]. The term "late onset hepatic failure" was used to describe a group of similar patients in whom this interval ranged from nine to 26 weeks[359]. Since then, the definition of ALF has been challenged and redefined by many[37, 38, 128, 253]. For example, Bernuau *et al.* first proposed the term fulminant hepatic failure (FHF) to encompass a subgroup of patients who developed HE within 2 weeks of the onset of jaundice[37]. Another subgroup of patients with a more insidious onset of disease and a delayed presentation of HE (up to 8 to 12 weeks) were classified as having subfulminant hepatic failure (SFHF)[37].

Another classification system for ALF was suggested in 1993 by John O'Grady[253]. In this system, liver failure is also classified according to the time, which elapses from jaundice to HE, which is 1 week in hyperacute liver failure, 8-28 days in acute liver failure and 4-12 weeks in subacute liver failure[253].

Young children with ALF may not develop HE until very late. As a consequence, it is widely accepted that HE is not essential in the diagnosis of ALF in children[39, 86]. Currently, the general definition of ALF in adults has also encompassed patients who have not yet developed HE despite rapidly failing liver function (increasing liver enzyme and bilirubin levels and/or decreasing coagulation factor levels) without previous liver disease[47, 101, 107, 382].

Incidence

ALF is a rare condition[204]. Moreover, a multitude of liver diseases can cause ALF and their relative frequency varies throughout the world[202]. Throughout the world, including Finland, the exact numbers of the incidence of ALF are missing mainly because the ICD-10 classification does not include specific codes for ALF. Therefore, a precise assessment of morbidity or mortality due to ALF in our country is not possible. According to the Finnish liver

transplantation registry (FLTR) during 2000-2008, 6-10 ALF patients were transplanted each year. The national numbers of the treated ALF patients are not available due to the lack of studies. In the United States, the incidence of ALF has been estimated at 0.7 patients/100 000 inhabitants/year. Today, numbers are available from the U.S. Acute Liver Failure Study Group, which was formed in 1997 as a consortium of tertiary liver units, and this group estimates that 2,800 new ALF cases occur per year[178].

Patophysiology and cellular mechanisms of hepatocyte and liver damage

The mechanisms of liver failure and liver cell (i.e. hepatocyte) damage has been studied mainly in in-vivo models because the hepatocytes in the in-vitro experiments do not remain viable and differentiated for long periods of time[183, 293].

It is believed that in ALF, the primary insult to the hepatocytes is caused by a noxious stimulus (e.g. virus, drug, and ischemia) and followed by a secondary injury induced by the release of proinflammatory cytokines (e.g. interleukin-1, interleukin-6 and the tumor necrosis factor alpha) and cytotoxic mediators from the damaged liver cells. The subsequent neutrophil migration to the liver tissue results in the discharge of free oxygen radicals and proteinases, causing a breakdown of the cell membranes and organelles. These cascades finally trigger apoptotic pathways which then cause cell death and on a larger scale, liver necrosis[120, 217, 259].

The impairment of normal metabolic and the detoxification function of the liver cells leads to the accumulation of various albumin-bound and water-soluble toxins (bilirubin, ammonia, lactate, bile acids, aromatic amino acids, fatty acids, mercaptans, phenols and endogenous benzodiazepines) [319, 339]. As the concentration of accumulating albumin-bound toxins exceeds the critical binding capacity of the albumin molecule, the free fraction of the toxins in plasma begins to rise, causing direct damage to the different organ systems. Thus, the accumulation of toxic metabolites is considered to be the main cause of the patophysiological signs and end-organ damage in liver failure[319, 339]. In fact, studies have shown an increased activation of the pro-apoptotic pathways when plasma from the patients suffering from ALF of AOCLF has been added to the human hepatocyte cultures[225, 297].

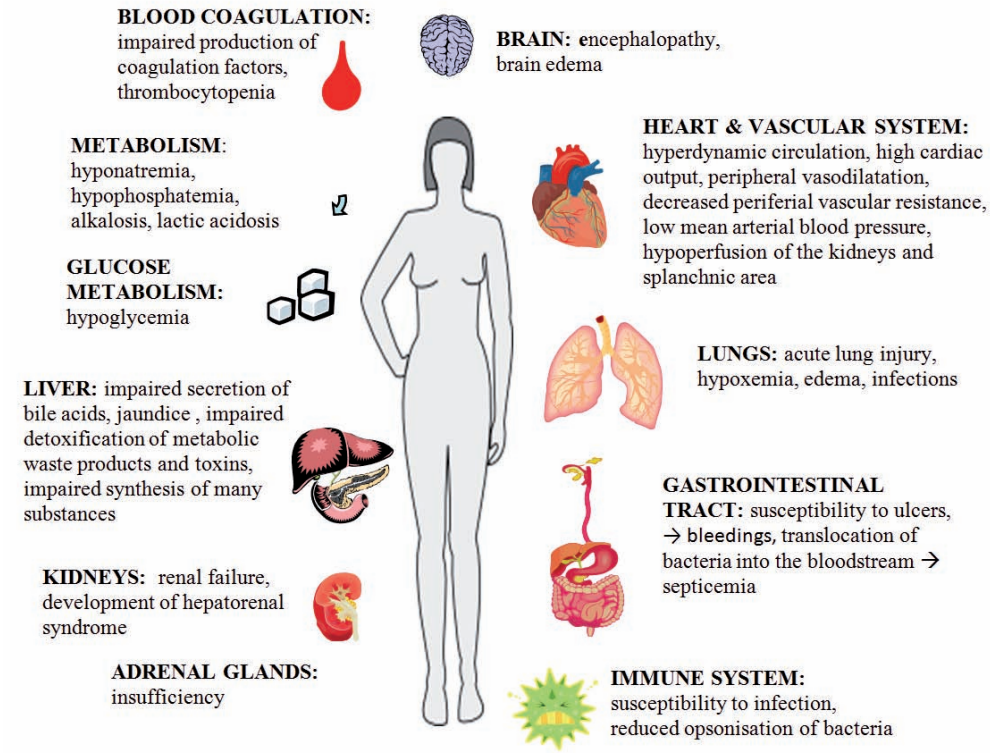
The injury of the endothelial cells of the liver causes impairment in microcirculation, perfusion and oxygen delivery, resulting in further tissue hypoxia. As the detoxification capacity of the hepatocytes is lowered, the circulating endotoxins induce the remaining cells to produce more vasoconstrictor and proinflammatory mediators, thus perpetuating the vicious circle of further cell injury[288].

Clinical characteristics

In clinical practice, the prodromal symptoms of ALF (fatigue, fever, nausea and abdominal pain) are usually very ambiguous and develop gradually over days or weeks. ALF is characterized by the development of jaundice, altered mental status, coagulopathy, susceptibility to infection[282, 285], acid-base and electrolyte imbalance[385], renal failure[279], hypoglycemia and hemodynamic instability[96]. Furthermore, once the clinical condition begins to deteriorate, and especially when HE sets in, the patient should be transferred to an ICU. The rationale for this is the characteristically rapid worsening of HE and the development of multiple organ damage, which might prevent future transportation to a liver ICU, and an Ltx center[204, 270].

The leading causes of death in ALF are brain edema, leading to tentorial herniation, an uncontrollable septic infection and irreversible multi-organ failure [107, 258, 352]. In many patients, all of the aforementioned complications are present at the time of death. A summary of the effected organs in ALF are presented in Figure 2.

Figure 2. Effected organs and metabolic derangements in acute liver failure



Etiology

ALF can be caused by a heterogeneous range of noxious agents, which are summarized in Table 2. The etiology of liver failure is one of the most important factors determining the prognosis of the patient with ALF[202, 252, 258].

For this reason, when ALF is suspected, a thorough and rapid investigation of all causative agents should be carried out[204].

Table 2. Etiologies of acute liver failure

Etiologies of acute liver failure	
VIRAL	
hepatitis viruses	A, B, C, D and E
other viruses	varicella zoster, adeno, yellow fever, cytomegalo, human herpes virus 1 & 2, Epstein Barr
TOXIC	
Drug-induced	
dose-dependent	*Paracetamol, salicylates, iron*
idiocyncratic	*Valproate, tuberculosis medicines, nimesulide, allopurinol, statins, disulfiram, azatioprin Ketokonazole,sulfonamides, quinolones, methyl-dopa, anabolic steroids, phenytoin*
synergistic	*Alcohol + paracetamol, amoxicillin+clavulanate, isoniatzid+rifampizine, trimethoprim + sulfamethoxazole*
Poison	*Tetrahydrochloride, chlorobenzenes, yellow phosphorus, cocaine, extacy*
Toxin	*Amanita phalloides, many herbal products (Chaparral, Chelidonium majus)*
VASCULAR	*Budd-Chiari syndrome*
PREGNANCY	*Fatty liver of pregnancy, HELLP-syndrome*
SYSTEMIC	
Copper metabolism	*Wilson's disease*
Autoimmune	*Autoimmune hepatitis*
OTHER	
Malignancies	*Malignant infiltration (lymphoma/acute myeloid leukemia)*
Trauma	
Cardiovascular	*Shock liver due to ischemia (resuscitation, acute myocardial infarction, cardiac tamponade)*
Heatshock	
Infection	*Sepsis*
UNKNOWN	*No definable cause can be found in extensive investigations*

The etiological distribution of causative agents in ALF varies markedly with geography[149, 202, 203]. In the North America and some European countries, toxic etiology is the most common cause of ALF[204, 255, 258] as opposed to many Southern European and developing countries, as well as Japan, where a viral origin is dominant[5]. During the past decades, a shift has occurred towards the more benign etiologies of ALF such as paracetamol-intoxications resulting in higher overall survival and native liver recovery rates[31, 175, 198, 203, 204].

Worldwide, the most common cause of ALF is an infection caused by a hepatitis virus. Only 0.2-4% of the hepatitis virus infections progress to ALF, and even in the high endemic areas (Africa, Asia and South-America), viral hepatitis tends to have a chronic clinical course[255]. The most common viral etiology of ALF are hepatitis virus B, A and E. Hepatitis virus E is most commonly seen in the developing countries, pregnant women and in immuno-compromised patients[9]. However, hepatitis virus C rarely causes ALF [149, 255]. In Finland and other Scandinavian countries, hepatitis virus induced ALF is rare and therefore, is hardly ever the indication for an Ltx [47].

In toxic drug-induced ALF, liver damage can occur in a dose-dependent manner if the drug has a narrow therapeutic range (e.g. paracetamol). ALF can also develop as an immunologically mediated idiosyncratic reaction, which usually takes place within the first 4-6 weeks of the initiation of a new treatment (e.g. isoniatzid/rifampicin, azatioprin)[126]. The list of potentially hepatotoxic drugs is long but the most common drug triggering ALF in the developed countries is the suicidal ingestion or unintentional overdose of paracetamol[31, 202, 204]. In addition to drugs, various exogenous toxins and herbal products can also cause ALF[242] (Table 1).

Paracetamol-intoxication occurs when the liver's metabolizing capacity is saturated, when the glutathione stores are depleted and when the cumulating toxic metabolite (N-acetyl-p-bentzoquinoneimine) of paracetamol causes hepatocyte necrosis and renal tubular damage[217, 271]. Usually the single-dose of paracetamol required to cause ALF is over 12g/day but much lower quantities have been reported to cause toxicity[215] especially if used continuously and by patients with altered metabolic states[74, 254, 258]. The factors, which can facilitate the development of drug-induced hepatotoxicity, include malnutrition, very young or old age, impaired renal function, simultaneous intake of P-450 cytochrome inducing drugs, alcohol or other hepatotoxic substances[44, 254, 379].

Amanita phalloides mushroom intoxications and the pharmacokinetics of the amatoxins have been studied in both human poisoning patients and dogs[108, 159, 372]. Here the ingestion of even a single full-grown mushroom can lead to ALF with a high associated mortality without treatment[99]. The amatoxins are rapidly adsorbed from the GI tract and quickly disappear from the blood stream as they enter hepatocytes and bind to the cellular RNA-polymerase II. While the exact molecular cascades which lead to cell death are not fully understood, it is known that amatoxins, such as the alpha-amanitin, inhibit protein synthesis in the affected cells[372]. The amatoxins taken-up by the hepatocytes are then excreted into the bile and subsequently reabsorbed from the GI tract, constituting an enterohepatic cycle. Approximately 90% of the ingested amatoxins are excreted via urine unmetabolized[99]. In fact, due to this rapid distribution into other body compartments, the reported plasma half-life of amatoxins is 37-50 min in dogs[108]. In human studies, amatoxins are rarely detected in plasma 36h

after intoxication[159]. Therefore, serious *Amanita* poisoning can occur even if serum samples taken 24h after the mushroom ingestion do not show detectable levels of amatoxin[168, 171].

The diagnosis of unknown or indeterminate etiology ALF can be established only after a thorough examination and exclusion of the known causes. The percentage of ALF patients having indeterminate etiology has been reported at 17% in the U.S.[258], 27% in Canada[352], 32% in Spain and France[42, 101] and 62% in India [5]. In a Scandinavian study, a large portion of the transplanted ALF patients (~40%) had an unknown etiology of ALF[47]. According to the most recent statistics from FLTR (1982-2009), 62% (90/147) of all the transplanted ALF patients had an unknown etiology.

Current conservative management of acute liver failure

The rarity and great variance in etiology and severity of ALF make it difficult to compare treatments or to carry out sufficiently powered studies. For the same reason, although general management guidelines have been recommended, the standardized intensive care treatment protocols for ALF have not been established [34, 270, 347].

The treatment of ALF aims at preventing the development of complications and irreversible organ damage (such as brain herniation) while waiting for native liver regeneration or for a suitable liver graft for an Ltx[34, 166, 270, 347]. The first line of treatment for ALF entails conservative medical and pharmacological management in an ICU setting. The next step, if conservative treatment fails to improve the clinical condition of the patient, is to use liver-assist devices (e.g. MARS). Finally, if native liver recovery is not expected, an Ltx remains the treatment of choice[32, 204].

The standard medical therapy (SMT) usually starts with an extensive evaluation of the origin and severity of ALF[34] (Table 3.). The potentially rapid progression of ALF necessitates an early consultation of the nearest transplant center. Furthermore, a patient exhibiting any signs of mental abnormality should be immediately transferred to the nearest Ltx center and have the suitability for an Ltx evaluated[204, 270]. The next chapters describe in detail the SMT of the ALF patients treated in an ICU setting.

Table 3. Investigations required to establish the etiology of the acute liver failure and clinical follow-up of the patient

The preliminary investigations in acute liver failure
ETIOLOGY OF LIVER DISEASE
Medical history (if the patient is incapacitated, family members/friends will be asked)
onset of symptoms, family history of liver disease
Risk behavior
blood transfusions, sexual contacts, body piercings/tattoos, occupation, travel history
ingestion of alcohol/illicit drugs/anabolic steroids/ medication/herbal products/mushrooms
Specific etiology
viral etiology: viral hepatitis serology
systemic disease: Ceruloplasmine, urine copper
autoimmune markers: antinuclear and smooth muscle antibodies
toxic screens: paracetamol levels, blood/urine toxicology and drug screen
Radiological and histological diagnosis
Abdominal doppler-ultrasound, computerized tomography and liver biopsy when necessary
CLINICAL FOLLOW-UP & MONITORING
vital signs
assessment of mental status (transcranial doppler ultasound and EEG if necessary)
signs of jaundice, ascites, bruising, bleeding and size of the liver
cardiovascular status (EKG and invasive blood pressure monitoring)
chest x-ray
urine output and presence of edema
LABORATORY INVESTIGATIONS, SEVERITY OF THE DISEASE
liver cell damage: liver transaminases (ALT, AST)
cholestasis: bilirubin, alkaline phosphatase, γ-glutamyl transpeptidase (GGT)
coagulation status: coagulation factor panel (INR, factor V)
synthetic capacity of the liver: albumin, prealbumin
kidney function: creatinine, urea, cystatin-c
general: complete blood count, blood type
infection parameters: C-reactive protein
metabolic state; Na, K, Ca-ion, acid-base balance, lactate, ammonia
glucose metabolism: blood glucose
liver function tests: galactose T½, indocyanine green, etc.

Monitoring in the ICU

Basic monitoring includes the EKG, arterial and central venous blood pressure, pulseoxymetry and urinary output measurement. In the

encephalopathic patients, the cerebral blood flow velocity can be estimated by using the transcranial-doppler ultrasound[10, 41]. It is important to note that invasive intracranial pressure monitoring devices are no longer used in many ICUs due to the risk of bleeding complications[45, 369]. Moreover, the use of these devices has not been shown to increase survival[369]. In a hemodynamically unstable patient requiring massive inotropic support, pulmonary artery cathetrization should be considered[270]. The nasogastric tube is also used for enteral nutrition in patients with altered mental status.

Hemodynamics

The hemodynamic instability in ALF is characterized by hyperdynamic circulation with high cardiac output, low vascular resistance and low mean arterial pressure (MAP). During the initial stages of ALF, vasodilatation is usually generalized and may respond to fluid replacement therapy. Later, through the activation of the neurohumoral system, vasodilatation is regionalized to peripheral and splanchnic vascular beds, which also become hyporesponsive to vasoconstrictive stimuli. The resulting relative hypovolemia and renal vasoconstriction cause lowered renal perfusion pressure[166]. It has been speculated that these abnormalities are due to the high concentration of circulating endotoxins and vasodilatory substances such as nitric oxide, which are normally removed by the healthy liver[126]. Relative adrenal insufficiency has also been suggested as one of the possible factors causing cardiovascular collapse in ALF patients[136].

The most commonly used vasoactive agent to maintain adequate MAP is noradrenalin infusion[34, 347]. Terlipressin, a vasopressin analog, has also been investigated but is not currently recommended because it seems to increase the intracranial pressure (ICP) in ALF patients[324, 347].

Hepatic encephalopathy, brain edema and neuroactive amino acids

One of the hallmark features of ALF is HE. It is defined as a potentially reversible neuropsychiatric disorder presenting as a decreased level of consciousness associated with liver failure[67, 111]. Although a direct positive correlation between the grade of HE and ICP has not been established, one study showed that approximately 80% of the ALF patients with a grade 4 HE also had significant cerebral edema[94]. Furthermore, the presence of brain edema is usually more common in hyperacute, fulminant, and in acute forms of ALF than in the subacute type[37, 253].

The correct management of HE and brain edema is extremely important because one of the leading causes of death in ALF is brain herniation resulting from increased ICP[258, 352]. For this reason, the mental status of each liver failure patient should be closely monitored by using the West-Haven criteria (Table 4.)[67, 111].

Table 4. The grading of hepatic encephalopathy according to the West-Haven Criteria

Encephalopathy grade	Mental status & clinical signs
0	Normal
1	Shortened attention span, anxiety or euphoria, drowsiness
2	Lethargy, personality changes, disorientation of space and time, slurred speech, hyperreflexia
3	Confusion, gross disorientation and bizarre behavior, still responsive to verbal stimuli
4	Unconscious, may be unresponsive or respond to strong painful stimuli, comatose

Patophysiological mechanisms causing hepatic encephalopathy

The patophysiological mechanisms causing HE and brain edema are likely to be multifactorial. According to current knowledge, HE is believed to result from the accumulation of various endotoxins in the brain tissue, causing a loss of autoregulation and impairing oxygen and glucose metabolism. Other possible mechanisms explaining the pathogenesis of HE have also been proposed, such as the development of false neurotransmitters, release of inflammatory mediators and oxygen radicals and imbalance in the ratios of plasma and intracerebral amino acids[112, 139, 323, 370].

In healthy humans, cerebral blood flow is tightly autoregulated and maintained to be constant through a wide range of systemic blood pressure variation (MAP 60-150mmHg) [263]. In ALF, the encephalopathic patients have lost this autoregulatory mechanism and their cerebral blood flow is directly proportional to their systemic blood pressure[82, 194]. Therefore, it is important to monitor and maintain normal MAP (~ 60-80mmHg) in these patients to prevent them from developing either hypo- or hyperperfusion of the brain. The consequences of brain hypo- or hyperperfusion are brain ischemia or hyperemia, respectively. Hyperemia of the brain results in intracranial hypertension and decreased cerebral perfusion pressure. This cascade eventually leads to further impairment of the brain cell metabolism, to tissue swelling and finally to herniation[165].

