Investigations into the pathophysiology, prognosis and clinical management of acute liver failure

UNIVERSITY OF BIRMINGHAM

by

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Abstract

In this thesis I will describe how my work has contributed to the understanding and management of acute liver failure (ALF). There are three main themes of my work:

1. Understanding the pathophysiological mechanisms in ALF
2. Defining prognosis in ALF.
3. Management of ALF, in particular focusing on the prevention of intracranial hypertension (ICH).

Acute liver failure (ALF) is a rare syndrome, estimated to result in less than 10 cases per million in the West (1). In severe form it results in critical illness with multiple organ failure. It occurs in all age groups and usually presents in young adults without any previous liver disease. It has a high mortality and for those with a severe presentation the only treatment option is liver transplantation, however, the shortage of donor organs means that is only an option for selected cases (2).

The ability to predict outcome early in the course of the illness is of critical importance when considering transplantation; there is only a short window of clinical opportunity during which liver transplantation is a viable option before the condition of the patient deteriorates to the point where transplantation becomes futile (3). Like other forms of critical illness, the progression to multiple organ failure dictates outcome. Additionally, patients with ALF can develop intracranial hypertension (ICH) due to cerebral oedema which has a further, significant, impact on morbidity and mortality. Management strategies to reduce the incidence and impact of ICH are, alongside accurate prognostication, an essential part of intensive care unit management of patients with ALF (4).

Furthermore, performing clinical research in patients with ALF is very difficult for a number of reasons. It is a rare condition thus studies take a long time to recruit sufficient numbers. Patients are also extremely sick with the illness following a fulminating course and so often die early or are transplanted, confounding trial outcomes. Consequently, the number of randomised clinical trials in ALF is very small internationally and collaborations of liver centres have been developed with only varying degrees of success. The US ALF study group has produced 68 papers over its 22-year history. To this end, I was a founding member of the European ALF initiative in 2010, which initiated a number of surveys in clinical practice across Europe (5,6).

The work that I authored with colleagues has had a significant impact on the understanding and management of ALF. It has directly influenced international management guidelines (7,8) in both Europe and the USA through the national hepatology associations (EASL & AASLD). My works covers the following principal themes:

1. Pathophysiology

Lactic acidosis was recognised as a prominent feature of ALF during the 1960s and the importance of it as a prognostic marker was elucidated during the late 1980s (9,10). The metabolic acidosis seen in ALF is due to a combination of lactic acidosis and acute kidney injury, both of which contribute to prognostic indices. **I have directly contributed to the understanding of lactate metabolism in ALF** through innovative work revealing the changes in lactate metabolism that occur during and following liver transplantation. The liver becomes a net producer of lactate in ALF instead of a consumer and I demonstrated that splanchnic lactate production comes

from both the gut and the liver in ALF. The accompanying editorial noted that due to the design of the study which enabled sampling of portal vein lactate we were uniquely placed to distinguish between splanchnic and liver metabolism (11). The net production of lactate from the liver reverses following emergency liver transplantation, contributing to the rapid fall in serum lactate seen (12,13). This is significant as it helps to explain why a high lactate has prognostic value. The lactate produced within the liver represents the activation of inflammatory cells which use glycolysis aerobically and is a marker for the inflammatory burden. This inflammatory burden contributes to liver damage and to the systemic inflammatory response and organ failure seen (11).

As well as work into lactate metabolism in ALF I have worked with colleagues in the Centre for Liver and GI Research in Birmingham to delineate the immunological mechanisms perpetuating liver damage. In particular, we demonstrated T-cell recruitment and activation within the liver during ALF (14). This work adds to the understanding of how the migration to and activation of inflammatory cells, stimulates the lactate production within the liver and result in systemic inflammation and organ failure.

2. Prognosis in ALF

Bernal and colleagues showed that serum lactate, which contributes to the metabolic acidosis in ALF, was also a prognostic marker in ALF due to paracetamol toxicity**. I led work with colleagues in Birmingham, extending this work and demonstrated that it held true for non-paracetamol causes of ALF** as well (15,16). This study confirmed and supports the use of lactate as a prognostic marker in a different cohort of patients with ALF, strengthening the evidence for its use. In

our centre lactate levels at 12 hours following admission was shown, on multivariate analysis, to be a significant differentiator for survival. The 12 hour interval is important as it differentiates those patients in whom arterial lactate falls following initial resuscitation from those with persistent hyperlactataemia.

I have continued to maintain an interest in prognostic models in ALF and contributed data and was an author on a recent publication examining dynamic models of predicting outcome in paracetamol induced ALF (17). The dynamic models look at both day one and day 2 markers. The lead time effect of early critical care intervention on organ failure increases the fidelity of the model and increases confidence in predication.

3. Management of ALF

Cerebral ischaemia and herniation related to cerebral swelling remains a significant cause of death in ALF (18).

The recognition that cerebral oedema was a significant cause of death in ALF led to the introduction of intracranial pressure (ICP) monitoring and strategies to reduce it in patients with established intracranial hypertension (19,20). The use of osmotherapy with mannitol became the main therapy for cerebral oedema complicating ALF. Once established ICH is difficult to treat and tends to recure despite therapy.

In novel work I initiated and studied, I looked at alternative types of osmotherapy in ALF by considering the use of hypertonic saline. It can increase blood osmolality and as sodium does not cross the blood brain barrier it serves to reduce brain water. In fact, because of the active nature of the barrier, sodium is actively pumped out,

making its reflection coefficient (the tendency to not cross the blood brain barrier) higher than that of mannitol (21). ICH has a very poor prognosis and so prevention is a logical approach.

In a landmark study I, together with colleagues investigated the prophylactic use of hypertonic saline infusions in patients with ALF in a randomised controlled trial. We demonstrated that maintaining serum sodium at approximately 150 mmol/l, prevented the increase in intracranial pressure seen in the control group. This study has now been cited over 340 times and has been adopted as standard therapy internationally (22).

I have had a long-standing interest in the use of extracorporeal liver support in ALF, with the concept of liver dialysis being particularly appealing. Many systems have been investigated including the use of albumin dialysis via the molecular absorbent recirculating system (MARS). Together with colleagues **I investigated the effects of the MARS extracorporeal liver support on physiological parameters in a series of patients with ALF** (23). The study demonstrated that MARS therapy improved haemodynamic stability and decreased jugular bulb oxygen saturation reflecting an increase in cerebrovascular tone during the sessions. Notably, intracranial pressure did not change significantly over the treatment period suggesting that factors, not cleared via MARS, are important in the pathology.

More recently, I helped develop the concept and design of an international collaboration, with colleagues in King's College London and the Rigshospitalet (Copenhagen), to investigate the effects of prophylactic hypothermia on intracranial pressure in ALF (24). This international, multicentre, randomised controlled clinical trial compared moderate hypothermia with standard of care in patients with ALF at

risk of cerebral oedema. Whilst the study was unable to show any difference in the rates of ICH the accompanying editorial noted that this “landmark study” highlighted the need to determine the correct target temperature to manage patients with ALF and how further experimental research is needed (25).

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Acknowledgments

I Would like to thank Professor Phil Newsome who encouraged me and gave me the confidence to pursue this MD by publication. I want to acknowledge the contribution colleagues have made in the research presented. Investigations in ALF are difficult due the rarity of the syndrome. Collaboration is essential if sufficiently powered studies are to be completed in the future.

I would like to acknowledge the patients and their relatives who consented for them to be enrolled in clinical research during critical illness with a significant uncertainty about their survival.

Contents

Chapter 1

Aims and structure of the thesis

In this thesis I will present a body of published work related to acute liver failure. This will start with a general introduction published as a book chapter in a major textbook of critical care medicine, describing ALF, its causes and general management. The following chapters represent a series of investigations into the pathophysiology, early prognostic markers and management of ALF. The publications presented follow a logical pattern. As a total it represents a significant contribution to the understanding of and has enhanced the management of ALF.

I will follow with a general discussion of how the work relates to current literature and how the principles and recommendations presented in the papers have influenced international clinical practice.

Performing clinical research in patients with ALF is difficult for a number of reasons. It is a rare condition with an estimated annual incidence of about 1 per 100000 of the population per year in the UK. The numbers presenting with ALF have fallen from a peak in the late 1990s following legislation limiting availability and pack size of over-the-counter paracetamol. Patients are often extremely sick at presentation to a specialist centre with multiple organ failure. They frequently die soon after presentation or receive a liver transplant, confounding trial outcomes. Consequently, recruitment to studies is slow and the number of randomised clinical trials published in ALF is very small. Various collaborations of liver centres have been developed to enhance recruitment to prospective studies and to pool data for observational examination in the UK and Europe and in the USA. Over time there has been a gradual advancement in the understanding of the pathophysiology and management

of patients with ALF improving mortality. This has been steady for the most part with a jump following the introduction of transplantation into the pathway during the early 1990s.

List of publications

Murphy N. Diagnosis and Management of Liver Failure in the Adult. In: Critical Care Medicine: Principles of Diagnosis and Management. 4th ed. Elsevier; 2014. p. 1309–33.

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The diagnosis and management of liver failure in the adult

Dr Nick Murphy

Acute liver failure is a syndrome manifest by the rapid cessation of normal function in individuals with previously normal livers. The rate of decline in function dictates the manner in which the syndrome manifests and influences the outcome. The aetiology is the main influence on the rate of progression and the likelihood of spontaneous recovery (1).

The pathological basis of the massive hepatic necrosis was described in detail by Lucké and Mallory following the Second World War in 1946 (2). The presence of the American army in East Asia and Africa resulted to exposure to both epidemic and serum hepatitis and data regarding the clinical course of the syndrome and pathology was collated via the army medical services.

In 1970 Trey and Davidson introduced the term fulminant hepatic failure (FHF) to encompass the current clinico-pathological understanding of the syndrome (3). This definition was an attempt to encapsulate the clinical course and to differentiate it from decompensation of chronic liver disease. They described a syndrome of rapidly progressing liver failure (within 8 weeks) in which the defining point was the onset of hepatic encephalopathy following the onset of symptoms, in someone without previous liver disease. They make the point that the syndrome is potentially reversible in some patients. This definition is still used today; however, it has become clear that this definition is too narrow and that subgroups exist.

Speed of progression to encephalopathy from the onset of jaundice or other initial symptoms is used to define subgroups. This is because the rate of progression of the syndrome is directly related to the aetiology of the underlying cause of liver failure. For example, patients with significant acetaminophen induced hepatotoxicity will generally present with liver failure within seven days of ingestion, unless

ingestion was staggered over a period of time when the timing of liver insult is difficult to define. Patients often present with cardiovascular collapse and renal failure before they become encephalopathic. In contrast, patients presenting with sero-negative hepatitis (unknown aetiology) can have a prolonged illness, over a period of months, resulting in a patient that is deeply jaundiced with evidence of portal hypertension, such as ascites, at the onset of encephalopathy. It is the two extreme ends of the syndrome that split the group into hyperacute, acute and subacute liver failure. Interestingly, it is the hyperacute group that have the best chance for spontaneous recovery, although this group has the highest risk of cerebral oedema. It is the subacute group that has the worse prognosis with medical management alone (1). (37)

- Hyperacute liver failure
    - Jaundice to encephalopathy within 7 days – usually due to paracetamol hepatotoxicity
- Acute liver failure
    - Jaundice to encephalopathy from 8 to 28 days
- Subacute liver failure
    - Jaundice to encephalopathy from 29 days to six months

Aetiology of Acute Liver Failure

Worldwide, and in the developing world in particular, approximately 95-100% of patients presenting with ALF will have viral hepatitis (4). Within the UK and as recently reported in the USA, paracetamol hepatotoxicity toxicity is the leading cause of ALF. This is followed by liver failure of unknown aetiology or seronegative hepatitis (5,6).

The pattern of ALF within the UK and USA has been changing over the last thirty years (5,6). Up until the late 1990's the rate of hospital admission due to paracetamol ingestion had risen year by year. In the UK paracetamol overdose (POD) is usually due to deliberate self-harm. In contrast the USA data suggests that over half of all patients with ALF due to POD were due to therapeutic misadventure. Some doubts have been expressed regarding this interpretation as some of the patients in the misadventure group may be, in fact, be occult suicide attempts (6,7). In 1998 legislation was introduced in the UK to restrict the over-the-counter sale of paracetamol to 16 tablets from most retail outlets and 32 from pharmacies in the form of blister packs. Since then the rate of admissions to hospital, severe liver toxicity and transplantation for POD have fallen (8).

In the United Kingdom and the USA, the incidence of acute hepatitis A virus (HAV) and hepatitis B virus (HBV) infection has fallen dramatically since the 1980s (5,9). Less than 1% of acute hepatitis A or B progress to ALF (5). In the USA and UK, as a proportion of the total, the number of admissions with ALF due to viral hepatitis has fallen steadily and is currently responsible for less than 5% of all admissions in the UK and 11% in the USA (5,9).

Indeterminate hepatitis (non-A to E hepatitis, seronegative ALF, non-A/non-B hepatitis), is often presumed to be viral in origin and is the most common presentation excluding POD in the UK and USA and viral hepatitis in the developing world. It is a diagnosis of exclusion and as diagnostic capabilities improve is falling in incidence in some centres (5).

Acetaminophen (paracetamol)

Acetaminophen induced liver failure is the cause of the vast majority of hyperacute liver failure. Acetaminophen poisoning is a common cause of presentation to acute and emergency departments in the UK and USA; however, the case progression to ALF following paracetamol ingestion is rare at just 0.6% of all presentations in the UK (10) .

It was in the mid 1960's that the main mechanisms of paracetamol induced liver injury were elucidated. The scheme outline by various groups revealed the production of electrophilic quinone imine (N-acetyl-p-benzoquinone imine, NAPQI), which covalently binds to hepatic proteins, was central to the resultant centrolobular necrosis seen following poisoning. The two major pathways for metabolism are the glucuronidation and sulphation of the phenolic group with the metabolites produced excreted in the urine. In therapeutic doses approximately 80% of the drug is metabolized via these two pathways and 5-10% is excreted unchanged in the urine. The remainder is metabolized via the hepatic mixed function oxidase, cytochrome P450 to produce NAPQI.

Following poisoning the half-life of acetaminophen is greatly prolonged because of the saturation of glucuronidation and sulphate conjugation. As a result there is an increase in the quantity of NAPQI produced. NAPQI is extremely reactive in

biological systems and has a short half-life. Following poisoning, reaction of NAPQI occurs within the centrilobular portions of the liver and leads to necrosis in experimental models. It reacts with cellular constituents in a covalent and non-covalent manner. The exact mechanisms by which NAPQI induces cell death are incompletely understood but include the deactivation of critical cellular proteins; the induction of reactive oxygen species and the activation of Kupffer cells (11). The loss of regulatory protein function results in abnormal calcium homeostasis and resultant energy failure within the cell and mitochondria (11). Other events such as non-covalent interaction with intracellular signalling and lipid peroxidation also contribute to the toxicity of this molecule. Following this primary toxic phase there is a secondary or extrinsic phase. This extrinsic phase is equated with the recruitment of immune cells to the liver. The liver is one of the major immune organs of the body. Up to 35% of the liver is made up of non-parenchymal cells, including endothelium, Kupffer cells and resident lymphocytes. These, together with macrophages within the liver perform a major role in immune regulation and in the filtering of antigens from the gut contents via the portal circulation. They are also implicated in the pathological processes that occur following liver insult. Massive activation of immune cells in response to the intrinsic cellular damage induces the release of cytokines and chemokines both locally and into the systemic circulation (12).

Immune effects following the direct toxic effects of NAPQI result in the secondary effects of poisoning such as the release of cytokines from the liver and the induction of organ failure.

Following a significant intake of paracetamol the symptoms over the following 24 hours, irrespective of amount ingested, are usually nausea and vomiting. The minimum dose that can induce hepatic damage appears to be about 125 mg/kg. This

represents 15 500mg tablets in a 60 kg individual, although hepatic necrosis has been recorded at much lower doses especially if associated with hepatic enzyme induction. Doses above 250 mg/kg (30, 500mg tablets in 60kg individual) will often produce damage and doses in excess of 350 mg/kg invariably produce significant damage (13). During the following four to five days, if liver failure ensues, there is a gradual worsening of the patient's general condition. Those with significant overdose should be admitted to the hospital and monitored closely.

The antidote for acetaminophen poisoning is N-acetylcysteine (NAC). It provides complete protection against hepatotoxicity if given within 12h of nonstaggered ingestion (14). Within 12 hours, if the time from ingestion is known with certainty and a plasma acetaminophen level is obtained reference can be made to nomogram to see if the potential for hepatotoxicity is present. The nomograms are unreliable if the time from ingestion is uncertain or if there was staggered ingestion over a period of time as often occurs with therapeutic misadventure or repeated overdose. The use of alcohol often accompanies POD making timing unreliable. Situations that alter normal cytochrome P450 function such as drug induction (chronic ethanol use, phenytoin, and isoniazid) again render the information unreliable (15). The use of the nomogram as the only basis for the decision to withhold NAC therapy is to be discouraged because of the uncertainty associated with this timing and the catastrophic potential if NAC is erroneously withheld.

The main effect of NAC is to increase hepatic glutathione production. This promotes the conjugation of NAPQI and its subsequent excretion. In addition NAC may act as an antioxidant within and outside the liver. It is most effective if given within the first 8 hours following overdose but is effective following this, although less so. There is some evidence that NAC is effective when administered to the patient up to 72 hours

following poisoning, although the mechanism of action is unclear and probably relates to antioxidant effects rather than to any effect on acetaminophen metabolism (16). The role of NAC in established ALF from any aetiology is more controversial and despite widespread use its role is not established (17,18).

Viral hepatitis

Both epidemic and serum hepatitis were recognized well before the viral aetiology was discovered. In the seminal work of Lucké and Mallory in 1946, they describe 196 patients that died of acute liver failure following both epidemic hepatitis and serum hepatitis, related to the administration of blood products during World War II (2).

Acute liver failure following acute viral hepatitis is uncommon with a reported incidence of 0.2 – 4 % depending on the underlying aetiology (19). Liver failure following viral hepatitis tends to run an acute or hyperacute course with the onset of encephalopathy occurring within days or weeks of the first symptoms (20).

Hepatitis A is now rare in the USA and Western Europe but is a common form of acute enterally transmitted hepatitis in the underdeveloped world where it is mainly a mild and self-limiting illness of children (20,21). The progression to acute liver failure is the lowest of all the hepatotrophic viruses. In the west the incidence of ALF following hepatitis A appears to be higher than in the endemic areas. It occurs more commonly in adults and is more severe. Persistent infection with hepatitis A has also been reported (22) even recurring following liver transplantation (23). Diagnosis is made on the basis of IgM antibodies at the time of hospitalization although false negatives can occur (24).

Hepatitis B may lead to ALF is several settings. It occurs most commonly following acute infection but can occur following an acute increase in viral replication following

immunosuppressive therapy such as cancer chemotherapy or steroids as well as with co-infection with other viral agents such as delta virus. The host immune response is thought to be responsible for the severity of reaction to the virus, subsequent clearance and the induction of acute liver failure. This can be seen following the withdrawal of immunosuppressive therapy when there is a very active immune response to the increased viral load. In acute infection surface antigen (HBsAg) is often negative but IgM antibodies to the viral core (HBcAb) will usually be positive. Mutations to the precore stop codon or the core promotor region of the viral genome may be associated with a higher incidence of ALF (25). These particular genes code for HBeAg and lack of this antigen is associated with a more profound immune response. There have been reports of a very high incidence of ALF associated with outbreaks of acute hepatitis B in the setting of intravenous drug use and chronic hepatitis C infection (26).

Hepatitis C, as a cause of acute liver failure, is rare in Northern Europe and the USA but has been described (27). There is a wide spectrum of clinical presentation associated with acute infection with the more florid presentation associated with a more rapid clearance rate suggesting the magnitude of the initial immune response is important (28). Liver failure associated with acute infection appears to be more common in India and the Far East (29). Acute infection may contribute to decompensation in patients with pre-existing liver disease and hepatitis C seropositivity may predispose to liver failure when co-infection with another hepatotrophic virus is present (29).

Hepatitis E is likely the most common cause of acute liver failure worldwide and is certainly true for the Indian subcontinent (29). In the Far East acute hepatitis B infection is the most common cause of ALF due to the high levels of endemicity (30).

The existence of hepatitis E was inferred before serological evidence was available by a process of exclusion. It was long assumed that most if not all epidemic enteric hepatitis was due to the A virus. When serological markers for hepatitis A became available in the early 1980's it was apparent that the majority of waterborne epidemic hepatitis were due to other agents, producing a syndrome similar clinically to hepatitis A (31). Hepatitis E does not produce a chronic infection and in the vast majority is a self-limiting infection that occurs most commonly in young adults, in contrast to hepatitis A, which is primarily an infection of children. The incidence of hepatitis E associated with ALF is small with a case related mortality reported at about 0.5 to 4% in the general population but with a much higher mortality in pregnancy, as high as 20% in the third trimester. Pregnancy itself appears to be a risk factor for ALF, with a quarter of all infected female patients reported as pregnant in one series. However, this may not be particular to hepatitis E but rather due to the high incidence of epidemic hepatitis E in a relatively immunosuppressed state (29). In the West, travel to endemic areas is a risk factor but sporadic cases are now being seen more commonly in the developed world. Some of these cases have been associated with contact with animals (32).

Sero-negative hepatitis is the second most common cause of ALF worldwide in most published series. In Northern Europe and USA it comes in behind paracetamol toxicity and in the developing world it is second to acute viral hepatitis. See figures 1 & 2.

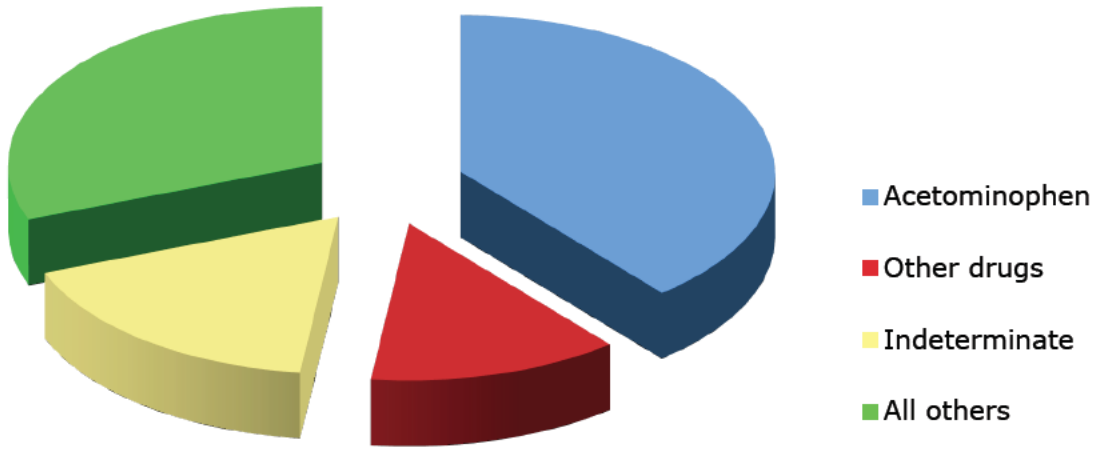

Figure 1. Aetiology of Acute Liver Failure in USA 1998 - 2001 based on 17 centres.

Ostapowicz Ann Intern Med. 2002;137:947-954

Sero-negative hepatitis can be conveniently thought of as a single entity. In reality it is probably an amalgam of various causes including acute presentations of autoimmune hepatitis, idiosyncratic drug reactions and viruses (33,34). Sero-negative hepatitis has a variable clinical presentation including a slow insidious onset of general malaise, jaundice and ascites followed by progressive signs and

symptoms of liver failure. At presentation the patient may be deeply jaundiced, already have ascites and splenomegaly. It can also present with a hyperacute picture. The pattern of signs, symptoms and organ failure is dictated by the rate of progression. In subacute sero-negative hepatitis the presenting clinical picture can be similar to that of decompensated chronic liver disease, causing occasional diagnostic difficulty. A liver biopsy is sometimes needed to differentiate between the two.

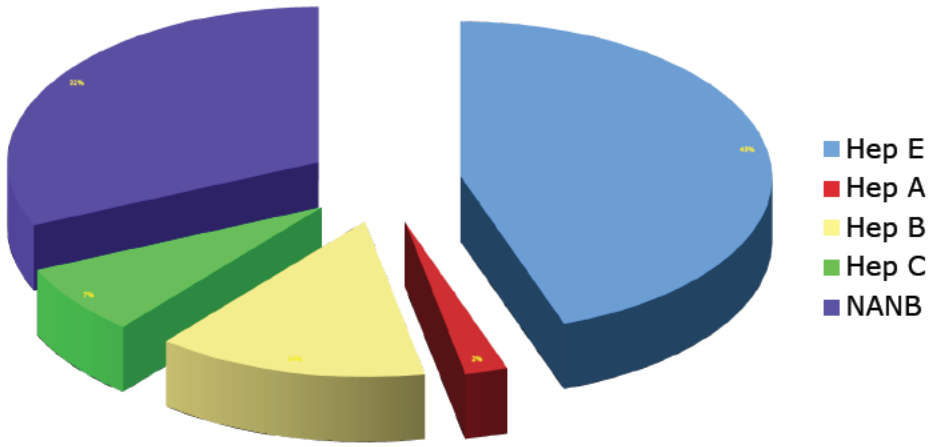

Figure 2 Etiology of Acute Liver Failure in an Indian center 89-96. Khuroo & Kamili J Viral Hepatitis 2003 10(3)

Acute presentation of autoimmune hepatitis.

Autoimmune hepatitis can occur at any time of life from childhood until old age. It is more common in women with a male to female ratio of 1:3. Presentation can be

asymptomatic, discovered following routine laboratory testing, or more commonly, present with jaundice and general malaise. In rare cases autoimmune hepatitis can present as acute liver failure (35). Unfortunately there are no serological tests with sufficient sensitivity or specificity to make the diagnosis certain. Patterns of markers in the right clinical setting and in the absence of other causes, may be useful (36). The diagnosis of autoimmune hepatitis in the setting of acute liver failure is difficult and a degree of uncertainty often lasts. Classically there is a combination of elevated immunoglobulin levels, autoantibodies and confirmatory histological evidence of hepatitis in the absence of active viral markers. Elevation of autoantibodies are often seen in patients with ALF due to other causes such as drug induced liver disease (35). Liver failure should be assessed in the same way as for other aetiologies and once signs of encephalopathy become apparent standard prognostic criteria apply. The role of steroids is unproven but should be considered in patients prior to the onset of encephalopathy. Once listed for transplantation steroid use is controversial because of increased risk of infection.

Drug Induced liver disease

The liver is a major site for drug metabolism in the body. Metabolism of xenobiotics (a chemical which is found in an organism but which is not normally produced or expected to be present in it (Wikipedia)) takes place in a series of specialized enzymes that increase the water solubility of lipophilic molecules by the incorporation of polar groups. Drug metabolism often involves a number of processes. Intermediates produced during the process are sometimes more toxic than the parent drug and can cause liver damage by a number of mechanisms.

Drug induced liver disease may be due to a known dose dependent toxicity as with paracetamol. Alternatively, unpredictable, rare, idiosyncratic reactions can occur with

any drug with a frequency of about 1 in 1000 to 1 in 100000 patient prescriptions (15). Drug induced liver failure can mimic all forms of acute and chronic liver disease. However the predominant clinical presentation consists of either acute hepatitis or cholestatic liver disease. The former has a reported mortality of 10% irrespective of the drug.

The patterns of injury associated with these idiosyncratic reactions relate to the mechanism of damage and the cells involved (37). Many patterns have been described but it is massive hepatic necrosis that most often presents as ALF. Liver failure associated with severe cholestasis and veno-occlusive disease is also seen (37).

Liver injury usually is seen within six months following the initiation of therapy. Even if the diagnosis of drug induced liver failure is considered, a search for other possible aetiologies should be performed. Although any drug is capable of inducing liver injury more common causes include herbal remedies and recreationally used drugs such as “ecstasy” and cocaine. If suspected, a comprehensive history with a timetable of drug initiation should be constructed. Management includes stopping the offending drug and supportive care. Transplantation should be considered once liver failure occurs as the outcome from drug induced liver failure, other than that induced by paracetamol can be poor (38). Presentation profiles for various drugs causing ALF are shown in Table 1.

Type of Reaction	Effect on Cells	Examples of Drugs
Hepatoce u ar	D rect effect or product on by enzyme drug adduct eads to ce dysfunct on, membrane dysfunct on, cytotox c T ce response	Ison az d, trazodone, d c ofenac, nefazodone, ven afax ne, ovastat n
Cho estas s	Injury to cana cu ar membrane and transporters	Ch orpromaz ne, estrogen, erythromyc n and ts der vat ves
Immunoa erg c	Enzyme drug adducts on ce surface nduce IgE response	Ha othane, phenyto n, su famethoxazo e
Granu omatous	Macrophages, ymphocytes nf trate hepat c obu e	D t azem, su fa drugs, qu n d ne
M croves cu ar fat	A tered m tochondr a resp rat on, *b* ox dat on eads to act c ac dos s and tr g ycer de accumu at on	D danos ne, tetracyc ne, acety sa cy c ac d, va pro c ac d
Steatohepat t s	Mu t factor a	Am odarone, tamox fen
Auto mmune	Cytotox c ymphocyte response d rected at hepatocyte membrane components	N trofuranto n, methy dopa, ovastat n, m nocyc ne
F bros s	Act vat on of ste ate ce s	Methotrexate, excess v tam n A
Vascu ar co apse	Causes schem c or hypox c njury	N cot n c ac d, coca ne, methy ened oxymethamphetam ne
Oncogenes s	Encourages tumor format on	Ora contracept ves, androgens
M xed	Cytop asm c and cana cu ar njury, d rect damage to b e ducts	Amox c n c avu anate, carbamazep ne, herbs, cyc ospor ne, meth mazo e, trog tazone

Table 1 Idiosyncratic drug reactions and the cells that are affected (15)

Pregnancy induced liver disease

In general, pregnancy associated liver failure has the best prognosis when compared to all other causes of acute liver failure and prompt recovery can be expected with delivery of the fetus in most cases, if recognized early enough. Pregnancy associated liver failure can present in several ways including the syndromes of pre-eclampsia and the HELLP syndrome (hemolysis, elevated liver function tests and low platelets), liver rupture and acute fatty liver of pregnancy. Any cause of ALF can occur during pregnancy and in particular viral hepatitis can be particularly fulminant in its course. This is especially true for hepatitis E and herpes simplex virus infection.

Although originally thought to be a variant of pre-eclampsia there is evidence that HELLP syndrome may be a separate entity and in fact more related to acute fatty liver of pregnancy (39). Both HELLP and pre-eclampsia appear to be related to an endothelial injury, possibly immunologically initiated with activation of the coagulation and complement cascades and an imbalance of prostaglandin and thromboxane resulting in increased vascular tone, microangiopathic haemolytic anaemia and vascular thrombosis (40). HELLP is thought to occur in approximately 1 in every 1000 live deliveries and patients usually present in the 3rd trimester with non-specific signs often seen in pre-eclampsia such as weight gain due to oedema and hypertension. In addition, right upper quadrant pain accompanied by nausea and vomiting is commonly seen. Laboratory abnormalities include hyperbilirubinemia due to liver dysfunction and evidence of haemolysis. Transaminases are modestly raised and the platelet count is usually less than 100,000. Liver biopsy, while commonly normal in pre-eclampsia shows specific changes of periportal necrosis and fibrin microthrombi. Microvascular steatosis may also be present (40). The liver failure associated with HELLP is manifest as a prolonged prothrombin time and ascites.

Renal failure is common. Maternal mortality is low but foetal mortality is high between 20 and 60% (40,41). Treatment of choice is delivery of the baby. Conservative therapy is associated with an increase in both maternal and foetal complications.

Spontaneous rupture of the liver can occur in the setting of both pre-eclampsia and the HELLP syndrome although it can occur de-novo. It often presents with sudden onset of right upper quadrant pain accompanied by signs of hypovolaemia or shock and is more common in multiparous women (40). Spontaneous rupture of the liver has a high maternal and foetal mortality. Its pathogenesis is unclear but it appears that periportal haemorrhage associated with HELLP syndrome may occur close to the capsule resulting in lifting and bleeding into the potential space. These areas of the capsule then coalesce and rupture. Management of this devastating complication includes prompt delivery of the foetus, local surgical control with packs and aggressive management of the accompanying coagulopathy. Embolization of any feeding vessels in the liver may be of utility if such skills are available. Hepatectomy followed by liver transplantation can be lifesaving and has been performed.

Acute fatty liver of pregnancy (AFLP) occurs during the 3rd trimester of pregnancy and should be considered in any patient exhibiting signs of liver dysfunction. It is uncommon with an incidence of approximately 1 in 6659 live births (42). If left untreated maternal and foetal mortality are high. The treatment of choice is delivery and prompt recovery can then be expected. There is usually a prodromal illness over a couple of weeks with non-specific symptoms progressing to jaundice and encephalopathy. Symptoms and signs of pre-eclampsia or HELLP syndrome are seen in a third of cases and there is some evidence of a common aetiology due to a foetal fatty acid metabolism disorder (39). Diagnosis is critical and it should be

differentiated from viral hepatitis or hepatic failure due to other causes. Liver biopsy can aid in the diagnosis and can be performed via the jugular route if coagulopathy precludes the conventional approach. Characteristic zone 3 microvesicular steatosis is seen. Delivery is the best treatment if diagnosed early. Characteristic features include normoblasts on blood smears and high serum urate. Bleeding can be a major problem during operative delivery. On occasion transplantation may be the only viable option.

Wilson's disease

Wilson’s disease is a rare autosomal recessive disorder resulting from copper toxicity with primarily brain and liver manifestation. It usually presents in the second or third decade of life, although it can present from early childhood until late middle age (43). The disease can present with predominately liver or neurological symptoms. Neurological symptoms relate to the distribution of copper to the basal ganglia and result in movement disorders. Patients with liver disease may present with an active hepatitis, established cirrhosis or with acute liver failure. Other signs such as Kayser-Fleisher rings are associated but not pathognomonic for Wilson’s disease. These are greenish-brown rings in the cornea resulting from deposition of copper.

Acute liver failure due to Wilson’s Disease can present at any age but commonly present in the early 20’s. High urinary copper excretion is possibly the most predictable laboratory finding, although the patient may be anuric on presentation. A low serum ceruloplasmin is an additional indicator, but again is unreliable in ALF. A high serum bilirubin in combination with modest elevations of transaminases and alkaline phosphatase is often seen, as is intravascular haemolysis, contributing to the raised bilirubin level. Patients with severe liver failure due to Wilson’s disease

have an almost 100% mortality without liver transplantation, which should be considered as soon as the diagnosis is made (43). There is debate as to whether liver failure secondary to Wilson's Disease is truly acute liver failure, as it does not fit the definition because cirrhosis is invariably present on liver biopsy at the time of presentation. Nevertheless, many patients have not been diagnosed at this point and the presentation of often acute and catastrophic. In this sense, the timing of the onset and the lack of previous symptoms, place fulminant Wilson's Disease in the ALF group.

Neoplastic infiltration

Infiltrating into the liver can occur with some cancers resulting in liver failure. In the majority the diagnosis is apparent as part of end stage carcinomatosis but rarely infiltration into the liver can occur de-novo and be the initial presentation. This occurs typically with lymphomas, but has been reported with other forms of cancer. Hepatosplenomegaly and a raised alkaline phosphatase are often present. Other stigmata including palpable lymphadenopathy and marrow or peripheral blood film changes may be present. Imaging may be diagnostic especially with massive hepatomegaly.

Budd-Chiari syndrome

ALF secondary to Budd-Chiari syndrome is usually fatal without transplantation. The syndrome is defined as outflow obstruction to the hepatic veins and the underlying pathogenesis is thrombosis in the majority but tumour invasion or vascular membrane obstruction may be the cause. This is a rare disorder that occurs predominantly in young adults and affects more women than men. Overall, 5-year survival varies from 50% to 80% in different series (44). Many patients with the syndrome have a predisposing disease state, either congenital or acquired, leading

to a clotting abnormality such as a malignancy, myeloproliferative disorders, protein C or S deficiency, polycythemia rubra vera, lupus anticoagulant, antithrombin III deficiency, antiphospholipid syndrome etc. (45). The fulminant form of the syndrome in which the patient develops encephalopathy within eight weeks of the onset of symptoms is rare and it is much more common for Budd-Chiari syndrome to present in a sub-acute form over a three or four month period, characterized by ascites, abdominal pain and hepatomegaly, jaundice, coagulopathy, raised AST and alkaline phosphatase. Others present with signs of portal hypertension, including refractory ascites and variceal bleeding, and relatively intact hepatocellular function. The diagnosis is made with a combination of clinical presentation and imaging, including Doppler ultrasound studies of the hepatic vessels, plus or minus liver histology, usually via the jugular route because of coagulopathy. The management of the syndrome depends on the manner in which it presents and the underlying cause. Medical management of the syndrome involves the use of anticoagulants and diuretics in an attempt to control ascites. Thrombolysis can be attempted in selected patients with recent onset disease (46). In patients in whom there is progression to signs and symptoms of liver cell failure some sort of porto-systemic shunting procedure may reduce symptoms and prevent progression of the disease, allowing time for collateral vessels to develop. Transjugular intrahepatic portosystemic shunt (TIPSS) is most often attempted unless there is evidence of a hypertrophied caudate lobe and IVC compression, making meso-atrial shunting a better option. In patients with signs of liver failure care must be used when considering a TIPSS procedure as this can precipitate decompensation and rapid progression to ALF (47). Liver transplantation is ultimately the only option in many patients in which there is a failure of medical and shunt therapy as well as in the fulminant presentation of the

syndrome (48). Anticoagulation is usually necessary in the immediate postoperative period.

Veno-occLusive disease of the liver

Veno-occlusive disease (VOD) of the liver is a relatively common complication of myeloablative chemotherapy induction regimens in preparation for bone marrow transplantation (BMT) being seen in up to 54% in some series (49). It was first described in children following the ingestion of herbal teas in South Africa. Pathologically it results from the non-thrombotic fibrous occlusion of centrolobular hepatic venules and it represents a non-specific response to certain nocuous stimuli. Certain induction regimens such as high dose cyclophosphamide and total body irradiation are implicated in the pathogenesis. The more aggressive induction regimens appear to result in a higher incidence of VOD. Recent reduction in incidence may be due to a reduction in the use of myeloablative regimens and better monitoring of plasma drug concentrations (50). The symptoms of VOD usually occur within two weeks of BMT. The development of jaundice, hepatomegaly, abdominal pain and encephalopathy in the setting of recent BMT strongly suggest the diagnosis. Severe cases are characterized by evidence of hepatocellular necrosis and a high AST concentration. In 25% of cases, which are characterized as severe, the syndrome is progressive leading to ALF (51). Treatment options in these patients are limited as the outcome is poor with medical management or liver transplantation, although there is hope that newer therapies such as defibrotide, if initiated early before the onset of multiple organ failure, may improve the outlook (50).

Ischemic hepatitis

In patients with chronic congestive heart failure (CCHF), liver congestion is common. This is usually manifest as mild abnormalities of liver function tests, a prolonged

prothrombin time and mild ascites in some. Hepatic congestion is usually clinically unapparent unless jaundice is present, which can occur following multiple bouts of CCHF. Chronic congestion can lead to fibrosis and ultimately cirrhosis in some patients. Acute rises in serum transaminase and prolongation in prothrombin time, representing acute hepatic necrosis, most commonly develops in patients with CCHF where there is a sudden drop in cardiac output due to an event such as an arrhythmia or myocardial infarction. It is relatively uncommon for the liver to become involved during shock states because of the huge redundancy in blood supply and the ability of the portal system to compensate for any reduction in hepatic arterial flow. If however, portal flow is already compromised due to passive congestion then an acute drop in hepatic blood flow can result in ischaemia. It is uncommon for ischaemic hepatitis to occur without a recognizable precipitant but it can occur due to a silent MI or arrhythmia for example. It can occur before the diagnosis of CCHF has been made or be the primary diagnostic event in a younger patient with a cardiomyopathy. Clinically, severe ischaemic hepatitis becomes apparent between 24 and 48 hours following an event and is manifest as huge rises in serum transaminase (up to 10 – 20 times normal). There is prolongation of the prothrombin time, encephalopathy, hypoglycaemia, jaundice and renal failure. The syndrome is usually self-limiting once the hemodynamic disturbance has receded and there is a rapid fall in serum transaminases (usually 50% in the first 72 hours) (52). Occasionally there is progressive liver cellular failure leading rapidly to death. Management should be aimed at investigating and supporting the cardiovascular system.

Heat stroke

Exertional heat stroke may occur in new recruits to the army or police force engaging in physical initiation programs. It can also occur in unacclimatized athletes in hot conditions or with drug overdoses such as cocaine. It is a potentially devastating syndrome that can lead to multiple organ failure and death. Liver involvement is usually seen as part of multi-system disorder. It ranges from mild involvement to ALF and is seen to develop during the first few days following the event. Management is supportive and the majority improve over a period of days to weeks. Liver transplantation has been used in severe cases but the outcome is poor due to coincident organ failure (53).

Mushroom poisoning

There are many types of poisonous fungi in the world that are responsible for a variety of disorders that can be classified according to the type of poisoning and the timing of onset (54).

There are a number of Fungi associated with the induction of liver failure following ingestion. Of these the most common and most deadly are of the genus *Amanita*. *Amanita* associated hepatotoxicity follows a triphasic response after ingestion. The first is a self-limiting, non-specific, gastrointestinal upset that occurs within the first 6 to 24 hours. Nausea and vomiting, abdominal cramps and diarrhoea are often seen and it can mimic food poisoning. This followed by a period of recovery and a few days later by progressive liver failure.

Management is essentially supportive, although, many specific therapies have been tried in the treatment of *amanita* toxicity such as high dose penicillin, silibinin, cimetidine and NAC, none have been shown to improve outcome. Liver

transplantation should be considered for patients with severe liver failure and standard criteria apply (54).

Early management of ALF

History and examination

The presentation and clinical course of acute liver failure depends on its aetiology and the rate of progression of the syndrome. This varies widely from admission via an emergency department following a paracetamol overdose or acute viral hepatitis versus admission from a hepatology outpatient department in a patient with progressive jaundice and ascites. Due to its relative rarity the diagnosis can often be missed during initial contact and this may detrimentally influence the outcome.

The history will initially focus on the rate of progression of the illness and any clues to the aetiology. The first symptoms are often the onset of jaundice (typically noticed by a relative). Any history of foreign travel or high-risk behavioural activity (IV drug use or unprotected intercourse) associated with the contraction of viral hepatitis should be investigated. A thorough drug history should be taken for prescribed and recreational drug use for the preceding six to ten weeks. Drug therapy, or recreational use that can induce or inhibit hepatic enzymes should be noted. If the patient is unable to provide this information their relatives, and the patients primary care physician should be contacted for help.

The history may be self-evident in the case of paracetamol hepatotoxicity, especially if the patient is self-presenting following deliberate self-harm. However, therapeutic misadventure, which is a relatively common cause of hepatotoxicity, may not be obvious unless considered by the physician. Any patient presenting with coma should have a drug screen (including paracetamol).

Subacute liver failure is, by definition, a more insidious onset. Jaundice is often preceded by non-specific symptoms of fatigue and general malaise. Abnormal liver enzymes will often be revealed during initial workup. Subsequent investigations will include viral, iron, copper and genetic studies and, in addition, attempt to rule out decompensation of chronic liver disease as a cause the current symptoms and signs. Liver biopsy may be considered in sub-acute liver failure.

Initial resuscitation and emergency care

Patients presenting with acute or hyperacute liver failure tend to follow a similar course irrespective of aetiology. Initial therapy and emergency care will be dictated by the condition of the patient at presentation. Liver failure is a multi-system disease and can progress very rapidly to multiple organ failure.

Patients presenting with rapidly progressive hepatic encephalopathy via the emergency care system will often be intubated and ventilated, at the time of or shortly after admission. The emergency physicians often perform a head CT scan because of diagnostic uncertainty (55).

The symptoms and signs of hepatic encephalopathy in acute liver failure are subjectively different from that seen in decompensated chronic liver disease or in patients with stable chronic encephalopathy. Agitation and aggressive behaviour is more common in acute liver failure. This may be based on an increase turnover of the excitatory neurotransmitter glutamate in ALF associated with an acute increase in cerebral ammonia uptake (56).

The West Haven criteria, designed to assess coma grade in patients with cirrhosis are often applied to patients with ALF and is useful because of its familiarity (57). However, once the patient becomes unconscious at grade IV encephalopathy, the

scale does not provide any further information and is too crude to provide a clinically useful description of the level of consciousness. The Glasgow coma score, while not assessed specifically in this setting is useful in relating clinical information to others (58). Encephalopathy grade can progress very quickly, especially with hyperacute presentation. Intubation and sedation is recommended once grade III encephalopathy is achieved because of the attendant risks to airway and possibilities of raised intracranial pressure see below.

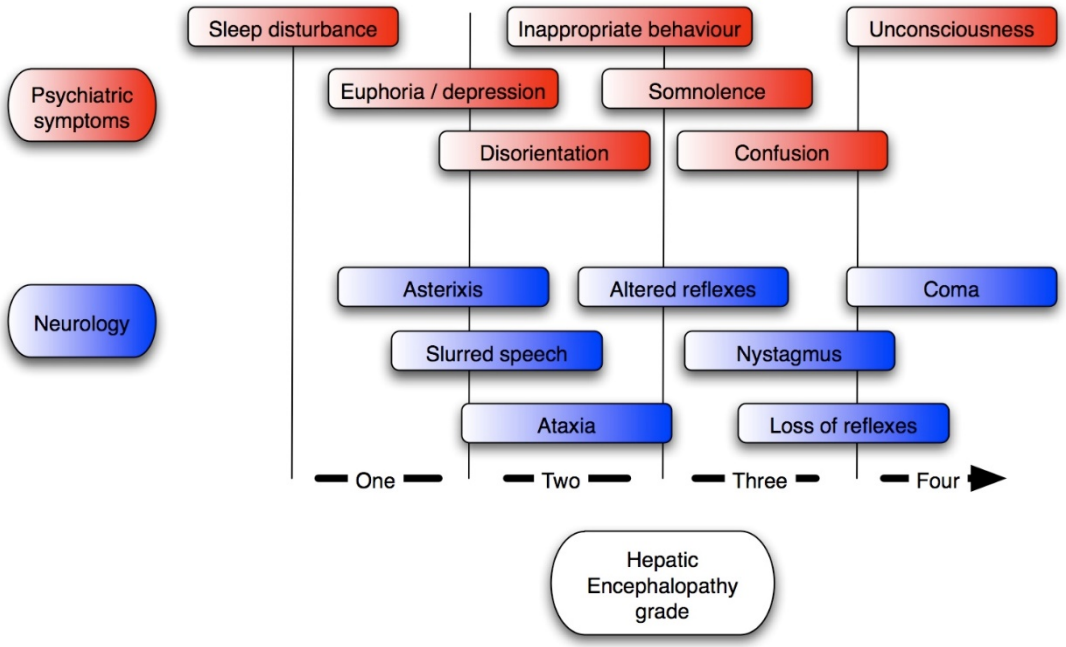

figure 3 Hepatic encephalopathy grade in ALF

Early contact with a regional liver centre should be made in any patient with signs of acute severe liver dysfunction as serial blood results and clinical signs can be relayed to the centre over the phone. POD patients can often be managed over the phone but plans for transfer can be made well in advance if necessary. As liver

failure progresses over the next several days, a number of organ systems may become affected.

Following the safe and appropriate management of airway and breathing focus can be directed to the correction of any circulatory dysfunction. Patients with ALF can progress rapidly to circulatory shock. The circulatory changes associated with ALF are predictable, usually associated with systemic vasodilatation and relative hypovolemia. This can be profound and require significant volume resuscitation. It is easy to underestimate the amount of fluid required in the early stages of ALF and the use of prognostic markers is unreliable before adequate circulatory function is resorted.. Monitoring at this stage should include a central venous catheter and direct arterial access for both blood pressure monitoring and for repeated blood tests. Care must be given to serum electrolytes because they often become deranged during this initial period. In particular hyponatraemia and hypophosphatemia are common (59–61). This may be due to excess quantities of hypotonic fluid administration. A typical flow sheet of investigations of a patient presenting with paracetamol poisoning from a referring hospital is shown in table 2.

Dates	7/10/2003	8/10/2003 am	8/10/2003 pm	9/10/2003 am
INR	2.08	3.17	3.79	6.2
Bilirubin	12	37		35
AST/ALT	273/223	8535/4254		15734/10060
Alk Phos				100
ALB	52	45		30
Glucose	4.1		7.5	5.2
Urea		5.5		3.0
Creatinine		86	83	128
pH		7.38	7.41	7.39
Lactate			5.4	

Table 2. 20 year old women intravenous drug user who took a 250 tablet overdose (500mg acetaminophen) the day before presentation at hospital. She received n-acetylcysteine 25 hours following OD. The patient was transferred and listed for transplantation.

Regardless of aetiology, patients with significant liver damage or signs of secondary organ dysfunction should be managed within the ICU. The speed at which patients deteriorate can be very rapid and it is not uncommon for hepatic encephalopathy to progress from grade 0 to grade IV over several hours.

For prognostic reasons it is important not to avoid correcting any biochemical coagulopathy at this stage if possible. Despite quite significant prolongation of prothrombin time, bleeding from line sites is uncommon in the initial stages of acute liver failure. In fact there is evidence that the patients are prothrombotic in vivo (62).

Investigation in a patient with suspected acute liver failure.

Initial investigations provide a baseline from which important diagnostic and prognostic information is taken. Serial blood test measurements should be performed as severity and prognosis are assessed over time from initial presentation (table 3). Dependent on the manner and speed of presentation, a complete blood count, clotting, liver function tests, arterial blood gases and ammonia should be performed twice daily, if not more often, in the early stages to assess progression of the illness. A liver ultra sound scan to assess liver size should be performed.

Investigations	**Common findings**
Complete blood count	Platelets often low
Urea and electrolytes	Serum sodium often low, especially in paracetamol hepatotoxicity. Urea often low due to reduced production. Serum creatinine is a useful prognostic marker
Liver enzymes	AST/ALT variable, very high in paracetamol toxicity. Bilirubin is a prognostic marker in non-paracetamol liver failure. Can also be high in Wilson's due to haemolysis.
Phosphate	Often low. Especially in paracetamol toxicity. Poor prognostic sign if high, suggests lack of regeneration.
Magnesium	Often low
Prothrombin time, international normalised ratio (INR)	Very sensitive indicator of liver function. Has prognostic significance in all forms of acute liver failure. May improve with vitamin K if deficient.
Viral serology for all known hepatotrophic viruses	
Urinary copper, plasma ceruloplasmin if Wilson's is suspected	Ceruloplasmin level often very low but can be normal. Urine copper is usually high.
Arterial ammonia	Has some prognostic value and may be an indicator of cerebral edema in patients at risk.
Arterial whole blood lactate	Very sensitive marker for of liver function. Particularly in paracetamol toxicity. High levels

	indicative of poor prognosis.
Serum glucose	Hypoglycaemia common
Beta HCG in women	Unwanted pregnancy can be a precipitant of deliberate self-harm

Table 3 Initial investigation and common findings

Transfer criteria guide

Acute liver failure is a sporadic syndrome with a case incidence of about 2000 per year in the USA (63). Management is complex and liver transplantation is the only effective therapy in severe cases. Transfer to a unit with experience in the management of these patients, preferably one with a liver transplant program will provide optimal care. It is often difficult to decide when to or whether to transfer a patient with acute liver dysfunction and this will vary according to aetiology and presentation. The best described clinical course is that following severe paracetamol poisoning after a deliberate over dose (1). Staggered overdose and therapeutic misadventure are less well defined but the clinical and laboratory signs of severe disease are still present.

The following list of criteria for transfer are based on expert opinion and have not been subjected to rigorous study and errs on the side of caution (64).

- The INR is greater than 3.0 in non-paracetamol aetiologies.
- A prothrombin time in seconds greater than hours since paracetamol overdose. For example INR 2.6 at 24 hours, 4.9 at 48 hours, 7.8 at 72 hours post overdose.
- If the INR is still rising from day 3 and 4 following a timed paracetamol overdose.

- If there is an elevated creatinine (>200 μmol/l) with a significantly raised INR (>3) or acidosis in any patient with acute liver failure.
- Significant hypoglycaemia in any patient with ALF.
- Any evidence of encephalopathy in any patient with ALF.
- Anyone who is hypotensive (mean arterial pressure < 60mmHg) following fluid resuscitation (mean blood pressure = (systolic + 2 x diastolic/3).
- Anyone who has evidence of a persistent metabolic acidosis (pH<7.3), a significant base deficit (< -3), or a raised lactate, following volume resuscitation.

In patients with a subacute presentation it is better to transfer patients before they have progressed to Grade II encephalopathy because of the additional complications associated with transferring ventilated patients.

