STEM CELL THERAPY IN LIVER CIRRHOSIS

By

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ABSTRACT

Liver cirrhosis is the fifth leading cause of death worldwide and the definitive treatment for liver cirrhosis is liver transplantation although there are limitations such as organ availability and surgical risks. Therefore, alternative therapies have been studied extensively and stem cell therapies have shown some promising results although most studies are small and not randomised.

The aim of this thesis was to explore the effectiveness of stem cell therapy in patients with chronic liver disease as well as explore the mechanism behind fibrosis resolution achieved with cell therapy.

There were three parts to the thesis: firstly, I examined the mechanistic actions behind fibrosis reduction by the infusion of bone marrow derived haematopoietic stem cells (HSC) in mice chronic fibrosis liver injury model. I worked on both immune-histochemical staining and qPCR to measure the effect oval cell response, matrix metalloproteinases and macrophage subsets within the liver with HSC therapies.

Secondly, I recruited patients with chronic liver diseases for a multicentre, randomised, controlled trial to assess the clinical effectiveness of either subcutaneous granulocyte-colony stimulating factor (GCSF) or GCSF with repeated HSC infusions. The co-primary outcomes were improvement in severity of liver disease measured by model for end stage live disease (MELD) at 3 months and the trend of MELD change over time. The results showed that neither of the treatments improved the clinical outcomes.

Lastly, I performed a systematic review of current published studies of stem cells therapies in liver diseases. The results showed that stem cells improved patients' clinical parameters in the short term (<6 months) but had no benefit on long term outcomes.

In conclusion, bone marrow derived stem cell therapy did not seem to be effective in liver cirrhosis.

DEDICATION

Without the support and encouragement of my family, I would not have been able to complete this thesis.

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ABBREVIATIONS

Abx: Antibiotics

ARLD: Alcohol related liver disease

ACLF: Acute on chronic liver failure

AFP: Alpha feta protein

AIH: Autoimmune hepatitis

ALT: Alanine transaminase

AST: Aspartate transaminase

AMA: Anti-mitochondrial antibody

Alb: Albumin

APRI: AST: platelet ratio index

AE: Adverse events

Bx: Biopsy

Bil: Bilirubin

BM: Bone marrow

BMI: Body mass index

BMSC: Bone marrow stem cell

BMC: Bone marrow cell

CC: Cryptogenic cirrhosis

CPS: Child Pugh score

CLD: Chronic liver disease

CCl: Carbon tetrachloride

CLDQ: Chronic liver disease questionnaire

CI: Confidence interval

DAB: Diaminobenzidine

ELF: Enhanced liver fibrosis

ECM: Extracellular matrix

FU: Follow up

GI: Gastrointestinal

GGT: Gamma-glutamyl transferase

GCSF: Granulocyte colony stimulating factor

Hx: History

HA: Hepatic artery

HBV: Hepatitis B virus

HCV: Hepatitis C virus

HCC: Hepatocellular carcinoma

HE: Hepatic encephalopathy

HSCs: Hepatic stellate cells

HSC: Haematopoietic stem cells

HA: Hyaluronic acid

HIV: Human immunodeficiency virus

HRS: Hepatorenal syndrome

HTLV: Human T-cell lymphotropic virus

INR: International normalised ratio

IL- Interleukin

kPa: Kilopascals

KSL: c-kit+ sca1+ lin-

LFT: Liver function tests

LPC: Liver progenitor cells

LT: Liver transplantation

MD: Mean difference

MELD: Model for end stage liver disease

MOF: Multi-organ failure

MMP: Matrix metalloproteinases

MSC: Mesenchymal stem cell

Non-RCT: Non-randomised controlled trial

NAFLD: Non-alcoholic fatty liver disease

NHS: National health service

OR: Odds ratio

PBSC: Peripheral blood stem cell

Plt: Platelet

Pan-CK: Pan cytokeratin

PIIINP: Pro-collagen type III

PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses

PT: Prothrombin time

PV: Portal vein

qPCR: Quantitative polymerase chain reaction

QoL: Quality of life

RCT: Randomised controlled trial

SAE: Serious adverse events

SBP: Spontaneous bacterial peritonitis

SMD: Standardised mean difference

Sox-9: Sry/sex determining region Y-box 9

SOFA: Sequential organ failure assessment

SMT: Standard medical therapy

TBS: Tris buffered saline

TBS-T: Tris-buffered saline- Tween

TIPSS: Transient intra-hepatic portosystemic shunt

T2DM: Type 2 diabetes mellitus

TE: Transient elastography

TIMPs: Tissue inhibitors of metalloproteinases

UK: United Kingdom

UKELD: United Kingdom Model for end stage liver disease

ULN: Upper limit of normal

UC: Umbilical cord

WBC: White blood cell

WHO: World Health Organisation

1.INTRODUCTION

1.1: Anatomy of the liver

The liver is the largest organ in the body and is found in the right upper abdominal region. There are 2 anatomical lobes in liver, as in the right and left lobe. Within the right lobe segment, the caudate lobe is found on the posterior surface of the liver and the quadrate lobe is on the inferior surface (Figure 1) (1). The functional liver unit consists of the hepatic lobule which has a central vein and hexagonal portal trials including portal vein, hepatic artery and bile duct (2). The central vein is connected to the portal trails vis sinusoids that run through the hepatic plates (2). The liver has dual blood supplies; 80% of the blood supply comes from the portal vein and 20% from the hepatic artery (1). The portal vein brings venous blood from the gut, pancreas and spleen and supplies low oxygenation venous blood which is high in nutrition and toxin content (1) . The liver receives arterial blood from the hepatic artery which arises from the coeliac axis (1). Bloods drain from sinusoids between the hepatocytes into the hepatic vein and subsequently into the inferior vena cava (Figure 2) (1).

1.2: Functions of the liver

The liver is essential for a wide variety of functions including glucose metabolism through glycogenolysis and gluconeogenesis, synthesis and excretion of bile acids, synthesis of plasma proteins such as albumin, production of clotting factors, metabolism of nitrogenous waste products, cholesterol and steroid hormone metabolism (1, 2). In addition, the liver removes toxins from the blood stream, metabolises pharmacological drugs and regulates the immune system (1, 2). Peri-portal hepatocytes are involved mainly in oxidative liver functions such as gluconeogenesis, beta-oxidation of fatty acids, cholesterol synthesis while peri-central hepatocytes are more specialised for glycolysis, lipogenesis and cytochrome p450 based drug detoxification (2).

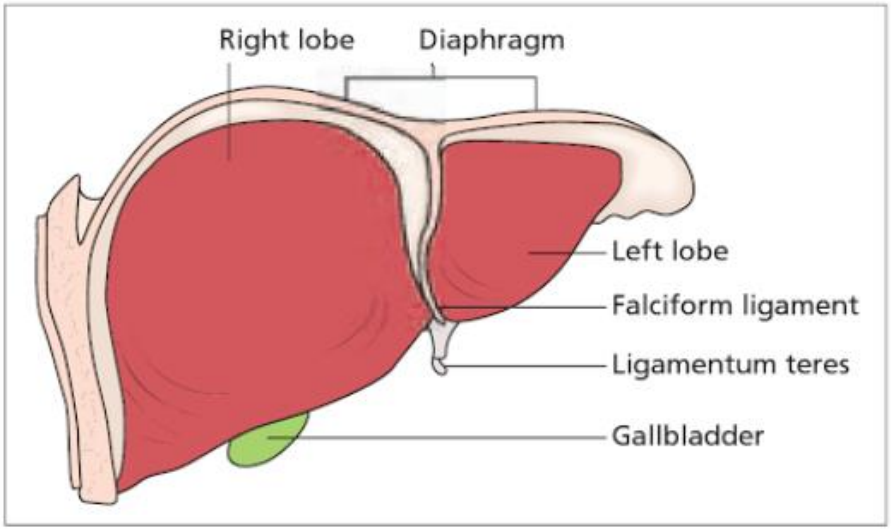


Figure 1: Anatomy of the anterior view of the liver (1)

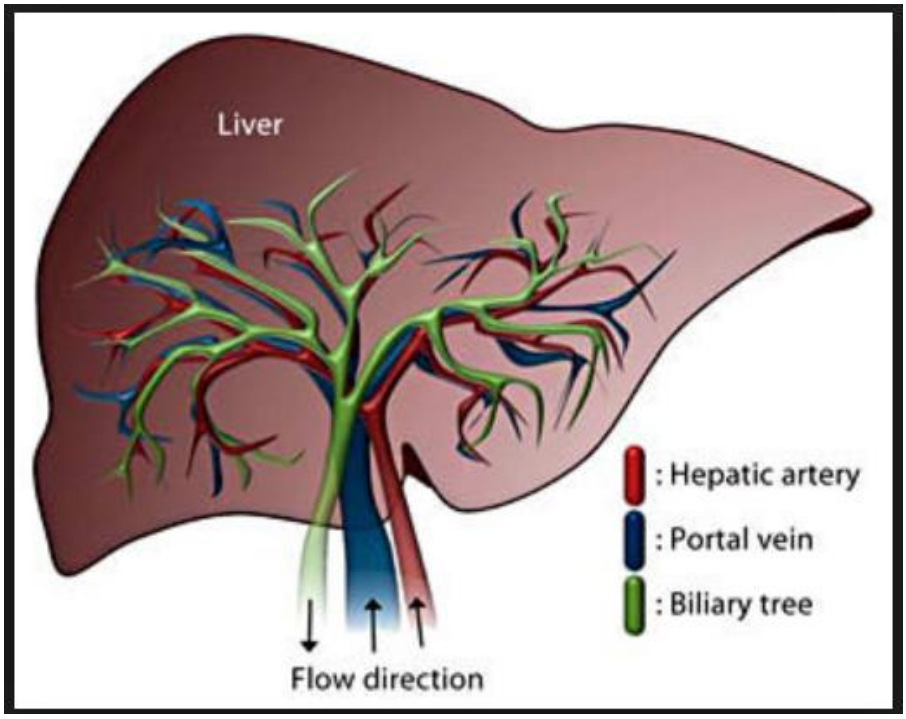


Figure 2: Vasculature of the liver (3)

2: LIVER DISEASE

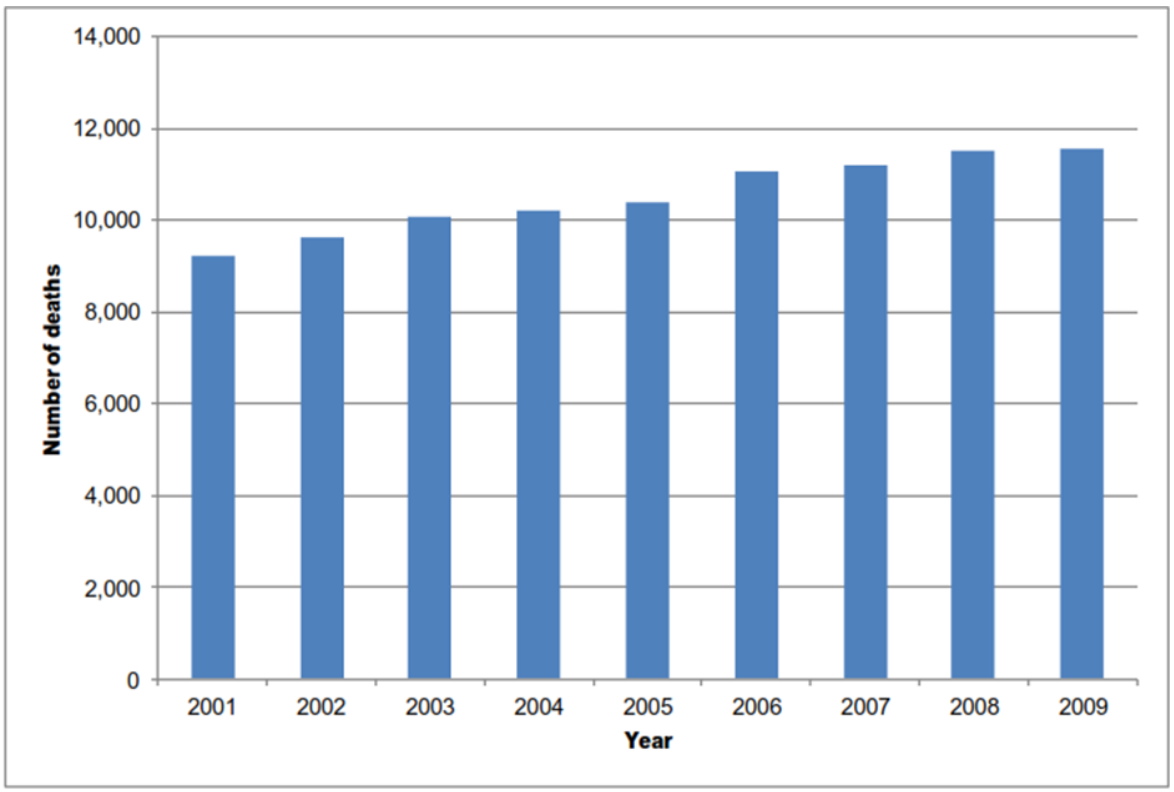
2.1: Introduction

In the United Kingdom (UK), liver disease is currently the third commonest cause of premature death and the rate is considerably higher than other European countries (4). Liver injury is caused by either an acute insult (as in paracetamol overdose) or ongoing chronic injury. Acute liver injury results occurs when there is a rapid, massive hepatocyte cell deaths which leads to significant impairment in liver function and hence is associated with a high mortality rate (5). In chronic liver injury, there is a progressive loss of hepatocytes that lasts from months to decades (5).

According to World Health Organisation (WHO) data, liver cirrhosis accounted for 1.8% of all deaths in Europe, causing around 170,000 deaths per year (6). Among northern European countries, there was a 2-fold increase in the rate of liver cirrhosis in UK and Ireland (6). Liver disease causes approximately 2% of all deaths (7) and liver disease related mortality has been rising steadily since 2001 (Figure 3). Hepatocellular carcinoma (HCC) is the fifth common cause of cancer and accounts for 70-90% of primary liver cancer (6). Without any treatment, 5-year survival of patient with HCC is around 5% (6).

Liver cirrhosis represents end stage liver damage irrespective of the underlying aetiology. In the UK, alcohol related liver disease (ARLD) accounts for well over a third of liver disease [6] although in recent years, the incidence of non-alcoholic fatty liver disease (NAFLD) has risen due to the increased incidence of obesity worldwide. NAFLD is usually associated with other metabolic risk factors such as diabetes and hypercholesterolemia [7, 8]. The underlying causes for liver cirrhosis had mentioned in Table 1.

Currently, the definitive treatment for acute liver failure, decompensated liver cirrhosis and liver cancer is liver transplantation (LT) although it is associated with certain limitations.



Source: ONS mortality data

Figure 3: Mortality caused by liver cirrhosis in England from year 2001 to 2009 (Reproduced from source: ONS Mortality Data)

Table 1: Causes of liver cirrhosis

Causes of liver cirrhosis

▪ Alcohol excess
▪ Chronic viral hepatitis
  o Hepatitis B and C
▪ Autoimmune liver disease
  o Autoimmune hepatitis
  o Primary biliary cholangitis
  o Primary sclerosing cholangitis
▪ Non-alcoholic fatty liver disease (associated with metabolic syndrome: diabetes, hypercholesterolemia, obesity and hypertension)
▪ Genetic
  o Haemochromatosis
▪ Drugs induced
▪ Vascular causes
  o Budd Chiari

2.2: Pathophysiology of liver fibrosis and regeneration

The liver is a unique organ with an extraordinary ability to regenerate when exposed to various insults and the regeneration is contributed mostly by mature hepatocytes with their rapid cell turnover (2, 5). Regenerative capacity of the liver is well known and in 1993, Higgins and Anderson (8) developed an experimental model of liver regeneration in which they surgically removed two thirds of liver mass. Since then, partial hepatectomy is routinely performed as a standard surgical procedure in clinical practice. In the case of hepatectomy, liver regenerate to compensate for the volume loss, also known as compensatory hyperplasia (2).

The pathogenesis behind liver fibrosis a complex mechanism but in recent years, there has been a better understanding of liver fibrogensis as well as fibrinolysis process. In a healthy liver, extracellular matrix (ECM) is present within the space of Disse, the space between

endothelial cells ad hepatocytes, mainly consists of collagen IV and V [8, 10]. ECM is a highly dynamic non-cellular structure that undergoes controlled remodelling constantly and it interacts with epithelial cells to regulate functions such as proliferation, migration and differentiation (9). This process of remodelling is complex and need to be regulated tightly to maintain tissue homeostasis, especially in response to injury (9). The cleavage of ECM components is the main process during ECM remodelling and is important for regulating ECM composition and structure(9). If there is dysregulation of ECM remodelling, the disease progress further to fibrosis and cancer (9). The homeostasis of ECM is tightly regulated by a balance between matrix metalloproteinases (MMPs) and their inhibitors known as tissue inhibitors of metalloproteinases (TIMPSs) [11]. MMPs are secreted as zymogens and are usually low in normal liver but increased during repair or remodelling process in injured tissue (9). Upregulation of TIMP-1 in the fibrotic liver contribute to collagen deposition by inhibiting the resolution of ECM (2).

After an acute insult to the liver, the necrotic or apoptotic cells will be replaced by regenerative parenchymal cells [8]. If the injury is transient, the cells activated by inflammation settle eventually, followed by the resolution of ECM and revascularisation (2). However, in some cases, the process of injury far exceeds the capacity of regeneration and in that circumstance, liver progenitor cells (LPC), also known as oval cells, that are present within the canals of Hering are activated to take over the role of regeneration (5, 10). LPC are bipotential cells and they can proliferate into either hepatocyte or cholangiocytes depending on the nature of injury (5, 11). The LPC environment includes epithelial cells, hepatic stellate cells, immune cells known as Kupffer cells and the ECM (5). Upon amplification, LPC infiltrate along the liver plate towards the central vein and differentiate into hepatocytes to restore liver function and cell mass (5).

In chronic liver injury, hepatic stellate cells (HSCs) became activated and differentiate into fibroblast like cells leading to an excessive deposition of collagen [11]. Activation of HSCs is stimulated by damaged hepatocytes through release of reactive oxygen species, cytokines and chemokines [10]. In activated HSCs, the expression of TIMP-1 is upregulated leading to the inhibition of MMP activity which leads to the accumulation of matrix proteins in the extracellular space [8, 11]. One of the pathological features of liver fibrosis is the increased expression of collagen, fibronectins, proteoglycans, structural glycoproteins, hyaluronan leading to persistent formation on new ECM [11]. In advanced stages of liver disease, there is a substantial change is in the deposition of collagens mainly type I, III and IV, fibronectin, laminin, hyaluronic acid and proteoglycans which are increased in fibrotic ECM up to 10-fold more than normal livers [8, 10, 11]. The simplified diagram of liver fibrosis and resolution of is shown in figure 4.