In the development of HE, the role of the plasma amino acids, among other neurotoxic substances, has been recognized from the 1970s[110, 113]. It has been hypothesized that the dysbalance between the excitatory and inhibitory amino acids might play a central role in the development of HE[52, 53, 209, 313]. As noted by Fischer *et al.*, liver failure patients tend to have an increased plasma concentration of tryptophan, tyrosine, and phenylalanine, which are aromatic amino acids (AAAs). In contrast, the concentration of valine, isoleucine and leucine, which are branched chain amino acids (BCAAs), is reduced. This characteristic amino acid dysbalance results in a low Fischer's ratio, which is defined as the ratio between the BCAAs and the AAAs[113].

It has been speculated that in liver failure patients, the low levels of the BCAAs result in a decreased production of excitatory neurotransmitters, and the high levels of the AAAs increase inhibitory neurotransmitter production. In addition, encephalopathic patients have been shown to have increased levels of the amino acid methionine, which is a neurotoxic substance released into the blood stream during liver necrosis[147]. The increased plasma concentration of other neuroinhibitory or neurotoxic substances, such as endogenous benzodiazepines, manganese, copper, phenols, mercaptans, pro-inflammatory cytokines and bilirubin, might also play a part in the pathogenesis of HE[53, 209].

The ammonia-glutamine theory proposes that an increased concentration of arterial ammonia causes disturbances in the re-uptake of glutamate and in the metabolism of glutamine in the brain[243, 323]. In liver failure, the detoxification of ammonia is impaired and the astrocytes in the brain act as an alternative metabolic pathway to convert the excess ammonia and glutamate into the amino acid glutamine[220, 323]. Accumulating glutamine then acts as an organic osmolyte, favoring the movement of water into the brain tissue and thus, causing astrocyte swelling. In addition, high ammonia concentration has a direct toxic effect on the nervous system[323]. To support this theory, a recently published study demonstrated a correlation between brain glutamine, arterial hyper-ammonemia and increased ICP in human subjects with ALF[355]. Another cause for the swelling of the brain tissue might be the loss of cerebral autoregulation, resulting in an increased cerebral blood flow and blood volume[194, 197]. Regardless of the original cause, the swelling of the astrocytes causes brain edema. Due to the limited space within the bony skull, brain edema results in increased ICP, which can lead to tentorial herniation and death[355].

There is also evidence supporting the "toxic liver" hypothesis that toxic substances released from the necrotic liver cause an increase in the ICP. This theory is supported by the clinical observations that ICP usually normalizes during the anhepatic phase of an Ltx[83, 161].

In summary, the various patophysiological mechanisms causing HE in liver failure patients include the dysbalance between the BCAAs and the AAAs[52, 53, 209, 313], the elevated levels of other neuroinhibitory and the neurotoxic amino acids and other substances[53, 113, 147, 209], the abnormal ammonia-glutamate metabolism[165, 243, 323] and the loss of cerebral blood flow autoregulation[194, 197].

Management of hepatic encephalopathy

The medical management of HE and brain edema aim at maintaining the ICP below 20mmHg and the mean cerebral perfusion pressure (=MAP-ICP) between 50-70mmHg[34, 347]. The avoidance and correction of any factor which might increase ICP, such as hypo- or hyperglycemia, fever, hypoxia, hypercapnia and electrolyte imbalance, is essential[68, 165, 239]. Furthermore,

environmental stimuli should be kept to a minimum and the patient should be adequately medicated before any procedure[239].

All sedative medication should be discontinued in non-intubated patients in order to adequately assess their level of consciousness. According to recent guidelines, if the grade of HE reaches three or four, the patient should be sedated, intubated and mechanically ventilated to prevent hypercarbia, hypoxia, and aspiration[34, 347]. In intubated patients, adequate sedation should be administered to prevent the patient from coughing or straining against mechanical ventilation[270]. Whereas hyperventilation has been shown to reduce ICP, this effect is usually temporary. Therefore, hyperventilation is only used, if life-threatening ICP emergency is suspected[94].

The pharmacological management of brain edema aims at reducing ammonia production (synthesized mainly by gut bacteria). Bowel decontaminating antibiotics (e.g. ciprofloxacin) and lactulose (which acts as a prokinetic inhibiting the overgrowth of GI bacteria) have been used to reduce ammonia production and the incidence of brain edema[13, 24]. In refractory intracranial pressure emergencies mannitol[55], hypertonic sodium chloride-infusion[240], phenytoin, temporary hyperventilation[94], propofol[384], moderate hypothermia (32-33°C)[160, 161, 164], thiopental-infusions[117], and even hepatectomy[289] have been used as a last resort to decrease ICP and to reduce brain edema.

Renal failure and the hepatorenal syndrome

Renal dysfunction or failure occurs in 30-70% of the ALF patients[258, 279]. Renal failure is particularly common in the paracetamol intoxications due to the direct toxic effect of the paracetamol metabolites causing acute tubular necrosis[44, 71].

Hepatorenal syndrome (HRS) refers to a reversible functional renal impairment that occurs mostly in advanced cirrhosis but also in ALF patients. Type 1 HRS is defined as the doubling of serum creatinine or reduction in creatinine clearance in less than two weeks in a patient with liver failure. In addition, all other possible causes for post- and prerenal renal failure have to be ruled out and fluid replacement and withdrawal of the nephrotoxic drugs does not improve renal function[16].

The cause of HRS is as of yet unknown but various theories about the underlying patophysiological mechanism have been proposed. For instance, the peripheral vasodilatation hypothesis suggests that type 1 HRS and functional renal failure are caused by the vasodilatation of the splanchnic and peripheral capillary beds leading to relative hypovolemia, renal vasoconstriction and reduced renal perfusion[17, 279, 374].

The prevention of renal failure entails the maintenance of adequate systemic blood pressure and the avoidance of nephrotoxic agents[347]. The mainstay of treatment in renal failure in ALF is early continuous renal replacement therapy[34, 77, 78] and cautious fluid management[347]. The

continuous renal replacement therapies are preferred in treating ALF patients, as they cause less fluctuation in hemodynamics and ICP than the intermittent therapy[76, 78].

Coagulopathy and bleedings

Profound coagulopathy (INR > 1.5) is always present in ALF patients due to their diminished production and increased consumption of coagulation factors, hypofibrinogenemia and thrombocytopenia[96, 292]. Coagulation abnormalities are usually not treated unless the patient is actively bleeding or an invasive procedure is contemplated[347]. Fresh frozen plasma should be administered with care in these patients due to the possibility of volume overload and a subsequent rise in ICP[270]. Furthermore, if the blood components are given, the trend in the plasma levels of the coagulation factors indicating the synthesizing capacity of the liver is temporarily lost[123].

Other complications, which can also occur during severe ALF, are GI bleeding and stress ulcers. For this reason, every patient is given a standard regime of proton-pump inhibitors (e.g. omeprazole) or histamine H_2-receptor antagonists (e.g. ranitidine)[213, 270, 347].

Infections

ALF patients are prone to both bacterial and fungal infections[282, 284, 285, 373]. In a study by Rolando *et al*., one-third of all ALF patients had a culture-positive fungal infection[283]. Moreover, the hemodynamic changes in the splanchnic vascular bed are believed to cause relative hypovolemia, which leads to hypoperfusion and tissue hypoxia of the GI mucosa. This enables the translocation of gut bacteria into the blood stream, causing disseminating endotoxemia[23]. The diminished opsonisation of bacteria and the production of complement factors, as well as the breakdown of natural endothelial barriers, all lead to the patient having an increased susceptibility to infection[278].Vigilant surveillance and prompt treatment of the infections are essential because one of the main causes of death in ALF is an uncontrollable septic infection[285].

Metabolic abnormalities

Metabolic abnormalities commonly associated with ALF include hyponatremia, hypokalemia, hypophosphatemia and hypoglycemia [79, 385]. In addition, alkalosis and acidosis may both occur[96, 385]. Patients with paracetamol-related ALF frequently have a combination of hyperlactatemia coupled with acidosis, which is considered to be a poor prognostic sign[251]. Electrolyte and acid-base imbalance should be corrected promptly, as these abnormalities can increase ICP and cause further hemodynamic instability[239]

Etiology-specific treatments

Only some etiologies of ALF have specific treatments available, for example, the N-acetylcystein (NAC) in paracetamol poisoning[48], prednisolone in autoimmune hepatitis[72], and penicillamine and other copper chelators in Wilson's disease[306].

In particular, the beneficial effect of the NAC treatment has been reported in paracetamol intoxications[48, 134, 174] because this treatment protects the liver cells by acting as an antidote against the toxic metabolites of paracetamol (N-acetyl-p-bentzoquinoneimine, NAPQI)[49]. NAC has been shown to replenish hepatic glutathione stores and to improve systemic hemodynamic parameters by acting as a vasodilator and thereby increasing the blood flow and oxygen delivery to the liver[135, 276, 329]. NAC is also used in other etiologies of ALF[329]. According to a recent RCT[205], NAC also improved transplant-free survival when given intravenously in the early stages of non-paracetamol-related ALF.

Prognosis

Without Ltx option, the mortality in ALF can reach up to 60-95%, depending on the etiology[149]. During recent decades, the prognosis in ALF has improved significantly as a result of the possibility for a Ltx and advanced ICU management. Currently, the mortality for ALF is about 20-40% and approximately 25-45% of ALF patients undergo Ltx[202, 204, 258, 352]. A spontaneous liver recovery rate of 45% has been reported in a recent U.S. study[204].

In general, patients with hyperacute liver failure and FHF tend to have a better prognosis as compared to those with SFHF. Although many patients with FHF die, their probability for native liver recovery without complications is higher than for the SFHF patients[37, 253]. For paracetamol-intoxication, acute hepatitis A and pregnancy-related ALF, the prognosis is relatively good with a good possibility of native liver recovery (~60%), whereas the prognosis is poor and native liver recovery is unlikely (~0-20%) in acute hepatitis B, idiosyncratic drug-induced or unknown etiology ALF, Wilson's disease and autoimmune hepatitis[202-204, 258].

ACUTE-ON-CHRONIC LIVER FAILURE

Definition

AOCLF is usually defined as a condition in which a previously stable patient with chronic liver disease experiences a rapid deterioration of liver function. The acute exacerbation of liver function is usually caused by a triggering event, such as GI bleeding, infection, or ingestion of a hepatotoxic substance such as alcohol[319].

Incidence

The incidence of AOCLF is difficult to approximate due to lack of a universally accepted definition for the condition. However, due to the high incidence of chronic liver diseases and cirrhosis worldwide, AOCLF is much more common condition when compared to ALF[315].

Patophysiology and clinical characteristics

In AOCLF, the same life-threatening organ failures occur as in ALF, including HE, renal failure, cardiovascular complications and severe cholestasis. However, some of the pathophysiological mechanisms behind these organ manifestations are somewhat different[319].

Characteristic cardiovascular changes in cirrhotic patients include an increased cardiac output, vasodilatation of the splanchnic and peripheral circulation, reduced renal blood flow, portal hypertension and cirrhotic cardiomyopathy. These changes become more pronounced during the acute decompensation of liver function[17]. Proposed as the mediators of these changes in the vascular tone have been elevated renin-angiotensin II secretion, sodium retention and altered nitric oxide production[17, 364].

Portal hypertension in cirrhotic patients causes shunting of the blood past the liver through alternative routes and this creates varices. Due to this portocaval shunting, gut-derived and other endogenous metabolic toxins escape the detoxification by the liver. Consequently, rising levels of toxins (e.g. neurotoxic amino acids, bilirubin, ammonia) can induce HE[53, 163, 323]. In spite of this, a marked rise in ICP and cerebral edema is rarely seen in AOCLF patients[323].

In cirrhotic patients, the hemodynamic changes described above develop slowly and the body has time to create compensatory mechanisms. For this reason, the alterations in the hemodynamic status of AOCLF patients are usually less pronounced than those seen in ALF patients[166]. Some studies have also demonstrated a decreased diastolic function and an impaired myocardial contractility in cirrhotic patients, which is a phenomenon called cirrhotic cardiomyopathy[12, 35, 272].

Etiology

Chronic liver disease can originate from numerous causes. The most common cause of liver cirrhosis in the Finnish population and in many other Western countries is the excessive use of alcohol[315]. Worldwide, chronic viral hepatitis is the number one cause of liver cirrhosis[315].

The most common causes of chronic liver disease leading to Ltx in Finland are primary biliary cirrhosis, primary sclerosing cholangitis, alcoholic cirrhosis and biliary atresia (FLTR 1982-30.6.2009). Other rarer causes include chronic hepatitis B or C infections and non-alcoholic steatohepatitis, autoimmune hepatitis, and various inherited metabolic diseases (Wilson's disease and alfa-1-antitrypsin deficiency).

Special features in the conservative management of AOCLF

The SMT of AOCLF aims at treating the triggering cause of the acute exacerbation episode and sustaining vital organ functions until compensated liver function is once again restored[319].

If the patient is a candidate for Ltx and is admitted to an ICU, the SMT follows the same guidelines as in ALF. However, the management of AOCLF has a few special features distinct from ALF, which are described next.

Hepatorenal syndrome in cirrhotics

In cirrhotics, HRS can develop spontaneously but it more commonly occurs after a precipitating event, such as an infection, or GI bleeding[17]. The management of type 1 HRS usually entails a discontinuation of diuretic medication and a paracenthesis of ascites fluid with concomitant fluid replacement (20% albumin solution) and a terlipressin-infusion[17, 221, 257]. Some studies have reported significant improvement in renal parameters after the simultaneous administration of terlipressin and albumin solution[221, 257]. One RCT including cirrhotic type 1 HRS patients showed a significant improvement in survival and kidney function with the MARS treatment[231].

Type 2 HRS is only seen in AOCLF patients. The onset of HRS type 2 renal failure is more insidious and can take several months to develop[17].

Spontaneous bacterial peritonitis

Spontaneous bacterial peritonitis (SBP) is defined as a bacterial infection of the ascites fluid in the absence of visceral perforation or other infectious focus[66, 261]. The diagnosis of SBP demands the presence of >250 polymorphonuclear cells/mm^3 in the ascites fluid[261]. The most common microbiological etiology in SBP is gram-negative GI bacteria, usually *E.coli* and *Klebsiella* species[361]. It has been hypothesized that in liver failure, the translocation of the gut bacteria into the blood stream occurs due to the dysfunctional gut-associated lymphoid tissue, as well as to the hypoperfusion and tissue hypoxia of the GI mucosa.[23, 46]. The resulting endotoxemia and colonization of the ascites fluid have been proposed as the principal mechanisms by which SBP develops[261]. Therefore, a prompt commencement of the correct antibiotic regime is essential, as failure to do so significantly increases mortality[361].

Prognosis

Recent studies on ICU patients have shown that the long-term prognosis of cirrhotic and AOCLF patients, even in the absence of complications, is poor and the 1-year mortality can reach up to 70%[8, 125, 214]. Prognosis in AOCLF depends mainly on the complications which arise and the severity of the underlying liver damage[214]. Short-term mortality to both GI bleeding and SBP in a cirrhotic patient has been reported ~20-30%[75, 261] whereas in type 1 HRS, mortality can reach up to ~90-100% within 6 months [17, 129]. According to one study, the development of progressive renal failure in

association with SPB in cirrhotic patients can predict a short-term mortality up to even 100%[116].

In patients with end-stage cirrhosis, the liver does not have the capacity to regenerate but this ability is still present in a steatotic and enlarged liver[183, 206]. Therefore, in end-stage cirrhosis without regenerative capacity, Ltx remains the only long-term possibility for survival[315].

LIVER TRANSPLANTATION

Despite the introduction of various liver assist devices during the past two decades, Ltx has still retained its place as the only validated treatment option in critical ALF, AOCLF and GF if conservative treatment fails[32, 149, 204, 258]. Even though the first Ltx was performed 46 years ago[341], it has not been the until the past two decades that the 1-year overall patient survival has exceeded 80%[353].

The first Ltx in Finland was performed in 1982 by professors Scheinin and Höckerstedt and their team. Since then, 777 Ltx procedures have been performed, including re-transplantations (FLTR, 30.6.2009). In Finland, the main indications for the primary Ltx have been chronic liver disease (71%), ALF (21%) and liver tumors (8%). In chronic liver disease, the most common indication for Ltx were primary biliary cirrhosis (24%), primary sclerosing cholangitis (20%), alcoholic cirrhosis (14%) and biliary atresia (8%). In transplanted ALF patients, 62% had unknown etiology and 14% had drug-induced liver failure. In liver tumors, hepatocellular carcinoma is the number one diagnosis (75%) in transplanted patients (FLTR, 30.6.2009).

For Ltx, correct patient selection is crucial. This is because a delay in the decision to transplant may lead to the development of complications and a deterioration in the clinical condition to such a point that the patient becomes untransplantable and Ltx is then contra-indicated. On the other hand, an inaccurate choice to transplant a patient who could have recovered without a Ltx leads to costly unnecessary major surgery and life-long immunosupression treatment with a possibility of serious side-effects and an increased risk of malignancy[93, 266, 395, 396].

Listing criteria and prognostic factors in ALF

The traditional and validated prognostic indicators and the Ltx listing criteria in ALF are the King's College criteria[251] and the Clichy criteria[36](Table 5.). The most widely used listing criteria, the King's College criteria, were introduced in 1989. The King's College study was based on a cohort of 588 ALF patients who were stratified into two distinct groups: the paracetamol and non-paracetamol-related ALF. According to a meta-analysis, these criteria carry a specificity of 92% and a sensitivity of 69% in predicting mortality in paracetamol-induced ALF[22]. In other words, due to the low

sensitivity of the King's college criteria, they may fail to recognize some patients who require Ltx to survive. Some transplantation centers also use the Clichy criteria, which were introduced already in 1986[36]. The Clichy criteria are based on a multivariate analysis of 115 patients with fulminant hepatitis B infection.

Table 5. The King's College and Clichy prognostic and transplantation listing criteria in acute liver failure

Poor prognostic signs in acute liver failure			
King's College Criteria			
Paracetamol-induced			
	pH < 7.3	**OR all of the following**	INR > 6.5
			creatinine > 300µmol/l
			hepatic encephalopathy grade III-IV
Other causes			
	INR > 6.5	**OR 3 of the following**	40 < age < 10
			bilirubin > 300µmol/l
			duration of jaundice before encephalopathy > 7 days
			etiology: nonA, nonB or drug-induced hepatitis
			INR < 3.5
The Clichy criteria			
hepatic encephalopathy	**AND** age < 30 and FV < 20%	**OR**	age > 30 and FV < 30%

In ALF, a number of other prognostic factors have been proposed with variable success and these include early arterial lactate [30], the APACHE II scoring system [179, 198], alpha fetoprotein[308, 314], the MELD-score [186], the ratio of the coagulation factors VIII and V[267], serum Gc (group-specific or vitamin D-binding) protein[201], serum phosphate levels [311] and liver size[322].

Listing criteria and prognostic factors in AOCLF

The severity of chronic liver disease and the need for Ltx can be assessed by using the Child-Pugh score[61, 211, 273] and the model for the end-stage liver disease (MELD)-score[118, 362, 380]. The MELD-score is used as a donor allocation criteria for Ltx in many countries[210].

The Child-Pugh-score was originally created to predict 1-year survival after surgery in cirrhotic patients. This score incorporates bilirubin, INR and albumin values and the presence of ascites and encephalopathy. Depending on the Child-Pugh-score, patients are assigned to three categories from A

to C. The predicted 1-year post-surgery survival rates are 100% in Class A, 81% in Class B an 45% in Class C[61, 273].