Patients with acute or hyperacute liver failure showing signs of early encephalopathy should be intubated and ventilated prior to transfer. This can occasionally result in disagreement between referring hospitals and receiving liver units on how the transfer should be managed. There are many anecdotal stories from liver units around the world describing patients that become unmanageable during transfer, in planes, helicopters and road ambulances resulting in injury to patients and staff.

Heroic transfers are almost always inappropriate. Patients should not be considered for transfer if they are on rapidly accelerating inotropic support, have severe hypoxemia or already have fixed dilated pupils. There is little point a patient dying in an ambulance.

Specialist Management

Supportive Care in the ICU

Airway and Ventilation

Patients with ALF that reach grade II or III encephalopathy require intubation and ventilation. This is usually necessary to provide safe management in the setting of increasing agitation, protection of the airway from stomach contents or transfer from peripheral hospitals. In patients with severe liver failure it can be expected that encephalopathy grade will progress and so waiting until some predefined stage before intubation often delays adequate resuscitation and monitoring.

Controlled ventilation to a normal $PaCO_2$ is recommended at this stage. The use of hyperventilation is not indicated without further monitoring (65,66).

Circulation

The circulatory changes associated with acute liver failure can be profound (67). The pathological basis for this are incompletely understood, but similar in many ways to changes observed in patients with a systemic inflammatory response due to sepsis or trauma. The pattern observed depends on the aetiology and the rate of onset of the syndrome. In hyperacute liver failure due to paracetamol OD, patients can develop fulminant peripheral cardiovascular collapse that can be an early mode of death (68). Others with a more subacute onset can develop peripheral vascular changes similar to those with decompensated chronic liver disease and hepatorenal failure. There is a loss of vascular tone resulting in peripheral vasodilatation. When compounded by vomiting, especially in POD, this results in both relative and actual hypovolaemia and hypotension.

Fluid resuscitation should be commenced as soon as possible after presentation and should be directed by invasive monitoring. The type of fluid used for resuscitation has not been subjected to controlled trial in this setting but it has been shown that hypertonic saline infusion reduces the incidence of intracranial hypertension in patients with ALF (59). Hypoglycaemia should be avoided.

If following adequate fluid resuscitation mean arterial pressure remains less than 65 mmHg a vasopressor should be commenced. Norepinephrine is the vasopressor of choice as it induces vasoconstriction without the induction of lactate production as often seen with the use of epinephrine. Although epinephrine has been used in this setting successfully.

Vasopressin and its longer acting analogue terlipressin have been used as vasopressors in septic and cardiogenic shock (69,70). Terlipressin results in profound systemic vasoconstriction and has the potential to result in a worsening in oxygen delivery and consumption. It has been shown to increase intracranial hypertension in ALF due to an increase in cerebral blood volume because of the breakdown in cerebro-vascular autoregulation (71). Due to its relatively long half-life and difficulty with dosing, and the association with increased intracranial hypertension the use of terlipressin cannot be recommended.

The majority of patients presenting with ALF are young adults and as a result comorbid cardiac dysfunction is usually absent. Cardiac depression has been reported in association with POD (68). On occasion cardiac disease can present as acute liver failure in the case of ischaemic hepatitis. (Previously discussed)

Relative adrenal insufficiency has been shown to occur in patients with septic shock and more recently in patients with decompensated chronic liver disease and ALF

(72–75). The use of stress doses of corticosteroids in ALF has been shown to reduce norepinephrine requirements in patients with ALF (76).

Renal

Renal failure is common in acute liver failure with a reported incidence of up to 70% (77). Paracetamol causes direct renal tubular dysfunction, possibly by effecting membrane protein function and occasionally renal failure is the predominant organ effected (78,79). As a result renal failure can be expected in this setting. Although it is most commonly associated with POD the incidence in other aetiologies, such as Wilson's disease due to the direct toxic effects of copper, is also high.

There is a lack of consensus regarding the classification and definition of renal dysfunction in the critically ill although attempts are being made to remedy this situation (80). The term "acute tubular necrosis" is inadequate in the setting of critical illness where it fails to describe the full spectrum of renal dysfunction. This is even truer in the setting of acute liver failure. In addition, the use of the term hepatorenal syndrome in acute liver failure is also inappropriate because this definition does not adequately describe the often rapid in clinical deterioration. This is not to say that altered systemic and renal haemodynamics do not contribute to the pathophysiology of renal failure as they do in critical illness in general. In the subacute presentation of this syndrome early renal dysfunction can be similar in presentation to that seen in end stage chronic liver disease with sodium and water retention in the absence of intrinsic renal damage (81).

Management of renal failure is essentially the same as in ALF and other forms of multiple organ failure and consists of maintenance of intravascular volume, cardiac output and mean arterial pressure (63).

Extracorporeal renal support is necessary in most patients with ALF at some time. Continuous veno-venous hemofiltration (CVVH) is the most efficient and safest method for renal support and should be started early (82). Indications for the early use of renal support are not just limited to oliguria. CVVH has been shown to improve haemodynamic stability in patients with critical illness and has been used as salvage therapy in patients on high dose vasopressor. Survival advantage relative to dose has also been shown (83). In addition it can be used to induce hypothermia in the setting of raised intracranial pressure. It also enables the infusions of large quantities of blood products and drugs, often required during intensive care, helping to maintain fluid balance. High volume haemofiltration (4000 ml/hr ultrafiltrate exchange) has been studied in ALF and shown to reduce serum lactate, base deficit and norepinephrine requirements, when compared to historical controls, although survival benefit has not been shown (84).

Patients with liver failure do not tolerate the lactate load associated with lactate buffered replacement fluid during CVVH. In fact the rapid infusion of lactate can induce a systemic acidosis in this setting (85). Also the infusion of large quantities of lactate containing fluid reduces the utility of serum lactate as one of the most important prognostic indicators (86). As a consequence the use of bicarbonate haemofiltration is recommended (87).

CVVH can often be performed successfully without anticoagulation in ALF. Patients with ALF appear to be hypercoagulable, in vivo, despite prolonged coagulation parameters. This may be due to a reduction in protein C production (62). If required, a loading dose of 2000 units of heparin and thereafter 500 to 1000 units an hour depending on the activated clotting time, can be used if needed to allow CVVH. If bleeding is a problem or there is severe thrombocytopaenia, epoprostenol should be

instituted (2.5 - 5ng/kg/min) either alone or in association with low dose heparin (100units/hr). Blood flow rates of 200 ml/min and above will help with filter life.

Cerebral care

Aetiology

Cerebral oedema was noted and commented on in the seminal clinico-pathological review of servicemen presenting with fulminant epidemic hepatitis during the East Asian campaign of the Second World War (2). However the recognition that cerebral oedema was a distinct clinical entity and cause of death associated with ALF did not become clear until much later (88,89).

The recognition that brain swelling is an important component of ALF is now well established. Management strategies place the risk of intracranial hypertension at the forefront of care and prophylactic therapy, monitoring and treatment and are widely debated in the literature (90,91).

The pathology of cerebral oedema and intracranial hypertension in ALF are not completely understood but in recent years progress has been made into the processes involved (92). Ammonia has long been implicated as important in hepatic encephalopathy and, as the evidence mounts, increasingly recognized has an important factor in the aetiology of cerebral oedema in ALF (93). Recent work has shown that whole blood ammonia predicts outcome and the chances of cerebral herniation (94,95). Electron-microscopic analysis of post-mortem brain biopsy together with gravimetric analysis of brain water content, in animal models, have shown that swelling occurs in the grey matter and that astrocytes are the main target (96,97). Ammonia is detoxified in astrocytes by combining with glutamate to produce glutamine. Astrocytes are the only cell type in the brain that contains glutamine

synthetase and this normal pathway maintains the ratio of glutamate to glutamine within astrocytes and neurons. The raised serum ammonia induces a build-up of glutamine within astrocytes, increasing the osmotic potential, absorption of water and an increase in volume. Evidence of this effect can be inferred by the fact that inhibition of glutamine synthetase, which catalyses the reaction, prevents brain swelling in experimental models (98). An osmotic effect may not be the only mechanism by which glutamine induces cellular swelling however, but there is circumstantial evidence that it occurs to some extent. In subacute and chronic liver disease there is time for adaptation to the increase in astrocyte osmotic potential by the excretion of intracellular osmolytes such as myo-inositol (99). In ALF there is no time for adaptation because of the rapid increase in intracellular glutamine. Recently, however, the direct correlation between astrocyte glutamine levels and cell volume has been questioned. Experimental data has not been able to show a correlation between the two, with peak glutamine levels occurring before cellular volume reaches its maximum (100,101). Clinical study using cerebral microdialysis has shown abnormal glutamate/glutamine trafficking within the brain extracellular space with initial high levels of glutamate but then a reduction to very low levels without any correlation with intracranial pressure (56). Extracellular lactate concentration was shown to rise prior to surges in ICP, however (56).

A breakdown in cellular energy metabolism induced by an increase in intracellular glutamine and ammonia is another possible mechanism for the cellular swelling. Impairment of alpha-ketoglutarate dehydrogenase and pyruvate dehydrogenase induced by oxidative stress and ammonia results in a breakdown in astrocyte energy production, and an increase in glycolysis. This is supported by an increase in

extracellular lactate and brain lactate flux in clinical studies (56,65). Mitochondrial dysfunction induced by ammonia may also play a role (93).

In addition to the cytotoxic oedema seen early in ALF, changes in cerebral blood volume may play an important role in the development of intracranial hypertension. Cerebral blood flow shows wide variation in patients with ALF and the normal close relationship between cerebral metabolism and flow appears to be lost (102). In animal models of liver failure there is a gradual increase in cerebral blood flow but this situation is less clear in human studies (92,103). Cerebral metabolism is generally reduced in high-grade encephalopathy because of the breakdown in autoregulation. There is evidence that relative or absolute cerebral hyperaemia can contribute to intracranial hypertension (71,104). An increase in cerebral blood flow may induce intracranial hypertension by a number of mechanisms and induce further cerebral swelling. This could occur due to an increase flux of potentially toxic metabolites such as ammonia or by an increase in cerebral water content because of an increase in cerebrovascular hydrostatic pressure. Neither of these hypotheses has been proven and the evidence suggests that the blood brain barrier is relatively intact in ALF suggesting that hydrostatic or vasogenic oedema is uncommon (97). Autoregulation is lost in ALF (103,105). The cause is unknown but a gradual cerebral vasoparesis concurs with clinical observation. Autoregulation can be restored by hyperventilation and mild hypothermia but not by the use of indomethacin (106). It has been suggested that the increase in astrocyte glutamine plays a role in this gradual vasoparalysis and loss of autoregulation by the induction of local nitric oxide or carbon monoxide (92). It is a relatively common to find a patient with a high ICP and jugular venous oxygen saturation above 80%. This suggests a state of “luxury perfusion” (unregulated blood supply in excess of demand) in which an increase in

cerebral blood volume in an already swollen brain accounts for the associated increase in intracranial pressure (107).

Management

In the management of intracranial hypertension (IHT) in acute liver failure it is important to target those at risk. Intracranial hypertension remains a leading cause of death despite advances in the understanding of aetiology and management of patients (108,109). Monitoring the brain is difficult and invasive. Despite investigation a reliable non-invasive method for the evaluation of cerebral blood flow, cerebral oxygenation, and intracranial pressure remains elusive (110). There are important points that need to be considered when managing a patient with ALF and possible raised intracranial pressure:

- Prediction of patients at risk of IHT
- How to monitor the brain
- Prophylactic management
- Treatment of established IHT

Predicting IHT

In acute liver failure the development of cerebral oedema is seen in patients with the shortest time between the development of jaundice and the onset of encephalopathy (1). A fulminating presentation, lack of time for cerebral adaptation and systemic burden of a necrotic liver appear to be the likely reasons. Patients with paracetamol toxicity make up the largest number in this group. Other aetiologies can fall into the hyperacute group including patients with ALF due to hepatotrophic viruses particularly hepatitis B. In contrast patients with subacute liver failure due to NANB have a smaller risk.

It has been suggested that the incidence of intracranial hypertension following paracetamol toxicity has fallen since the mid nineteen eighties (111). However it still represents a significant complication in this setting. Recent work from King's College Hospital in London suggests an incidence of 20 – 30% in all patients with ALF (108). Data from our own unit suggests that intracranial hypertension is implicated in the death of 25% of all patients with ALF and 35% of those following paracetamol induced toxicity (109).

Young age has consistently been found to be a risk factor (88,108). Arterial ammonia concentration has been shown to correlate with death and cerebral herniation (94,95,112). Recent commentary suggests that arterial ammonia should be measured serially in all patients with ALF and intracranial pressure monitoring be instituted if the concentration is greater or equal to 150 μmol/l (107).

Monitoring the brain

There are a number of monitoring devices and methods that can be undertaken to screen for raised intracranial pressure or cerebral ischaemia or both. Some of these are more invasive than others and the possible risks and benefits need to be understood.

Computerized tomography is a standard investigation in any patient with suspected intracranial pathology. Cerebral oedema can be recognized in CT scans of patients with ALF and the severity correlates crudely to encephalopathy grade but the correlation between imaging and severity of intracranial pressure measurement is poor (113,114). As little additional information is gained very careful consideration should be undertaken before transporting this very sick group of patients to the CT

scanner. Occasionally there are diagnostic difficulties or a suspected complication of ICP bolt insertion and CT scanning might be considered.

Functional brain imaging, using single positron emission tomography (SPECT) has been used to investigate the distribution of cerebral blood flow in ALF and MRI scanning has been used to investigate the distribution of intracerebral water but neither has found a place in clinical practice (66,115).

In patients with suspected intracranial hypertension the direct monitoring of cerebral oxygenation and blood flow are appealing but current methods have technical and clinical limitations. Tissue PO_2 and interstitial metabolites, using intra-parenchymal probes, have been investigated in traumatic brain injury and to a limited extent in ALF (56,116). They have the advantage in traumatic injury of providing localized information around the area of injury. The use of cerebral microdialysis (sampling of the cerebral interstitial fluid using a micro-coaxial catheter with a semi-permeable membrane) remains a research tool in ALF at the present time.

Methods used for the estimation of global cerebral oxygenation include the sampling of jugular venous (JV) blood for oxygen saturation, and products of metabolism such as lactate. A jugular venous saturation of less than 55% suggests an ischaemic brain. This can be due to a reduction in blood flow in excess of demand because of brain swelling or cerebral vasoconstriction due to hypocarbia, if the patient is being hyperventilated. An increase in demand due to seizure activity can also manifest as a reduction in jugular venous saturation (117). High jugular saturation (> 80%) may represent a hyperaemic brain and steps can be made to reduce cerebral blood volume if ICP is raised. Very high JV saturation is often seen as a terminal event and may represent a complete loss of oxygen extraction by the brain.

Near infrared spectroscopy is a non-invasive technique used to assess the oxygen content of various organs. It can be used to determine cerebral oxygenation and changes in cerebral perfusion in ALF and warrants further investigation (118).

Non-invasive measurement of cerebral blood flow using transcranial Doppler has been investigated in ALF and found to be predictive of changes in cerebral blood flow induced by hyperventilation; however the technique does not provide data on cerebral oxygenation and so could not be recommended without the addition of a jugular venous catheter (119).

The recognition that intracranial pressure is raised in a significant proportion of patients with acute liver failure and that this is implicated in significant morbidity and mortality has led to the use of direct measurement of intracranial pressure with various forms of monitor (88,120). These techniques, while fully supported internationally in traumatic brain injury, are controversial in the field of acute liver failure and there remains a dichotomy of opinion in most countries with some units using them and others not (90,91,121).

Controversy revolves around the lack of evidence of improved outcome with the monitoring of intracranial pressure and the risk of intracranial bleeding complicating insertion. The reported risk of bleeding, from survey data, is between 10 and 20% overall, the majority of which is not clinically significant. Mortality has been reported at between 1 and 3%, (Alistair Lee, Edinburgh UK, personal communication) (120,121). The risk of bleeding following placement is higher than that seen following traumatic brain injury. There is uncontrolled evidence that activated factor VII can reduce this risk (122).

It has not been possible to prove that intracranial pressure monitoring improves survival in ALF, as a RCCT has not been performed to evaluate it. However, it is generally accepted that medical intervention can reduce intracranial pressure and prevent cerebral ischaemia and brain herniation in patients with acute liver failure (107). Published data suggests that having an ICP monitor increases the intervention rate compared to patients without, and increases the length of survival in the critical care unit, if not overall survival (123). The majority of patients with ALF die of multiple organ failure due to sepsis. Intervention to reduce ICP may just prevent early cerebral death.

While the risks of monitoring are documented the risks of not monitoring are less clear. Without monitoring intracranial pressure there is a tendency toward therapeutic paralysis because of uncertainty and to manage all patients as if they had raised intracranial pressure. The reassurance of a normal ICP enables a reduction in sedation and paralysis. It enables tracheal suctioning and other nursing care without the uncertainty of worsening an unknown ICP. With ICP monitoring modest increases in ICP can be treated early before clinical signs suggest impending brain herniation. Monitoring ICP enables the calculation of cerebral perfusion pressure and together with the monitoring of jugular oxygen saturation allows a more complete picture of cerebral perfusion and oxygenation. The use of intracranial pressure monitoring has been advocated in the setting of liver transplantation for ALF and, of course, enables continued clinical research into the management of cerebral oedema.

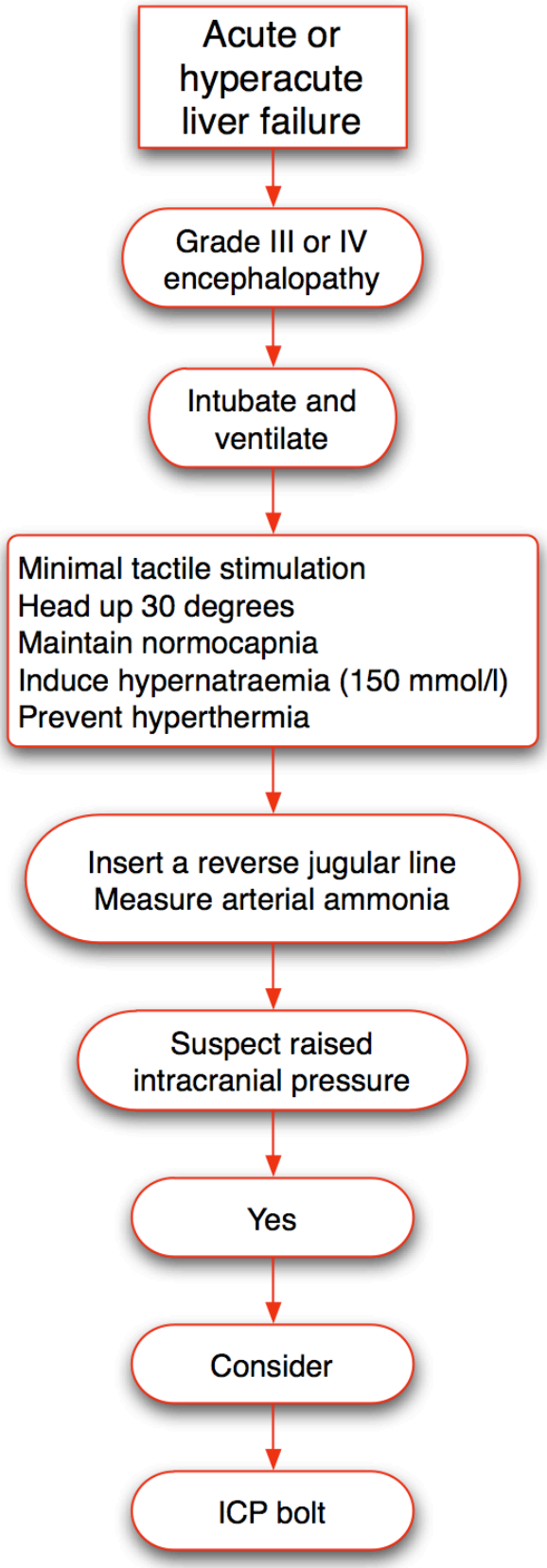

Figure 4 Initial management of patient with high-grade encephalopathy

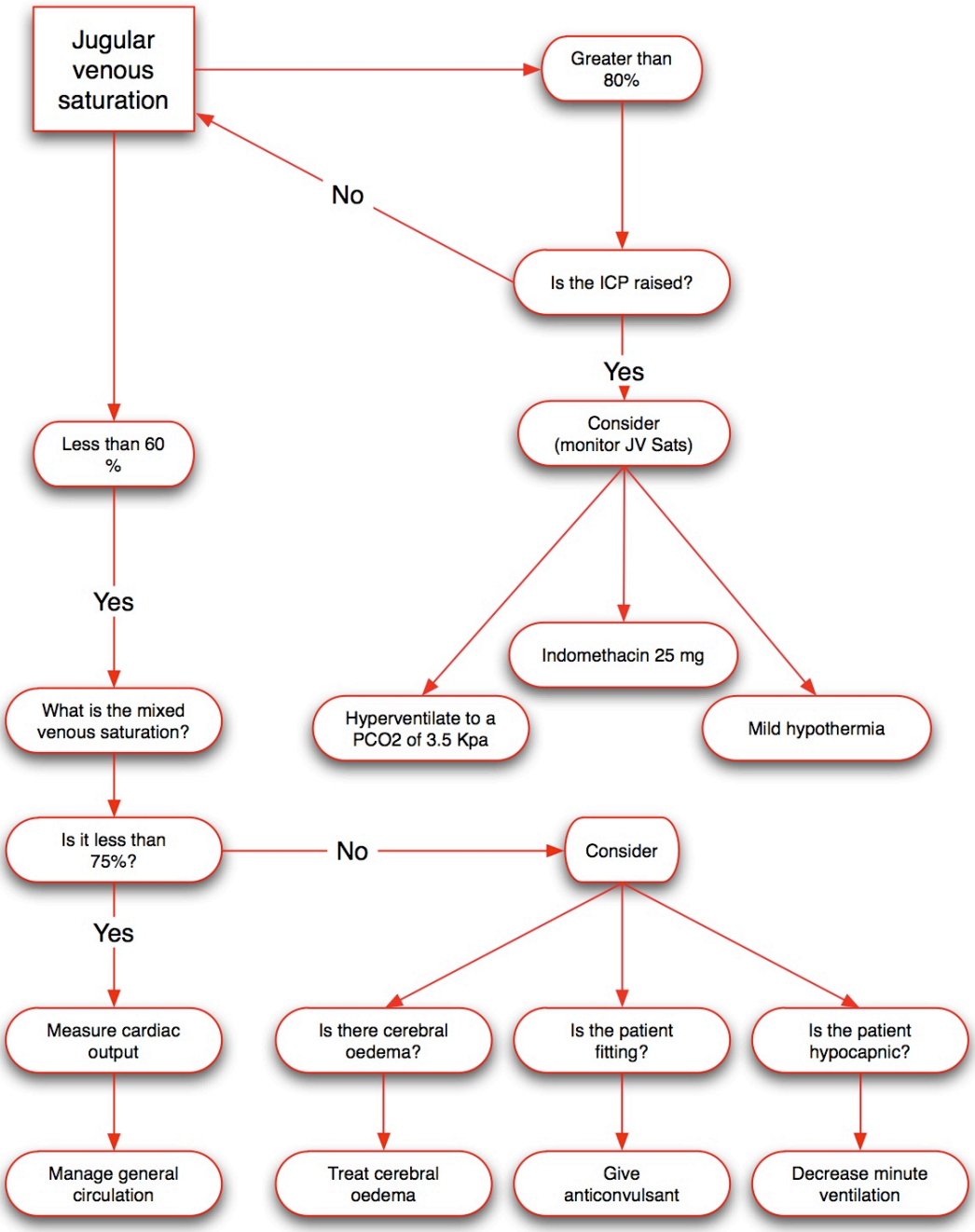

Figure 5 Monitoring cerebral oxygenation with jugular bulb sampling

Prophylactic measures

In patients at high risk of cerebral oedema a number of prophylactic interventions have been shown to reduce the incidence of intracranial hypertension.

Serum sodium is often low in patients with ALF. In a consecutive group of patients with ALF from POD admitted to King's liver intensive care unit, 65% were hyponatraemic on arrival (Will Bernal personal communication). Hyponatremia is associated with a poor outcome in ALF (124). Based on retrospective data showing an inverse relationship between intracranial pressure and serum sodium in patients with ALF, moderate hypernatraemia was investigated as a possible prophylactic intervention (59,125). Maintaining serum sodium between, 145 to 155 mmol/l using hypertonic saline was found to reduce ICP from baseline and reduce the incidence of surges in intracranial pressure (59). Hypothermia improves outcome following out of hospital cardiac arrest and has been investigated in patients with traumatic brain injury. Early reports suggest hypothermia can reduce ICP and ammonia production in patients with ALF. Prophylactic hypothermia is currently being investigated in this setting. Simple measures such as raising the head of the bed to a thirty-degree angle and the avoidance of excessive stimulation are also prudent.

Cerebral perfusion pressure

In traumatic brain injury there is a consensus of opinion supporting the use of cerebral perfusion pressure (CPP) as a treatment goal (126). In ALF the concept of CPP directed therapy is less useful. To assume a correlation with cerebral blood flow here has to be a consistent cerebrovascular resistance and this is not the case in ALF (107). This is due to the loss of cerebrovascular autoregulation and attempts to increase cerebral perfusion are often unsuccessful as the use of a vasopressor results in an increase in ICP as brain blood volume increases (71,127). However this is not to say that CPP should be ignored entirely but the safe lower limit of CPP has yet to be defined, as there are many reports of patients surviving with normal cerebral function despite a low CCP (128). The normal lower limit of cerebral

autoregulation is reached at a mean arterial blood pressure of about 50 mmHg, below which flow becomes pressure dependent. In patients with absent autoregulation, such as in ALF, CCP should probably be maintained above 40 mmHg (the normal lower limit of autoregulation with an ICP of 10 mmHg or less) but no data exists to back this statement up. Maintenance of CPP in ALF is best achieved by decreasing ICP and aiming for a mean arterial pressure with fluid and vasopressor that does not increase ICP above 25 mHg. Attempting to improve cerebral oxygen balance is also attractive in this setting. This may be attempted with intravenous indomethacin (has been shown to improve CPP without compromising cerebral oxygenation), hypothermia, increased sedation and hyperventilation (106,129–131). Monitoring cerebral oxygenation is very useful during such a manoeuvre (107).

General management of patients with raised intracranial pressure

In patients at risk of or with suspected cerebral oedema prophylactic measures should be instituted. The decision to insert an ICP bolt or not will have to be made by the clinical team. If inserted there is the potential to manage intracranial pressure.

Intracranial pressure is normally less than 15 mmHg in an adult. The definition of intracranial hypertension is not precise and will vary between patients. Available data are derived from patients with traumatic brain injury (TBI) where observational studies suggest that intervention to reduce pressure should be instituted between 20 and 25 mmHg, although pupillary abnormalities and brain herniation can occur at lower pressures (132). There have not been any studies investigating treatment threshold in ALF and so similar thresholds to TBI are used.

The management of intracranial hypertension is usually escalated along standard algorithms [see figures 4,5,6]. Elevate the patient to an angle of 30 degrees and avoid tight straps around the neck to encourage venous drainage. ICP tends to increase during nursing intervention. If this takes more than a couple of minutes to recover it can suggest poor intracranial compliance. Treatment is usually instituted for a sustained rise in ICP (> 5 to 10 mmHg minutes) or clinical signs suggesting cerebral ischaemia or impending herniation. Sedation should be increased. Propofol is probably the agent of choice (110). Osmotherapy is the mainstay of treatment following these simple measures. Mannitol as a rapid infusion (0.5 – 1.0 g/kg) has been shown to reduce ICP reliably in ALF (133). The dose can be repeated but care must be used in renal failure due to accumulation and multiple administrations can result in a hyperosmolar syndrome. Plasma osmolality should be monitored if multiple doses are used. Current practice is to remove 500 ml of ultrafiltrate via CVVH following each bolus dose of mannitol. Bolus doses of 20ml hypertonic saline (30%) has a similar effect to mannitol in this setting (personal observation). Hypertonic saline has a higher reflectance coefficient at the blood brain barrier (BBB) compared to mannitol and there appears to be less tachyphylaxis to multiple administration (59).

In patients with a raised ICP and cerebral hyperaemia, suggested by a jugular venous oxygen saturation of 80% or greater (luxury perfusion), short-term hyperventilation will induce cerebral vasoconstriction and reduce blood volume. This manoeuvre has been shown not to impair cerebral oxygenation but close monitoring of cerebral oxygenation should be employed if it is attempted (131). Short-term hyperventilation has not been shown to improve outcome in ALF but does prolong survival in the intensive care unit (134). Hyperventilation may be lifesaving and buy

time for definitive treatment (transplantation). Indomethacin induces cerebral vasoconstriction and reduces ICP in patients with both TBI and ALF without impairing cerebral oxygenation although confirmatory studies are needed (106).

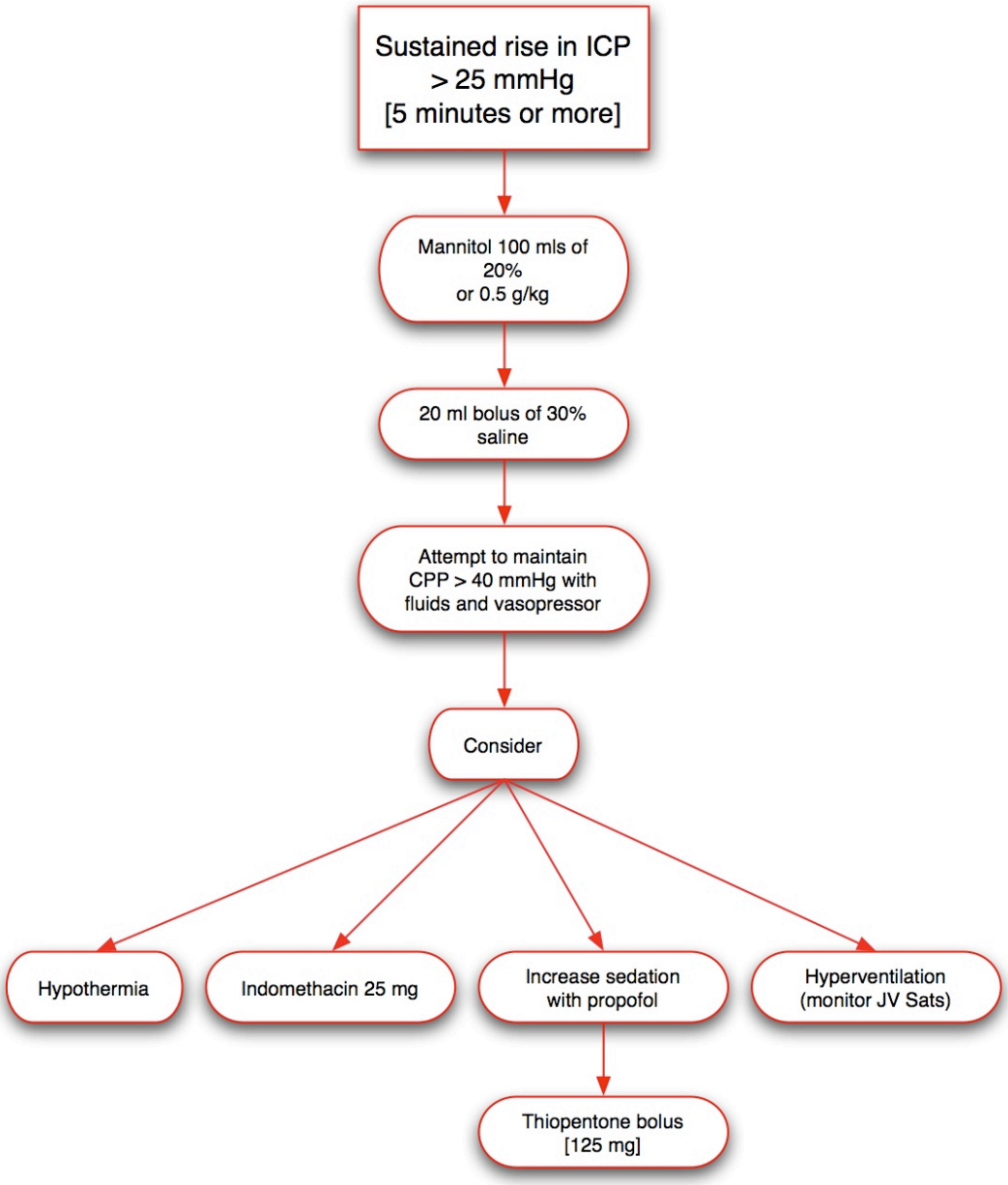

Figure 6 management of a sustained rise in ICP

Seizures

Ammonia toxicity and cerebral oedema are associated with seizure activity and it has been recognized that sub-clinical seizures occur more commonly than previously thought (117). The use of mechanical ventilation facilitated by sedatives and muscular paralysis can mask clinical signs. Seizures adversely affect cerebral oxygen consumption and may contribute to cerebral oedema and are a cause of low jugular oxygen saturation. It has been suggested that prophylactic phenytoin be used in all patients with ALF and high-grade encephalopathy. Others have questioned this approach because of the significant side effects and apparent lack of effect on outcome (135). If confirmed seizure activity should be managed along standard management guidelines.

Infection and immunosuppression

Patients with acute liver failure have multiple immune defects and are susceptible to infections (136). Infection is a common cause of progression and complication in ALF (137). Antibiotic prophylaxis has been shown to reduce the incidence of infection and enable transplantation to proceed but has not been shown to improve survival. Selective decontamination of the digestive tract has not been shown to be superior to IV antibiotics alone (136,138).

It is current practice to prescribe broad spectrum antibacterial and antifungal medication to patients with acute liver failure depending on local sensitivities. There is a general trend to the timing of infection with Gram-positive infections occurring earlier than Gram negative. Early Gram negative infections tend to be less resistant “endogenous” bacteria followed later by resistant hospital acquired organisms (139).

Nutrition

Within the general intensive care literature there is a consensus toward enteral nutrition (EN) as the route of choice (140). There is little additional data on which to base decisions in patients with ALF. There is however wide regional variation in the prescribing of parenteral nutrition (PN) compared to EN. The reason for this is unclear as EN is associated with a reduction in infectious complications (140). However it is clear some centres prefer PN (141).

Nutritional requirements in ALF are not well understood. Energy expenditure is raised during ALF. This is surprising considering the normal contribution of the liver, a large metabolically active organ, on the overall energy expenditure, illustrated by the effects of hepatectomy on energy expenditure during elective liver transplantation (142). There is organ specific lactate production in both the liver and the lungs and evidence of a systemic inflammatory response in many patients (137,143,144). The cause of systemic inflammation in ALF is activation of inflammatory cells (leucocytes and endothelium) and the release of systemic cytokines. This may be initiated by infection (137). Patients in the early stages of ALF are markedly catabolic with insulin resistance despite the often presence of a hypoglycaemic state (145). The Harris-Benedict equation is inaccurate in ALF and indirect calorimetry should be used if energy requirements are sought.

Hypoglycaemia is common due to the failure of both glycogenolysis and gluconeogenesis. Massive hepatic necrosis can result in a precipitous fall in serum glucose concentration. For this reason an infusion of 50% glucose should be used at least until feeding is established. Low volume, high concentrations of glucose are preferred to reduce the infusion of large quantities of water and the resultant hyponatraemia if lower concentrations are used.

Serum amino acids are consistently deranged during liver failure. There is a low or normal concentration of glutamine, branched chain amino acids and tryptophan and an increase in others (146). High brain concentration of ammonia is thought to contribute to cerebral oedema and encephalopathy in ALF. Manipulation of ingested amino acids has been investigated in an attempt to reduce ammonia concentration. L-ornithine & L-arginine (LOLA) infusion encourage alternate pathway metabolism and reduce hepatic encephalopathy in chronic liver disease (147). LOLA has been investigated in animal models of ALF and resulted in normalization of plasma ammonia concentration, significant delay in the onset of encephalopathy and a reduction in brain water concentration (148). This simple treatment method has not been investigated in ALF but would seem to warrant further investigation. N-acetylcysteine (NAC) is primarily used as a glutathione precursor and antidote in paracetamol poisoning but is used widely in patients with ALF induced by other causes and later on in its subsequent course. Evidence is lacking for its use outside the first twenty-four hours post paracetamol poisoning.

There is little evidence that protein restriction has any role in ALF and protein requirements based on total calorie ingestion should be used. It has been suggested that lactulose therapy may reduce ammonia concentration and increase survival time in patients with ALF but this needs confirmation and cannot be recommended at this time (149).

Serum electrolytes are often deranged in the early stages of ALF. Hypophosphatemia is common in patients with acute liver failure and is a good prognostic sign. High or normal phosphate may indicate a lack of hepatic regeneration and renal impairment and is associated with a poor outcome (60). Hypomagnesaemia is also common and should be corrected.

Artificial liver support

At the present time liver transplantation is the only form of definitive therapy for severe hepatic failure. However, the scarcity of organs and potential for delay in transplantation, together with a proportion of patients that will make a full recovery if supported while the liver regenerates suggest that there would be a role for some kind of liver support system.

The liver is a complex organ and to be an ideal liver replacement any system has to support a wide range of biosynthetic and metabolic functions (see box: functions of the liver, page 69). Any working system will also have to counter the systemic toxic effects of the dying liver (55).

Artificial liver support can be split into two main approaches. In the first there is an attempt to simulate or replace all or most of the functions of the liver. These systems include hepatocytes either from human or animal sources. Another view of liver failure suggests that toxins either excreted by the dying liver or not metabolised because of an acute reduction in function are responsible for the majority of the signs and symptoms. In this view extracorporeal blood purification with dialysis or adsorption techniques are employed and serum proteins not produced are replaced with plasma.

Functions of the liver

- Excretion of bilirubin, cholesterol, hormones, and drugs
- Metabolism of fats, proteins, and carbohydrates
- Enzyme activation
- Storage of glycogen, vitamins, and minerals,

Biological systems

Biological systems consist of a bioreactor within which the cellular biomass is contained, and a mechanism of containing the biomass away from the circulation of the patient. They require an extracorporeal system to deliver blood or plasma to the bioreactor and may also contain an adsorption or dialysis component. Data from liver resection suggests that approximately 250 ml of liver by volume is required to prevent death from liver failure. This typically represents 20 – 30% of liver mass (150). More may be needed to counter the systemic effects of a dying necrotic liver.

Possible cell types to use as the biomass include animal or human primary hepatocytes or other forms of cell line with hepatocyte phenotype. Primary hepatocytes outperform other cells lines but are of limited availability and tend to have a time dependent loss of hepatic phenotype. In addition, scaling up production with primary hepatocytes is difficult as they do not readily undergo cell division under lab conditions. Instead they have to be directly seeded into the bioreactor either immediately following harvest or following a period of storage and cryo-preservation. Human cells are limited in availability but can be obtained from unused livers and cut

down grafts. Theoretically, animal cells are readily available but uncertainty about possible cross infection from animal pathogen to the patient with organisms such as PERV (porcine endogenous retrovirus) and incompatibility of secreted antigens render them far from ideal and regulatory authorities are often reluctant sanction their use.

Immortalized cell lines that proliferate in culture, while retaining some liver specific functionality can be used in an attempt to overcome the limitations of primary hepatocytes. Many lines have been created by retroviral transfection with regulatory genes that stimulate cell division. The insertion of “terminator” genes that give the cells a limited life or enable switching of the immortalizing gene off have also been developed to improve safety (151). Other sources of immortal and readily cultured cells are tumour derived such as the ubiquitous Hep G2/C3A hepatoblastoma line. Finally, stem cell sources appear to offer the most hope in terms of a readily available and functional supply of differentiated hepatocytes (152).

Clinical trials in the use of bioartificial liver support have been relatively disappointing. The Bio-Artificial Liver (BAL) uses porcine hepatocytes and a charcoal column in series. The largest trial published so far in the field was powered for survival advantage in ALF and primary non-function following liver transplantation (153). The study was terminated early by the data and safety-monitoring board because the trail was likely to be futile based on the results at interim analysis (153). Post hoc analysis suggested some effect in the ALF group alone. The Extracorporeal Liver Assist Device (ELAD) system uses Hep G2/C3A hepatoblastoma line and has been investigated in a number of phase 1 studies designed to report safety and activity (154,155). The most recent was a randomized controlled study, not powered for mortality, and showed a trend for improved survival in the treatment group (155).

Other small case series and controlled trials with various systems have been reported over the last ten years (156–158).

Non-biological

Many of the molecules that accumulate within the blood during ALF are small or middle-sized (55). These can be targeted by a variety of extracorporeal purification techniques, including dialysis through various types of membrane and adsorption onto carriers such as charcoal, resins or albumin.

Non-biological systems are attractive as they are relatively inexpensive (compared to biological) and logistically much easier to implement.

Early work with haemodialysis was unsuccessful and was largely abandoned with the conclusion that the toxaemia of ALF was not due solely to small water-soluble molecules. The advent of synthetic membranes in the 1970's rekindled the interest in convective therapy for ALF. These allowed larger molecule to pass through compared to the cuprophane alternatives. Opolon reported clinical improvement with the use of high-permeability membrane haemodialysis and haemofiltration in patients with ALF (159). Haemofiltration, as a form of renal support, is used in most liver critical care units as part of general supportive care and there is some evidence that increased convective exchange, with high volume (> 35 ml/kg/hr) haemofiltration, is associated with improved haemodynamic stability and improved encephalopathy scores (84,159).

Charcoal hemoperfusion has been extensively investigated. Initial trials were encouraging but latter larger randomized controlled trials were unable to show an improvement in outcome (77). Large volume plasma exchange showed some improvement in haemodynamics and other parameters in initial studies (160). The

therapy is logistically difficult to perform and like total exchange transfusion is an inefficient method of clearing the toxaemia of ALF.

In an attempt to improve the efficacy of dialysis techniques adsorbents have been added to the dialysis fluid to widen the range of molecules removed. The two most extensively studied are charcoal suspension in the BioLogic-DT™ system and 20% albumin in the MARS system.

Both the BioLogic-DT and MARS have been shown to improve blood pressure, increase vascular resistance and improve short term encephalopathy scores in patients with acute on chronic liver failure (AOCLF). The results of studies into their utility in ALF is less clear cut with inconsistent results from small uncontrolled series and case reports. With MARS™ therapy there appears to be an increase in peripheral vascular resistance and concomitant reduction in cardiac index in the short term. There does not appear to be any consistent effect on intracranial hypertension (161,162). Similar results have been reproduced with high volume hemofiltration pointing to the need for comparative randomized controlled trials (84).

Meta-analysis of all randomized controlled trials in non-biological liver support concluded that there is an improvement in short term mortality in acute on chronic liver failure but that this could not be shown in ALF (163,164).

Liver transplantation

When and whom to transplant

Liver transplantation for ALF was used sporadically during the 1980's but gained pace from the late 80's and has a huge impact. It remains the only definitive form of therapy for some.

Timing is important, and in those with severe liver injury there is a window of opportunity beyond which transplantation often becomes futile because of deteriorating organ function (165). It was recognized early in the history of transplantation for ALF that the challenge was to develop robust prognostic indicators. These have to be sensitive, early enough to provide maximum advantage to the patient and specific enough not to result in unnecessary transplants.

A “super-urgent” designation exists in the national transplant sharing scheme in the UK and a similar “category 1A” designation in the USA – see http://www.unos.org/ for details. These categories recognize the role of early transplantation in ALF and the detrimental effect of delay in this setting.

In the UK, the super-urgent designation (see table 5) is closely linked to the prognostic score developed in King’s College Hospital in the late 80’s using retrospective multivariate analysis with prospective validation (166). The prognostic criteria have been subsequently validated in other centres and shown to be robust (167). Other criteria have been developed (168).

The criteria are not perfect. Firstly, despite their good specificity i.e. if the patient achieves criteria they are likely to die, the sensitivity and negative predictive value are not as good and there is a substantial proportion of patients that will die without ever reaching transplant criteria. In addition awaiting positive criteria can lead to delay in listing and worsening of organ failure that often then precludes listing. This contributes to the fact that published rates of transplantation in those that reach criteria are only 50% following POD (169). Clinical practice has changed since this designation was first defined. For example, it is rare to see a patient following POD with a pH < 7.3 or a creatinine > 300 mmol/l because of improved resuscitation and

early renal support at the referring hospital. Because of these factors ongoing efforts to establish markers that increase sensitivity and occur even earlier in the course of the syndrome, while maintaining good specificity and not reducing the positive predictive value to unacceptable levels leading to unnecessary transplants, continue.

Serum phosphate levels are higher in non-survivors following both POD and in other causes of ALF (60,170). However, there appears to be an unacceptable overlap and others have suggested the use of serum phosphate does not provide any additional benefit to existing markers (61,171,172). Other factors investigated include alpha-fetoprotein levels and NMR analysis of peripheral blood (60,173). Acute physiology scoring as a basis for prediction has also been utilized (174).

The liver plays a central role in lactate metabolism. In fact in patients with severe liver necrosis the liver changes from being a consumer of lactate to being a net producer (178). Arterial blood lactate levels have been shown to improve the sensitivity and maintain the specificity if added to the original KCH criteria and are achieved earlier in the course of the syndrome (119).

On the practical issue of actually managing patients with FHF some room for clinical interpretation has been included in the super-urgent listing rules. For example there is a group of patients that do not achieve KCH criteria but subsequently die - usually of cerebral oedema or multiple organ failure secondary to sepsis (109,111). These patients often have worse acute physiology scores compared to survivors (169,174). As a result the UK super-urgent criteria allow an assessment of deteriorating acute physiology based on cardiovascular, respiratory or cerebral pathology. Similarly the UNOS 1A criteria allow for patients “not expected to survive a further 7 days”.

Category 1: Paracetamol: pH <7.25 more than 24 hours after overdose and after fluid resuscitation

Category 2: Paracetamol: Coexisting prothrombin time >100 seconds or INR >6.5, serum creatinine >300 μmol/l or anuria, grade 3/4 encephalopathy

Category 3: Paracetamol: Serum lactate >3.5 mmol/l on admission or >3.0 mmol/l more than 24 hours after overdose and after fluid resuscitation

Category 4: Paracetamol: Two of three criteria from category 2 with clinical evidence of deterioration (eg increased ICP, FiO2 >50%, increasing inotrope requirements) in the absence of clinical sepsis

Category 5: Aetiology: hepatitis A, hepatitis B, idiosyncratic drug reaction, seronegative hepatitis. Prothrombin time >100 seconds or INR >6.5 and any grade of encephalopathy

Outcome from transplantation

In Europe the one-year survival rate following transplantation for ALF is worse than that seen in chronic liver disease. The excess mortality is in the first month or so post transplant. This represents the severity of organ dysfunction seen prior to transplantation in ALF. Following this initial period, the curve flattens and the survival rate is actually better than that of patients with chronic liver disease [see Figure 5]. This probably represents a younger age group and less disease recurrence. There is a huge degree of heterogeneity in ALF and those transplanted with sero-negative hepatitis display a better survival profile than patients transplanted for other causes, although they exhibit a similar early mortality while in the intensive care unit (34).

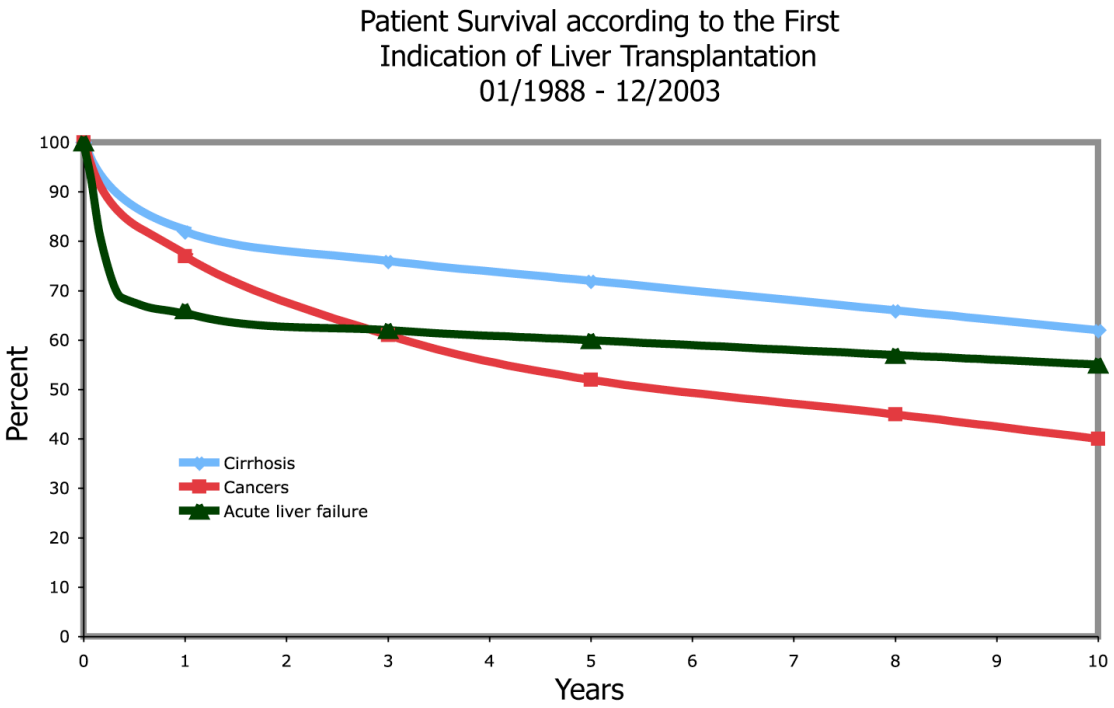

Figure 7 Patient survival according to the first indication for liver transplant (http:www.eltr.org)

A tremendous amount of research effort has been put into the search for prognostic criteria on which to base the decision to transplant patients with ALF however, much less information is available about the prediction of mortality following transplantation. This is important, as decisions to withdraw from the waiting list based on severity are difficult.

There appears to be both recipient and donor factors that help predict the outcome from transplantation in ALF (34,169,175,176). In theory the severity of illness and organ dysfunction prior to transplantation should predict outcome. However, because unstable patients tend to be either not listed or withdrawn from the waiting list there is inherent bias in retrospective analysis. Age of the recipient is a significant factor – certainly for seronegative ALF, but also in POD where age is often used to exclude listing (169). In non-paracetamol ALF, serum creatinine at the time of transplantation

is a predictor of two-month survival. Following paracetamol overdose, time from ingestion to transplantation has been shown to be a good predictor of two-month survival, all patients transplanted later than six days from ingestion died. APACHE III score at transplantation and the severity of metabolic acidosis are also predictive (175).

Donor factors found to be important are the use of reduced size grafts in paracetamol induced ALF and evidence of early graft dysfunction as defined by a high AST or INR in the early postoperative period. In addition a high donor body mass index (BMI) is a risk factor for death in sero-negative hepatitis (34,169).

The conclusions from this data are difficult to interpret with confidence but suggest that older recipients with severe preoperative organ dysfunction are less able to tolerate poor early graft function, often seen with marginal grafts. Therefore, in order to achieve the best graft and patient survival there should be matching of the organ to the recipient, as has been suggested in both chronic liver disease and ALF (176,177). This is not often an option due to time constraints.

Auxiliary transplantation

Auxiliary partial liver transplantation has many theoretical advantages compared to standard orthotopic transplantation in ALF. It can be performed orthotopically i.e. in the same place as the original liver or heterotopically e.g. in the left iliac fossa. These days it is always performed as a partial orthotopic transplant with a native left lobe in situ in adults and a right lobe in children using an adult left lobe graft, depending on size.

It provides the potential to support the patient during the acute phase of liver failure enabling the regeneration of the native liver. This is attractive as in a number of

patients immunosuppressive drugs can be withdrawn, allow the graft to atrophy or be removed and eliminate the risks associated with lifelong immunosuppression. Data on this procedure has been accumulating over the last 10 years. Initial reports suggested that the procedure was associated with a high incidence of technical problems, primary dysfunction and retransplantation. Later reports, however, suggest that many of these issues are resolving with greater experience, patient and graft selection. The best outcome has been seen in patients aged less than 40 years with either acute viral hepatitis or paracetamol hepatotoxicity where one year graft and patient survival is similar to standard transplantation for ALF. Withdrawal of immunosuppression can be achieved in 30 to 70 % of patients transplanted (178–180).

Living related lobe donation

In many countries the only chance of transplantation for ALF is in a living related donation of a liver lobe. This is most often performed in children where an adult left lobe can often be used. In adults a right lobe is usually required increasing the risk to the donor. With the worldwide shortage of donor organs living related transplantation for ALF is widely accepted in many countries but not all. There are significant issues related to living related transplantation in ALF including donor mortality of 1% and major morbidity of 40 – 60%. There are also ethical implications of adequately preparing the donor, medically and psychologically, in a time of acute crisis (181).

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Chapter 2 Pathophysiology of ALF

Liver and intestinal lactate metabolism in patients with acute hepatic failure undergoing liver transplantation.