When fibrosis is established, structural changes in the form of extensive capillarisation of the liver sinusoids and the formation of intrahepatic vascular shunts occurs as well as functional changes such as endothelial dysfunction which leads to the development of regenerative nodules and portal hypertension [10, 11]. The clinical manifestations of this are the development of varices, ascites and hepatic encephalopathy (12). The loss of hepatocyte mass results in hepatic synthetic dysfunction with low levels of albumin and raised clotting factors on blood investigations (12). The progression of liver disease is illustrated in Figure 5.

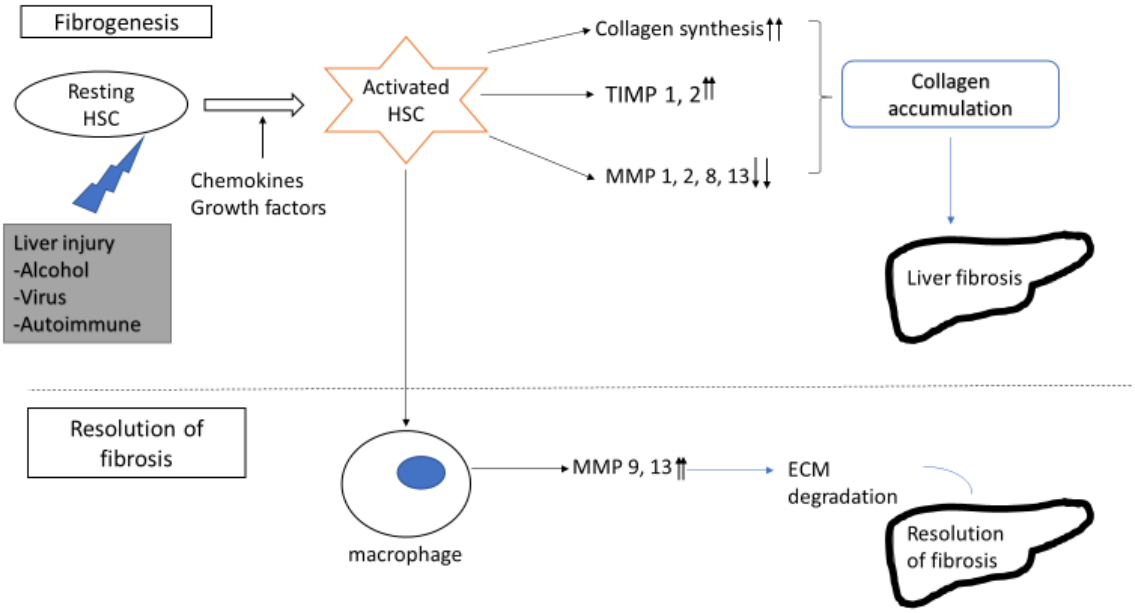


Figure 4: Simplified schematic view of liver fibrosis pathogenesis and resolution of fibrosis

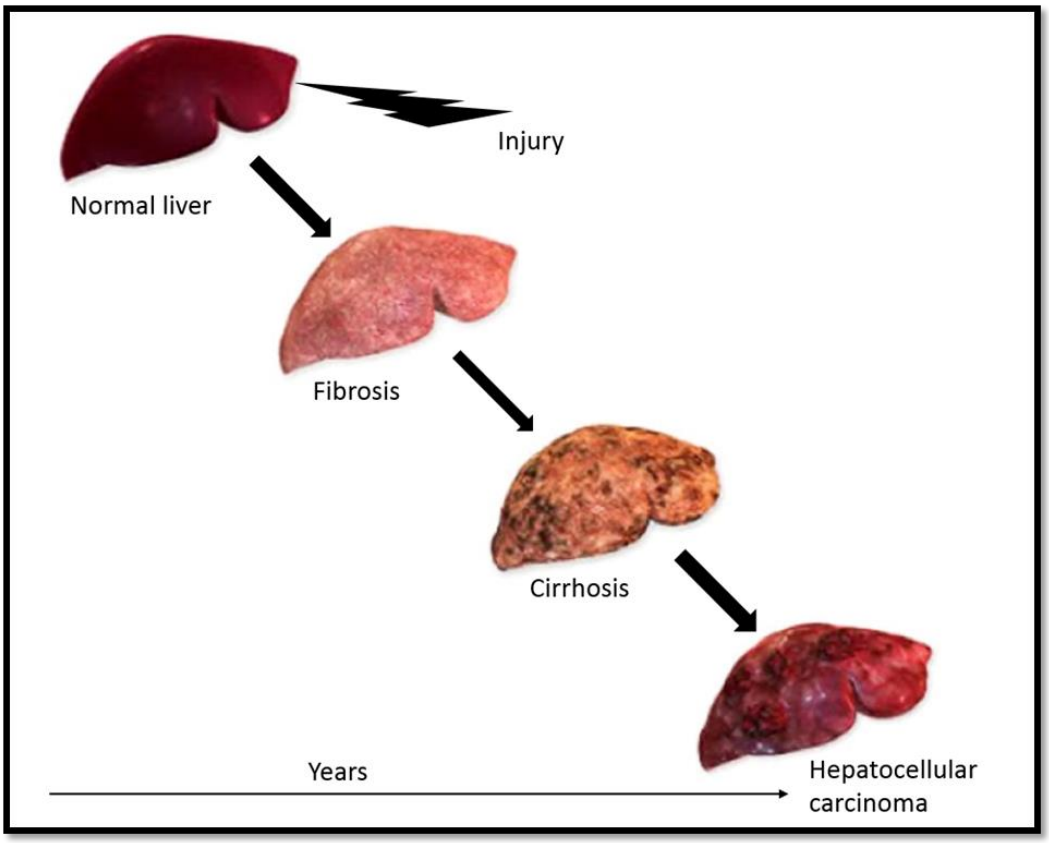


Figure 5: Progression of liver disease after injury to the liver

2.3: Clinical features of liver cirrhosis

In compensated cirrhosis, patients have underlying liver cirrhosis but no overt clinical features of liver disease. Patients with decompensated cirrhosis present with clinical features such as jaundice, ascites, variceal bleeding or hepatic encephalopathy which are the consequence of portal hypertension. The transition from compensated to decompensated cirrhosis occurs at a rate of approximately 5 to 7% per year (10). Table 2 shows the mortality associated with stages of liver cirrhosis based on their clinical features.

Table 2: Four stages of liver cirrhosis classification (12)

	Compensated liver cirrhosis		**Decompensated liver cirrhosis**	
	Stage 1	**Stage 2**	**Stage 3**	**Stage 4**
Clinical	No varices No ascites	Varices No ascites	Varices +/- Ascites	Bleeding +/- Ascites
Mortality at 1 year	1%	3%	20%	57%

2.4: Assessment of liver disease progression

The progression and severity of liver disease can be monitored in variety of ways; either invasive or non-invasive investigations. The methods used in assessing patients include clinical assessment, biochemical measurement, serum fibrosis markers and radiological assessment and these are usually used in combination to determine the severity of liver disease. It is important to recognise the disease stage as early as possible to prevent the progression of liver fibrosis and clinical deterioration.

Non-invasive investigations include blood tests, radiological assessment including ultrasound, computed tomography, magnetic resonance imaging and transient elastography (TE). Routine biochemical blood tests that used in patients with liver disease include alanine transaminase (ALT), aspartate transaminase (AST), serum bilirubin, serum albumin, international

normalised ratio (INR) and platelets. These blood tests are then used to calculate scores that can predict the survival of patients. The commonly used scoring systems in clinical settings are Model for End-Stage Liver Disease (MELD), United Kingdom Model for End-Stage Liver Disease (UKELD) and Child -Turcotte-Pugh Score (CPS). In recent years, TE has been used in clinical settings to measure the stiffness of the liver as a surrogate markers of liver fibrosis.

There are two broad types of serum markers; direct and indirect that are used as a surrogate to predict liver fibrosis (13). Direct biomarkers measure the components of ECM as well as the enzymes which regulate the matrix: including MMPs, subtypes of collagen and hyaluronic acid (14, 15). Indirect markers include parameters such as platelet count as marker of portal hypertension, AST and ALT as marker of liver cell inflammation and injury and INR as a marker of liver synthetic dysfunction (16, 17). The commonly used biomarkers in clinical settings are included in Table 3.

Table 3: Indirect and direct serum biomarkers of liver fibrosis (13, 18)

Test	**Components**	**Formula**	**References**
Indirect markers of liver fibrosis			
APRI: (AST to Platelet Ratio Index)	AST/Platelet count	[AST (U/L)/upper limit of normal (U/L)] × 100/platelets (10^9/L)	(17)
BARD score	Body mass index (BMI), AST/ALT ration, Diabetes	Sum obtained from three variables (BMI≥ 28= 1 point, AST/ALT ratio≥ 0.8= 2 points, diabetes =1-point, scale varies from 0 to 4)	(19)
FIB-4	Age, AST, ALT, Platelet	Age × AST (U/L)/ [platelets (10^9/L) × $ALT^{1/2}$ (U/L)]	(20)

Test	Components	Formula	References
FibroTest	A2 macroglobulin, Haptoglobin, Apolipoprotein A1, Gamma-glutamyl transpeptidase, total bilirubin	4.467× $\log_{10}$ [α_2-macroglobulin(g/L)]- 1.357×$\log_{10}$[Haptoglobin(g/L)] + 1.017×$\log_{10}$[GGT(IU/L)] +0.0281×[Age(years)] + 1.737× $\log_{10}$[Bilirubin (μmol/L)]- 1.184×[ApoA1*(g/L)] +0.301×Sex (female = 0, male = 1) - 5.54	(21)
NAFLD Fibrosis score	Age, Body mass index, Diabetes, AST, ALT, Platelet, Albumin	=−1.675 + 0.037×age (years) + 0.094×BMI (kg/m^2) + 1.13× IFG/diabetes (yes = 1, no = 0) + 0.99×AST/ALT ratio – .013× platelet (× 10^9/l) – 0.66×albumin (g/dl)	(22)
Direct markers of liver fibrosis			
Enhanced Liver Fibrosis Score (ELF)	Hyaluronic acid, Pro collagen III amino terminal Peptide, TIMP-1 level	−7.412 + [ln(HA)*0.681] + [ln(PIIINP)*0.775] + [ln(TIMP1) *0.494] + 10	(23)

2.4 (i): Model for End-Stage Liver Disease (MELD)

Mode for end stage liver disease (MELD) score was originally developed to predict survival of cirrhotic patients undergoing elective trans-jugular intrahepatic portosystemic shunt (TIPSS) procedure (24). MELD score is calculated using a combination of blood tests: serum creatinine, serum bilirubin and international normalised ratio (INR). The score determines the severity of underlying CLD and is used to predict how likely the patient will need liver transplantation (LT) within the next 3 months (25, 26), as it has been shown to be an accurate predictor of

survival in patients with liver disease not undergoing LT (25, 27). The score ranges from a minimum of 6 (mild disease) to a maximum of 40 (severe disease) (28). The higher the score, the more likely that the patient will have a worse survival (Figure 6) and hence, a higher likelihood of requiring liver transplantation. Patients waiting for an LT with a score of 40 have a 300-fold increased risk of mortality compared to patients with a score of less than 12 (25, 29).

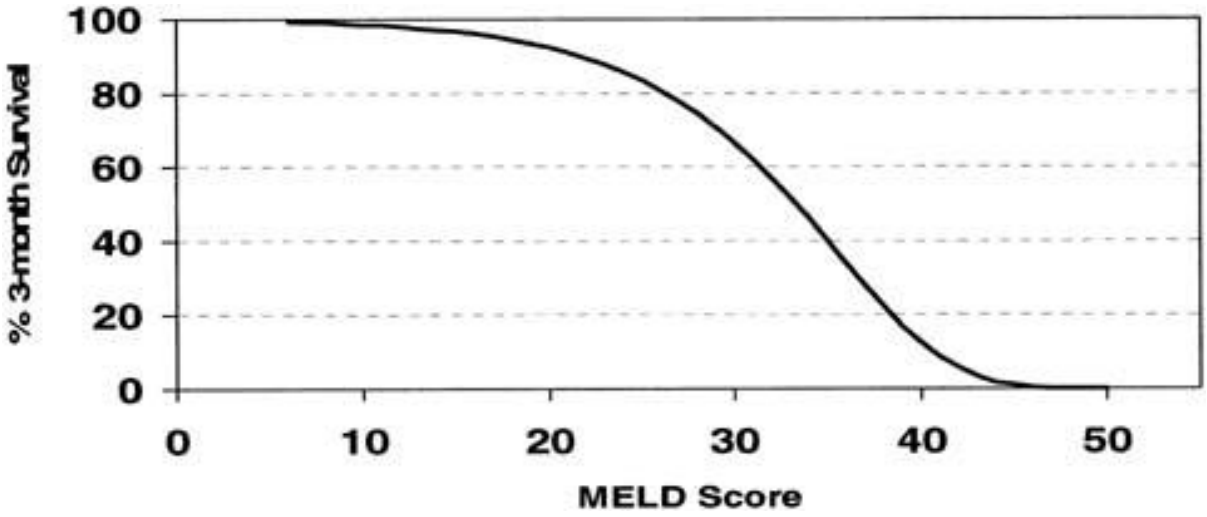


Figure 6: Three-month mortality based on model for end stage liver disease (MELD) score (25)

2.4 (ii): United Kingdom Model for End Stage Liver Disease (UKELD)

UKELD (United Kingdom Model for End-stage Liver Disease) is an another scoring which has been used widely in UK to help determine the need for patients to have LT (30). It was developed in 2008 and was based on MELD score with incorporation of the serum sodium level (30). A UKELD score of 49 indicates a 9% 1-year mortality risk and the minimum score required to be added to the LT waiting list in the UK (30, 31). A UKELD score of 60 indicates a 50% one year survival rate (31).

2.4 (iii): Child-Turcotte Pugh Classification

Child Pugh classification is calculated from a combination of objective parameters [serum bilirubin, INR or prothrombin time, serum albumin] and subjective parameters [clinical assessment of ascites and hepatic encephalopathy] (32, 33). Chid Pugh score (CPS) varies between 7 and 15 and the mortality is increased with a higher CPS (34). In table 4 demonstrated the parameters that involved in Child Pugh Scoring. The scoring system showed that patients with Child C have higher mortality compared to Child A or Child B with 1-year survival of 45% (Figure 7) (12)**.**

Table 4: Child-Turcotte Pugh Score classification (33)

Child Pugh Classification for severity of liver cirrhosis			
	Points		
Parameters	**1**	**2**	**3**
Hepatic Encephalopathy	None	Grade 1-2	Grade 3-4
Ascites	None	Mild-Moderate (Diuretic responsive)	Severe (Diuretic refractory)
Bilirubin (umol/L)	<34	34-51	>51
Albumin (g/dL)	>35	28-35	<28
Prothrombin time (seconds)	<4	4-6	>6
Class A: 5-6 points (least severe)			
Class B: 7-9 points (moderately severe)			
Class C: 10-15 points (most severe)			

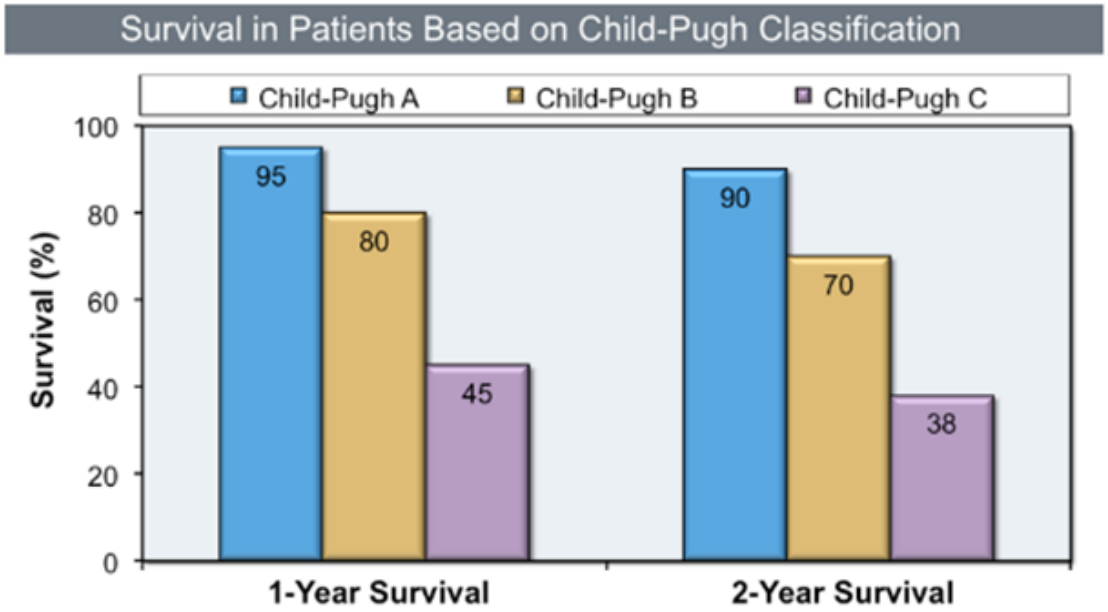


Figure 7: Survival in patients with liver diseases as per Child-Turcotte Pugh grades (12)

2.4 (iv): Enhanced liver fibrosis test (ELF)

Enhanced liver fibrosis test (ELF) test is a validated test which includes panels of highly sensitive ELISA assays measuring matrix components and enzymes [Hyaluronic acid (HA), TIMP-1 and Pro-Collagen Type III aminoterminal peptide (PIIINP)] (23). The values for each of these markers is combined in an algorithm which produces a discriminant ELF score. The score is related to the level or degree of fibrosis. The high score relates to the worse fibrosis stage.

2.4 (v): Transient elastography (TE)

Transient elastography (TE) is used in the Fibroscan device (Figure 8) and it measures the velocity of the ultrasonic sheer sound wave passing through the liver which then converts into a measurement of liver stiffness in kilopascals (kPa) (35). The probe of the Fibroscan device is positioned in an intercostal space near the right side of the liver. The procedure can be

performed in outpatient setting as part of bed-site clinical assessment and it is non-invasive. The higher readings correlate with worsening degree of liver stiffness. It can also be measured sequentially to monitor the progression of liver fibrosis.