The MELD-score, which is calculated using the INR, bilirubin and creatinine-values, was originally designed to predict the outcome in patients undergoing a transjugular intrahepatic portosystemic shunt operation[61, 273]. Since then, it has been validated in various patient populations and seems to give a reliable estimate of the short-term mortality risk of a cirrhotic patient[118, 170, 186, 380]. If the MELD-score exceeds 40, the patient has a less than 20% probability of surviving the next 3 months without Ltx[118, 170, 380]. The Child-Pugh- and MELD-score calculators are available on the internet[362, 363].

Contra-indications to liver transplantation

The list of absolute contra-indications to Ltx has undergone many changes over the past decades and differs among various Ltx centers[371]. Today, the only absolute contra-indications are an extrahepatic malignancy, a life-threatening systemic disease (in which the patient is not expected to survive the surgery or the following ICU treatment period) or an uncontrollable septic infection. The relative contra-indications include portal thrombosis, pulmonary hypertension (mean pulmonary artery blood pressure > 35mmHg), active alcohol or drug abuse, and psychiatric disorders causing an inability to comply with strict immunosuppressant protocols[210, 219, 346].

Surgical techniques

The most commonly used technique for Ltx is a full organ orthotopic transplant from a brain-dead donor. This method has been used since 1963[341], but recently new techniques using living donors and split liver grafts have emerged as well[216]. Auxiliary liver transplants have also been used with encouraging results in some patients [153, 368]. Auxiliary Ltx has mainly been employed in FHF cases when, given enough time, the patient's own liver is expected to recover. This technique provides the possibility of withdrawing immunosuppressant medication and sacrificing the graft once the patient's own liver has regenerated.

Deaths on the waiting list

Due to the shortage of available organs and the development of complications, some listed patients become untransplantable or die while waiting for a suitable organ[118, 202, 258, 303]. In the Scandinavian countries, 4% (14/351) of all listed patients died on the Ltx waiting list in 2008. When considering ALF patients only, the percentage of patients dying on the waiting list is clearly higher at ~15%[42, 47, 303]. In Finland, the annual number of deaths on the Ltx waiting list (including both the acute and chronic patients) has varied from 0 to 6 (0% -11%) during 2002-2007[303].

Prognosis after transplantation

The survival in transplanted ALF patients is somewhat lower compared with the elective Ltx patients even though ALF patients tend to be younger[6, 303, 353]. This is because the ALF patients have a higher risk of dying due to their higher incidence of multiple organ failure and increased ICP. Nowadays, in chronic hepatic disorders leading to cirrhosis, there is a tendency to perform a Ltx before the development of an AOCLF episode or multiple organ complications[210].

During the past decade, the 1-year patient and graft survival rates in ALF after a Ltx have been reported at 61-81% by various transplantation registries[303, 353] and studies[6, 107, 294]. In Finland, according to FLTR, the 1-year survival in ALF after Ltx is 86%. In comparison, 1-year survival rates in elective Ltx for chronic liver disease are over 90% in Finland and in many European centers[303, 353].

One of the most important factors determining the prognosis after Ltx is the etiology of liver failure[6, 51, 303, 353] as well as the recipient's clinical condition, renal function and encephalopathy grade prior to the procedure in ALF[42, 84]. Other important predictors of outcome are the quality of the graft, cold and warm ischemic time[20, 42, 51] and donor/recipient's age as well as the surgical technique used[6, 51, 150, 269].

Re-transplantation

The re-transplantation rates after primary Ltx have been reported to be around 10%, depending on the center[7, 173, 303, 353]. In Finland, 8% (65/777) of all transplantations have been re-transplantations during 1982-2009. The most common indications for re-Ltx have been a chronic rejection (43%), arterial thrombosis of the primary graft (20%), other specific complications (8%), a primary non-functioning graft (5%), a recurrence of original disease (4%) and biliary complications (4%) (FLTR 30.6.2009). In fact, many studies have shown that the 1-year survival after re-Ltx is significantly lower (~50-60%) when compared to primary procedures[6, 353].

LIVER GRAFT FAILURE

After a successful Ltx, the new graft starts to function immediately. The stabilization of hyperdynamic circulation and disappearance of encephalopathy occur within the first few postoperative hours and days[210].

Any form of graft dysfunction has been associated with an increased morbidity and graft rejection[151, 346]. Early liver allograft dysfunction has been divided into primary non-function (PNF) and primary dysfunctioning graft (PDG). PNF is defined as a complete loss of graft function leading to patient death or re-transplantation within a week of Ltx. Its incidence has been reported at 2-7%[73, 346]. PDG lacks a commonly accepted definition,

but is generally characterized by a rise in liver enzyme levels (AST or ALT) over 1500 U/l within the first three postoperative days[346]. Multiple factors can contribute to the development of both PNF and PDG, including a recipient's clinical condition prior to Ltx, donor factors (age, degree of steatosis of the graft, etc.), organ preservation (cold and warm ischemia time, etc.) and ischemia-reperfusion-injury[119, 236]. In Finland, only 0.6 % (5/777) of all transplanted patients have been re-transplanted due to PNF or PDG (FLTR 30.6.2009) .

Late graft dysfunction, which occurs at the earliest some months after Ltx, usually develops as a result of the reactivation of the primary disease (autoimmune disease, hepatocellular cancer, hepatitis, metabolic disease, etc.) or chronic rejection[381].

LIVER-ASSISTING DEVICES

There is a need for alternatives to Ltx due to a shortage of donor organs. Furthermore, some critically ill patients have a contra-indication that prevents Ltx. This has resulted in the innovative development and research of various liver assist devices during the past decades[321]. These liver assist-devices are generally divided in two categories: (I) the bioartificial cell-based systems which provide both metabolic support and detoxification function, and (II) the artificial non-cell based detoxification systems.

I Bioartificial liver assist devices

The principle goal of the bioartificial liver assist devices (BAL) is to provide an extracorporeal functioning liver mass connected to the patient's circulation.

The history of bioartificial liver assist devices

- In the late 1960s, the first attempts at creating a biological liver assist treatment included cross-circulating the patient's blood against that of a baboon, pig liver or a healthy human liver[1-3, 50, 275].However, it was soon realized that during human-to-human cross-circulation, the healthy person suffered from major toxic side-effects and subsequent trials were abandoned[50]. In animal-to-human cross-circulation, severe allergic and anaphylactic reactions prevented the further development of these techniques. Transient improvement in HE and other clinical variables, as well as a decrease in ammonia levels, were observed with the above-mentioned methods. The first hybrid BAL technique was introduced in 1986. This technique was based on suspension-cultured rabbit hepatocytes combined with a hemodialysis chamber[222]

Functional principles

The basic functioning principle of all BAL devices is that the hepatocytes in the bioreactor come into direct contact with the patient's plasma or blood through a membrane of selected pore size. This allows metabolic interaction and the passage of protein carriers but prevents the translocation of viruses, immunoglobulins, complements and cells[367]. In BAL systems, the patient's blood is perfused across a column of hollow-fiber capillaries lined with hepatocytes within the extracapillary spaces either alone or attached to microcarriers. To remove the toxic metabolic byproducts of the BAL system (bilirubin, ammonia, etc.) a detoxification module is incorporated into the BAL device.

The exact quantity of viable hepatocytes needed to support a patient with a failing liver is yet unknown but based on data with hepatic resection patients, it has been speculated that the required hepatocyte mass is approximately 150-450g[241, 345]. The hepatocytes in BAL devices are derived from either discarded human donor livers[300], immortalized human hepatoma cell lines[224, 225, 249, 349], porcine[59, 80, 301, 365], rat[249] or rabbit[222] origin.

Clinical studies on the bioartificial liver assist devices

The five cell-based systems have been clinically assessed mainly in the phase I studies: ELAD (extracorporeal liver assist device) system[97, 229], HepatAssist [80], MELS (Modular extracorporeal liver support) [300, 301], BLSS (Bioartificial Liver Support System)[223] and AMC-BAL (Amsterdam medical center - Bioartificial liver) [365]. One of the most studied BAL systems, the HepatAssist (Cedar-Sinai Medical Center, Los Angeles, California, USA) using porcine hepatocytes, has been evaluated in small case series[377] and in a large multi-center RCT[80]. These studies have shown some success in improving laboratory variables and HE. Survival was also improved with HepatAssist therapy, even though the survival benefit did not achieve statistical significance except in a subgroup of patients with paracetamol-related toxicity[80]. The first BAL systems, including the hepatoma cell line based ELAD, have also been investigated in RCTs[98, 229] and other studies[60, 301, 365] with similar results. A summary of all the RCTs on BAL devices is presented in Table 6.

Table 6. Randomized controlled studies on bioartificial liver assist devices

Study	Patient population	System	Study design	End point	Follow-up	Outcome	Reference number
Ellis et al. (1996)	ALF (n=24). Group I: not fulfilling Ltx criteria (n=17); Group II: fulfilled criteria for Ltx (n=7)	ELAD (hepatoma cells)	Single center RCT	Survival, Ltx	In hospital	**Survival:** Group I: ELAD 78% vs. controls 75% (P=ns); Group II: ELAD 33% vs. controls 25% (P=ns)	97
Millis et al. (2001)	ALF (n=24) (19 listed for LTx, 5 not listed)	ELAD (hepatoma cells)	Phase I RCT	Survival, Ltx	30 days	**Survival:** listed for LTx: ELAD 83% vs. controls 43% (P=0.12); transplanted ELAD 92% vs controls 43% (P<0.05)	229
Demetriou et al. (2004)	ALF (n=147), primary graft non-function (n=24)	The HepatAssist (porcine cells)	Multi-center RCT	Survival, Ltx	30 days	**Survival:** BAL 71% vs. controls 62% (P=0.26). Subgroups: ALF due to paracetamol: BAL 70% vs. controls 37% (P<0.05)	80

ALF = acute liver failure, ELAD=extracorporeal liver assist device, RCT = randomized controlled trial, Ltx = liver transplantation

Limitations and future prospects of the BAL devices

There are limitations and specific concerns associated with each cell type used in the BAL devices. Human hepatocytes, which would be ideal to use, are difficult to grow in cultures and rapidly lose their liver-specific functions[293]. Cells from discarded human donor livers would be the second best option but these are in short supply and have low viability[230]. Immortalized hepatoma cells might present a risk of tumorgenity and the cells of animal origin are feared to spread xenozoonoses or to provoke immunological responses in patients[262]. The most used cell type at present is of porcine origin because it is widely available and can be easily cryopreserved[390]. Today, bioartificial devices are still only used in clinical studies but not in everyday clinical practice. One important obstacle is the extremely high price of these devices. The high costs, safety concerns and difficulty in obtaining or culturing hepatocytes, have led to the development of artificial extracorporeal liver assist devices.

The future prospects of the BAL devices lie in the development of human stem cells which could be induced to differentiate into functional hepatocytes[321]. In addition, intrasplenic, intraperitoneal and intrahepatic hepatocyte transplantations have been investigated with some success in animals and humans[40, 85, 114, 133, 348].

II Artificial liver assist devices

The main goal of the extracorporeal artificial liver-assist devices is to remove both endo- and exogenous toxins from the patient's blood and thus compensate for the loss of the detoxification function of the liver through an extracorporeal module[334].

The history of artificial liver assist devices

In the 1950s, before the transplantation era, the first attempts to support failing liver function were made by experimenting with a technology used in renal failure, hemodialysis[177, 228]. The next steps toward a more efficient toxin removal were made with blood exchange transfusion techniques[199] and hemoperfusion[304]. Later in the 1960s and 1970s, more advanced artificial blood purification methods were developed using hemoperfusion over charcoal[127, 250, 391] and plasma exchange (e.i. plasmapheresis)[131, 296]. The plasmapheresis technique was introduced in 1968[296] and it is based on the filtration and removal of the patient's own plasma and then the replacement of it with an equal volume of fresh donated plasma from healthy individuals. With these techniques, a short-lived improvement of HE and a decreased in the levels of ammonia and bilirubin concentrations were demonstrated, but no true survival benefit in the subsequent studies has been shown. In 1988, an RCT with 137 FHF patients treated with charcoal hemoperfusion was published[250]. It concluded that the hemoperfusion and SMT control groups did not differ in survival and that the etiology of FHF was the most important factor in determining the fate of the patient[250].

More recently, in the early 1990s, high-volume plasmaferesis (HVPF)[64, 182, 193, 195, 196] and single pass albumin dialysis (SPAD)[58, 65, 302] have also been investigated. The results from an ongoing European multi-center RCT with more than a hundred patients treated with HVPF has not been published yet.

Limitations of the artificial liver assist devices

The major downfall of the plasma exchange was the non-selective removal of physiologically important molecules such as growth and coagulation factors. In plasmapheresis and single pass albumin dialysis, the substantial amounts of plasma or albumin needed restricted the usefulness of these methods.

In general, the artificial liver assist devices merely act as mechanical filters that remove toxins from the patient's blood. These devices are unable to compensate for the complex metabolic and synthetic functions of the healthy liver. Therefore, they can only be used as supportive therapies to

enable native liver recovery or as bridging treatments until a suitable organ is found.

MARS TREATMENT

The new age of the artificial liver-assist devices began in the early 1990s with the introduction of the MARS albumin dialysis[336-339]. This was developed by Stange and Mitzner at the University of Rostock Germany and the first publications appeared in 1993[336, 337]. At present, MARS has been in clinical use for over ten years since 1998.

The aim of the MARS treatment is to enable:

1) Native liver recovery in ALF

2) Recovery back to the compensated status in the AOCLF patients

3) Bridging to Ltx

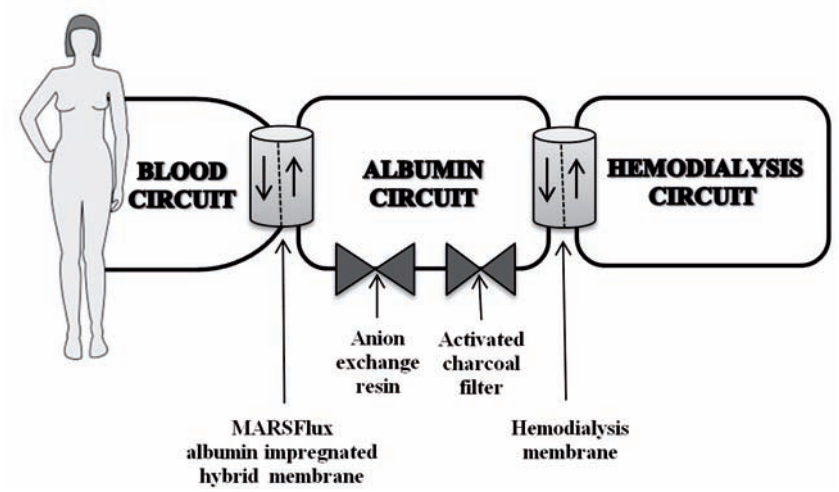
4) Stabilizing the patient prior to Ltx

Operational principles of the MARS machine

The operation of the MARS machine is based on the selective removal of albumin-bound toxic substances and metabolites (e.g. bilirubin, bile acids, neuroactive amino acids) from the blood. Moreover, the MARS device is always coupled with a conventional hemodialysis or hemofiltration unit which can remove water-soluble substances (e.g. ammonia, lactate and urea) from the blood.

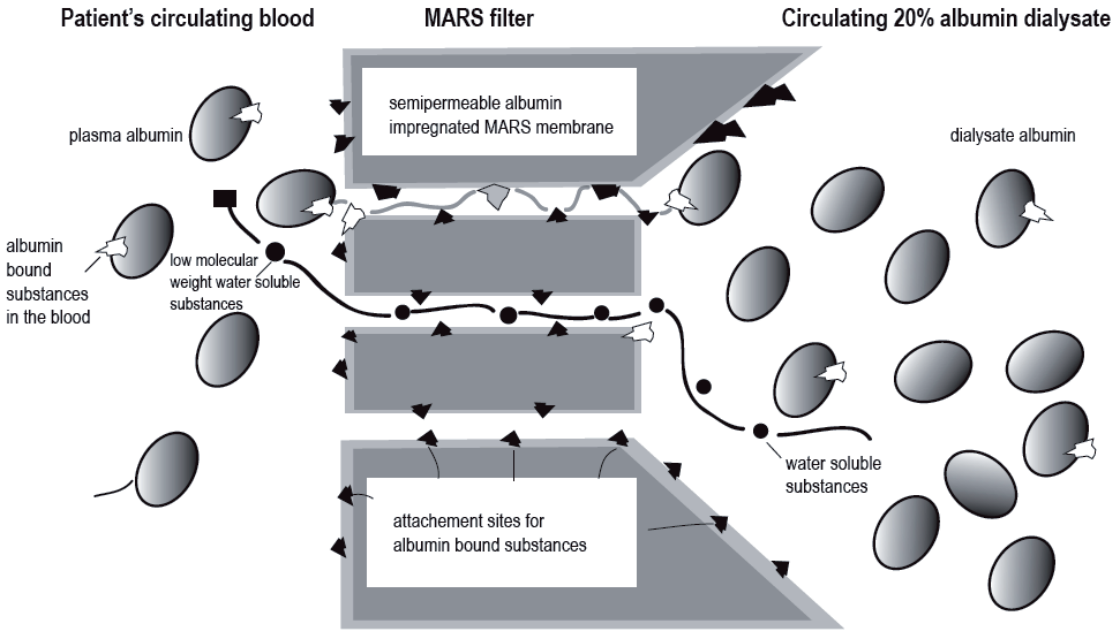
The MARS system is composed of three compartments - a blood circuit, a closed albumin circuit and an open single-pass dialysate circuit (Figure 3.). Venous access to the patient is usually achieved with a temporary dialysis catheter inserted into the internal jugular vein, or more rarely, into the subclavian or femoral vein.

Figure 3. The MARS circuit



The patient's blood is perfused through a hollow fiber dialysis module (MARSflux) across an albumin-impregnated hybrid membrane (pore size 50kD) against a circulating albumin dialysate which is composed of 20% human albumin solution (Figure 4.). This membrane enables the free passage of small- and medium-sized molecules but prevents the translocation of over 50kD molecules such as hormones, clotting factors, immunoglobulins and cells. The endo- and exogenous toxins detach from the blood albumin and bind with greater affinity to the albumin impregnated in the MARSflux membrane and consequently pass through to the circulating albumin dialysate along with the concentration gradient[338].

Figure 4. The structure and movement of substances across the MARS membrane



[Courtesy by Prof. Marja-Leena Mäkelä, Docent Helena Isoniemi and Suomen lääkärilehti [157]]

The fixed volume of 600ml of the albumin dialysate is composed of a 20% human albumin solution. The albumin dialysate is continuously detoxified by first circulating it through a conventional dialysis filtration column, thereby removing the water-soluble toxins. Next, the albumin dialysate is circulated through a charcoal perfusion and anion exchange resin columns and these remove the albumin-bound toxins (Figure 3.). In this way, a continuous concentration gradient is created and the purified albumin dialysate is once again created and ready to adsorb the toxins from the patient's blood. The recommended blood flow rate is 150-250 ml/min, depending of the hemodynamic status of the patient and the flow rate of the albumin circuit is set at 150-180 ml/min[232].

MARS treatment in clinical practice

The initiation criteria and length of a single MARS session vary greatly between the different centers. The only absolute contra-indication to the MARS treatment is active bleeding. In some centers, extracorporeal treatments are carried out in separate dialysis units which only operate during office hours but depending on the initiation criteria, the MARS treatment can also be given in an ICU setting. Usually, the reported length of a single MARS session has varied between 6-8h[95, 138, 141, 231, 320, 333].

The most studied etiological patient subgroup by far has been the AOCLF[56, 63, 89, 105, 138, 141, 145, 231, 232, 320, 375, 388]. The impact of MARS treatment on survival and on the surrogate end-points such as the clinical variables and laboratory values has been studied in many other liver failure etiologies with small patient groups including those induced by cardiogenic shock[95], following hepatic resection[156, 366], associated with sepsis and multiple organ failure[142, 212, 264, 281, 354], liver graft failure[122, 146, 248], and pediatric patients[18, 69, 148, 247, 291, 354]. Refractory pruritus has also been treated successfully by MARS[4, 26, 27, 91, 234].