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Objective: To determine the relative contribution of the gastrointestinal tract (GIT) and the liver in lactate metabolism in patients with acute liver failure (ALF) and the effect of liver transplantation upon this. We hypothesised that the liver and gut are net producers of lactate in ALF and that this is reversed following liver transplantation.

Setting: A university affiliated specialist liver transplant operating theatre.

Subjects: Eleven patients with acute liver failure (ALF) undergoing liver transplantation.

Measurements and Interventions: Following ethical approval, eleven patients with ALF listed for orthotopic hepatic transplantation (OLT) were studied. Whole blood was analysed for lactate concentration from radial artery (RA) catheter, portal vein (PV) and hepatic vein (HV) during the dissection phase and was repeated post reperfusion of the liver graft. Gradients across the gut and the liver were calculated to see if there was net production or consumption.

Results: HV lactate was significantly higher than arterial (p=0.028) in patients with ALF before liver transplantation suggesting splanchnic production of lactate. Total splanchnic lactate gradient (HV-RA) is positive in ALF. Both the gut (PV-RA) and the liver (HV-PV) were net producers of lactate. Following liver transplantation hepatic venous lactate falls below arterial levels but not significantly. The gradient across the gut (PV-RA) remained positive, but the trans-hepatic gradient (HV-PV) became significantly negative showing consumption by the graft (p=0.021). The magnitude of lactate consumption following transplantation correlated positively with portal venous lactate concentration (p=0.029) and inversely with graft cold ischaemic time (p=0.007)

Conclusion: The liver is a net producer of lactate in acute liver failure. Following liver transplantation the graft becomes a consumer of lactate as shown by the negative lactate gradient. The degree of consumption is dependent on portal venous lactate concentration and cold ischaemic time.

Key words: Lactate; acidosis; liver transplant; acute liver failure; critical illness; splanchnic; liver; gut; lactate consumption; lactate production

Introduction

Hyperlactataemia is common in critical illness and has been shown to have prognostic significance in septic, trauma and patients with acute liver failure (ALF) (1–4). The cause of hyperlactataemia in critical illness is incompletely understood but recent evidence has challenged the view that lactic acidosis is always the result of cellular hypoxia or hypoperfusion with resulting anaerobic metabolism particularly in patients with sepsis or the systemic inflammatory response syndrome (SIRS) (5).

Under normal conditions lactic acid is produced in the periphery (muscle, skin, brain, red blood cells) and is metabolised, following systemic circulation, to pyruvate predominantly in the liver and kidney, in the presence of oxygen, into glucose. Lactate build-up may be seen if pyruvate production increases. This may be caused by several reasons. I) The redox state of the cell, which depends on adequate oxygen supplies. II) Respiratory chain poisons such as biguanides or cyanide can also compromise it. III) An increase in glycolysis, independent of the redox state of the cell, may also induce an increase in lactate production (6) as may be observed following catacholamine therapy. IV) Hyperlactataemia may also be caused by a failure of consumption of lactate.

Hyperlactataemia is a prominent feature of acute liver failure and but it is unclear as to whether there is increased production which exceeds the capacity for metabolic clearance or whether reduced clearance on its own is responsible. In patients with sepsis and multiple organ dysfunction an increase in hepatic venous lactate has been noted but whether this is due to increased gut production, decreased liver extraction or a combination of both is unclear (7). In acute liver failure the capacity of the liver to metabolise lactate may be compromised (8). Indeed the liver may

become a net producer of lactate during acute liver failure as has been shown in hypoxic and ischaemic models of acute liver injury (9–11).

The aims of this study were to investigate total splanchnic lactate gradient in patients with acute liver failure undergoing liver transplantation and to characterise the contribution of both the gut and the liver to the hyperlactataemic state by the simultaneous sampling of portal venous, radial arterial and hepatic venous blood.

Methods

Following approval from our hospital ethical committee eleven patients with ALF proceeding to liver transplantation were prospectively enrolled into the study (12). The nature of the illness precluded consent from the patients and so written informed assent was obtained from the patients' relatives. Nine females and three male patients were studied. The aetiology of ALF was acetaminophen toxicity in seven patients; one patient hepatitis B; one seronegative hepatitis; one pregnancy induced acute liver failure and one an acute drug reaction to the recreational drug "ecstasy" (3,4 methylenedioxymetamphetamine). All patients had grade III or IV encephalopathy, were intubated and ventilated and sedated with midazolam and fentanyl.

All patients were managed in intensive care in a university-affiliated hospital and transplanted in a specialist liver transplant theatre. Anaesthetic management was similar in all patients. On arrival in the operating theatre all patients were converted to inhalational anaesthesia with isoflurane in air and oxygen and the fentanyl infusion was continued. Vascular access included two 6 fr introducers in the right internal jugular vein, triple lumen central line and radial arterial line. Further invasive monitoring varied between patients. All patients were supported with catacholamines

to maintain MAP at approximately 60 mmHg. Seven of the eleven patients received noradrenaline, one boluses of adrenaline. Nine of the eleven received dopamine and two patients dopexamine. The doses varied between patients and during the procedure. No correlation could be found between catacholamine dosage and baseline lactate levels.

Blood products and fluids were given as indicated throughout the surgical procedure to maintain a haemoglobin concentration of approximately 10 g/dl and an international normalised ratio (INR) of less than two. Whole blood lactate was sampled from radial artery, portal vein and hepatic vein simultaneously during the dissection phase of the operative procedure and analysed immediately. The three blood samples were repeated following hepatic transplantation toward the end of the procedure. All samples were obtained off veno-venous bypass. The initial samples were taken on exposure of the native liver before any clamping of vessels took place. The second set of samples were taken following reperfusion and haemostasis and following restoration of native circulation.

To measure lactate production or consumption by the gut and liver both before and following hepatic transplantation gradients were calculated. Total splanchnic lactate gradient was calculated as hepatic venous lactate (HV) minus radial arterial lactate (RA) in mmol/L. Gastrointestinal lactate gradient was calculated as portal venous (PV) minus RA in mmol/L. Hepatic lactate gradient was the sum of HV minus PV lactate in mmol/L. Changes in lactate gradients are expressed in tabular form and graphically. Grafts were assessed grossly by the surgeon performing the transplant into moderately fatty, mildly fatty or normal. Biopsies of the graft were undertaken at the discretion of the operating surgeon and were not part of the experimental protocol.

Whole blood lactate was measured using an analyser (YSI 2300 Stat Plus; Yellow Springs Instrument Co; Yellow Springs, OH) that employs a technique based on membrane-bound enzyme electrode methodology (13). Instrument performance was checked against standards on a daily basis. The machine self-calibrates every five samples. Repeatability of whole blood lactate measurements in critically ill patients was tested, as has been reported previously (14).

Results are reported as median and inter-quartile range. Intrapatient comparisons were performed using nonparametric analysis of variance (Friedman’s two-way ANOVA). Pairwise comparison was with Tukey’s honestly significant difference test. Paired data before and following liver transplantation are compared with Wilcoxon sign rank test. Correlation between data was tested with Spearman’s' rank correlation. P is considered significant at 0.05.

Results

Pre-transplant

Hepatic venous (HV) whole blood lactate (5.44 ± 0.8 mmol/L) was significantly higher than radial arterial (RA) lactate (5.11 ± 0.92 mmol/L) in ten of the eleven patients studied with acute hepatic failure ($p = 0.006$). Portal venous (PV) lactate was higher than RA lactate but not significantly. Total splanchnic lactate gradient (HV-RA) was positive in all but one patient in acute liver failure (0.12 ± 0.165 mmol/L). Both the gut (PV-RA) and the liver (HV-PV) were net producers of lactate (0.06 ± 0.05, 0.08 ± 0.14 mmol/L respectively). Table 1 & 3. One patient died during the operative procedure following hepatic reperfusion and so the second part of the study could not be completed. This patients result were included in the analysis of splanchnic lactate gradients in ALF but not in the analysis of change in gradients following transplantation.

No correlation could be found between noradrenaline dose at the start of the procedure and whole blood lactate. There was not any correlation between noradrenaline dose and total splanchnic or transhepatic lactate gradient in those receiving it.

Post-transplantation

In the ten patients that completed the study whole blood portal venous lactate rose significantly following hepatic transplantation compared to pretransplant levels (6.44 ± 1.14, 5.34 ± 0.91 mmol/L, $p = 0.021$) and radial artery to portal venous lactate gradient remained positive (0.04 ± 0.135 mmol/L). When compared with pretransplant, total splanchnic lactate gradient remained unchanged overall following reperfusion, but the majority of patients show either consumption or a decrease in

production of lactate. Overall the gut continues to produce lactate and so the gradients do not change significantly.

Hepatic lactate gradient changed significantly from pretransplant gradient with the graft consuming lactate rather than producing lactate (-0.07 ± 0.135 mmol/L, p = 0.014). See figures 1, 2 & 3. In two of the patients (patient 5 and patient 2) hepatic lactate gradient increased in the initial post transplantation period suggesting lactate production by the graft possibly related to graft dysfunction. Because of the gross appearance of the graft transplanted in patient 2 a pre-operative biopsy was performed which showed microvesicular steatosis. Patient 1 received an auxiliary graft thus retaining an injured lobe with possible continued production of lactate. After insertion of the auxiliary graft, the trans-hepatic lactate gradient falls to zero, suggesting either a decreased production of lactate by the necrotic lobe or consumption by the graft or both. Despite the partial transplant, there is an overall improvement in lactate metabolism. If patient 1's results are excluded from the analysis the change in gradients following transplantation remains significant (p=0.02 Wilcoxon sign rank test).

The hepatic lactate gradient following transplantation correlates inversely with portal venous lactate concentration in all but one patient (patient 1) $r^2 = 0.37$, $p = 0.029$, figure 4. If this patient is excluded from the regression analysis, then $r^2 = 0.81$, $p = 0.0005$, figure 2. Examination of hepatic lactate consumption showed it to decease with increasing cold ischaemic time $r^2 = 0.54$, $p = 0.007$, figure 5.

The terms *veno-venous (V-V), auxiliary* and *piggyback* were used to describe the operative technique used to see if this had any effect on liver or gut lactate production following transplantation. Piggyback transplantation involves side

clamping the vena cava and clamping the portal vein; it avoids veno-venous bypass. It was hypothesised that this may have caused gut ischaemia during the procedure because of venous stasis. The term V-V (Veno-venous bypass) was used to denote insertion of graft plus a section of vena cava. This technique requires isolation of a portion of native vena cava. During this procedure, the cava is clamped above and below the anastomosis and venous return is provided via the bypass technique. The use of bypass provides venous drainage to the splanchnic circulation and so potentially reduced venous stasis. The type of operative procedure did not influence post-transplant portal vein lactate.

Repeatability of whole blood lactate was examined by plotting the mean of three blood lactate concentrations against their standard deviation from 30 critically ill patients. The samples were analysed in the same accredited intensive care laboratory that performed the assays for the study. Although it can be seen that reproducibility declined with increasing lactate concentration as previously reported (14), the measured variation between samples was small throughout the range of lactate tested, figure 6.

Discussion

The main findings of the study are that both the gut and the liver are net producers of lactate in patients with ALF who fulfilled criteria for liver transplantation. After transplantation, the gastrointestinal tract continues to produce lactate in the immediate postreperfusion period while the liver graft consumes lactate. The lactate gradient across the liver after transplantation correlates positively with the PV lactate concentration and inversely with the length of cold ischemic time.

ALF describes a constellation of clinical symptoms associated with sudden cessation of normal hepatic function. The defining state is hepatic encephalopathy and the development of a coagulopathy with subsequent jaundice (15). The systemic inflammatory response syndrome that accompanies ALF is similar to sepsis in that vasoparesis leading to hypotension and an increased cardiac output are common and progression to multiple organ failure is characteristic.

Hyperlactatemia is prominent in both sepsis and ALF but whole blood levels tend to be higher in ALF.

In critical illness, lactic acidosis has traditionally been explained on the basis of cellular hypoxia. However, efforts to prove this have been difficult and evidence that has emerged over the last 10 years suggests that the causes are more complex and not just to do with simple substrate limitation. On the contrary, global oxygen delivery is increased in both the systemic inflammatory response syndrome and ALF, and efforts to increase oxygen delivery do not have predictable effects on lactate levels (16). Attempts to find evidence of regional anaerobic metabolism caused by ischemia or hypoxia have not proven fruitful in either animal or human experiments. Indeed, tissue oxygen content has tended to be higher than normal in both septic patients and animal models (17,18).

Early studies in ALF inversely correlated mixed venous lactate concentrations with mean arterial pressure, systemic vascular resistance and oxygen extraction ratio (4). The explanation ascribed to these phenomena was that the lactic acidosis seen was, in part, the consequence of tissue hypoxia developing because of arteriovenous shunting, reflected in the reduction in systemic vascular resistance. However, the increases found in oxygen consumption associated with the augmentation of oxygen

delivery may have been the result of mathematical coupling rather than any real effect (19). A recent comparison of the Fick method and indirect calorimetry for the measurement of oxygen consumption in ALF failed to show a good correlation between methods and poor reproducibility of the Fick method in the calculation of oxygen consumption (20).

If anaerobic metabolism resulting from global or regional supply-dependent oxygen consumption is not the predominant cause of hyperlactatemia in patients with ALF, then what are the alternatives? There is evidence for both increased production and decreased clearance. Energy expenditure is increased in both ALF and the systemic inflammatory response syndrome (21,22). This increase in metabolic rate may stimulate lactate production even in the absence of anaerobic metabolism (13). Catecholamine infusions increase lactate production by stimulating glycolysis. This increases the production of pyruvate that is converted to lactate by mass effect via the enzyme lactate dehydrogenase (13,23).

Recent studies designed to detect individual organ production of lactate have shown increased lactate production in the lung of both patients with acute lung injury and in patients without acute lung injury but with ALF (14,24). In the patients with acute lung injury, lactate production was proportional to the severity of injury (25). Others have also demonstrated pulmonary lactate production and have ruled out tissue hypoxia as the cause (26). Douzinas et al. (7) suggested the organs with the most severe dysfunction in a group of patients with multiple organ failure were the site of increased lactate production as measured by organ lactate fluxes. De Jonghe et al. (27) have shown in a group of 92 patients with acute circulatory failure that early hepatic dysfunction (defined as a serum bilirubin >60 μmol/L or serum glutamic-oxaloacetic transaminase >100 IU/L) was associated with an elevated serum lactate

concentration. They did not however, distinguish between increased production or impaired clearance as the cause. A recent study addressed this question in stable septic patients by the analysis of infused lactate kinetics. The group with an elevated baseline blood lactate concentration showed reduced clearance compared with the group with normal blood lactate levels. Production was the same in both groups (28). Further evidence suggesting hepatic dysfunction to be important in the aetiology of hyperlactatemia comes from studies of lactate elimination in patients with hepatic cirrhosis. Woll and Record (29) compared lactate clearance between cirrhotics and normal volunteers and found a prolonged lactate elimination half-life. Forearm clearance was the same in both groups. Almenoff et al. (30) showed that lactate was eliminated three times more slowly after exercise in cirrhotic patients compared with healthy aged-matched volunteers. Chiolero et al. (31) studied the effect of major hepatic resection on lactate metabolism compared with healthy controls. They found similar basal lactate levels and similar clearance and endogenous production but a prolonged terminal half-life. They suggested that this might be caused by decreased hepatic uptake and conversion into glucose by the liver. The normal basal lactate concentration in patients after the loss of about 50% of liver mass suggests a large reserve of hepatic clearance. This implies that, with normal levels of production, loss of metabolic capacity needs to be greater to result in raised basal lactate levels. However, the loss of functional hepatocytes may be >50% in some patients with ALF.

Lactate kinetics have also been studied in ALF. Record et al. (8), using a lactate infusion load test, found that lactate elimination was markedly reduced in patients with paracetamol-induced liver damage whereas forearm clearance was increased. They suggested that decreased clearance by the liver was the cause of raised basal

lactate levels in the patients with paracetamol-induced hepatotoxicity. We found that overall there was net production of lactate from the liver in ALF when HV concentrations were compared with PV and RA concentrations.

The cause of splanchnic lactate production in ALF has been investigated recently. Clemmesen et al. (32), using hepatic venous catheters, found that lactate was released from the splanchnic circulation in patients with ALF compared with consumption in controls. They suggested that increased lactate production was not the result of hypoxia within the splanchnic region because of the normal HV lactate to pyruvate ratio found. The lactate to pyruvate ratio is a marker of the redox status of the cell and describes the adequacy of cellular oxygenation. They speculated that lactate production was the result of accelerated glycolysis within the splanchnic region stimulated possibly by catecholamines. They did not, however, differentiate between the liver and intestine as the source of increased production. In our study, we did not find any correlation between catecholamine dose and whole blood lactate at the beginning of the procedure or between lactate gradients across either the gut or liver.

The fall in lactate from the HV to the RA found in our study could be interpreted as pulmonary consumption of lactate; however, all studies in critically ill patients including ALF, looking at lactate gradients across the lungs, have shown lactate production. This suggests that there must be consumption of lactate in sites other than the liver, lungs, and gut. An increase in muscle consumption of lactate has been reported in ALF and this fact may explain the HV-RA lactate gradient both pre- and post-transplant (8).

Lactate flux was not calculated, as liver blood flow was not measured in our study. Liver blood flow is difficult to measure with accuracy in ALF because the standard methodology relies on the Fick principle and hepatic clearance of the usual marker (indocyanine green) is reduced (33). Recent reports using sorbitol clearance have suggested that liver blood flow is increased in ALF. Clemmesen et al. (34) estimated hepatic blood flow at 1.78 - 0.78 L/min in 14 patients with ALF. This combined with the range of hepatic lactate gradients from our study would give a hepatic lactate flux ranging from production of lactate at a rate of 42 mmol/hr pretransplant to consumption at a rate of 80 mmol/hr post-transplant.

The gut has received attention as a focus for organ dysfunction early in critical illness, however, its reported contribution to hyperlactatemia is conflicting. Bellomo et al. (35), investigating the effects of early endotoxemia on transvisceral lactate fluxes in dogs, found consumption of lactate in the gut with the lungs the main contributor to hyperlactatemia. We found a positive gradient across the gastrointestinal tract (PV-RA) in the majority of patients with ALF and this persisted post-transplant regardless of whether veno-venous bypass or portal venous side-clamping was used; there was marked interindividual variation, however.

After orthotopic liver transplantation, the majority of hepatic lactate gradients fell, with most grafts consuming lactate. Consumption of lactate correlated with PV lactate concentration, suggesting flow limited kinetics within the grafts. This correlation, however, did not hold for the patient that received an auxiliary right hepatic lobe, possibly because of continued production of lactate by the retained injured lobe. If graft factors are analysed, cold ischemic time can be seen to correlate inversely with lactate consumption. The association of cold ischemic time to lactate consumption after liver transplantation has been shown previously in an experimental model. Two

groups of pigs were transplanted with livers differentiated by cold ischemic time—the first <4 hrs, the second >25 hrs. The group with the prolonged preservation time not only ceased to metabolize lactate, but they generated lactate (36). We observed similar findings with grafts following long cold ischemic times.

The causes of hyperlactatemia in ALF are complex, with increased production shown in the lungs (14), gut, and liver and a decrease in total body clearance (8). We have shown that not only is there reduced hepatic clearance of lactate in ALF, as would be expected, but that there is hepatic production. What is the site of this increased production within the liver? The data from Clemmesen and colleagues (32)—lactate production from the splanchnic bed with a normal hepatic venous lactate to pyruvate ratio—suggest the source of the lactate is an increase in aerobic metabolic activity within the liver. In animal models of early sepsis, mapping of glucose and lactate metabolism show the sites of highest turnover to be areas associated with inflammatory cell populations such as the spleen and the lungs. Given that the sites of inflammation correlate to the sites of glucose utilization and lactate production, the implication is that the inflammatory cells themselves are the source of this excess production. The respiratory burst of phagocytic cells accounts for this increase in metabolic activity and is independent of mitochondrial electron transport. Instead, the energy for this comes from an increase in glycolysis through the hexose monophosphate shunt (37). Haji-Michael and colleagues (38) have shown that peritoneal leukocytes taken from a polymicrobial sepsis model have a higher basal lactate production compared with sham animals and that this is a result of glycolysis. They suggest that little of the lactate produced by leukocytes is channelled into the Krebs cycle and that the majority of the pyruvate produced from glycolysis is diverted away from oxidative metabolism within the cell and transported via the blood pool as

lactate in the Cori cycle. In addition, they report that during sepsis the leukocytes are, on a weight-for-weight basis, the single largest source of lactate within the body. Others have shown that glucose utilization by the liver in an endotoxin rat model is predominantly by nonparenchymal cells. They have also shown the rate of glucose utilization is enhanced 6.7-fold in endotoxin- stimulated Kupffer cells (39).

The conclusion that can be drawn from our work and the studies described is that the inflammatory process within the liver and other organs as a by-product of the increased metabolic rate produces lactate as a result of enhanced glycolysis. This, coupled with reduced clearance, contributes to the high whole blood lactate levels seen in ALF. After liver transplantation, the transhepatic lactate gradient is reversed. The degree of reversal is dependent on host factors, the substrate load (PV lactate concentration), and graft factors, one of which is cold ischemic time.

Tables

Pre transplantation

Patient	Diagnosis	pH	Bilirubin	Creatinine	INR	RA	PV	HV[a]
1	POD	7.50	3.3(57)	1.6(142)	>15	10.1	10.2	10.6
2	NANB	7.09	20.8(356)	1.5(140)	2.9	4.91	4.96	4.79
3	POD	7.29	7.1(123)	3.6(325)	>15	6.21	6.28	6.33
4	POD	7.30	1.6(29)	1.9(168)	>15	5.21	5.18	5.44
5	POD	7.20	2.5(43)	6.3(563)	5.9	1.68	1.74	1.73
6	Pregnancy	6.98	5.0(87)	2.0(181)	1.88	6.3	6.19	6.42
7	POD	7.21	6.6(113)	2.2(201)	5	5	5.5	5.6
8	Hep B	7.33	30.8(527)	1.4(128)	6.8	6.88	6.93	6.91
9	Ecstasy	7.37	19.6(366)	0.9(83)	8.7	3.72	3.83	3.91
10	POD	7.36	8.7(149)	4.3(382)	6.9	4.46	4.46	4.82
11	POD	7.06	2.8(49)	1.5(133)	7.2	5.11	5.24	5.13

INR = international normalised ratio; RA = radial artery; PV = portal vein; HV = hepatic vein

Units: lactate mmol/L; Bilirubin mg/dl (μmol/L); Creatinine mg/dl (μmol/L)

[a] $p = 0.006$ compared to RA (Friedman two way ANOVA)

Table 1**: Patient characteristics and dissection phase lactate concentrations**

Post transplant

Patient	Graft	CIT	Op	RA	PV[a]	HA	INR-24	INR-48
1	mild fatty	12.2	Aux	11.9	11.7	11.7	1.57	1.39
2	good	14.5	VV	4.6	4.6	4.6	1.18	1.15
3	fatty	8.85	PB	6.48	6.82	6.5	1.14	1.04
4	good	10.3	PB	5	5.33	5.4	1.35	1.00
5	fatty	11	PB	2.41	2.52	2.63	1.34	1.17
6	mild fatty	17	VV	6.38	6.38	6.32	1.24	1.18
7	good	5.08	PB	6.5	6.5	6.3	1.23	0.90
8	good	4.08	VV	7.01	7.09	6.61	1.80	1.36
9	good	6.59	PB	7.72	7.42	6.97	1.37	1.05
10	mild fatty	9.4	VV	4.55	4.82	4.74	1.57	1.19

Cold IT = graft cold ischaemic time in hours; RA = radial artery; PV = portal vein; INR = international normalised ratio; Op type = operative technique, (Aux = auxiliary; VV = veno-venous bypass; PB = piggy back)

a, p = 0.021 compared to pre transplant (Wilcoxan sign rank test)

Lactate concentrations are in mmol/L

Table 2: **Graft characteristics, post reperfusion lactate concentrations, day one and two INR post transplant**

Lactate gradients

Patient	Pre HV-PV	Pre PV-RA	Pre HV-RA	Post HV-PV[a]	Post PV-RA	Post HV-RA
1	0.4	0.1	0.5	0	-0.2	-0.2
2	-0.17	0.05	-0.12	0	0	0
3	0.05	0.07	0.12	-0.32	0.34	0.02
4	0.26	-0.03	0.23	0.07	0.33	0.4
5	-0.01	0.06	0.05	0.11	0.11	0.22
6	0.23	-0.11	0.12	-0.06	0	-0.06
7	0.1	0.5	0.6	-0.2	0	-0.2
8	-0.02	0.05	0.03	-0.48	0.08	-0.4
9	0.08	0.11	0.19	-0.45	-0.3	-0.75
10	0.36	0	0.36	-0.08	0.27	0.19

Pre = dissection phase; Post = post reperfusion.

a, p = 0.014 compared to dissection phase (Wilcoxan sign rank test)

Lactate concentrations are in mmol/L

Table 3: **Total splanchnic, gastrointestinal and hepatic lactate gradients during the dissection phase and following liver transplantation**

Figures

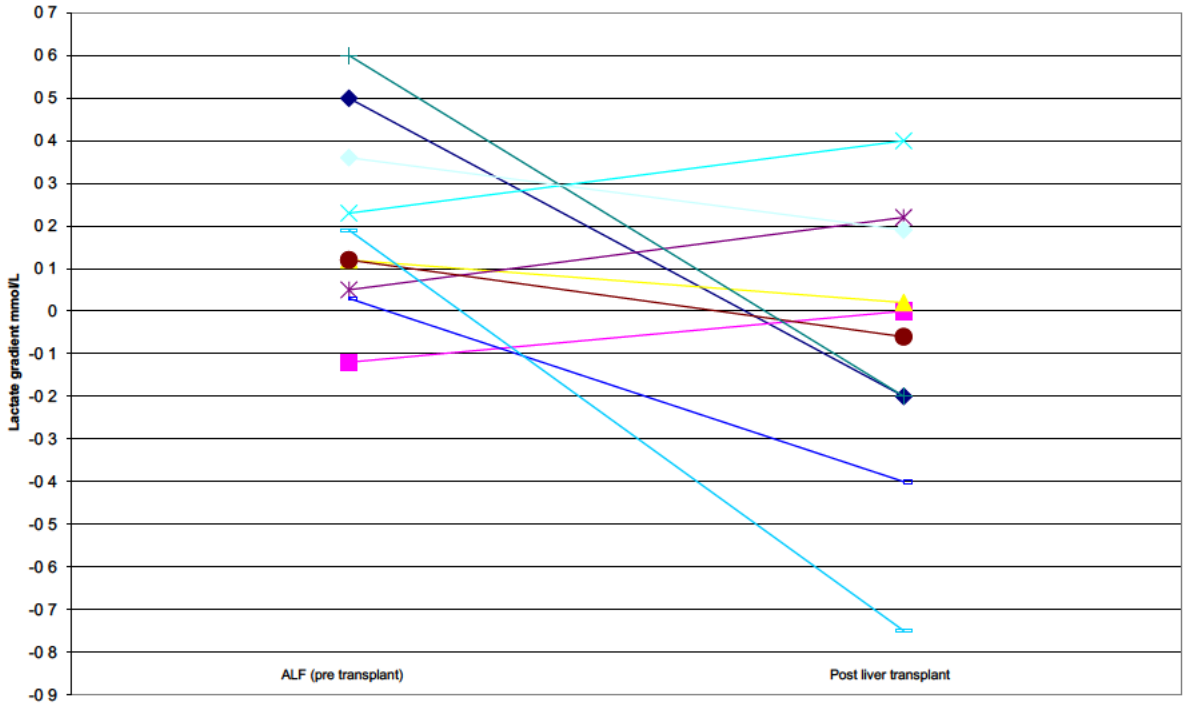


Figure 1: Total splanchnic lactate gradient (HV-RA) pre and post liver transplant for the 10 patients. A trans splanchnic lactate gradient of greater than zero represents lactate production; a splanchnic lactate gradient of less than zero represents consumption. Change in splanchnic lactate gradient was not significant.

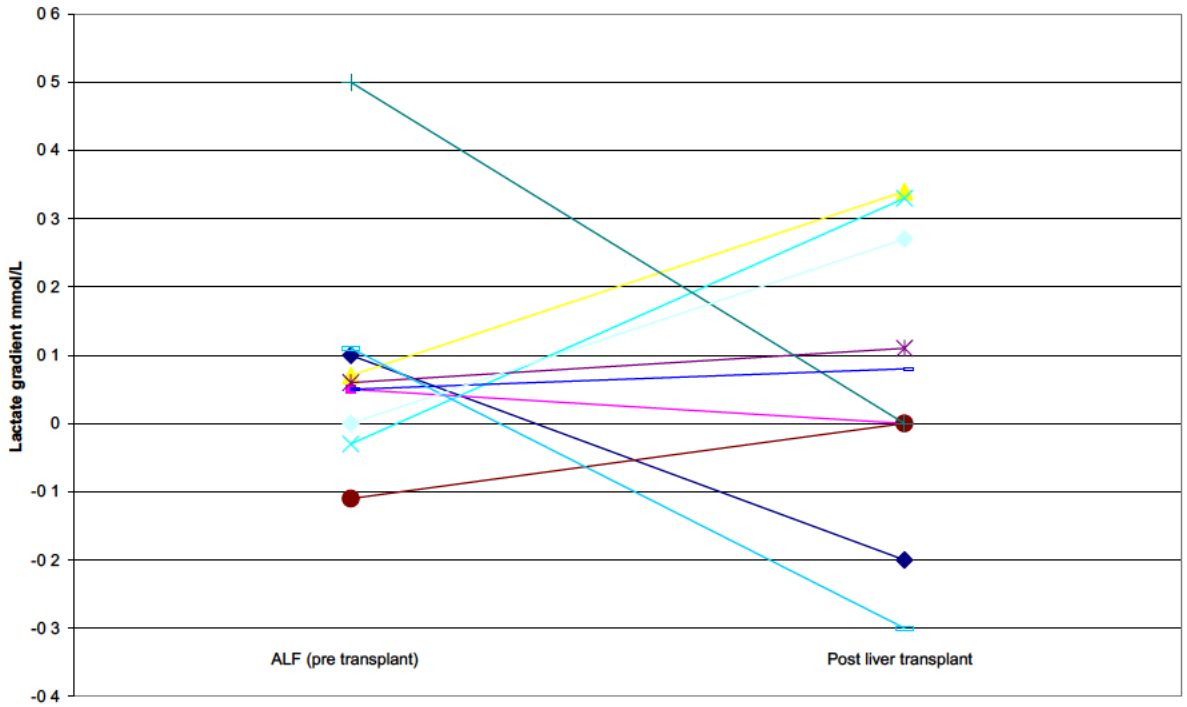


Figure 2: Gastrointestinal lactate gradient (PV-RA) pre and post liver transplant for the 10 patients. A gut lactate gradient of greater than zero represents production; a gut lactate gradient of less than zero represents consumption. Change in lactate gradient was not significant.

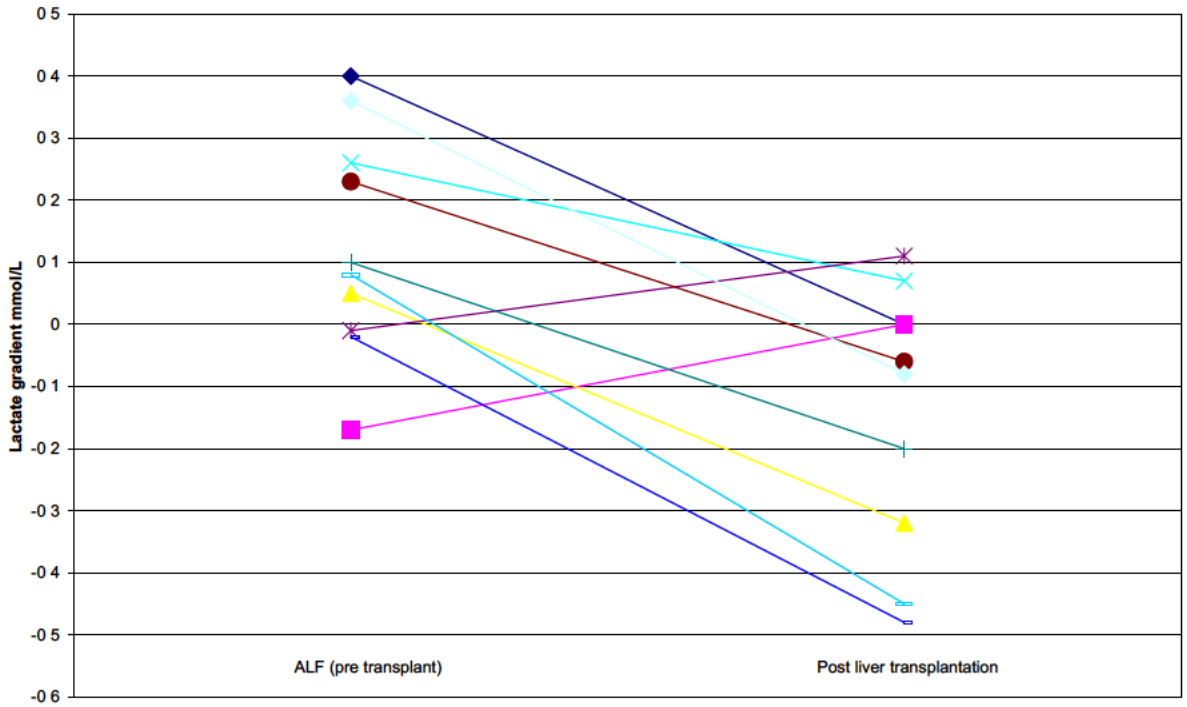


Figure 3: Trans hepatic lactate gradient (HV-PV) pre and post liver transplant for the 10 patients. A hepatic lactate gradient of greater than zero represents production; a hepatic lactate gradient of less than zero represents consumption. Change in lactate gradient from dissection phase to post reperfusion was significant (p = 0.014, Wilcoxon sign rank test)

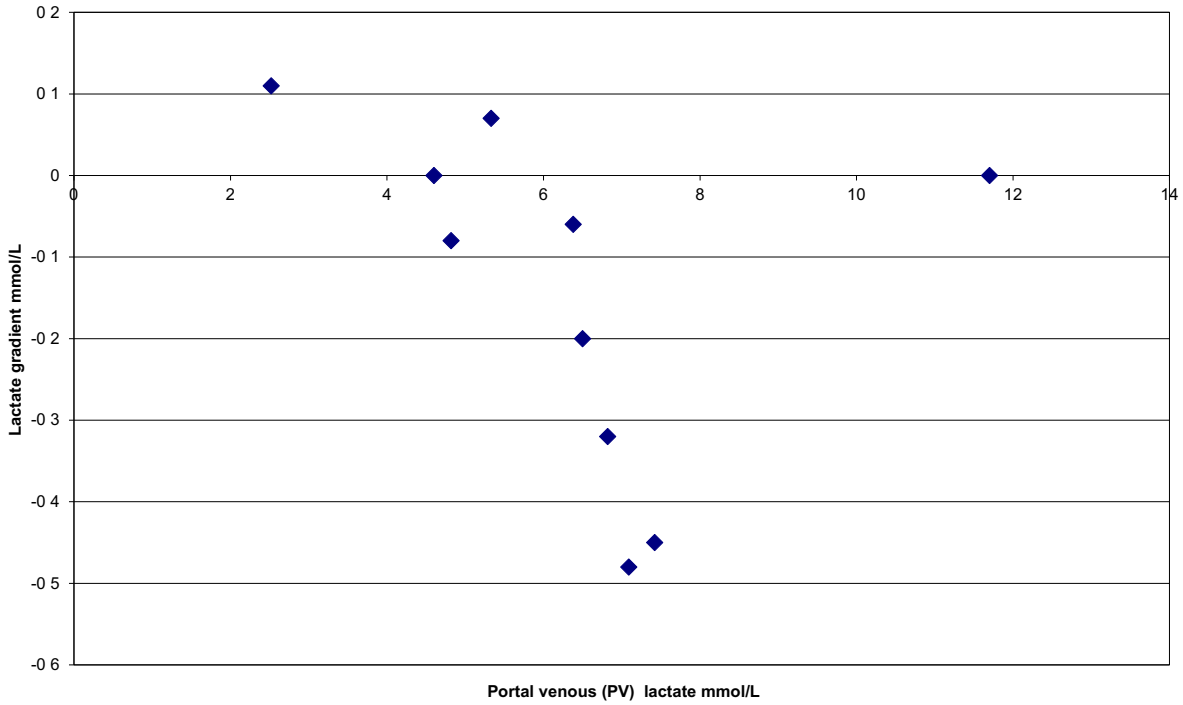
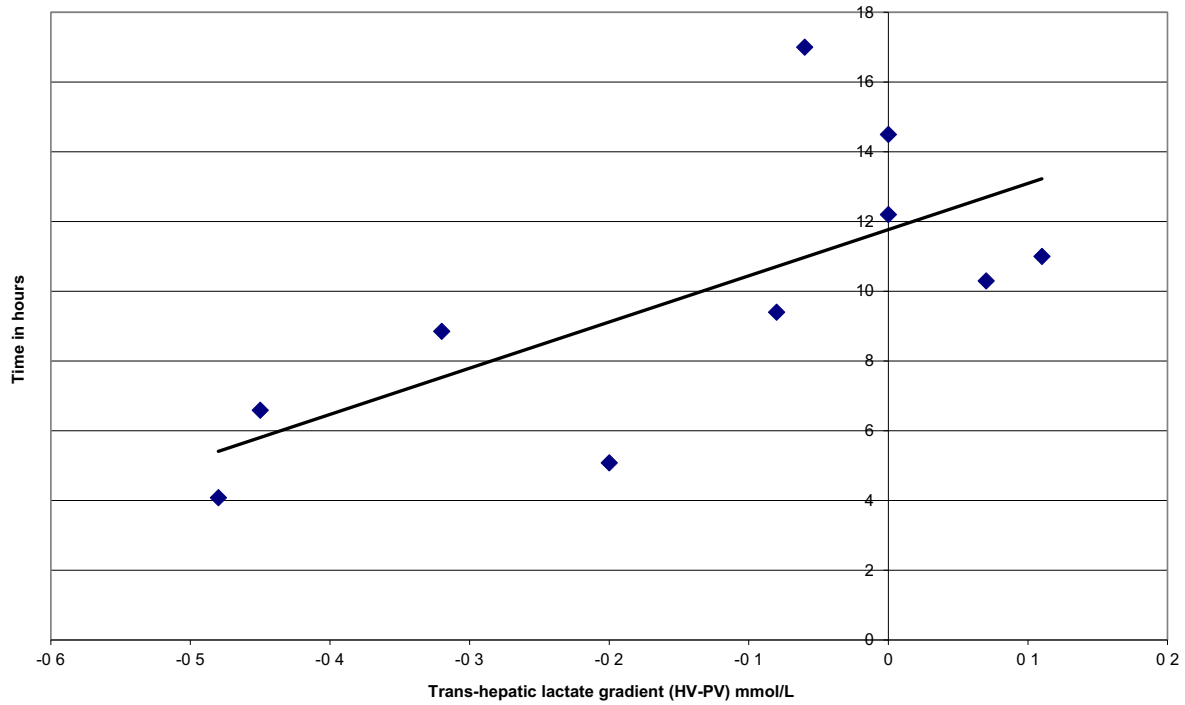


Figure 4: Post reperfusion hepatic lactate gradient (HV-PV) verses portal venous lactate concentration (PV) for the 10 patients. Hepatic lactate consumption increases significantly as PV lactate concentration increases ($r^2 = 0.37$, $p = 0.029$). The relationship does not hold for the patient following auxilary transplant, if this patient is excluded from the analysis the relationship is stronger ($r^2 = 0.81$, $p = 0.0005$, Spearman rank correlation)



Figures 5: Post reperfusion hepatic lactate gradient (HV-PV) verses graft cold ischaemic time in hours for the 10 patients. The graft lactate consumption increases significantly the shorter the cold ischaemic time, ($r^2 = 0.54$, $p = 0.007$, Spearman rank correlation).

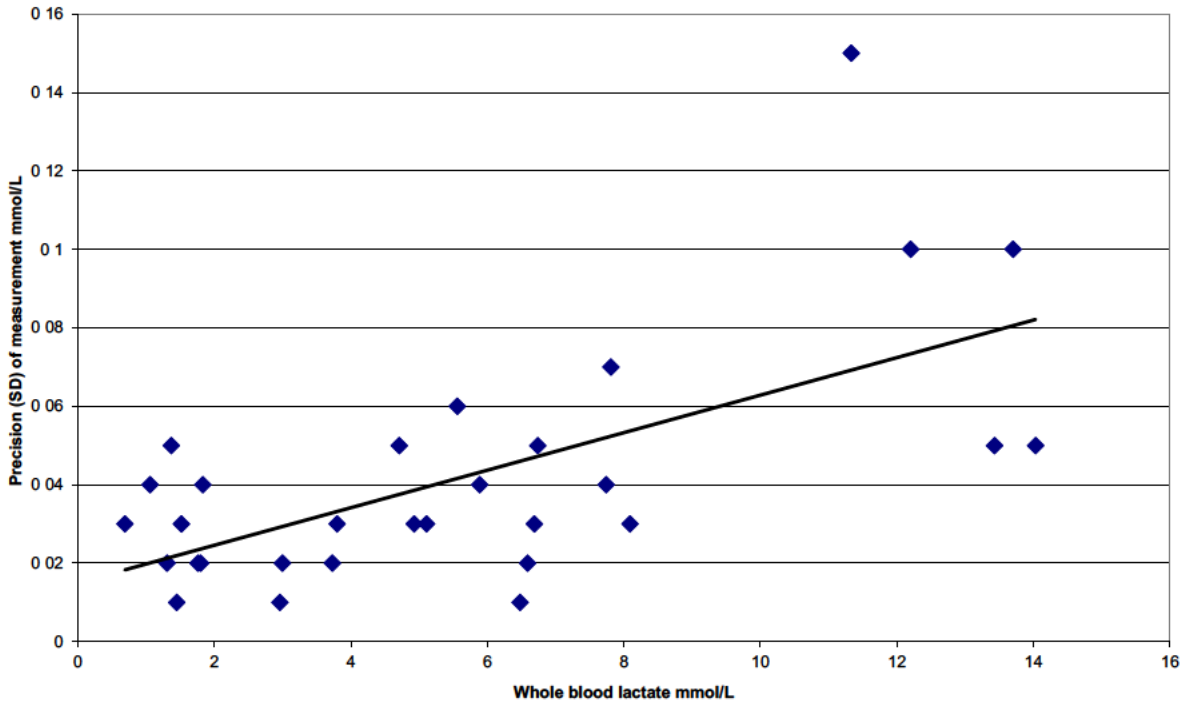


Figure 6: The precision of arterial whole-blood lactate measurements from 30 critically ill patients. For each data point, the mean and standard deviation of three measurements on the same 2 ml sample are plotted. Reliability decreases with increasing lactate concentration, (r^2 = 0.4).

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The regulation of T-cell recruitment to the human liver during acute liver failure

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Abstract

Background & Aims: Acute liver failure (ALF) is a rare clinical syndrome with high mortality resulting from hepatocellular necrosis and loss of function. In seronegative hepatitis (SNH), a T-cell-rich infiltrate leads to immune mediated hepatocyte destruction, whereas in paracetamol poisoning, toxic metabolites induce hepatocyte necrosis, followed by a macrophage-rich, lymphocytic infiltrate that is an important factor in driving repair and regeneration. The nature of the hepatic inflammatory infiltrate, key to ALF pathogenesis and outcome, is determined by the recruitment of effector cells from blood, but the molecular basis of recruitment is poorly understood. To determine the phenotype of circulating and hepatic lymphocytes in patients with

ALF secondary to paracetamol overdose (POD) or SNH and investigate the molecular basis of lymphocyte recruitment. Methods: We used FACS, immunohistochemistry and flow-based adhesion assays to determine the regulation of lymphocyte adhesion. Results: SNH and POD intrahepatic lymphocytes were $\alpha L\beta 2^{h}$, $CD69^{h}$ and $CD38^{h}$ with a distinct homing phenotype being L-selectin^{o}, $CXCR3^{h}$ and $CCR5^{+}$. Expression of chemokine ligands for the receptors CCR5, CXCR3 and CXCR6 and the adhesion molecules ICAM-1, VCAM-1 and VAP-1 was markedly increased in the liver in ALF. Lymphocytes isolated from the livers of patients with SNH showed enhanced chemokine-dependent adhesion and transmigration across the human hepatic endothelium in vitro under flow and used a combination of β1 and β2 integrins to adhere to endothelium and β2 integrins, CD31 and VAP-1 to transmigrate. Conclusion: Aetiology-dependent combinations of adhesion molecules and chemokines expressed within tissue during ALF recruit lymphocytes with a distinct homing phenotype.

Introduction

Acute liver failure (ALF) is a rare clinical syndrome resulting in severe hepatic injury and loss of normal liver function in the absence of previous liver disease (1, 2). The aetiology of ALF varies with geographical location; hepatitis viruses are the major cause in developing countries, whereas drug-induced hepatotoxicity secondary to paracetamol overdose (POD) is the predominant cause in the UK. In the UK, approximately 20% of cases are caused by fulminant hepatitis of unknown causation, the so-called seronegative hepatitis (SNH). The clinical features are similar to ALF induced by viral hepatitis, but no aetiological agent has been defined

(1). SNH is associated with a lymphocytic infiltrate of effector T cells, NK cells and to a lesser extent B cells which drive immune-mediated cytotoxic damage to hepatocytes as a consequence of the activation of several cell death pathways (1, 3–5). If this persists, it leads to massive hepatic necrosis and liver failure. This contrasts with the pathogenesis of paracetamol toxicity, where toxic metabolites of paracetamol induce direct hepatocyte damage and necrosis which activates innate immune pathways (6) and a secondary inflammatory response involving the recruitment of monocytes, macrophages and to a lesser extent lymphocytes. The recruited immune cells themselves can then contribute to further hepatocyte damage mediated via direct activation of death receptors or the secretion of cytokines (1, 7). Thus, mechanisms designed to orchestrate liver regeneration and tissue repair can further amplify liver injury and the progression of liver failure (8, 9). To date, the mechanisms that lead to the recruitment of disease modifying, immune cell populations in human ALF have not been well described. A clear understanding of the molecular mechanisms underlying lymphocyte recruitment and how this contributes to the pathogenesis of ALF would facilitate the development of targeted anti-adhesive therapies, which may reduce the need for transplantation in patients with fulminant hepatitis. The nature of the inflammatory stimulus determines whether portal inflammation or lobular hepatitis is predominant and this is regulated by the differential expression of adhesion molecules and chemokines on vascular and sinusoidal endothelium which act together to compartmentalize infiltrating lymphocytes. Vessels in the normal liver express low levels of adhesion molecules and chemokines, which are induced and/or upregulated in response to injury and liver inflammation. These include CC- and CXC-chemokines associated with T helper (Th)-1 immunity and classical adhesion molecules such as intercellular

adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 as well as the atypical adhesion receptor vascular adhesion protein (VAP)-1 (10, 11). In mice, recruitment of liver-infiltrating lymphocytes (LIL) decreases with the blockade of ICAM-1, VCAM-1, E-selectin (12, 13) or VAP-1 (14) in ConA hepatitis models, which have some similarity with human ALF (15). Liver injury is significantly inhibited in the absence of lymphocyte function- associated antigen-1 [LFA-1 (αLβ2)] (16) or both ICAM-1 and L-selectin (17). Similarly, the intrahepatic expression of chemokines CCL3-5 and CXCL9-11 increases in response to the ConA challenge resulting in the recruitment of CCR5 and CXCR3-expressing lymphocytes (18–20). Thus, multiple molecular interactions between liver endothelial cells and circulating lymphocytes mediate recruitment to the liver during ALF in mice, but little is known about the pathways involved in human ALF. In this study, liver tissue, peripheral blood lymphocytes (PBL) and LIL were collected from human patients with ALF secondary to POD or SNH and the expression of endothelial adhesion molecules and their lymphocyte ligands was determined. This permitted us to understand the differences in adhesive phenotype between peripheral and hepatic immune cell populations and to provide insight into potential recruitment cues. The contribution of these receptors to the recruitment was then investigated by studying the interactions between peripheral and hepatic lymphocytes and cytokine activated human hepatic sinusoidal endothelial cells (HSEC) under physiological flow using function-blocking antibodies against endothelial adhesion molecules, their receptors on lymphocytes and specific chemokine receptors.

Materials and methods

Sample collection and preparation

Peripheral blood and explanted liver tissue from patients with ALF secondary to POD or SNH, donor liver tissue surplus to transplantation requirements, explanted liver tissue from transplant patients with Child Pugh C scoring chronic cirrhotic liver disease (primary biliary cirrhosis and alcoholic liver disease) and blood from normal donors were all obtained from the Queen Elizabeth Hospital, Birmingham, UK. All patients with ALF had either SNH or POD, fulfilled the UK liver transplant ALF criteria, were super-urgently listed and transplanted upon admission. Matched pre transplant peripheral blood and explanted liver tissue were used. All patient samples were collected with the approval of the local research ethics committee and with informed consent. LIL were isolated from approximately 100 g of tissue using a non-enzymatic method as described previously (21). PBL from patients or normal donors were isolated using Lympholyte® density gradient (Cedarlane®; VH BIO Ltd, Gateshead, UK) according to the manufacturer's instructions and contaminating monocytes were depleted by virtue of their adhesion to plastic. All functional assays and cytometry were performed upon cells within 4 h of initial isolation from blood or tissue, or on cells which were immediately cryopreserved after isolation to minimize effects of manipulation upon cell phenotype (22) Cryosections (5–10 μm) for histological analysis were prepared from snap-frozen tissue and stored at -20°C until use. Sinusoidal endothelial cells were isolated from fresh tissue and cultured as described previously (23, 24).

Immunohistochemistry

Acetone fixed frozen liver sections (5 μm) were blocked with 20% (v/v) normal rabbit serum (Dako, High Wycombe, UK) and then incubated with mouse primary antibodies at optimal concentrations [ICAM-1:6.5B5; 4 μg/ml,VCAM-1:1.4C3; 7 μg/ml, CD31:JC70A; 7 μg/ml or isotype matched control antibody (all from Dako)]

and VAP-1:1B2; 10 μg/ml (gift of David Smith, Biotie, Finland). Primary antibody binding was visualized using peroxidase-conjugated secondary antibodies according to standard protocols (25). Chemokine staining was performed using ChemMate™ DAKO EnVision™ detection kit according to the manufacturer's instructions, and polyclonal goat antibodies (CCL4:AF-271-NA; 5 μg/ml, CCL5:AF-278-NA; 5 μg/ml, CXCL16:AF976; 1 μg/ml) or mouse monoclonal antibodies [CXCL9: 49106.11; 10 μg/ml, CXCL10:33036; 15 μg/ml, CXCL11: 87328; 5 μg/ml (all from R&D Systems, Abingdon, UK)] were applied at room temperature for 40 min. Sections were washed with TBS pH 7.6/0.01% (v/v) Tween®20 and endogenous peroxidase activity was blocked using methanol/0.5% (v/v) H_2O_2 (Sigma, Dorset, UK). All slides were incubated with appropriate EnVision™ secondary antibody reagent before application of EnVision™ substrate solution as directed by the manufacturer. Sections were counterstained with haematoxylin and visualized microscopically. A semiquantitative scoring system was applied to compare staining on specific features between normal and diseased livers with a + or - to indicate the presence or absence of staining, respectively, moderately increased intensity signified by ++ and +++ to indicate very intense staining.

Flow cytometry

Lymphocytes were incubated with optimal concentrations of fluorescently conjugated mouse monoclonal antibody targeting activation markers [CD38-FITC: AT13/5; 2 μg/ml, CD69-PE:FN50; 5 μg/ml (both Dako) 12G10: 5 μg/ml; gift of Martin J. Humphries, University of Manchester, UK], adhesion molecules [aL-FITC: MHM24; 2 μg/ml, α4-FITC:44H6; 2 μg/ml, L-selectin- FITC:FMC46; 4 μg/ml (all from Dako)], chemokine receptors [CCR2-PE:48607.211; 1.5 μg/ml, CCR5-PE: CTC5; 2 μg/ml, CCR6-PE:53103.111; 1.2 μg/ml, CXCR1- PE:42705.111; 2.5 μg/ml, CXCR3-

PE:49801; 1.5 μg/ml, CXCR4-PE:12G5; 2.5 μg/ml, CXCR6-PE:56811; 1.2 μg/ ml (all from R&D Systems)] or isotype-matched control antibody. FITC or PE conjugated mouse anti-human CD3 monoclonal antibody (UCHT1; 5 μg/ml, Dako) was also used to label T lymphocytes. Lymphocytes were further stained with 7-amino-actinomycin D (7-AAD) according to the manufacturer's instructions (BD Biosciences, Oxford, UK) before analysis to discriminate dead cells from the live cell population on the cytometer. 7-AAD$^+$ cells were excluded and median channel fluorescence values and percentage positivity were determined for each marker.