Figure 8: Fibroscan/Transient Elastography machine (Picture from www.intechopen.com)

2.4 (vi): Liver biopsy

Despite availability of non-invasive tests, liver biopsy is still the gold standard investigation in measuring liver fibrosis. Liver biopsy can be obtained via transcutaneous or trans-jugular method under radiological guidance. The trans-jugular route is preferred in patients with significant coagulopathy with less risk of bleeding. Liver biopsy (Figure 9-10) has been used to evaluate the underlying cause of liver disease, to stage disease severity in the case of viral hepatitis and to determine whether treatment given has been effective especially in patients with autoimmune hepatitis. The main disadvantage of the procedure is that the patient need to come into hospital as day case procedure and it is associated with significant complications

such as pain, bleeding, injury to internal organs such as perforation (35). In some cases, though biopsy will not give a desired diagnosis due to sampling error which can lead to either over-staging or under-staging the disease (35).

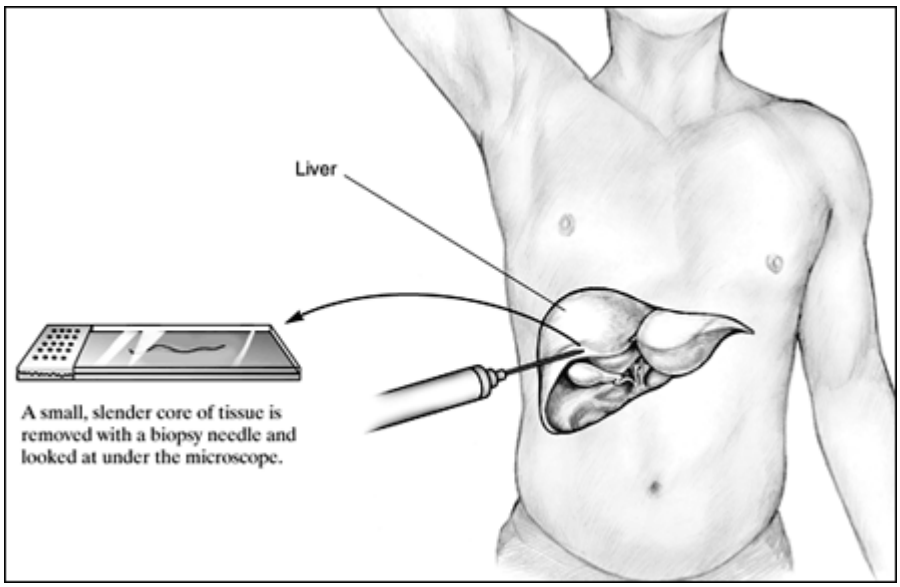


Figure 9: Liver biopsy procedure (http://fattyliversite.com)

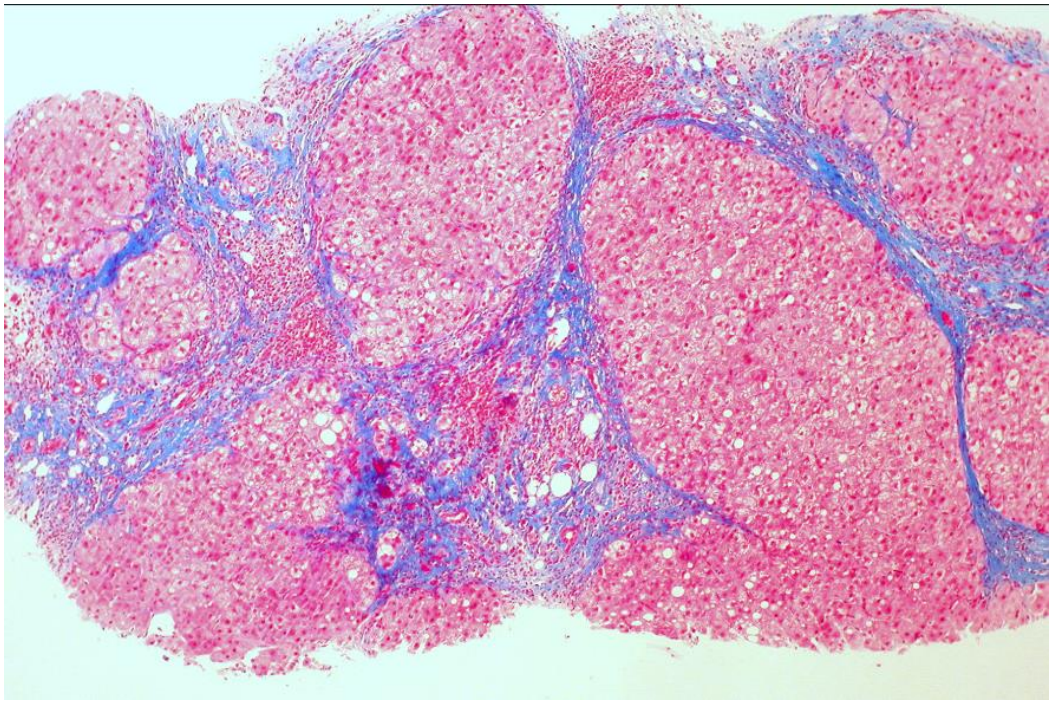


Figure 10: Histology (H&E staining) showed significant fibrosis of the liver (blue colour showed scarring) (36)

3: LIVER DISEASE MANAGEMENT

3.1: Current standard management of liver cirrhosis

Liver transplantation (LT) is the only curative treatment available for patients with decompensated cirrhosis and has a favourable 5-year survival rate (37). However, LT is not without risk and the major drawbacks are availability of donor organs, high operative risks and complications from lifelong immunosuppression (38). Due to those factors, the current approach is to identify patients who are at risk of developing liver cirrhosis and treat the underlying conditions as in alcohol management, treatment of viral hepatitis and autoimmune conditions to halt the progress of the disease pathogenesis.

Despite those interventions, some patients may progress further and develop decompensated cirrhosis. In patients with decompensated cirrhosis, the treatment is mainly focused on managing the presenting clinical symptoms as well as treating the precipitating cause of those symptoms to develop. The common reasons for decompensation include infection, bleeding, constipation, drug, electrolyte disturbances, venous thromboembolism and hepatocellular carcinoma. The management is mainly based on treating underlying cause of liver disease as in abstinence of alcohol in patients with alcohol related liver disease and anti-viral therapies in patients with viral hepatitis. They should also be managed based on the clinical presentation.

In patients with ascites, treatment options are diuretics, large volume paracentesis in patients who did not respond or tolerate diuretics and shunt insertion. The commonly performed shunt is TIPSS which is usually performed by an interventional radiologist. In patients with bleeding, the mainstay of management is endoscopy with banding for oesophageal varices and glue or thrombin injection for gastric varices. In patients with hepatic encephalopathy, the treatment is based on the underlying contributing factors. The mortality increases significantly once

patients develop liver decompensation and therefore they should be considered for suitability of liver transplantation and referred early to a liver transplant unit if deemed appropriate.

With the increased demand of patients requiring LT, there is an urgent need to develop alternative treatment strategies for the treatment of decompensated chronic liver disease (CLD) (39) and stem cell therapies have therefore been developed in the field of liver diseases.

3:2: Stem cells

Stem cells are undifferentiated, pluripotent cells that can differentiate into multiple cell lineages except for primordial germ cells (40, 41). They retain the capacity to generate and renew themselves throughout life (42). They are divided into 4 different groups according to their potential for differentiation: totipotent, pluripotent, multipotent or unipotent (41). Stem cells can be classified into 2 broad categories: adult stem cells and embryonic stem cells.

Embryonic stem cells are pluripotent in nature and they are derived from the inner cell mass of the embryo which can form any cells types of the three embryonic germ layers; ectoderm, mesoderm or endoderm (43). There are limitations in using embryonic stem cells due to its potential of high immune reaction as well as the ethical concerns (43). Adult stem cells are multipotent cells with a limited differential potential and can form different cell types within the tissue although the exact mechanism of differentiation are not fully known (43). There are many sources of adult stem cells such as bone marrow, umbilical cord blood, fetal and adult liver progenitor cells, mature hepatocytes and placenta although the major populations of adult stem cells are obtained from bone marrow due to easy accessibility (40).

Bone marrow contains at least two main populations of stem/stromal cells, haematopoietic stem cells (HSC) and mesenchymal stromal cells (MSC), which provide stromal support for HSC

(39). There had been concern about injecting unsorted bone marrow stem cells (BMSC) into patients with liver cirrhosis as these contain MSC which can potentially differentiate into scar forming myofibroblasts and worsen the underlying fibrosis (44), although more recent work suggests this occurs infrequently (45).

Experimental studies of stem cell therapy in rodent models of cirrhosis have shown encouraging results (46-49). The first report came from Petersen and colleagues who performed sex mismatched bone marrow transplant in mice with liver injury and they demonstrated the presence of donor derived hepatocytes in the livers of transplanted mice (50). Sakaida and colleagues investigated the effect of bone marrow cell (BMC) tail vein infusion half way through an 8-week long mouse model of carbon tetrachloride (CCl4) induced liver fibrosis (11, 48). The study found that mice the received BMC infusions had reductions in liver fibrosis as well as improvements in survival compared to control mice due to rise in levels of MMP-9 (11, 48). Thomas and colleagues also demonstrated that infusion of syngeneic macrophages in a mouse model of CCl4-induced liver fibrosis led to a reduction in fibrosis, which was in marked contrast to the increase seen with unfractionated BM cells with control injured mice (11, 51).

Due to those encouraging results, many clinical studies have been conducted in recent years to study for the potential benefit of stem cell therapy in liver disease (39, 52-55). Earlier studies were mostly feasibility and safety studies. Gordon and colleagues studied the safety and tolerability of injecting autologous CD34+ stem cells mobilised by granulocyte colony stimulating factor (GCSF) into 5 patients with liver insufficiency and showed that there were no complications or side effects related to the procedure (53). The study showed an improvement in serum bilirubin and albumin in some patients (53). In recent years, there had been few randomised controlled trials that studied the clinical effects of stem cells therapy in liver cirrhosis with variable results. Mohamadnejad and colleagues (56) conducted a randomised, placebo-controlled trial to evaluate the efficacy of peripheral infusion of

autologous bone marrow MSC in cirrhosis and the study showed that there were no beneficial effects in patients. Recent study by Lin and colleagues showed that peripheral infusion of allogenic BM-MSC is safe and significantly increased the 24-week survival in hepatitis B viral (HBV) related acute on chronic liver failure (ACLF) (57).

3.2 (i): Haematopoietic stem cells (HSC)

Haematopoietic stem cells (HSC) are the main stem cell population within the bone marrow (BM) and give rise to all mature blood lineages such as red blood cells, white blood cells and platelets (42) and account for approximately 0.01% of the total cells in the bone marrow (58). In addition to the bone marrow, HSC can be harvested from peripheral blood after mobilising with GCSF and umbilical cord bleed, making it possible to harvest from patients in adequate amounts using relatively non-invasive methods (59). In 1999, Petersen el al (50) first showed that hepatic cells derived from bone marrow regenerated the livers of lethally irradiated rats. A phase I study from Gordon et al (53) on 5 patients with decompensated liver cirrhosis proved that peripherally mobilised CD34+ HSC cells could be used in human and it was safe to do so. Since then, there had been many clinical trials on studying the effect of HSC in patients with liver diseases (55, 60, 61).

3.2 (ii): Granulocyte colony stimulating factor (GCSF)

Granulocyte-colony stimulating factor (GCSF), a protein containing 175 amino acids, was routinely used in haematological malignancies for BMC transplantation purposes and had potent mobilisation capacity of haematopoietic stem cells in donors and chronic haematological patients (62, 63). The effects of GCSF are not limited to the mobilisation of bone marrow derived cells but also due to autocrine and paracrine effects within the liver, promoting and enhancing the oval cell reaction (64). This synergistic effect of BMSC and oval cells might be

responsible for improvement of liver function post GCSF therapy (64). GCSF also improves neutrophil activity which can improve the severe immunological dysfunction that characterises the pathophysiology behind ACLF, prevent sepsis and reduce mortality (64).

GCSF treatment significantly improved survival and liver histology in chemically injured mice, predominantly by promoting endogenous repair mechanisms as per the study from Yannaki and colleagues (65). They studied the possibility that GCSF mobilised bone marrow cells could home to the injured liver of mice model and promote tissue repair (65). The study showed that GCSF administration increased BM-derived hepatocytes that caused acceleration in the regeneration process with significant improvement in survival (65). Another study showed that 5 days treated with GCSF decreased the hepatic inflammation and improved survival in acute hepatic injury rat model (66).

Those studies led to clinical studies and GCSF has also been used in patients with both chronic liver cirrhosis and ACLF with some promising outcomes (52, 67, 68). Gaia and colleagues studied the effect of GCSF in 8 patients with liver cirrhosis and showed that GCSF was well tolerated with no serious adverse events (68). Recent study from Singh and colleagues showed that GCSF administration improved CPS, MELD score and 90-day survival rate in patients with severe alcoholic hepatitis (69). The differentiation of bone marrow stem cells had been illustrated in Figure 11.

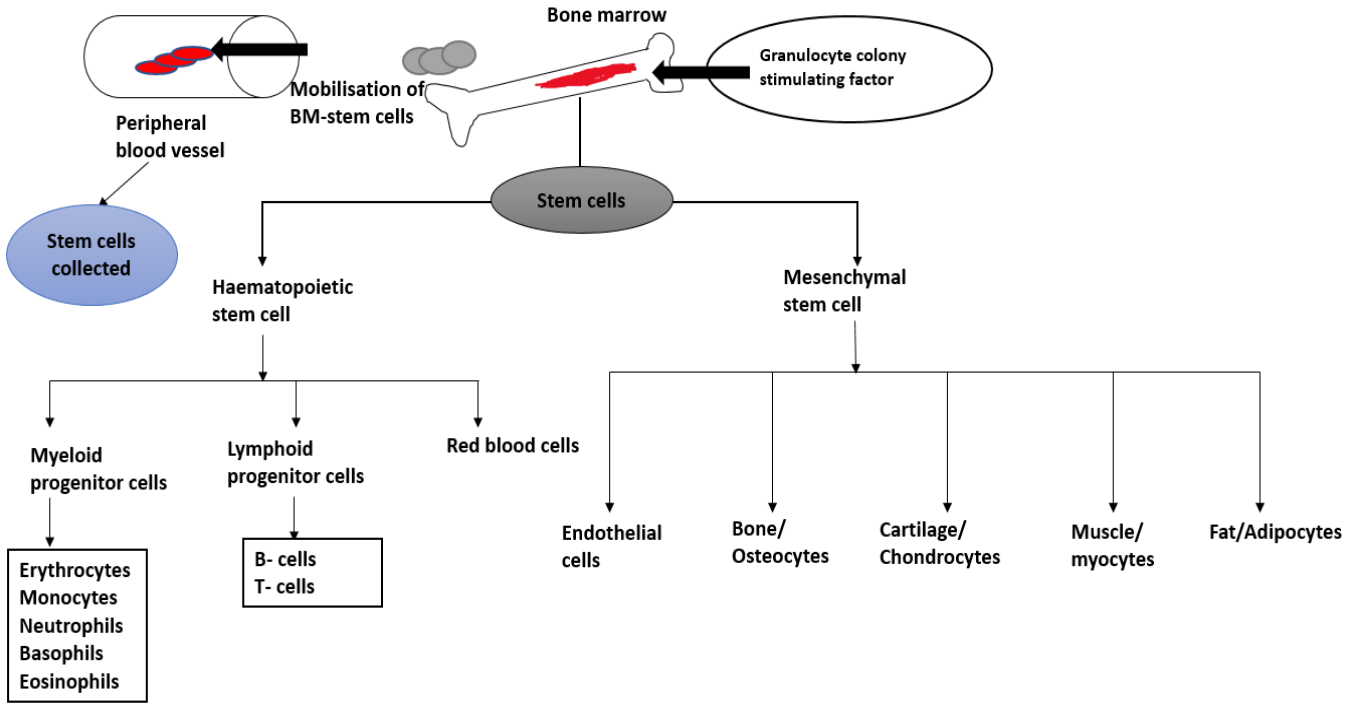


Figure 11: Types of bone marrow stem cells in human

3.3 (iii): Methods for stem cell therapy

Stem cells can be administered in different ways: via hepatic artery infusion (70), intrahepatic injection (38), portal vein injection (54), intra-splenic injection (38) or peripheral infusion (71, 72). Each route has their own benefits and risks associated with it and the ideal way to deliver cell therapy should be based on the following factors: easy to perform, minimally invasive to the patients, less traumatic procedure with very few side effects (73).

4: MECHANISTIC ACTION OF HAEMATOPOIETIC STEM CELL THERAPY IN MICE MODEL OF LIVER FIBROSIS

4.1: Rationale behind animal work

4.1 (i): My role within this project

My lab-based project was an extension of previous project completed by Dr Andrew King (PhD fellow). The frozen mouse tissue samples and fixed paraffin slides were handed over to me to complete the project. During my research time, I learnt to perform immuno-histochemistry staining and analysis as well as techniques in performing RNA extraction, cDNA synthesis, running qPCR panel and interpretation of the results.

4.2: Overview of previous completed data

The results of this study had been published and I was one of the author (74) and the section below are from that published manuscript as well as from Dr King's PhD Thesis.

4.2 (i): Ethic approval for the animal work

This section was reproduced from Dr Andrew King PhD thesis

All animal experiments performed were conducted in accordance with the Animals (Scientific Procedures) Act 1986 under a Project Licence (PPL 40/3201) (74). Approval from the University of Birmingham Ethics Committee was obtained. All animals were housed in an approved animal facility (Biomedical Services Unit, University of Birmingham) under conditions of 12-hour light/dark cycles and with unlimited access to food and water (74). C57/Bl6 mice were supplied directly to the Biomedical Services Unit by Harlan UK and used for cell isolation at 6-8 weeks of age and for experimental procedures at 8 weeks of age (74). BoyJ (B6.SJL-*Ptprca Pep3b*/BoyJ) mice were supplied from a colony maintained in the

Biomedical Services Unit and used for experimental procedures at 8 weeks of age (74). At the completion of experimental procedures mice were euthanased under terminal inhalational anaesthesia (3% Isoflurane) allowing blood sampling via cardiac puncture to be performed prior to death. Mice used for cell isolation were euthanased using cervical dislocation as approved under Schedule1 of the Animals (Scientific Procedures) Act.