A recent Finnish systematic literature review on the MARS treatment found 22 uncontrolled studies which included more than 20 patients[157]. These studies encompassed a total of 160 ALF, 600 AOCLF and 100 other liver failure patients. However, the etiology of the patients was not stated clearly in all the publications. In 19 of the 22 studies, the survival rates were reported, but most lacked sufficient data concerning the follow-up time or the follow-up time was very short (e.g. 30 days or in-hospital survival)[157].

Only a few small uncontrolled case series[69, 190, 200, 317, 393] and one controlled prospective study[312] have been published solely on ALF patients but mostly these patients have been represented as a subgroup in larger studies of heterogeneous etiologic origins[54, 87, 246, 313, 343, 344, 376].

In few case reports or small case series, MARS has been used to treat teophylline poisoning[185] and ALF induced by leptospirosis[70], dengue fever[265] and *Amanita phalloides* mushroom poisoning[69, 207, 291, 316, 317, 389].

MARS studies

Randomized, controlled trials and the meta-analysis

Thus far only 8 RCTs[95, 138, 141, 191, 231, 320, 333, 335] have been conducted on the MARS treatment with a total number of 96 MARS patients. The study design and results of these studies are summarized in Table 7. In addition, a recently published abstract on the preliminary results from a French RCT is included in this table[298]. This is the only RCT including the ALF patients.

Only six RCT studies report survival figures[95, 138, 141, 231, 298, 320] and four studies showed a survival benefit favoring the MARS group to the SMT group[95, 141, 231, 298]. The reported 30-day survival of the MARS-treated AOCLF patients in RCTs has ranged from 25%[231] to 92%[141]. One study with 3 months follow-up showed 44% survival which was identical with the control group[320]. Two RCTs have been prematurely discontinued by ethical committees due to the increased mortality in the control group in the interim analysis[141, 231].

The majority of the RCTs conducted on the MARS patients[138, 141, 191, 231, 320, 333, 335] have contained only the AOCLF patients with the exception of one study which comprised of patients with liver failure due to cardiogenic shock after cardiac surgery[95]. In the unpublished French multi-center study[298], the MARS-treated ALF patients had a higher survival as compared to the controls but statistical significance was not reached[298].

So far, only one meta-analysis has been published on the MARS treatment[176]. This analysis concluded that MARS does not offer a significant survival benefit when compared to SMT. However, it also stated that at the moment, definite conclusions as to the efficacy of the MARS treatment cannot be made due to the small number of high quality studies[176].

Table 7. Randomized controlled studies on the MARS treatment

Study	Patient population	System used	Study design	Length of MARS session	End point	Follow-up time	Outcome	Reference number
Mitzner et al. (2000)	AOCLF and type I HRS (N=13)	MARS (N=8)	Two center RCT	6-8h	Survival	30 days	Survival: MARS 25% vs. 0% controls (P<0.01)	231
Heemann et al. (2002)	AOCLF with cirrhosis (N=24)	ECAD (N=12)	Two center RCT	6h	1) Bilirubin 2) 30 day survival 3) Encephalopathy	30 days	1) Significant decrease in bilirubin levels with MARS 2) Survival: 11/12 MARS vs. 6/11 controls (P<0.05) 3) significant improvement of encephalopathy with MARS	141
El Banayosy et al. (2004)	Hypoxic liver failure after cardiogeic shock (N=27)	MARS (N=14)	Single center RCT	8h	1) Survival 2) Length of stay at ICU/ventilator	Hospital discharge (~30days)	1) Survival: MARS 50% vs. controls 32% (P=ns) 2) length of stay at ICU: MARS 42days vs. controls 27days (P=ns) Ventilatory support: MARS 9days vs. 20days controls (P=ns)	95
Sen et al. (2004)	Alcohol-related AOCLF (N=18)	MARS (N=9)	Single center RCT	8h	Cytokine profile, oxidative stress, NO, ammonia	7 days	No change in cytokines/ammonia. Significant decrease in NO (P<0.05) and improvement in encephalopathy with MARS (P<0.01) In-hospital mortality: 5/9 in both groups.	320
Laleman et al. (2006)	Alcoholic AOCLF (N=18)	MARS (N=6) Prometheus (N=6)	Single center RCT	6h	Hemodynamic changes	Treatment session	MARS significantly attenuated hyperdynamic circulation whereas Prometheus did not.	191
Stadlbauer et al. (2006)	AOCLF (N=8)	MARS vs Prometheus (N=8)	Randomized cross-over study	6h	Clearance of cytokines	Treatment session	Cytokines were eliminated by both systems. No change in cytokine profiles during treatment.	333
Stadlauer et al. (2007)	AOCLF (N=8)	MARS vs Prometheus (N=8)	Randomized cross-over study	6h	Elimination of bile acids	Treatment session	Both devices eliminate bile acids to a similar extent.	335
Hassanein et al. (2007)	Cirrhosis with HE grade 3-4 (N=70)	MARS (N=39)	Multicenter RCT	6h	Improvement in encephalopathy	5 days	The mean improvement proportion of HE in MARS 34% vs controls 19%, (P=0.044)	138
Saliba et al. (2008) abstract	ALF (N=102)	MARS (N=53)	Multicenter RCT		Survival	6 months	Survival: MARS 85% vs controls 76% (P=ns); in paracetamol etiology: MARS 85% vs controls 69% (P=ns)	298

AOCLF=acute-on-chronic liver failure, ECAD= extracorporeal albumin dialysis, HRS=hepatorenal syndrome, HE =hepatic encephalopathy, NO=nitric oxide, RCT=randomized controlled study

Outcome and survival in the uncontrolled studies

Survival of the MARS-treated patients has been reported by a vast number of uncontrolled studies[162, 246, 344, 388, 393]. However, comparison between these results is challenging due to the considerable variation in the follow-up time from 30 days to 3 years as well as the unequal availability of transplant organs in the different countries. Furthermore, indications for the MARS treatment and thus the severity of liver failure differ substantially between the centers and studies. In addition, many reports lack sufficient data concerning follow-up time, and outcome has been reported for a heterogeneous group of patients without distinguishing between different etiologies (AOCLF, ALF and GF). Moreover, some centers classify alcoholic hepatitis, acute exacerbation episodes of chronic viral hepatitis and posthepatectomy dysfunction as ALF[54, 246, 376].

Outcome and survival in the MARS-treated ALF patients: Overall survival (including both transplanted and non-transplanted patients) has been reported around 60-70%[54, 62, 87, 190, 200, 244, 246, 344, 393]. Due to the low prevalence of ALF, most published reports are small case series with very few patients. The outcome of the MARS-treated ALF patients in different studies are presented in Table 8. excluding studies containing children[247], case series of pure *Amanita*-intoxications[69, 316, 317], or missing outcome data[109, 274, 313, 343, 387].

For comparison, a large national U.S. study including 17 centers reported the overall survival of the ALF patients at 67% at 3 weeks. In this U.S. study, 43% of the patients survived without Ltx. The short-term transplant-free survival varied greatly, from 68% for the patients with paracetamol-related liver failure to 25% and 17% for those with other drug reactions and an indeterminate cause, respectively[258]. Another study reported that 45% of the adult ALF patients experienced spontaneous liver recovery, 25% underwent Ltx and 30% died without Ltx[204].

The mortality from *Amanita* poisoning related ALF is estimated to be around 6-18% with many patients requiring Ltx[99, 121, 159]. A few case reports[152, 207, 291, 389]and small case series[69, 316, 317] have been published on the MARS treatment in severe *Amanita* poisoning with survival results ranging from 67% (4/6)[69] to 71% (10/14)[317].

Table 8. The outcome of the acute liver failure patients in observational studies

Study	Patient population	Study design	Follow-up time	Length of MARS session	Ltx & Survival	Other results	Reference number
Novelli at al. (2002)	Total 34; ALF 9	Case series	Not reported	8h	67% of the ALF patients transplanted. Survival: 67% in transplanted and 100% in non-transplanted patients	Significant decrease in bilirubin and ammonia.	244
Steiner at al. (2002)	Total 176; ALF 38	Case series	Variable	Variable	Overall survival: 50%, transplant-free survival: 34%	Significant improvement in HE and the MELD-score and a decrease in bilirubin, creatinine, urea, albumin and ammonia.	344
Zhou at al. (2004)	drug induced ALF 14	Case series	6 months	8h	Overall survival and native liver recovery rate 79%. None were transplanted.	Significant improvement in most laboratory variables.	393
Di Campli et al. (2005)	Total 20; ALF 3	Case series	3 months	Not reported	2 transplanted patients survived and 1 non-transplanted patient died.	Significant improvement in HE. Significant decrease in bilirubin, bile acids, creatinine and ammonia.	87
Lai et al. (2005)	ALF 10	Case series	Not reported	8h	Overall survival: 30%, 2 patients received Ltx and died.	Significant increase in systemic vascular index and reduction in cardiac index, urea and creatinine.	190
Lee et al. (2005)	drug induced ALF 13	Case series		6h	2 patients survived of which the other received Ltx. The other 11 patients died without Ltx.	Significant decrease in bilirubin.	200
Novelli et al. (2005)	Total 116; ALF 24	Case series	Not reported	9h	75% of the ALF patients transplanted. Survival 58% in the ALF patients	Significant decrease in bilirubin, ammonia, creatinine.	246
Camus at al. (2006)	Total 22; ALF 14	Case series	30 days	8h	Survival in the ALF patients only not reported. The overall spontaneous liver recovery rate 32% and survival 68%. Survival 89% in listed and 54% in non-listed patients.	Significant decrease in bilirubin and increase in prothrombin-index.	54
Chiu et al. (2006)	Total 22; ALF 2	Case series	30 days	6h	1 patient transplanted and survived, 1 patient survived without Ltx.	Significant decrease in bilirubin, ammonia, and urea.	62

ALF=acute liver failure, MELD= end-stage liver disease score, HE= hepatic encephalopathy

Outcome and survival in the MARS-treated AOCLF patients: In case series, the short-term (30 day - 3 month) survival of mostly nontransplanted MARS-treated AOCLF patients has been reported between 30-65%[87, 331, 339, 340, 344]. The out-of-hospital survival rates for the nontransplanted alcohol-related AOCLF patients vary between 21%-63% [162, 388] and a 1-year survival was reported at 58% by Hessel *et al.*[145].

Outcome and survival in the MARS-treated graft failure patients: A recent study on the MARS-treated early GF patients reported a 28% transplant-free survival rate and a 60% survival in the re-transplanted patients[248]. In the other small uncontrolled studies, the short-term survival in early and late GF patients who received MARS treatment has been reported between 25% to 67%[62, 122, 146, 344]. The survival in the MARS-treated post-hepatectomy liver failure patients has varied significantly between centers (0-71%)[62, 156, 366].

Effect on encephalopathy, cerebral perfusion and plasma amino acids

Significant improvement in neurological function, HE and the Glascow Coma Scale has been reported in a vast number of the MARS studies[54, 63, 232, 260, 339, 344] including three RCTs[138, 141, 320]. In the RCTs, the grade of the HE decreased significantly whereas in the controls, it increased[138, 141, 320]. Some studies have even described a complete resolution of the HE grade 4 coma with the MARS treatment[132, 189]. In addition, normalization of the cerebral perfusion pressure[28, 245, 310] has been noted during the MARS treatment.

A few reports have described a decrease in the plasma AAAs and in the tryptophan concentration, and a subsequent improvement in the Fischer's ratio in the encephalopathic AOCLF and the ALF patients treated with MARS[209, 260, 313]. It has been speculated that the improvement in the amino acid dysbalance might correlate with the simultaneous improvement in HE[209, 260, 313].

Effect on the hemodynamic variables

The stabilization and improvement in the hemodynamic parameters and the attenuation of hyperdynamic circulation have been reported during MARS[56, 89, 132, 190, 191, 248, 274, 309]. In an RCT by Laleman *et al.*, a significant improvement of the MAP and systemic vascular resistance index were noted with MARS treatment while the cardiac index remained unchanged. Furthermore, a simultaneous decrease was recorded in plasma renin activity, aldosterone, norepinehrine, vasopressin and nitrate[191]. Another prospective controlled study on MARS-treated ALF patients reported a significant decrease in cardiac index and heart rate, whereas the systemic vascular resistance index and MAP increased when compared to the controls[312]. Uncontrolled case series have also reported a decrease in portal hypertension and renal/splenic resistance indexes after the MARS treatment in the AOCLF patients[56, 89]. Another benefit of the MARS treatment is a reduced need of vasoactive medication[248]. These changes have been speculated to arise from the removal of the albumin-bound vasoactive substances such as nitric oxide[89, 132].

Effect on the albumin-bound and water-soluble substances/toxins

In the first in vivo and in vitro studies, it was noted that the MARS treatment was able to remove the low- and middle- molecular weight albumin-bound substances (such as copper, bilirubin, bile acids, aromatic amino acids, tryptophan, endogenous benzodiazepine-like substances and nitric oxide) as well as the water-soluble toxins (ammonia, creatinine, lactate and urea) from the patient's blood[336, 337, 339]. These findings have been reproduced by RCTs[95, 138, 141, 191, 231, 320, 333, 335] and other MARS studies[62, 102, 344, 360, 388].

A clinically and statistically significant reduction in the plasma levels of bilirubin, creatinine, urea and ammonia have been noted in most of the previously quoted MARS studies. On the other hand, the in vitro studies using the plasma from ALF patients have been shown to induce apoptotic pathways in human hepatocyte cultures as compared to the plasma from healthy patient[225]. This apoptosis-inducing ability did not decrease after the MARS treatment, suggesting that there are some residual toxic mediators in the plasma of the ALF patients which cannot be removed by MARS[297].

Effect on the plasma cytokines

Elevated plasma cytokine levels have been proposed as one of the mediators of hepatocyte necrosis and the development of HE[139]. The cytokine profiles of MARS-treated ALF and AOCLF have been investigated in a number of studies[14, 18, 88, 154, 155, 158, 320, 333] with conflicting results. Most studies, including our own center's research[154, 155, 158], have concluded that the MARS treatment is able to remove cytokines from the blood but it does not change the cytokine profile of the patient in a clinically significant manner.

The cost-utility and impact on the health-related quality of life

The cost-utility of the MARS treatment and its impact on hospitalization costs has been investigated in a few small nonrandomized studies containing only AOCLF patients[137, 143-145]. Moreover, three of these studies have been conducted in the same center in Essen Germany[143-145]. The health-related quality of life (HRQoL) or cost-effectiveness of MARS treatment in ALF patients has not been studied previously.

The study by Hassanein *et al.* contained only 12 patients and it compared the direct in-hospital medical costs associated with the MARS treatment to a control group receiving SMT in the alcohol-related cirrhotic AOCLF patients in the U.S.. The hospitalization costs in the MARS-treated surviving patients were $32,036 which was $4,000$ less than in the control group[137].

A recent cost-utility study on alcohol-related AOCLF patients in Germany concluded that the mean direct medical costs during 3 years were 40,032€ with a survival rate of 52% in the MARS treatment group. The incremental costs per life-year gained was 31,400€ and the incremental costs per quality adjusted life year (QALY) gained was 47,200€[145]. However, the aforementioned study excluded all patients who underwent Ltx or had a serious concomitant co-morbidity.

Side-effects and safety considerations

The most common side-effects reported in association with the MARS treatment include decrease in platelet count and/or moderate bleeding complications (mucous bleeding or bleeding from the insertion site of the dialysis catheter) and catheter infections[4, 54, 104, 109, 191, 231, 237, 274]. One case of non-cardiogenic pulmonary edema has been reported[90] but other serious life-threatening complications have not been documented. In review articles MARS has been considered a safe treatment[318, 351].

Comparison between the MARS and the Prometheus albumin dialysis systems

The introduction of the MARS device was followed shortly thereafter by the presentation of another albumin dialysis system in 1999; the Fractioned plasma separation, adsorption and dialysis (i.e. Prometheus)[106, 277]. Although both the MARS and the Prometheus systems are basically albumin dialysis machines, there are two fundamental differences between them. Firstly, in the MARS machine, the albumin dialysate is circulated in a separate closed-loop system, whereas in Prometheus, the patient's plasma and albumin are separated from the blood for filtration. Secondly, the Prometheus device has a much bigger filtration pore size as compared to the MARS (250kD vs. 50kD) which allows the passage of larger molecules such as albumin (68kD) into the secondary circuit[106, 336, 337]. Due to the larger pore size in the Prometheus system, the filtration and adsorption of some coagulation factors can lead to major coagulation disturbances and consequently to repeated thrombosis[227]. A significant prolongation of the prothrombin time[104] and the reduction of several coagulation factors (II, X and protein S and C) have been reported with the use of the Prometheus system[227], while the levels of factor V and VIII have remained unchanged due to the bigger molecular weight (>300kD) of these molecules[227, 277].

Some comparative studies have been carried out between the MARS and the Prometheus systems[104, 187], including three RCTs[191, 333, 335]. Both devices have been shown to decrease water- soluble and albumin-bound toxin levels in the blood much in the same manner[103, 191, 333, 335]. A recent study of 18 patients found that the Prometheus demonstrated a higher removal rate of most toxins (bilirubin, urea, creatinine) as compared to the MARS treatment[104]. On the other hand, Laleman *et al.* found that the MARS treatment was able to improve the hemodynamic profile in the cirrhotic AOCLF patients, whereas Prometheus did not[191].

AIMS OF THE STUDY

The main goal of the present studies (I-VI) was to evaluate the outcome of 195 MARS-treated patients in Finland and to answer the most important and clinically relevant question associated with this treatment.

The specific aims of this thesis were:

1) To study the effect of the MARS treatment on patient outcome (survival, native liver recovery and need for Ltx) in different liver failure etiologies (II, III, IV, V, VI). And also, to determine which patients stand to gain and who do not benefit from MARS treatment.

2) To explore prognostic factors predicting survival in MARS-treated patients in various liver failure etiologies (V).

3) To investigate the impact of MARS treatment on the plasma amino acid profile (I) and encephalopathy (I, II, IV, V) in liver failure patients.

4) To investigate the impact of MARS treatment on laboratory values and albumin bound toxin levels (I, V).

5) To examine the direct medical costs and the cost-utility of MARS treatment and its impact on the HRQoL of the patient in an ICU setting (VI).

PATIENTS AND SETTING

This thesis includes 188 MARS-treated liver failure patients in Finland during May 2001-March 2007. Four of these patients were treated both before Ltx due to their liver failure and afterwards, due to their graft failure and they are considered to be individual treatment cases as categorized by etiology. Study III and IV featured seven additional ALF patients treated during April 2007 - September 2007. The total number of MARS-treated patients in this thesis was 195.

A historical control group of 46 consecutive ALF patients treated during January 1995 - April 2001 was included in Study II. This patient population and time period was selected to include a satisfactory number of patients for a comparative analysis within such a time interval that the SMT of the liver failure patients would be similar to the MARS era. In Study VI, a more contemporary control group was used of 17 ALF patients treated during March 2000 – April 2001. This was done in order to compare the treatment costs within a relatively small time window to minimize the impact on the final results of inflation, price changes and the currency conversion. In addition, from the year 2000 onwards, the HUCH district began to use the clinical patient-administration database Ecomed® (Datawell Ltd., Espoo, Finland) for registering treatment costs.

During the past decade, a handful of patients has also received The MARS treatment at the University hospitals of Tampere and Oulu in collaboration with the Surgical Hospital[157], but generally, the MARS machine in Tampere has been used in research projects. Moreover, in a few cases, nurses from the Surgical Hospital who are specialized in giving the MARS treatments, have performed the MARS treatments in the Children's Hospital and the Cardiac ICU of HUCH. However, only the patients treated at the liver ICU of HUCH were included in this thesis.