Flow-based adhesion assay

For flow-based adhesion assays, HSEC were grown to confluence in glass microcapillary tubes (Camlab Ltd, Cambridge, UK), stimulated with TNF-α and IFN-γ (both at 10 ng/ml; Peprotech, London, UK) for 24 h and connected to the flow system (23, 25). Lymphocytes were resuspended at a concentration of 5 x 10^5 viable cells/ml in basal media containing 0.1% (v/v) BSA and perfused over HSEC at a constant wall shear stress of 0.05 Pa. Lymphocyte adhesion was visualized using phase contrast video microscopy and recordings were made for offline analysis. The total number of adherent lymphocytes was normalized and expressed as cells/ $mm^2/10^6$ perfused, and migrated cells were also counted and calculated as a percentage of total adherent cells as described previously (23). Function blocking antibodies were applied to HSEC [anti-ICAM-1:84H10; Immunotech, Marseille, France, anti-VCAM-1:1G11; Immunotech, anti-E-selectin:1.2B6; Dako, anti-VAP-1:TK8-14; gift of David Smith, Biotie, anti-CD31:HEC-7; Endogen, Pierce Biotechnology Inc., Rockford, IL, USA, and isotype matched control mAb (Dako)] prior to perfusion of lymphocytes. Similarly, lymphocytes were incubated with antibody raised against β2 integrin (7E4; Immunotech) or the chemokine receptors

CCR5 (45531), CXCR3 (49801), CXCR4 (44716) (all from R&D Systems) and CXCR6 (7F3; gift of Mike Briskin, Millennium Pharmaceuticals Inc., Cambridge, MA, USA). All antibodies were applied at 10–20 μg/ml for 20 min at 37°C. The inhibitor of VAP-1/SSAO 2-bromoethylamine hydrobromide (Sigma) was incubated with lymphocytes and HSEC at 200uM for 20 min, 37°C, and maintained in lymphocyte suspensions and wash buffer throughout the experimental assay. Pertussis toxin (Sigma) was used to inhibit G-protein linked chemokine signalling by incubating lymphocytes (100 ng/ml, 90 min at 37°C) before perfusion over HSEC.

Statistical analysis

Data were assessed for normal distribution using the Kolmogorov–Smirnov test. Paired samples were analysed using the paired samples t-test in the case of parametric data. For unrelated samples, either independent samples t-test (for parametric data) or Mann–Whitney U-test (for non-parametric data) was employed. A P-value of less than 0.05 was considered to be statistically significant throughout the analysis.

Results

Adhesion molecule and chemokine staining in liver tissue

The liver removed at transplantation from patients with ALF exhibited varying degrees of necrosis and hepatocyte ballooning associated with the loss of normal lobular architecture. Tissue from patients with POD exhibited the greatest degree of central necrosis consistent with the toxic nature of the injury. Increased numbers of inflammatory cells were observed in both POD and SNH samples compared with non-diseased liver tissues. The parenchyma from both POD and SNH patients included areas of surviving hepatocytes and necrotic areas in which few, if any,

structures, including blood vessels could be recognized. Lymphocytic infiltration was particularly prominent in SNH whereas parenchymal necrosis tended to be more extensive in the POD cases. Interestingly, we did note residual adhesion molecule expression within necrotic areas in ALF which were particularly abundant in patients with POD (Figure S1).

Endothelial adhesion molecules

We scored expression of adhesion molecules on all vessels and within necrotic areas for all patients (see Table S1), but were particularly interested in sinusoidal areas that represent the major site for inflammatory cell recruitment (26). ICAM-1 was expressed at basal levels on sinusoidal endothelium in non-diseased liver tissue and levels increased markedly on sinusoids in both POD and SNH (Fig. 1). It was largely absent from portal vessels; although diffuse staining was observed in necrotic areas (Figure S1B). Hepatocyte membranes stained positively for ICAM-1 in SNH (Fig. 1). CD31, VCAM-1 and VAP-1 were upregulated on sinusoidal endothelium (Fig. 1) during ALF, but again little expression was detected on portal vessels. Necrotic areas in both POD and SNH were diffusely positive for CD31, VCAM-1 and VAP-1 (Figure S1B). In general, E- and P-selectin were not detected on sinusoidal endothelium as reported previously (27), although importantly we noted that occasional patients with ALF demonstrated infrequent, localized areas of E-selectin staining on sinusoidal or portal vessels (Figure S1A).

Hepatic chemokine expression

Little staining for CXCR3 ligands CXCL9, CXC10 and CXCL11 was detected on HSEC in normal liver, but all three chemokines were detected in ALF liver tissue with strong expression of CXCL9 and CXCL10 on sinusoids and occasionally on

hepatocyte membranes. The intensity of CXCL10 staining was highest at sites of leucocyte infiltration (Fig. 2A see arrow on panel). CCL4, CCL5 and CXCL16 were all detected in both sinusoids and hepatocytes in all livers with markedly increased staining in SNH (Fig. 2A,B). Strong staining of chemokines was seen in necrotic regions in ALF (Figure S2).

Integrin and chemokine receptor expression on lymphocytes

Flow cytometric analysis of lymphocytes isolated from peripheral blood and liver demonstrated a reversal of the CD4/CD8 ratio in tissue (SNH CD4 blood 47.6 ± 12.4% and liver 24.1 ± 4.3 CD8 blood 18.5 ± 7% and liver 51.9 ± 16.6%; POD CD4 blood 37.3 ± 5.8% and liver 21.3 ± 2.4% CD8 blood 17.1 ± 2.2% and liver 47.7 ± 17.7% n = 4 donors for each). We did not see significant differences between PBL from healthy donors and those with ALF with regard to integrins, chemokine receptors or activation markers. Liver derived lymphocytes expressed high levels of β2 integrins, low levels of L-selectin and high levels of CD69 when compared with matched blood samples. No significant difference was observed with CD38 (Table 1 and Fig. 3). Although LIL were enriched by a population of $\alpha L\beta 2^{h}$ cells, a small population of $\alpha L\beta 2^{o}$ cells was also seen in SNH (data not shown) and we demonstrated the expression of the β1-integrin activation reporter epitope in LIL and PBL during SNH (91 ± 1.26% positive SNH LIL vs 88 ± 2% positive on SNH PBL). L-selectin was expressed by approximately 60% of patient PBL but significantly reduced on liver-derived cells in ALF (Table 1). CXCR3 expression was increased in liver-derived lymphocytes compared with blood, whereas CXCR4 was highly expressed in all populations (Fig. 4). CCR5 (Table 1 and Figure S3) and CXCR6

were present at relatively low levels on lymphocytes in blood and liver with a modest increase in the expression of SNH LIL.

Lymphocyte adhesion to human sinusoidal endothelium in vitro

We used a flow-based adhesion assay incorporating cytokine-activated HSEC [TNF-α and IFN-γ (28) treatment] to model lymphocyte interactions with inflamed endothelium. We compared the behaviour of PBL from patients with POD or SNH with liver-derived lymphocytes from patients with SNH. Poor yields of viable lymphocytes from livers explanted from POD patients meant that these cells could not be studied in the adhesion assays.

Peripheral blood lymphocytes from SNH and POD patients behaved similarly, with adhesion supported by β1 and β2 integrins (Fig. 5A,B). A combination of blocking reagents was necessary to reduce adhesion by >60%. We then studied the proportion of adherent cells that went on to transmigrate through the endothelial monolayer under flow (Figs 6 and 7). The proportion migrating was similar to that found in our previous studies in healthy volunteers, but we saw less inhibition of TEM with anti-integrin antibodies. When LIL were studied, we saw a significant reduction in TEM after antibody blockade of VAP-1 or inhibition of VAP-1/ SSAO activity with enzyme inhibitors (23) VCAM-1, ICAM-1 and CD31 also contributing (Figs 6A and 7). Migration was reduced by pertussis toxin and small reductions were seen in the presence of blocking antibodies against CCR5, CXCR3 and CXCR4 (Figs 5 and 6).

Discussion

Current animal models of acute liver injury are limited by their inability to fully reproduce the features of human ALF (29). Immune cell recruitment from the circulation is an early feature of ALF and the accumulation of cells such as

macrophages (30), neutrophils (31) and lymphocytes alters the local cytokine environment, and promotes inflammation and hepatocyte apoptosis (32, 33). This led us to study the mechanisms underlying lymphocyte recruitment using patient lymphocytes and liver samples. Our data suggest that a marked increase in the local hepatic expression of chemokines and adhesion molecules is responsible for the recruitment of lymphocytes to the liver in patients with fulminant hepatitis. Acute paracetamol toxicity results in confluent cell necrosis (Figure S1) with a secondary inflammatory infiltrate composed of monocytes and lymphocytes (32). The syndrome is dominated by direct toxic damage and inflammation is a secondary event. This contrasts with SNH in which a heavy lymphocyte-rich infiltrate is responsible for hepatocyte damage mediated via direct activation of death receptors or the secretion of cytokines (1, 7). Consistent with previous studies, we found that the infiltrate in SNH is dominated by CD8 T cells in portal tracts, and in the parenchyma where effector T cells were seen in close contact with hepatocytes (34–37). NK cells and CD8+ T lymphocytes can directly kill hepatocytes by activating TNF-receptor superfamily-mediated cell death pathways (33, 38). Liver-derived lymphocytes in our study expressed a phenotype associated with activated effector T cells with high surface levels of CD69, $\alpha 4\beta 1$ and $\alpha L\beta 2$, and low levels of L-selectin (39, 40) and high levels of CXCR3 consistent with our previous observations in chronic hepatitis and liver disease (21, 28, 41). The $CXCR3^{h}$ $\alpha L\beta 2^{h}$ $CD69^{h}$ phenotype is consistent with effector cells. We did not detect enrichment of $CCR6^{h}$ cells suggesting that Th17 cells do not dominate in the infiltrate. In vitro, studies have demonstrated that the expression of CCR5 is independent from that of CXCR3, but correlates with CXCR6 in both $CD4^{+}$ and $CD8^{+}$ lymphocytes (42, 43). Moreover, acutely activated lymphocytes in vitro express CXCR3 with no detectable CCR5 expression, but cell

surface levels of both receptors increase gradually in long-term cultures (44, 45). Thus, differences in the levels of CCR5 in ALF and chronic hepatitis may be a consequence of the duration of lymphocyte activation in an environment dominated by persistent chronic inflammation. Intravital studies have demonstrated that most leucocyte entry into the inflamed liver occurs in the sinusoids in a selectin-independent process (27). Generally, the sinusoidal endothelium of the human liver does not express E-/P-selectins even with inflammation (10), although consistent with previous observations in alcoholic hepatitis we detected infrequent, focal sinusoidal E-selectin expression in ALF (Figure S1). E-selectin dependent ligation of leucocyte P-selectin glycoprotein ligand-1 modifies the avidity of αLβ2-integrin (46) which may result in strengthening of αLβ2-integrin and ICAM-1-mediated adhesion of lymphocytes within hepatic sinusoids.

The αLβ2-integrin predominates in antigen-dependent adhesion of $CD8^+$ T cells in the liver (47), whereas $CD4^+$ Th1 cells use α4β1-integrin to adhere within the hepatic sinusoids (14). Their ligands, ICAM-1 and VCAM-1, have been shown to mediate lymphocyte adhesion to human hepatic endothelium, and ICAM-1 is important for transmigration across HSEC under flow (28, 39). In this study, significant ICAM-1 expression and VCAM-1 induction were seen in cases with severe liver inflammation (11), and lymphocyte adhesion to HSEC under flow was dependent on ICAM-1 and VCAM-1 and could be reduced by inhibiting G-protein coupled receptors with pertussis toxin suggesting that chemokines are involved. Transmigration of LIL from SNH involved β2-integrins and VAP-1/SSAO. Interestingly, the migration of PBL from SNH patients was enhanced when VCAM-1 (with or without ICAM-1) was inhibited, suggesting an inhibitory role for VCAM- 1 in transmigration of these lymphocytes. This has been shown to occur only when α4β1-integrin on lymphocytes is expressed

in the fully active state (48) and we detected a distinct population of circulating lymphocytes expressing high-affinity β1-integrins by staining for mAb 12G10, which recognizes a ligand-induced activation epitope on β1-integrins (49). Moreover, integrin ligand specificity is governed by activation state and fully activated α4β1-integrin can bind fibronectin in addition to VCAM-1 (50). Thus, in SNH, highly activated lymphocyte integrins may support alternative routes of adhesion. Inhibition of α4-integrins in ConA-dependent hepatitis has been observed to worsen hepatic inflammation (51).

VAP-1 protein and enzymatic activity have a role in both adhesion and transmigration of lymphocytes across cytokine-activated HSEC under flow and in migration through the hepatic sinusoids in vivo (52). In vivo, the recruitment of activated $CD4^+$ and $CD8^+$ lymphocytes in rat liver allograft rejection (53) and CD4+ Th2 cells in ConA hepatitis (14) is dependent on VAP-1 showing that VAP-1 regulates adhesion of different lymphocyte subsets in the liver depending on the nature of inflammatory stimulus. In the present study, although antibody blockade of VAP-1 or inhibition of SSAO activity (data not shown) had no effect on total adhesion, the transmigration of LIL from SNH patients was reduced by either antibody blockade of VAP-1 or inhibition of SSAO activity. Taken together with our findings of increased sinusoidal VAP-1 expression in ALF, it is likely that VAP-1 contributes to the recruitment of effector lymphocytes in ALF.

Similarly, hepatic expression of CD31 increased in both the portal and sinusoidal endothelium in ALF, and CD31 blockade reduced transmigration of LIL in patients with SNH. CD31 can engage with both heterotypic and homotypic ligands and has been shown to mediate leucocyte migration through endothelial cell junctions and

basement membrane (54, 55). Therefore, homotypic CD31 interactions between HSEC and lymphocytes may promote lymphocyte interactions with endothelial ligands as shown previously for $CD8^+$ (56) and NK cells, or elevated expression during ALF may promote heterotypic interactions with CD38 (57), which are detected in a population of LIL.

In chronic hepatitis, CXCR3 ligands are increased in the sinusoidal endothelium and hepatocytes resulting in the recruitment of $CXCR3^+$ lymphocytes to the liver (28, 41, 58) and CXCL16 is expressed in the hepatic parenchyma (21) and on bile ducts, whereas CCR5 ligands are largely restricted to portal tracts (41). However, in ALF, we detected significant expression of CCR5 ligands in the sinusoids (36). The highest levels of CCR5, CXCR3 and CXCR6 ligands were seen in patients with SNH and only in SNH did we detect increased numbers of CCR5 and CXCR6 cells in the liver. Interestingly, CXCR3 ligands were not detected on the sinusoids in POD which might be a consequence of their dependence on IFN-γ for maximal expression (26) which comes from infiltrating lymphocytes in ALF (34). Thus, POD maybe associated with lower intrahepatic IFN-γ levels (58). The chemokine receptors CCR5, CXCR3 and CXCR6 have been shown to specifically mediate the recruitment of effector $CD8^+$ T cells to the liver in mice models of graft vs host disease and murine cytomegalovirus infection (59–61). Our group has shown that CXCR6 triggers β1-integrin-dependent adhesion of lymphocytes in liver (21) whereas CXCR3 mediates both adhesion and transmigration of effector lymphocytes across HSEC through ICAM-1-dependent pathway under flow (28). In this study, the inhibition of CCR5 and CXCR3 with mAb had a moderate effect on the transmigration of lymphocytes from either POD or SNH patients consistent with their expression of activated integrins that do not require chemokine receptor-mediated triggering of adhesion. In summary,

we demonstrate that during ALF, adhesion molecules (ICAM-1, VCAM-1, VAP-1 and CD31) and chemokines (CXCL9-11, CXCL16 and CCL4) are locally upregulated within the hepatic parenchyma. We also demonstrate that lymphocytes isolated from this environment have an activated phenotype with corresponding upregulation of specific chemokine receptors such as CXCR3. Our functional assays confirm that VCAM-1, ICAM-1 VAP-1 and CD31 contribute to the recruitment of lymphocytes to the liver in SNH and suggest that individual chemokines have a relatively modest role. Further studies are necessary to determine whether specific combinations of pro-adhesive signals drive subset- specific recruitment, and to test whether inhibition of key regulatory cells may modulate the outcome of acute hepatic inflammation for patient benefit.

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%Positive cells	Normal PBL	POD PBL	POD LIL	SNH PBL	SNH LIL
aL	99.5 ± 0.2	98.2 ± 0.5	99.7 ± 0.1	99.4 ± 0.08	99.2 ± 0.4
a4	85.3 ± 4.9	77.0 ± 5.0	85.7 ± 5.0	80.4 ± 6.4	84.9 ± 6.1
L-selectin	63.9 ± 1.0	66.5 ± 3.8	23.2 ± 2.3***	59.1 ± 11.7	25.9 ± 2.6*
CD 38	58.0 ± 10.6	63.3 ± 3.7	47.0 ± 12.6	69.6 ± 6.7	81.3 ± 3.1
CD 69	24.0 ± 5 6	14.8 ± 1.4	60.8 ± 5.4**	17.3 ± 3.9	73.6 ± 11.1***
CCR2	3.5 ± 0.3	3.5 ± 0.4	2.7 ± 0.7	3.2 ± 0.4	4.4 ± 0.9
CCR5	3.0 ± 0.3	3.9 ± 0.4	5.5 ± 1.4	4.1 ± 0.3* vs NL	10.2 ± 3.7
CCR6	4.3 ± 0.7	4.4 ± 0.4	2.4 ± 0.3*	4.7 ± 0.7	4.1 ±1.1
CXCR1	4.3 ± 0.2	6.4 ± 1.5	4.5 ± 1.1	3.7 ± 0.5	5.1 ± 1.1

CXCR3	45.1 ± 6.9	25.5 ± 6.3	47.5 ± 13.9	32.0 ± 8.1	61.0 ± 12.4
CXCR4	78.1 ± 5.1	81.7 ± 2.4	73.4 ± 9.9	78.5 ± 5.1	83.6 ± 4.9
CXCR6	3.4 ± 0.4	3.7 ± 0.7	2.8 ± 0.7	3.1 ± 0.2	7.5 ± 1.9 * vs POD LIL
*P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001.					

Table 1. The percentage of peripheral blood lymphocytes (PBL) or liver-infiltrating lymphocytes (LIL) staining positively for adhesion molecules, activation markers and chemokine receptors is summarized for normal donors and patients with paracetamol overdose (POD) or seronegative hepatitis (SNH). Data represent mean percentage of positive cells ± SEM for n = 3–7 donors. Statistical significance was calculated by independent samples t-test for different PBL or LIL groups and PBL and LIL populations of the same aetiology

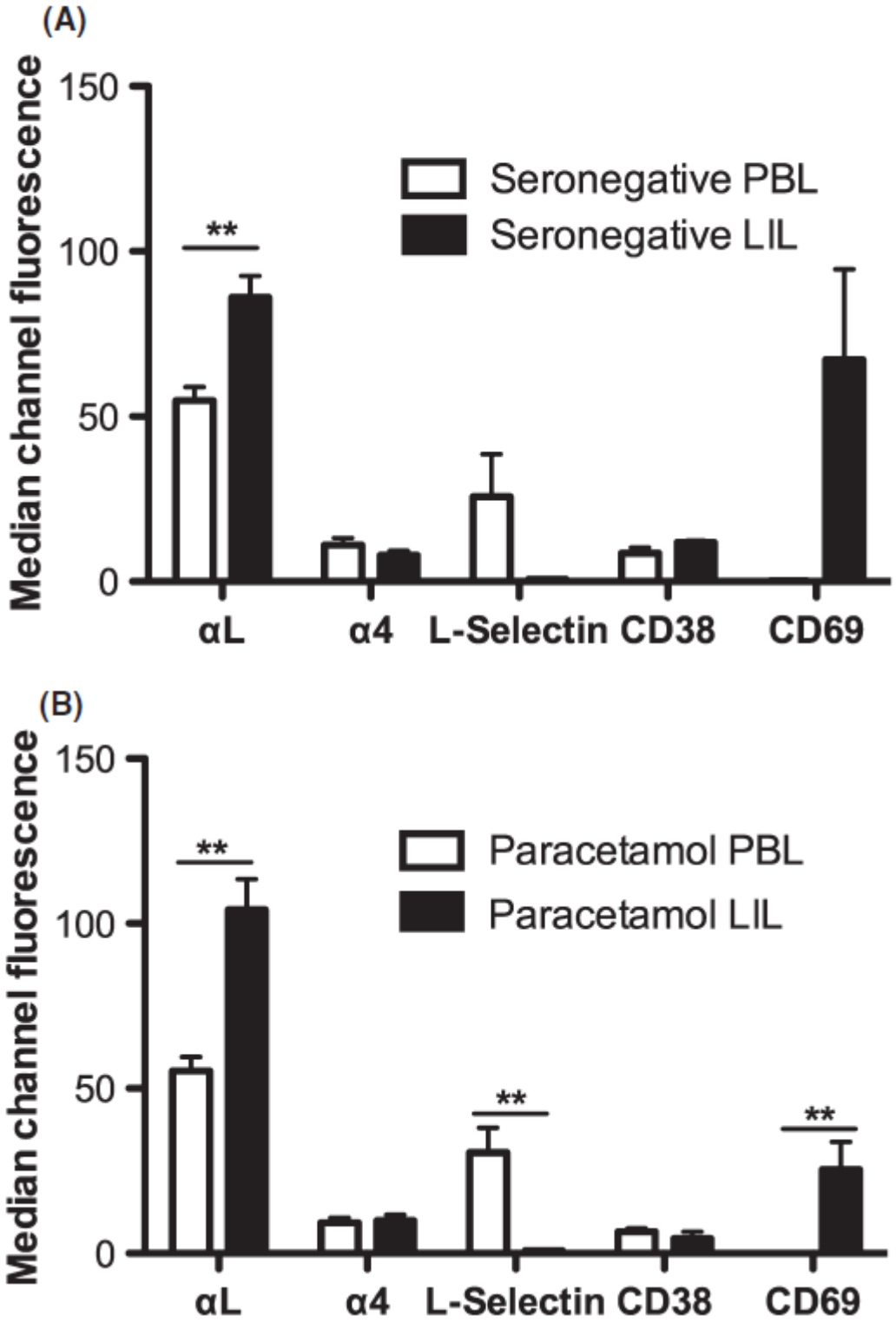


Fig. 3. Adhesion molecule (αL integrin, α4 integrin and L-selectin) and activation marker (CD38 and CD69) expression on peripheral blood and liver-infiltrating

lymphocytes is shown for patients with (A) seronegative hepatitis and (B) paracetamol overdose. Data are expressed as median channel fluorescence values and represent mean ± SEM of at least three experiments for each lymphocyte group studied where cells were isolated from different donors for each experiment. Independent samples t-test or Mann–Whitney U-test was used to calculate statistical significance (**$P \leq 0.01$) and compared median channel fluorescence values between lymphocyte populations.

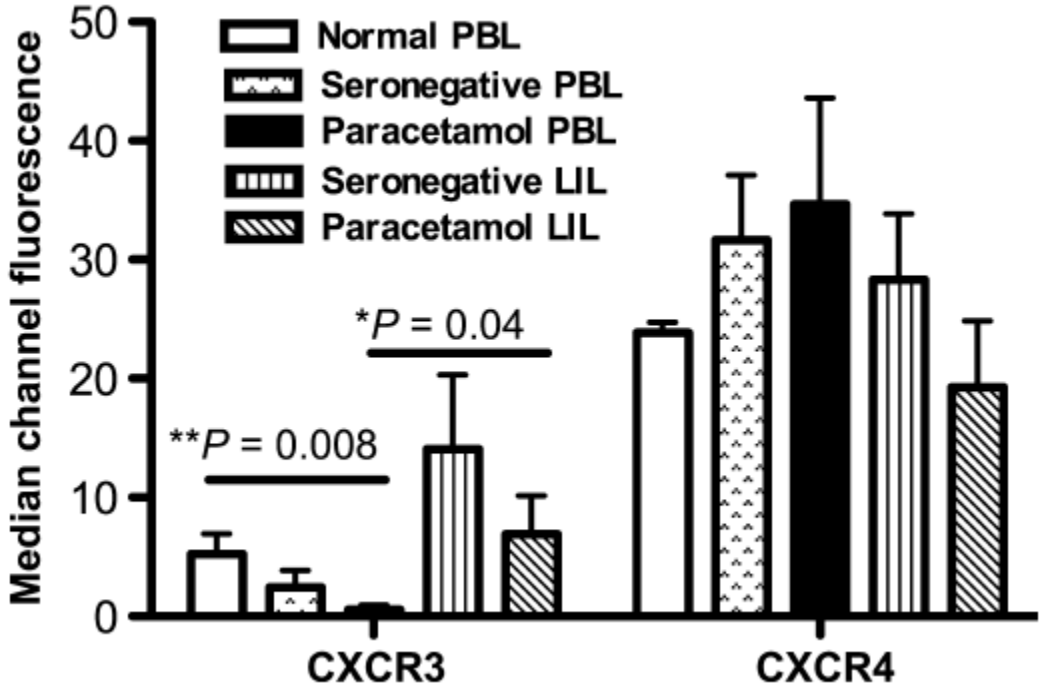


Fig. 4. The expression of chemokine receptors CXCR3 and CXCR4 on circulating and liver-derived lymphocytes from normal donors and patients with acute liver failure (POD; paracetamol overdose, SNH; seronegative hepatitis) was investigated by flow cytometry. Data represent mean ± SEM of median channel fluorescence values from at least four separate experiments using different donors for each. Independent samples t-test was used to compare the expression of CXCR3 and CXCR4 between different PBL groups, LIL groups or PBL and LIL populations of the same aetiology (*P ≤ 0.05; normal vs POD PBL, **P ≤ 0.01; POD PBL vs POD LIL).

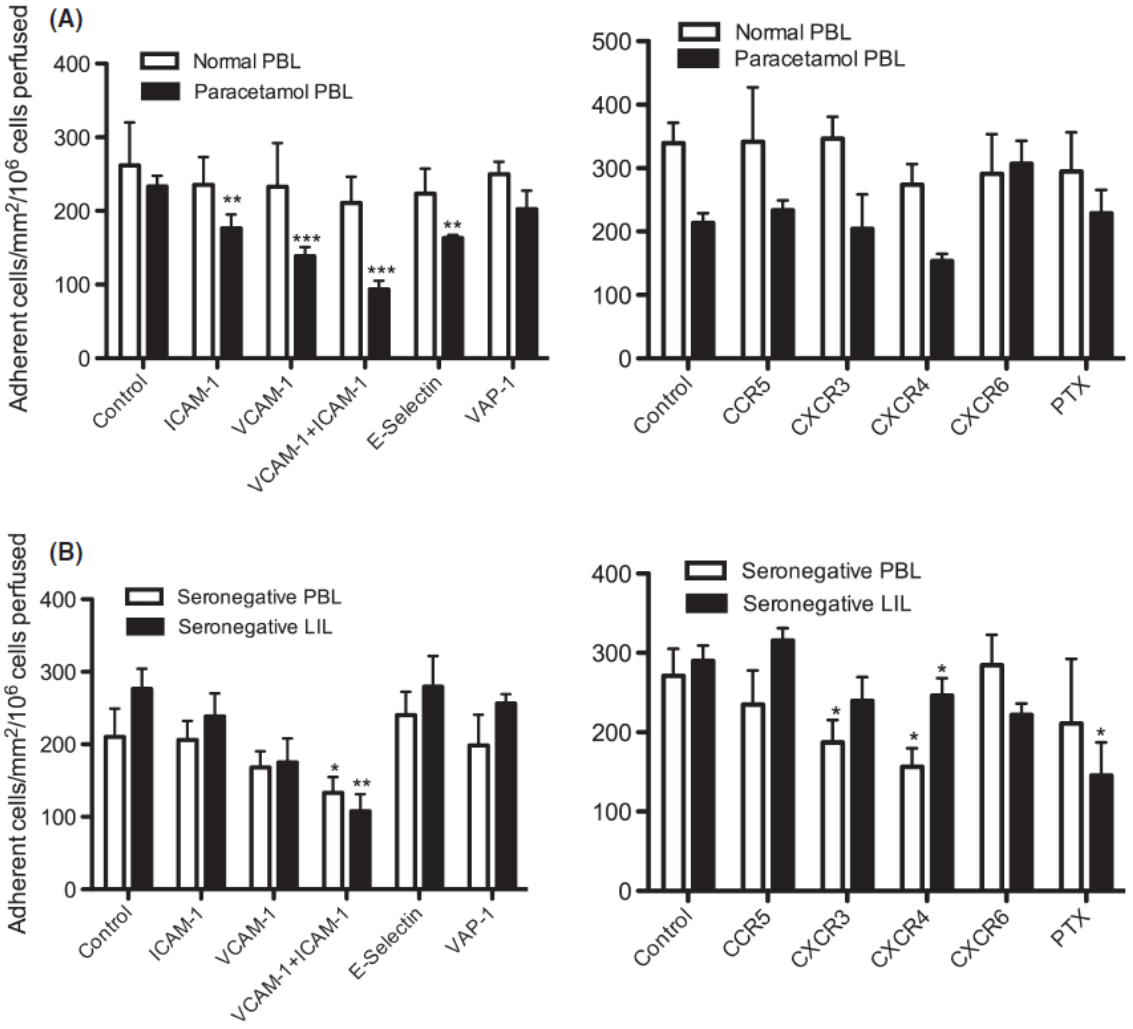


Fig. 5. The role of endothelial adhesion molecules and chemokine receptors on adhesion of patient lymphocytes to HSEC was studied using a flow-based adhesion assay. HSEC stimulated with TNF-α and IFN-γ (10 ng/ml; for 24 h before assay), were treated with function blocking monoclonal antibodies targeting ICAM-1, VCAM-1, E-selectin, VAP-1 or isotype-matched control antibody prior to perfusion of lymphocytes at a shear stress of 0.05 Pa. Alternately, lymphocytes were incubated with function blocking monoclonal antibody targeting chemokine receptors as indicated, pertussis toxin (PTX), or an isotype-matched control antibody before perfusion over HSEC. The total adhesion of lymphocytes is expressed as adherent cells/mm^2/10^6 perfused. Data represent mean ± SEM of four experiments using

lymphocytes from different patients for each replicate. Paired samples t-test was used to calculate statistical significance (*P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001) between the adhesion in the presence of function blocking monoclonal antibody or PTX and the adhesion in the presence of isotype-matched control antibody or control (PBL, peripheral blood lymphocytes; LIL, liver-infiltrating lymphocytes).

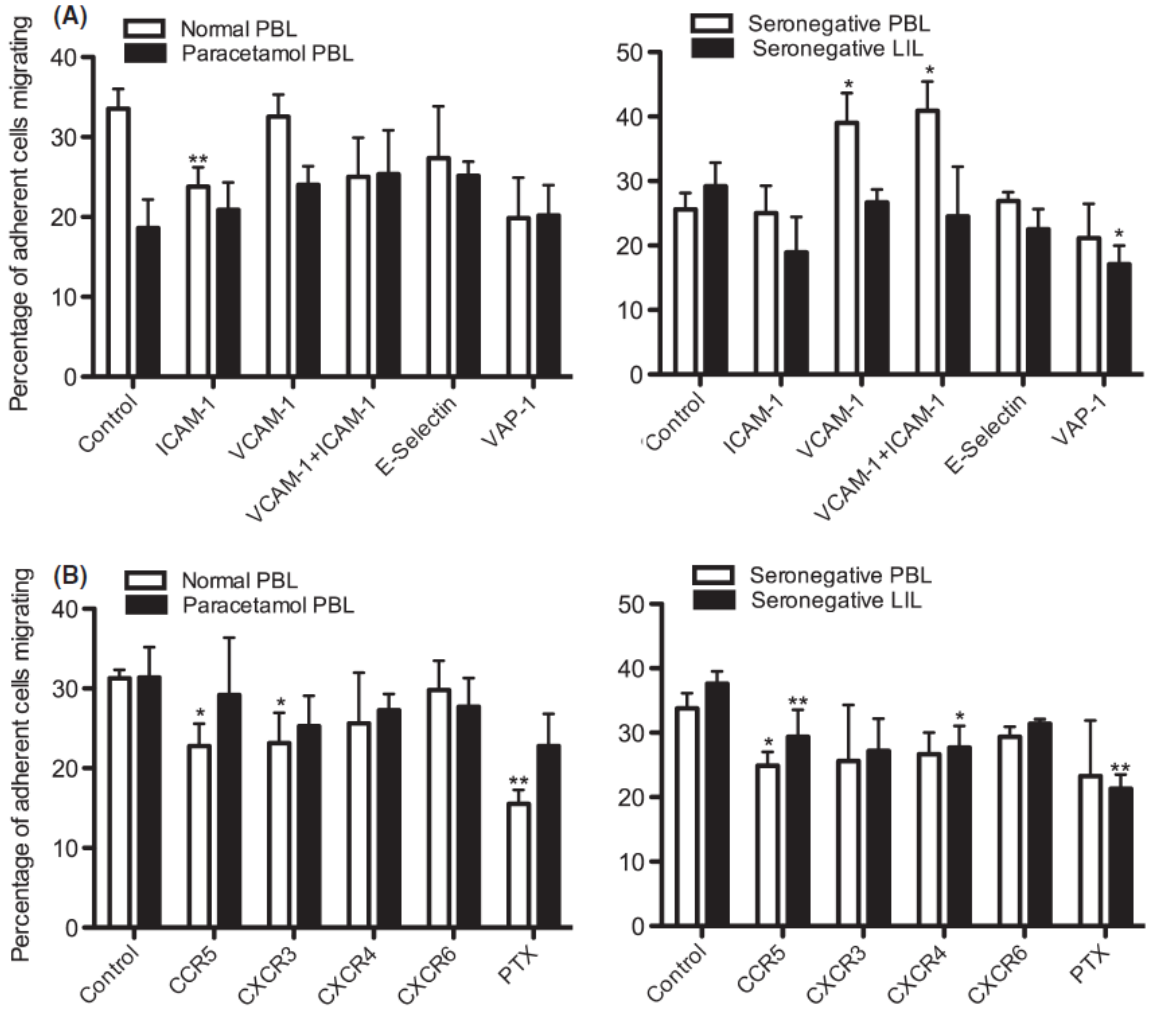


Fig. 6. The effect of endothelial adhesion molecules and chemokine receptors on transendothelial migration of lymphocytes from patients with acute liver failure secondary to paracetamol overdose or seronegative hepatitis was investigated under flow conditions (0.05 Pa). Lymphocytes that underwent transmigration across HSEC are expressed as a percentage of total adhesion. HSEC was prestimulated with TNFα and IFN-γ (10 ng/ml, 24 h) and treated with function blocking monoclonal antibodies or isotype-matched control antibody, as indicated. Alternately lymphocytes were incubated with function blocking monoclonal antibody targeting chemokine receptors as indicated, pertussis toxin (PTX), or an isotype matched control antibody before perfusion over HSEC at a constant shear stress of 0.05 Pa.

Data represent mean ± SEM from four separate experiments each using lymphocytes from different patients. Paired samples t-test indicated statistical significance (*P ≤ 0.05; **P ≤ 0.01) between transmigration in the presence of function blocking monoclonal antibody and isotype-matched control (PBL, peripheral blood lymphocytes; LIL, liver-infiltrating lymphocytes).

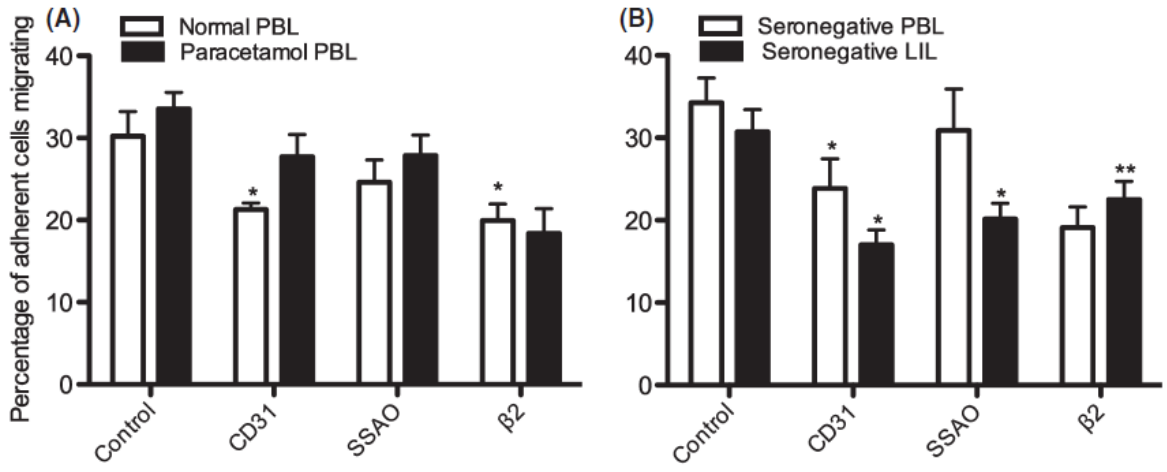


Fig. 7. Transmigration of PBL from patients with paracetamol overdose compared to that of PBL and LIL from patients with seronegative hepatitis. Lymphocytes that underwent transmigration across TNFα and IFN-γ stimulated HSEC are expressed as a percentage of total adhesion in the absence or presence of function blocking monoclonal antibodies (CD31 and b2 integrin, to treat HSEC and lymphocytes respectively) or VAP-1 enzyme activity inhibitor (Semicarbazide, SSAO). Data represent mean ± SEM of four separate experiments each using lymphocytes from different patients. Paired samples t test indicated statistical significance (*P ≤ 0.05; **P ≤ 0.01) between the transmigration in the presence of functional inhibition vs control cells (PBL, peripheral blood lymphocytes; LIL, liver-infiltrating lymphocytes).

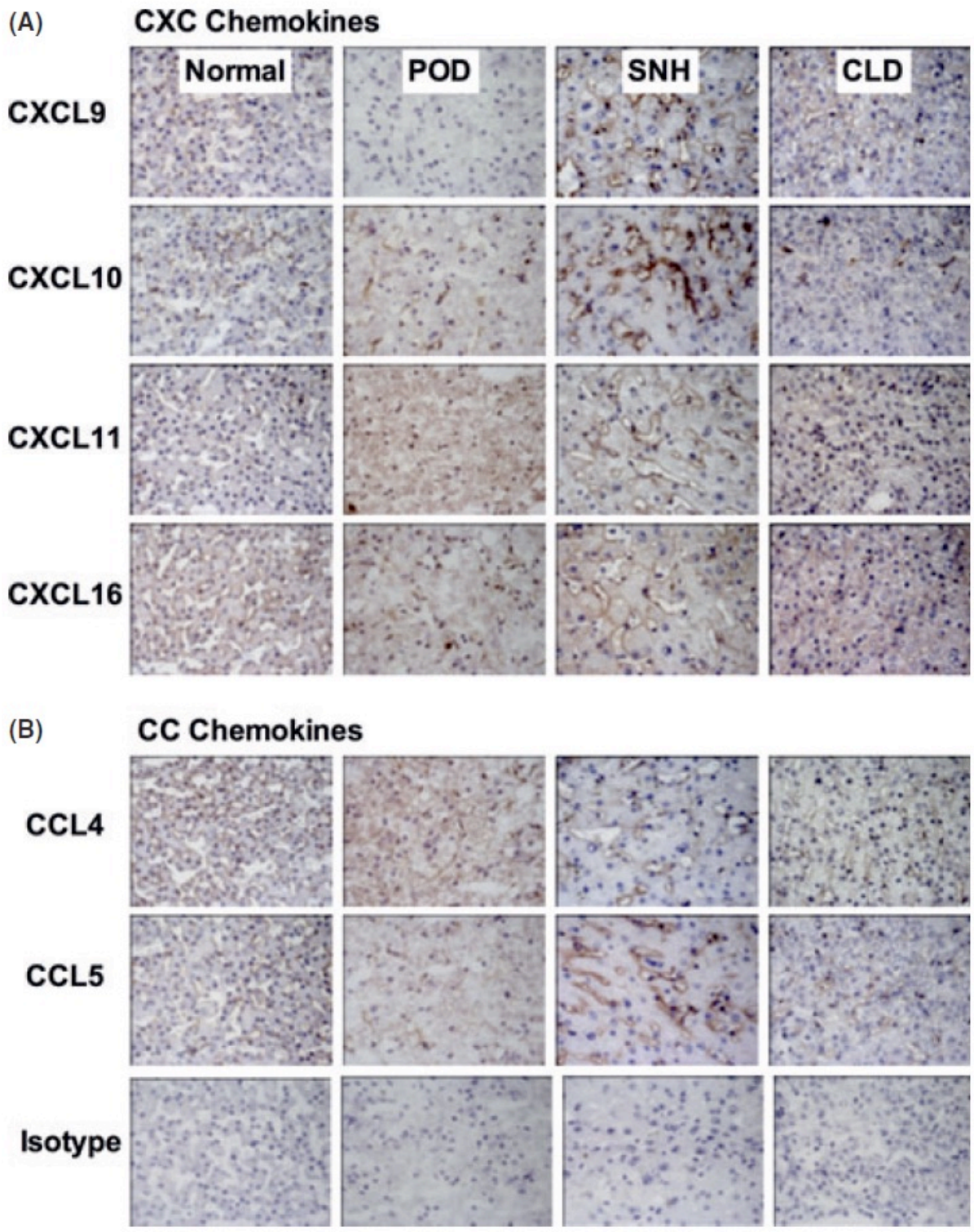
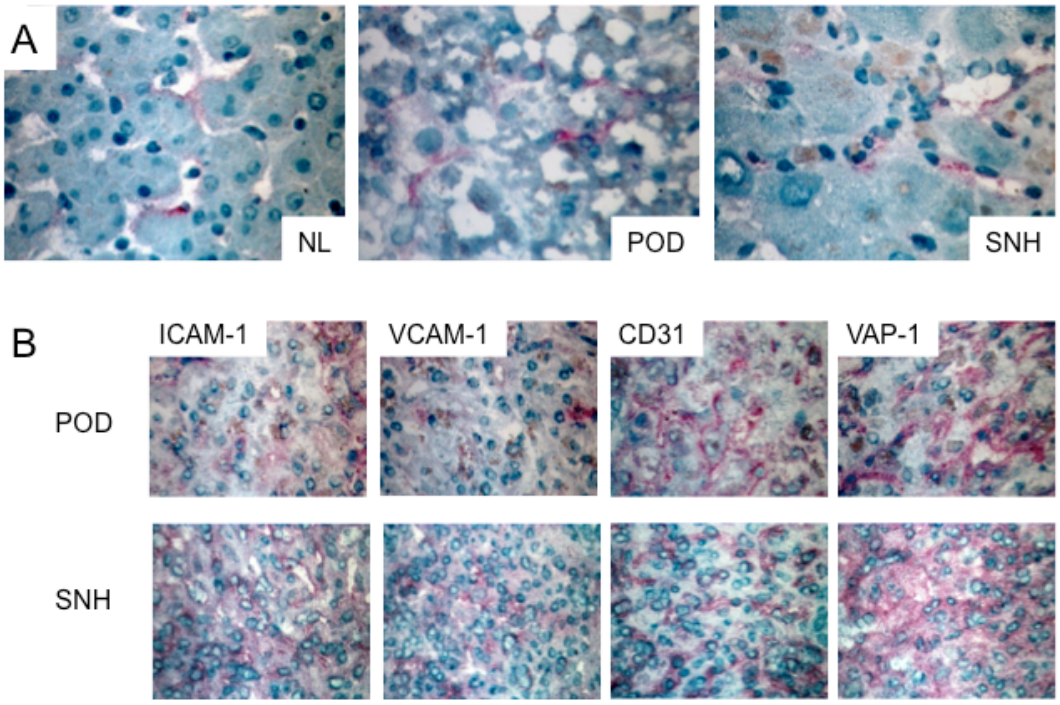


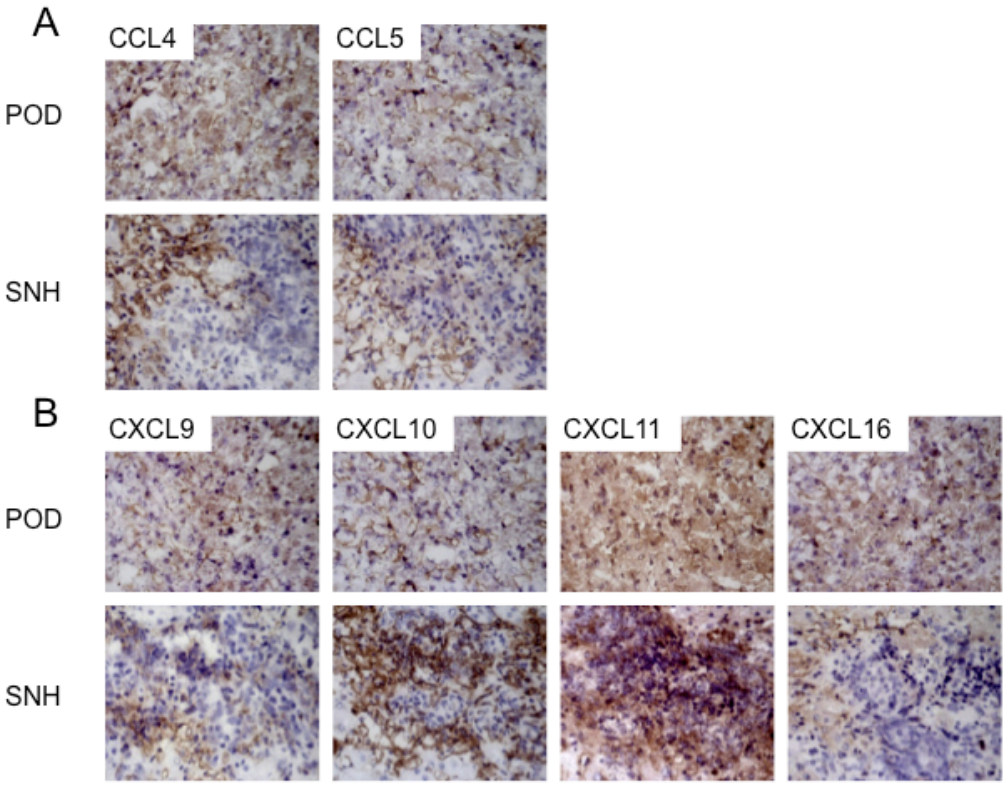
Fig. 2. Sinusoidal staining for (A) CXC-chemokines and (B) CC-chemokines was compared between livers from normal donors, patients with paracetamol overdose (POD), seronegative hepatitis (SNH) or chronic liver disease (CLD; representative images from a patient with primary biliary cirrhosis). Chemokine expression is

indicated by brown staining and isotype-matched antibody was used as control. Data are representative images from one of four replicate experiments using different donors. Original magnification 400x.



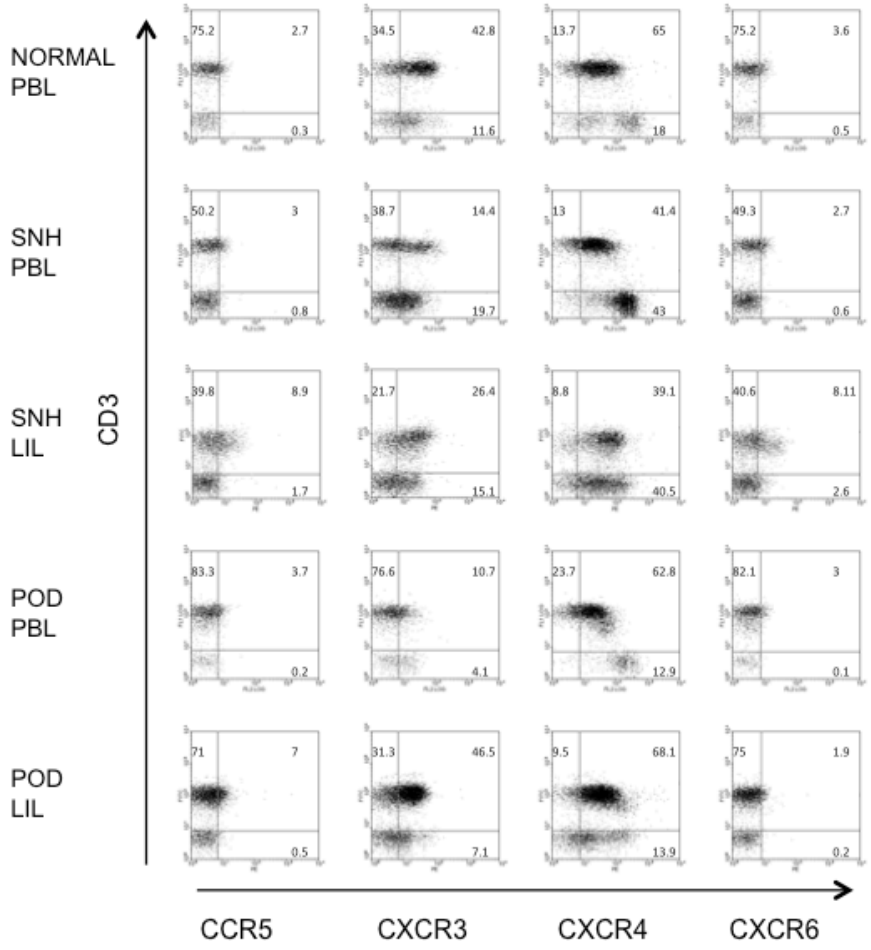
(A) Exemplary images of immunohistochemical staining for E-selectin in sinusoids of tissue sections from normal liver, paracetamol overdose (POD) liver, and seronegative hepatitis (SNH) liver illustrating occasional focal positive staining. *(B)* Images from immunohistochemical staining show the expression of *indicated* adhesion molecules (red) in necrotic areas of liver sections from patients with acute liver failure secondary to POD and SNH. Data present representative images from 1 of at least 4 different donors, 400X magnification.

Supplemental Figure 1



Representative images from immunohistochemical staining show the expression of *(A)* CC-chemokines and *(B)* CXC-chemokines (brown), as indicated, in the necrotic areas of liver sections from patients with acute liver failure secondary to paracetamol overdose (POD) and seronegative hepatitis (SNH). Data present representative images from 1 of at least 4 separate donors, 400X magnification.

Supplemental Figure 2



Representative dot plots for chemokine receptor expression on typical donors of PBL and LIL populations gated according to forward scatter and side scatter characteristics. Dot plots illustrate variables FL1 ($CD3^+$–FITC) & FL2 (chemokine receptor–PE) from single typical donors. Values in each quadrant represent percentage of cells from 1 experiment.

Supplemental Figure 3

Table 1 : Summary of Adhesion Molecule staining data

LIVER TYPE	ICAM-1	VCAM-1	CD31	VAP-1	E/P-Selectin
Expression of Adhesion Molecules on Surviving Sinusoidal EC					
NORMAL	+	+	+	+	–/–
CLD	++	+	+	+	–/–
POD	+++	++	++	+ to ++	–/–
SNH	+++	++	++	+ to ++	–/–
Expression of Adhesion Molecules on Necrotic Tissue					
POD	++	+	+++	+++	–/–
SNH	++	+ to ++	+++	+++	–/–
Expression of Adhesion Molecules on Vascular EC of Portal Tracts					
NORMAL	–	–	+	+	–/–
CLD	–	–	+	+	–/–
POD	–	–	++	+	–/–
SNH	–	–	++	+	–/–

Semiquantitative scoring of the staining data for adhesion molecule expression on different tissue structures of normal (n=4) and diseased livers (n=4+ for each disease type). Baseline expression of each molecule in normal livers was compared with the expression of each adhesion molecule in the diseased livers. The expression of adhesion molecules on necrotic tissue of ALF livers was compared to the expression of each molecule in the surviving areas of the matching tissue sections. + or – sign indicates the presence or absence of the specific molecule, respectively. ++ shows an increase in the expression whereas +++ represents high levels of upregulation of the molecule of interest (CLD; chronic liver disease, POD; paracetamol overdose, SNH; seronegative hepatitis).

Supplemental Table 1

Chapter 3 Prediction models in ALF

Blood Lactate But Not Serum Phosphate Levels Can Predict Patient Outcome in Fulminant Hepatic Failure

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Abstract

Early identification of those patients with fulminant hepatic liver failure (FHF) who need a transplant greatly helps in their management. A number of prognostic criteria have recently been proposed, including arterial blood lactate and serum phosphate concentrations. To validate their use, we retrospectively studied 83 consecutive patients with FHF admitted to our intensive treatment unit between August 2000 and March 2003. A total of 48 patients (58%) survived with medical management only (group I) and 35 patients (42%) failed to survive spontaneously (group II). This group included 19 patients (23%) who underwent orthotopic liver transplantation (LT), and 16 patients (19%) who died without undergoing LT (group IIa). A total of 5 patients (6%) who underwent liver transplantation died. Within paracetamol overdose (POD) and non-POD subgroups, phosphate concentrations were not significantly higher in group II patients ($P = 0.08$ and $P = 0.27$, respectively), when compared to group I patients. In multivariate analysis, post admission 12-hour lactate level was the only

predictor of survival for the POD subgroup, whereas in non-POD patients, 12-hour lactate and admission bilirubin levels were significant in predicting patients' outcome. In conclusion, we found that while serum phosphate concentrations have limited clinical utility as prognostic markers, persistently elevated arterial blood lactate levels despite adequate fluid resuscitation are indicators of a poor prognosis in FHF.