4.2 (ii): Results generated by previous researcher

This section was reproduced from Dr Andrew King PhD thesis

Dr King used the carbon tetrachloride model of liver injury to investigate the effect of chronic liver injury on c-kit+ sca1+ lin- (KSL) HSC mobilisation and recruitment to the liver. Carbon tetrachloride was used in age and sex matched C57/Bl6 mice at a dose of 1mg/kg body weight (diluted 1:4 in mineral oil vehicle) and administered to mice by intra peritoneal injection and control mice received twice weekly intraperitoneal injections of mineral oil. After 8 weeks of treatment, a significant chronic liver injury developed. Mice were then sacrificed, and bone marrow, peripheral blood and liver obtained. Significantly higher numbers of HSC determined by KSL surface phenotype were found in the livers (Injury 1163 +/- 173 KSL cells per liver vs Control 258.5+/-22.2 KSL cells per liver, $p<0.01$) and peripheral blood (Injury 0.397+/-0.055 KSL cells per μl blood vs Control 0.0674+/-0.011 KSL cells per μl blood, $p<0.001$) of mice with liver injury compared with control mice (Figure 12 A, B) (74). The numbers of HSC within the bone marrow remained constant (Injury 2323+/-48.9 KSL cells per femur vs Control 2327+/-64.3 KSL cells per femur, p=not significant) (Figure 12 C) (74).

BoyJ mice were allocated randomly to either treated or control group (n=8 in each group). KSL cells from donor mice C57/BL6 mice were injected via tail vein into the mice in the treated group at the start of weeks 7, 8 and 9 (74) (Figure 13). Quantitative morphometric analysis of

fibrosis in the liver using picrosirius red staining (Figure 14 A, B) revealed significantly lower levels of collagen deposition within the liver with a 49.7% reduction in staining in the KSL cell treatment group compared with controls (KSL 2.207+/- 0.119 % staining area vs Control 4.388+/-0.27 %staining area, p<0.0001) (74). Serum albumin levels were measured as a marker of liver synthetic function and in the treatment group, the levels were significantly higher than those in the control group (KSL 4.06 +/- 0.37 vs Control 3.14+/-0.39, p<0.01) (Figure 14 C) (74).

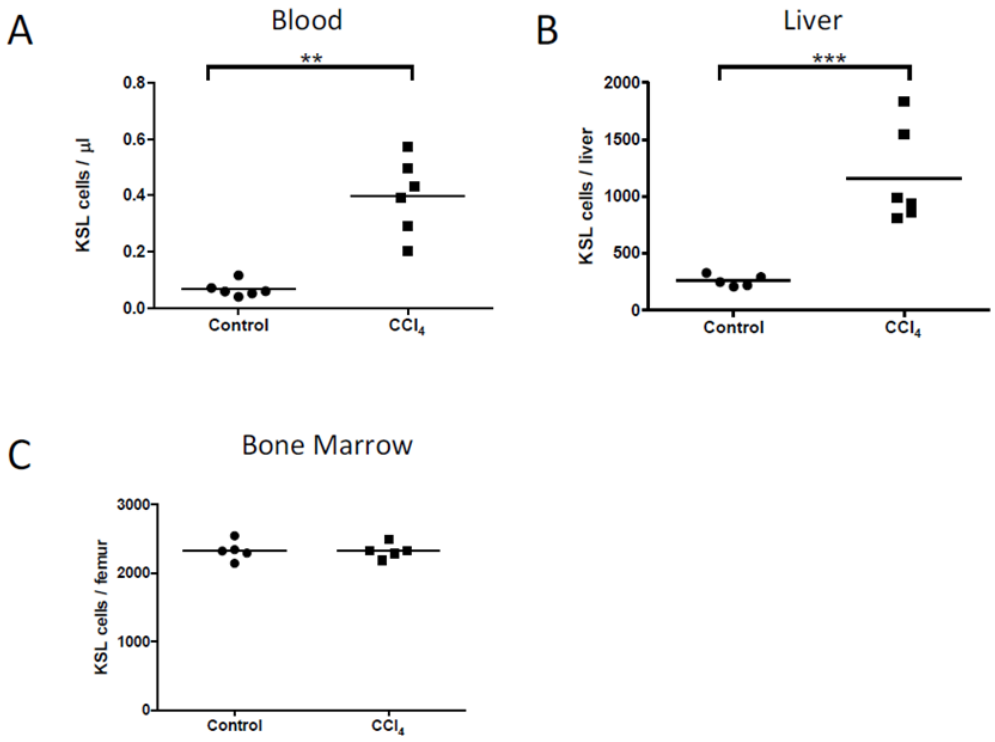


Figure 12 (A-C): The numbers of haematopoietic stem cells (HSC) determined by c-kit+ sca1+ lin- (KSL) surface phenotype were found in the peripheral blood, livers and bone marrow (74), **p<0.01 ***p<0.001 (two tailed, unpaired students t-test) (Reproduced from Dr Andrew King's PhD Thesis)

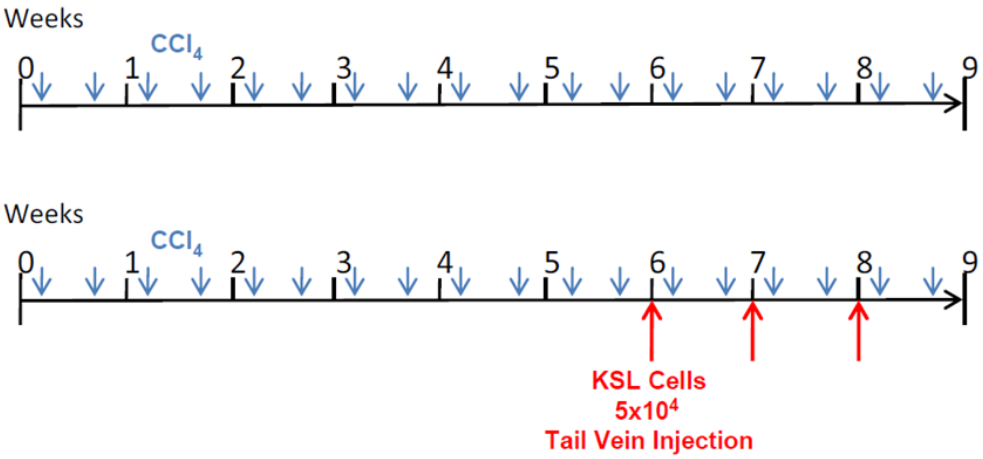


Figure 13: Time frame of c-kit+ sca1+ lin- (KSL) cells injection in carbon tetrachloride (CCL4) model of mouse liver fibrosis (Reproduced from Dr Andrew King's PhD Thesis and (74))

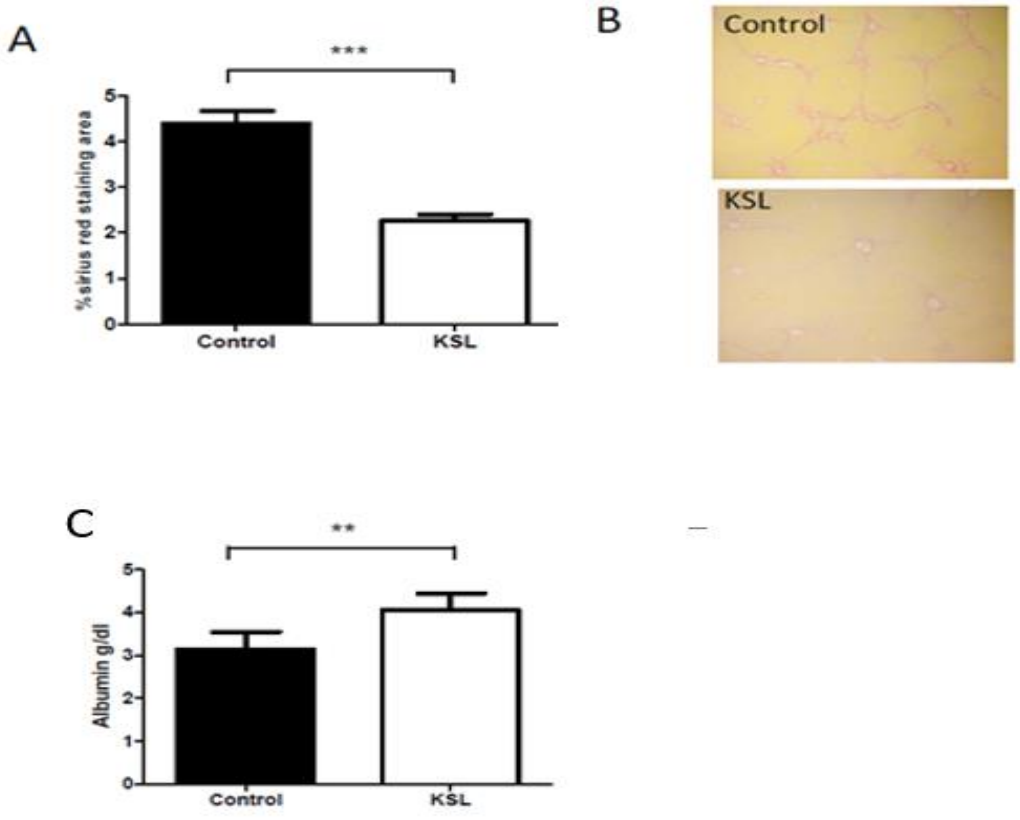


Figure 14 (A-C): Comparison between c-kit+ sca1+ lin- (KSL) treated mice and control mice (A, B: Percentage Sirius red staining quantification, C: Albumin level) (74), **p<0.01

***p<0.001(two tailed, unpaired students t-test) (Reproduced from Dr Andrew King's PhD Thesis)

4.3: Hypothesis of the current project

In chronic liver injury, there was an increased accumulation/turnover of ECM which is predominantly driven by hepatic stellate cells (75). MMPs and TIMPs are the main regulators of ECM turnover in liver fibrosis (75) and MMP activities are under close control by TIMPs (76). The roles of MMPs and TIMPs in hepatic fibrosis have been well studied in CCL4 induced mouse liver injury with increased expression of several MMPs especially MMP-1, 8, 9 and 1 (75, 76).

The rationale behind the project was to understand the nature of MMP expression in the model of mouse liver injury that received purified KSL cells infusion. MMP9 and MMP13 expression was studied in the livers of control and KSL cells treated mice. The reason behind choosing these MMPs was that both MMPs are involved in remodelling of ECM after liver injury as mentioned above due to their fibrinolytic nature. Previous study also showed that mice undergoing CCl4-induced injury had macrophage-rich infiltrates and a blunted oval cell response (77) with stem cells therapy considered to be able to increase the oval cells response. To identify oval cell reactions, immune-histochemical staining for pan-cytokeratin (Pan-CK) and Sry/sex determining region Y-box 9 (Sox-9) was performed.

4.4: Immunohistochemistry Staining

4.4 (i): Methodology

Staining of formalin fixed paraffin embedded sections started with de-waxing the sections through series of graded solutions: washed twice with xylene, washed twice with alcohol and

finally washed in deionised water. Sections were then placed in plastic humidifier chamber and 150 µl of readymade Peroxidase-blocking solution, DAKO real (S2023) was added to each section and then incubated on rocker for 40 minutes to block endogenous peroxidase activity, followed by washing two times in TBS (Tris buffered saline) and 0.1% Tween-20 (TBS-T) for 5 minutes. Antigen retrieval was performed using the following steps: mixing 10 ml of high pH antigen unmasking solution (H-3301, Vector Lab) with 990 ml of distilled tap water in a plastic container and heated at high power to 95-100˚C for 5 minutes in a microwave oven; the sections were then immersed and heated for further 15 minutes at full power The sections were allowed to cool slowly to room temperature and then washed twice in TBS-T. Nonspecific binding of antibodies to the sections was blocked by incubating the sections for 30 minutes with 150 ul of Casein buffer solution (x10) which was diluted to 1x with distilled water prior to use. The casein solution was tipped off the slide and the primary antibody was added diluted in TBS to the appropriate concentration (Table 5) and incubated for 1 hour at room temperature in a humidified chamber on a rocker. The sections were then washed two times in TBS-T followed by addition of the relevant horse radish peroxidise conjugated secondary antibody (ImmPRESS Peroxidase Anti-Rabbit IgG or ImmPRESS Peroxidase Anti-Rat (Mouse Adsorbed) IgG, Vector Labs) for 30 minutes, followed by washing in TBS-T twice. Diaminobenzidine (DAB) was used as the peroxidase substrate and DAB substrate was prepared by adding 1 drop of DAB to 1 ml of substrate buffer and mixed gently. The sections were incubated with ImmPACT DAB reagent (VectorLabs) until the tissue turned brown (varied between 20 second to 2 minutes). Sections were then washed once in water for 5 minutes and counterstained with Mayer's haematoxylin for 20 seconds, washed again in tap water for 2 minutes and 30 seconds and afterwards, run under warm tap water for further 2 minutes and 30 seconds. The sections were dehydrated through graded alcohols /xylene solutions and coverslips were mounted using DPX mountant.

Table 5: Antibodies used in immunohistochemistry staining

Antibody	Origin	Target	Isotype	Supplier	Dilution	Antigen Retrieval
Anti-Sox-9	Rabbit	Mouse	Polyclonal	Millipore	1: 1000	High pH (VectorLab)
Pan CK/ Anti-Cytokeratin, Wide Spectrum Screening (WSS)	Rabbit	Mouse	Polyclonal	DAKO	1: 500	High pH (VectorLab)

4.4 (ii): Immuno-staining analysis

The number of individual stained cells in 6 random, non-overlapping fields of view (x100) was counted using Image J software. The data were then analysed with Prism GraphPad software (version 6) using non-parametric Wilcoxon test. P valve of <0.05 was considered as statistically significant.

4.4 (iii): Immuno-staining results

Quantification of hepatic oval cell numbers, as indicated by pan-cytokeratin staining in CCL4 injured mice (control) and KSL-cells treated mice had higher percentage of pan CK positive staining in tissue compared to control mice ($p<0.01$) (Figure 15) (74).

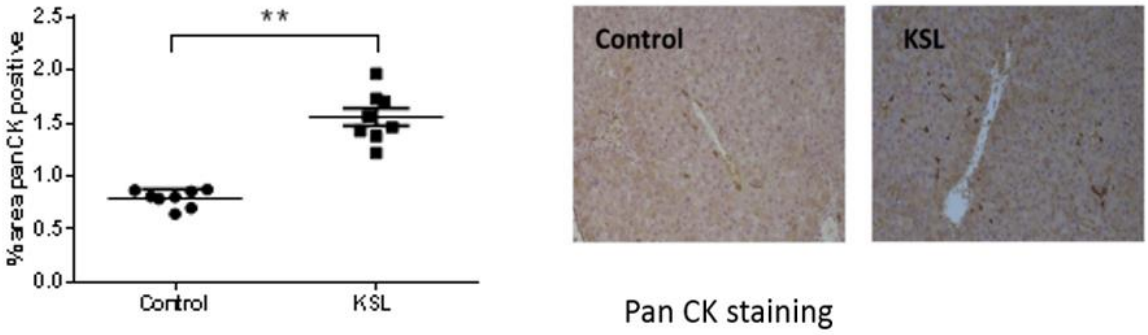


Figure 15: Comparison in qualitative analysis of Pan-CK staining between control and c-kit+ sca1+ lin- (KSL) treated mice (74), **p<0.01 (two tailed, unpaired students t-test)

In the adult organ, *Sox9* marks the precursor cell population during physiological cell replacement and/or during the regenerative process after injury (78). Sox9 expression had been detected in cytokeratin 7–positive bile duct cells of the liver, including the canals of Hering, but not in hepatocytes (78). When damage occurs in the liver, a Sox9-dependent process causes HSCs to become activated through TGF-β signaling in type 1 collagen production (79). In the study, KSL administration did not result in a difference between the two groups in Sox-9 staining (p=0.31) (Figure 16). In addition, immune staining for both MMP-9 and MMP-13 showed an increased expression of both in KSL treated groups (Figure 17) (74).

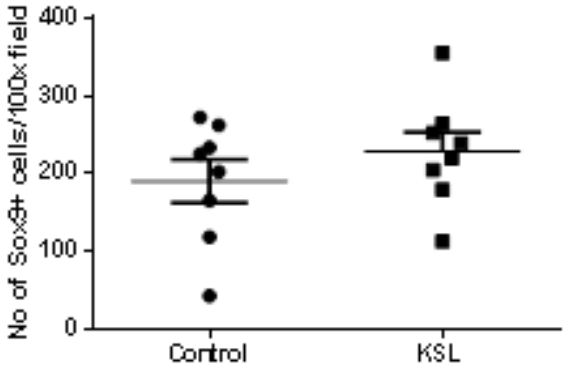


Figure 16: Comparison in SOX-9 staining in both control and c-kit+ sca1+ lin- (KSL) treated mice

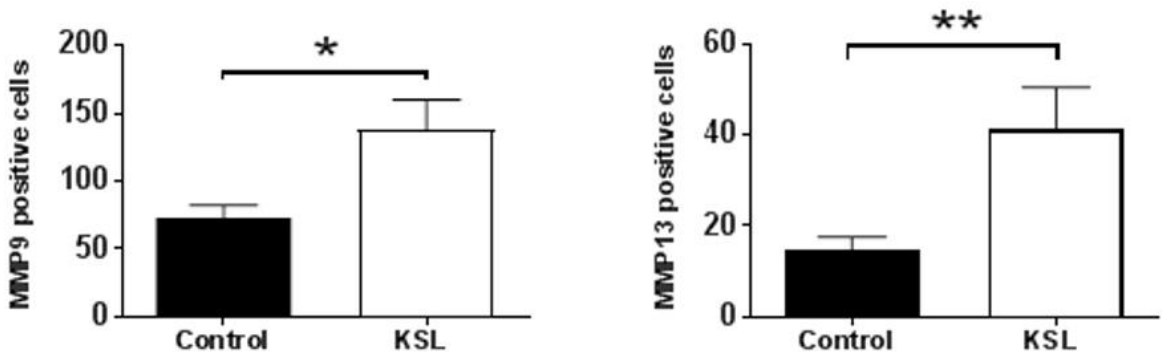


Figure 17: Comparison of MMP 9 and 13 staining in control and c-kit+ sca1+ lin- KSL treated mice (74), *p<0.05, **p<0.01 (two tailed, unpaired students t-test)

4.5: Quantitative polymerase chain reaction (qPCR)

4.5 (i): Methodology

*RNA extraction***:** RNA Isolation was performed using the RNeasy Mini Kit (Qiagen). For RNA isolation from tissue, approximately 25 to 30mg of the relevant tissue was placed in a GentleMACS M tube (Miltenyi Biotec) together with 600µl of RLT buffer supplemented with 10 µl/ml 2-mercaptoethanol (Sigma). Samples were processed on the Miltenyi GentleMACS processor using program *RNA.01_01* producing a tissue homogenate ready for RNA extraction. Tissue lysate was transferred to Eppendorf tube and centrifuged for 3 minutes at 1300 RPM. 600 ul of 50% Ethanol was added to the supernatant and immediately mixed by pipetting and this mixture was transferred to a RNeasy Spin Column and centrifuged at >10,000 rpm for 15 seconds. The column flow through was discarded and 350µl RW1 buffer was added to the spin column and centrifuged at >10,000 rpm for 15 seconds. 80µl of RNase free DNase solution (10 ul of DNAase stock solution to 70 ul of Buffer RDD) was then added directly to the spin column membrane and left to incubate for 15 minutes at room temperature. A further 350µl RW1 buffer was added to wash the spin column and centrifuged at >10,000 rpm for 15 seconds.