Defining liver failure categories

The term ALF is defined in this dissertation as a deteriorating liver function (increasing liver enzyme and bilirubin levels and/or decreasing coagulation factor levels) with or without encephalopathy in a person with no previous history of liver disease. Furthermore, ALF is used as an umbrella term encompassing the hyperacute, acute and subfulminant subtypes. The patients in Studies I-VI are subgrouped according to the specific etiology of their liver failure.

AOCLF was defined as an acute decompensation episode of liver function in a previously well-compensated chronic liver disease patient. AOCLF

often developed rapidly due to a triggering cause (such as an infection or GI bleeding).

The GF patients included both those that had early and late graft dysfunctions. The group of miscellaneous etiologies contained one case of acute hemorrhagic pancreatitis, one case of ischemic injury to the liver following myocardial infarction, one multiple trauma patient with chronic hepatitis C and three post-liver resection hepatic failures.

MARS treatment indications and the treatment protocols in Finland

The initiation criteria for the MARS treatment in Finland depend on the etiology of the liver failure (Table 9.). The recommended flow rates of blood, albumin and dialysate circuits in our unit are presented in Table 10. In some patients, the MARS treatment was started without signs of HE, particularly in ALF patients who had ingested a lethal amount of toxin (e.g. paracetamol, *Amanita*), or if laboratory parameters indicated progressive liver failure (increasing liver enzyme and bilirubin levels and/or decreasing coagulation factor levels) despite the best possible SMT.

The diagnosis of *Amanita phalloides* poisoning (III) and the decision to start the MARS treatment was based on 1) the information given by the patient (all had eaten white mushrooms resembling *Amanita phalloides)* and 2) the appearance of typical symptoms within 24h of mushroom ingestion (i.e. vomiting, abdominal pain and diarrhea) .

In critical ALF, treatment was usually discontinued only to replace a new dialysis absorption kit to the machine. As a general rule, we only treated the first exacerbation episode of chronic alcoholic liver disease.

Table 9. MARS treatment initiation criteria and treatment protocols in Finland.

Etiology	MARS treatment initiation criteria	Treatment protocol
Acute liver failure	Rapid deterioration of the clinical condition and hepatic synthetic function despite standard medical therapy	22 hour sessions daily until:
	AND one of the following criteria:	1) Native liver recovers
	1) Ingestion of a lethal dose of known hepatotoxin (mushroom, paracetamol, iron, etc.)	2) Suitable transplant organ is found
	2) Patient fulfills the criteria for high urgent Ltx	3) Irreversible multi-organ damage occurs
Acute-on-chronic liver failure	Rapid deterioration of the clinical condition and hepatic synthetic function despite standard medical therapy	6-8 hour sessions based on the daily assessment by the surgeon and anesthesiologist until:
	AND two of the following criteria:	1) Patient's clinical condition improves
	1) Hyperbilirubinemia, bilirubin >400µmol/l	2) Suitable transplant organ is found
	2) Type 1 Hepatorenal syndrome	3) Irreversible multi-organ damage occurs
	3) Progressive hepatic encephalopathy	
Graft failure	No set criteria, depends on the assessment of the transplant surgeon and anesthesiologist	No set protocol, based on the daily assessment by the surgeon and anesthesiologist

Table 10. The flow rates of the MARS circuit

Bilirubin	Creatinine	Ammonia	Blood flow (ml/min)	MARS circuit (ml/min)	Dialysate flow (ml/min)	Ultrafiltration (min. ml/min)
↔	↔	↔	150-180	150	500	400
↑	↔	↔	180	180	500	400
↔	↑	↑	150-180	150	500-800	1000-1200
↑	↑	↑	180	180	500-800	1000-1200

↔ = plasma level within normal range, ↑= plasma level elevated

Electrolyte balance and anticoagulation during the MARS treatment

The blood gas analysis and the plasma levels of potassium, magnesium, and phosphate are controlled every 6 hours to ensure optimal electrolyte balance. Hypokalemia and hypomagnesemia are promptly corrected with a potassium chloride-infusion and magnesium sulphate-boluses, respectively.

The blood phosphate-ion–balance is controlled by a continuous infusion of a solution containing phosphorus. The saturation of the MARS filters is monitored by taking bilirubin samples taken every 6 hours.

Anticoagulation is used if permitted by the coagulation status and platelet count to prevent the clotting of the MARS filters. If the patient's INR is below 3, dalteparin can be used. An epoprostenol-infusion (ad 5 ng/kg/min) is used either alone or in combination with dalteparin if the platelet count is over $50x10^9$/l. If coagulation problems persist despite the dalteparin/epoprostenol medication, and antithrombin III levels decrease below 15%, an antithrombin III preparation is given. If the patient shows signs of bleeding, the dosage of anticoagulants is reduced or the medication is discontinued. If the INR is above 3 and the platelet count below $50x10^9$/l, anticoagulation is not given.

Standard medical therapy of the liver failure patients

All patients received a similar SMT at the same liver disease specialized 4-bed ICU and Ltx unit at the Surgical Hospital of HUCH. The SMT follows the same guidelines as presented in the chapters describing the current conservative management of ALF and AOCLF. The only major exception to the general SMT was the use of HVPF and invasive ICP monitoring in some control group patients before the MARS era (II).

METHODS

Prospectively collected data:
Since the introduction of the MARS treatment in Finland in May 2001, the information regarding each patient and treatment session has been prospectively collected to a specifically designed data collection sheet. The patients were stratified before treatment into etiological subgroups: ALF, AOCLF, graft failure, postresection or other indications. The cause of liver failure was recorded in more detail. Pre-existing liver disease-related complications (HRS, encephalopathy, ascites, GI bleeding, SBP, hyperbilirubinemia) were also recorded.

In all patients, the baseline demographics, laboratory (cell counts, coagulation factor levels, liver transaminases, bilirubin, ammonia, urea, creatinine, blood gases, and electrolytes) and clinical variables (grade of HE, renal failure, ascites, MAP, need for mechanical ventilation and vasoactive-infusions) were recorded at the beginning of the first and after the last MARS session. MARS treatment-related data were also collected for the same case record forms (e.g. timing, number and duration of sessions, flow rates of the albumin, blood and dialysate circuits and treatment related complications). At baseline, the MELD-score was calculated for each patient using the standard formula by the United Network for Organ Sharing[362].

The same data variables were collected retrospectively from each control group patient (II, VI). In addition, the use of other extracorporeal treatments (e.g. HVPF) was recorded. The baseline data was collected upon the patient's admittance to the ICU. The post-treatment values were obtained before leaving the ICU, Ltx or before death.

In Studies II-VI, the rates of survival, native liver recovery and Ltx were determined after the follow-up period. Table 11. offers a summary of the study type, number of patients, and the main outcome variables of studies I-VI.

Table 11. The summary of patients and methods used in studies I-VI

			Number of patients			
	Study type	Time period	MARS	Controls	Main outcome variables	Follow-up
I	**Observational, prospective**	5/2001 - 4/2003	50 ALF 32 AOCLF	-	1) Plasma concentration of neuroactive amino acids 2) Encephalopathy grade before and after the MARS treatment	-
II	**Controlled, retrospective**	5/2001 - 3/2007 (MARS) 1/1995 - 4/2001 (Controls)	113 ALF	46 ALF	1) Outcome: Survival, native liver recovery rate, need for Ltx 2) Laboratory / clinical variables/ encephalopathy before and after treatment	6 months
III	**Observational, case series**	5/2001 - 8/2007	10 Amanita poisonings	-	1) Outcome: Survival, native liver recovery rate, need for Ltx	1 year
IV	**Observational, case series**	5/2001 - 9/2007	37 ALF high urgently transplanted	-	1) Percentage of liver necrosis in the explanted liver 2) Waiting time for Ltx 3) Survival 4) Encephalopathy before and after treatment	30 days, 1 year
V	**Observational, prospective**	5/2001 - 3/2007	113 ALF 62 AOCLF 11 GF 6 others	-	1) Survival, native liver recovery rate, need for Ltx 2) Laboratory / clinical variables/ encephalopathy before and after treatment	1 year
VI	**Controlled, retrospective**	5/2001 - 10/2005 (MARS) 3/2000 - 4/2001 (Controls)	90 ALF	17 ALF	1) Total direct medical costs during 3.5 years and 2) HRQoL before and after the MARS treatment 3) Cost-utility	3 years

ALF=acute liver failure, AOCLF=acute-on-chronic liver failure, GF= graft failure, Ltx=liver transplantation, HRQoL= health-related quality of life, QALY= quality-adjusted life years

The specific methodology used in Studies I-VI:

Study I: The plasma concentration of the neuroactive amino acids were measured using a liquid chromatography (and the Fischer's ratio determined) before and after the MARS treatment. Statistical methods were then used to analyze the differences in the amino acid levels before and after the MARS treatment and also their correlation to the grade of HE were determined in different etiologies. The levels of amino acids before and after treatment were compared between the ALF and AOCLF patients.

Study II: For the comparison of outcomes (survival, native liver recovery and need for Ltx) between the MARS and the historical control ALF group, patients were subdivided into etiological subgroups. This was a way to match up the patients who had similar prognoses. All patients received the same SMT with the exception of the HVPF which was used in 12 control group patients.

Study III: Every patient received the standard SMT for mushroom poisoning (including fluid resuscitation, NAC, and activated charcoal). The time which had elapsed was determined from the mushroom ingestion to 1) the appearance of symptoms 2) the first aid at local hospital and 3) the beginning of MARS treatment.

Study IV: The time from the listing and the MARS treatment to the Ltx were determined. The pathologist then determined the weight, histopathology and degree of necrosis in the explanted liver. Patients were analyzed and subgrouped according to degree of necrosis in their explanted liver: group I: 100% (n=10), group II: >80% (n=9) and group III: <80% (n=18) necrosis.

Study V: All patients were categorized according to their liver failure etiology. A stepwise logistic regression analysis was used to search for prognostic factors predicting 6-month survival in each etiological subgroup. The variables analyzed included all the collected demographic, clinical and treatment-related variables and all the laboratory parameters at baseline.

Study VI:. All the direct liver disease-related costs were obtained from the Ecomed-database for the period of 6 months prior to treatment until 3 years afterwards. Only those patients living in the Helsinki Uusimaa hospital district were included in the cost analysis. The costs in the Ecomed-database include the ICU and ward costs, ambulatory visits, laboratory and radiology costs, surgery and procedure costs, expensive medicine and blood products.

The HRQoL of MARS patients was measured with a generic self-administered questionnaire, the 15D instrument, using the questions related to 15 dimensions: moving, seeing, hearing, breathing, sleeping, eating, speech, eliminating, usual activities, mental function, discomfort and symptoms, depression, distress, vitality, and sexual activity. For each dimension, the patient or doctor selected one of five levels that best described the patient's current state of health. A set of utility weights was used to generate the 15D score, ranging from 0 to 1 (1=healthy state, 0=dead)[325]. For most of the important properties, i.e. the reliability, content validity,

discriminatory power, and responsiveness to change, the 15D compares favorably to other similar HRQoL instruments such as the EQ-5D, SF-6D, HUI3, and AqoL [140, 235, 326-328, 342].

Upon ICU admittance, the HRQoL of the patient was estimated by three ICU doctors, retrospectively, by using the 15D instrument and the patients' clinical documents. The post-treatment HRQoL was measured by sending the 15D questionnaire to all the surviving MARS-treated patients. A 15D score three years after the first MARS treatment was estimated for each patient using a linear regression analysis.

In the cost-utility analysis, the effectiveness of the MARS and SMT was measured as the quality-adjusted life years (QALYs) gained. The QALYs gained were estimated by determining the difference in the HRQoL before and after treatment and the resulting survival benefit within 3,5 years time. Also, the total direct medical costs and outcomes of MARS treatment were compared with those in the control group over a 3.5-year time horizon from the perspective of the health care provider. The incremental cost-effectiveness ratio (ICER) was determined by dividing the difference in costs by the difference in QALYs.

Statistical methods

Data in Studies I and IV was analyzed with StatView5 for Windows (SAS Institute Inc.). All other data in Studies II, III, V and VI were analyzed by the SPSS for Windows version 15.0. and 16.0 (SPSS, Chicago, IL). The $P \leq 0.05$ was considered to be statistically significant. The results were presented as a mean ($\pm$ standard deviation) or a median (minimum-maximum). The missing laboratory values were replaced by the median value of that laboratory result in all patients. The median value and the use of non-parametric tests were selected due to the skewed distribution of most results.

The following statistical methods were used in this thesis.

Wilcoxon signed rank-test	Repeated scale measurements (laboratory results, etc.) before and after treatment within a single treatment group.
Mann-Whitney-*U* –test	Comparison of the scale measurements between two independent treatment groups (MARS vs control).
Kruskall-Wallis test	Comparison of the scale measurements between three on more independent groups.
Pearsons χ^2 and Fisher's exact tests	Comparison of the outcomes and bionominal results between independent groups.

Additional statistical methods

Study I: Variance analysis

Study II: To compare the survival of the MARS and the control group, the Kaplan-Meier method and the log-rank test for cumulative survival over time was used.

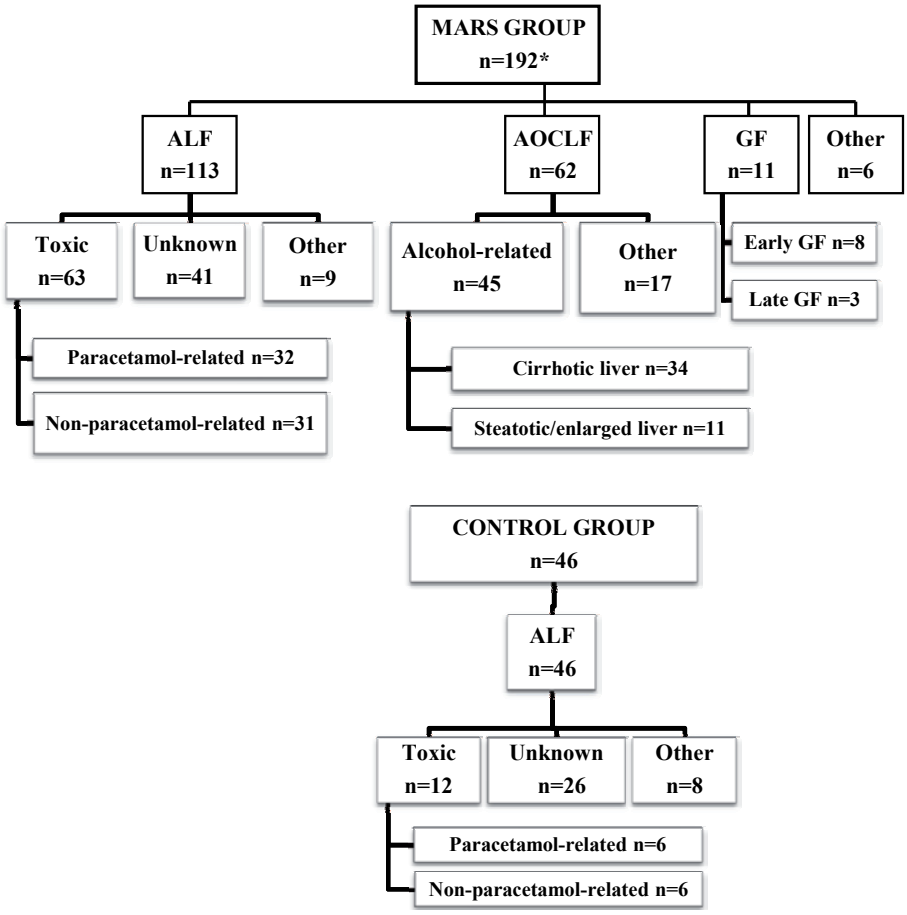
Study V: To explore the factors predicting the survival in each etiological subgroup, a stepwise binary logistic regression analysis was used. The odds ratio (OR) and confidence interval (C.I.) for each predictive variable was calculated. The best combination of the significant predictive variables was selected using the R^2-score and Hosmer-Lemeshow Goodness of Fit-test.

Study IV: To estimate the effect of the model parameter uncertainty on the results, a one-way and probabilistic sensitivity analysis were used. In the one-way sensitivity analysis, the discount rate of costs, the mean costs and QALYs of both MARS and control treatment, the probability of Ltx, and survival rates, were varied. A regression analysis was used to estimate the overall costs and HRQoL 3 years after treatment for all patients.

RESULTS

The distribution of the liver failure etiologies in the MARS group (V) and the ALF control group (II) are shown in Figure 5. The other ALF subgroup contained a heterogeneous combination of rare ALF etiologies (e.g. pregnancy related ALF, acute viral hepatitis, ischemia, etc.). The fourth main group, other etiologies, included miscellaneous liver failure patients who were difficult to categorize under the ALF, AOCLF or GF (e.g. pancreatitis, post-resection hepatic failure, trauma, etc.).

Figure 5. Distribution of liver failure etiologies



ALF=acute liver failure, AOCLF=acute-on-chronic liver failure, GF= graft failure * = 4 patients were included in both the liver failure and GF groups.

Baseline characteristics of the MARS-treated patients

The baseline characteristics of the 192 MARS-treatment cases in Study V are summarized in Table 12. All AOCLF patients belonged to the Child-Pugh class C.

Table 12. Demographic, clinical, and treatment data at the beginning of the MARS treatment in Study V.

Baseline demographic & clinical data	ALL CASES	ALF toxic	ALF unknown	ALF other	AOCLF alcohol-related	AOCLF other	GRAFT FAILURE
Number of patients	192	63	41	9	45	17	11
Age in years	49 (14-81)	41 (14-81)	51 (19-68)	43 (32-58)	52 (30-71)	54 (16-75)	47 (18-62)
Sex %, male	48% (93)	48% (30)	32% (13)	33% (3)	69% (31)	47% (8)	36% (4)
MARS sessions/ patients	2 (1-13)	2 (1-8)	3 (1-12)	3 (1-9)	2 (1-13)	2 (1-9)	2 (1-4)
Duration of MARS session, hours	16.5 (4-22.5)	15.0 (5.5-22)	16.8 (4-22)	16.0 (6.4-22)	20.1 (7.8-22)	18.3 (4.5-22.5)	17.5 (9.5-22)
Mechanically ventilated %	36% (69)	29% (18)	34% (14)	56% (5)	29% (13)	41% (7)	73% (8)
Vasoactive-infusion used %	43% (82)	33% (21)	27% (11)	33% (3)	47% (21)	82% (14)	63% (7)
Renal insufficiency %	49% (94)	33% (21)	37% (15)	44% (4)	60% (27)	76% (13)	73% (8)
Contra-indication to Ltx prior to treatment %	35% (67)	17% (10)	10% (4)	22% (2)	93% (42)	18% (3)	9% (1)
MELD-score	32 (5-52)	27 (5-48)	32 (23-50)	27 (23-46)	39 (17-52)	36 (27-44)	26 (20-47)

All demographic values are expressed as median (range) or as a percentage % of patients (number of patients). ALF = acute liver failure, AOCLF = acute-on-chronic liver failure, MELD = Model for end-stage liver disease-score, Ltx = liver transplantation

The comparison of the baseline characteristics between the MARS and the control ALF patients (II) are presented in Table 13.

Table 13. Comparison of baseline data between the MARS and the control group ALF patients in study II.