Introduction

Fulminant hepatic failure (FHF) is the indication for 6% to 7% of liver transplants.(1) Liver transplantation (LT) has significantly improved the survival in this group of patients and remains the cornerstone of treatment in selected patients with FHF. (2,3) The decision to offer liver replacement in this group of patients is difficult since some will return to normal structure and function without liver replacement and the need for lifelong immunosuppression (4,5); early and accurate identification of those who will die without transplantation is of central importance.(6) Among the various prognostic criteria proposed, the King's College Hospital (KCH) criteria (7) have been the most widely applied, (8) with a clinically acceptable specificity in identifying patients with a poor prognosis.(6,9) However, their sensitivity is limited by their failure to identify a significant proportion of patients who will not survive without transplantation. (9-11) Hyperlactatemia develops in approximately 80% of those with FHF.(12) The pathogenesis may involve enhanced hepatic and extrahepatic aerobic glycolytic activity and lactate production, coupled with impaired hepatocyte function, resulting in net hepatic lactate production.(13) The sensitivity of the KCH criteria has recently been improved by a modification to include post-resuscitation arterial blood lactate (>3.0 mmol/L) as an early predictor of outcome in paracetamol-induced FHF.(14) Abnormalities in phosphate homeostasis have been observed for many years in FHF,(15,16) but its prognostic significance has been studied only

recently.(6,17,18) Hyperphosphatemia appears to portend a poor prognosis, while hypophosphatemia was noted in patients who recover without LT.(6) Thus, the introduction of both lactate and phosphate measurements into selection criteria should await further confirmation of their performance in appropriately conducted validation studies.(19) We evaluated retrospectively the prognostic significance of admission serum phosphate and serial arterial blood lactate levels in the setting of FHF.

Patients and Methods

Patients with FHF referred to The Liver Unit, Queen Elizabeth Hospital, Birmingham, between August 2000 and March 2003, were retrospectively analysed. The diagnosis of FHF was made according to the criteria of Trey and Davidson. (20) Inclusion criteria were available measurements of admission serum phosphate and serial arterial blood lactate levels, and hospitalization in the intensive treatment unit for more than 24 hours before a terminating event (recovery, LT, or death). Patients were treated and monitored in accordance with the department protocol and were considered for transplantation if they meet the KCH criteria. (7) Patients were grouped as survivors with medical management (group I), or nonsurvivors (LT and/or death: group II). In an attempt to reduce bias, nonsurvivors excluding those who underwent LT were also analysed (group IIa). Patients were also categorized according to the aetiology of their FHF: paracetamol overdose (POD) and non-POD subgroups. Each patient was evaluated for the static variables including age, gender, race, and aetiology of liver disease. Dynamic variables analysed on admission included serum bilirubin levels, prothrombin time, arterial pH, serum creatinine, serum phosphate levels, and serial arterial blood lactate concentrations (on admission, and 4, 8, and 12 hours post admission). Arterial blood lactate levels were

measured hourly during the first 12 hours postadmission with an automated analyser (YSI 2300 STAT Plus; Yellow Springs Instrument, Co. Inc. [YSI] Environmental Science, Yellow Springs, OH).

During the study period, 83 patients were identified who fulfilled the inclusion criteria for the study. The mean age was 37.8 years, with a range of 17 to 65 years. A total of 58% of patients were female, and 92% were Caucasian. POD was the most common aetiology, accounting for 56 cases (67%). Aetiology for non-POD patients (n = 27) included seronegative hepatitis (n = 12), drugs (n = 6), viral hepatitis (n = 5), indeterminate (n = 3), and veno-occlusive disease (n = 1).

Statistics

Two dichotomous outcome variables were analysed: for the first variable the 2 categories of patients were “survived without transplantation” (group I) and “survived with transplantation or died” (group II); for the second outcome variable, patients who underwent transplantation were excluded and the others were categorized as “survived” (group I) or “died” (group IIa). Analyses were performed on the whole group of patients and on the 2 subgroups formed by including and excluding those who had taken a paracetamol overdose (POD and non-POD patients). Categorical variables were analysed using Fisher’s exact test. The logarithms of prothrombin time, creatinine, bilirubin, lactate, and phosphate levels were used in the analysis, as these were normally distributed and the untransformed levels were not. Unpaired *t* tests were used in the univariate analyses and stepwise binary logistic regression in the multivariate analyses. As all the significant variables in the multivariate analyses had been log transformed, the odds ratio (OR) corresponding to a 10% increase in the value of the untransformed variable was calculated ($OR_{10\%}$), together with the

associated 95% confidence interval (95% CI). All statistical analysis was performed with SPSS for Windows version 10.0.7 (SPSS Inc., Chicago, IL).

Results

All patients admitted following a POD were started on *N*-acetyl-cysteine in their local hospital. All patients developed at least grade 1 hepatic encephalopathy in association with markedly abnormal liver function tests (data not shown). A total of 55 patients (66%) were ventilated prior to admission (POD group: n = 34 [61%]; non POD group: n = 21 [78%]).

Univariate Analysis of Predictors of Nonsurvival

Of the entire cohort, 48 patients (58%) survived with medical management alone (group I) and 35 patients (42%) failed medical management (group II). Of those 35 patients, 19 (23%) underwent LT (14 patients survived, and 5 patients died). A total of 16 patients (19%) died without undergoing LT (group IIa). Patients in group I were younger and a higher proportion were Caucasian compared with those in group II (Table 1). POD-related FHF was associated with a significantly higher spontaneous recovery rate; 73% of those patients recovered with medical management alone compared with 26% among non-POD patients ($P \leq 0.001$). This was also reflected in the number of patients undergoing LT in each group (POD: n = 6 [11%] of 56; non-POD: n = 13 [48%] of 27).

Analysis of dynamic variables on admission showed that serum bilirubin was significantly lower in group I than in group II or IIa patients ($P \leq 0.001$ and $P = 0.022$, respectively); however, serum creatinine, arterial pH, and prothrombin time were not significantly different (Table 1). Within each etiological group, a considerable overlap

in bilirubin concentrations was observed for the different clinical outcomes (data not shown).

Serum admission phosphate levels were significantly higher in group II ($P = 0.005$) than in group I patients (Table 1). While serum phosphate levels were also higher when group IIa patients were compared to group I patients, the difference was not statistically significant (Table 1). Within POD and non-POD subgroups, there was no significant difference in serum admission phosphate levels between group I, II, and IIa patients (Table 2).

Lactate concentrations were significantly higher ($P \leq 0.001$) at 12 hours in group II and IIa patients when compared to group I patients (Table 1). Within the POD subgroup, admission, 4-, 8-, and 12-hour postadmission lactate concentrations were significantly higher ($P \leq 0.004$) in group II and IIa patients compared with group I patients (Table 2A). Within the non-POD patients, 4- and 12-hour lactate levels were significantly higher ($P \leq 0.04$) in group II and IIa patients compared with group I patients (Table 2B).

The 12-hour arterial blood lactate levels for the different clinical outcomes are shown in Figure 1.

Multivariate Analysis of Predictors of Nonsurvival

In the stepwise logistic regression analysis, the 12-hour lactate level was the only significant predictor of survival in multivariate analysis for POD patients ($OR_{10\%}$: 1.29; 95% CI: 1.12-1.49; $P \leq 0.001$). When patients undergoing LT were excluded from the analysis (group IIa), 12-hour lactate level was a significant predictor of survival in multivariate analysis for POD and non-POD patients (POD: $OR_{10\%}$: 1.26; 95% CI: 1.09- 1.45; $P \leq 0.001$; non-POD: $OR_{10\%}$: 1.32; 95% CI: 1.01-1.71; $P =$

0.009). The 12-hour lactate level ($OR_{10\%}$: 1.24; 95% CI: 1.01-1.54; P = 0.042) and admission bilirubin levels ($OR_{10\%}$: 1.19; 95% CI: 1.00-1.42; P = 0.032) were significant predictors of nonsurvival in non-POD patients (group II). Figure 2 shows how the estimated probability of nonsurvival (group II and IIa patients) varies with blood lactate concentrations (note that the curve for the non-POD patients indicates the estimated probability when the bilirubin level is not taken into account). At the same blood lactate level the aetiology of FHF greatly influenced the predicted probability, with higher probability of nonsurvival among non-POD patients. For the POD subgroup (Fig. 2; curve c), if a predicted probability of > 0.25 is taken to predict nonsurvival and < 0.25 is taken to predict survival then the negative predictive value is 94%; i.e., 94% of patients with a probability < 0.25 do survive.

In the non-POD subgroup of patients, multivariate analysis showed that bilirubin and 12-hour blood lactate were both significantly associated with a poor prognosis. The results are represented as a probability contour plot as seen in Figure 3. If a patient has a 12-hour blood lactate and admission bilirubin values that are anywhere above or to the right of the P = 0.75 line, their predicted probability of nonsurvival (group II) is greater than 0.75. This cut-off value of 0.75 to predict nonsurvival has a sensitivity of 80%, specificity of 86%, positive predictive value of 94%, and negative predictive value of 60%. When the analysis was restricted to group IIa patients, a cut-off value of 0.50 of predicted probability of death had a sensitivity of 86%, specificity of 71%, positive predictive value of 75%, and negative predictive value of 83%. Figure 4 shows the receiver operating characteristic curves for 12-hour lactate, predicting nonsurvival from the stepwise logistic regression analyses for the POD and non-POD patients. The areas under the receiver operating characteristic curve were 0.86

and 0.86 for POD and non-POD patients, respectively (group II), and 0.82 and 0.92 for POD and non-POD patients, respectively (group IIa).

Discussion

In this retrospective study, we examined the prognostic significance of admission serum phosphate and early serial arterial blood lactate levels in a cohort of patients with FHF. We attempted to avoid potential biases by evaluating patients admitted to the intensive treatment unit over a period of less than 3 years, comparing patients who survived with medical management only (group I) with those who failed to survive spontaneously (group II) and with those who died without undergoing LT (group IIa patients). We avoided the comparison against KCH criteria, as these criteria are validated (10) and used in our unit for selection of LT candidates.

The possible prognostic value of serum phosphate measurements has been the subject of several recent studies. Early hyperphosphatemia in severe POD-induced hepatotoxicity had been reported to be significantly associated with a poor prognosis.(17) Issues raised from this study regarding the relatively small number of patients with FHF,(21) and the relatively low prevalence of patients who died or underwent LT,(22) complicate the interpretation of this interesting observation. In a retrospective study by Chung et al.,(6) the prognostic significance of serum phosphate was investigated in 38 patients with FHF. They reported hyperphosphatemia as a predictor of mortality, and when used alone, was equivalent to KCH criteria. However, the study did not specify the timing of serum phosphate measurements, or if there was correction of hypophosphatemia. Baquerizo et al.(18) studied 112 patients with FHF retrospectively and found that hyperphosphatemia

was a predictor of poor recovery, whereas hypophosphatemia and early phosphate administration was associated with a good prognosis.

In our study, the relative hyperphosphatemia on admission was a significant predictive marker of poor outcome within the entire cohort of patients with FHF. However, when we used admission phosphate levels to predict outcome within the POD and non-POD subgroups, it was not useful. Our results are compatible with a recent report from Edinburgh, which found serum phosphate to be an unreliable predictor of outcome in POD-induced hepatotoxicity.(23)

Arterial blood lactate measurements in POD-induced FHF has been proposed to improve the speed and accuracy of selection of LT candidates.(14) In this series from KCH, blood lactate levels alone, with cut-off values of 3.5 mmol/L early after admission and 3.0 mmol/L after adequate resuscitation, identified patients with poor prognosis early with equivalent accuracy to KCH criteria. The prognostic significance of high admission blood lactate with albumin and pyruvate in non-POD-induced FHF has been addressed recently by another group from the UK.(24) The novel technique used for the measurement of admission lactate levels in this study (nuclear magnetic resonance spectroscopy) may limit its practical application.

Our findings are in keeping with those in previous reports regarding the prognostic significance of arterial blood lactate levels in the setting of FHF.(14) All our patients had been treated at a referring hospital prior to intensive treatment unit transfer. It is interesting to note that admission arterial blood lactate concentrations on univariate analysis were significantly different between survivors and nonsurvivors in the POD and non-POD subgroups, whereas arterial pH, serum creatinine, and prothrombin time were not (although there was a trend towards a lower arterial pH in the POD

nonsurvivors). It is not known whether this reflected earlier arrival to our institution, or earlier treatment with N-acetyl-cysteine and fluid resuscitation at the referring hospital. It is also recognized that with modern intensive care management of FHF patients, there is sometimes a delay in fulfilling KCH criteria following admission and measurement of lactate levels may identify patients earlier who have a poor prognosis.(14)

On multivariate analysis, post admission 12-hour blood lactate was a significant predictor of survival among POD patients, along with admission bilirubin in the non-POD group. We propose clinically applicable curves created from logistic regression analysis that can predict poor outcome in both groups. When we applied the lactate criteria proposed by Bernal et al.(14) to our patients, it had a substantially lower performance in predicting outcome. The blood lactate cut-off value of 3.0 mmol/L after resuscitation had a predicted probability < 0.25 for nonsurvival in POD patients. Several factors may affect the clinical utility of specific cut-off values for arterial blood lactate measurements. First, hyperlactatemia in FHF results from a complex interplay between increased production (25) and reduced hepatic clearance.(13) Second, the timing and degree of fluid resuscitation needed to correct hypovolemia may vary between liver units. Our data has showed that serial blood lactate measurements were higher in nonsurvivors, with increasing significance in relation to time. This raises the importance of close monitoring of serial arterial blood lactate levels. The large values for the area under the receiver operating characteristic curve do suggest that 12-hour lactate is a good predictor of outcome in FHF and that it will be a useful addition to the KCH criteria for identifying patients early who have a poor prognosis with medical management alone. It remains to be determined whether 12-hour postadmission arterial blood lactate measurements alone can be used as a

single marker to identify patients destined for LT. In addition, the prognostic value of 24-hour and 36-hour arterial blood lactate measurements needs to be explored in FHF.

In conclusion, serum phosphate concentration has limited clinical utility as a prognostic marker. We developed clinically applicable curves based on 12-hour lactate and admission serum bilirubin levels, which may be an adjuvant to the KCH criteria in prediction of the outcome in FHF. The proposed curves need to be validated in further prospective studies.

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Table 1. Univariate analysis of predictors of non-survival in the study cohort

Predictor*	Group I (n=48)	Group II (n=35)	P I v. II	Group IIa (n=16)	P I v. IIa
Age (y)	34 (31–37)	42 (38–47)	<0.01	46 (38–53)	<0.01
Gender (% female)	27 (56.3%)	21 (60.0%)	0.82	13 (81.3%)	0.08
Ethnic origin (% Caucasian)	47 (97.9%)	29 (82.9%)	0.038	15 (93.8%)	0.44
Aetiology (% POD)	41 (85.4%)	15 (42.9%)	<0.001	9 (56.3%)	0.031
Ventilated (% yes)	26 (55.1%)	29 (82.9%)	0.009	13 (81.3%)	0.08
Phosphate (mmol/L)	0.65 (0.54–0.77)	1.02 (0.77–1.36)	0.005	0.77 (0.47–1.26)	0.40
Lactate (mmol/L)	2.97 (2.49–3.53)	4.12 (3.29–5.17)	0.029	5.17 (3.60–7.41)	0.004
4 hour lactate	2.56 (2.15–3.04)	3.85 (3.05–4.86)	0.005	4.89 (3.47–6.89)	<0.001

8 hour lactate	2.35 (2.01–2.75)	3.50 (2.80–4.39)	0.004	4.48 (3.09–6.48)	< 0.001
12 hour lactate	2.25 (1.92–2.65)	3.81 (2.98–4.89)	<0.001	5.16 (3.60–7.40)	<0.001
Creatinine (μmol/L)	145 (121–173)	147 (122–177)	0.82	148 (110–200)	0.82
Bilirubin (μmol/L)	77 (61–97)	169 (124–230)	<0.001	137 (84–223)	0.022
pH	7.42 (7.40–7.44)	7.41 (7.36–7.46)	0.76	7.41 (7.32–7.50)	0.80
PT	35 (31–40)	42 (36–49)	0.12	36 (30–43)	1.0

*Data on admission; and lactate at 4, 8, and 12h post-admission .

Age and pH values are arithmetic means with 95% confidence limits in parentheses. All other values are geometric means with 95% confidence limits in parentheses. P values relate to t tests of the transformed data, except for age and pH where they relate to t tests of the untransformed data.

Table 2A. Dynamic variables assessment in POD patients (56 patients).

Predictors	Group I (n=41)	Group II (n= 15)	P I vs II	Group IIa (n=7)	P I vs II
Phosphate	0.63 (0.52–0.77)	0.93 (0.56–1.55)	0.08	0.70 (0.33–1.48)	0.70
Lactate	3.19 (2.62–3.89)	6.09 (4.15–8.93)	0.002	6.51 (3.85 11.00)	0.004
4 hour lactate	2.75 (2.28–3.31)	6.06 (4.35–8.45)	<0.001	6.18 (3.85–9.92)	<0.001
8 hour lactate	2.52 (2.13–2.99)	5.42 (3.88–7.56)	<0.001	5.60 (3.15–9.97)	<0.001
12 hour lactate	2.44 (2.06–2.90)	5.65 (4.18–7.63)	<0.001	5.89 (3.48–9.99)	<0.001
Creatinine	154 (127–188)	169 (124–230)	0.62	139 (90–214)	0.64
Bilirubin	67 (53–85)	74 (59–92)	0.66	73 (53–102)	0.75
PH	7.42 (7.39–7.44)	7.32 (7.23–7.42)	0.06	7.34 (7.22–7.47)	0.22
PT	36 (31–41)	49 (36–66)	0.036	37 (28–48)	0.82

Table 2B. Dynamic variables assessment in non-POD patients (27patients).

Predictors	Group I (n= 7)	Group II (n=20)	P I vs II	Group IIa (n=7)	P I vs II
Phosphate	0.77 (0.37–1.63)	1.11 (0.75–1.64)	0.27	0.91 (0.18–4.59)	0.71
Lactate	2.20 (1.68–2.28)	3.08 (2.48–3.82)	0.09	3.84 (2.24–6.61)	0.044
4 hour lactate	1.69 (1.07–2.67)	2.82 (2.20–3.61)	0.039	3.62 (2.10–6.23)	0.025
8 hour lactate	1.71 (1.14–2.55)	2.53 (2.01–3.18)	0.07	3.36 (2.05–5.50)	0.023
12 hour lactate	1.53 (0.99–2.38)	2.84 (2.04–3.96)	0.043	4.34 (2.33–8.12)	0.006
Creatinine	89 (65–122)	132 (104–169)	0.07	161(92–281)	0.043
Bilirubin	181 (99–330)	314 (234–422)	0.06	305 (154–604)	0.19
pH	7.43 (7.39–7.48)	7.48 (7.43–7.52)	0.29	7.49 (7.35–7.63)	0.39
PT	36 (22–60)	37 (32–44)	0.87	34 (25–47)	0.83

* pH values are arithmetic means with 95% confidence limits in parentheses. All other values are geometric means with 95% confidence limits in parentheses. P values relate to t tests of the transformed data, except for pH where they relate to t tests of the untransformed data.

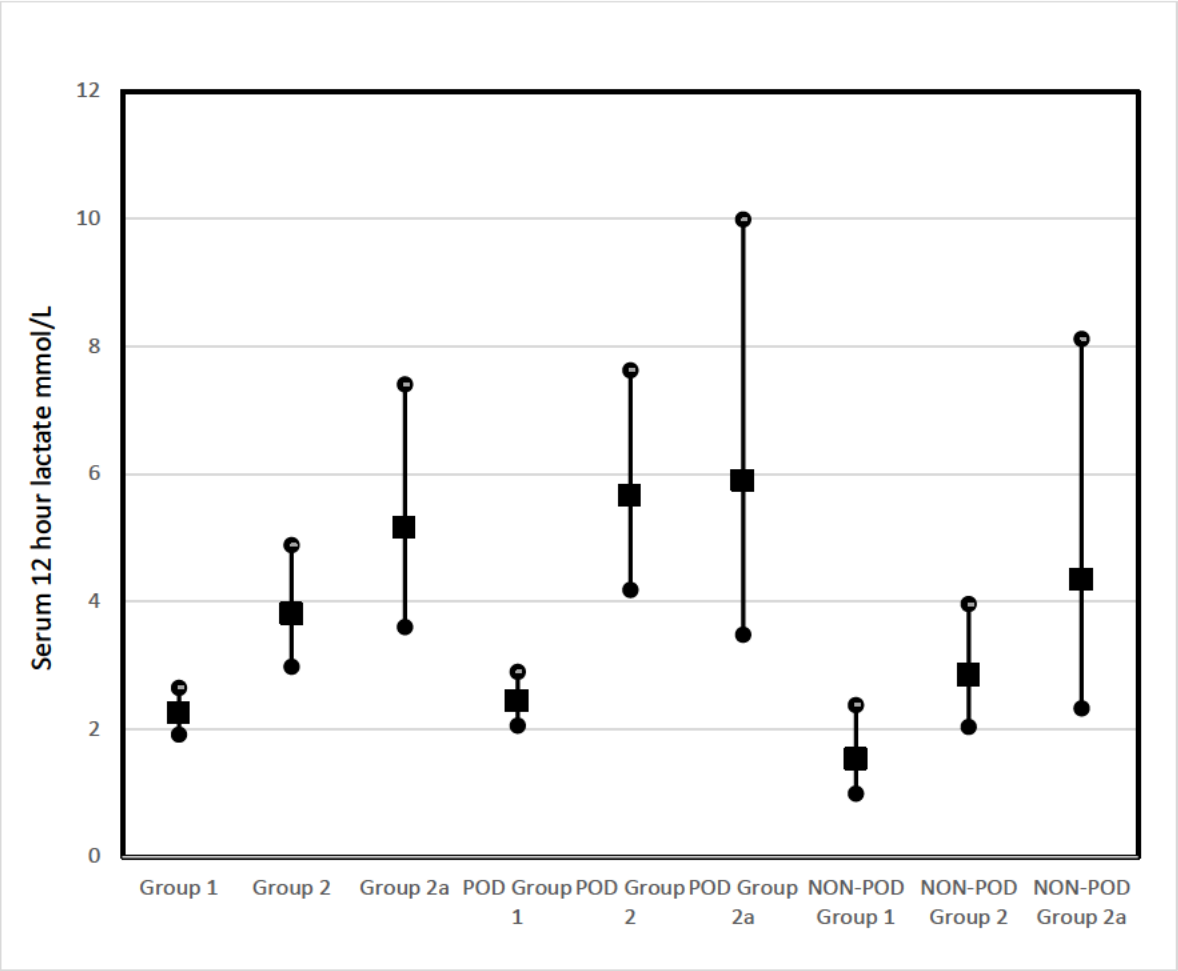


Figure 1. Arterial blood lactate concentrations (mmol/L) levels for the different clinical outcomes. Values shown are geometric means with corresponding 95% CIs.

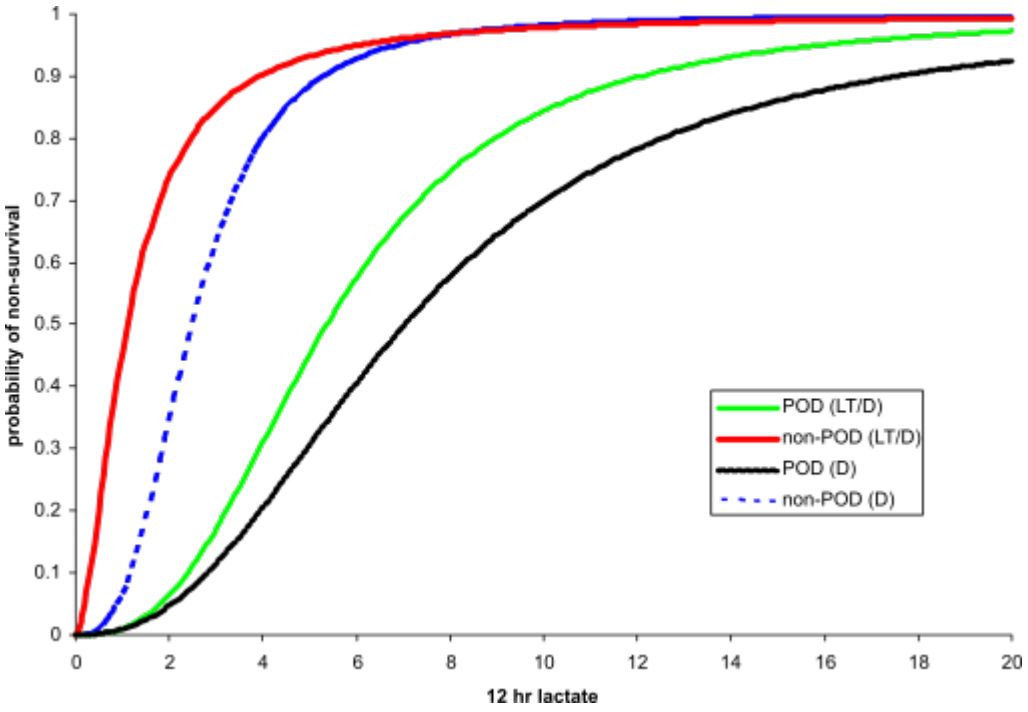


Figure 2. Predicted probability of nonsurvival based on 12-hour lactate value (POD and non-POD patients).

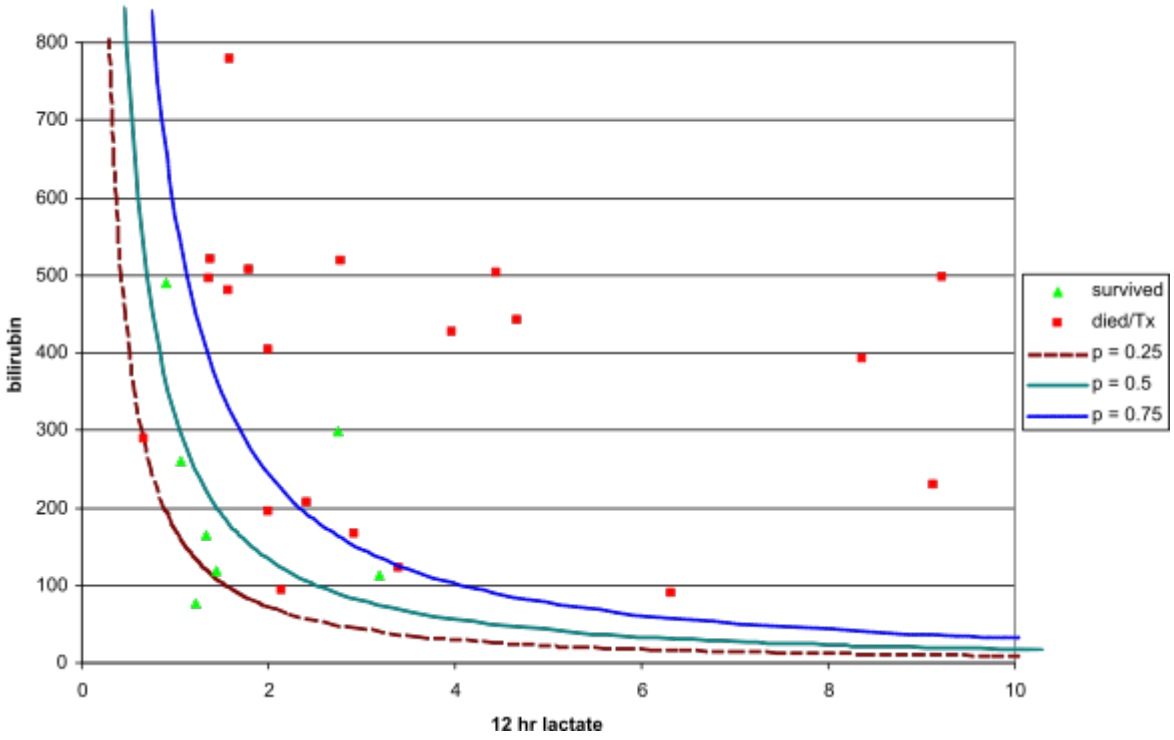
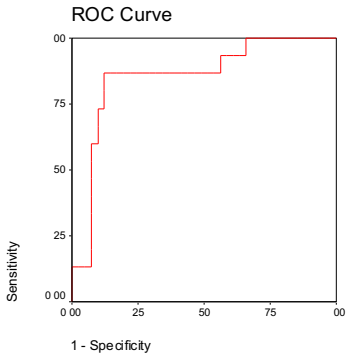
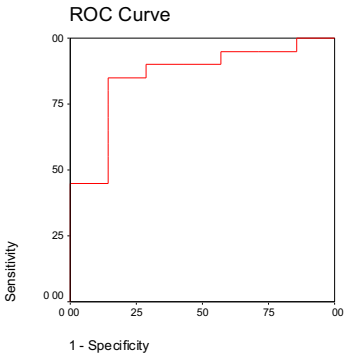


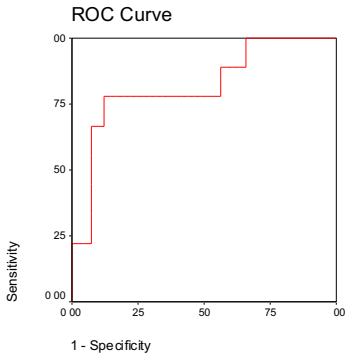
Figure 3. Graphical representation of results of logistic regression for non-POD patients. *P* is the predicted probability of liver transplantation or death (group II patients).



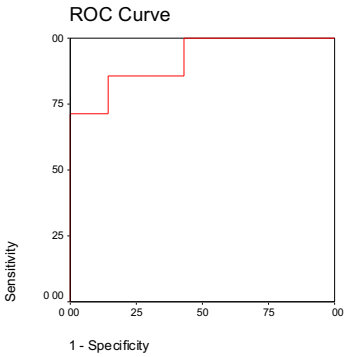
POD (including LT)



Non-POD (including LT)



POD (excluding LT)



Non-POD (excluding LT)

Figure 4. (A,C,D) The receiver operating characteristic curves for 12-hour lactate predicting nonsurvival for group II patients (A), and group IIa patients (C-D). (B) The receiver operating characteristic curve for the combination of 12-hour lactate and admission bilirubin levels predicting nonsurvival in the non-POD subgroup (group II patients).

Development and validation of a dynamic outcome prediction model for paracetamol-induced acute liver failure: a cohort study

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Abstract

Background

Early, accurate prediction of survival is central to management of patients with paracetamol-induced acute liver failure to identify those needing emergency liver transplantation. Current prognostic tools are confounded by recent improvements in outcome independent of emergency liver transplantation, and constrained by static binary outcome prediction. We aimed to develop a simple prognostic tool to reflect current outcomes and generate a dynamic updated estimation of risk of death.

Methods

Patients with paracetamol-induced acute liver failure managed at intensive care units in the UK (London, Birmingham, and Edinburgh) and Denmark (Copenhagen) were studied. We developed prognostic models, excluding patients who underwent transplantation, using Cox proportional hazards in a derivation dataset, and tested in initial and recent external validation datasets. Mortality was estimated in patients who had emergency liver transplantation. Model discrimination was assessed using area under receiver operating characteristic curve (AUROC) and calibration by root mean square error (RMSE). Admission (day 1) variables of age, Glasgow coma scale, arterial pH and lactate, creatinine, international normalised ratio (INR), and cardiovascular failure were used to derive an initial predictive model, with a second (day 2) model including additional changes in INR and lactate.

Findings

We developed and validated new high-performance statistical models to support decision making in patients with paracetamol-induced acute liver failure. Applied to the derivation dataset (n=350), the AUROC for 30-day survival was 0·92 (95% CI

0·88–0·96) using the day 1 model and 0·93 (0·88–0·97) using the day 2 model. In the initial validation dataset (n=150), the AUROC for 30-day survival was 0·89 (0·84–0·95) using the day 1 model and 0·90 (0·85–0·95) using the day 2 model. Assessment of calibration using RMSE in prediction of 30-day survival gave values of 0·1642 for the day 1 model and 0·0626 for the day 2 model. In the external validation dataset (n=412), the AUROC for 30-day survival was 0·91 (0·87–0·94) using the day 1 model and 0·91 (0·88–0·95) using the day 2 model, and assessment of calibration using RMSE gave values of 0·079 for the day 1 model and 0·107 for the day 2 model. Applied to patients who underwent emergency liver transplantation (n=116), median predicted 30-day survival was 51% (95% CI 33–85).

Interpretation

The models developed here show very good discrimination and calibration, confirmed in independent datasets, and suggest that many patients undergoing transplantation based on existing criteria might have survived with medical management alone. The role and indications for emergency liver transplantation in paracetamol induced acute liver failure require re-evaluation.

Introduction

Acute liver failure is a rare critical illness, and in many countries, including the UK, paracetamol-induced hepatotoxicity is its most frequent cause.(1,2) The illness may follow a rapidly progressive course with severe hepatic necrosis quickly followed by the development of encephalopathy, multiple extrahepatic organ failures, and death.(3) However, the condition is one in which there is substantial variation in clinical course. In some patients, recovery with medical therapy alone might be possible despite severe multiple organ failure, whereas in others survival might be

impossible without emergency liver transplantation.(4,5) The early and accurate evaluation of expected prognosis is key to effective management, to enable successful transplantation in the narrow window of opportunity for those who will benefit from it, and to avoid unnecessary surgery in those who will survive with medical therapy alone. Tools for prognostic evaluation are available, but their limitations are increasingly apparent.(6–8)

Prognosis in paracetamol-induced acute liver failure is most commonly assessed using the King's College Criteria. The criteria were derived from the analysis of patients managed in a single centre between 1973 and 1987 and have been in use to select transplant candidates for more than two decades.(6) Poor prognosis criteria include an arterial pH of less than 7.3 after volume resuscitation, or the combined findings of high-grade encephalopathy, creatinine of more than 300 μmol/L and international normalised ratio (INR) of 6.5 or higher occurring within a narrow timeframe.(6) Early meta-analysis of the diagnostic performance of the King's College Criteria in paracetamol-induced liver failure suggested high specificity but more limited sensitivity; introduction of arterial blood lactate concentration as a supplemental marker was proposed to address this issue.(7–9) However, the increasing success of non-transplantation medical therapies alone is likely to have affected the performance of the King's College Criteria in patients with paracetamol-induced liver injury because substantial improvements in survival with medical care alone have been made for many causes of acute liver failure in the past three decades, particularly cases resulting from paracetamol-induced hepatotoxicity.(3) These improvements in non-transplant outcomes have not been reflected in changes in recognised indications for emergency liver transplantation.

Experience has also shown practical issues in the use of the King's College Criteria. The criteria were intended for use in a transplant centre and not early after presentation in the emergency room where intravenous fluid resuscitation has not been undertaken and the effect of high blood levels of paracetamol might contribute to a reversible lactic acidosis.(10,11) The criteria perform less accurately in accidental or intentional staggered overdoses of the drug than after single timepoint deliberate ingestions, and are difficult to interpret because their component variables might be confounded by alteration by medical interventions.(12) Since the criteria provide a binary outcome prediction rather than a continuum of risk, their application can also be difficult, and they do not address a key clinical question in wait listed patients who show signs of improvement—is it better to remove them from the list or to proceed with transplantation?

In exploring the development of a novel model for the prediction of death without transplantation in patients with paracetamol-induced acute liver failure, we sought to address the limitations of the present King's College Criteria. By studying more recent cohorts we hoped to reflect the current outcomes of the illness and develop a model using readily available clinical variables and standardised definitions, with sequential assessment to detect changing prognosis over time.(13) Our objective was to develop a decision support tool that was simple to use and gave a continuous and updated estimation of risk of death rather than a static binary outcome prediction. We hoped to avoid the reference bias that might complicate studies of prognosis in acute liver failure by excluding patients who underwent transplantation, and to assess reproducibility and transportability by the use of several validation cohorts.

Methods

Study design and participants In this study, we included patients with severe paracetamol-induced hepatotoxicity managed at four specialist liver transplantation intensive care units in the UK and Denmark. In all cases, a history of drug ingestion or detectable blood paracetamol was present, with the exclusion of other causes of acute liver injury. Other inclusion criteria were an INR of 1.5 or higher and absence of a history and clinical or radiological findings of previous liver disease.

The primary derivation and initial internal validation test cohorts were derived from consecutive patients with severe paracetamol-induced hepatotoxicity who had not undergone transplantation and who were admitted during the period 2000–12 to the Liver Intensive Therapy Unit at King's College Hospital, London, UK. The external validation cohorts included patients who did not undergo transplantation admitted to the Rikshopitalet Liver Unit, Copenhagen, Denmark in 2011–13; the Scottish Liver Transplant Unit at Edinburgh Royal Infirmary, Edinburgh, UK in 2008–14; the intensive care unit at Queen Elizabeth Hospital, Birmingham, UK, in 2004–13; and the Liver Intensive Therapy Unit at King's College Hospital in 2012–14. Validating mortality in paracetamol-induced acute liver failure is rarely an issue since almost without exception death in patients who have not undergone transplantation occurs during a single hospital admission, most commonly during the first week in the intensive care unit, with rapid recovery seen in those who survive.(14)

A common approach to clinical management was applied in all units, with emergency liver transplantation considered in patients who fulfilled the standard King's College Criteria. Standard medical care applied has been detailed elsewhere.(3) Briefly, patients who developed encephalopathy at grade 3 or higher were intubated, sedated, and mechanically ventilated. Guided restoration of circulating volume was commenced immediately on admission and used invasive haemodynamic

monitoring. Coagulopathy was not supported unless active haemorrhage was present.(15) Norepinephrine was the primary vasopressor used and dobutamine the primary ionotropic agent with adjunctive use of intravenous low-dose hydrocortisone and vasopressin. Renal replacement therapy used continuous veno-venous haemofiltration. Indications for use of renal replacement therapy included not only those standard for patients with acute kidney injury with anuria but also for relative oliguria, metabolic stabilisation, and control of acidosis and hyperammonaemia. Sedation was achieved with fentanyl and propofol infusions, with rare use of paralysis. Treatment for intracranial pressure crises was with bolus intravenous mannitol, hypertonic saline, and increased sedation using thiopentone in refractory cases. Intravenous N-acetyl cysteine was administered to all patients with an infusion of 100 mg/kg every 24 h for a maximum of 5 days or until the INR was lower than 2.

Datasets and statistical methods

The derivation and initial validation patient datasets were taken from the King's College Hospital Liver Intensive Therapy Unit clinical database in which demographic, physiological, and laboratory variables are prospectively collected daily for all patients by specialist audit nurses. These variables included those required for the Sequential Organ Failure Assessment (SOFA) and Acute Physiology and Chronic Health Evaluation scores (appendix p 1). Fewer than 1% of cases had missing values.

We used standardised scores assessing level of consciousness and grade of encephalopathy using the Glasgow coma scale, and cardiovascular dysfunction by the SOFA cardiovascular component score (SOFA CVS; appendix p 2), with a score

of 3 or more considered to represent cardiovascular failure.(3,16,17) The Glasgow coma scale score was assessed in patients who had not received sedative agents, and we used the lowest score before their administration, except if signs of intracranial hypertension were present, where a score of 3 was assigned.(3,18) Survival time was from date of admission to date of death. Continuous variables are summarised as mean (SD) or median (IQR) and categorical data as count (percentage). Student's *t* test, Mann-Whitney *U*, and Wilcoxon signed-rank tests were used to test differences in continuous variables where appropriate, and the χ^2 test used for proportions. Multiple survival analyses were undertaken using Cox proportional hazards models to determine the prognostic and predictive value of demographic factors and clinical variables. All-cause mortality was the primary event studied and patients who underwent transplantation were excluded from model development and primary testing. Variable selection and model fitting were conducted through backward stepwise regression based on p values. The model started with a range of clinical variables of important prognostic value suggested by literature review (appendix p 1), and then went through extensive backward stepwise model and variable selection process. The final models consisted of variables most strongly associated with death in the multiple Cox proportional hazards regressions. The proportional hazards assumption for each covariate was tested using the scaled Schoenfeld residuals. The proportionality test showed that all the covariates followed the proportional hazards assumption ($p>0.05$).

To develop and validate the predictive survival models, we divided the King's College Hospital derivation set (n=350) and an initial validation set (n=150) with random case selection. Survival models using day 1 and day 2 were first built from derivation dataset separately and then validated in the validation dataset. The

performance and predictive accuracy of the models were assessed using receiver operating characteristic (ROC) analysis. The ROC curves and values were compared among different models and also between derivation and validation stages. Model calibration was assessed comparing observed and predicted survival using root mean square error (RMSE).(19) All tests were two-tailed, and p values lower than 0.05 were considered statistically significant. ROC values with 95% CIs for all the survival models and hazard ratios (HR) with 95% CIs for the chosen clinical variables were calculated. Statistical analyses were done with statistical software R, version 2.11.1, and SPSS™, version 22.0.0. All data were fully de-identified before exchange and analysis, and their use was approved by the Research Ethics Committee of King's College Hospital.

Role of the funding source

This study was supported by an unrestricted grant from the Foundation for Liver Research. The funding source had no role in study design, collection, analysis, or interpretation of the data, or in the writing of the report. Authors had access to the raw data from their individual centres and WB and YW to all de-identified datasets. The corresponding author had final responsibility for the decision to submit for publication.

Results

We included 500 consecutive patients who had not undergone transplantation with severe paracetamol induced hepatotoxicity admitted during the period 2000–12 to the Liver Intensive Therapy Unit at King's College Hospital in the primary derivation (n=350) and initial internal validation test cohorts (n=150). External validation cohorts included 151 patients admitted during the period 2011–13 from the Rikshopitalet

Liver Unit, 90 patients admitted in 2008–14 to the Scottish Liver Transplant Unit at the Edinburgh Royal Infirmary, 72 patients admitted in 2004–13 to the intensive care unit of Queen Elizabeth Hospital, Birmingham, and 99 patients treated at King's College Hospital Liver Intensive Therapy Unit between 2012 and 2014. Characteristics of the derivation and initial validation sets are shown in table 1, and features on admission of the external validation cohorts are shown in table 2. 78 (22%) of 350 patients in the derivation set died during their hospital stay; median day of death was 9 days (IQR 2–14) after admission. Patients who died were older and, on admission, had evidence of more severe liver dysfunction with higher INR and arterial lactate concentrations, and worse extra-hepatic organ failure with more severe encephalopathy, cardiovascular, and renal dysfunction than did survivors (appendix p 3). Of note, 49 (46%) of 106 patients with a Glasgow coma score of 9 or less on day 1 died versus 29 (12%) of 244 with a Glasgow coma score above this threshold (relative risk 3.9 [2.6–5.8]; $p<0.0001$).

After extensive model fitting and variable selection, admission (day 1) variables of age, Glasgow coma score, arterial pH and lactate, creatinine, INR, and SOFA cardiovascular failure were identified as the best clinical predictors. Hazard ratios for these component variables on admission are shown in table 3 and the predictive equation in the appendix p 4. Based on the day 1 model, we explored a day 2 model with additional changes in all clinical variables between day 1 and day 2, and, after backward stepwise variable selection, found only changes in blood lactate and INR to be significantly associated with survival. A dynamic day 2 survival model was thus further developed based on clinical variables on day 1 plus changes in blood lactate concentration and INR between day 1 and 2 to reflect the changing patterns in these crucial variables (table 3, appendix p 4). Exploration of models using data up to 7

days after admission did not show significant improvements in model performance above the dynamic day 2 model (data not shown).

When applied to the derivation set, AUROC for prediction of death using the day 1 model was 0.95 (95% CI 0.91–0.99) at 7 days, 0.94 (0.90–0.97) at 15 days, and 0.92 (0.88–0.96) at 30 days, and for the dynamic day 2 model was 0.96 (0.93–1.0) at 7 days, 0.95 (0.91–0.98) at 15 days, and 0.93 (0.88–0.97) at 30 days (figure 1). Assessment of calibration using RMSE in prediction of 30-day survival gave values of 0·1123 for the day 1 model and 0.1317 for the dynamic day 2 model for the derivation set.

35 (23%) of the 150 patients in the initial validation set died during their hospital stay. Using the day 1 model, AUROC for the prediction of death was 0·93 (95% CI 0·86–1·00) at 7 days, 0·91 (0·85–0·96) at 15 days, and 0·89 (0·84–0·95) at 30 days, and with the dynamic day 2 model was 0·94 (0·88–1·0) at 7 days, 0·91 (0·86–0·96) at 15 days, and 0·90 (0·85–0·95) at 30 days (figure 2). Assessment of calibration using RMSE in prediction of 30-day survival gave values of 0·1642 using the day 1 model and 0·0626 for the day 2 model (figure 2). Based on the proposed survival models, individual survival curves were calculated and updated during the first 2 days of intensive care unit admission (figure 3).

Characteristics of the four validation sets differed significantly from one another and from the primary cohorts. Median age of the Danish patients was higher than that of the other cohorts, and the Birmingham cohort had more severe acidosis, lactate, and creatinine, more severe encephalopathy, and higher mortality than the other cohorts (table 2). Overall, the day 1 single component variables were missing in 19 (5%) of

412 cases and day 2 variables in 141 (34%). Where day 2 INR and lactate variables were missing, values were categorised as showing no improvement.

AUROC and RMSE for the individual validation sets are shown in table 4. In the combined external validation sets, AUROC for 30-day survival was 0·91 (95% CI 0·87–0·94) for the day 1 model and 0·91 (0·88–0·95) for the day 2 model and RMSE was 0·079 for the day 1 model and 0·107 for the day 2 model (figure 4).

During the study periods of the derivation and validation sets, 116 patients underwent emergency liver transplantation: 84 at King's College Hospital, 23 at Queen Elizabeth Hospital, and eight at Edinburgh Royal Infirmary. Comparison of admission variables of patients who died with those who underwent transplantation showed significant differences, with patients who died being older and with higher arterial lactate concentrations, and lower Glasgow coma scores and INR values (appendix p 5). Using the day 1 model, median predicted 30-day survival for the cohort who underwent transplantation was 51% (95% CI 33–85), and in patients with severe hepatic encephalopathy (Glasgow coma score ≤9; n=70), predicted survival was 36% (18–73).

Discussion

We present the development and validation of high-performance statistical models to support decision making in the care of patients with paracetamol-induced acute liver failure. The models proposed show very good discrimination and calibration, confirmed on application to several independent external validation datasets with patients with a range of illness severity, and show substantial promise as prognostic tools. We developed models both on (day 1) and after (day 2) admission because their combined use provides practical support for key clinical decisions not

addressed by existing single time point prognostic criteria. In management of paracetamol induced acute liver failure the first 2 days are crucially important for the selection of transplant candidates; early prediction is required and is directly clinically relevant.(14) Experience has shown that the clinical condition of patients with paracetamol-induced liver failure might change rapidly after admission and initial therapy—with improvement in some patients and deterioration in others. The two-stage assessment of prognosis enables quantitation of this change, objectively reassessing prognosis in patients who show signs of improvement. Assessment of test performance showed both models to have high discrimination but importantly calibration was improved in the day 2 model. This sequential approach, derivation from recent patient cohorts, and use of novel important prognostic variables represent clear advances beyond the original King's College Criteria.

There has been criticism in the statistical literature of the use of stepwise regression for model fitting relating to its biases and suboptimal results. However, we used stepwise regression for its simplicity and in conjunction with expert opinion to decide which variables to include in the model. Our predictive models were robustly trained and validated on both internal and external datasets, which resulted in excellent model performance. Nevertheless, there are alternatives to stepwise regression, which include partial least squares regression and least absolute shrinkage and selection operator. These alternatives can overcome some of the shortcomings of stepwise regression, although they have their own limitations.(20) We will explore these novel methods in future research to further enhance the performance of our predictive models.

We chose to exclude patients who underwent emergency liver transplantation from model derivation and initial validation. Valid criticism can be made both of the

inclusion or exclusion of patients who underwent transplantation in this process, and both have been used in the scientific literature. Our main concern was risk of reference bias in view of the drastic improvements in survival with medical management alone in patients with paracetamol-induced liver failure, and the possibility that detail of transplantation practice varied between centres.(3,5) Although this approach could introduce changes in cohort composition, it is unlikely to have yielded a less unwell sample since, in key respects, those who later died were more severely ill. Predictions of a survival model derived in a population that did not undergo transplantation applied to one in which patients underwent transplantation should be interpreted with caution. However, this survival estimate is derived from the analysis of a population of patients with illness of the same cause, including cases that had similar acuity of illness and with a model that uses variables derived solely from the pre-transplant phase of illness.

When applying the day 1 model to patients who underwent transplantation during the study period, the median predicted survival was slightly more than 50% but fell to 36% when only those with severe encephalopathy were considered. These observations reinforce the key prognostic importance of development of hepatic encephalopathy and that transplantation should not be considered in the absence of high-grade encephalopathy. Importantly, a substantial proportion of patients who underwent transplantation might have survived with medical management alone. These findings quantify the substantial improvements in survival independent of liver transplantation that have occurred over time, and suggest that transplantation as an intervention in paracetamol-induced acute liver failure requires comprehensive re-evaluation.(3,21)

In considering the application of these models in a clinical setting, limitations to their potential practical use should be assessed. In other critical illness scoring systems, use of individual patient outcome prediction has historically been with caution, because the primary purpose of most such scores was for group outcome assessment and quality assurance.(22–24) However, there is clear precedent within hepatology for use of scores, including Model for End-Stage Liver Disease (MELD), for individual decision making in relation to transplantation—and in the widely accepted use of the King's College Criteria and other poor prognosis criteria for selection of patients with acute liver failure for emergency liver transplantation.(24,25)

We see these models as tools to quantify the risk of death and support expert clinical judgment and decision making; experience in other areas of acute and critical care medicine suggests that combining an objective prognosis measure with a physician's clinical estimate results in the most accurate assessment of actual prognosis.(23,26,27) Further, the sequential assessment of our models provide is likely to be of benefit because, in critically ill patients with and without liver disease, trends in illness severity provide additional prognostic value over single static determinations.(23,28–30) Rather than considering a single timepoint survival estimate, transplantation wait listing decisions might best be made from observations of the dynamic course of the illness. An obvious issue is that of the threshold of estimated survival that should trigger addition of a patient to the waiting list; a figure of 25% demonstrated in recent reports of early transplantation for acute alcoholic hepatitis provides a useful parallel.(31) A website has been developed to use the new paracetamol prediction models in a form that is accessible and easy to use, and

whose output will provide real-time estimates of expected survival, while accumulating a further prospective confirmatory validation set.

In developing these models we deliberately chose not to rely on the reported timing of drug ingestion in our patient selection and survival modelling. In practice, this information is often inconsistent or unavailable and, in the case of overdoses, staggered over days, making definition of a specific timepoint of ingestion impossible. Even without reliance on this information, the model functioned well. However, it is important to recognise that these patients were assessed in liver transplantation centres usually days after drug ingestion, with established and clinically significant hepatic necrosis and after receiving initial resuscitative measures at their receiving hospitals before transfer. Use of the models to predict survival in patients soon after overdose or at early after fi rst presentation has not been assessed.

These models were primarily designed to identify patients whose survival would be enhanced by emergency liver transplantation. However, identification as having a poor prognosis does not necessarily mean that survival will be improved by liver transplantation. The models are designed to predict survival without transplantation but not survival after surgery, when factors not considered in our model are of prognostic importance.(32) In many cases contraindications to emergency liver transplantation might be present, and here the models might rather serve to guide patients and family members in the expected outcome of illness.

Contributors

The study was conceived by WB and JW. YW and WB developed the predictive model and WB wrote the first draft of the paper. WB, JM, NM, AE, DH, KS, and FSL

contributed data, and all authors contributed in detail to the writing of the final version of the report.

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Table 1. Demographics, admission clinical and laboratory findings and outcome of Training and Test Cohorts.

		Training		Test		p
n		350		150		
Age (years)		37 (27-48)		36 (26-47)		0.38
Gender (F)		188 (54%)		94 (63%)		0.06
INR		4.0 (2.6-6.2)		3.8 (2.8-6.0)		0.40
Bilirubin (μMol/l)		68 (44-97)		72 (44-104)		0.44
AST (IU/l)		5776 (2601-9809)		5215 (2089-9315)		0.51
Creatinine ((μMol/l)		162 (92-267)		172 (93-281)		0.75
Arterial pH		7.40 (7.31-7.4)		7.4 (7.31-7.43)		0.29
Arterial Lactate (mmol/L)		2.8 (1.9-4.5)		2.6 (1.8-4.9)		0.32
HE Grade ≥3		149 (43%)		64 (43%)		0.98
Glasgow Coma Score		13 (8-15)		14 (8-15)		0.88
CVS Failure (n (%))		83 (24%)		35 (23%)		0.93
Mean Arterial Pressure (mmHg)		74 (63-95)		70 (61-90)		0.32
Mortality (%)		78 (22%)		35 (23%)		0.80
Day 2						
INR		3.0 (2.1-4.6)		3.0(2.2-4.9)		
Arterial Lactate (mmol/L)		2.0 (1.4-3.2)		2.1(1.4-3.2)		

Note; INR; International Normalised Ratio, AST; Aspartate Transaminase, HE; Hepatic Encephalopathy.