500µl RPE buffer was twice added to the spin column and centrifuged at >10,000 rpm firstly for 15 seconds then for 2 minutes. The columns were transferred to a new collection tube and centrifuged for 1 minute to allow solvent to evaporate from the membrane. After this, 50µl of RNase free water was added to the membrane and centrifuged at >10,000 rpm for 1 minute to elute the RNA and this step was repeated. All RNA samples were assessed for quantity and purity of RNA using a Nanodrop-100 spectrophotometer. Samples were used for cDNA synthesis if the ratio of absorbance at 260nm (A260) to absorbance at 280nm (A280) was between 1.8 and 2.0, indicating low levels of genomic DNA contamination. RNA samples were aliquoted and stored at -80˚C pending use in further applications.

*cDNA Synthesis***:** Each cDNA synthesis reaction was run in a thin walled PCR tube and consisted of 4.5µg RNA template added to 0.5 ul Random primers (Promega) and 1ul of dNTP Mix (100mm, 500 ul, Bioline). The mixture was made up to total volume on 12 ul with RNase DNAse free water (Qiagen). The reaction mix was incubated in a thermal cycler at 65˚C for 5 minutes and after that, the mixture was chilled on ice quickly. 5x First Strand Buffer, 0.1M DTT and Superscript IIRT was from Superscript II reverse transcriptase 10,000 U Invitrogen (Life Technologies) 4 ul of 5x First Stand Buffer, 2 ul of 0.1 M DTT and 1 ul of RNase DNase free water (Qiagen) were added to each tube. They were mixed gently and incubated at 25◦C for 2 minutes. 1 ul of Superscript II RT was added to each mixture and mixed gently, followed by incubation in a thermal cycler at 25◦C for 10 minutes, 42◦C for 50 minutes and finally the RI was inactivated by incubation at 70◦C for 15 minutes. The reaction was then cooled to 10°C and either used immediately for further applications or stored at -80◦C.

*Quantitative PCR***:** Polymerase chain reaction is a process by which samples of DNA undergo geometric amplification, yielding many copies of the original DNA sequence. The reaction is

catalysed by a heat stable DNA polymerase (Taq polymerase). Taqman gene expression assays were used in this work. Taqman fluorescent probes have the reporter dye FAM at the 5' end and the quencher MGB at the 3' end. The intact probes do not fluoresce as the reporter and quencher are in close proximity. As the reaction mixture cools during each cycle after the denaturation step the probe is able to hybridise with its target sequence. During the elongation step which follows the polymerase encounters the bound probe and the 5' nuclease activity of the enzyme separates the reporter from the quencher, thus rendering the reporter fluorescent. The free reporter molecules are excited by the detection instrument which detects emission of fluorescence. The amount of fluorescence is proportional to the number of copies of the target sequence.

Individual Taqman Gene Expression Assays used in this work are listed in Table 6. Each of these assays were inventoried and validated by the manufacturer. For each experiment, PCR mixture was made according to the following ratio: 800 ul of Taqman 2x Universal PCR Master Mix (Applied Biosystems), 625 ul of RNase DNAase free water (Qiagen) and either 40 ul of target Taqman Gene Expression Assay or GAPDH (Table 6). Reactions were performed on a 96 well PCR plate (Life Technologies) and 14 ul of PCR mixture and 1 ul of cDNA sample was added to each well of the plate to make up the total volume of 15 ul. PCR was performed using LightCycler 480 instrument and the programme used consisted of polymerase activation at 95°C for 10 minutes followed by 40 cycles of denaturing (95°C for 10 seconds) and annealing (60°C for 30 seconds and 72°C for 1 second) and cooling (40° C for 10 seconds).

The fluorescence of each reaction was recorded at the end of each cycle. Each reaction was performed in triplicate and control reactions which did not contain any cDNA template were also included. Cycle threshold (cT) values were obtained and the difference between the

reference and target gene calculated (ΔcT = cT target – cT reference). Relative differences between samples was expressed using the 2-ΔΔCT, where ΔΔcT = ΔcT sample1 – ΔcT sample2. The use of the ΔΔcT method to determine relative differences between samples under varying conditions is reliant upon there being no differences in the amplification efficiencies of the target and reference genes. The analysis was performed automatically on the software of LightCycler 480 which was then crossed checked with manual calculation.

Table 6: Gene expression assay used for PCR

Target (Murine)	**Gene Expression Assay**
Matrix Metalloproteinase 2	Mm00439498_m1
Matrix Metalloproteinase 8	Mm00439509_m1
Matrix Metalloproteinase 9	Mm00442991_m1
Matrix Metalloproteinase 12	Mm00500554_m1
Matrix Metalloproteinase 13	Mm00439491_m1
Inducible Nitric Oxide Synthase (iNOS) or NOS 2	Mm00440502_m1
Arginase-1	Mm00475988_m1
Glyceraldehyde 3-phosphate Dehydrogenase	Mm99999915_ g 1

4.5 (ii): Results for qPCR

The results showed that expression of MMP 2, 8, 12 and were similar between the two groups (Figure 18). MMP 9 was significantly higher in KSL treated group compared to control group ($p<0.05$). There is a reduction in the Arg-1/Nos 2 ratio in KSL treated mice (74).

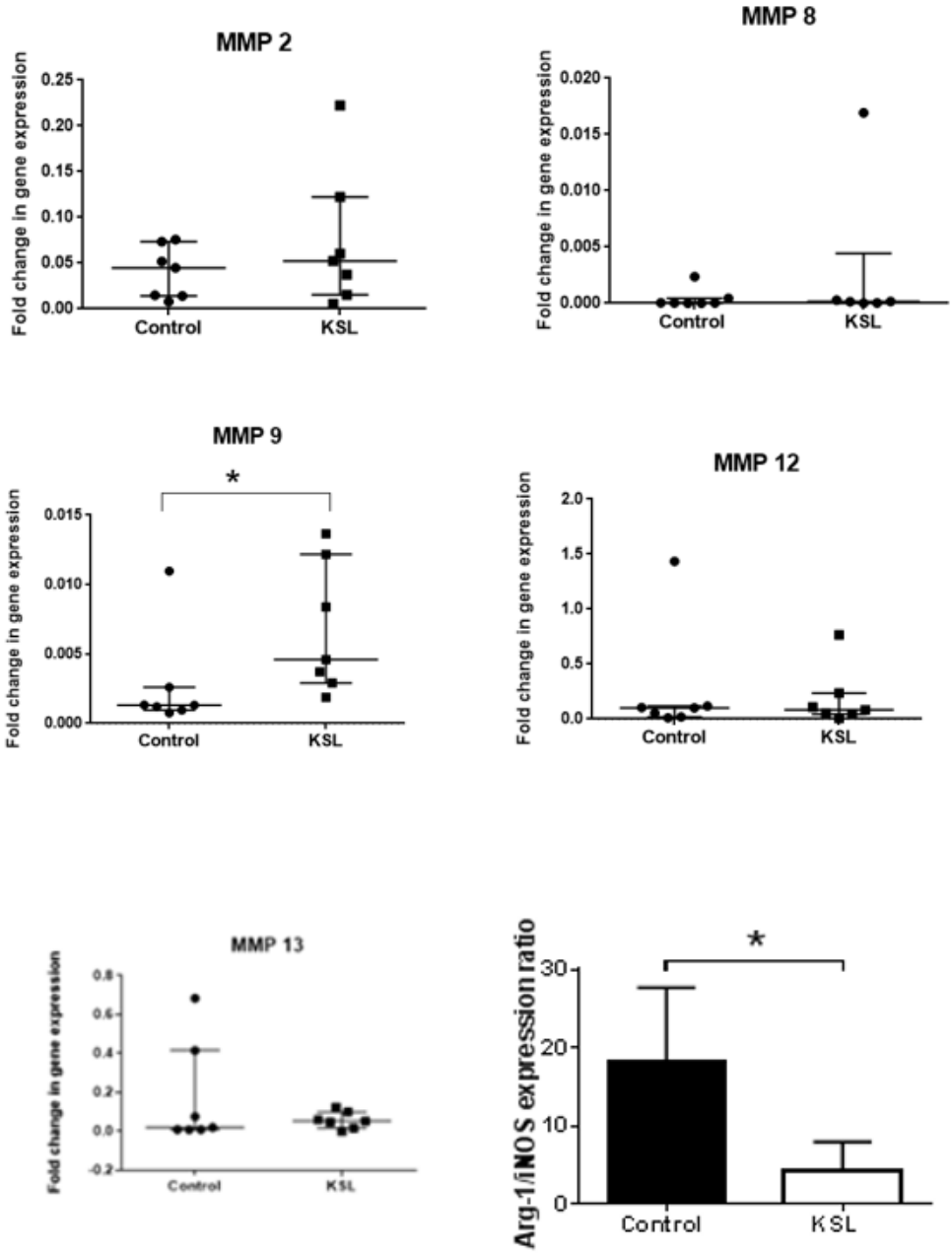


Figure 18: Quantitative polymerase chain reaction (qPCR) results of MMP2, MMP 8, MMP 9, MMP 12, MMP 13, Arg 1/NOS-2 expression ratio (74) * $P<0.05$ (two tailed, unpaired students t-test)

4.6: Discussion

Bone marrow stem cell therapy in liver disease is emerging but there is still uncertainty in relation to their efficacy as well as the type of stem cells required (74). In this study, we showed

that repeated injections of purified haematopoietic stem cells resulted in marked resolution of fibrosis by promoting the recruitment of endogenous macrophages and neutrophils with associated increases in MMP 9 and 13 expressions in KSL treated mice (74). We also examined the effect of oval cell response in KSL treated mice by staining the liver tissues with pan-CK and Sox-9. CK are expressed in epithelial cells and their functions include providing mechanical strength to the epithelial cells and also transport membrane proteins (80). Breakdown of CK by caspases plays a role in apoptotic process (80). CK7 and CK19 are strongly expressed in interlobular bile ducts, intraportal and intralobular bile ductules, the biliary epithelial cells and hepatic progenitor cells (80). Our study showed that there was a significant increase in number of pan-CK positive cells in the KSL treated group but not for Sox-9 staining. Both are used as markers of oval cell response to injury but with stem cell treatment, the positive finding was only seen in pan-CK staining. Tarlow et al mentioned that Sox9+ ductal proliferation makes only a minor contribution to parenchymal regeneration in liver injuries (81). It is possible that Sox 9 staining did not showed any positive response since Sox 9+ cells contribute only a minor response towards the hepatic regeneration process. KSL treated mice had significantly higher levels of MMP-9 and 12 compared to control mice which potentially increased the process of breaking down fibrosis in ECM remodelling.

For qPCR, there was an increased expression of MMP-9 in KSL treated mice compared to the control group but not in any other forms of MMPs. We examined the macrophage subsets in the livers of KSL treated mice since previous studies mentioned that bone marrow cells can differentiate into myofibroblast like cells and worsen the fibrosis process. There was no difference in the proportion of individual macrophage subsets (Arg-1 and Nos-2) from qPCR analysis although there was a reduction of Arg-1/Nos-2 ratio compared to the control group indication that there was no obvious increased risk seen in KSL treated mice. This change in ratio may be indicative of a change towards an anti-fibrotic milieu.

In summary, this work showed that repeated infusions of HSCs in CCL4 injured mice were safe and associated with an antifibrotic effect as supported by increases in the expression of MMP9 and 13 as well as an improvement in oval cell response as evidence by increased pan-CK staining in KSL treated mice. Due to prior data we designed and conducted a clinical trial to examine the effect of repeated haematopoietic stem cell infusions in patients with chronic liver disease. Our pre-clinical data reinforced this decision.

5: CLINICAL TRIAL

5.1: Role within the clinical trial

During the two and a half years of research fellowship, my main aim was to recruit patients to complete the clinical trial. The trial was initially started in 2009 and the first draft of the protocol was completed by previous research fellow, Dr King. I was then involved in the process of amending the clinical trial protocol.

My responsibilities that within this trial include:

1) Involved in the process of amending clinical trial protocol (final amendment was made on 5th March 2015)
2) Weekly screening of patients' electronic clinical records (clinical letters, imagining and blood tests) and identifying suitability
3) Contacting suitable patients either in person at clinic or via telephone conversation to explain about the clinical trial.
4) Keeping track of patients that had been approached and contacting them in a timely manner to recruit.
5) Contacting other hospitals within the West Midlands region to ensure that patients from secondary non-specialised hospitals were involved in the trial.
6) Getting a written consent from the patients, followed by detailed clinical assessments (clinical examination, review of blood tests/ imaging and electrocardiogram)
7) Assessing patients' clinical conditions regularly during their treatment and follow-up visits throughout.
8) Involving in addressing patients' queries and concerns throughout the study period.
9) Working together with clinical research team and helping them with data cleaning especially in documentation of adverse events.

When I first started, 37 patients had already been recruited into the trial and the recruitment was completed in March 2015, 6 months prior to completing my post graduate study.

5.2: Clinical trial methodology

The manuscript for the clinical trial protocol had been published (I was an author) and the following chapters were from the published manuscript (72).

5.2 (i): Research aim

The aim of the study was to access the safety and efficacy of GCSF and haematopoietic stem cells (HSC) infusions in patients with liver cirrhosis (72, 82).

5.2 (ii): Methods and analysis

The REALISTIC trial was a multicentre, open-label phase II randomised controlled trial of two different therapies: 1) administration of GCSF alone and 2) administration of GCSF, followed by isolation of CD133+ HSC and repeated infusion of those cells. Those therapies are compared with standard management of compensated cirrhosis according to local, national and international guidelines (72).

5.2 (iii): Trial organisation

The trial was an investigator led and designed trial, co-ordinated by the Liver Research group within the Cancer Research Clinical Trials Unit at the University of Birmingham (72). The University of Birmingham was the trial sponsor and the trial was funded by NIHR (National Institute for Health Research) Biomedical Research Unit for Liver Disease, Birmingham and the Sir Jules Thorn Trust (72). The trial was run at three sites in the United Kingdom (Birmingham, Edinburgh and Nottingham) and recruitment began in May 2010 (72).

The trail was registered at Current Controlled Trials (http://www. Controlled-trials.com) on 18 November 2009 (ISRCTN number 91288089, EuDRACT number 2009-010335-41) (72). The

procurement, processing, storage and distribution of the Autologous CD133+ HSC were performed in accordance with Tissue Quality and Safety Regulations by establishments holding Human Tissue Authority licenses (72).

5.2 (iv): Inclusion and exclusion criteria

The details of the inclusion and exclusion criteria are listed in table 7 and 8.

Table 7: Inclusion criteria of the clinical trial (72, 82)

Inclusion criteria	**Details**
Age	18 to 75 years
MELD at randomisation	11.0 and 15.5
Aetiology of liver diseases (one or more of)	○ Alcohol related liver disease: features (clinical, biochemical, histological or radiological) of chronic liver disease with a compatible history of alcohol excess >80g/day), in the absence of other causes of chronic liver disease. ○ Hepatitis C with positive HCV antibody and who are not currently on antiviral treatment ○ Hepatitis B with positive HBsAg and Anti-HBc and who are on established antiviral therapy with adequate viral suppression. ○ Primary biliary cholangitis with 2 out of: cholestatic LFTs, positive AMA (>1:40), compatible histology. If already receiving ursodeoxycholic acid, the treatment must be established on current dose for >3 months prior to enrolment ○ Genetic haemochromatosis with diagnosis made on basis of compatible biochemistry (transferrin sat >60%, ferritin >400), genotype (homozygous C282Y or H63D, compound heterozygote) or Histology

Inclusion criteria	Details
	○ Cryptogenic cirrhosis Diagnosis of cirrhosis un-attributable to any other cause ○ Non-alcoholic fatty liver disease (NAFLD) with diagnosis by either the presence of histological evidence of steatosis in the absence of other liver diseases or radiological evidence compatible with NAFLD (e.g. Fatty infiltration of liver) and one or more risk factors (e.g., elevated BMI, T2DM, hypertriglyceridemia, hypertension) and the absence of significant alcohol consumption (<20 g/day) and no evidence of other causes of chronic liver disease ○ α-1 anti-trypsin deficiency with diagnosis based on compatible genetic, phenotypic or histological testing
Cirrhosis as defined as	○ previous liver biopsy confirming histological features of cirrhosis ○ Fibroscan >16 kPa ○ Clinical and radiological features correlate with diagnosis of cirrhosis (as per opinion of investigator) ○ AST: platelet ration index (APRI)>2.0 (APRI= (([AST]/ULN*[Plt]) X100)

Table 8: Exclusion criteria of the clinical trial (72)

Exclusion criteria	Details
General	○ Refusal or inability to give informed consent ○ Any situation that in the Investigator's opinion may interfere with optimal study participation such as alcohol or drug abuse, domicile too distant from study site, potential non-compliance or inability to co-operate ○ Participation in any clinical study of an investigational agent within 30 days of randomisation ○ The presence of clinically relevant cardiovascular, pulmonary, gastrointestinal, renal, metabolic, haematological, neurological, psychiatric, systemic, ocular, gynaecological or any acute infectious disease or signs of acute illness that in the opinion of the investigator might compromise the patient's safe participation in the study ○ Presence or history of cancer within past 5 years with exception of adequately treated localised basal cell carcinoma of the skin, in situ cervical cancer or solid malignancy surgically excised in total without recurrence for 5 years
Pregnancy or breast feeding	Women of childbearing potential and men who have partners of childbearing potential who are not willing to practise effective contraception for the duration of the study and for 12 months (females) and 6 months (males) after the last study drug administration
Liver specific	○ Alcohol ingestion >21 units/week in male and >14 units/week in female

Exclusion criteria	Details
	○ Aetiology of chronic liver disease (not listed in the inclusion criteria) ○ Ascites—unless minimal and well controlled with no changes to diuretic therapy in the last 3 months ○ Encephalopathy—current or requiring hospitalisation in last 3 months ○ Portal hypertensive bleeding—active or requiring hospitalisation in past 3 months ○ Hepatocellular carcinoma—current or previous ○ Liver transplantation—previous or on waiting list
Granulocyte colony stimulating factor (GCSF) related	Recent history of pulmonary infiltrates or pneumonia: patients should have completely recovered from any previous episodes, both clinically and radiologically

5.2 (v): Screening

Patients were identified and recruited at the participating trial site and gave written informed consent at the beginning of the screening visit prior to undergoing any tests or procedures needed to assess their eligibility (72). Patient's eligibility for the study was determined by clinical assessment including a full medical history followed by thorough clinical examination, blood tests, complete liver aetiology screen if not previously performed, 12 lead E.C.G (Electro cardiogram), chest X-ray, abdominal ultrasound scan, liver stiffness evaluation by Fibroscan, baseline chronic liver disease questionnaire (CLDQ) and urine pregnancy tests for female patients of child bearing age (72). As required by National Health Service (NHS) Blood and Transplant Standard Operating Procedures, prior to processing and storage of cellular products,

patients were tested for viral infections: viral hepatitis B and C, human Immunodeficiency virus (HIV), human T-cell lymphotrophic virus (HTLV)-1 and 2 and syphilis (72).