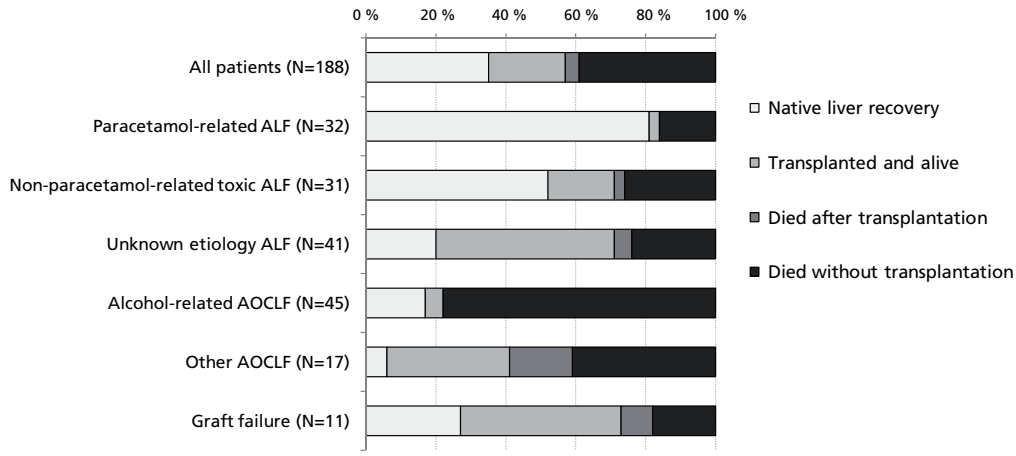
	ALF PATIENTS		
Baseline demographic & clinical data	**MARS**	**Control**	**P**
Number of patients	113	46	
Age in years	45 (14-81)	45 (21-72)	0.952
Sex %, male	41% (46)	35% (16)	0.591
Contraindication to Ltx prior to treatment %	14% (16)	13% (6)	0.853
Mechanically ventilated %	33% (37)	33% (15)	1.000
Vasoactive-infusion used %	31% (35)	33% (15)	0.853
Renal insuffiency %	35% (40)	44% (20)	0.370
MELD-score	31 (5-50)	34 (19-51)	0.020
Encephalopathy grade before treatment	1.8 (1.5)	2.0 (1.4)	0.433
Platelets x109	135 (11-511)	137 (18-448)	0.459
Creatinine µmol/l	83 (35-1318)	84 (35-673)	0.505
NH4-ion µmol/l	75 (8-512)	94 (14-407)	0.169
Bilirubin µmol/l	290 (4-761)	368 (38-880)	0.083
ALT U/l	943 (11-12500)	905 (47-12280)	0.899
TT %	20 (6-80)	16 (6-51)	0.069

All values are expressed as a median (range) or as a percentage % of patients (number of patients). The encephalopathy grades are expressed as a mean (±standard deviation). MELD = Model for end-stage liver disease-score, Ltx = liver transplantation, NH4-ion = ammonium-ion, ALT = Alanine aminotransferase, TT % = thrombin time (includes coagulation factors II, VII, X).

Patient outcome

Figure 6 shows the 1-year outcome of the 188 MARS treated patients in Study V in terms of their native liver recovery, survival and need for Ltx.

Figure 6. The 1-year outcome of 188 MARS treated patients in study V.

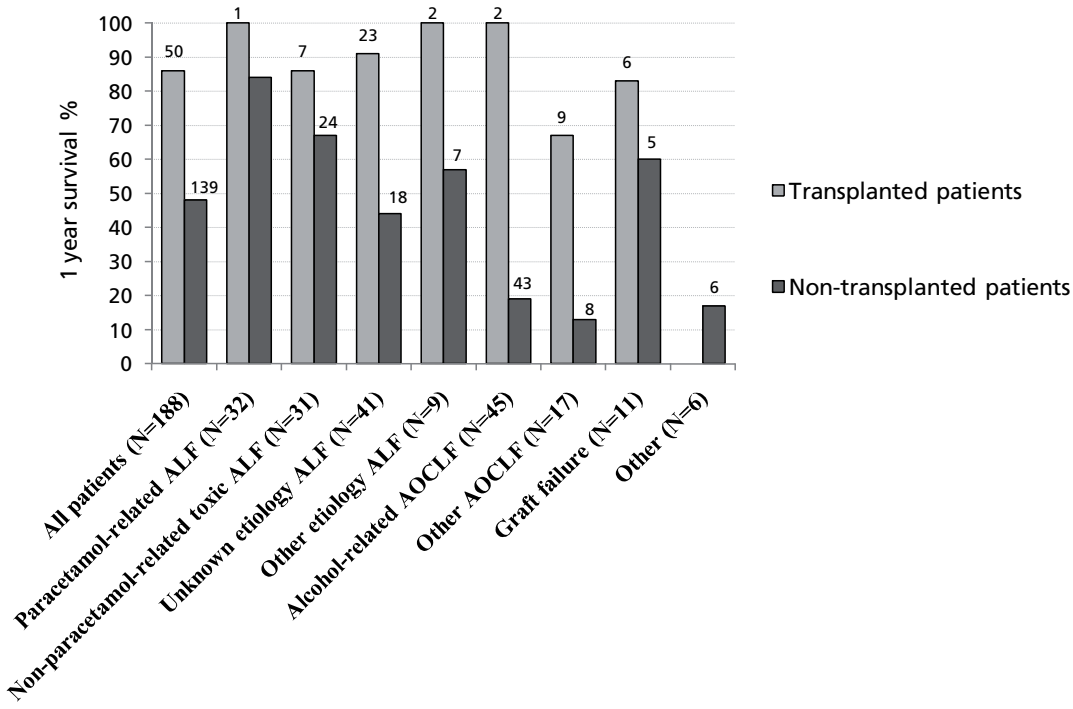


ALF = acute liver failure, AOCLF = acute-on-chronic liver failure

Overall survival

The 1-year survival of all 188 MARS-treated patients was 57%. The highest overall survival was observed in the toxic etiology ALF subgroup (79%) and the lowest survival in the alcohol-related AOCLF (22%), and for the other miscellaneous etiology liver failure subgroups (17%). The 1-year survival of the transplanted and non-transplanted patients in Study V were 86% and 47%, respectively (Figure 7.).

Figure 7. The 1-year survival of the transplanted and non-transplanted MARS-treated patients



The total sumber of patients in each subgroup is presented above the corresponding column.
ALF = acute liver failure, AOCLF = acute-on-chronic liver failure

ALF

In Study II, the 6-month survival of both the transplanted (94% vs. 77%, P= 0.06) and non-transplanted (66% vs. 40%, P=0.03) ALF patients was higher in the MARS treatment group as compared to the controls. Furthermore, the percentage of patients who died during treatment due to tentorial herniation was significantly lower in the MARS group (4% vs. 15%, P=0.014). However, it was noted that the etiological distribution of the ALF patients who have been referred to our transplantation ICU, has changed markedly over the past ten years (P=0.002) toward the more benign toxic etiologies (Figure 5.). The baseline comparison of the demographic, clinical and laboratory data before treatment shows that despite significant differences in the etiological distribution and the MELD-scores, the groups are otherwise similar (Table 13.)

In the subgroup analysis of Study II, the patients with unknown etiology ALF in the MARS and the control groups were comparable at all measured baseline variables. There was a trend towards higher 6-month survival in the MARS group (71% vs. 50%, P=0.09) even though statistical significance was not achieved. In the toxic etiology subgroups, the MARS and the control

groups differed significantly at the baseline HE grades and MELD-scores. Moreover, all patients with *Amanita* mushroom poisoning survived the 1-year follow-up (III).

In Study IV, the 1-year survival of ALF patients who underwent high urgent Ltx was 92%. In Study VI, the percentage of ALF patients who survived over 3 years after treatment was significantly higher in the MARS treatment group as compared to the controls (78% vs. 41%, P= 0.002) in Study VI.

AOCLF

The 1-year survival of the transplanted and non-transplanted patients were 100% and 19% in the alcohol-related AOCLF, and 67% and 13% in the other etiology AOCLF (V). In the alcohol-related AOCLF, the patients exhibiting signs of steatosis and enlargement of the liver had significantly higher 1-year survival when compared to those with end-stage cirrhosis (55% vs. 6%, P=0.002).

GF

The overall 1-year survival rate was 73% in 11 patients. The 1-year survival of the re-transplanted and non-re-transplanted patients were 83% and 60%, respectively. Sixty-two percent of the early GF patients survived and four were re-transplanted. All 3 patients with late GF survived and two were re-transplanted successfully.

Native liver recovery and Ltx

In Study V, 26% of the 188 patients were transplanted. Native liver recovery, which was defined as a survival of over 3 months without Ltx, occurred in 35% of the patients (Figure 6).

ALF

In Study II, the percentage of patients who experienced native liver recovery was significantly higher in the MARS group as compared to the controls (49% vs. 17%, P<0.001). Furthermore, the percentage of Ltx was lower in the MARS treatment group (29% vs. 57%, P=0.001). In the subgroup analysis of the toxic etiology ALF, the rate of native liver recovery was also higher (67% vs. 33%, P=0.05) and the need for Ltx was significantly lower (13% vs. 50%, P=0.002) in the MARS treatment group. In the unknown etiology ALF subgroup, a similar tendency was noted towards higher native liver recovery (20% vs. 8%, P=0.294) in the MARS-treated patients.

AOCLF

In the alcohol-related AOCLF group, two abstinent patients (4% of the entire group) received transplants. In the other-etiology AOCLF, nine patients out of 17 were transplanted.

Re-transplantation in GF

In total, six GF patients underwent re-Ltx (4 early GF patients and 2 late GF patients).

Survival predicting factors

According to the results from Study V, the etiology of liver failure was the most significant factor predicting survival (P<0.0001) with the highest mortality observed in the alcohol-related AOCLF subgroup. In the subgroup analysis, the most significant predicting factor for survival in paracetamol-related toxic ALF was the grade of HE (OR 0.345; 95% CI, 0.154-0.774; P=0.001) prior to treatment. In the non-paracetamol-related toxic etiology ALF, the prognostic factors were thrombin time, (TT%) (OR, 1.103; 95% CI, 1.000-1.217; P=0.049), and the grade of HE before treatment (OR 0.562; 95% CI, 0.305-1.035; P=0.064).

In the unknown etiology ALF, the predicting factors were the coagulation factor V levels (OR, 1.052; 95% CI, 1.007-1.099; P=0.02) and alanine aminotransferase (ALT) plasma levels (OR, 1.001; 95% CI, 1.000-1.001; P=0.013). The mathematical formulae predicting a 6-month survival without Ltx in different ALF etiologies are summarized in Table 14.

Table 14. The survival predicting formulae in the different etiological ALF subgroups.

Paracetamol-related toxic ALF

$$p = 100 \times \left[1/(1 + e^{-(3{,}831 - HE * 1.064)})\right]$$

Non-paracetamol-related toxic ALF

$$p = 100 \times \left[1/(1 + e^{-(-1{,}120 + TT * 0.098 - HE * 0.577)})\right]$$

Unknown etiology ALF

$$p = 100 \times \left[1/(1 + e^{-(-4{,}894 + ALT * 0{,}001 + FV * 0{,}051)})\right]$$

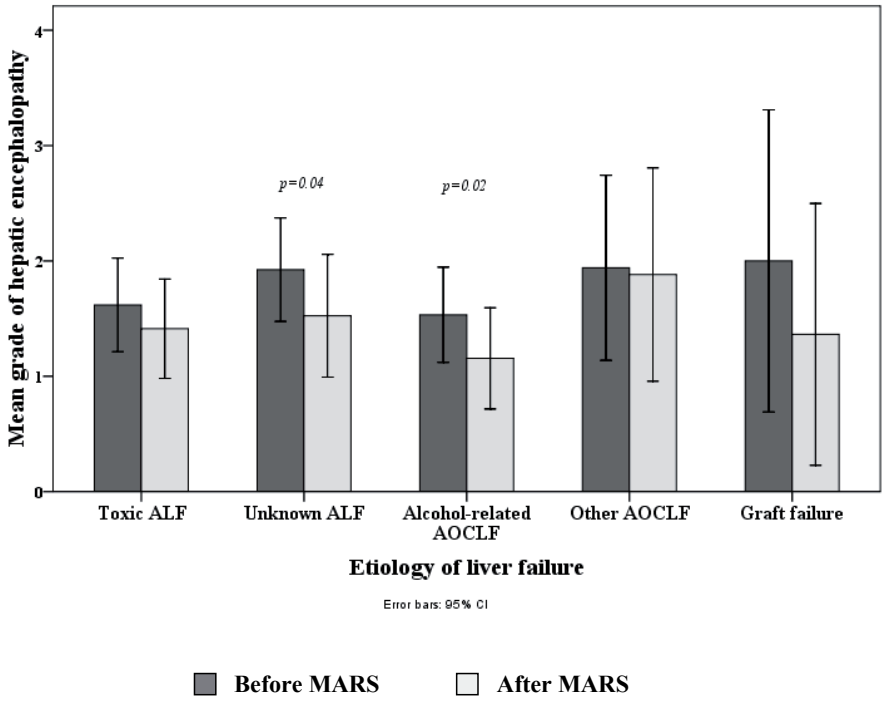
HE=grade of hepatic encephalopathy, TT%=trombin time, ALT= alanine aminotransferase, coagulation factor V

Hepatic encephalopathy and the neuroactive amino acids

During the MARS treatment, the grade of HE was significantly decreased (or the worsening of HE was halted) in most patients (I,II,IV,V) (Figure

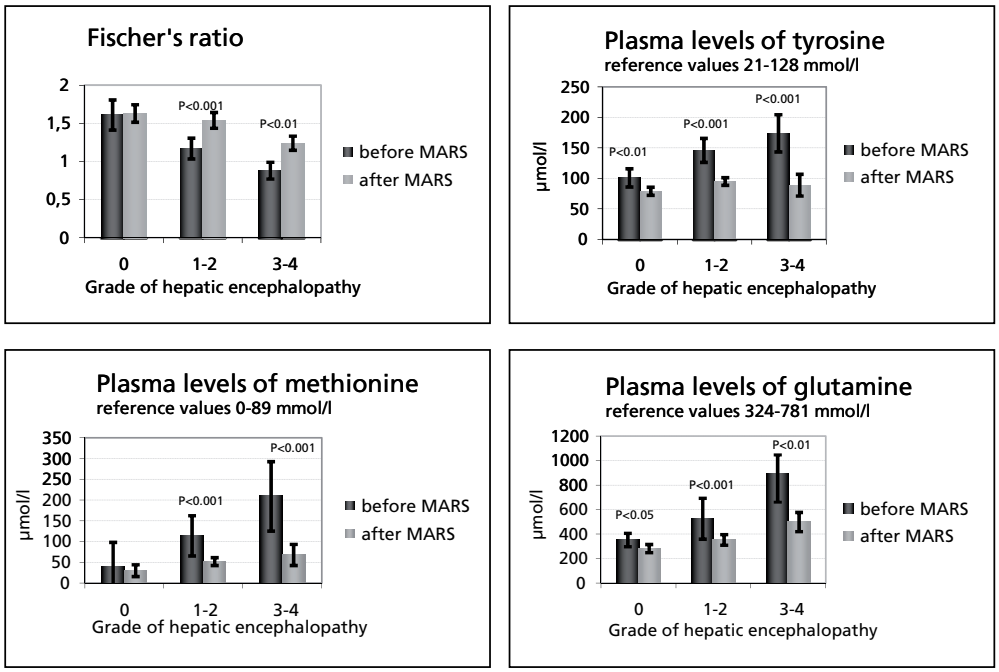
8.). In Study V, including the 192 treatment cases, the mean grade of HE in all patients decreased significantly during the MARS treatment (the mean HE grade before MARS 1.8 vs. after MARS 1.4, P<0.001). The opposite occurred in the historical control ALF group and the grade of HE increased significantly (P=0.032) during SMT (II). Also, a number of the MARS-treated ALF (4/19) patients with over 80% liver necrosis showed no signs of HE before Ltx (IV).

Figure 8. The grade of encephalopathy before and after the MARS treatment in the different liver failure etiological subgroups.



With the majority of measured amino acids, the plasma concentrations were comparable between the ALF and AOCLF patients before the MARS treatment was started (I). The plasma levels of phenylalanine (P<0.05), methionine (P<0.05), glutamine (P<0.01), and histidine (P<0.001) and also ammonia (P<0.001) were higher in the patients with the HE grade ≥1 when compared to non-encephalopathic patients. The Fischer's ratio improved in all the encephalopathic patients with the MARS treatment. In all patients, regardless of the HE grade upon admission, the plasma levels of AAAs (tryptophane, tyrosine and phenylalanine) decreased significantly during the MARS treatment. In addition, in encephalopathic patients, the levels of methionine, glutamine and histidine decreased significantly during MARS (Figure 9.).

Figure 9. Neuroactive amino acids and the Fischer's ratio according to the encephalopathy grade, before and after the MARS treatments in Study I.



Data is presented as median with standard error bars (95% C.I.). Fischer's ratio = valine+isoleucine+leucine / phenylalanine+tyrosine

Measured laboratory variables and toxin removal

The median plasma concentration of most toxic metabolites (bilirubin, ammonia and creatinine) as well as some neuroactive amino acids, trombocytes, hemoglobin and liver enzymes were significantly reduced during the MARS treatments (I, II, III, V). The median coagulation factor levels remained unaffected throughout the MARS treatment. The changes in key laboratory variables during the MARS treatment are summarized in Table 15.

Table 15. The changes in the laboratory variables in 188 patients during the MARS treatment in Study V.

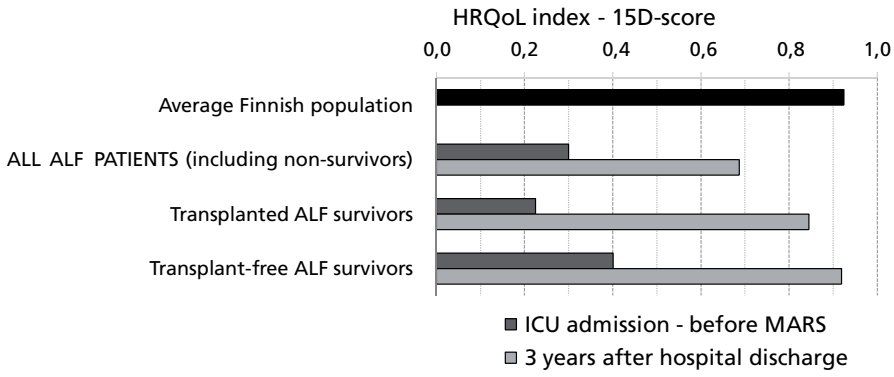
Laboratory variable (normal range)	Before MARS	After MARS	P-value
Hemoglobin g/l (125-180)	104 (59-170)	95 (59-136)	<0.001
Leucocytes x109 (3.3-8.2)	10.3 (1.0-41.0)	9.6 (1.3-45.7)	0.200
Platelets x109 (145-360)	120 (11-511)	77 (6-349)	<0.001
Sodium mmol/l (137-146)	135 (115-150)	134 (128-145)	0.103
Potassium mmol/l (3.3-4.9)	3.8 (2.0-5.6)	4.0 (2.9-6.4)	<0.001
C-reactive protein g/l (<10)	15 (5-356)	25 (5-215)	<0.001
Creatinine µmol/l (0-110)	110 (29-1318)	55 (17-585)	<0.001
Urea mmol/l (2.5-8.5)	8.8 (0.8-56.5)	2.5 (0.2-58)	<0.001
NH4-ion µmol/l (10-65)	74 (8-512)	55 (4-309)	<0.001
Bilirubin µmol/l (2-20)	410 (4-909)	243 (6-570)	<0.001
AST U/l (<40)	313 (15-24360)	156 (13-87380)	<0.001
ALT U/l (<50)	303 (9-12500)	159 (5-25120)	<0.001
γ-GT U/l (5-80)	110 (8-2385)	65 (5-1619)	<0.001
FV % (70-139)	44 (5-201)	44 (5-149)	0.053
AT3 % (70-130)	34 (13-137)	32 (15-122)	<0.001
TT % (70-130)	22 (6-139)	22 (6-113)	0.334
INR (0.7-1.1)	2.5 (0.9-9.9)	2.4 (1.0-10.0)	0.262
Albumin g/l (35-55)	25.0 (11.5-46.4)	27.3 (15.7-48.1)	<0.001

All laboratory values are expressed as a median (range). NH4-ion = ammonium-ion , AST = Aspartate aminotransferase, ALT = Alanine aminotransferase, γ-GT = gamma glutamyltransferase, FV = Coagulation factor V, AT3 = Antitrombin III, TT (%) = thrombin time (includes coagulation factors II, VII, X), INR=international normalized ratio

Health-related quality of life

The estimated mean HRQoL of all the MARS-treated and control ALF patients prior to treatment was very low when compared to the age and gender-standardized reference population of Finland[15] (0.30-0.27 vs. 0.92). The highest 15D scores and the mean QALY gains after treatment were observed in the ALF patients who did not have a contra-indication to Ltx and recovered without it (Figure 10.). The HRQoL of a surviving transplanted MARS patients was generally very good, although it was still somewhat lower than that of the age-standardized general population [15] (0.84 vs. 0.92).