Table 2. Demographics, admission laboratory findings and outcome of validation sets.

	Copenhagen	Edinburgh	Birmingham	Kings	All
n	151	90	72	99	412
Age (years)	52 (36-61)	35 (27-46)	40 (31-46)	39 (30-50)	42 (31-53)
Gender (F)	93 (61%)	49 (54%)	39 (54%)	59 (59%)	240 (58%)
INR	3.0 (2.2-4.3)	5.3 (3.9-7.2)	4.9 (3-7.1)	6.2 (3.8-9.7)	3.9 (2.7-6.0)
Arterial pH	7.42 (7.36-7.46)	7.41 (7.31-7.47)	7.29 (7.19-7.38)	7.40 (7.35-7.45)	7.40 (7.30-7.45)
Creatinine (µmol/L)	82 (56-153)	119 (73-216)	186 (96-297)	146 (76-273)	118 (66-236)
Arterial Lactate (mmol/L)	2.6 (1.7-4.9)	2.9 (1.8-5.2)	5.6 (3.9-10.9)	3.3 (2.3-7.3)	3.5 (2.1-7.1)
Glasgow Coma Score	15 (8-15)	15 (14-15)	9 (9-10)	14 (9-15)	14 (9-15)
CVS Failure (SOFA ≥ 3)	26 (17%)	17 (19%)	57 (79%)	59 (60%)	159 (39%)
Mortality (%)	23 (15%)	13 (14%)	36 (50%)	27 (27%)	99 (24%)

Note; INR; International Normalised Ratio.

Table 3 (a). Component variables and adjusted hazard ratios for admission (D1) predictive model in the Training dataset (n=350).

		Hazard Ratio	95% CI		p
Age (5yrs)		1.07	(0.17-0.18)		0.17
Day 1					
CVS Failure		2.41	(1.39-4.17)		0.0018
Glasgow Coma Score		0.90	(0.84-0.98)		0.009
Arterial pH		0.06	(0.01-0.61)		0.018
Log (Creatinine (per 10 units))		1.74	(1.12-2.7)		0.013
Log (INR)		1.53	(1.04—2.24)		0.029
Sqrt (Arterial Lactate)		2.01	(1.53-2.63)		<0.0001

Table 3 (b). Component variables and adjusted hazard ratios for Dynamic (D2) predictive model in the Training dataset (n=350).

		Hazard Ratio	95% CI		p
Age (5yrs)		1.07	(0.17-0.18)		0.17
Day 1					
CVS Failure		3.14	(1.8-5.49)		<0.0001
Glasgow Coma Score		0.90	(0.83-0.97)		0.005
Arterial pH		0.09	(0.01-0.95)		0.044

Log (Creatinine(per 10 units))		1.70	(1.08-2.67)		0.022
Log (INR)		1.97	(1.33—2.91)		<0.0001
Sqrt (Arterial Lactate)		1.78	(1.37-2.32)		<0.0001
Day 2					
Lower Arterial Lactate		0.31	(0.13-0.69)		0.0045
Lower INR		0.54	(0.29-0.99)		0.046

Table 4. Diagnostic test performance of D1 and D2 models for predicting 30-day survival applied to individual and combined external validation sets.

		D1 Model				D2 Model		
Set	n	AUROC	(95% CI)	RMSE		AUROC	95% CI	RMSE
Copenhagen	151	0.93	(0.88-0.98)	0.140		0.94	(0.87-1.00)	0.208
Edinburgh	90	0.88	(0.79-0.96)	0.281		0.89	(0.81-0.97)	0.239
Birmingham	72	0.84	(0.74-0.94)	0.053		0.83	(0.73-0.93)	0.111
Kings	99	0.92	(0.85-0.99)	0.117		0.93	(0.86-0.99)	0.185
Combined	412	0.91	(0.87-0.94)	0.079		0.91	(0.88-0.95)	0.107

Note: AUROC; Area Under Receiver Operating Characteristic Curve, RMSE; Root-Mean-Square Error.

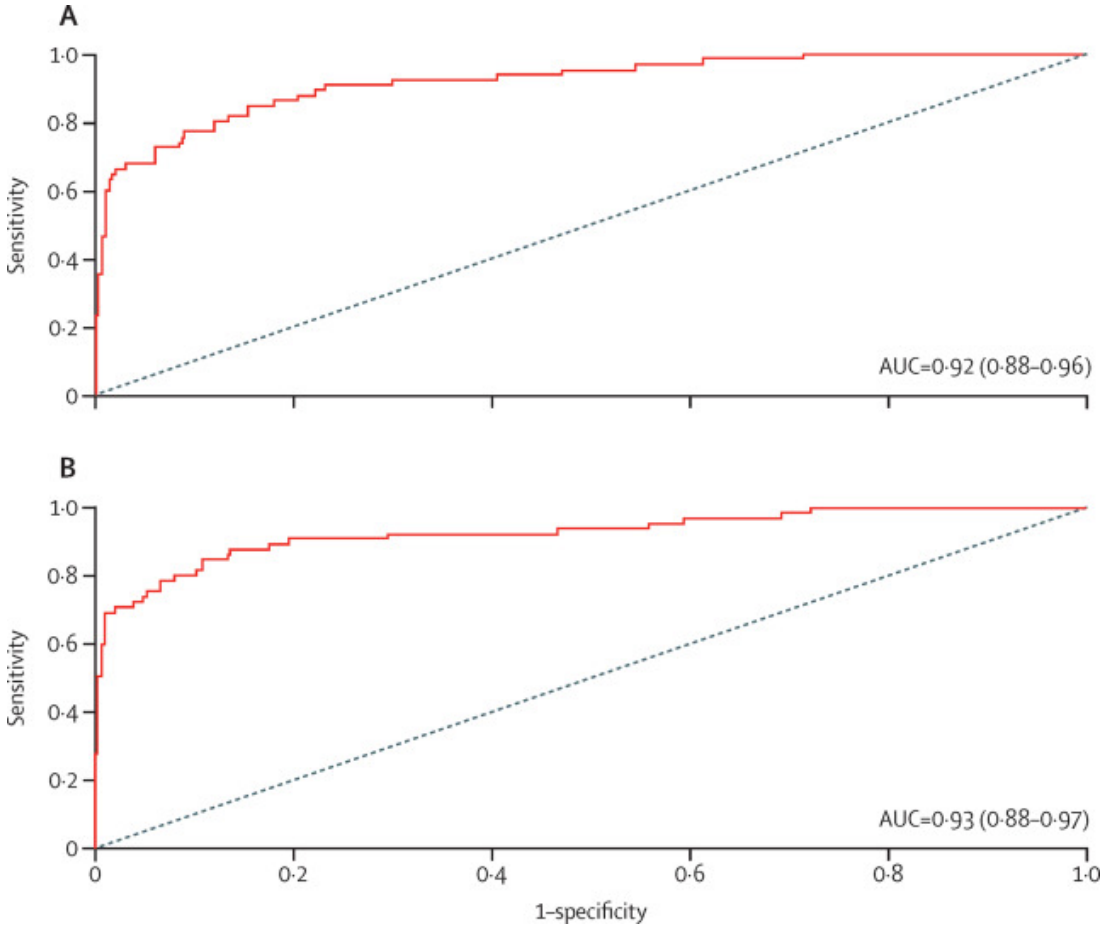

Figure 3 Area under receiver operating characteristic curve for the derivation dataset

(A) Day 1 model and (B) day 2 model. Data from the King's College Hospital derivation set (n=350).

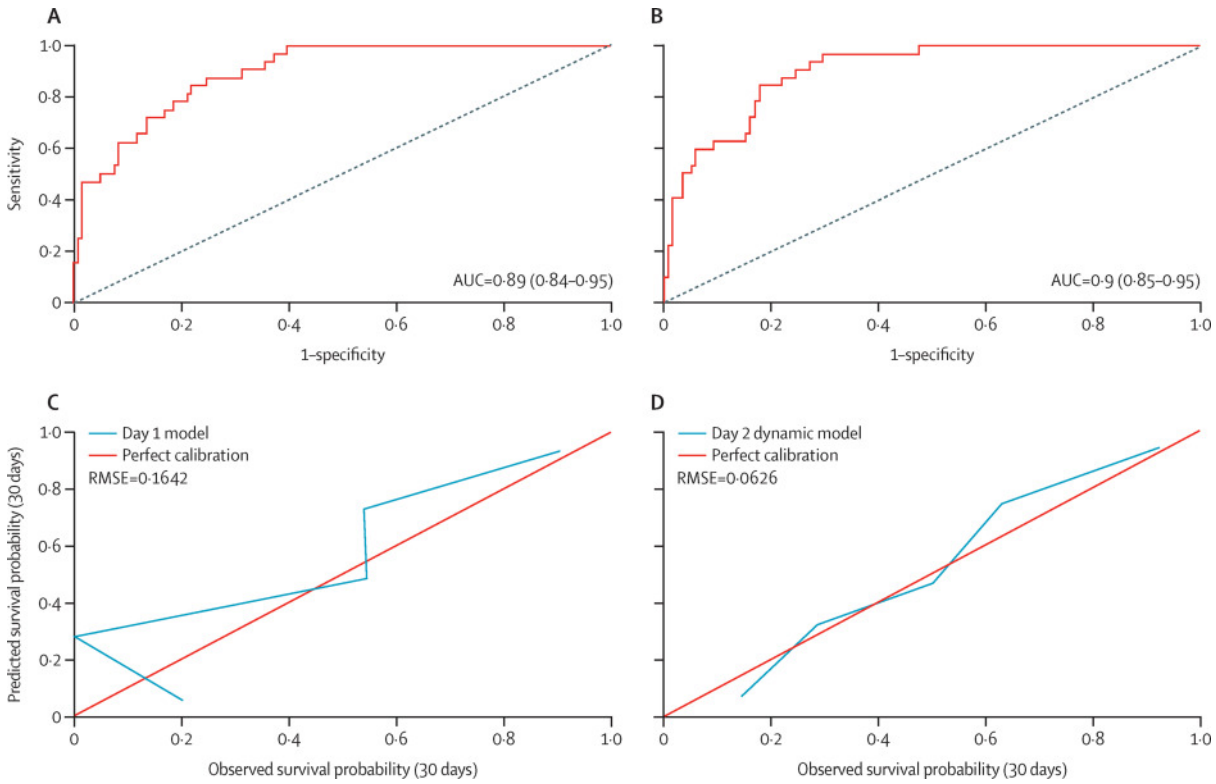

Figure 4: Discrimination and calibration of day 1 and day 2 models in the initial validation dataset

(A) Area under receiver operating characteristic curve (AUROC) for the day 1 model. (B) AUROC for day 2 model. (C) Calibration curve for the day 1 model. (D) Calibration curve for day 2 model. Data from the King's College Hospital initial validation cohort (n=150). RMSE=root mean square error.

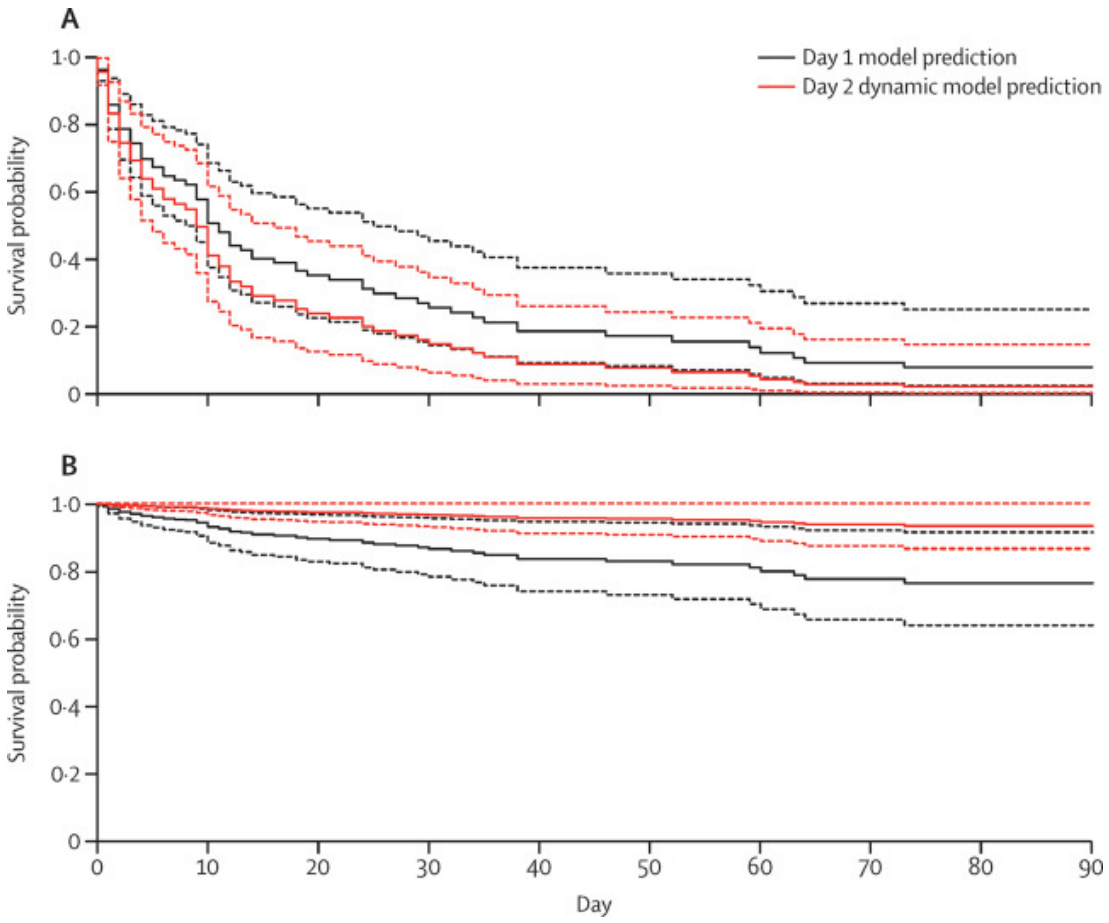

Figure 5: Predicted survival curves by day 1 and day 2 models for King's College Hospital patients

(A) Died at 23 days. (B) Patient survived to hospital discharge. Broken lines represent 95% CI.

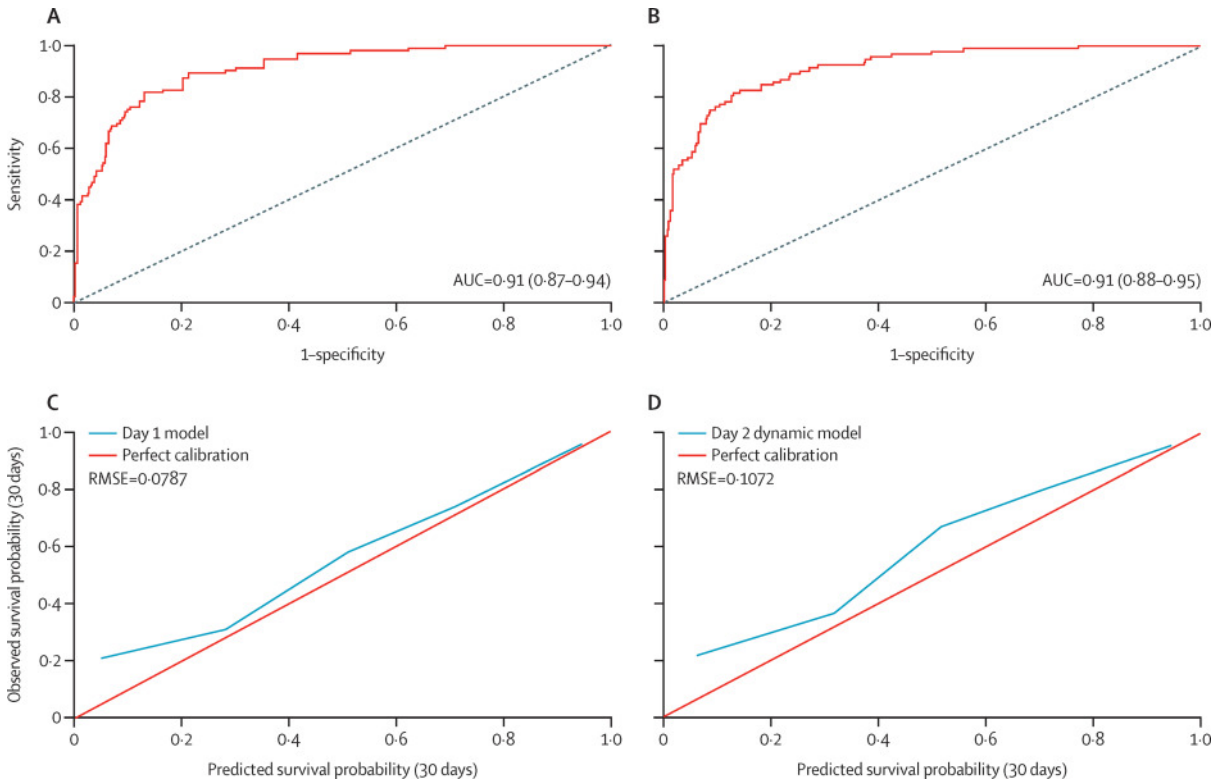

Figure 6: Discrimination and calibration of day 1 and day 2 models in combined external validation sets

(A) Area under receiver operating characteristic curve (AUROC) for day 1 model. (b) AUROC for day 2 model. (C) Calibration curve for day 1 model. (D) Calibration curve for day 2 model. Data from combined external validation datasets (n=412).

Appendix

Supplemental Materials 1.

Variables assessed in predictive model derivation:

Age

Gender

Heart rate

Mean Arterial Pressure

Systolic Blood Pressure

Diastolic Blood Pressure

SOFA Cardiovascular Failure

Respiratory rate

PaO_2/FiO_2 ratio

$PaCO_2$

Arterial pH

Arterial $[HCO_3]$

Arterial [lactate]

Blood concentrations:

Sodium

Potassium

Creatinine

Urea

Leucocyte count

Platelet count

INR

Bilirubin

Aspartate Transaminase

Albumin

Glucose

Glasgow Coma Scale

Urine Output

Supplemental Materials 2.

Predictive Equations for D1 and D2 Models

Predictive equation for D1 model:

Survival function (integration of hazard function over time 0 to t) is used to calculate the survival probability *S(t)* for any case at time t:

Based on the estimation results (Hazard ratios) from the training dataset, we were able to calculate the survival probability for any patient at time t:

$$S(t) = S_0(t)^K$$

$$K \quad e\text{^}PI \quad \llbracket \exp(b \mid age * age + b\ cardio * Cardio + b\ GCS * GCS + b\ pH * pH + b\ creatin$$
$$* log(creatin) + b\ INR * log(INR) \ \llbracket + b \rrbracket \ lactate * \sqrt{}(2\&lactate))$$
$$1.07 \rrbracket\ \text{^}age + \llbracket 2.41 \rrbracket\ \text{^}cardio + \llbracket 0.90 \rrbracket\ \text{^}GCS + \llbracket 0.06 \rrbracket\ \text{^}pH$$
$$+ \llbracket 1.74 \rrbracket\ \text{^}\log(creatin) + \llbracket 1.53 \rrbracket\ \text{^}\log(INR) + \llbracket 2.01 \rrbracket\ \text{^}\sqrt{}(2\&lactate)$$

$S_0(t)$ can be estimated through the baseline cumulative hazard *$H_0(t)$* and need help of statistical software.

Predictive equation for D2 model:

Based on the estimation results (Hazard ratios) from the training dataset, we were able to calculate the survival probability for any patient at time t:

$$S(t) = S_0(t)^K$$

$$K \quad e\text{^}PI \quad \llbracket \exp(b \mid age * age + b\ cardio * Cardio + b\ GCS * GCS + b\ pH * pH + b\ creatin$$
$$* \log(creatin) + b\ INR * \log(INR) \ \llbracket + b \rrbracket \ lactate * \sqrt{}(2\&lactate)$$
$$+ b\ dynamiclactate * (dynamiclactate) + b\ dynamicINR * (dynamicINR))$$
$$1.07 \rrbracket\ \text{^}age + \llbracket 3.14 \rrbracket\ \text{^}cardio + \llbracket 0.90 \rrbracket\ \text{^}GCS + \llbracket 0.09 \rrbracket\ \text{^}pH$$
$$+ \llbracket 1.70 \rrbracket\ \text{^}\log(creatin) + \llbracket 1.97 \rrbracket\ \text{^}\log(INR) + \llbracket 2.01 \rrbracket\ \text{^}\sqrt{}(2\&lactate)$$
$$+ \llbracket 0.31 \rrbracket\ \text{^}((dyamiclactate)) + \llbracket 0.54 \rrbracket\ \text{^}((dynamicINR))$$

$S_0(t)$ can be estimated through the baseline cumulative hazard $H_0(t)$ and need help of statistical software.

Supplementary Materials 3. Comparison of demographics and admission laboratory findings of patients who died or underwent transplantation.

		Died		Transplanted	p
n		185		116	
Age (years)		44 (35-54)		33 (24-40)	<0.001
Gender (F)		104 (56%)		75 (65%)	0.15
INR		5.0 (3.3-8.7)		6.9 (4.4-10)	0.003
Arterial pH		7.27 (7.15-7.38)		7.28 (7.20-7.38)	0.39
Glasgow Coma Score		8 (6-10)		9 (7-12)	0.005
Creatinine (μmol/L)		200 (128-282)		197 (129-298)	0.96
Arterial Lactate (mmol/L)		8.1 (4.3-12.7)		5.9 (4-9.7)	0.01
CVS Failure		151 (82%)		89 (77%)	0.30

Note: Note; INR; International Normalised Ratio.

Chapter 4 Management of ALF

The effect of molecular adsorbent recirculating system on pathophysiological parameters in patients with acute liver failure

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Abstract

Objective: To investigate the effect of the molecular adsorbent recirculating system (MARS) on physiological variables in patients with acute liver failure. Design: A prospective, observational study of MARS in addition to standard medical therapy in the management of patients presenting with acute liver failure.

Patients: Ten consecutive patients admitted with acute liver failure with a grade III or IV hepatic encephalopathy. MARS therapy was used for 8 hours on 2 consecutive days. Standard monitoring included the use of a pulmonary artery catheter and an intracranial pressure monitor.

Results: During the first MARS treatment there was a significant increase in systemic vascular resistance index (SVRI) from 1114 ± 196 to 1432 ± 245 dyne s^{1} cm^{5} m^{2} with a reduction in cardiac index from 5.5 ± 0.6 to 4.2 ± 0.4 l min^{1} m^{2}. The changes were maintained between the start of the first and second sessions but not to the end of second. Significant clearance of urea and creatinine was observed. Intracranial pressure did not change during the treatments. Overall mortality was 70%.

Conclusions: MARS therapy was well tolerated, with significant increases in vascular tone during the first session. This increase was not sustained over the duration of the study with a return to baseline values by the end of the second session. Based on our experience we cannot recommend the routine use of MARS therapy in acute liver failure outside of a clinical trial.

Keywords: Acute liver failure · Albumin dialysis · Molecular adsorbent recirculating system · Paracetamol

Introduction

Acute liver failure (ALF) is a devastating syndrome which triggers a cascade of events often leading to multiple organ failure and death [1, 2]. Despite recent advances in intensive care and organ support mortality rates remain high. In patients with high-grade encephalopathy the chances of survival are less than 15% with medical management alone [2, 3]. Early deaths in ALF are often caused by cerebral oedema or unsupportable cardiovascular collapse [4, 5]. In addition to a failure of synthetic function the clinical syndrome of acute liver failure is caused by a reduction in its excretory capacity and the accumulation of circulating toxins. It is thought that this leads to hepatic encephalopathy and reduced peripheral vascular tone, resulting in a hyperdynamic circulation with systemic hypotension, renal failure and cerebral oedema. Conventional blood purification techniques such as renal dialysis clear small water soluble molecules. The molecular adsorbent recirculating system (MARS), however, is an albumin-based dialysis system that removes both protein-bound water-insoluble and water-soluble molecules [6, 7]. It has been developed as a non-biological liver support system designed to enhance the excretory function of a failing liver. Furthermore investigators have demonstrated the removal of vasoactive substances which are thought to be responsible for the changes in measured haemodynamic parameters [8, 9].

The efficacy of MARS therapy in patients with acute decompensation of chronic liver disease has been extensively studied [10, 11, 12, 13, 14, 15]. There are, however, few data assessing the role of MARS in patients with ALF. Hence the aim of this

study was to systematically examine the impact of MARS on the physiology of patients presenting with ALF [16].

Materials and methods

Patients

Ten consecutive patients with grade III or IV hepatic encephalopathy were recruited into the study (median age 43 years, range 19– 62; six women, four men). All patients were assessed for transplantation, and the decision to transplant was independent of the patient's participation in this study. The local research ethics committee approved the study in accordance with the Declaration of Helsinki (1989). Due to the nature of the illness consent was precluded. Written informed assent was obtained from the next of kin. The patients' clinical and biochemical data before the first MARS treatment are shown in Table 1. Eight of the patients had paracetamol induced hepatotoxicity, one had seronegative (non-A to E) hepatitis and one liver failure induced by an idiosyncratic drug reaction to isoniazid. Patients were managed according to unit protocols; this included intubation of the trachea and ventilation to maintain normocapnia. All patients were sedated with morphine and midazolam. Standard monitoring included insertion of a peripheral arterial line and a pulmonary artery catheter. A subdural intracranial pressure (ICP) monitor (Integra Neurosciences) was inserted over the non-dominant frontal-parietal region following correction of any coagulopathy with fresh frozen plasma and platelets aiming for an international normalised ratio (INR) less than 2 and a platelet count greater than 100 $\times 10^9$/L. A retrograde venous catheter was inserted into an internal jugular vein to monitor jugular bulb oxygen saturation. The plasma sodium was maintained between 145 - 150 mmol/L with hypertonic saline. Haemofiltration is an integral part of MARS

therapy, and all patients received 2 L/h ultrafiltration and replacement during MARS therapy. Haemofiltration was continued between the two MARS sessions at the same rate.

Study protocol

All ten patients with high-grade encephalopathy associated with acute liver failure were fully resuscitated and stabilised prior to commencing the first scheduled session of MARS. The study protocol intended two MARS session per patient, each of 8 h duration, and a proposed interval between treatments of 8 h. Median interval between sessions was 10 h (range 6 - 12 h). Clinical and biochemical data were collected before the start of each session and at 2 - h intervals. Clinical data included mean arterial pressure, central venous pressure, pulmonary artery wedge pressure, jugular bulb venous saturations, cardiac index (CI), systemic vascular resistance index (SVRI) and intracranial pressure. The haematological and biochemical data included full blood count, clotting studies, liver function tests, lactate levels, urea and creatinine. Nonparametric statistical analysis of data before and after the MARS treatment was performed using Wilcoxon rank sum test. SPSS 11.0 for windows (SPSS, Chicago, Ill., USA) was used.

Results

Nine patients met either the King's College Hospital criteria (KCH) and / or lactate criteria for liver transplantation [2, 17]. Patient no. 5 had taken a staggered overdose with a late presentation and did not fulfil either set of criteria. However, he had established anuric renal failure, an INR of 4.2 and a lactate of 2.93 mmol/l after resuscitation. Two patients (nos. 1, 8) received liver transplantation while two patients (nos. 4, 9) deteriorated rapidly following admission and were deemed too

unstable to undergo transplantation. Transplantation was excluded in the remaining six patients due to significant psychiatric morbidity (nos. 5, 6, 7, 10), sepsis (no.2) or clinical improvement (no. 3). All ten patients had a first session of MARS, and eight patients survived to receive the second scheduled session. Two patients died during the initial treatment period (nos. 4, 9) and received MARS therapy for a duration of 4 and 8 h, respectively. Both died because of cardiovascular failure with unsupportable hypotension. Patient no. 10 also received inotropic support requiring 4 mg/h norepinephrine during the 1st h of MARS, reducing to 2.1 mg/h by the end of session 1 and 0.3 mg/h by the end of session 2. All patients were assessed for transplantation, and the decision to transplant was independent of the patient's participation in this study. Hence the timing of transplantation was unaffected by the study. Patient no. 1 died of multi-organ dysfunction and sepsis 3 months post-transplantation whilst patient no. 8 had primary non-function and died 3 days after the second transplant with multi-organ dysfunction. Two patients survived in the group of seven that met criteria but were not transplanted.

Comparison of haemodynamic variables before and following each MARS treatment revealed an increase in SVRI following the first session ($p=0.02$). CI was noted to decrease over the same time period ($p=0.01$; Table 2). The changes in SVRI and CI were sustained between the two sessions, remaining significantly different at the commencement of the second session (SVRI $p=0.012$, CI $p=0.05$). However, these changes were not sustained over the course of the second treatment session; the SVRI was not significantly different between the start of the study and the end of the second session ($p=0.09$; Fig. 1). There was a decline in SVRI during the second session, with the CI remaining stable both of which did not reach statistical significance. The increase in SVRI and reduction in CI after the first session

remained significant after excluding the two patients who died during the first treatment (p=0.03).

ICP did not change during MARS therapy (Fig. 2). There was a trend towards decreasing jugular venous saturations over the two sessions; however, this did not reach statistical significance (Fig. 3). There were two complications related to MARS, both due to perforation in the MARS membrane. Blood was noted within the MARS circuit and the treatment session was terminated at this point; this occurred without any clinical consequences. There was a significant decrease in haemoglobin (p=0.02), platelets (p=0.002), INR (p=0.004), urea (p=0.02) and creatinine (p=0.002) following MARS therapy (see Table 3).

Discussion

In this study we set out to systematically assess the effect of MARS therapy in ten patients presenting with ALF, of whom all but one fulfilled the KCH or lactate criteria for transplantation [2, 17]. Those patients that completed the study received two sessions consecutively over a 2-day period. We observed a significant increase in vascular tone during the first session and the effects were sustained until the start of the second session. These findings are consistent with other reports on the efficacy of MARS in improving the haemodynamic stability in patients with ALF during a single 8-h session [18, 19]. We were able to show, however, that the effects were not sustained and had reverted to baseline by the end of the second MARS session.

ICP did not change significantly in our patients over the treatment period. In addition, evidence of a consistent trend toward a reduction in ICP was lacking. This contradicts some earlier case reports [20]. The lack of effect noted may be due to the relatively low ICP measured in the patient group and because of the standard use of

hypertonic saline prophylaxis against cerebral oedema, but it may also represent absent efficacy of MARS in this setting [21]. Hence the usefulness of MARS in the management of raised ICP remains to be elucidated. In our study there was a trend, however, towards a decrease in the measured jugular bulb saturations without any significant changes observed in mean arterial pressure and ICP. This might reflect an improvement in cerebral vascular tone whilst on MARS. It has been noted previously that MARS therapy increases cerebral blood flow velocity during a single session in acute on chronic liver failure [22]. However, this effect may also represent the global reduction in cardiac output previously noted [23, 24].

The treatment was well tolerated with no clinically significant complications associated with the MARS therapy. There was a significant reduction in platelet count, which has been observed in other studies using MARS therapy without any adverse consequences [7]. The significant reduction in INR seen was due to correction with fresh frozen plasma prior to insertion of the intracranial pressure monitor.

Two patients (nos. 4 and 9) who presented with the highest INR and lactate deteriorated very rapidly and died within 8 h of admission despite MARS therapy and intensive support. As MARS functions by dialysis against an albumin gradient, it shares the same disadvantage as other extracorporeal blood purification systems in that there is inevitably a lag between start of treatment and any possible effect or clinical benefit and in these two patients progressive deterioration was seen. Moreover we showed a reduction in effect over time demonstrated by the diminished response during the second MARS session in the eight patients who survived to finish the study. Even though the decline in vascular resistance and CI during the second session did not reach statistical significance, caution needs to be exercised

when considering prolonged use of MARS as evidence of sustained response is lacking from this pilot study. Previous studies in this setting which used only a single session have shown a similar pattern to our study [19]. The reduction in effect over the second session is, however, an important observation as it suggests that any treatment effect may be short lived.

As MARS does not support liver synthetic function, the long-term outcome of these patients still depends on the availability of liver transplantation or recovery of native liver function. Although this pilot study demonstrated that MARS therapy increases vascular tone in patients presenting with ALF, further studies are needed to determine whether these haemodynamic changes can translate into better patient survival, as our study suggests that treatment effect is short lived, with haemodynamic parameters reverting to baseline during the second treatment session. In addition, we did not observe an effect on intracranial hypertension despite initial enthusiasm for MARS in this setting. Randomised controlled trials are clearly needed to confirm the effect in one way or the other especially in comparison to other extracorporeal modalities such as high volume haemofiltration before MARS can be recommended as standard medical therapy in patients with ALF due to the additional costs estimated at UK £1500 per day.

In conclusion, we found that MARS therapy increased vascular resistance and reduced CI in the majority of patients treated during the first 8-h session. This increase was not sustained over the duration of the study, with a return to baseline values by the end of the second session. There were no significant changes in the other measured parameters. Based on our experience we cannot recommend the routine use of MARS therapy in ALF outside of a clinical trial.

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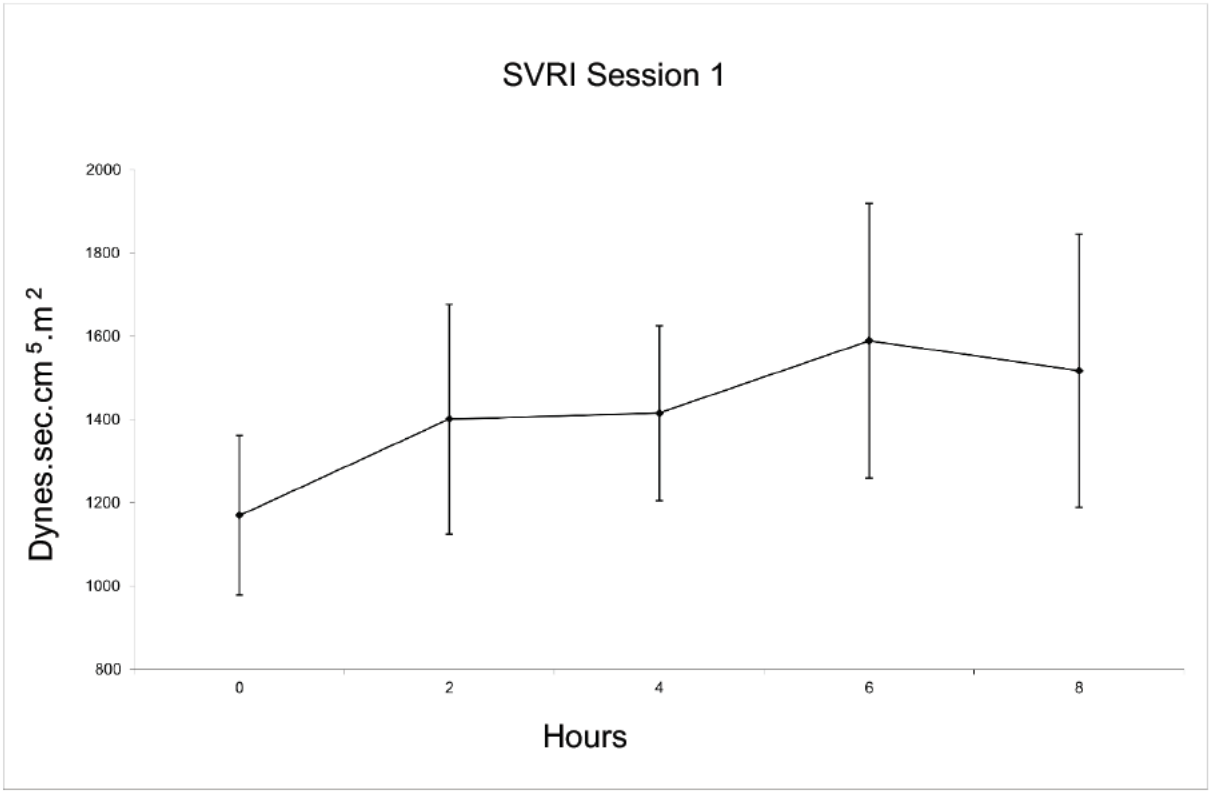
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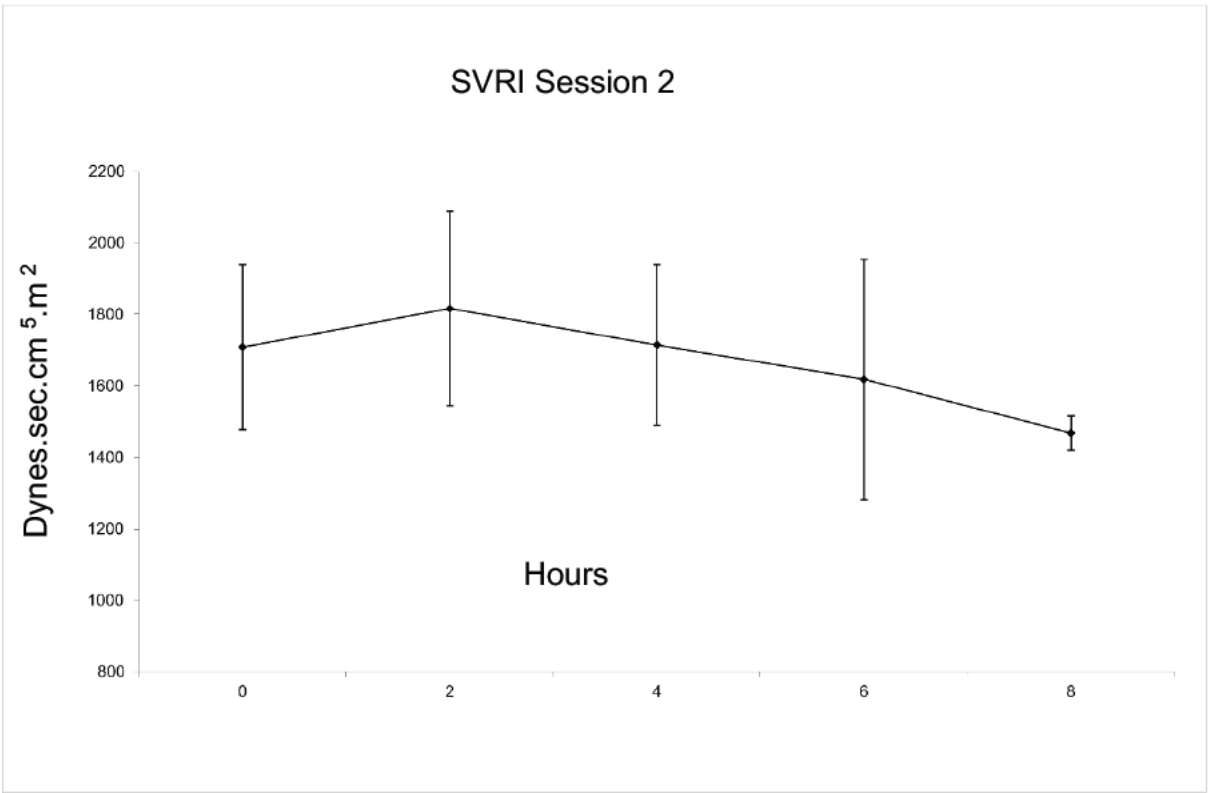
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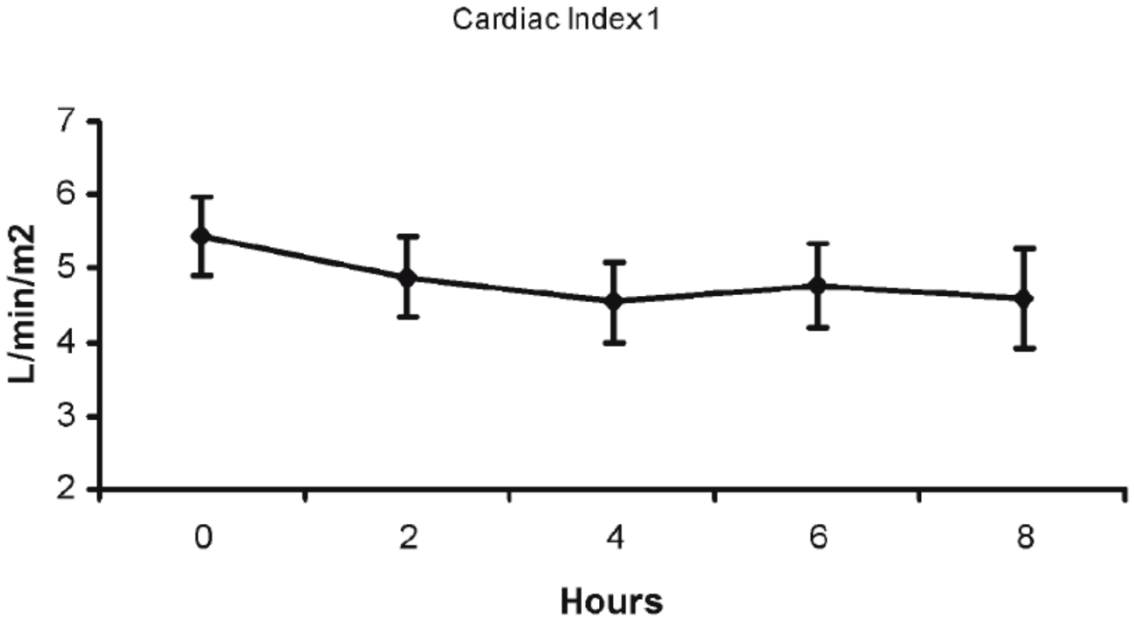


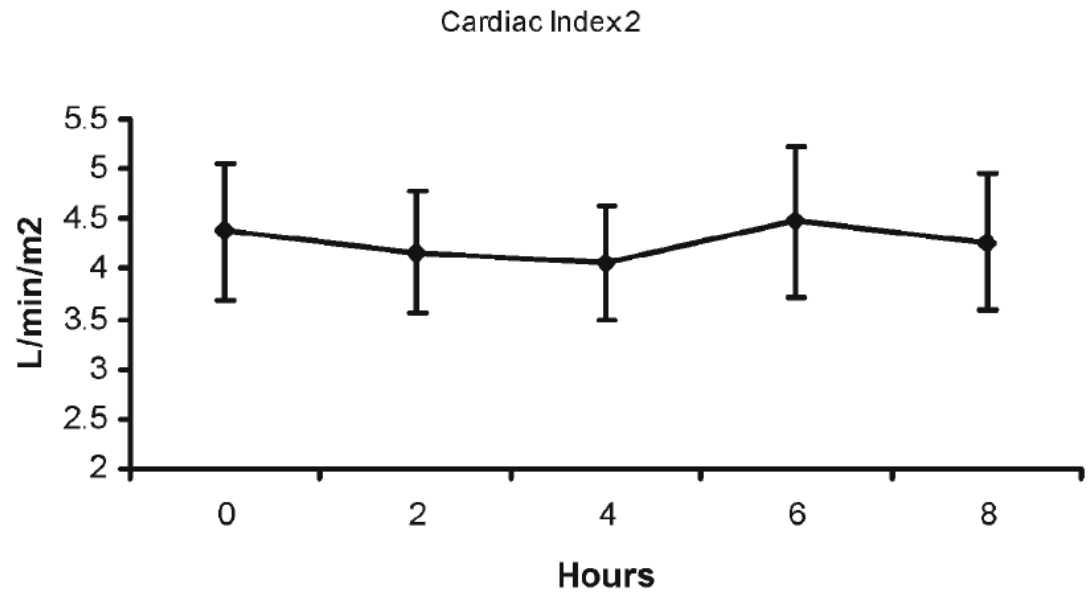
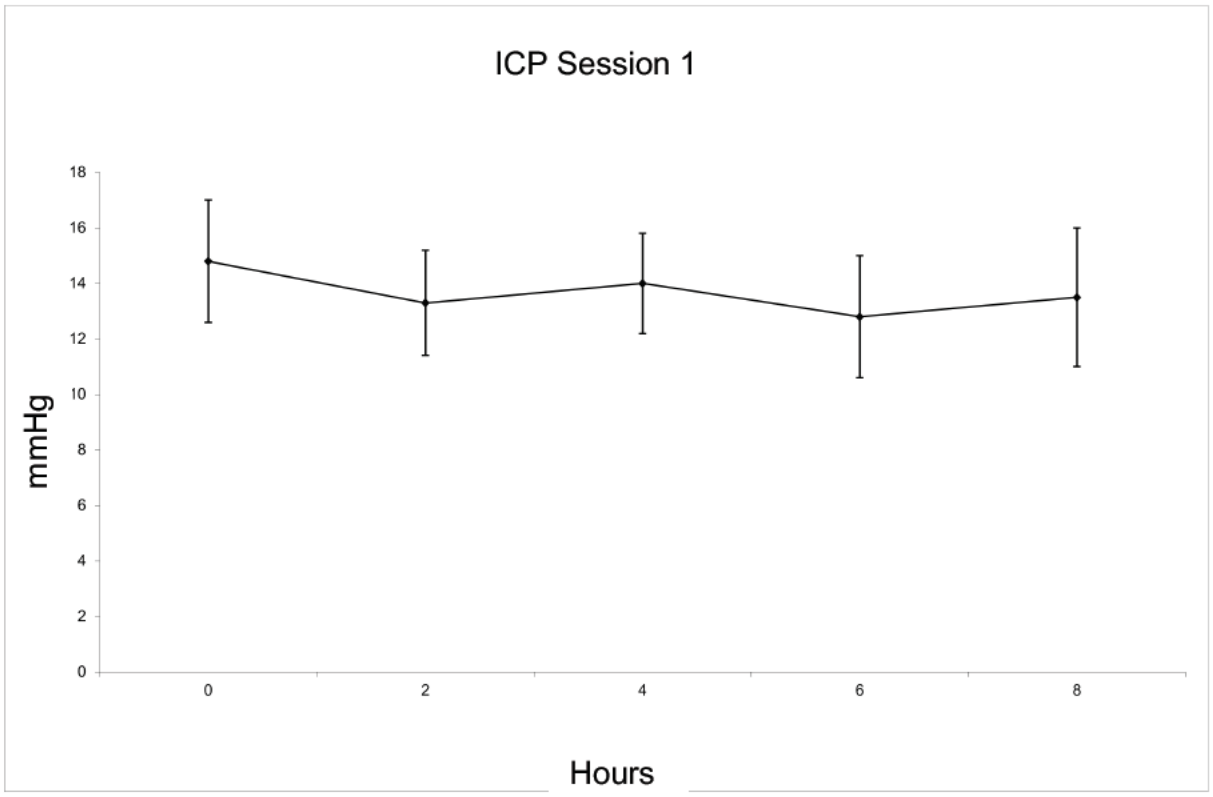


Fig. 1 Changes in mean SVRI and CI with SEM before and after each session of MARS treatment. Significant improvements in SVRI (p=0.02) and CI (p=0.01) observed between start and end of first session



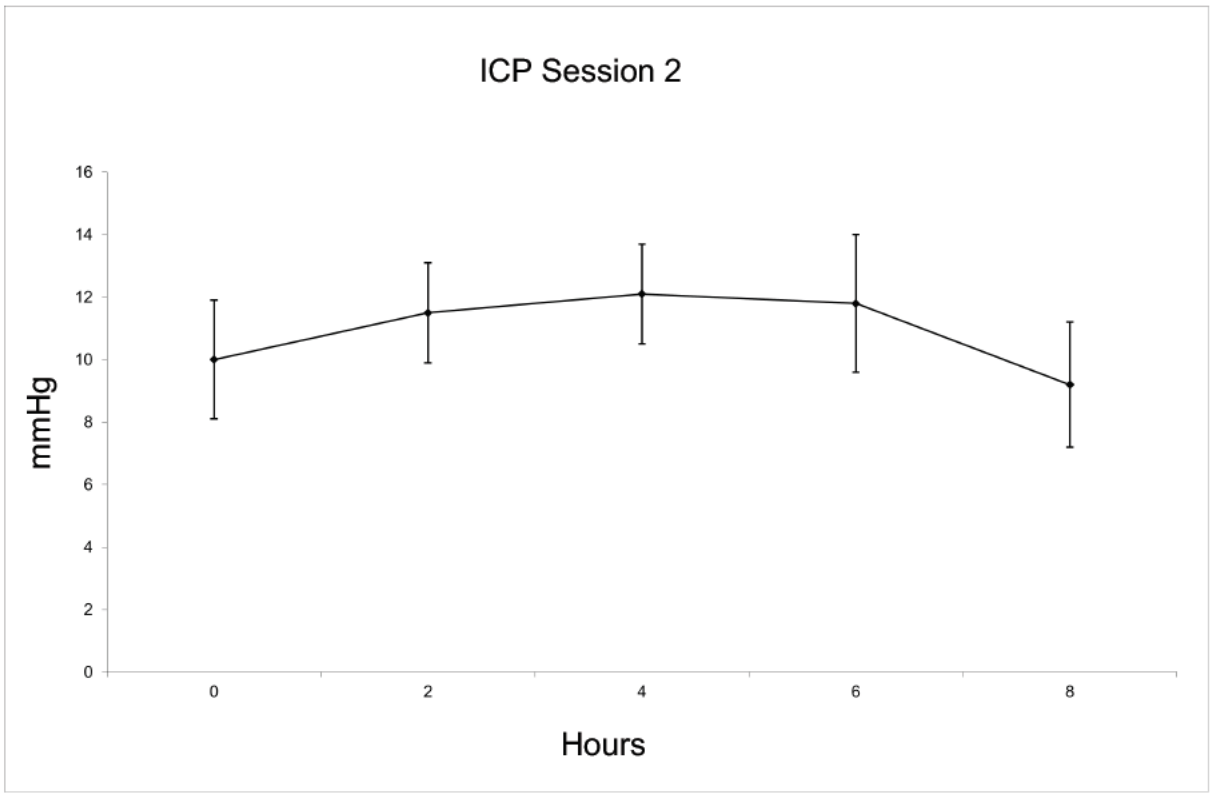


Fig. 2 Mean intracranial pressure with SEM during each MARS session

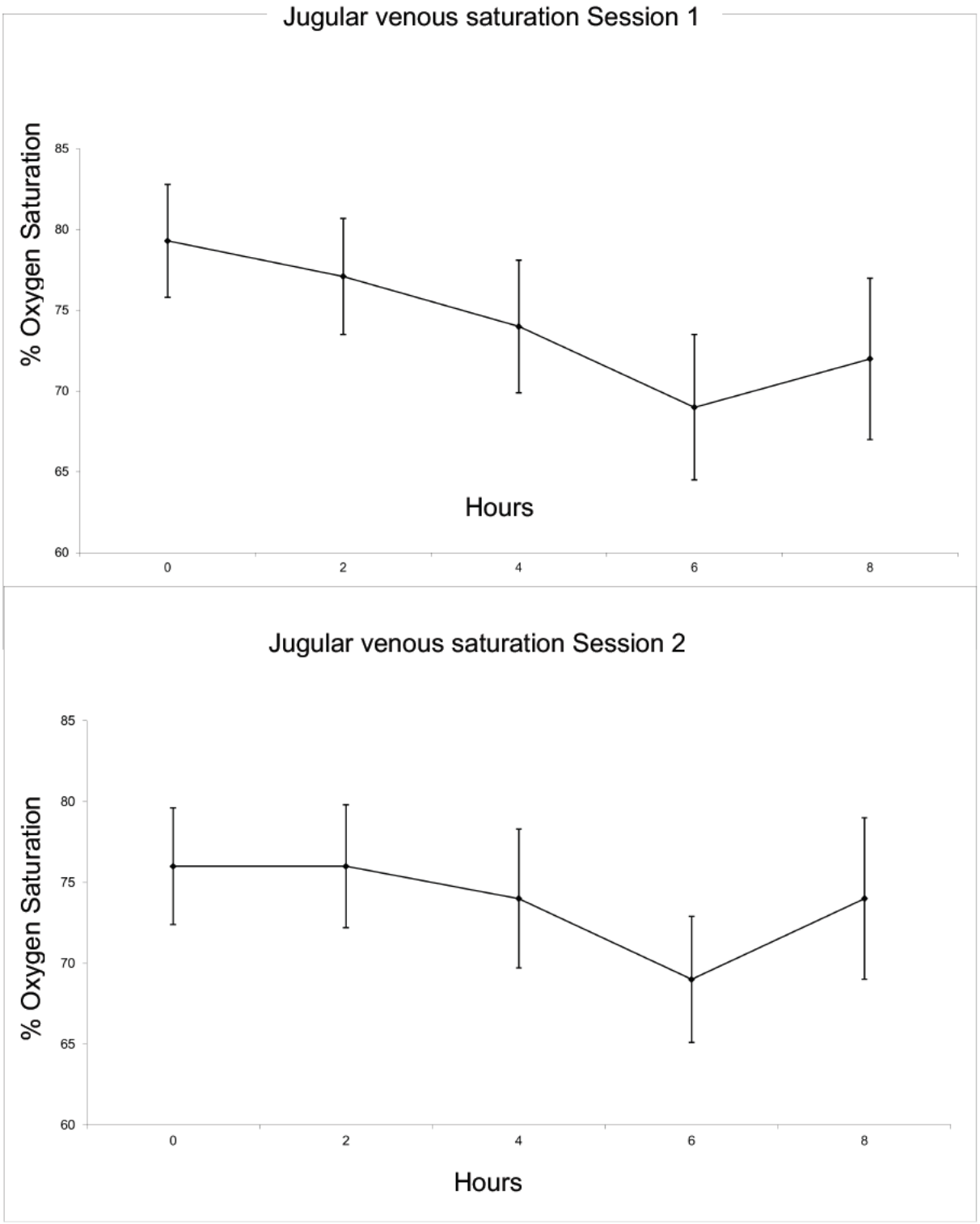


Fig. 3 Mean jugular bulb saturations with SEM during each MARS session

Table 1
Clinical and biochemical status before MARS treatment and outcome (POD paracetamol overdose, NANB non-A/non-B seronegative hepatitis, OLT orthotopic liver transplant)

Patient no.	Sex	Age (years)	Cause	Mars session	ICP	INR	pH	Lactate (mmol/l)	Bilirubin (μmol/l)	Albumin (g/l)	Creatinine (μmol/l)	Outcome
1	F	42	POD	2	6	6.4	7.30	4.35	53	26	171	OLT/died
2	F	62	NANB	2	15	3.5	7.59	2.77	518	20	131	No OLT/died
3	M	19	POD	2	18	2.2	7.40	3.59	128	29	940	No OLT/alive
4	M	53	POD	1	12	>15	7.22	6.34	38	12	167	No OLT/died
5	M	44	POD	2	19	4.2	7.45	2.93	144	20	592	No OLT/alive
6	F	35	POD	2	14	2.9	7.35	3.00	100	23	153	No OLT/died
7	F	53	POD	2	7	2.0	7.28	4.00	5	20	384	No OLT/died

8	M	60	Isoniazid	2	7	3.9	7.49	4.38	166	20	61	OLT/died
9	F	31	POD	1	25	2.7	7.18	11.39	55	25	271	No OLT/died
10	F	39	POD	2	25	5.1	7.20	8.60	38	14	226	No OLT/alive

Table 2 Haemodynamic variables, intracranial pressure and serum lactate before and after each MARS treatment: means (parentheses range) (MAP mean arterial pressure, CVP central venous pressure, PACWP pulmonary arterial wedge pressure, ICP intracranial pressure, SVRI systemic vascular resistance index, CI cardiac index, JBS jugular bulb saturations)

	Session 1			Session 2		
	Pre MARS	Post MARS	P	Pre MARS	Post MARS	P
MAP (mmHg)	75 (65–115)	79 (60–103)	NS	85.5 (77–124)	83 (73–124)	NS
CVP (mmHg)	12.5 (6–21)	10.5 (7–19)	NS	11 (6–12)	10 (8–16)	NS
PACWP (mmHg)	14.5 (9–22)	13.5 (10–20)	NS	13.5 (9–16)	14 (11–17)	NS
SVRI (dyne s^{-1} cm^{-5} m^{-2})	1114 (562–2614)	1432 (734–2489)	0.02	1614 (1021–2868)	1535 (1045–1356)	NS

CI (l min^{-1} m^{-2})	6.14 (3.18–7.7)	4.65 (2.05–6.4)	0.01	3.65 (2.37–7.1)	3.73 (2.71–7.11)	NS
JBS (% oxygen saturation)	83.45 (56.5–92.9)	73.6 (55.5–89)	NS	81.05 (57.9–85)	75.45 (60–84.8)	NS
ICP (mmHg)	14.5 (7–25) 1	4 (3–25)	NS	7 (3–19)	13 (2–20)	NS
Lactate (mmol/L)	3.97 (1.89–11.29)	3.25 (1.83–13.6)	NS	3.02 (1.9–5.2) 2.5	(1.45–5.46)	NS

Table 3 Biochemical variables before and after first session of MARS treatment: means (parentheses range) (INR international normalised ratio)

	Pre MARS	Post MARS	P
Urea (mmol/l)	8.6 (5.6–37.5)	6.6 (4.1–29.1)	0.023
Creatinine (_mol/l)	177 (80–970)	165 (74–604)	0.002
Bilirubin (_mol/l)	77.5 (21–518)	76 (29–293)	NS
Albumin (g/l)	20 (14–29)	22 (13–26)	NS

Haemoglobin (g/dl)	9.1 (7.7–11.8)	8.4 (7.3–9.7)	0.028
Platelets (x109/l)	118 (76–223)	90 (62–163)	0.002
INR	3.2 (1.6–15)	2.3 (1.6–4.4)	0.004

The Effect of Hypertonic Sodium Chloride on Intracranial Pressure in Patients With Acute Liver Failure

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Abstract

Acute liver failure (ALF) is a rare condition characterized by the development of encephalopathy in the absence of chronic liver disease. Cerebral oedema occurs in up to 80% of patients with Grade IV encephalopathy. In the current prospective randomized controlled clinical trial, we examined the effect of induced hypernatremia on the incidence of intracranial hypertension (IH) in patients with ALF. Thirty patients with ALF and Grade III or IV encephalopathy were randomized. Patients in Group 1 (n = 15) received the normal standard of care. Patients in Group 2 (n = 15) received standard care and hypertonic saline (30%) via infusion to maintain serum sodium levels of 145–155 mmol/L. Intracranial pressure (ICP) was monitored in all patients with a subdural catheter (Camino Systems, San Diego, CA) for up to 72 hours after inclusion. Serum sodium levels became significantly different from the levels observed in the control group at 6 hours (P<.01). Over the first 24 hours, norepinephrine dose increased relative to baseline in the control group (P < .001; 13 patients)

but not in the treatment group. ICP decreased significantly relative to baseline over the first 24 hours in the treatment group (*P* = .003; 13 patients) but not in the control group. The incidence of IH, defined as a sustained increase in ICP to a level of 25mmHg or greater, was significantly higher in the control group (*P* = .04).