5.2 (vi): Randomisation

Centre-delegated staff telephoned randomisation officers at the Cancer Research UK Clinical Trials Unit (Birmingham, UK), who used a computer-generated, centrally administered procedure to randomly assign eligible patients (1:1:1) to one of three treatment arms (82). Randomisation was based on a minimisation algorithm (prepared and validated by the CRCTU programming and statistical team) and patients were also stratified by (i) trial site (ii) aetiology of disease (82). The patient was then allocated a unique patient trial number and scheduled for treatment and follow up visits (82). The local site staff could not pre-determine treatment allocation (82). Since the study was an open label, both clinicians and local site staff including the patient were aware of which treatment had been allocated (82). After randomisation/treatment and depending on allocated arm, patients returned for study visits at days 30, 60, 90, 180 and 360 (end of study) (82).

5.2 (vii): Study treatment

During the trial period, all patients received standard medical management of underlying liver cirrhosis, which may include disease specific treatment (antiviral treatment for hepatitis B, ursodeoxycholic acid for primary biliary cholangitis) and treatments for the complications of cirrhosis (72). Concomitant medications were used at the discretion of the site investigator with the exception of the introduction of antiviral therapy for chronic HCV infection, changes to medications for chronic HBV infection, the introduction of UDCA for PBC and participation in another clinical trial of an investigational product (72).

Patients were randomised into:

Group 1: Patients randomised to group 1 received standard medical therapy (control group).

Group 2: Patients randomised to group 2 received GCSF (Lenograstim) therapy without CD133+ HSC infusions. The treatment began within 7 days of randomisation and patients received subcutaneous (SC) injection of GCSF 15 ug/kg of body weight for 5 days, in addition to their standard medical therapy.

Group 3: Patients randomised to group 3 received SC injection of GCSF 15ug/kg for 5 days within 7 days of randomisation, in addition to their standard medical treatment. On the fifth day of GCSF treatment, leukapharesis was performed according to the standard operating procedure in place at each site and CD133+ cells were collected. If there was insufficient number of CD133+ cells, a second leukapharesis was performed on day 6. The cells were reinfused on days 6, at 1 month and 2 months from randomisation. The randomisation was illustrated in Figure 19.

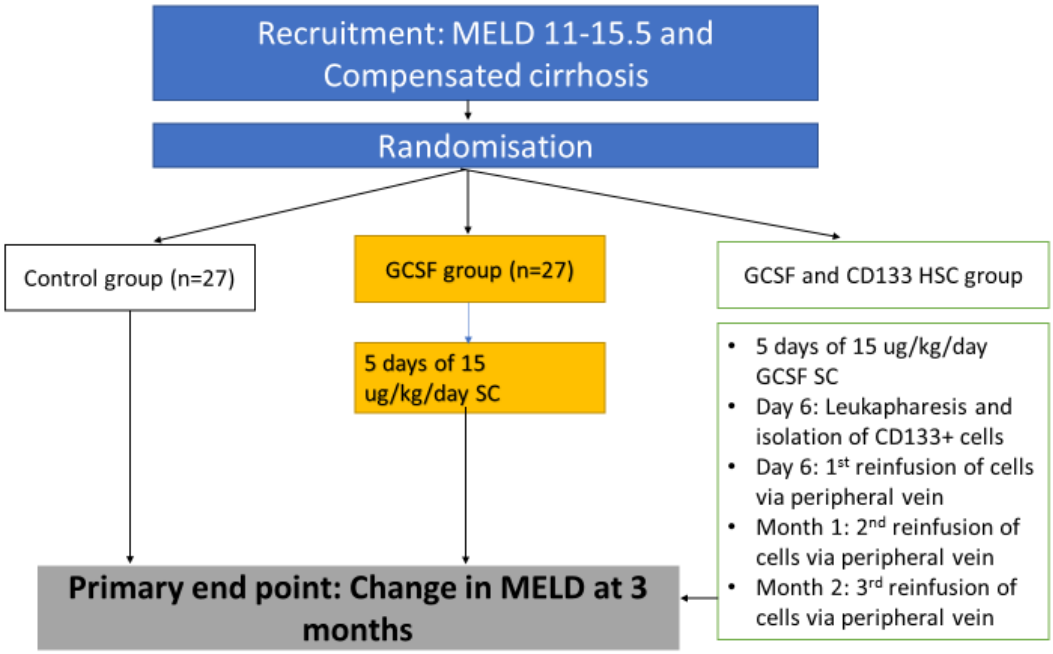


Figure 19: Outline of clinical trial and the randomization pathway within the trial (72, 82)

CD133+ HSC were isolated from the harvested peripheral blood mononuclear cells (MNC) under aseptic conditions within clean room facilities in accordance with good manufacturing practice regulations (Medicines and Healthcare Products Regulatory Agency, MHRS/HTA, UK) (72). CD133+ HSC are isolated through immunomagnetic positive selection using super paramagnetic iron dextran particles directly conjugated to CD133 antibodies (72). This is performed in close, sterile system providing clinical grade enrichment (CliniMACS Plus, Miltenyi Biotec, Germany) (72). The CliniMACS system has been shown to provide CD133+ cells at up to 97% high purity and yields up to 81% (82-84) with passive depletion of unwanted cells. The CliniMACS Plus instrument is CE-marked for clinical use in Europe (72).

Isolated CD133+ HSC were aliquoted in three portions in the required quantities, one portion is available for immediate re-infusion and other two portions were cryopreserved according to site protocols for later reinfusion at day 30 and day 60 from randomisation (72). The dose of CD133+ HSC re-infused was 0.2×10^6 cells/kg for each of the three infusions and hence, the minimum cells required for the collection was 0.6×10^6 cells/kg (72). If there were insufficient cells for three doses, the cells were allocated preferentially to the first, then second and third dose (72).

5.2 (viii): Primary outcomes

The primary objective was to demonstrate an improvement in liver function and reduction in liver fibrosis in patients receiving therapies compared with those receiving standard medical therapies (72). This objective was measured by the change in MELD score, also known as delta MELD, at day 90 from the day of randomisation, noted as day 1 (72). The trend of treatment activity by incorporating MELD measured at baseline and days 30, 60 and 90 was also included as co-primary 2 outcomes (72). The protocol was updated in March 2015 to include co-primary 2 (72). Co-primary 2 measured the effect of treatment on the change in MELD that was also

explored through linear mixed-effects models to find a most appropriate parsimonious model which incorporated measurements taken at baseline and at days 30, 60 and 90. Random effects were assigned at the patient level (82).

5.2 (ix): Secondary outcomes

The secondary objectives were treatment related adverse events, liver fibrosis, disease related quality of life, liver related clinical events and transplant free survival. Liver fibrosis was measure by non-invasive methods and those included 1) Fibroscan TM, Echosens, France and ELF test (72). ELF was CE marked and approved for use in research and now in clinical practice (72). The serum ELF samples were sent to Southampton for analysis (72). Fibroscan was performed at randomisation (day 1), and repeated at day 90, day 180 and day 360 (72). Blood tests for ELF test were collected at randomisation/day 1, day 30, day 90, day 180 and day 360 (72).

Disease related quality of life was measured by "Chronic Liver Disease Questionnaire (CLDQ)" (72). In CLDQ, there were 29 items that was divided into 6 quality of life domains (abdominal symptoms, fatigue, systemic symptoms, activity, emotional function and worry) (72). These items were ranked on a 1 to 7 scale, providing a range of scores from 29 (worse quality of life) to highest 203 (best quality of life) (72). Patient completed CLDQ questionnaire at randomisation (day 1), day 90, day 180 and day 360 (72). The example of CLDQ questionnaire used in this study was shown in appendix 1. UKELD score was also calculated at randomisation (day 1), day 90, day 180 and day 160 (72).

Patients were followed up for 12 months from the day of randomisation for survival (72). During the follow up, treatment related adverse events and liver related clinical events were monitored and recorded at each visit and any unscheduled visits (72). The liver related clinical events recorded were 1) Newly developed clinical significant ascites or worsening of

established ascites (confirmed radiologically) 2) Encephalopathy that require introduction of treatment or hospitalisation 3) Portal hypertensive bleeding (confirmed at endoscopic examination) 4) Spontaneous bacterial peritonitis (confirmed on ascitic fluid sample with polymorphonuclear cell count of >250cm^3 5) Development of hepato-renal syndrome 6) Listing for liver transplantation 7) Diagnosis of liver cancer and 8) Death.

5.*2 (x): Recruitment*

The trail recruitment was started on 18th May 2010 and was completed on 26th February 2015. The detail outlined of the trial schedule is documented in appendix 2.

6: CLINICAL TRIAL RESULTS

6.1: Overview

The clinical trial manuscript has now been published (I was one of the author) and the following chapters are from the published manuscript (82).

6.2: Results

6.2 (i): Statistical analysis

Statistical analysis was performed by trial statistician and consent was obtained for this section to be used.

The sample size calculation was based on the change in MELD score from baseline to 90 days' post-randomisation (82). With pooled standard deviation assumed in this controlled setting to be 1.25, this trial aimed to detect a standardised effect size of at least 0.8 between treatment arms and control, equating to a 1-point reduction in MELD (82). Error rates based on two sided $\alpha=0.05$ ($\alpha=0.10$ split equally between the two hypotheses) and 80% power, required recruitment of 27 patients into each arm (72, 82).

The trial was designed as a three-armed study with one control arm and powered to compare each treatment to control with respect to co-primary 1 but was not powered to detect differences between the two treatment arms (82). Analysis of primary outcome measure: the hypothesis was designed to assess activity and as such all analyses were carried out in the modified intention to treat (mITT) population. Modified intention to treat (mITT) population included participants who were 1) protocol violator 2) ineligible participants 3) participants who received at least 1 day of GCSF at 15ug/kg in G2,4) participants who received one infusion of 0.17x106 cells/kg for G3 (82) . The safety population was defined based on actual treatment received (82).

Co-primary 1: Change in MELD from baseline to day 90 was calculated for each participant and arms 1 and 2 were compared to control using the non-parametric two-sample Wilcoxon test. Exploratory linear regression models, with transformed MELD where required, were fitted to enable adjustment of co-primary 1 estimates (82).

Co-primary 2: The effect of treatment on the change in MELD was also explored through linear mixed-effects models for modelling approach to find a most appropriate parsimonious model which incorporated measurements taken at baseline and at days 30, 60 and 90. Random effects were assigned at the patient level (82).

Co-primary 1 is conditional on availability of day 90 MELD measure, whereas co-primary 2 is not (82).

Sensitivity analyses included adjustment of model- based analyses for known prognosticators, such as alcohol and aetiology, minimisation factors and for differences observed at baseline (82). UKELD was analysed as per co-primary 1 and 2, and CLDQ responses were assessed through area-under-curve analyses with a set of varied assumptions applied to address censoring, and, as a sensitivity analysis, removing those participants experiencing major events (82). Long-term MELD and UKELD, measured to day 360, were assessed as per co-primaries 1 and 2 (82). Average change in liver parameters from baseline was compared using a test appropriate to the data (82). Other outcome measures were reported descriptively (82). Stata v14 was used for all analyses (82).

6.2 (ii): Trial population

Patients were recruited from liver clinics (82). All participants gave written informed consent and the study was conducted by site investigators, and data were gathered by specifically trained personnel (82). Of 153 patients with liver cirrhosis who were screened, 81 underwent randomisation between 18 May 2010 and 26 Feb 2015 (82). Participants were recruited from

UK sites as follows: Birmingham (n=58), Edinburgh (n=19) and Nottingham (n=4) (82). Among 81 patients, 27, 26 and 28 patients were randomised into group 1 (standard care), group 2 (GCSF only) and group 3 (GCSF and CD133+ cells) respectively. At day 90, primary outcomes were not available in 4 patients from G1, none from G2 and 2 from G3 due to death, deviation, and withdrawal from treatment (Figure 20) (82).

One patient never received GCSF (withdrew from study), one patient received GCSF but no CD133+ cells (unable to obtain venous access for leukapheresis) and six patients received GCSF but did not receive sufficient cells to complete 3 full doses and therefore followed the protocol (82). Based on treatment received the modified intention to treat (mITT) population comprised 27, 26 and 26 patients belonging to Groups 1, 2 and 3 respectively (82).

6.2 (iii) General characteristics of included patients

Participant characteristics recorded at baseline (Table 9) indicated an imbalance in gender as women were underrepresented in group 3, whereas no differences were observed in age, MELD, UKELD, blood analyses, non-invasive measures of liver fibrosis and quality of life (82). Median MELD scores were 13.1 (12.4, 13.8), 12.7 (12.0, 13.1) and 13.2 (12.1, 13.9) across groups 1, 2 and 3 respectively (82). Patients had features of liver decompensation (ascites, encephalopathy and prior variceal bleeding) although these were mild and responsive to standard medical treatment (82).

Table 9: Baseline characteristics of the patients' groups included in the clinical trial (82)

	Standard care (n=27)	**GCSF only (n=26)**	**GCSF+ CD133 cell infusion (n= 28)**
Demographics			
Age (years)	52.0 (47.0, 60.0)	54.0 (49.0, 61.0)	56.5 (47.5, 62.5)
Sex: Male (n, (%))	13 (48.1)	18 (69.2)	22 (78.6)
Liver disease aetiology (n, (%))			
Alcohol related liver disease	12 (44.4)	12 (46.2)	14 (50.0)
Hepatitis C	4 (14.8)	3 (11.5)	3 (10.7)
Non-alcoholic fatty liver disease (NAFLD)	5 (18.5)	3 (11.5)	5 (17.9)
Primary biliary cholangitis (PBC)	5 (18.5)	7 (26.9)	3 (10.7)
Cryptogenic cirrhosis	0 (0)	1 (3.8)	2 (7.1)
Mixed	1 (3.7)	0 (0)	1 (3.6)
Liver disease severity			
MELD	13.1 (12.4, 13.8)	12.7 (12.0, 13.1)	13.2 (12.1, 13.9)
UKELD	51.5 (49.8, 54.2)	51.1 (50.0, 52.5)	52.0 (50.9, 53.5)
Child Pugh score	7.0 (7.0, 8.0)	7.0 (6.0, 8.0)	7.0 (6.0, 8.0)
Child Pugh Class			
A	6 (22.2%)	7 (26.9%)	11 (39.3%)
B	21 (74.1%)	19 (73.1%)	16 (57.1%)
C	1 (3.7%0	0 (0.0%)	0 (0.0%)
Unknown	0 (0.0%)	0 (0.0%)	1 (3.6%)
Liver co-morbidities (n (%))			
Ascites	12 (48.1)	10 (38.5)	14 (50.0)
Variceal bleeding	7 (25.9)	11 (42.3)	11 (39.3)
Encephalopathy	3 (11.1)	3 (11.5)	7 (25.0)
Full blood count			
Haemoglobin (g/dL)	12.9 (11.8, 13.8)	13.1 (11.6, 14.3)	12.9 (12.1, 14.3)
WBC (*10^9/L)	4.2 (3.3, 5.4)	4.3 (3.4, 5.2)	4.3 (3.3, 5.3)
Platelets (*10^9/L)	77.0 (57.0, 92.0)	90.5 (54.0, 116.0)	78.5 (57.0, 106.5)
Biochemistry			
Sodium (mmol/L)	140.0 (137.0, 142.0)	140.0 (137.0, 142.0)	139.0 (137.0, 140.0)
Potassium (mmol/L)	3.9 (3.7, 4.4)	4.0 (3.7, 4.2)	4.1 (4.0, 4.2)
Urea (mmol/L)	3.7 (2.7, 4.2)	3.8 (2.9, 4.8)	4.8 (3.7, 5.3)
Creatinine (mmol/L)	62.0 (52.0, 74.0)	63.0 (56.0, 75.0)	71.0 (64.0, 90.0)
Liver function tests (LFTs)			
Bilirubin (mmol/L)	38.0 (30.0, 53.0)	44.0 (34.0, 53.0)	41.5 (33.0, 51.0)
Albumin (g/L)	33.0 (30.0, 37.0)	36.0 (30.0, 39.0)	35.5 (33.5, 39.0)
AST (U/L)	44.0 (35.0, 62.0)	50.5 (37.0, 82.0)	48.0 (37.0, 62.0)