Figure 10. The 15D scores of the MARS-treated patients before and 3 years after treatment in Study VI.

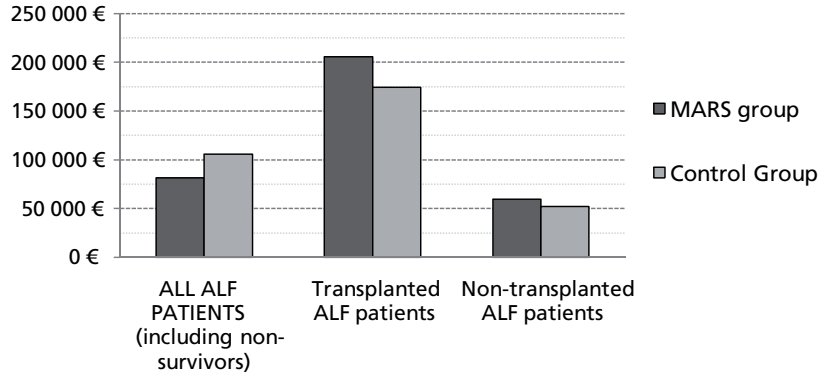


Costs and cost-utility

The costs of the MARS treatment in Finland: Currently the MARS machine and dialysis sets are manufactured by Gambro. One MARS treatment session costs 4,900€, including the wages of the essential nursing staff (HUCH service tariff catalog 2009). The cost of a treatment day in a liver ICU setting is 3,720€ (HUCH service tariff catalog 2009). In comparison, the price of a Ltx operation (either urgent or elective) is 48,000€ including both the donor and recipient operations (HUCH service tariff catalog 2009). However, substantial additional costs are associated with the Ltx procedure, as all patients require ICU and hospital treatment afterwards for a varying period of time (ranging from a few days to weeks).

Most of the liver disease-related direct medical costs incurred within the first year of the treatment (VI). The mean overall direct medical costs over 3.5 years in all patients were significantly lower in the MARS treatment group when compared to the controls (79,745€ vs. 105,820€). The costs of the transplanted and non-transplanted ALF patients in the MARS group and the control group are shown in Figure 11.

Figure 11. The mean overall direct medical costs over 3.5 years per patient in the MARS-treated and control ALF patients in Study VI.



The cost-utility of the MARS treatment: The incremental cost of the standard medical treatment alone compared to the MARS was 10,928€, and the incremental number of the QALYs gained by the MARS was 0.66. In the sensitivity analysis, the MARS treatment remained the dominant treatment strategy even though the difference in the etiological distribution between the MARS and the historical control group was taken into account and adjusted for. Table 16 shows a summary of the survival, cost and QALY data of Study VI.

Table 16. Summary of cost-utility data in Study VI

	3 years survival			Total costs, €				Mean 15D-score		
Treatment group	N	%	Mean, days	N	Mean	Min	Max	N	Before treatment	At 3 years or death
MARS	90	78	866	31	79 745	11 961	370 573	90	0.30	0.70
Control	17	41	460	16	105 820	16 862	262 481	17	0.27	0.36

N = number of patients, 15D-score = measure of the health-related quality of life

Side-effects

No serious or fatal side-effects were noted during the MARS treatment of 195 patients. The development of significant trombocytopenia (P<0.001) was recorded during the MARS treatment (Table 15.), but no fatal bleedings occurred. Minor bleeding complications have occurred, such as the catheter puncture site oozing and mucosal bleeding problems, causing adjustment in the anticoagulation regime.

DISCUSSION

The present thesis evaluated the impact and effectiveness of the MARS treatment in a comprehensive manner by incorporating both outcome (survival, native liver recovery and need for Ltx), clinical (HE, survival predicting factors), biochemical (effect on plasma amino-acids and laboratory variables), psychological (HRQoL studies), as well as the economic aspects (direct medical costs and cost-utility analysis) of the 195 MARS-treated patients in Finland. Previously, only a few small uncontrolled pilot studies have addressed this patient population in our country[154, 155, 180, 189].

The strengths and main findings of this thesis

The 195 patients in this thesis represent the largest MARS-treated patient population studied in a single center. Study II is also the first published controlled MARS study that investigates the survival and outcome of ALF patients. In addition, Study VI represents the first cost-utility and HRQoL study which has been conducted on the MARS-treated ALF patients. Despite the limited number of patients in this thesis, the 195 MARS-treated patients including a total of 120 ALF patients, represent a large patient population when considering the rarity and high mortality in ALF, the population base of Finland, and other previous MARS studies. Furthermore, the follow-up time which ranged from 6 months (II) up to 3 years in (VI), was exceptionally long when compared with most other previously published MARS studies[141, 231, 339].

The main findings of this thesis were that the outcome of the MARS-treated ALF patients, in terms of 6 months survival, native liver recovery rate and need for Ltx, was better when compared to a historical control group (II). In MARS-treated AOCLF patients with end-stage cirrhosis, mortality was very high without Ltx (V). The MARS treatment was successfully used as a bridging treatment to Ltx (II-VI) and in some cases, it seemed to facilitate native liver recovery (II,III,V,VI). Some patients with critical ALF and massive liver necrosis requiring a high urgent Ltx also seemed to endure a prolonged waiting time to Ltx with the MARS treatment (IV). Encephalopathy was often alleviated with the MARS treatment (I-V) and the percentage of ALF patients dying from tentorial herniation decreased significantly during the MARS treatment era when compared to historical controls (II). Moreover, the removal of toxic substances such as ammonia, bilirubin and neuroactive amino acids and an improvement in the amino acid profile of the patients was observed with the MARS treatment (I, II and V). In cost-utility analysis, MARS treatment of ALF patients was less costly and more cost-efficient (VI) than SMT alone.

Outcome

ALF

One might speculate that implementation of the MARS treatment in Finland has led to a trend of improved 6-month survival in both non-transplanted (66% vs. 40%) and transplanted (94% vs. 77%) ALF patients when compared to the historical controls from our own unit. The 1-year survival rate of our transplanted MARS-treated ALF patients also compares favorably to the international and European Ltx registries and previous studies (91% vs. 62-81%)[42, 107, 294, 303, 307, 352, 353]. The overall ALF patient survival rate (including both transplanted and non-transplanted patients) in previous MARS studies has been reported at 50-79%[54, 246, 344, 393], which is similar to our 1-year overall survival rate of 74%. Substantially poorer survival (~15-30%) with the MARS treatment has also been reported by some centers with limited possibility to Ltx[200] and ALF with high grade HE[190].

Study II of this thesis is the largest and the first published controlled MARS study in the world investigating the outcome of ALF patients in terms of survival, native liver recovery and need for Ltx. Due to the rarity and high mortality in ALF, as well as the varying availability and waiting times of transplant organs, randomized trials are difficult to carry out. Therefore, only a few RCTs including more than 100 ALF patients have been published in the context of liver-assist devices[80, 250] and only one RCT concerning the MARS treatment of ALF patients has been published in an abstract form thus far[298]. In the yet published RCT study by Saliba *et al.*, the randomization of patients was done after the listing for the high urgent Ltx and the results showed a trend of higher overall survival in the transplanted MARS-treated patients compared with the controls receiving SMT (85% vs. 76%, P=ns)[298]. However, the patient population in this French study[298] was quite different from ours, as all the ALF patients in the French study were listed for Ltx and 75% of the patients received a graft within 24h of listing. The mean waiting time for Ltx in the French study[298] was only 16.2h, meaning that the total number and duration of the MARS sessions which the patient received was also lower when compared to our patients. In our MARS-treated high urgently listed ALF patients (IV), the median waiting time for a graft was 5 days and the range was from 1 to 11 days. It is important to note that the median waiting time for high urgent Ltx in the Scandinavian countries is much longer[47] than the 1-2 days which is typically observed in the Eurotransplant-area[42, 298], Canada[352], and in the U.S.[107, 204, 294]. In fact, the median waiting time for a high urgent Ltx in the Scandinavian countries varies between 1.7-6.6 days, depending on the recipient's blood type[47]. Even though high urgent ALF patients have a priority for the first available organ within three days of listing in the Scandiatransplant area, only about 60% of the patients receive a graft within this time period[43].

In Study II, the etiological factors causing ALF differed significantly between the MARS and the control group (P=0.002). The unknown etiology ALF, which represented the majority of patients in the control group, is known to carry a poor prognosis, and has a low tendency of native liver recovery[204]. In contrast, the toxic and especially the paracetamol-related ALF patients who dominated the MARS group carry a substantially better prognosis and tendency for native liver recovery[202-204, 258]. For this reason, the subgroup analysis were carried out to compare patients with the same etiology.

In a subgroup of the unknown etiology ALF, 56% of our MARS patients were transplanted as compared to 62% in the control group and the native liver recovery rates were 20% and 8%, respectively. In contrast, a large multi-center U.S. study reported a 51% transplantation rate and a 17% native liver recovery rate in the unknown etiology ALF[258]. The 28-day survival of all our MARS-treated unknown etiology patients was higher when compared to the U.S. study (78% vs. 64%)[258]. In Study II it is also noteworthy that the survival of the transplanted MARS-treated unknown etiology ALF patients was higher when compared to the transplanted controls (91% vs. 69%) even though statistical significance was not achieved (P=0.1). One previous study has reported 80% survival in the transplanted ALF patients with an unknown etiology[382].

In toxic etiology ALF, including both paracetamol and other drug-induced cases, a significantly lower rate of Ltx (13% vs. 50%; P=0.002) and a higher tendency for native liver recovery (67% vs. 33%; P=0.05) were noted in the MARS-treated patients when compared to the historical controls (II, VI). However, in our material, the MARS-treated toxic etiology ALF patients were in a better clinical condition prior to their treatment (i.e. a lower median MELD score and a higher percentage of non-encephalopathic patients), which might partly explain the favorable outcome. In other MARS studies, the spontaneous recovery rates in the toxic ALF patients have been reported at 33-79%[190, 393]. In comparison to a recent U.S. study on non-MARS-treated patients, the native liver recovery rate with MARS was higher in both paracetamol patients (81% vs. 65%), and in other drug-induced ALF patients (52% vs. 29%)[204].

In Finland, only three patients have been transplanted due to pure paracetamol-intoxication during the past 27 years (FLTR 30.6.2009). In general, paracetamol-related ALF is probably not as common in Finland as in the U.S. and the United Kingdom[31, 175, 202, 203]. In the near future, however, this might change, as according to the new legislation in Finland, the amount of paracetamol which can be freely purchased over the counter without prescription is now 15g/client/day, as opposed to the previous 6g/client/day.

AOCLF

The first MARS related articles[141, 231, 339] and a few subsequent studies[145, 344] reported surprisingly high survival rates of 52-92% for the AOCLF patients. In addition, two RCTs have shown a survival benefit favoring the MARS treatment as compared to the SMT[141, 231]. Other studies on cirrhotic AOCLF patients without MARS treatment have reported substantially lower 1-year survival rates of ~20-30%[125, 214] and even lower survival rates in the presence of co-existing complications such as HRS I and SBP[116].

Despite the initial encouraging survival results in the MARS-treated AOCLF patients, the majority of the subsequent studies have reported much lower overall survival rates ranging from 0 to 30%, especially in the patients lacking the Ltx option[62, 63, 87, 231, 237, 246, 376]. Our results support the findings of these less enthusiastic survival observations as the 1-year survival of our non-transplanted AOCLF patients was only 18%.

When interpreting the results from the initial MARS studies, one must bear in mind that firstly, the follow-up time in some studies was extremely short (30 days or in-hospital survival)[141, 231, 339] and secondly, patients with serious co-morbidities and AOCLF-associated complications (such as HRS I, GI bleeding or hemodynamic instability) were excluded from other studies[141, 145, 191, 320]. Furthermore, the RCTs presenting favorable results only contained a total of 20 MARS-treated patients[141, 231]. Even though most AOCLF patients in the aforementioned studies were not transplanted due to contra-indications, the lack of the Ltx option in some countries undoubtedly has a negative effect on the overall survival results[376].

A survival subgroup analysis (V) showed that for the alcohol-related AOCLF patients with end-stage cirrhosis who are not eligible for Ltx, the MARS treatment is futile in terms of prognosis. This is because the 6 month survival of these patients was less than 10%. However, if the alcohol liver exhibited signs of residual regenerative capacity (e.g. steatosis and enlargement with milder forms of chronic changes), the MARS treatment seemed to be more beneficial, even in the absence of an Ltx option, as native liver recovery was theoretically possible. Other MARS studies on alcohol-related AOCLF have reported short-term survival of ~50% [144, 162]. In contrast, a recent study by Wolff *et al.* revealed comparable survival results to our data, presenting 21% out-of-hospital survival rates[388].

All of the previously discussed AOCLF studies clearly demonstrate that the patient selection and exclusion criteria for the MARS treatment are one of the key elements in determining the prognosis of a patient. When interpreting the results from Study V, one must keep in mind that the indications in AOCLF patients for the MARS treatment at our center necessitated the presence of two of the following criteria: hyperbilirubinemia (bilirubin>400umol/l), type 1 HRS or progressive HE. Most of the non-transplanted AOCLF patients in our study were in such poor clinical condition prior to treatment that they could only be temporarily sustained with MARS. In fact, the median survival

time of the non-transplanted non-surviving AOCLF patients was only 8 days after the initiation of the MARS treatment. After albumin dialysis was discontinued, most patients succumbed quickly to new complications and died. Therefore, it seems that the only long term possibility for the survival of the AOCLF patients with end-stage cirrhosis is Ltx. In a clinical setting, these findings have changed the existing MARS treatment indications in Finland. For the AOCLF patients with end-stage cirrhosis and no signs of regenerative capacity, the MARS treatment is only justified if the patient is eligible for Ltx and the MARS is used as a bridging treatment.

GF

The overall survival rate in this subgroup was high (8/11). In early GF, 63% of our MARS-treated patients survived 1-year, which is comparable to other small MARS studies reporting survival figures around 60%[122, 146, 248]. However, due to the small number of GF patients in our study and other reports, it is not possible to draw conclusions as to the efficacy of the MARS treatment in this patient sub-population.

Prognostic factors

To our knowledge, the 195 MARS patients contained in this thesis represents the largest MARS population investigated so far in a single center. It also contains the first study to analyze the prognostic factors in the MARS-treated patients in the different liver failure etiological subgroups (V). When evaluating prognosis, the stratification of patients into distinct etiological subgroups is essential. Based on previous studies, it is known that the etiology of liver failure is one of the most important determinants of outcome[202, 204, 252, 258] and this was also noted in our study (V). Previously, only one study has examined prognostic factors in MARS-treated patients[392]. The study by Yuan *et al.* contained a heterogeneous group of 50 miscellaneous liver failure patients who were MARS-treated before Ltx. The conclusion was that MARS treatment had favorably affected the known risk factors of early postoperative mortality (sequential organ failure (SOFA) score, creatinine, INR, tumor necrosis factor-α, and interleukin-10)[392].

Our results (V) as well as a previous study by O'Grady *et al.*[251]have shown that the grade of HE is a significant predictor of survival in toxic etiology ALF. Interestingly, in our MARS-treated patients, the grade of HE was not a significant prognostic factor in unknown etiology ALF. This finding is noteworthy since the most used transplantation criteria in non-paracetamol-related ALF, the King's college criteria[251], include the presence of HE as one of the listing criteria.

In Study V, the coagulation variables were found to be significant predictors of survival in non-paracetamol-related and unknown-etiology ALF. Other studies on ALF patients have also proposed that prothrombin time[251] and factor V level[36, 267] to be significant predictors of survival. The coagulation status of the patient

is a reflection of the synthetic capacity of the liver. For this reason, coagulation factor levels usually serve as accurate indicators of the severity of the liver damage and decreasing levels indicate poor prognosis[36, 123, 251, 267], as noted in our study.

The higher plasma level of the liver enzyme ALT was found to predict a better outcome in unknown etiology ALF. One explanation for this might be that necrotic liver cells do not produce enzymes. In fact, the simultaneous decrease in the ALT and factor V levels in unknown etiology patient are signs of the declining synthetic capacity of the remaining liver mass. As more liver tissue is destroyed, the regenerative capacity and the probability of transplantation-free survival diminish.

Other previously identified prognostic factors in ALF such as the early lactate[30], APACHE II scoring system[179, 198], alpha fetoprotein[308, 314], MELD-score[186], ratio of coagulation factors VIII and V[267], serum Gc protein[201], serum phosphate levels[311] and liver size[322], were not analyzed in the study V.

In Study V, survival predicting factors could not be found in other liver failure etiologies. The most probable explanation for this is that the remaining subgroups contained such a few patients that a statistical analysis was not able to identify the existing prognostic factors. Also, factors predicting survival could not be identified in alcohol-related AOCLF because most patients died within 6 months.

Hepatic encephalopathy and amino acids

The definition of ALF before the era of Ltx required the presence of HE[359]. However, times are changing as is the definition of ALF and few studies have already reported adult ALF patients without HE[47, 101, 107, 382]. In Finland, the initiation criteria for the MARS treatment in ALF do not necessitate the presence of HE if the patient has rapidly progressive liver damage (a deterioration of clinical condition or of the liver's synthetic function despite the best possible SMT). Moreover, in some intoxication patients (e.g. *Amanita phalloides* poisoning or lethal dose of known hepatotoxin without antidote), MARS is started as soon as possible without waiting for HE or for liver failure to develop.

According to the results of the present study as well as of many other studies[54, 138, 141, 232, 320], the degree of HE decreased significantly during MARS treatment (I,II,V) whereas the grade of HE increased in the control group receiving SMT (II). Additionally, Study II showed that the percentage of ALF patients who died as a result of tentorial herniation was significantly lower in the MARS treatment group when compared to the controls (II). In fact, four high urgently transplanted MARS-treated ALF patients with over 80% necrosis of the liver showed no signs of HE before Ltx (IV). This was exceptional considering that there were only a few or no viable hepatocytes in the explanted livers. It seems that by removing neurotoxic substances from the blood, the MARS therapy can prevent or delay the development of HE and brain edema in these critical patients. These are significant findings since

one of the leading causes of death in ALF patients is tentorial herniation caused by critical brain edema[352]. By reducing HE grade and thus, the risk of brain edema, the MARS-treated patients might have better chance of survival as noted in the Studies II,IV and V.

The waiting time for a high urgent Ltx in the Scandinavian countries can sometimes be too long for a critically ill patient. According to one Scandinavian study before MARS was available, the high urgently listed ALF patients who received their Ltx within 1-3 days of listing had a significantly higher survival (77% vs. 48%) when compared to those with longer waiting time (4-10days)[43]. Another Scandinavian study demonstrated that high urgently listed ALF patients who were non-encephalopathic at the time of listing had a mortality of 65% if they were not transplanted[47]. In our study IV, the survival in the MARS treated ALF patients did not differ in relation to the Ltx waiting time. According to these observations, early aggressive MARS treatment of ALF patients might improve the prognosis of the listed patients by minimizing the risk of developing critical brain edema and herniation.

Earlier studies on the patophysiological mechanisms of HE have reported elevated levels of neuroinhibitory and neurotoxic substances and their precursors in the plasma of encephalopathic liver failure patients[52, 113, 147, 358, 29]. Our results (I) also showed that the concentration of phenylalanine, methionine, histidine and glutamine were significantly higher in patients with HE when compared to non-encephalopathic patients.