In conclusion, induction and maintenance of hypernatremia can reduce the incidence and severity of IH in patients presenting with ALF.

Introduction

Acute liver failure (ALF) is defined as the acute cessation of normal liver function in a patient without a history or clinical signs of chronic liver disease. The defining state is the onset of hepatic encephalopathy. The classification of ALF into hyperacute, acute, and subacute categories based on the time from the onset of jaundice to the development of encephalopathy aids in predicting outcome.(1) Patients with rapidly progressive liver failure have a greater risk of developing cerebral oedema and intracranial hypertension (IH).(2,3)

Although IH in ALF is not completely understood, there are thought to be two main pathologic processes that contribute to this condition. These processes are brain swelling caused by water influx down an osmotic gradient into astrocytes (cytotoxic oedema) and cerebrovascular vasodilation, which results in increased cerebral blood volume.(4)

Under normal conditions, ammonia, which is produced mainly in the gut, kidney, and pancreas, is metabolized in the liver to both urea and glutamine.

When the liver fails, there is an increase in circulating ammonia levels. Increased metabolism occurs at alternative sites, such as skeletal muscle and the brain, during liver failure.(5) Within the brain, ammonia is detoxified in astrocytes. Here, glutamine is produced when ammonia combines with the excitatory neurotransmitter glutamate via the enzyme glutamine synthetase. The ammonium load associated with liver failure fuels this reaction, and the glutamine produced increases the osmotic potential within astrocytes. In fact, inhibition of glutamine synthetase ameliorates brain oedema and improves survival results in animal models of ALF.(6) The rapidity of onset of ALF reduces the time available for cellular adaptation. In contrast, in chronic liver disease, astrocytes have time to adapt to the increase in circulating ammonia levels.(3)

The glutamine hypothesis(7) suggests that water diffuses across the blood-brain barrier and into astrocytes, resulting in cerebral oedema and IH. Thus, it can be expected that agents that increase extracellular osmolality, such as mannitol, will reduce IH(8) and that in contrast, a decrease in extracellular fluid osmolality will be associated with an increase in brain swelling.(3)

In the current prospective randomized controlled clinical trial, we investigated the effects of systemic hypernatremia (via hypertonic saline infusion) on the incidence of IH in patients with ALF and Grade III or IV encephalopathy. We hypothesized that systemic hypernatremia may reduce the incidence of cerebral oedema and IH.

Patients and Methods

After approval was granted by the local hospital ethics committee, 30 patients with ALF and Grade III or IV encephalopathy were entered into the current trial. Due to the nature of the disease, consent requirements were eschewed and informed assent was obtained from next of kin. The sample size was based on previously reported data.(9) A difference of 10 mm Hg in mean intracranial pressure (ICP) between groups was anticipated, based on previous anecdotal experience using hypertonic saline to treat patients with ALF. This expectation, together with an α value of 0.05 and a β value of 0.2, yielded a target sample size of 29 patients.

On entry into the trial, patients were randomized into 2 groups using sealed envelopes. Group 1 (*n* = 15) received standard care. Group 2 (*n* = 15) received standard care plus a 30% hypertonic saline infusion via a syringe driver. The infusion rate was titrated between 5 and 20 mL per hour to maintain serum sodium levels at 145–155 mmol/L.

The primary endpoint of the study was onset of IH, which was defined as a sustained rise (lasting 10 minutes or longer) in ICP to 25 mm Hg or greater.(9–11) Data were recorded for up to 72 hours after inclusion.

Management of both groups was standardized. Ventilation was provided using a volume-controlled mode, with minute volume adjusted to achieve a pCO_2 of 4.5– 5.5 kilopascals (34–42mmHg). All patients were nursed with 15–20 degrees of head elevation to facilitate venous drainage and sedated with fentanyl and midazolam. ICP was measured with a subdural ICP probe (Integra Neuro- Sciences, Plainsboro, NJ) inserted into the nondominant frontoparietal region. Before insertion of the probe, fresh-frozen plasma and

platelets were administered. A reverse jugular venous catheter was inserted into the right or left internal jugular vein. Irrespective of cause, all patients received *N*-acetylcysteine (150 mg/kg per day) via continuous intravenous infusion. Hemodynamic monitoring included arterial access via a radial artery for arterial blood gas measurement and central venous access. Further hemodynamic monitoring was determined by clinical need. If mean arterial blood pressure decreased below 70 mm Hg, the patient was adequately volume-loaded and norepinephrine administration was initiated and titrated to maintain a mean blood pressure of 75–80 mm Hg. All patients were fed enterally and given prophylactic antibiotics. Haemoglobin levels were maintained at 8–10 g/dL with packed red cells. Endotracheal suction and patient turning were minimized to reduce the risk of bringing about cerebral oedema.

Group 1, the control group, consisted of 12 patients with acetaminophen hepatotoxicity, 1 patient with non-A, non-B hepatitis (seronegative hepatitis), 1 patient with acute hepatitis B, and 1 patient following acute hepatotoxicity in response to antiretroviral therapy. Group 2, the treatment group, consisted of 12 patients following acetaminophen hepatotoxicity, 1 patient with acute hepatitis B, 1 patient with hepatotoxicity due to use of the recreational drug ‘ecstasy’ (3,4-methylenedioxymethamphetamine), and 1 patient following hepatotoxicity due to a traditional Chinese medication.

Censoring occurred after death, liver transplantation, or resolution of illness. Physiologic data were collected at six hourly intervals. Patients with ICP ≥ 25 mm Hg received an escalating treatment regimen. Sedation was increased and followed by mannitol, 0.5 g/kg, over 10 minutes in conjunction with the

removal of 500 ml of ultrafiltrate via hemofiltration if the patient was in anuric renal failure. Surface cooling was performed for patients with ICP that was resistant to initial measures. Two patients in the control group had resistant IH and received 20 mL bolus injections of 30% hypertonic saline. Both of these patients died with intractable IH.

Statistical analysis was performed using SPSS Version 10 (SPSS Inc., Chicago, IL) and Unistat 4.5 (London, United Kingdom). Study data are presented as mean values with standard errors. Within-group analysis consisted of nonparametric ANOVA (Quade two-way ANOVA). Between-group data were compared using the Mann– Whitney *U* test. Kaplan–Meier analysis with Breslow's test for significance was used to compare the incidence of clinically significant IH between groups. Spearman's test was used to evaluate correlations. A *P* value of less than .05 was considered significant.

Results

The average time from admission to the hospital until entry into the trial was 36.5 hours, with a range of 3–73 hours. Sixteen of the 30 patients in the trial had encephalopathy on arrival at the intensive care unit; for these patients, the exact timing of the onset of encephalopathy was unclear. For the remaining 14 patients, the average time from the onset of encephalopathy to entry into the trial was 31 hours, with a range of 5–55 hours.

Seven patients in the control group and 9 patients in the treatment group had sodium levels of less than 135 mmol/L on admission into the intensive care unit. One patient in the control group had a serum sodium level of less than

130 mmol/L, compared with 6 patients in the treatment group. On admission to the trial, no patient in either group had a serum sodium level of less than 130 mmol/L; 1 patient in each group, however, had a serum sodium level of less than 135 mmol/L. The relation between serum sodium level at admission and the first available ICP measurement was investigated in all patients (Fig. 1); a weak but significant inverse correlation was observed ($r^2 = 0.06$; $P = .02$). At the time of enrolment into the trial, there were no significant differences between the two groups (Table 1).

Over the study period, there was no difference between the two groups in terms of the volume of fluids administered or the amount of blood transfused. Eight patients in the control group required mannitol, compared with five in the treatment group; this difference was not significant. The number of interventions also did not differ significantly between the two groups. Fourteen of 15 patients in the control group and 13 of 15 patients in the treatment group underwent continuous venovenous hemofiltration, with a blood flow rate of 200 mL per minute and a median ultrafiltrate exchange rate of 4 L per hour in both groups. For all patients, the indication for continuous venovenous hemofiltration was oliguric or anuric renal failure.

Serum sodium levels increased in both groups between admission to the intensive care unit and entry into the trial. These increases resulted from the use of hemofiltration for the majority of patients in both groups. In the treatment group, serum sodium concentration increased significantly from its initial levels and, at 6 hours, became significantly different from the levels observed in the control group ($P < .01$) (Fig. 2). During the first 24 hours, the treatment group received an average of 6.5 mL of 30% saline per hour. Each

10 mL ampoule of 30% saline contains 50 mmol of sodium chloride; thus, the average sodium load during the first 24 hours was approximately 780 mmol. The mean peak serum sodium concentration for patients in the treatment group was 153 ± 0.8 mmol/L.

Mean arterial pressure did not differ between the two groups, because of the management protocol. Vasopressor use was compared over time within each group and between groups. Norepinephrine dose increased significantly compared with baseline over the first 24 hours in the control group ($P < .001$; 13 patients). This increase continued to be observed at 36 hours ($P < .001$; 11 patients) but was no longer apparent at 48 hours (9 patients). In the treatment group, there was a nonsignificant increase in norepinephrine dose from baseline over the first 24 hours. The difference between groups was not significant (Fig. 3).

ICP data from the study were analysed within each group, over time, and between groups. Over the first 24 hours, there was a significant reduction in ICP relative to admission values in the treatment group ($P = .003$; 13 patients). ICP remained significantly decreased at 48 hours ($P = .001$; 11 patients). In the control group, ICP increased over these time periods, but not significantly (13 patients at 24 hours; 8 patients at 48 hours) (Fig. 4). The difference in ICP between groups became significant at 42 hours ($P = .04$).

The primary outcome, incidence of IH, also was compared between groups. Seven patients in the control group had ICP ≥ 25 mm Hg, compared with 3 patients in the treatment group (P = not significant; Fisher's exact test). Using survival analysis to account for case censoring (due to death, transplantation,

or recovery) (Fig. 5), the cumulative risk of having ICP ≥ 25 mm Hg during the 72-hour trial was found to be significantly greater in the control group (P = .04). No difference in terms of cerebral perfusion pressure or jugular venous saturation data was found between the two groups.

Seven patients in the control group reached the primary endpoint of the trial, an ICP of 25 mm Hg. Eight patients did not reach the primary endpoint; 5 of these 8 were censored (2 due to early death and 3 due to transplantation). Overall, there were 7 transplantations and 6 early deaths in the control group at 72 hours. Three of the 6 deaths resulted from IH, and the remaining 3 resulted from unsupportable hypotension.

Three patients in the treatment group reached the primary endpoint of the trial, and 12 patients did not. Of the 12 patients who did not reach the primary endpoint, 7 were censored (3 due to early death, 1 due to transplantation, and 3 due to early removal of ICP bolts because of improved medical condition). ICP bolts were removed at the discretion of the attending medical team; one patient's ICP monitor was removed at 66 hours, another patient's monitor was removed at 48 hours, and the third patient's monitor was removed at 60 hours. Overall, there were 5 transplantations and 5 early deaths at 72 hours. Two of the five deaths resulted from IH, and the remaining three resulted from unsupportable hypotension.

After termination of the trial, seven patients in the control group and eight patients in the treatment group died while in intensive care. Late deaths were due to sepsis.

Discussion

Slightly more than half of all patients in the current study had hyponatremia on admission to the intensive care unit. In our experience, hyponatremia is common in ALF, especially in cases of acetaminophen hepatotoxicity. In a consecutive group of 240 patients with acetaminophen toxicity who presented to the liver unit at our institution, 62% had admission serum sodium levels of 135 mmol/L or less, and 32% had serum sodium levels of 130 mmol/L or less (Bernel W, unpublished data, 1998). Hyponatremia previously has been reported to be a predictor of poor outcome for patients with ALF.(12,13) We found a weak correlation between serum sodium concentration at admission and initial ICP, and it may be the case that hyponatremia exerts its negative impact by exacerbating cerebral oedema in patients whose brains are at risk. It is noteworthy that chronic hyponatremia recently was shown to have a negative impact on cerebral oedema in an animal model of liver failure.(3)

After entry into the trial, a significant increase in serum sodium levels was observed in the treatment group. The goal of the current study was to investigate the effects of moderate hypernatremia on cerebral oedema and IH. Serum sodium concentration, rather than serum osmolality, was chosen as the treatment target because of the anticipated use of hemofiltration. The high ultrafiltration rate (median, 4 L per hour) demanded a rapid feedback mechanism for the control of serum sodium concentration and osmolality. Serum sodium concentration, measured by near-patient blood gas analysis, was determined to be the most appropriate target. The total volume of hypertonic saline infused varied among patients in the treatment group, but the total quantity in mmol of sodium administered was not used as an indicator of osmolar load, because of the daily plasma water (and sodium

chloride) exchange of as much as 96 L during hemofiltration. Under these conditions, the total dose is heavily dependent upon the ultrafiltration rate. In the setting of ALF with high-grade encephalopathy, renal failure is quite common, and the majority of patients in the current study were managed with renal replacement therapy. In fact, the results of this study may only apply to patients with ALF and renal failure.

The sodium loads associated with the treatment group were very high. A 24-hour infusion of 30% saline at 10 mL per hour results in a sodium load of 1200 mmol. Without hemofiltration to buffer the sodium load, rapid changes in serum sodium concentration can occur; thus, 30% saline infusions should be used with caution, especially in patients with abnormal renal function.

In the treatment group, we found that induction of moderate hypernatremia reduced ICP from its baseline level. ICP decreased significantly during the first 6 hours of the study. In contrast, there was a nonsignificant increase in ICP in the control group. We defined IH as a sustained increase in ICP to 25 mm Hg or greater for 10 minutes or more; this criterion was the treatment trigger in use at our institution at the time of the study and was based on previously published work.(9–11) When comparing the two groups directly, the difference in number of patients reaching the primary endpoint (7 in the control group vs. 3 in the treatment group) was not significant. This lack of significance may stem from the relatively small sample size. After controlling for withdrawal from the study, however, the difference in incidence of IH was significant.

There are several possible mechanisms by which hypertonic saline and the induction of hypernatremia may prevent IH in patients with ALF. One such mechanism is the osmotic effect. The blood-brain barrier prevents the bulk flow of water and solute from the circulation into the brain and occurs as a result of tight junctions between endothelial cells in brain capillaries. Water crosses the barrier by diffusion, in contrast to filtration across ordinary fenestrated capillary endothelium, in which the flow may be 50 times greater than the flow across the blood-brain barrier. Water flow in either direction across the blood-brain barrier is influenced by both hydrostatic and osmotic gradients. Most commonly, it is changes in osmotically active particles on either side of the blood-brain barrier that influence water flow.(14)

The permeability of the blood-brain barrier to sodium is low. Studies suggest that the majority of sodium transport from the blood into the brain matter occurs across endothelial cells via channels or carriers.(15) In fact, sodium is actively pumped out of all cells via the action of the ubiquitous sodium/potassium adenosine triphosphatase.(16) This pumping results in a high reflection coefficient, which represents the selectivity of the blood-brain barrier to a particular substance. Sodium has a higher reflection coefficient than does mannitol.(17) Consequently, hypertonic saline acts as a dehydrating agent; it has been demonstrated that hypertonic saline promptly reduces brain water content in experimental models of brain injury and successfully reduces ICP and tentorial herniation in both patients and animals with resistant IH.(17,18) To our knowledge, the current study is the first to use hypertonic saline in the management of IH and in the prevention of cerebral oedema for patients with ALF.

Hypertonic saline and induced hypernatremia also may prevent IH by affecting cerebral blood flow in patients with ALF. Hypertonic saline solutions can improve regional cerebral blood flow in patients with traumatic brain injury.(18) The probable mode of action involves reduction of endothelial swelling by dehydration and increased deformation of erythrocytes, which improves flow at a microvascular level.(19) Endothelial swelling has been shown to occur in cerebral blood vessels in patients with ALF, as well as in animal models of this disease.(20,21) Generally, a reduction in cerebral blood flow coupled with a reduction in cerebral metabolic rate early in the course of high-grade hepatic encephalopathy in ALF is followed by gradual cerebral vasodilation and an increase in cerebral blood volume in severe cases.(4,22) Variation in regional cerebral blood flow, however, has been reported in patients with ALF; this finding suggests that some areas of the brain may be more prone to ischemia.(23) Increased cerebral lactate efflux shortly after episodes of IH in patients with severe ALF also has been reported.(24) Whether hypertonic saline has any effect on cerebral blood flow and metabolism in patients with ALF currently is unknown and requires further investigation.

Other effects of hypertonic saline also may be important in controlling IH. Hypertonic saline solutions can restore normal resting potential in membranes and thereby potentially stabilize cerebral endothelial cell membranes, helping to inhibit the occurrence of vasogenic cerebral oedema.(25) The stability of resting membrane potential is important in the inhibition of seizure activity, which is not well characterized in patients with ALF.(26) Hypertonic saline has

been used successfully in the management of patients with status epilepticus induced by hyponatremia.(27)

The systemic inflammatory response syndrome has been implicated in the development of both hepatic encephalopathy and IH in ALF.(28) A correlation between serial cytokine levels and the severity of hepatic encephalopathy in patients with ALF also has been found.(29) Recent investigation in animals has demonstrated that the addition of lipopolysaccharide to a toxic liver trauma exacerbates liver injury and IH.(30) These findings suggest that some product of systemic inflammation either precipitates or (more probably) intensifies brain swelling in patients with ALF.

Hypertonic saline has profound effects on immune function.(31) Hypertonicity inhibits neutrophil activation by preventing adhesion and degranulation and has been shown to reduce neutrophil accumulation in the lungs in a haemorrhagic shock model.(32) Hypertonic saline also inhibits the production of proinflammatory cytokines (such as tumour necrosis factor) while enhancing the expression of anti-inflammatory cytokines (such as interleukin 10).(33) This effect on systemic inflammation may help reduce the severity of IH. Published findings on this topic are conflicting, however, and it may be that systemic inflammatory response syndrome and cerebral oedema are coincident phenomena.(34)

The improvement in systemic haemodynamics was an unexpected finding and is difficult to interpret given our study design. Mean arterial pressure was managed above protocol limits with intravenous fluid and vasopressor and thus did not differ between groups. The norepinephrine dose required to

maintain acceptable arterial pressure increased significantly in the control group over the first 6 hours and remained elevated for the following 24 hours. Hypertonic saline is known to have significant effects on the cardiovascular system. These effects include an increase in blood pressure mediated by the activation of the sympathetic nervous system, an increase in vasopressin release, and an increase in extracellular fluid volume. Peripheral infusion of hypertonic saline increases blood pressure and reduces heart rate via a baroreceptor-mediated reflex. In a rat model, some of the effects of hypertonic saline appear to operate centrally.(35) The use of hypertonic saline may induce a degree of hemodynamic stability in patients with ALF, although this possibility requires further investigation.

The induction of hypernatremia has been investigated in the setting of head injury. Hypernatremia induced with 3% saline in patients with resistant IH results in a significant reduction in ICP and an increase in cerebral perfusion pressure.(36) Simma et al.(37) performed a randomized controlled clinical trial investigating fluid therapy in paediatric head trauma. They compared hypertonic saline with lactated Ringer's maintenance fluid and concluded that hypertonic saline was superior in the management of severe head injury. These investigators also found that increased serum sodium concentration was significantly correlated with decreased ICP and increased cerebral perfusion pressure. In addition, they observed that fewer interventions, fewer complications, and a shorter time in intensive care were associated with hypertonic saline use.(37)

From the results of the current study, we conclude that inducing moderate hypernatremia with 30% hypertonic saline can decrease ICP relative to

baseline and reduce the incidence of clinically significant IH in patients with ALF. Larger trials are required to determine whether this simple intervention reduces the incidence of cerebral death or improves intensive care or hospital survival results.

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	Control	Hypertonic saline	Signifi-cance
Age	36	36	
Gender			
Male	6	9	
Female	9	6	
INR	4.7 ± 0.6	7.9 ± 1.4	NS
JV sats (%)	68.4 ± 7.3	71.7 ± 3.0	NS
Sodium (mmol/l)	135.3 ± 1.1	131.7 ± 1.4	NS
ICP on insertion (mmHg)	16.9 ± 3.5	17.2 ± 2.3	NS
Lactate (mmol/l)	5.51 ± 0.9	4.67 ± 1.1	NS
Base deficit (mmol/l)	-6.55 ± 2.3	-5.26 ± 1.7	NS
Bilirubin (μmol/l)	147 ± 62	110 ± 24	NS
Alk Phos	99 ± 10	116 ± 16	NS
AST	6828 ± 1273	6798 ± 1263	NS
γ–GT	76 ± 13	123 ± 23	NS
Creatinine (μmol/l)	256 ± 37	250 ± 34	NS

Table 1. Admission clinical data in the prospective cohort. Mean and standard error. (JV sats = jugular venous saturation, AST = Aspartate aminotransferase, Alk Phos = Alkaline phosphatase, γ-GT = glutamyl-transferase)

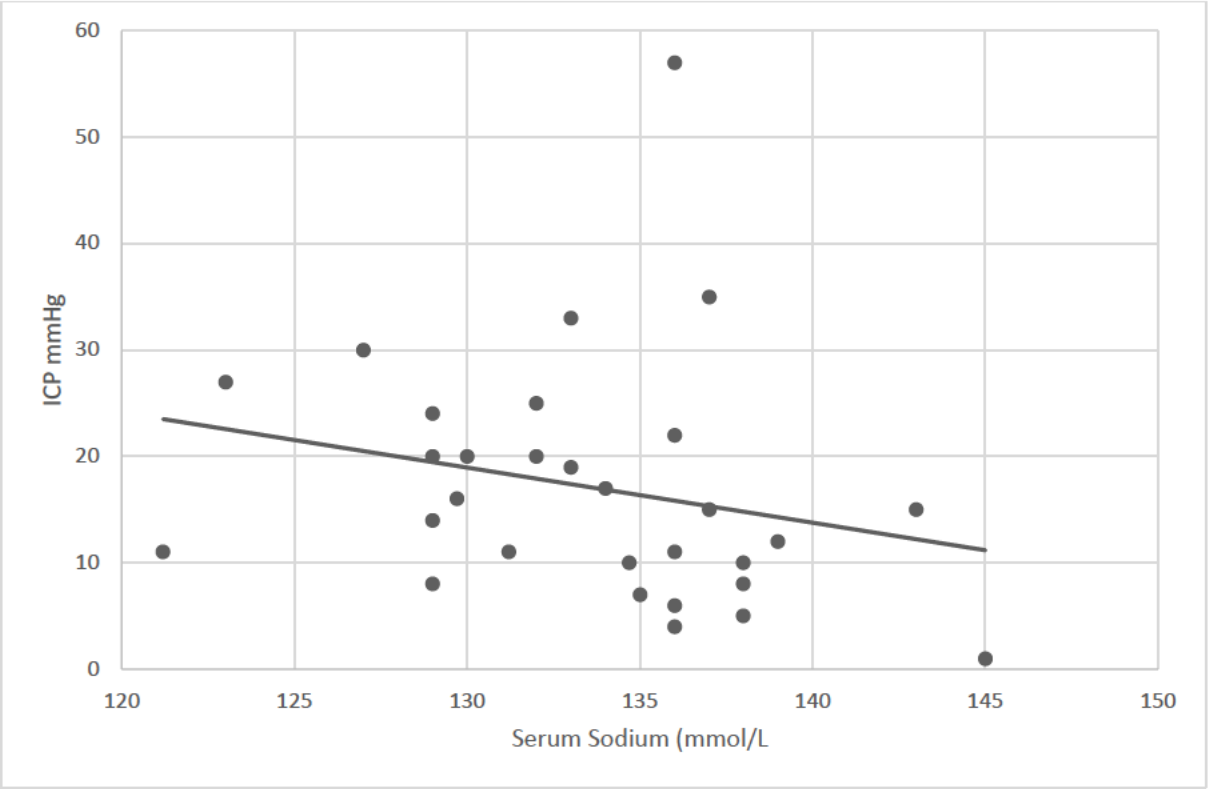

Fig. 1. Relation between serum sodium concentration at admission and initial ICP measurement (r^2 = 0.06; P = .02).

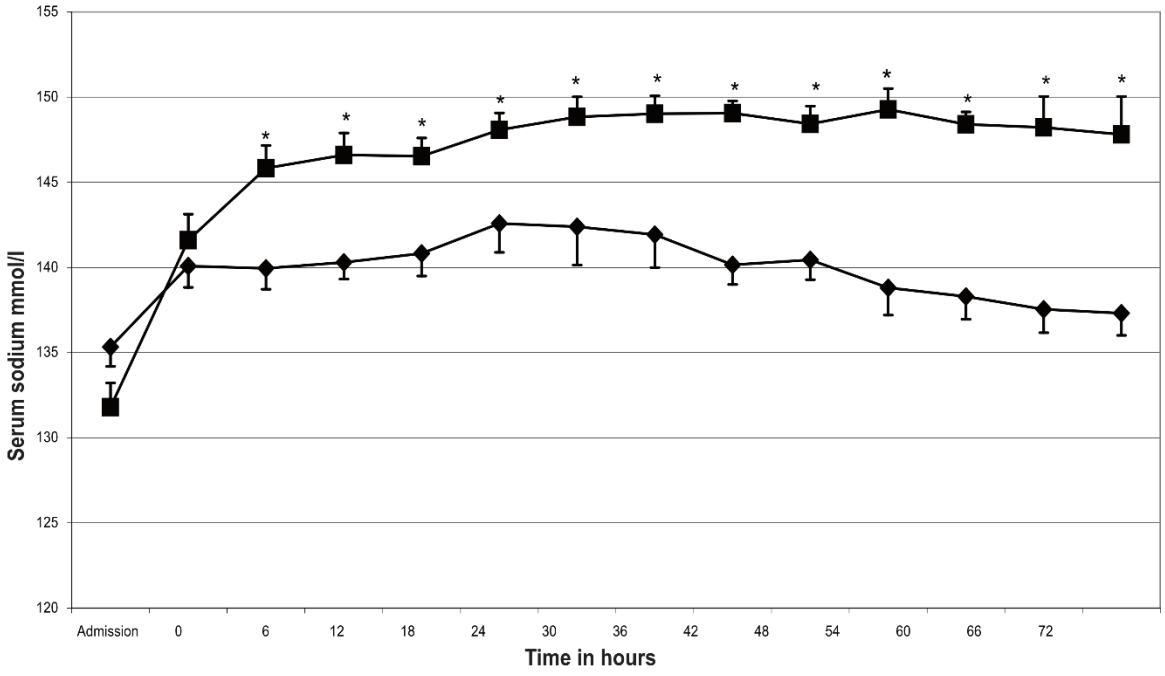

Fig. 2. Serum sodium concentration (mean values with standard errors) in the control (♦) and treatment (■) groups. Asterisks indicate that P < .01 (Mann–Whitney U test).

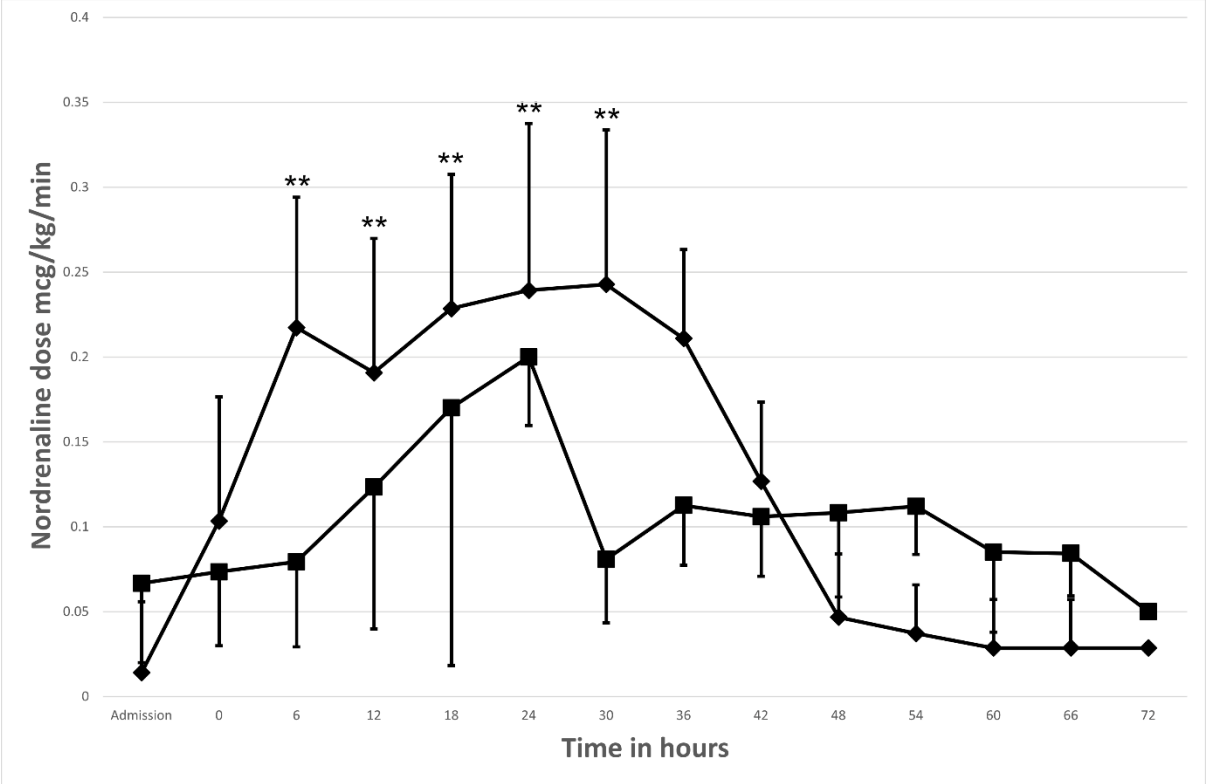

Fig. 3. Norepinephrine dose (mean values with standard errors) in the control (♦) and treatment (■) groups. Double asterisks indicate that P < .001 (Quade ANOVA).

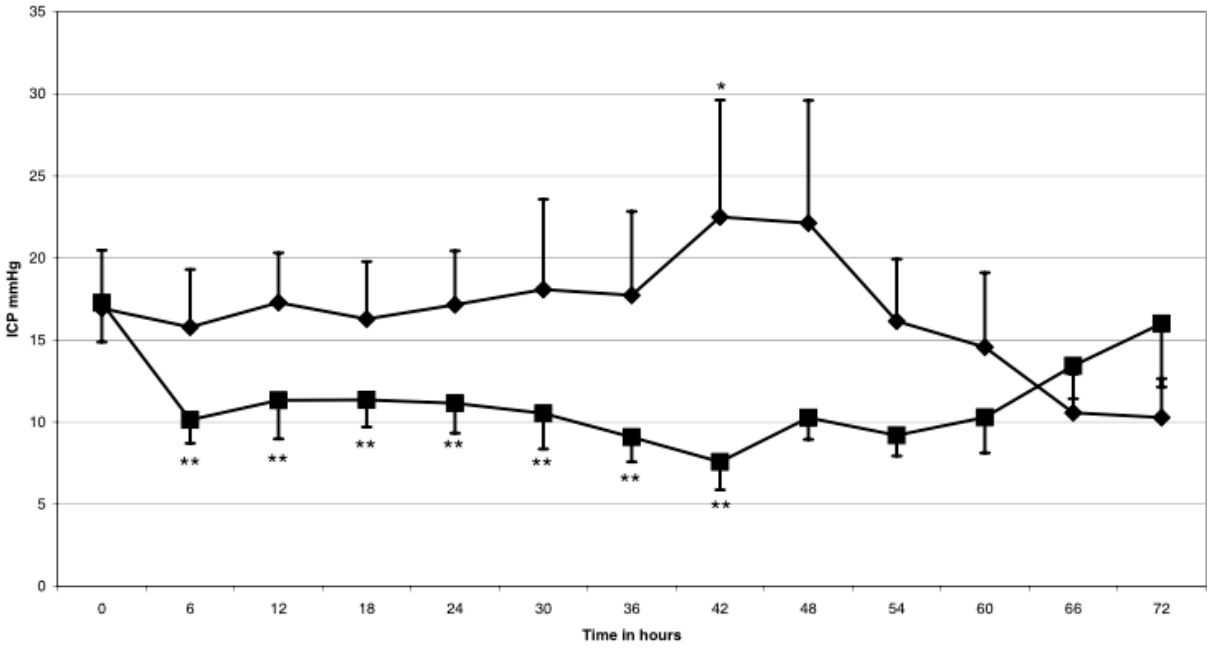

Fig. 4. ICP (mean values with standard errors) in the control (♦) and treatment (■) groups. Single asterisk indicates that P = .04 (Mann– Whitney U test) for between-groups analysis. Double asterisks indicate that P = .001 (Quade ANOVA) for within-group analysis.

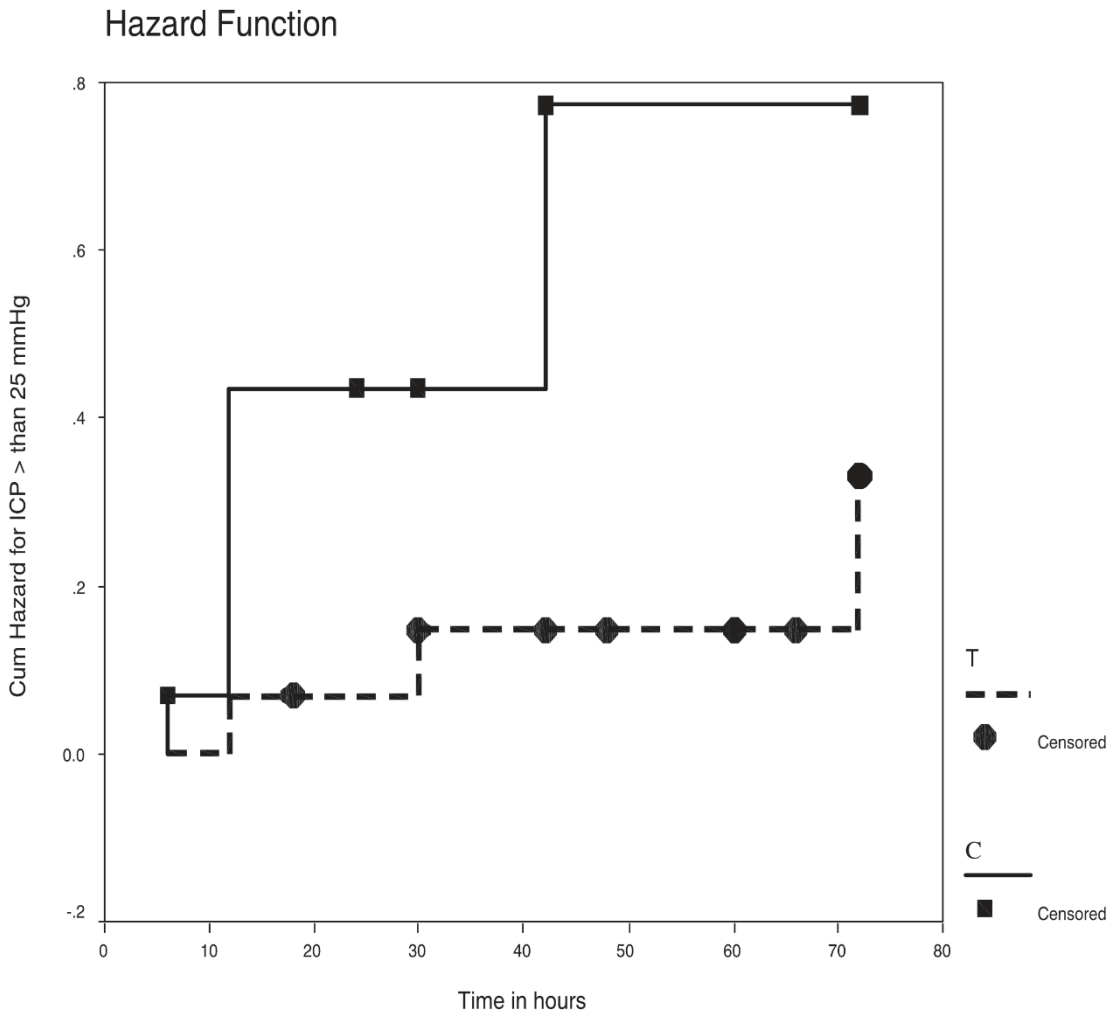

Fig. 5. Kaplan–Meier analysis. Cumulative incidence of ICP ≥ 25 mm Hg (P = .04; Breslow test) in the treatment (T) and control (C) groups. Circles and squares represent case censoring in the treatment and control groups, respectively.

A multicentre randomized controlled trial of moderate hypothermia to prevent intracranial hypertension in acute liver failure.

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Abstract

Background

Animal models and human case series of acute liver failure (ALF) suggest that moderate hypothermia (MH) may have protective effects against cerebral oedema (CO) development and intra-cranial hypertension (ICH) severity. However, the optimum temperature for patient management is unknown; we performed a multicenter randomised controlled trial to investigate this issue.

Patients and Methods

Patients with ALF**,** high-grade encephalopathy and intracranial pressure (ICP) monitoring in specialist intensive care units were randomized by sealed envelope to two targeted temperature management (TTM) groups of 34^{O}C (MH) or 36^{O}C (control) for a period of 72 hours. Investigators were not blinded to group assignment. The primary outcome was a sustained elevation in ICP >25mmHg, with secondary outcomes the occurrence of predefined serious adverse effects, magnitude of ICP elevations and cerebral and all-cause hospital mortality (with or without transplantation).

Results

A total of 46 patients were randomized, of whom 43 were studied. There was no significant difference between the TTM groups in the primary outcome during the study period (35% vs. 27%, p=0.41 for the MH (n=17) or control (n=26) groups respectively) The groups had similar incidence of adverse

events and no differences in overall mortality (41% vs.46%, p=0.75). In those transplanted, mortality was increased in the control group (0% vs. 36%, p=0.07).

Conclusions

In patients with ALF at high risk of ICH, moderate hypothermia at 34^{O}C did not confer a benefit above management at 36^{O}C in prevention of ICH or in overall survival. A trend toward improved outcome was seen in those undergoing transplantation. (ISRCTN registration number 74268282; no funding)

Introduction

Acute liver failure (ALF) is a rare critical illness that continues to have a high mortality, despite advances in supportive intensive care management and the utilization of emergency liver transplantation (ELT). It is characterized by an acute liver injury resulting from a wide variety of hepatic insults, in the absence of pre-existing liver disease, complicated by the development of hepatic encephalopathy (HE) and often by the simultaneous development of multiple organ systems failure [1].

In some patients, HE may progress with the development of cerebral oedema (CO) and elevated intracranial pressure (ICP), a feared complication and important cause of death. Approaches to the intensive care management of patients with ALF therefore include a package of neuroprotective measures designed to prevent its development or ameliorate its severity [1,2]. Targeted temperature management (TTM) and the induction of moderate hypothermia (MH) may be employed as part of this [3]. Animal models suggest it to have cerebral and systemic effects of particular benefit in this setting, preventing the development of CO and controlling refractory elevation of ICP when it has evolved [4 5 6 3]. The decrease in cerebral metabolic activity seen with induced hypothermia may lower the pathological metabolism of ammonia, the likely principal neurotoxin in the development of CO, and attenuate local and systemic inflammation, thought to have an important permissive effect [5,6 3]. Human case series suggest that MH may be effective in controlling otherwise refractory elevations in ICP, beneficially modulate local and systemic inflammatory status and associated with increased hemodynamic stability and improved cerebral perfusion [7 8 9].

However there are no human trial data to suggest an optimum temperature for patient management, or whether MH is effective in the preventing development of elevated ICP. Concerns also exist in relation to the induction by MH of potentially clinically significant adverse effects [3,10]. Studies in neurotrauma suggest that ICP may be lowered by application of MH but there is little evidence of improved outcome and an increase in adverse events; recent data on the use of TTM in patients who have suffered out of hospital cardiac arrest also showed no mortality benefit associated with hypothermia [11] [12] [13] [14]. In the setting of ALF adverse events might include worsening of already significant coagulopathy, impaired hepatic regeneration, and of increased susceptibility to infection - important as a contraindication to ELT and a key cause of death [15] [10,16].

A clear need therefore exists to determine the efficacy or otherwise of MH in ALF patients at high risk of developing ICH, examining its impact on development and severity of ICH and safety profile. Acute liver failure is a uniquely difficult condition in which to perform randomized controlled trials (RCT) relating to its rarity, severity, heterogeneity and the rapidity of illness progression which make participant recruitment and study challenging. The use of ELT further complicates interpretation as the duration of any intervention may be truncated and treatment effects difficult to ascertain. Consequently, few RCT of any sort have been performed in patients with ALF, and when undertaken may take many years to enrol [17,18].

Nonetheless, we performed a pragmatic multicentre RCT of MH in ALF, aiming to study patients at high risk of CO and investigate whether the onset

of clinically significant elevations of ICP might be prevented or delayed by its use.

Methods

Patients, Trial Setting and Design

The trial was a RCT recruiting patients with ALF in 3 specialist intensive care units (ICU) in the United Kingdom and Denmark and was approved by the ethics committees of each country and institution. The trial was registered with ISRCTN (ISRCTN74268282).

ALF was defined as: (1) an INR of ≥1·5; (2) absence of a previous history and clinical / radiologic findings of liver disease; and (3) illness ≤26 weeks duration and (4) overt encephalopathy of HE grade ≥3. We screened consecutive patients of 18 years or older with ALF who had developed HE of grade ≥3 who had been intubated, sedated and mechanically ventilated and who were considered at high risk of ICH such that the treating clinician had inserted a Camino intracranial pressure monitor. Informed assent was then obtained from the next-of-kin of all participants and consent confirmed in those patients who recovered; inclusion in the trial was within 12 hours of insertion of the ICP monitor. Exclusion criteria included pregnancy, evidence of brain stem death, ongoing or suspected haemorrhage, suspected or microbiologically confirmed severe sepsis such that hypothermia induction was considered inappropriate and no or withdrawn consent.

Supportive Care

Standard medical care applied has been detailed elsewhere [1]. In brief, guided restoration of circulating volume was commenced immediately on admission and supported through use of invasive hemodynamic monitoring. Norepinephrine was the primary vasopressor used and dobutamine the primary inotropic agent with adjunctive use of intravenous low dose hydrocortisone and vasopressin. Renal replacement therapy (RRT) was undertaken using continuous veno-venous hemofiltration (CVVHF). Indications for its use included not only those standard for patients with acute kidney injury but also anuria, relative oliguria, metabolic stabilization, control of acidosis and hyper-ammonaemia. Hypertonic saline was infused to maintain serum sodium at 140-150 mMol/l. Sedation utilized fentanyl and propofol infusions and neuromuscular blockade was not routinely undertaken.

Mean arterial pressure was maintained at > 65 mmHg, cerebral perfusion pressure was managed at the discretion of the clinician dependent upon the presence or absence of autoregulation. Arterial pC02 was maintained at 4-4.5 kPa.

Treatment for ICH crises as defined by sustained elevation of ICP > 25 mmHg or episodes of fixed/ sluggish pupils (> 6mm), was sequential use of sedation bolus whilst marinating adequate perfusion pressure then with bolus intravenous mannitol 20% (0.5g/kg) or hypertonic saline (30% 20 mls); with use of thiopentone and /or intravenous indomethacin in refractory cases. Intravenous N-acetyl Cysteine (NAC) was administered to all patients with an

infusion of 150 mg/kg/24 hrs for a maximum of 5 days or until the INR was <2. Intravenous broad-spectrum antibiotics were administered to all patients in the trial. In those patients transplanted, immunosuppression was with Tacrolimus and corticosteroids.

Randomization and Trial Intervention

After confirmation of eligibility and assent, patients were randomly assigned to TTM groups with a target core body temperature of either 34 ^{o}C (MH) or 36^{o}C (Controls). Randomization was performed nationally, by sealed envelope and in blocks of varying size by country. The intervention period of 72 hours commenced at the time of randomization; the treating clinicians were not blinded to the intervention.

Sedation and ventilation was mandated in all patients until the end of the intervention period but sedation targets and the drugs used to achieve targets were left to the discretion of the local clinicians. The goal was to achieve the assigned temperature as rapidly as possible with the use of surface temperature management devices (warming and cooling) and where present, control of blood temperature through continuous renal replacement therapy (RRT) extracorporeal circuits.

After the trial period, rewarming to 36-37^{o}C was commenced in both groups at the discretion of the treating physician and subsequent management was in accordance with standard medical practice including avoidance of fever (>37^{o}C). In those patients who underwent ELT rewarming was permitted prior

to surgery if there was concern with bleeding risk, and following return to ICU the patient was managed with a temperature of 36–37 ^{o}C.

Outcomes

The primary outcome was a sustained elevation in ICP >25mmHg for 5 minutes. Secondary outcomes included the occurrence of predefined serious adverse effects of thrombocytopenia, spontaneous haemorrhage, cardiac dysrhythmias, confirmed sepsis or acute pancreatitis. Other secondary outcomes considered included the absolute magnitude of ICP elevations and cerebral and all cause mortality. Follow-up was to hospital discharge.

Sample Size and Statistical Analysis

Case series from the participating centres at the trial inception suggested ICH to occur in 40% of patients with ALF and severe encephalopathy. The uncontrolled studies reporting the effect of MH suggested ICH to occur in in ≤10% of hypothermic patients. A sample size of 50 was thus planned to detect a difference in proportions of 40% in the control group and 15% in the 34^{o}C at a 5% significance level with 80% power [19]. In view of the potential for a powerful treatment effect, interim analysis was performed after enrolment of 20 patients, and then after the addition of each further 20.

The principal trial analyses were performed in a modified intention to treat population which included all randomly assigned patients, but subsequently excluded 3 patients all allocated as controls who were emergently treated with

therapeutic plasma exchange during the study period, at that time an experimental rescue therapy to control profound cardiovascular and metabolic disarray [17].

Comparison between groups for continuous variables was with the Mann-Whitney U test and for categorical variables the Chi-square and Fishers Exact tests. Kaplan –Meier survival curves were compared between the intervention groups with the use of the log-rank test. Analysis was performed using IBM SPSS Statistics version 22.0.0.

Results

A total of 46 patients were enrolled between January 2005 and March 2010. The modified intention to-treat population of 43 patients consisted of 17 patients assigned to the MH group and 26 as Controls. The two groups had similar characteristics at randomization (table 1). Overall, hospital mortality was 19/43 (44%) and 4/19 (21%) of deaths resulted from ICH. Eighteen (42%) patients underwent ELT a median of 2 (1-2) days after randomization of whom 4/18 (22%) died; hospital mortality in the non-transplanted patients was 15/25 (60%) ($p<0.05$). Flow of patients through the trial is shown in figure 1.

Temperature Intervention.