	Standard care (n=27)	GCSF only (n=26)	GCSF+ CD133 cell infusion (n= 28)
ALT (U/L)	28.0 (20.0, 39.0)	31.5 (21.0, 54.0)	31.0 (21.5, 45.0)
ALP (U/L)	160.0 (108.0, 255.0)	142.5 (118.0, 282.0)	138.5 (97.5, 244.0)
GGT (g/dL)	68.0 (49.0, 110.0)	86.0 (57.0, 198.0)	73.0 (41.0, 188.5)
AFP (IU/L)	3.0 (2.0, 6.0)	3.0 (2.0, 5.0)	3.0 (2.0, 5.0)
INR	1.4 (1.2, 1.4)	1.2 (1.2, 1.4)	1.3 (1.2, 1.4)
Non-invasive hepatic biomarkers			
Fibroscan (kPa)	32.5 (22.2, 44.8)	34.3 (26.1, 66.4)	28.9 (17.3, 45.2)
ELF score	12.0 (11.2, 13.0)	11.9 (11.4, 12.6)	12.1 (11.3, 13.0)
ELF: Hyaluronic acid	490.6 (265.2, 879.9)	476.9 (253.8, 722.2)	574.4 (366.8, 807.5)
ELF: Amino-terminal propeptide of type III procollagen	17.1 (12.0, 22.8)	18.2 (13.0, 25.7)	18.7 (13.2, 26.4)
ELF: Tissue inhibitor of metalloproteinases 1	329.3 (267.0, 399.1)	372.2 (289.5, 507.6)	322.9 (227.1, 412.2)
Quality of life - Chronic Liver Disease Questionnaire (CLDQ)			
Abdominal	6.3 (4.7, 7.0)	5.7 (4.0, 6.7)	6.0 (4.7, 7.0)
Fatigue	4.5 (2.8, 6.0)	3.5 (2.6, 5.2)	4.2 (3.6, 5.2)
Systemic	5.4 (4.0, 6.2)	5.4 (4.0, 6.0)	5.2 (4.4, 6.0)
Activity	6.0 (3.7, 7.0)	4.7 (3.2, 6.0)	5.8 (4.7, 6.8)
Emotion	5.6 (4.5, 6.8)	4.8 (3.7, 5.8)	5.4 (4.5, 6.1)
Worry	5.8 (3.9, 6.9)	5.1 (3.3, 6.6)	4.8 (3.6, 6.4)
Overall	5.5 (3.4, 6.3)	4.8 (3.5, 5.5)	5.0 (4.2, 6.1)

MELD: Model for End Stage Liver Disease, UKELD: United Kingdom Model for End Stage Liver Disease, AST: Aspartate transaminase, ALT: Alanine transaminase, ALP: Alkaline phosphatase, GGT: Gamma-Glutamyl Transferase, AFP: Alpha-feto protein, INR: International normalised ratio, ELF: Enhanced Liver Fibrosis,

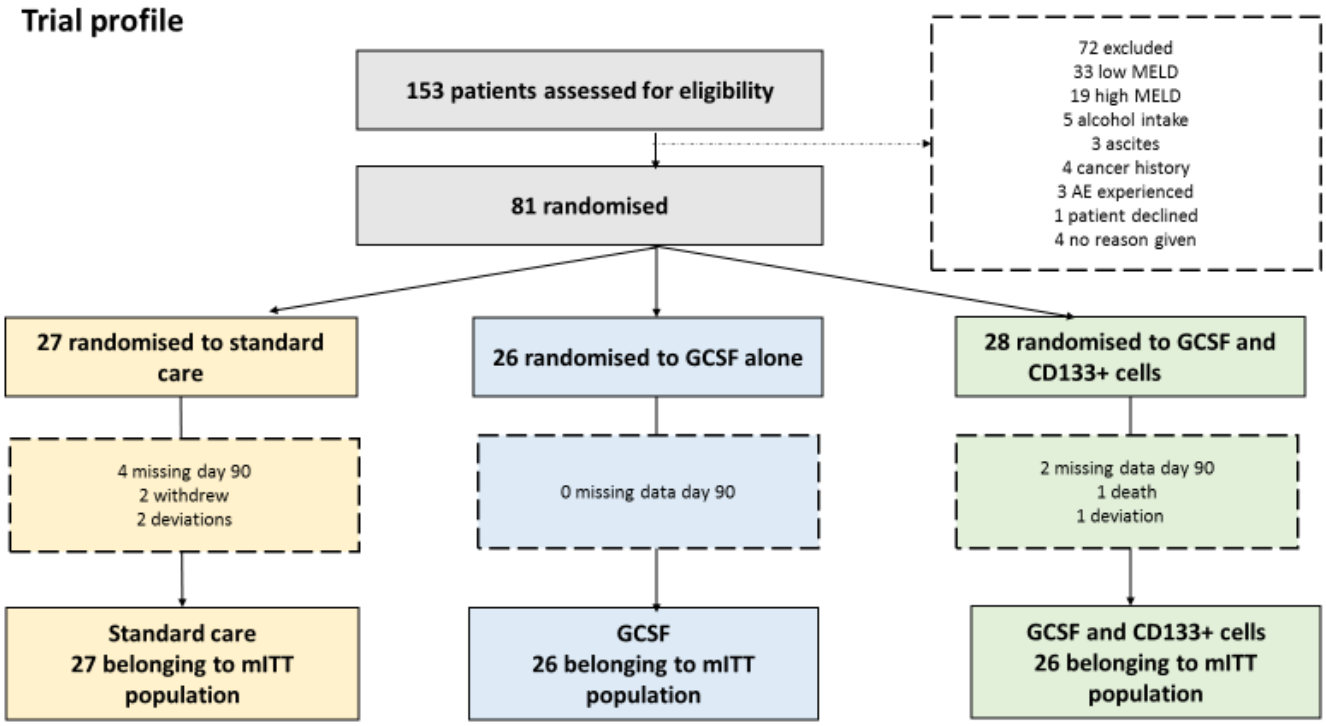


Figure 20: Flowchart of trial randomisation (82); Modified intention to treat (mITT) population was defined as participants who were protocol violator, ineligible participants, and participants receiving at least one-day GCSF at 15mcg/kg body weight in group 2, plus one infusion of 0.17x106 cells/kg for group 3, MELD: Model for End Stage Liver Disease, UKELD: United Kingdom Model for End Stage Liver Disease, AE: Adverse events

6.2 (iv): Safety and Adverse events

Safety and adverse events were assessed using standard reporting forms by trained investigators (82). The National Cancer Institute's common terminology criteria for AEs (CTCAE V.4.02 – 2010) was used to grade each AE (82). The reporting period for adverse event (AEs) started from the date of patient consent and continued throughout the study until visit 7 (Day 360) (82). Serious adverse events (SAE's) were reported from date of consent until 30 days after the last possible cell infusion (Day 90) for all treatment arms, therefore ensuring that the reporting period stayed the same for all treatment arms (82).

6.2 (v): Primary outcome

Of the mITT population a day 90 MELD score was recorded for 23, 26 and 26 patients in groups 1, 2 and 3 respectively (82). The median (IQR) change in MELD from day 0 to 90 for groups 1, 2 and 3 were -0.5 (-1.5, 1.1), -0.5 (-1.7, 0.5) and -0.5 (-1.3, 1.0) respectively (82). There was no difference MELD change between days 0 and 90 in groups 2 and 3 when compared to the control (p=0.718 and p=0.904 respectively) (82). There was no evidence of treatment activity in the per protocol population (82). Co-primary 2 was based on all participants belonging to the mITT population (82). Changes in MELD was shown in Figure 21A, Table 10.

6.2 (vi): Secondary outcomes

In none of the analyses of UKELD was evidence of a difference between groups detected and no differences were detected in either composite or individual markers of liver dysfunction across any of the groups (Figure 21 B, Table 10) (82). Moreover, there were no differences in markers of liver fibrosis (serum ELF or Fibroscan) nor in quality of life scores (CLDQ) (82).

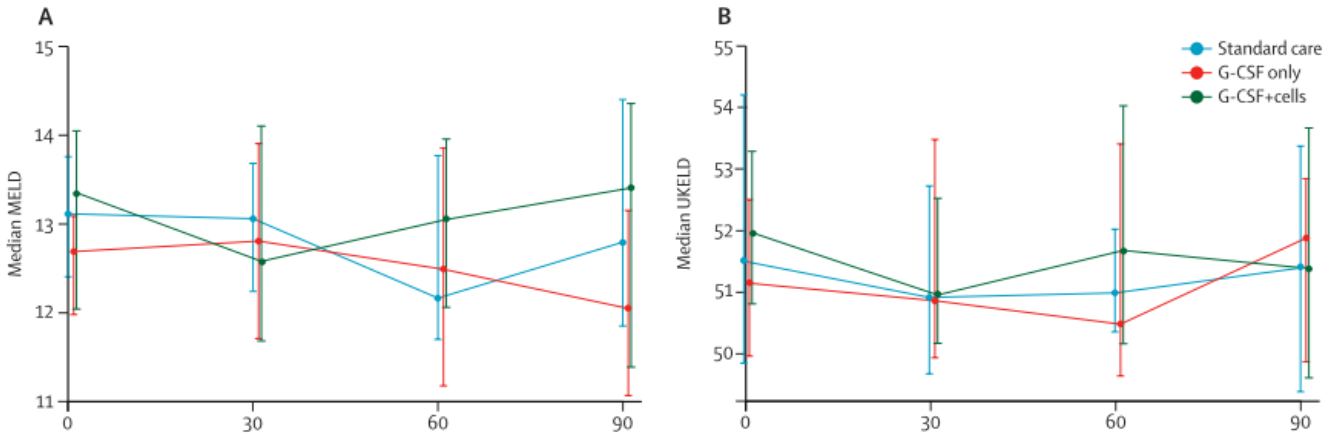


Figure 21: Median change in MELD (A) and UKELD scores (B) at day 30. 60 and 90 for the study population (82); MELD: Model for End Stage Liver Disease, UKELD: United Kingdom Model for End Stage Liver Disease

Table 10: Change in liver parameters from baseline to day 30 and day 90 (82)

	Group 1 (n=27) Standard care		Group 2 (n=26) GCSF only		Group 3 (n=28) GCSF and CD133 positive cells infusion	
	Day 30	**Day 90**	**Day 30**	**Day 90**	**Day 30**	**Day 90**
Liver disease severity						
MELD	-0.3 (-0.8, 0.2)	-0.5 (-1.5, 1.1)	0.0 (-1.0, 1.0)	-0.5 (-1.7, 0.5)	-0.1 (-2.2, 1.0)	-0.5 (-1.3, 1.0)
UKELD	-1.0 (-2.2, 0.4)	-0.1 (-3.2, 1.7)	-0.3 (-1.1, 1.0)	0.5 (-1.0, 1.4)	-1.0 (-1.9, -0.2)	-0.5 (-1.2, 0.5)
Child-Pugh score	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.0 (0.0, 1.0)	0.0 (0.0, 1.0)	0.0 (-1.0, 1.0)	0.0 (0.0, 1.0)
Full blood count						
Haemoglobin (g/dL)	-0.1 (-0.7, 0.4)	0.3 (-0.6, 0.5)	-1.0 (-1.4, -0.4) *	-0.3 (-0.6, 0.5)	-1.0 (-1.6, -0.4) †	-0.4 (-1.0, 0.4)
White cell count (*10^9/L)	0.4 (-0.3, 1.0)	0.3 (-0.2, 0.6)	-0.7 (-1.1, -0.2) ‡	-0.1 (-0.8, 0.6)	-1.0 (-1.6, -0.3) §	-0.2 (-1.1, 0.5)
Platelet count (*10^9/L)	2.0 (-7.0, 9.0)	-0.5 (-8.0, 9.0)	13.0 (2.0, 23.5) ¶	0.0 (-8.0, 7.0)	8.0 (-4.0, 30.0)	1.0 (-3.0, 5.0)
Biochemistry						
Sodium (mmol/L)	1.0 (-1.0, 4.0)	0.0 (-2.0, 3.0)	0.0 (-1.0, 2.0)	-1.0 (-2.0, 1.0)	1.0 (-1.0, 3.0)	0.0 (-1.0, 3.0)
Potassium (mmol/L)	0.0 (-0.2, 0.2)	0.1 (-0.1, 0.3)	0.0 (-0.2, 0.3)	0.2 (-0.2, 0.5)	-0.1 (-0.3, 0.2)	-0.1 (-0.3, 0.3)
Urea (mmol/L)	0.3 (-0.1, 0.9)	0.1 (-0.4, 1.0)	0.0 (-0.5, 0.7)	0.2 (-0.6, 0.9)	0.2 (-0.6, 0.5)	-0.1 (-1.0, 0.4)
Creatinine (mmol/L)	3.0 (-2.0, 5.0)	1.0 (-2.0, 10.0)	2.0 (-5.0, 4.0)	2.5 (-1.0, 10.0)	1.0 (-3.0, 8.0)	1.5 (-4.0, 9.0)
Liver function tests (LFTs)						
Total bilirubin (mmol/L)	-5.0 (-9.0, 3.0)	-5.0 (-12.0, 3.0)	-2.0 (-10.0, 8.0)	-6.0 (-12.0, -1.0)	-2.5 (-9.5, 10.0)	-2.0 (-9.0, 7.0)
Albumin (g/L)	0.0 (-3.0, 1.0)	0.0 (-3.0, 1.0)	-2.0 (-3.0, -1.0)	-2.0 (-3.0, 0.0)	-3.0 (-4.0, -1.0)	-1.0 (-4.0, 2.0)
International normalised ratio (seconds)	0.0 (0.0, 0.0)	0.0 (-0.1, 0.1)	0.0 (-0.1, 0.1)	0.0 (-0.1, 0.1)	0.0 (-0.1, 0.1)	0.0 (0.0, 0.0)
Alanine aminotransferase (U/L)	1.0 (-4.0, 3.0)	0.0 (-4.0, 4.0)	-3.0 (-7.0, 1.0)	-2.0 (-9.0, 4.0)	-2.5 (-5.0, 0.0)	-4.5 (-8.0, 0.0)
Aspartate aminotransferase (U/L)	-0.5 (-6.0, 5.0)	-2.0 (-6.0, 4.0)	-3.0 (-8.0, 2.0)	-4.0 (-13.5, 4.0)	-3.0 (-9.0, 1.0)	-2.0 (-9.0, 1.0)
Gamma-glutamyl transferase (U/L)	-1.0 (-7.0, 3.0)	-1.0 (-7.0, 3.0)	-8.0 (-22.5, 0.0)	-10.0 (-42.5, 0.5)	-5.0 (-22.0, 1.0)	-3.0 (-18.0, 1.0)
Alkaline phosphatase (U/L)	-8.0 (-28.0, 14.0)	-4.0 (-20.0, 11.0)	7.0 (-8.0, 21.0)	-5.0 (-16.0, 7.0)	8.0 (0.0, 11.0)	1.5 (-10.0, 24.0)
Alpha-feta protein (AFP)	0.0 (0.0, 0.0)	0.0 (-1.0, 1.0)	0.0 (-1.0, 0.0)	0.0 (-1.0, 0.0)	0.0 (-0.5, 0.0)	0.0 (-1.0, 0.0)
Non-invasive hepatic biomarkers						
Enhanced Liver Fibrosis (ELF)		-0.1 (-0.4, 0.6)		0.0 (-0.4, 1.0)		0.1 (-0.6, 0.5)

	Group 1 (n=27) Standard care		Group 2 (n=26) GCSF only		Group 3 (n=28) GCSF and CD133 positive cells infusion	
	Day 30	**Day 90**	**Day 30**	**Day 90**	**Day 30**	**Day 90**
Fibroscan (kPa)		0.0 (-1.6, 8.6)		0.0 (-11.9, 9.5)		0.5 (-3.8, 10.1)
Quality of life						
CLDQ, overall		0.2 (-0.1, 0.6)		-0.1 (-0.4, 0.3)		0.0 (-0.2, 0.2)

Values are recorded as a change in medians (interquartile range/IQR). Statistical comparisons were made between change at day 30 and 90 to baseline between the treatment groups, and unless indicated, there were no significant differences. *p=0·0017. †p=0·0006. ‡p=0·0024. §p=0·0002. ¶p=0·0053. MELD: Model for End Stage Liver Disease, UKELD: United Kingdom Model for End Stage Liver Disease, CLDQ: Chronic Liver Disease Questionnaire, GCSF: Granulocyte colony stimulating factor

6.2 (vii): Clinical outcomes and safety

None of the patients from treatment arm had a reduction in mortality nor admissions to hospital (82). One patient from group 1 died due to variceal bleed. No one died in GCSF group but 2 died in GCSF+ CD133 cell group because of myocardial infarction and progressive liver disease (82). One patient in standard care was assessed for LT but not listed due to cardiac arrhythmias. Four patients from GCSF group was assessed for LT and 3 were transplanted. In group 3, all 3 who were assessed for liver transplantation received the organ (82). There were more serious adverse events recorded in group 3 compared to group 2 and group 1 (Table 11) (82). None of the SAE were thought to be related to treatment (82). Patients were monitored for a year including assessment for LT, serious AEs, standard screening for hepatocellular carcinoma (6 monthly) and no malignancy was observed (82).

Table 11: Adverse events and clinical outcomes of the studied population in the trial (82)

Standard care	**GCSF only**	**GCSF and CD133+ cell infusion**
Deaths		
Variceal Bleed		Myocardial infarction
		Progressive liver disease
Assessed for liver transplantation		
Assessed but not listed due to ventricular arrhythmia	Assessed & listed due to decompensation Transplanted	Assessed & listed due to decompensation Transplanted
	Assessed & listed due to decompensation Transplanted	Assessed & listed due to decompensation Transplanted
	Assessed & listed due to poor synthetic function Listed but removed due to improvement	Assessed & listed due to decompensation Not transplanted
	Assessed & listed due to decompensation, Transplanted	
Serious adverse events		
Admission with hypoglycaemia– resolved with no sequelae	Admitted with oesophageal variceal bleed - resolved with no sequelae	Admitted with diarrhoea and pulmonary sepsis
Admitted with hepatic decompensation - died	Admitted with urinary retention and ascites - resolved with no sequelae	Admitted with sepsis and encephalopathy- resolved with no sequelae
	Admitted with hepatic decompensation and ascites - resolved with no sequelae	Admitted with acute kidney injury- resolved with no sequelae
		Admitted with cardiac failure - resolved with no sequelae
		Admitted with ascites and encephalopathy- resolved with no sequelae
		Admitted with ascites and encephalopathy - resolved with no sequelae
		Admitted with abdominal sepsis - resolved with no sequelae

Standard care	GCSF only	GCSF and CD133+ cell infusion
		Admitted with peripheral oedema- resolved with no sequelae
		Admitted with sepsis and ascites - resolved with no sequelae
		Admitted with encephalopathy - resolved with no sequelae

6.3: Discussion

This study did not show improvement in MELD in either GCSF or GCSF with stem cell infusion group at day 30 or day 90. For our study, MELD was chosen as a primary outcome since it was a validated objective scoring system used in clinical practice and it independently predicts risk of decompensation in patients with compensated cirrhosis (82). It is highly accurate in predicting mortality at 1 week, 3 months and 1 year (82). The change in MELD also predict the development of liver complications such as ascites and variceal bleeding (82). None of the secondary outcomes were shown significant across 3 groups. These findings contrast with studies that had been published before. Khan and colleagues studied the effect of CD34+ cells in 4 patients with liver insufficiency and they showed that all patients had improvement in biochemical markers (ALT, albumin, bilirubin and ALT) one month after the cell infusion (70). However, our study did not show any improvement in biochemical markers across all 3 groups. Andreone and colleagues (85) performed phase 1 trial to evaluate the feasibility and safety of CD133+ stem cells in patients with end stage liver disease and the study showed that among the patients had significant improvement in MELD at 2 months.