It has been proposed that the favorable effect of the MARS treatment on HE might be due to the changes in the plasma amino acid profile[19, 209]. In agreement with previously published investigations[19, 209, 313], results from our Study I also demonstrated an improvement in both HE grade, and Fischer's ratio during MARS treatment. The increase in the Fischer's ratio was mainly due to the removal of AAAs. The concentration of other neurotoxic and neuroinhibitory amino acids (e.g. methionine and glutamine) also decreased during the MARS treatment, and this decrease was more pronounced in patients with higher HE grades (I). The observed favorable effect on the HE and amino acid profile during MARS treatment could be explained by the removal of the excess of the neurotoxic amino acids by the MARS filters. On the other hand, it might also be attributable to improved liver function and thus, diminished toxin production or increased clearance.

Toxin removal

The observation that many albumin-bound and water-soluble toxic substances can be removed from patient's blood with the MARS treatment[336-338, 344] was also noted in our patients. The median bilirubin level of our MARS-treated patients was significantly decreased by -41% which is comparable to other reports ranging from -21% to -52%[141, 191, 231, 320, 344]. In our patients, there was also a significant reduction in their liver enzyme levels during treatment,

which was more pronounced in the ALF patients compared to the AOCLF patients (II). However, the interpretation of this finding is somewhat difficult because high enzyme levels are usually seen in the initial stages of ALF, whereas decreasing levels can either indicate recovery or progression to massive liver necrosis. On the other hand, cirrhotic patients may have normal or only slightly elevated liver enzyme levels despite acute decompensation. It should be kept in mind, however, that the reduction in the level of bilirubin, liver enzymes and other toxins does not necessarily correlate with survival, and thus the efficacy of the MARS treatment cannot be judged on these surrogate parameters alone.

In general, the coagulation parameters (with the exception of platelet count) remained unaffected throughout the MARS treatment, which can be expected as the MARS treatment does not synthesize or remove coagulation factors. The decrease in platelet count and hemoglobin might be partly caused by the direct mechanical sheer caused by the filters in the MARS machine [109]. However, in some of our MARS patients, the coagulation status improved during treatment, which might be a sign of recovering liver function.

The median reduction of ammonia was -26% during the MARS treatment in Study V, which is comparable to other reports[54, 62, 246, 344]. However, the removal of water soluble toxins (e.g. ammonia, creatinine and urea) is not related to albumin dialysis itself but rather to the hemodialysis or hemofiltration module attached to the MARS circuit. Furthermore, plasma electrolytes did not change significantly in our patients during the MARS treatment because of the vigilant surveillance, the regular blood controls and the prompt correction of abnormal values.

To date, the amatoxin removal capacity of the MARS treatment has remained unknown in the *Amanita phalloides* intoxications. During the past 20 years, many extracorporeal detoxification methods have been researched in the hope of finding a new cure for *Amanita* mushroom poisonings. The most frequently used methods include plasmapheresis, hemodialysis, hemofiltration and hemoperfusion[11, 167, 168, 332, 378]. Given that the α-amanitin molecule is a small oligopeptide (0,9kD) and not protein bound in plasma[115], it would seem that, in theory, it could be easily dialyzed. Surprisingly though, some studies have found that hemodialysis and hemoperfusion are ineffective in removing amatoxins from the circulation[184, 238]. Taking into consideration the short plasma half-life of amatoxins[108], it seems plausible that the efficacy of the MARS treatment in *Amanita phalloides* intoxications observed in our patients is not based on the removal of the amatoxins, but rather, is based on the removal of other toxic substances or some amatoxins metabolites which are yet unknown and have not been measured.

On a larger scale, we speculate that early MARS treatment in ALF is beneficial because it removes circulating endotoxins and other cytotoxic mediators from the patient's blood. According to current knowledge, these accumulating toxic mediators (bilirubin, ammonia, lactate, bile acids, aromatic amino acids, fatty acids,

mercaptans, phenols and endogenous benzodiazepines, various cytokines, and NO) are responsible for the end-organ damage (e.g. HE, hemodynamic instability) in liver failure [319, 339] as well as being a part of the vicious cycle inducing further hepatocyte injury. Perhaps the good 1-year survival rate (100%) of our *Amanita* intoxication, as well as of other ALF patients, is partly attributable to the early initiation of the MARS therapy. In this light, early aggressive MARS treatment of ALF seems advisable before the development of critical ALF and of irreversible end-organ damage .

Cost-utility and the health-related quality of life

From the perspective of the health care provider and society, it is important to determine which medical treatments are cost-efficient, effective and which provide as many high quality life-years as possible for the patient. These days, the fair allocation of existing financial resources is a demanding task, as costs and the number of patients are escalating and new expensive treatments are introduced. In the cost-utility analysis, the QALYs gained by a given treatment are used as the measuring units of efficacy. Thus, the QALYs and cost/QALY-ratios can be used to compare the different treatments in terms of length of life and quality of life[357]. Currently, there is no consensus as to how much a QALY gained can cost but a 50,000€ threshold has been suggested[350].

Study VI is the first published work on the cost-utility and HRQoL of the MARS-treated ALF patients. According to the cost-utility analysis, the MARS treatment seemed to provide a less expensive and more cost-efficient treatment option when compared to SMT alone in ALF patients (VI). This was mostly due to the decreased number of Ltx and therefore costs when compared to the controls. On average, the overall treatment costs of liver-failure patients in an ICU setting are considerable with or without MARS, due to the long ICU treatment periods and, in some cases, Ltx costs. In fact, any treatment which reduces the number of Ltx has a huge impact on the total costs. The sensitivity analysis of the cost-utility data showed that by increasing the percentage of transplanted patients in the MARS group up to 42% or higher, the expected costs of the MARS group would exceed those of the control group.

In Study VI, the cost/QALY was considerably lower in the MARS treatment group compared to control group (53,845€/QALY *vs.* 106,958€/QALY) during 3.5years. The incremental cost of SMT was 10,928€ when compared to the MARS treatment. Recent Finnish studies have evaluated the cost-utility of the ICU treatment of sepsis[172] and renal replacement therapy in acute renal failure[397]. The cost/QALY of these treatments was reported at 2,139€/QALY in sepsis and 220,000€/QALY in acute renal failure. In comparison, the cost/QALY of ICU treatment following cardiac arrest and cardiopulmonary resuscitation was 10,107€ in a German study[130], whereas the use of activated protein C in sepsis patients in an ICU setting cost 46,600$/QALY[218]. However, in the aforementioned QALY

studies, the number of QALYs gained by the treatment was assumed to last until the remaining lifetime of the patient. When evaluating the results of our Study VI, one must bear in mind that it dealt with a time window of 3.5-years, thus severely underestimating the QALYs gained during the remainder of the patients' lives. Alternatively, the lifetime expected costs and outcomes could have been extrapolated but this would have necessitated further assumptions and resulted in greater uncertainty. To demonstrate this, the theoretical cost-utility of the MARS treatment in ALF patients was calculated assuming that the surviving patients had the same lifespan as an average Finnish person. The resulting cost/QALY was 8,570€.

Previous studies on critically ill ICU patients have reported long-lasting negative effects on HRQoL following treatment[92, 169]. However, in our HRQoL analysis, the MARS and ICU treatment did not seem to have a lasting negative impact on the HRQoL of the ALF patient unless they were transplanted. Furthermore, the average HRQoL of the MARS-treated and transplanted ALF patients was only slightly lower when compared to the age-matched reference population in Finland[15]. Yet, this slight decrease probably has very small clinical relevance. Similar results have been obtained from previous studies on transplanted patients[356, 394].

The analysis of HRQoL and the cost-utility in ALF patients in Study VI is unique in the sense that all previous similar small studies have evaluated AOCLF patients only[137, 143-145]. In addition, most of these studies have been carried out by the same center in Germany and all patients with serious co-morbidities, liver disease- related complications or Ltx have been excluded from the analysis[143-145].

MARS treatment protocols

In Studies I-VI, the target duration for one MARS session in ALF was 22 hours, but in reality, the median duration of one MARS session was 16.5 hours according to Study V. This was mainly caused by the clotting of the dialysis filters which occurred even with an adequate anticoagulation regime and led to the premature cessation of the treatment in many cases. In our MARS-treated ALF patients, the median length of the treatment sessions were considerably longer when compared to most other MARS studies where the target has been only 6-8h sessions[95, 141, 144, 231, 258, 320, 393]. Another significant difference in the treatment protocol used at our center compared to other centers is that the MARS treatment was used continuously in the ALF patients instead of it being administered in intermittent treatments. The aforementioned differences in treatment protocol might explain our success with the MARS treatment in ALF patients.

Studies on acute kidney failure patients have shown that high intensity and an early aggressive initiation of conventional renal replacement therapy have a favorable effect on patient outcome[286, 287, 305]. The same analogy might apply to the MARS patients, as albumin dialysis is always coupled with a

hemodialysis circuit. Furthermore, extended removal of albumin-bound and water-soluble toxins in long treatment sessions may have an added favorable effect on the outcome in ALF. Our clinical experience has also shown that once the MARS treatment is initiated, even the most unstable patients can endure prolonged periods of dialysis. Similarly, earlier studies have concluded that continuous renal replacement therapies are preferred in ALF, as they cause less fluctuation in hemodynamics and ICP than the intermittent therapy[76, 78].

Safety considerations

A significant decrease in the platelet count was observed in our MARS-treated patients, although no fatal bleedings occurred. These findings are similar to other published reports by various MARS centers describing mild trombocytopenia and few minor bleeding complications[4, 21, 54, 62, 104, 109, 191, 231, 237, 360]. One study reported that even though platelet loss is observed during the MARS treatment, the function of the remaining tromobocytes remains intact[109]. In general, it is difficult to separate the possible complications due to the MARS treatment and those which are normally attributable to critically ill liver failure patients. In our patients, the MARS treatment appeared to be safe and only some minor bleeding complications occurred. However, intensive surveillance of the coagulation factors and the clinical signs of bleeding complications is essential to correctly adjust the anticoagulation therapy during the MARS treatment.

LIMITATIONS OF THIS THESIS

The major limitations of this thesis is the non-randomized nature of all of the Studies I-VI, and this reduces the scientific value of the results. Studies II and VI used historical control patients from the time prior to the MARS treatment. Although all patients received the same SMT at the same ICU, it is undeniable that some treatment and monitoring protocols (such as the HVPF and the invasive ICP monitoring) and patients referral patterns have changed over time.

One significant factor complicating the comparison between the MARS treatment group and the historical controls was the unequal distribution of the ALF etiologies between the groups in Studies II and VI. The MARS treatment group contained more patients with paracetamol-related intoxications with a favorable prognosis, whereas the control group contained a larger share of unknown etiology patients with less potential to native liver recovery[175, 258]. This was compensated for by comparing only patients with similar etiology in Study II and by adjustments in the sensitivity analysis in Study VI. However, in Study II, this led to a rather small number of patients in each etiological subgroup, complicating the statistical analysis. As ALF is an extremely rare condition, to gather a sample of even a few hundred patients with a similar etiology would take many decades in Finland.

The general applicability of these MARS treatment results in other scenarios may vary according to many significant factors contributing to the overall survival and management of the liver failure patients (e.g. the distribution of liver failure etiologies, availability of transplant organs, Ltx waiting times, transplant organ quality, and availability/quality of ICU management). For example, in Asian countries, the majority of the patients have a viral origin of liver failure[149, 203]. Our material contained only few hepatitis patients and therefore our overall survival results do not necessarily apply to the Asian or other populations with different etiological background.

In Study III, the presence of amatoxins in the blood or urine was not confirmed by a laboratory measurement. We do not have a test for the quantitative analysis of amatoxins in Finland because these intoxications are so rare. Therefore, complete certainty that all ten patients really had *Amanita phalloides* intoxication could not be reached even though typical clinical signs and history of eating white mushrooms strongly suggested it.

One obvious source of the bias in the HRQoL Study VI was the difficulty in estimating the pre-treatment baseline HRQoL of patients. This is a commonly recognized problem in the HRQoL studies of critically ill ICU patients[172, 397]. Most liver failure patients are encephalopathic if not

unconscious, and thus cannot fill-out the HRQoL questionnaires. For this reason, the pre-treatment HRQoL was assessed retrospectively by an expert panel in Study VI. However, previous studies have demonstrated that the subjective feelings of a patient, such as their pain or HRQoL, are usually misjudged or underestimated by the attending doctors, treatment staff, or even by close relatives[100, 295, 330]. It is also important to remember that the time-window of our HRQoL study was only 3.5-years and this severely underestimates the QALYs to be gained over the remaining lifetime.

CLINICAL IMPLICATIONS OF THIS THESIS

The results from Studies I-VI suggest several clinical implications.

Firstly, based on these results, it seems that MARS treatment might improve survival in ALF, and especially in the unknown etiology patients with a poor prognosis when compared to SMT. In toxic etiology ALF, the MARS treatment seems to promote native liver recovery and thus a larger portion of the MARS treated patients recovered without Ltx when compared to SMT. The reduced need for Ltx in the MARS-treated ALF patients lead to higher cost-utility when compared with SMT. As demonstrated in our studies, the progression of encephalopathy was halted and the concentration of circulating neurototoxins (e.g. neurotoxic amino acids) was reduced with the MARS treatment. In the light of these findings, it would seem plausible to recommend the early initiation of the MARS treatment in ALF before the development of life-threatening complications (such as progressive encephalopathy).

Secondly, a new definition of ALF should be adopted to also encompass those patients who do not develop encephalopathy when liver assist therapy is initiated early in the course of the disease. As Study IV clearly demonstrated, even patients with total or almost total necrosis of the liver, and thus evident ALF, can remain non-encephalopathic or improve their grade of HE if treated continuously with MARS.

Thirdly, in those AOCLF patients with end-stage cirrhosis who are not eligible for Ltx, the MARS treatment is not meaningful in terms of prognosis. Therefore, the results from Study V suggest that in the future, the treatment of AOCLF patients be restricted to situations where the patient can either be bridged to Ltx or the liver still shows some signs of residual regenerative capacity (e.g. alcohol hepatitis with an enlarged steatotic liver with minor chronic changes).

ETHICAL CONSIDERATIONS

As discussed earlier, the efficacy and survival benefit of the MARS treatment has not been substantiated with large RCTs. Most evidence supporting the beneficial effects of the MARS treatment originate from a few RCTs with AOCLF patients[138, 141, 191, 231, 320, 333, 335] and uncontrolled case series[54, 344] with a limited number of patients and a short follow-up. It has always been difficult, if not impossible, to conduct RCTs in patients with a life-threatening medical condition. Consequently, in these circumstances, large case series must serve as a justified means of gathering information for a lack of a better option, especially when it comes to ALF patients. So far, only two RCTs[80, 250] and one abstract[298] have been published containing more than 50 ALF patients. In these days, a study design with randomization to either SMT or the MARS treatment without a rescue Ltx option, would not be possible due to ethical considerations.

From point of view of society, the MARS treatment is a costly procedure which can only be applied to a carefully selected, small group of patients and therefore it has no great impact on the medical welfare of the entire population. On the other hand, the possible benefits of the MARS treatment can be of great consequence to an individual patient if a Ltx can be avoided or if the patient can be kept alive while waiting for a suitable transplant.

All studies in this thesis (I-VI) complied with the current ethical considerations and are in accord with the Declaration of Helsinki. In addition, the collection and analysis of this patient material was assessed and approved by the ethical committee of Helsinki University Hospital.

FUTURE OF THE MARS TREATMENT

In recent years, the usefulness, efficiency and survival benefit of liver assist devices, including the MARS treatment, have been discussed in many reviews and editorials[57, 81, 124, 188, 192, 226, 233, 256, 268, 280, 290, 299, 334, 383, 386]. All the papers have more or less arrived at the same conclusion that the current liver support devices have shown somewhat promising results, especially in improving the clinical variables such as the HE and hemodynamic profile in addition to a significant ability to remove toxins from the blood. However, as these surrogate endpoints do not directly correlate with survival or with other clinically important endpoints such as native liver recovery or the need for a Ltx, large RCTs with hard endpoints are needed.

Today, as randomized controlled and sufficiently powered studies on liver assist devices and MARS treatment are still scarce, definite conclusions cannot be drawn as to the survival benefit of these devices. A Cochrane review[208] evaluating the beneficial and harmful effects of the artificial and bioartificial liver devices stated that there is not enough supporting evidence that either BAL or artificial liver support systems have a significant effect on the outcome in ALF when compared to a SMT. However, according to this review, artificial liver support systems might be beneficial in AOCLF patients by reducing mortality and HE[208]. This review included only those studies which had been published until 2002. Future randomized controlled MARS trials are needed to demonstrate if the MARS treatment improves long-term survival.

As the indications for the MARS treatment have been expanding during the past decade, it is essential to determine which patient groups genuinely gain from it. A sufficiently large sample size for an adequately powered clinical trial could only be achieved with a multinational multi-center study with substantial financial resources. Other important questions which remain unanswered concern the optimal length of the MARS treatment session (continuous vs. intermittent treatment) and the optimal timing of treatment in the different liver failure etiologies

The liver assist device of the future

The development process towards the ultimate liver assist device, which could compensate for all the functions of a normal healthy liver, is far from over. An ideal liver support device would perform a wide range metabolic, synthetic and detoxification functions in addition to being reasonably priced, safe and easy to use in clinical practice.

The future hope rests on the continuous research on the hybrid liver assist device technology which combines the efficient toxin removal capacity of the MARS system and the synthetic capacity of the BAL devices. In the meanwhile, the MARS treatment seems to provide a possibility for temporary liver support until the patient's own liver regenerates or a suitable transplant organ is found.

CONCLUSIONS

No serious adverse side-effects or complications were observed during the MARS treatment of 195 patients.

Based on Studies I-VI, the major conclusions are:

1. The 1- and 3-years survival and the rate of native liver recovery were higher and the need for Ltx was lower in the MARS-treated ALF patients when compared to historical controls. As the etiological distribution of patients has changed markedly over the past decade, the outcome of these groups cannot be compared directly. In subgroup analysis, the results suggested a survival benefit in especially MARS-treated unknown etiology ALF patients when compared to historical controls.

   The survival of transplanted MARS patients was high and the patients who were bridged to Ltx gained from the MARS treatment independent of liver failure etiology. The AOCLF patients with end-stage cirrhosis and hyperbilirubinemia, HE or HRS, did not benefit from the MARS treatment if Ltx was contra-indicated. In AOCLF, if the liver was still enlarged and steatotic owing some regenerative capacity, the MARS treatment was more beneficial even in the absence of the Ltx option.

2. The most important predictor of survival was the etiology of liver failure. Other survival predicting factors were the grade of HE in the toxic etiology ALF and the plasma concentration of the coagulation factors and the liver enzyme ALT in unknown etiology ALF. In other liver failure etiologies this study was unable to detect survival predicting factors.

3. A favorable effect on the plasma amino acid profile, removal of neurotoxic amino acids and improvement in the Fischer's ratio was noted during the MARS treatment in both the ALF and AOCLF patients. The plasma concentration of most neuroactive amino acids, such as the aromatic amino acids and glutamine, decreased significantly during treatment.

   The grade of HE decreased in most liver failure etiological subgroups or remained unchanged during the MARS treatment Also, the number of patients who died due to tentotorial herniation was significantly lower in the MARS treatment group when compared to the historical controls. According to the results of this study, the early MARS treatment of ALF patients might improve prognosis by removing the neurotoxic substances from the blood and thereby decreasing the risk of critical brain edema and herniation.

4. The concentration of all measured albumin-bound (e.g. bilirubin) and water-soluble toxins (e.g. creatinine, urea and ammonia) decreased significantly during the MARS treatment while the coagulation factors remained unaffected or increased in some patients. The platelet count was also significantly reduced during treatment but did not cause any fatal bleeding complications.

5. In an ICU setting, the MARS treatment combined with SMT appears to be less costly and more cost-efficient than SMT alone in ALF patients. This was mainly caused by the decreased number of Ltx in the MARS group when compared to the historical controls.

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