At randomization the median body temperature was 35.4^{o}C (35.0-35.9) in the MH and 35.9^{o}C (35.0-36.6) control groups respectively ($p=0.2$). At 6 hours after starting TTM, the temperatures were 33.2^{o}C (32.4-34.1) and 35.9^{o}C

(35.5-36.4) (p<0.001), and at 24 hours 33.0 (32.5-34) vs. 36.1 (35.5-37) (p<0.001) ; temperature curves are depicted in figure 2. Temperatures were within 0.5 ^{o}C of target at 6 hours in 8/14 (57%) in the 34^{o}C group and 15/25 (60%) 36^{o}C group. Median duration of TTM was 66 hours (23-72); 21/43 (49%) patients did not complete the 72 hour intervention period; 8/17 (47%) and 13/26 (50%) in the MH and control groups respectively (p=0.65). In 14 cases this was as a result of undergoing transplantation (TTM period 30 hrs (14-42)), in 5 as due to development of adverse events (24 hrs (18-54)), and in 2 (45 hrs) after patient death.

Outcomes

Intracranial Pressure

Overall, 19 of 43 (44%) of patients developed sustained elevated ICP at a median of 20 (12-93) hours after randomization with a peak ICP of 33 (28-47) mmHg. Intracranial pressure through the TTM intervention period is shown in figure 3; there were no significant differences in mean ICP or cerebral perfusion pressure when the two intervention groups were compared (figures 3,4). At randomisation 11/17 (65%) of the MH group and 17/28 (61%) of the controls (p=0.96) were requiring vasopressors and at 24 hrs 4/17 (24%) and 12/26 (46%) respectively (p=0.13).

During the 72 hour TTM intervention period only, elevated ICP occurred in 6/17 (35%) and 7/26 (27%) in the MH and control groups respectively (p=0.41, table 2). Overall during hospitalisation, elevated ICP occurred in 10/17 (59%) and 9/26 (35%) in the MH and controls respectively (p=0.12,

table 2) Survival plots are shown in figure 5, p=0.094 for comparison of TTM Groups, log-Rank test. In those developing ICH, peak pressures did not differ significantly when the two groups were compared MH 33 (26-42) vs. controls 37 (32-50) mmHg, p=0.56.

Survival

Overall, mortality was 7/17 (41%) in the MH group and 12/26 (46%) in the controls (p=0.75). In the MH group 7/10 (70%) of those not transplanted died at a median of 4 (3-8) days after randomization. In two cases a tentorial herniation was responsible, and in the remainder hypotensive multiple organ failure (MOF). There were no deaths in the 7 cases transplanted. In the control group 8/15 (53%) non-transplanted patients died with MOF at 9 (4-21) days. Four of 11 (36%) transplanted died on the second post-transplantation day, two with tentorial herniation (p=0.07) and the remainder with MOF.

Adverse Events

A total of 23 adverse events were seen in 17 patients; 6 of 17 (35%) in the MH and 11 of 26 (42%) in the control groups respectively (p=0.83, table 3). Discontinuation of TTM was performed after adverse events in 5 cases, 3 (18%) in the MH group and 2 (7%) controls. Brady-dysthymias occurred in 2 patients in the MH group whose nadir temperatures reached 32.3^{o}C and 33.2^{o}C; one control patient developed an acute myocardial infarction. One patient in the MH group developed a right middle cerebral artery hemispheric infarction, unrelated to ICP monitor insertion. One control patient developed a

temporal lobe intra-parenchymal hemorrhage 9 days after undergoing transplantation. There were no other complications related to ICP monitoring and no differences between groups in the incidence of confirmed sepsis.

Discussion

In this randomized controlled trial of TTM in patients with ALF at high risk of cerebral oedema and ICH, we found that management of patients with a target body temperature at 34°C as compared to 36°C was not associated with a lower incidence of clinically significant elevations in ICP. Rather, there was evidence of a tendency toward an increase. These findings were unexpected and contrary to those suggested by numerous animal models of ALF, where MH appears extremely effective in prevention of ICH and in the reduction of its severity when it develops [3]. Lack of concordance between the results of animal and clinical studies of TTM suggests that the clinical complexity of development of ICH may not be fully reproduced in current animal models [11 14]. By example, our patients received therapies not commonly applied in animal models which have the potential to modulate risk of CO and ICH including intravenous NAC, antibiotic and renal replacement therapy [20 22].

Conducting interventional studies in ALF is uniquely difficult, and in the assessment of our findings potential sources of bias and confounding factors must be considered. Our intervention groups were unequal in size, reflecting differences in the size of randomization blocks in the UK and Denmark, but were very well matched in respect of baseline characteristics and in particular

those associated with increased risk of ICH. The differences in cohort size were identified late in the study at a planned analysis, at which time the tendency to an increased incidence of ICH in the MH group were also identified. The consensus view of investigators at that time was that with altered patient characteristics and a lower prevalence of ICH and use of ICP monitors, continuing the trial appeared impractical and recruitment was closed [23]. Though this decision likely reduced the power of the study, indications were not of any benefit from MH, but rather an increased risk of ICH.

It is possible that we might have failed to demonstrate a benefit of MH as the temperature induced was insufficient to have cerebral therapeutic effect. However, animal studies have suggested effective reduction of CO at temperatures of 34-36^{o}C [24 25]. In clinical studies demonstrating effective short-term control by MH of refractory ICH, median temperatures induced were generally colder at 32-33^{o}C than those we targeted [7]. However, we found cooling was easy to achieve, and in practice it proved more difficult to maintain a temperature of 34 than 36^{o}C; temperatures in the former group were often closer to 33 than 34^{o}C.

In our study, adverse effects were equally common in both treatment groups, though dysrhythmias were only seen in patients whose temperature fell below the lower target range. In ALF and in common with other studies of TTM in critical illness, adverse event rates appear to relate closely to the minimum temperature induced [10,26]. The recent retrospective review of the clinical outcomes of patients from the USALF cohort compared those managed with MH at a median temperature of 34.1^{o}C with those with standard management

at 36.6°C [27]. Use of MH was associated with increased rates of arrhythmias and in those cooled to below 33°C indications of increased risk of sepsis [27].

Our clinical impression is thus that the use of these lower temperatures is not justified for ICH prevention, though they may continue to be utilized as short-term rescue therapy in patients with refractory ICH or potentially in other specific clinical settings. In this study those patients managed at 36°C mortality after transplant – including deaths from ICH - was higher than that in patients managed at 34°C, though this difference did not reach statistical significance. Whilst we cannot discount a beneficial effect of MH in the peri-transplantation setting, it is important to recognize that the numbers of patients involved in this sub-group was very small and our analysis did not consider graft factors of established importance in determining survival after transplantation [28,29]. However, it is possible that hypothermia prior to transplantation could have a beneficial effect during the intra- and post-operative period of very high risk for ICH. Further studies will be required to confirm these observations before this approach can be endorsed.

Conclusions

Taking currently available data together, some conclusions can be reached in relation to the place of TTM in the management of patients with ALF and high grade encephalopathy. In such patients, moderate hypothermia can be induced rapidly and without the use of specialist cooling devices. In common with its use in other clinical settings, adverse effects are seen, with risk of development relating to the degree of cooling. The routine use of

temperatures below 34°C probably carries greater risk to patients and does not appear to have any benefit beyond that seen at 36°C in general protection from the development of ICH or improved survival.

This study failed to demonstrate any of the expected benefits of an intervention that in animal models shows powerful and apparently consistent effects against the development of ICH in high-risk ALF patients. It is clearly important to understand what aspects of the human illness these models apparently fail to replicate. Further, this study did not address how best to treat those patients in whom ICH has developed and which has failed to respond to standard measures. The application of TH in this setting may well be of benefit but the rarity of these patient sub-groups makes establishing the most effective means of its application even more difficult to determine. There is still need for coordinated international study of the condition and further multi-centre randomized trials.

Table 1. Characteristics of Study Patients at Randomisation.

	TTM Group			
	34ºC		**36ºC**	**p**
N	17		25	
Age (years)	39 (28-51)		38 (29-51)	0.75
Gender (F)	9 (53%)		16 (64%)	0.56
Etiology (APAP)*	10 (59%)		20 (80%)	0.21
KCC fulfilled	16 (94%)		22 (88%)	0.34
ICP (mmHg)	16 (9-19)		15 (8-21)	0.99
Temperature	35.4ºC (35.0-35.9)		35.9ºC (35.1-36.6)	0.2
HR (b/min)	95 (76-100)		87 (76-104)	0.88
MAP (mmHg)	81 (71-89)		80 (72-90)	0.98
Vasopressors	11 (61%)		17 (68%)	0.96
Creatinine (µMol/l)	163 (93-261)		232 (178-303)	0.08
RRT	11 (61%)		18 (72%)	0.76

FiO2 (%)	0.30 (0.26-39)		0.34 (0.30-42)	0.63
PaO2/FiO2	365 (305-417)		330 (277-394)	0.30
INR	4.8 (3.8-7.7)		5.4 (4.6-7.9)	0.23
Bilirubin	140 (67-256)		102 (78-223)	0.80
AST (IU/l)	1275 (326-4345)		2921 (904-5523)	0.13
Lactate (mMol/l)	2.8 (1.9-4.2)		2.5 (1.8-4.2)	0.68
Sodium (mMol/l)	143 (138-146)		143 (136-147)	0.85
WBC ($x10^9$/l)	7.3 (4.5-9.7)		8.2 (5-14)	0.45
Platelets ($x10^9$/l)	78 (46-124)		70 (56-125)	0.27
Arterial				
pH	7.39 (7.28-7.46)		7.40 (7.33-7.41)	0.89
$pC0_2$ (kPa)	4.9 (4.3-5.5)		5.0 (7.7-5.6)	0.45
HCO_3 (mMol/l)	22.4 (19.5-25.6)		23.1 (20.9-25.9)	0.56
pO_2 (kPa)	14.7 (14.1-19.2)		13.9 (12.8-14.2)	0.13
Ammonia (µMol/l)	159 (89-210)		171 (121-198)	0.53

Note; *Non-APAP aetiologies; 34°C (n=7) Indeterminate n=5, Wilsons Disease n=1, DILI n=1, 36°C (n=6) Indeterminate n=4, Viral n=2.

Abbreviations: KCC; Kings College Criteria for poor prognosis, WBC; white blood cell count, AST; Aspartate Transaminase, RRT; Renal Replacement Therapy, HR; heart rate, MAP; Mean Arterial Pressure, ICP; Intracranial Pressure.

Table 2. Primary and Secondary Outcomes.

		TTM Group				
						P
		34ºC		**36ºC**		
N		17		26		
Treatment Duration (hours)		66 (42-72)		66 (24-72)		
Intracranial Pressure (ICP)						
ICP >25 mmHg: onset <72 hours		6 (35%)		7 (27%)		0.41
ICP >25 mmHg: at any time		10 (59%)		9 (35%)		0.12
Peak ICP (mmHg)		33 (26-42)		37 (32-49)		0.43
Survival						
Transplanted		7 (41%)		11 (42%)		0.94
Of whom Died		0 (0%)		4 (36%)		0.07
Non-Transplanted		10 (59%)		15 (58%)		0.94
Of whom Died		7 (70%)		8 (53%)		0.41
Overall						
Died		7 (41%)		12 (46%)		0.75
Cause of Death						

Tentorial Herniation		2 (29%)		2 (17%)		0.98
Multiple Organ Failure		5 (71%)		10 (83%)		

Table 3. Adverse Events and reasons for failure to complete 72 hour TTM period.

		TTM Group				
		34ºC		**36ºC**		**p**
N		**17**		**26**		
Adverse Events						
Thrombocytopenia*		1 (6%)		5 (25%)		0.40
External Haemorrhage		0 (0%)		3 (12%)		0.38
Cerebrovascular Event		1 (6%)		1 (4%)		0.85
Sepsis		3 (18%)		5 (25%)		0.85
Cardiac		2 (12%)		1 (4%)		0.73
Pancreatitis		1 (6%)		1 (4%)		0.78
Any		6 (35%)		11 (42%)		0.83
Reason for Discontinuing TTM						
Transplanted		5 (29%)		9 (35%)		
Adverse events		2 (18%)		2 (8%)		
Died		0 (0%)		2 (8%)		
All		8 (47%)		13 (50%)		0.85

Note; *platelet count <30 $x10^9$/l

Figure 1. Flow of Patients through the Trial.

Note: MH; moderate hypothermia, CVS/ICP; cardiovascular/Intracranial pressure

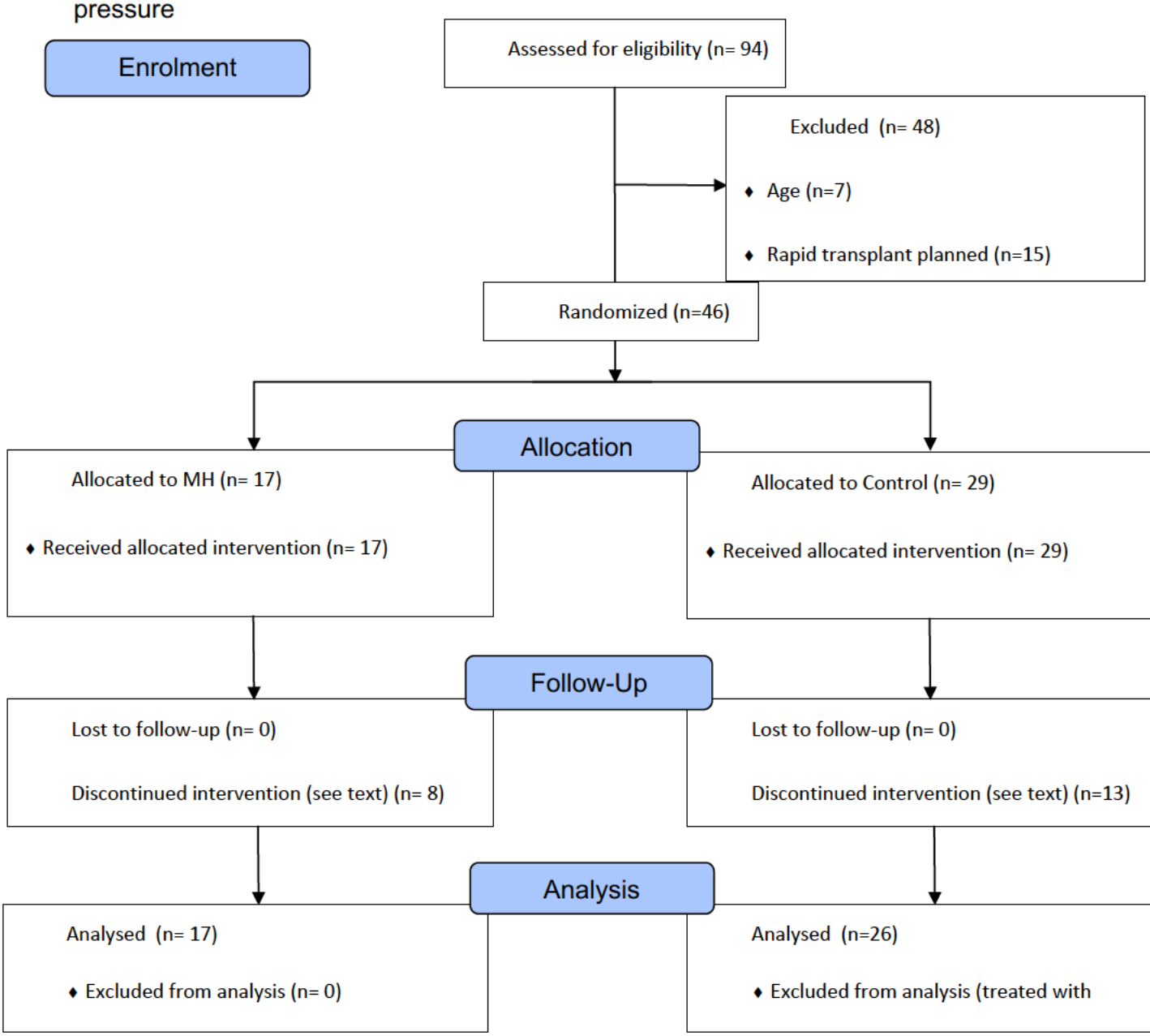
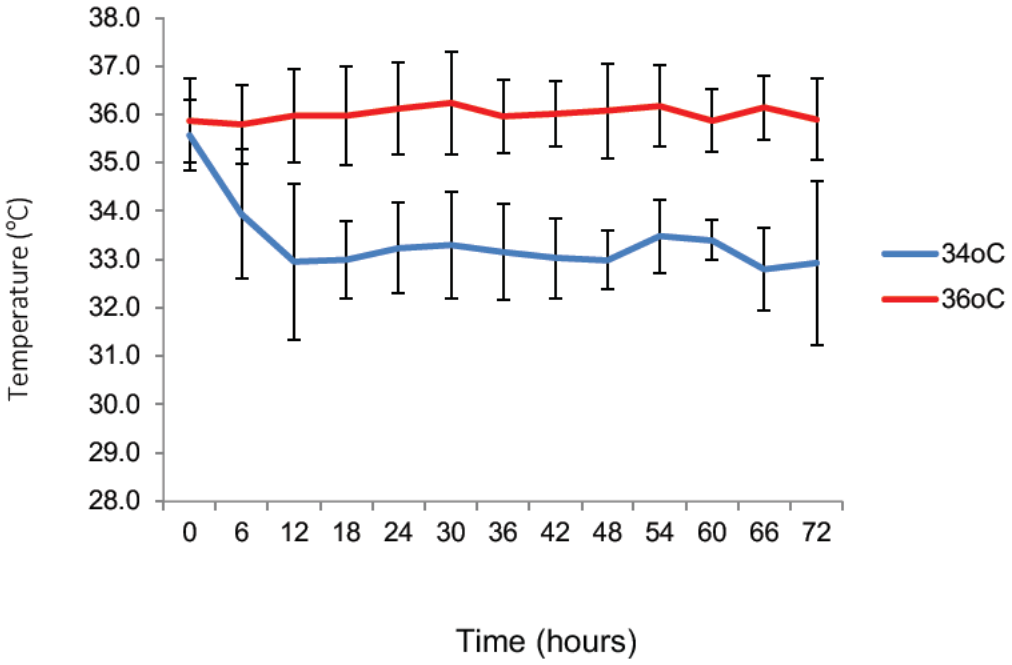
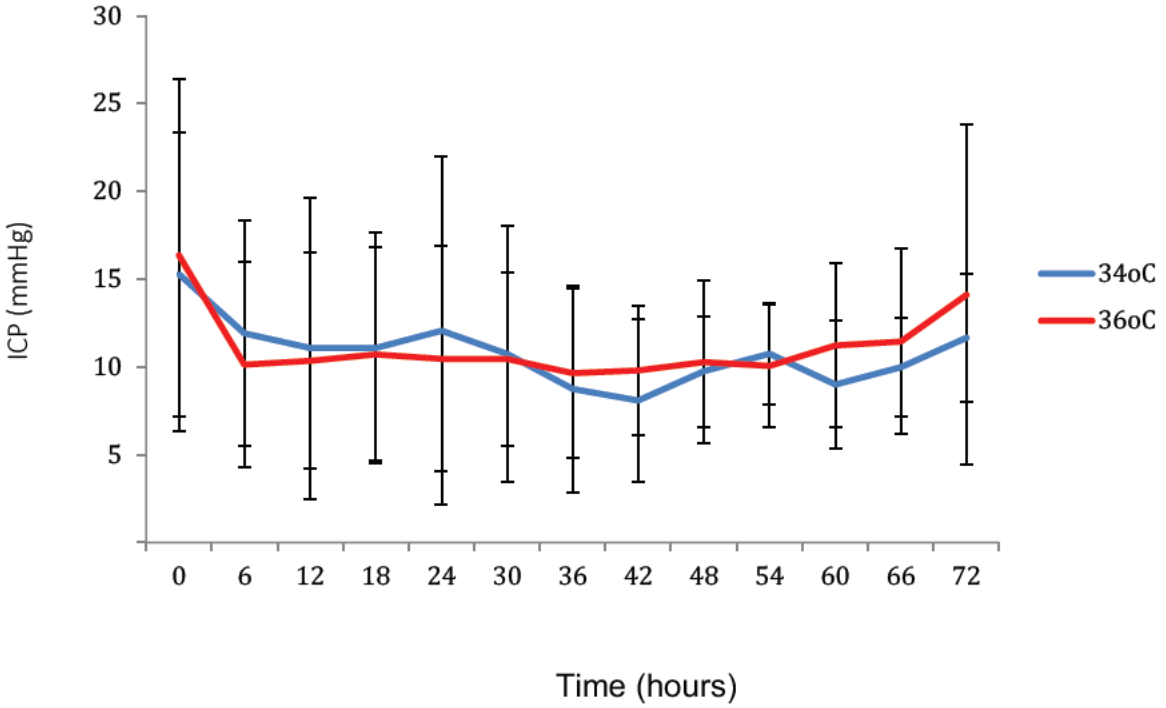


Figure 2. Temperature Profiles during study period in TTM groups.



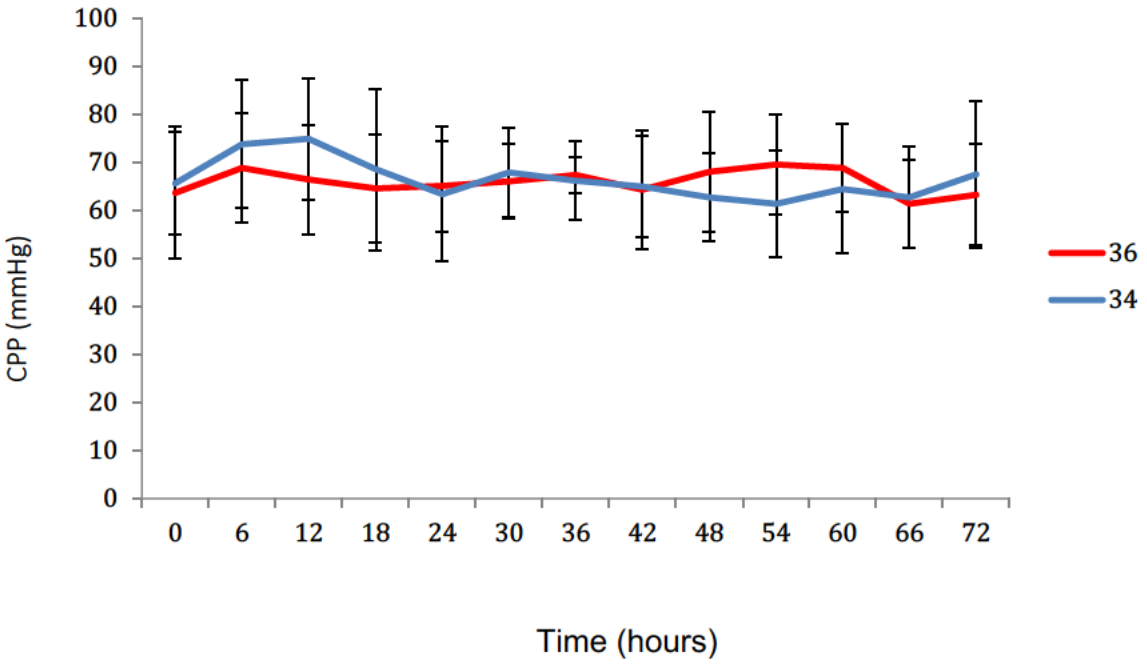
Note; figure shows mean (Error bar is +/- 1 standard deviation). $p<0.001$ for comparison of TTM groups at all time points except 0 hours.

Figure 3. Intracranial Pressure (ICP) during study period in TTM groups.



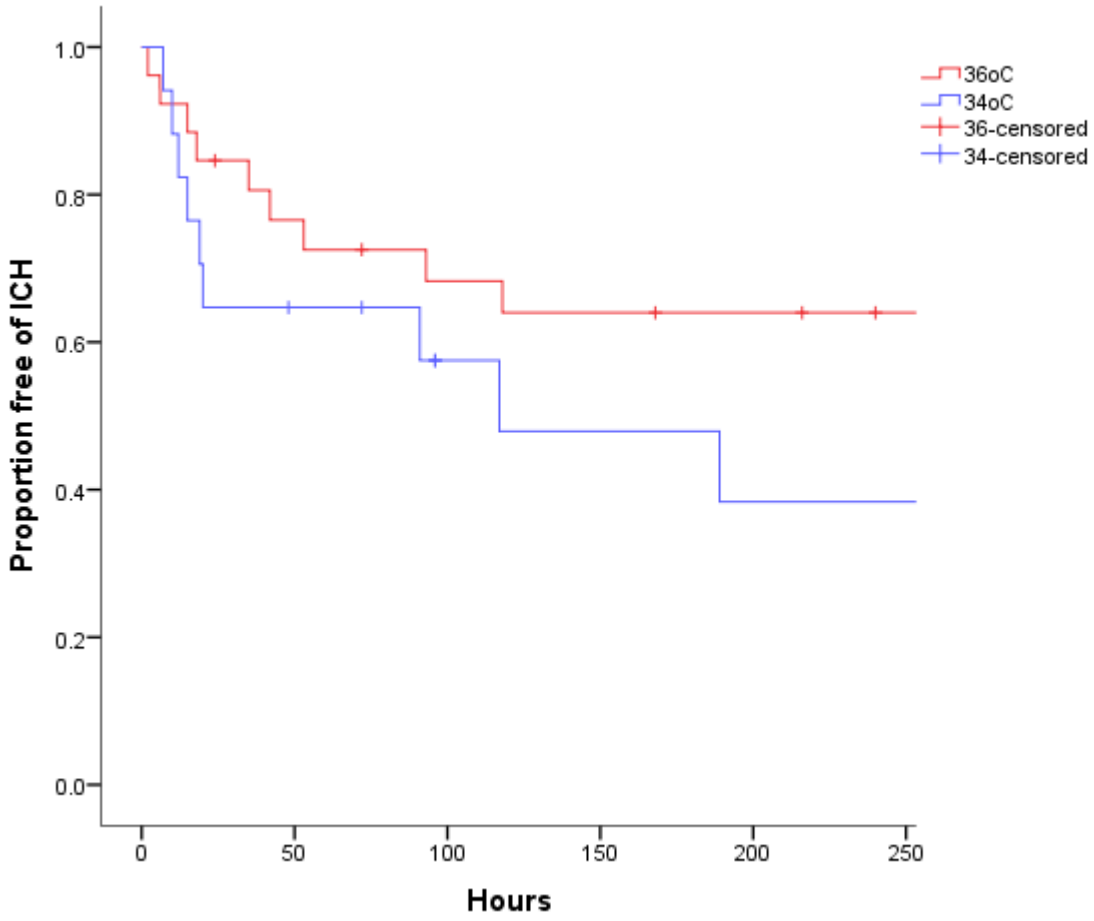
Note: figure shows mean (Error bar is +/- 1 standard deviation). P=non-significant for comparison of TTM groups at all time points.

Figure 4. Cerebral Perfusion Pressure (CPP) during study period in TTM groups.



Note: figure shows mean (Error bar is +/- 1 standard deviation). P<0.05 for comparison of TTM groups at 12 hours otherwise non-significant.

Figure 5. Kaplan-Meier Plot of freedom from intra-cranial hypertension by TTM Group



Note; Patients were censored at death. p=0.094 Log-Rank for comparison of TTM groups.

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Chapter 5 Discussion

Following a general introduction into aetiology and the management of ALF, this thesis describes a number of investigations into the pathophysiology, the prognosis and management of acute liver failure. More specifically it examines three main topics;

1. The pathophysiology of hyperlactatemia in ALF and the regulation of T-cell recruitment into the liver in human ALF
2. The prediction of outcome in ALF
3. The management of ALF. In particular, strategies to prevent intracranial hypertension and the investigation of albumin dialysis in patients with ALF.

Chapter 1 is a review of ALF, written as a commissioned chapter in a major textbook of critical care medicine; Critical Care Medicine: Principles of Diagnosis and Management in the Adult. Edited by Joseph E. Parrillo MD FCCM and R. Phillip Dellinger MD MS. It was taken from the fourth edition, 2014. The chapter is a comprehensive review on the aetiology, pathophysiology and management of ALF and provides an introduction for the thesis.

Chapter 2 examines aspects of the pathophysiology of ALF. Specifically into the causes of a high serum lactate seen in ALF, especially in those with hyperacute ALF, notably associated with paracetamol induced necrosis. Hyperlactataemia observed in ALF represents a complex interaction resulting from an increase in production and reduced clearance. In health the liver consumes lactate. The first study presented investigates the role of the liver in lactate flux in patients with ALF undergoing liver transplantation. It has been

speculated that one of the causes of the positive lactate gradient seen across the liver in ALF might be related to the influx and activation of non-parenchymal cells to the liver. The second study in this section looks at the regulation of T-cell recruitment into the liver during ALF.

Chapter 3 presents two studies investigating the predication of clinical outcome in ALF. ALF has a high mortality. Despite the advances in management of the past few decades it still has an overall mortality of approximately 50%. Liver transplantation in ALF was introduced as a treatment option during the late 1980's. Predicting which patients would likely benefit from this very limited resource became increasingly important. Foreseeing outcome with certainty, early enough in the course of the syndrome before significant collateral organ failure has become established, is important to maximise utility without doing unnecessary transplants. Since then prognostic markers have been developed from demographic data and specific biomarkers. The first study investigates candidate biomarkers in a population here in Birmingham the second is a multicentre investigation into a novel dynamic model.

Chapter 4 presents two studies investigating prophylactic management of intracranial hypertension. ALF stands out from other causes of multiple organ failure such as sepsis, burns or trauma (excluding direct traumatic brain injury), as a cause of cerebral swelling and ICH. In ALF intracranial hypertension is a significant cause or mortality and once established has a significant attributable mortality. Strategies to treat established ICH have been investigated since it was recognised to be an issue. However, the prevention of ICH had not been investigated before the publication on the use of

hypertonic saline. The second study investigated the prophylactic use of hypothermia in the same setting. The third study presented in this section investigated the physiological effects of albumin dialysis in ALF, including the effects on ICH.

Lactate metabolism in ALF

Arterial lactate is raised in ALF. The peak lactate concentration correlates with outcome (1). It was noticed in the 1960s that metabolic acidosis is a clinical feature of ALF, particularly in paracetamol poisoning, and that lactate is one of the main contributors of this (2,3).

For much of the last century lactate was considered a waste product of metabolism associated with anaerobic metabolism. It represented an oxygen debt that had to be repaid, and in illness was thought to represent an breakdown of the normal balance between oxygen delivery and consumption (4). Much of the research that prompted this interpretation was performed in isolated muscle preparations in which oxygen insufficiency was assumed to result in the observed lactate accumulation following exercise. This explanation was extremely resilient and influenced the interpretation of hyperlactatemia seen during other stresses such as severe illness (5).

It is now recognised that hyperlactataemia in critical illness is more complex. Lactate is in a continuous state of turnover within the body. It is produced during aerobic and anaerobic metabolism and is consumed both locally and remotely and it can be considered an energy shuttle molecule within and between organs (6). Lactate can accumulate when production increases or when consumption decreases or when both happen at the same time. It can

be a temporary event with rapid resolution, for example, following a 100 meter sprint or a seizure, or can be more prolonged as often seen in established liver failure or sepsis. The peak of the lactate per unit time curve has less significance, generally, than the duration of elevation, or failure of clearance in terms of severity and prognosis (7).

Hyperlactataemia is seen following a change in the redox state of the cell as a consequence of a mismatch between oxygen supply and demand. Respiratory chain poisons can limit aerobic metabolism, increasing pyruvate and hence lactate production, and stress induced aerobic glycolysis can be seen during severe illness (8).

The liver has a central role in intermediary metabolism. Glucose produced during the digestion of carbohydrates in the gut is transported to the liver via the portal circulation. Glucose recirculates or is taken up and converted to glycogen for use during a fast. The majority of recirculated glucose is converted to lactate in peripheral tissues via glycolysis. The circulating lactate has a high turnover and represents about 50% of the resting energy substrate but is the primary circulating fuel for energy metabolism during prolonged exercise and other stressful occasions such as critical illness (6). Circulating lactate is also consumed within the liver where it is converted to glucose and hence onto glycogen (the Cori cycle). Lactate, rather than carbohydrate digestion, is the main source of circulating glucose, which is in relatively constant supply via gluconeogenesis.

Hyperlactatemia in ALF represents the complex interaction between increased production and reduced consumption. There is evidence of

increased production in the lungs and the splanchnic organs. Walsh and Clemmesen both showed positive lactate flux across the lung and splanchnic bed (9,10). What was uncertain was the role of the liver in this process. It could be assumed that there is a reduction in hepatic consumption in ALF because of a loss of hepatocytes and studies have shown a reduction in whole body lactate consumption in ALF (11).

The positive lactate gradient across the whole splanchnic region when sampling from the hepatic vein is unable to distinguish between the relative contribution of the liver and this was unknown prior to the publication of our paper. Access to the portal vein is critical to clarify the role of both the gut and the liver to the overall flux. The design of the study enabled a comparison of the lactate gradients before and following transplantation. Just prior to the closure of the abdomen all the grafts became net consumers of lactate except one patient that had an auxiliary graft. In which a left lobe of the injured liver is retained in the hope of possible recovery, regeneration and a future withdrawal of immunosuppressive therapy (12). The fact that all gradients reversed following transplantation strongly suggests that some metabolic process within the injured livers produced the lactate.

The positive lactate flux produced within the liver most likely comes from activated non-parenchymal cells (13). Activation of resident and migration to the liver of non-parenchymal cells has been shown by a number of different groups in ALF (14,15). Activation in response to tissue injury or infection changes the metabolic pathway from oxidative to non-oxidative. This enables the rapid growth, proliferation and effector functioning of the cells (16).

The regulation of T-cell recruitment to the human liver during acute liver failure

The liver plays a is central role in immune surveillance in the body. It has a large duel blood supply and effectively screens the splanchnic outflow from the portal vein. It is characterised by slow moving blood within the fenestrated liver sinusoids and is constantly exposed to systemic and gut derived antigens (17). During health the liver is in a state of relative immune tolerance because of this exposure. However, immune activation occurs rapidly on the exposure of many liver cell types to particular molecular patterns or alarmins. These can be pathogen derived or related to cellular damage. Often referred to as PAMPS or DAMPS (pathogen or damage associated molecular patterns). They lead to the activation of many cell lines in the liver. This immune activation in response to damage is characterised by an inflammatory infiltrate within the liver. The particular pattern seen depends on the aetiology of the liver insult (18). Both innate and adaptive responses occur. Recruitment of lymphocytes into the liver is stimulated by the secretion of chemokines and by an upregulation of adhesion molecules in response to injury. We showed expression of adhesion molecules on the surface of sinusoidal endothelial cells is markedly increased in ALF as was expression of chemokines ligand. This was seen most strongly in areas of leucocyte infiltration.

Paracetamol toxicity results in cell necrosis, most prominently in zone 3, and inflammation is a secondary event. In seronegative hepatitis we observed a lymphocyte rich infiltration of CD8 T effector cells in the portal tracts. The activation and migration of non-parenchymal cells into the liver investigated by

us and others provide a plausible explanation for the positive lactate flux seen in ALF.

Validation of prognostic biomarkers in ALF

Since the introduction of liver transplantation as a possible treatment strategy for ALF the use of early prognostic markers has been investigated. The decision to embark on liver transplantation, in this very sick group of patients, is difficult. The challenge of surviving the additional insult of surgery followed by lifelong immunosuppression is considerable. Clinicians need to be able to accurately predict the likelihood of death before organ failures become severe enough to render the patient inoperable, as significant number of patients with ALF will recover with supportive care, especially those with paracetamol toxicity. Large observational studies identified demographic and causative factors that contribute to a poor outcome in ALF. In the initial attempts, biomarkers of severity were identified and when combined with demographics and aetiology can be used as simple scoring systems with enough reliability and reproducibility to be used in the clinical setting (19).

Factors associated with outcome in ALF can be split into number of categories. Aetiology of the liver failure is important, with POD being the most likely to recover spontaneously. Demographics are important with extremes of age implicated with a worse outcome. The loss of metabolic activity of the liver is significant, with both the degree of coagulation abnormalities and bilirubin present in most prognostic scores. The degree of loss of liver volume (measured on CT scan) is also significant in certain aetiologies as is the severity of extra-hepatic organ failure, particularly renal and cardiovascular. One organ failure stands out in its prognostic influence. High grade

encephalopathy has a significant weighting on outcome in all aetiologies of ALF .

Many laboratory tests are abnormal in ALF. The degree of abnormality often correlates with severity to a greater or lesser extent. Individually, the majority are poor predictors of outcome and have large confidence intervals rendering them unreliable. Many models, usually defined via regression analysis, of combinations of laboratory tests, demographics and other clinical parameters, have been investigated by different groups (20). The majority represent single centre investigation and often have methodological issues.

Internationally there are a number of established prognostic criteria that have entered clinical practice. In the UK the King's College Hospital criteria (KCC), developed at the end of the 1980's, have been shown to very robust and simple to use, with a high specificity (21).

Studies in the 1960's and 70's revealed marked acid-base abnormalities to be prevalent in ALF and a low pH is a component of the KCC (19,22). Respiratory alkalosis, associated with severe encephalopathy is rarely seen today because of early intubation and ventilation, but was commonly reported in the 1970's (22). There are two main components of the metabolic acidosis seen in ALF. Renal failure seen commonly, especially in paracetamol toxicity and hyperlactatemia (23). But pH is a composite marker and has become less useful with the introduction of early renal replacement therapy (RRT), which will normalise serum pH rapidly, especially when started in referring hospitals before arrival at a transplant centre. Lactate remains elevated even if the pH

has been normalised via early RRT and when added to the KCC improves the sensitivity (1).

Serum phosphate levels are often abnormal in patients with ALF. Hypophosphatemia is common especially in patients with paracetamol induced ALF (24). The causes of this are not well understood but it is associated with renal wasting of phosphate (25). The prognostic significance of abnormal phosphate levels have been investigated in observational studies. Initially it was thought that low levels correlated with a worse outcome. However, a large study in Demark found that in fact normal or high levels of phosphate were associated with a worse outcome. The high phosphate levels were associated with acute kidney injury (AKI) (24). It has been speculated that the high levels seen in non-survivors may be related to the degree of liver necrosis and a lack of regeneration and the degree of extrahepatic organ failure (26). Others have suggested that the high phosphate seen in patients with an ultimately poor outcome was more a marker of renal failure but because of the large overlap with survivors its use as an outcome predictor in ALF is unreliable (27).

We looked at both lactate and phosphate in a cohort of patients with ALF. This was a retrospective review over 3 years of patients with ALF due to a range of aetiologies. We split the cohort into groups as survivors with medical management alone and nonsurvivors (patients managed with a liver transplant were included in this group). We also examined the groups on the basis of aetiology into patients with paracetamol induced ALF and others.

Relative hyperphosphatemia was significantly higher on univariate examination but did not stand up to multivariant analysis whereas there was a significant separation between survivors and nonsurvivors with lactate on both. This held for all aetiologies. 12 hour lactate is a significant predictor of survival or death following admission to the critical care unit. Whereas admission lactate was much less reliable.

We found that elevated lactate had time related significance. Repeated high lactate levels over time following resuscitation and management on the ICU is a poor prognostic sign.

Development and validation of dynamic prediction model for paracetamol induced ALF

There are limitations to the current poor prognostic criteria when applied to liver transplantation in patients with ALF. This is especially true for patients with paracetamol induced ALF (28).

The KCH criteria were introduced 30 years ago and they have withstood repeated validation over the years. The specificity remains high but sensitivity is lower. The criteria don’t work as well with staggered overdoses when the time from liver injury is uncertain (29). Lead time treatment effects such as fluid resuscitation and the use of renal replacement therapy in referring hospitals can significantly alter admission biomarkers. Critical care management has improved since they were first developed and the overall survival in patients with paracetamol induced ALF managed medically is approaching that of patients managed with liver transplantation as a pathway (28).

There will remain a small number of patients in whom liver transplantation remains the best option. There is an imperative to update and improve prognostic criteria and move away from single binary criteria at a single time point and recognise the evolution of the syndrome and how the patient responds to supportive therapy.

Criteria derived from admission data aim to inform decisions about transplantation as early as possible in the patients stay at a transplant centre. Clinicians need to be confident that if patients pass the threshold of severity transplantation is the best option for the patient. This high specificity is at the expense of sensitivity, in that there will be patients who subsequently deteriorate, later in the course of their illness. Adding more criteria, such as lactate or other organ failure parameters, will increase sensitivity but will increase the number of transplants, inevitably making the criteria less specific (30). A way out of this problem is to use dynamic criteria which monitor parameters over time. By doing this, response to supportive care can be monitored and confidence increased. Authors in India, developed dynamic criteria, looking at how they evolved over three days. The criteria included arterial ammonia, INR, bilirubin and encephalopathy grade. The patient group consisted mainly of patients with viral hepatitis and indeterminant aetiology. Those that deteriorated or did not improve over the 3 days had better AUC compared either to the MELD score or KCC (31).

Together with colleagues at four liver transplant centres in the UK and Demark. Will Bernal, at King's, developed a dynamic outcome predication model in patients with ALF secondary to paracetamol toxicity. We here in

Birmingham provided data for the validation of the model and I helped construct the final paper for publication.

An initial cohort of 500 consecutive patients from King's College Hospital was used for the development of the model. 350 for derivation and 150 for validation. This was followed by an external validation cohort of 412 patients derived from Copenhagen, Edinburgh, Birmingham and King's.

A sequential model was chosen with day 1 and day 2 criteria. The idea was to see response to supportive care and evolution over time. The variables of age, Glasgow Coma Score (GCS), arterial pH, lactate, creatine, INR and SOFA cardiovascular score were identified and included. When day two variables were considered only reduction in INR and lactate improved prognosis and so the delta lactate and INR were added to the day one variables in the day two model. The dynamic model improves both discrimination and calibration when the day variables are included. This should increase confidence for clinicians making difficult decision about transplantation.

The evaluation of a non-biological extracorporeal liver assist device in patients with ALF.

The chance of spontaneous recovery from ALF varies according to aetiology. Patients with paracetamol induced hepatic necrosis have the highest chance of spontaneous recovery. Following injury hepatocyte regeneration starts and if the patient survives the associated extrahepatic organ failure normal liver structure and function can be anticipated in the future (32). The hyperacute

presentation is associated with the highest burden of organ failure and the highest incidence of intracranial hypertension.

As well as general supportive care in the intensive care unit, the search for effective liver support to provide lost metabolic function, reduce the burden of organ failure and bridge the patient to recovery or transplantation has remained a highly appealing concept (33).

There is a long history of investigations into both biological and non-biological liver assist devices. The non-biological systems assume that the systemic effects of ALF, at least in part, relate to reduced metabolic capacity and the accumulation of “toxins” within the blood. This concept remains ill-defined and specific toxins, apart from ammonia, have not been characterised.

The hyperammonaemia that occurs in ALF, is related to severity and is known to contribute to end organ damage (34). Ammonia is water soluble and can be cleared by dialysis or ultrafiltration. In fact, continuous, high volume haemofiltration or diafiltration is now a standard of care in patients with ALF, particularly those that have a hyperacute presentation (35,36).

Molecular adsorption and recirculation system (MARS) is an extracorporeal blood purification system that dialyses the blood against an albumin containing dialysis fluid. Early in its development it was shown that albumin dialysis can remove bilirubin and reduce jaundice in vitro and in patients with chronic liver disease (37,38). While dialysis is good at removing small water soluble molecules from plasma water, protein bound and lipid soluble molecules are less easily cleared. Albumin dialysis together with polysulphone

dialysis membranes has the ability to facilitate the transfer of protein bound molecules from the blood into the albumin containing dialysate fluid (39).

Initially single pass albumin dialysis was used but this was inefficient and expensive as the albumin was wasted. The MARS system was invented to recirculate and clean the albumin in the dialysate fluid through an ion exchange and activated charcoal columns. Ultrafiltration of the dialysate fluid is achieved with a second filter in series for control of fluid balance .

Data was accumulating for the effects of MARS in patients with acute on chronic liver disease but the effects on patients with ALF had not been systematically studied, outside of a few case reports and case series (40).

Our study was designed to systematically investigate the physiological effects of two MARS sessions of 8 hours each in patients with ALF. All patients had high grade encephalopathy and as well as cardiovascular monitoring all had an ICP bolt and a jugular venous catheter in situ to measure intracranial pressure and cerebral oxygen extraction.

We found that MARS therapy had modest effects on cardiovascular parameters, increasing vascular resistance with a consequent reduction in cardiac output. There was also a reduction in jugular venous oxygen saturation over the course of each session, which did not achieve significance, but probably represented an increase in extraction accounting for the fall in cardiac output. Intracranial pressure did not change over the sessions. We recommended further research but could not recommend clinical use based on our data. Since our study was published there has been a large multicentre randomised controlled clinical trial into the use of MARS in

ALF which did not show a mortality benefit (41). However, MARS therapy continues to be used and retrospective studies suggesting benefit continue to be published (41,42). MARS therapy has a biological effect, it reduces jaundice and alters haemodynamics, at least initially. The temptation to “do something” in patients with ALF is great and so it continues have advocates. It is expensive and until outcome benefit can be shown its use in ALF will remain controversial (43).

The prevention of intracranial hypertension in patients with ALF

Intracranial hypertension was noted to be prevalent in ALF during the second world war. Clinical descriptions of the cases and autopsy studies on US service men dying of acute viral hepatitis during the south east Asia campaigns revealed it to be a relatively common finding (44). it wasn’t until later that this became recognised as part of the clinicopathological syndrome of ALF (45,46).

The pathology of intracranial hypertension is complex and likely related to more than one factor. Age of the patient, the degree of organ support, the rate of onset of the syndrome and the arterial ammonia concentration have all been shown to increase the risk of developing ICH (34).

The neuro-psychiatric changes associated with ALF have been described in the introduction. And can be seen as a continuum in severity. Cerebral oedema is preceded by grade 4 encephalopathy.

The incidence of ICH has fallen since it was recognised as a significant cause of mortality in ALF (47). The causes of this is multifactorial and related to general improvements in management of patients while on the intensive care

unit. Explicit prophylactic interventions aimed at reducing the incidence of ICH were investigated in the papers presented. This was not a completely novel approach as dexamethasone prophylaxis had been investigated previously although the use of osmotic therapy and hypothermia in such a way had not. Although dexamethasone prophylaxis produced no effect on the incidence or the pattern of rises in intracranial pressure (48).

The use of osmotherapy for the management of brain swelling has been used for over 100 years (49). Its use in ALF was described from the early 1970s. Mannitol boluses were reported to result in a fall in ICP in the majority of cases but rebound effects where ICP was shown to rise after therapy was also noted (46). Mannitol therapy remained the mainstay of management, but repeated use will induce a hyperosmolar state and there is evidence of tachyphylaxis.

We investigated the effects of prophylactic hypertonic saline infusion in a randomised controlled clinical trial of in 30 patients with ALF. All participants had grade 3 or 4 encephalopathy and had an intracranial pressure monitor in-situ. We found that an infusion of 30% hypertonic saline significantly reduced ICP from baseline and reduced the number of surges in ICP over the next 5 day when compared to the control group.

Following this publication, the use of prophylactic hypertonic saline has been included in the American Association of Liver Disease (AASLD) position paper on the management of ALF and the European Association for the Study of the Liver (EASL) clinical practice guideline on the management of ALF (50,51). It has become a standard of care internationally.

Hypothermia has been noted to have life sustaining properties for millennia. In circumstances where death would normally be assured, incidental hypothermia was noted, anecdotally, to preserve vital functions on rewarming (52). This has lead to the use and investigation of therapeutic hypothermia in many circumstances. A case series was published in patients with ALF illustrating the effects of hypothermia on ICP in patients with ALF and subsequent studies, by the same group investigated the mechanism. It was suggested that this manoeuvre could support patients to recovery or liver transplant (53,54).

Together with colleagues from King's College Hospital in London and the Rigshospitalet, University Hospital in Copenhagen we set up and ran a multicentre international randomised controlled clinical trial into the use of prophylactic moderate therapeutic hypothermia in patients with ALF. All patients enrolled had high grade encephalopathy and had an ICP bolt in situ. I was involved with the concept, design and the delivery of the study. I led the local enrolment of patients. We enrolled 46 patients and were unable to show that reduction of temperature to 34 degrees centigrade, prophylactically, in patients with high ALF and high grade encephalopathy with an ICP monitoring reduced the baseline ICP or prevented surges. In fact there was a trend in the opposite direction.

The use of therapeutic hypothermia (TH) in critically ill patients following cardiac arrest or trauma, has been introduced over the last twenty years following initial encouraging studies (55) and TH has been embraced by neonatologists providing consistent results in neonatal hypoxic encephalopathy (56). However, following initial enthusiasm more recent

clinical trials and meta-analysis do not support TH as a treatment modality in these settings in adults (57,58). It is interesting that TH has followed a similar evolution in ALF. Theoretical and preclinical experiments point to the potential therapeutic benefits of TH but translating this to improved outcomes within the noise and heterogeneity of clinical practice has proven difficult (59).

Future directions

The incidence of viral hepatitis is low in the UK and the numbers of patients with ALF secondary to paracetamol toxicity has fallen dramatically from the peak in the mid 1990s. As a result of this there is significant challenge in running single centre investigation and clinical trials in ALF. Developing collaborative links between liver transplant centres, both nationally and internationally is crucial to enable future research. The US acute liver failure research group has been very successful over the last 20 years (https://www.utsouthwestern.edu/labs/acute-liver) publishing extensively.

Informal collaboration has been successful in Europe and the UK. In particular with clinical trials in high volume plasma exchange and therapeutic hypothermia, and the development of novel prognostic criteria, (60–62). The development of the UK BASL special interest group in acute liver failure in recent years will be a platform for closer collaborative work in the UK.

Despite the challenges in performing clinical research in ALF there is much to do. Basic research into the epidemiology and pathogenesis of ALF is the key understanding and to the development of future therapy (63)(64).

Effort to develop better prognostic models of liver failure continues but the adoption of dynamic scores, which appear to provide greater sensitivity

without discarding specificity is slow. The use interventions that measure metabolic capacity hold promise as a way of testing function in real time. LiMax, which measures the exhaled labelled markers of methacetin metabolism, has been investigated in ALF and the development of novel tests such as a lactate consumption test may provide more information through which clinicians can base difficult clinical decisions (65).

Other markers of cell damage such as microRNA have shown promise as early biomarkers of regeneration in ALF and may become clinically applicable (66). Serum bile acids have been shown to be raised in paracetamol induced toxicity and have received little attention until recently. Not only are they raised but are also toxic to hepatocytes and may be both prognostic and a target for intervention (67).

The management of ALF improves with general developments in intensive care medicine but there have also been specific advances. Ammonia is now recognised as pivotal in the development of cerebral swelling and intracranial hypertension. Controlling ammonia production, metabolism and removal remain active areas of research. Early haemofiltration is now a standard of care in hyperacute liver failure. It performs a number of management aims; such as, the control of volume status, serum sodium, support of renal function, and importantly, removal of ammonia. Studies have shown that haemofiltration removes ammonia, however the most efficient method is not known and should be a relatively simple investigation to undertake (36).

Monitoring for intracranial hypertension remains a thorny issue in the management of ALF. The use of pressure transducers is invasive and

dangerous. The incidence of intracranial bleeds is higher in ALF than in TBI and as a result the use in this setting has been questioned. Additionally, the original generation of enthusiast intensivist and hepatologist inserting ICP bolts in patients with ALF have either retired or nearing the end of their careers. The newer generation of clinicians has less experience, as the numbers of patients with ALF has decreased. At the same time, observational data suggest a reduction in incidence of ICH, but more importantly, no difference in outcome when patients with bolts are compared to those managed without (47). This creates a challenge as management algorithms published by national and international societies still suggest the use of ICP bolts in patients with hyperacute ALF. Clearly patients with ALF still develop ICH so the development of management algorithms not dependent on invasive pressure measurements are required. In the short-term national outcome data is being gathered through the BASL ALF-SIG and may help provide outcome data and also inform a way forward.

Each generation of clinician managing ALF is attracted by the promise of extracorporeal liver support. All of the possible techniques, including biological and none-biological perfusion, cross perfusion of whole organs, both xeno-grafts and human, have been investigated previously (68). Each generation builds on the previous with technological gains but good outcome data remains elusive. Recent advances with whole organ normothermic perfusion of whole human livers, turned down for transplant, is making extracorporeal support a realistic option again. Novel technology and protocols with normothermic blood perfusion makes the resuscitation and prolonged perfusion of discarded human livers possible. It is relatively easy next step to

see this as an option for extracorporeal cross perfusion of patients with ALF (69).

Access to transplantation for ALF in the UK is based on national criteria and consensus. However, listing decisions remain local to each transplant centre. How patients get onto the list or are excluded is not well understood and understanding if there is variation is an important area for future investigation.

Finally, long term health outcomes from ALF are poor compared to the general population. Transplantation, although lifesaving in some patients does not appear to improve long term outcomes when compared to spontaneous survivors (70). Further work attempting to understand long term health in ALF survivors is needed.

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