Prajapati and colleagues (86) studied the efficacy of GCSF in patients with decompensated cirrhosis and they used 300ug BD for 5 days and the dose was lower than what we used in our

study (e.g.: 15 ug/kg/day – 60kg will received 900ug). Their study showed that GCSF therapy improved overall survival and clinical outcomes at 6 months but in our cohort, who received GCSF therapy, there were no difference in clinical outcomes when compared to control group.

Strengths of the study

Despite not positive impact on the outcomes, the study had several strengths since it had been the largest and most rigorous randomised controlled trial on haematopoietic stem cell therapy for patients with liver cirrhosis (82). This study also had been powered prior to trial initiation to detect a clinically meaningful difference. Another strength in this study was inclusion of GCSF group and in direct comparison with group that had stem cell infusion. There were no serious complications related to either GCSF or GCSF- cell infusion was recorded during the study follow up. We recruited patients with compensated liver cirrhosis hoping that they had a greater potential to regenerate which then help with regression of liver fibrosis. In this study, we chose purified, autologous haematopoietic stem cells (CD133+) because it represented a more enriched subpopulation which can potentially have a greater impact on regression of liver fibrosis. In this study, stem cells were given via a peripheral vein and this mode of cells delivery had better safety profile compared to more advanced methods of delivery such as portal vein or hepatic artery.

Limitations of the study

There are few limitations with our study and one of them is not to have histological endpoint as part of the outcome. Performing liver biopsies pre- and post-therapy in the space of 12 months can put significant risk to the patients since most of the liver patients had coagulopathy. Even with liver biopsies, the interpretation can be misleading in cirrhotic patients due to presence of regenerative nodules and may not reflect true degree of disease severity. Liver

biopsies were also not without significant risk such as bleeding, infection, perforation and even death. Although MELD score is user friendly and widely accepted in hepatology community in risk stratifying patients with liver cirrhosis, it may not have been a good marker to detect a change in degree of liver fibrosis. The reason being is that to actually see a significant change in liver fibrosis, we will need significant reduction in MELD score. As per Figure 6, patient with MELD of 30 have about 60% survival compared to 85% survival in patient with MELD of 20 which means the patients need to have a 10-point reduction. Change in MELD of 1 or 2 points did not reflect the improvement of liver fibrosis.

In clinical settings, we use hepatic venous pressure gradient (HVPG) measurement to detect liver stiffness. In a study by Ripoll (87) showed that each 1mmHg rise in HVPG was associated with 3% mortality risk in patients. HVPG may be a better representative of liver fibrosis although most of the stem cell studies that had been conducted never included HVPG as an outcome of interest. Although there is a risk with the procedure of getting HVPG, the risk is much less than having a liver biopsy. For future studies of stem cells therapy, we need a better biomarkers or more precise imagining to detect a meaningful outcome.

The other limitation was that we did not track the cells *in vivo* to understand the homing of cells to the liver. The reasons for not doing so was due to technical and regulatory barriers as well as uncertainty in the viability and efficacy of the stems post labelling. Another limitation was the delivery of the cell therapy. We used peripheral route instead of the central route due to its ease in delivering the cells especially when we planned to give the cells in 3 separate infusions. Although the central route potentially can be more effective than peripheral route, it is invasive with serious risk as in thrombosis and bleeding (82).

In our study, we recruited patients with various causes of liver cirrhosis and felt that these groups of patients might have reflected the overall population of liver diseases in daily clinical practice. The assumption was that the final mechanism towards liver fibrosis may be the same irrespective of the causes of liver diseases. However, each cause of liver cirrhosis may have different pathway into pathogenesis of liver fibrosis. The other reason that haematopoietic stem cells were not effective in our cohort was likely to be related to the underlying diseased liver fibrosis. There was a possibility that the disease liver itself did not have the ability to repopulate the cell populations which were also failed despite giving external stimuli such as GCSF injection.

In summary, our study showed that GCSF alone or GCSF with stem cell infusions did not showed any clinical benefit in patients with liver cirrhosis. This result varies from currently published studies although most studies had less patients with less inferior study design (either phase I or II). Even with GCSF, we used the dose higher than most studies in literature, but we did not see any improvement in clinical outcomes. In mice model of liver injury, we showed that repeated HSC infusions improved liver fibrosis but in human study, the study did not show an improvement in liver fibrosis which was most likely due to underlying complexity in pathogenesis of liver fibrosis/cirrhosis in human.

If I have another opportunity to conduct this clinical trial again, I will consider recruiting dedicated cohort of patients with liver cirrhosis such as alcohol related liver cirrhosis or non-alcoholic fatty liver disease. There is a possibility that crosslinking pattern of collage in the liver may be different between patients with different causes of liver diseases as well as different stages of liver cirrhosis. By choosing particular group of liver disease will give us an opportunity to understand the underlying mechanism of liver fibrosis and how the stem cells

can have an effect on liver fibrosis. We should also attempt the basic science research alongside the clinical trial to understand the mechanistic action of stem cell in liver regeneration. I will also choose patients with lower and tighter range of MELD score for example MELD between 10 and 12 instead of our MELD score of 11.5 to 15.5. I felt that our range of MELD in this clinical trial was broad and as a result, we had a heterogenous populations with different stages of liver cirrhosis and stem cells have not have effect on patients with more advance fibrosis. By narrowing the range of MELD, patients recruited may have similar degree of fibrosis stage. We also other means to better define the underlying degree of liver damage such as having a liver biopsy prior to enrolling into the clinical trial or having a measurement of HVPG or even using a more robust biomarkers or imaging technique. Although 12 months is a good duration of follow up, we will need longer duration of follow up to actually see the benefit of stem cells on liver fibrosis although it may not be feasible due to timing with recruiting patients as well as costs of running the trials.

This is the first ever randomised controlled clinical trial and we did not see benefit in improvement of liver fibrosis. If we ever need to perform another clinical trial, we need to better define a population of liver cirrhosis and stem cells of interest. The trial should look into ways of tracking the stem cells which will give us better understanding of the viability and efficiency of the stem cells. Most studies have these clinical outcomes such as patient's survival, MELD score and liver elasticity although those outcomes did not detect any therapeutic effect of cell therapies. We should look into other outcomes such as changes of HVPG or even spleen elastography which are more representative of liver fibrosis.

Due to inconsistent findings of clinical outcomes with stem cell therapies, a systematic review and meta-analysis had been conducted to critique the current published literatures. In 2017,

there had been nine systematic reviews looking at stem cells in liver diseases mostly in "mesenchymal stem cells". The studies searched for those systematic reviews were performed only up to 2015 and hence, a new study is needed including the clinical trial that I was involved in. Our trial was a randomised controlled trial which involved largest number patients and hence, by conduction a systematic review including our data, it will give us better understandings on the efficacy of haematopoietic stem cells therapy in patients with liver cirrhosis. The detailed methodology and the results of the systematic review had been documented in Chapter 7 and 8 of this thesis.

Overall, we did not an improvement of liver fibrosis in our cohort of patients, we do not feel that patients with decompensated cirrhosis will achieve benefit from stem cells therapy. Stem cells therapy has a degree of short term benefit on the resolution liver fibrosis although we may need to perform studies with more robust, well designed clinical trials with alternative interested outcomes. It is also really important to track the cells in human trials to understand the nature of stem cells and hopefully will give us a better way to deliver the stem cells to improve the disease process.

7. SYSTEMATIC REVIEW AND META-ANALYSIS

7.1 Introduction

The systematic review protocol paper had been published recently (I was the author) and the session below were from the published manuscript (88).

The systematic review project has been registered with PROSPERO (International prospective register of systematic reviews) and the trial registration number is CRD42016016104.

7.1 (i): Role within the clinical trial

My responsibilities include

1) Writing the systematic review protocol

2) Registration of systematic review on the PROSPERO website

3) Performing thorough searches using MESH and free search terms on the databases mentioned in the protocol

4) Screening of titles and abstracts using the pre-identified study form

5) Screening of full articles

6) Performed data extraction, quality assessment of the included studies

7) Performed data analysis using “Revman” software

8) Final writing up of the results

7.2: Methodology

7.2 (i): Rationale behind systematic review

Up to September 2017, there had been nine systematic reviews and meta-analysis of stem cell therapies in patients with both chronic liver disease and ACLF (Table 12) (44, 64, 89-95). Across all the reviews, each study had their own strengths and limitations. Most studies mentioned both targeted population (either CLD or ACLF) and cell therapies (MSC or GCSF) that were of interest but one study in particular (95) was unclear on the target population but only mentioned of as liver failure patients which can be either acute, acute-on-chronic or chronic liver failure. Most studies combined different study designs when analysing data that can obscure the interested outcomes. Two studies (90, 93) used all types of stem cells and the outcomes can vary due to heterogeneity seen with different stem cells. Other issues noted from these current published studies were less elaborative search strategies, analysis of difference cell therapies together and lack of clarity on limitations of (or reason for not) conducting meta-analysis or subgroup analysis (88). The current published studies had been critically appraised using PRISMA 2009 checklist prior to starting this current review (appendix 5).

In current published systematic reviews, the searches of the studies were up to 2015. Since 2015, there had been few more original studies (57, 82, 86, 96, 97) and we felt that a new systematic review is needed. In this review, the searches were updated to September 2017. In addition, we hoped to address the limitations that was noted before. We aim to cluster studies according to the study designs, the populations and the stem cells of interest when performing meta-analysis.

Table 12: Current published systematic reviews and meta-analysis of stem cell therapies in liver disease – PubMed search

Author name	Place	Types of studies	Types of patients	Types of interventions	Type of outcomes measures	Number of studies	Number of patients
Liu Z 2016 **(90)**	China	Any types of studies	CLD	Any types of stem cells (HSC, bone marrow-MSC, Fetal liver-derived stem cell)	1) Short term and long-term efficacy 2) Assessment of liver function indices 3) Clinical outcomes during the follow-up	20 studies included in qualitative and quantitative analysis	507
Yang Q 2016 **(95)**	China	Randomised controlled trials, prospective cohort studies	Liver failure	Granulocyte-colony-stimulating factor	Clinical benefits of GCSF in patients with liver failure	5 studies included	206
Chavez-Tapia 2015 **(64)**	Mexico	Randomised controlled trials	ACLF	GCSF alone or in combination	**Primary outcomes** 1) Overall mortality 2) Mortality due to organ failure 3) Adverse events **Secondary outcomes** 1) Complications 2) Hospitalisation length 3) Liver transplantation 4) Changes in severity indices (CTP, MELD, SOFA) 5) Mortality secondary to gastrointestinal bleeding 6) Changes in peripheral leucocytes and/or neutrophils counts 7) Changes in peripheral and hepatic CD34+ count	2 studies included in qualitative and quantitative analysis	102
Kim G 2015 **(89)**	Korea	1) Before-after study	CLD	MSC	1) Safety and efficacy	14 studies included in	448

		2) Controlled trials 3) Case series			2) Changes in liver function tests and associated prognostic markers of liver disease such as the MELD score or CPS	qualitative and quantitative analysis	
Ma X-R 2015 **(91)**	China	Any types of studies but excluded case report, editorial, letter to editors, case series with only experimental arm	CLD	Autologous bone marrow MSC	Effectiveness of transplantation	7 studies included in quantitative analysis	489
Qi X 2015 **(93)**	China	Any type of studies	CLD	Any types of stem cells	1) Changes in liver function before and after therapy 2) Difference in liver function between stem cell therapy and conventional treatment 3) Difference in incidence of procedure related complications between stem cell therapy and conventional treatment 4) Difference in incidence of hepatocellular carcinoma between stem cell therapy and conventional treatment 5) Difference in incidence of death between stem cell therapy and conventional treatment	31 studies included	1247
Wang K 2015 **(94)**	United States	Randomised controlled trials, controlled trials without randomisation, cohort or case-control analytic studies	CLD	Autologous bone marrow stem cell	1) Analysis on common symptoms and sings 2) Analysis on levels of ALT, ALB, TBIL, PT/PTA/INR, MELD score, Child-Pugh score 3) Analysis on morbidity and mortality.	7 studies included	534

Moore J 2014 **(44)**	United Kingdom	Any type of studies	CLD	Autologous stem cell therapy (any)	**Primary outcomes** 1) Safety and feasibility of the therapy **Secondary outcomes** 1) Prognostic liver scores 2) Survival 3) Changes in liver function tests	33 studies included	598
Pan X-N 2014 **(92)**	China	Any type of studies	CLD	BM-MSC	Safety and efficacy of therapy	5 studies included	80

7.2 (ii): Aims and Objectives

The aim of this systematic review was to evaluate the clinical effectiveness of cell therapies in patients with CLD or ACLF (88).

Standard systematic review methodology aimed at minimising bias will be employed (88). Where data allowed, the intention was to consider, through sub-group analysis; the evidence of effect in different underlying disease populations (as in viral hepatitis or alcohol related liver diseases), the effect of each type of stem cells (HSC, MSC, unsorted stem cell or GCSF therapy alone), the source of the stem cells (autologous and allogeneic stem cells) and the route of administration of the cells such as peripheral or central route (88).

Determination of comparative effectiveness between cell types and routes of administration would be considered if there were direct comparisons in studies included in the reviews. In addition, the potential for indirect adjusted comparisons would be assessed (88).

7.2 (iii): Type of studies

All studies were included irrespective of their study designs (88). Controlled trials (either randomised or non-randomised) were included (88). All observational evidence was obtained, whether controlled or uncontrolled, in order to gain an overview of existing observational evidence (88). Uncontrolled observational studies were used where primary outcomes are not reported in the controlled studies, or, where uncontrolled studies had longer follow-up for these outcomes (88). Existing systematic reviews will be selected to identify any primary studies that were not identified by the searches (88).

7.2 (iv): Types of participants

Inclusion criteria: Adult patients (≥18 years old) with 1) CLD and 2) ACLF

Exclusion criteria: 1) Patient with acute liver failure (no evidence of liver cirrhosis) 2) Patient with cancer (unable to ascertain the effect of stem cells on tumour pathogenesis) (88).Studies on mixed populations of those defined under inclusion and exclusion criteria will only be included where the data for CLD or ACLF is presented separately (88).

7.2 (v): Types of interventions

1) Treatment with HSC of any dose, duration and mode of delivery with standard medical therapy with or without GCSF therapy to mobilise stem cells for collection/harvesting

2) Treatment with MSC from any source, any dose, duration and mode of delivery with standard medical therapy.

3) Treatment with unsorted stem cells (BMSC and/or BM-MNC) of any dose, duration and mode of delivery with standard medical therapy with or without GCSF.

4) Treatment with GCSF therapy only (without stem cell infusion) of any dose and duration with standard medical therapy.

7.2 (vi): Comparator

Comparator include standard care, placebo, other stem cells as a comparator or using different route as comparator.

7.2 (vii): Types of outcome measures

There was no restriction placed on the type of clinical outcomes or the duration of follow up for study selection to capture the additional evidence of adverse events occurring close to the time of stem cell infusion or GCSF injection (88). Primary and secondary outcomes interested were as followed (88):

Primary outcomes:

1) Overall patient survival

2) Liver transplant free survival

3) MELD

4) Quality of life

5) Adverse events specific to the intervention

Secondary outcomes

1) Liver function tests

2) Child Pugh Score

3) Events of liver decompensation as defined and reported by the study authors.

7.3: Search strategy

Cell therapy in liver diseases was first investigated in clinical phase studies in early 2000s and hence, the searches will be run from year 1990 onwards. The following databases will be searched to capture both published and unpublished studies.

1. Bibliographic databases - MEDLINE, MEDLINE in Process and EMBASE, Cochrane Library CENTRAL database for published studies and additionally for systematic reviews the Cochrane Library Database of Systematic Reviews, Health Technology Assessment database and The Database of Abstracts of Reviews of Effects

2. The International Standard Randomised Controlled Trial Number (ISRCTN) database, United Kingdom Clinical Research Network (UKCRN), WHO International Clinical Trials Registry Platform (WHO ICTRP) Portal and ClinicalTrials.gov for ongoing studies.

3. Hand searching of conference reports from the following databases between January 2012 and September 2017: The European Association for the study of Liver Disease, American association for the study of liver disease, Asian-Pacific association for liver disease, British association for the study of liver disease and British society of gastroenterology.

4. Screening of citation lists of included studies and relevant systematic reviews

Database were searched with a combination of MeSH terms and text words for the population and the interventions as appropriate (88). No language restrictions were applied to the searches (88). Study design filters was not used (88). The detail of the search strategy can be found in appendix 3. Search results are entered into Endnote version X7.02, Thomson Reuters to facilitate with record keeping, duplicate removal and study selection (88). Full-text articles were retrieved, and the data were entered into Microsoft Excel spreadsheet for data extraction and risk of bias assessment (88).

7.4: Data collection and analysis

7.4 (i): Selection of studies

To remove irrelevant articles, I screened all the titles and abstracts and to ensure consistency, another reviewer checked a proportion (minimum 50% of all articles) independently (88). This way of screening articles was a limitation of the study due to this project being unfunded. Hard copies of relevant articles were acquired and assessed independently against the inclusion criteria by two reviewers (88). Discrepancies between reviewers were resolved by discussion and by referring to a third reviewer if required (88). Full text selection was performed by myself and another reviewer independently (88). Where necessary, translation (full/part) of non-English language articles was planned to undertake to facilitate this process and subsequent reviewing (88). Due to time constraint, translations were not possible, and this limitation was

reported in discussion (88). Study selection process is illustrated using PRISMA flow diagram (Figure22).

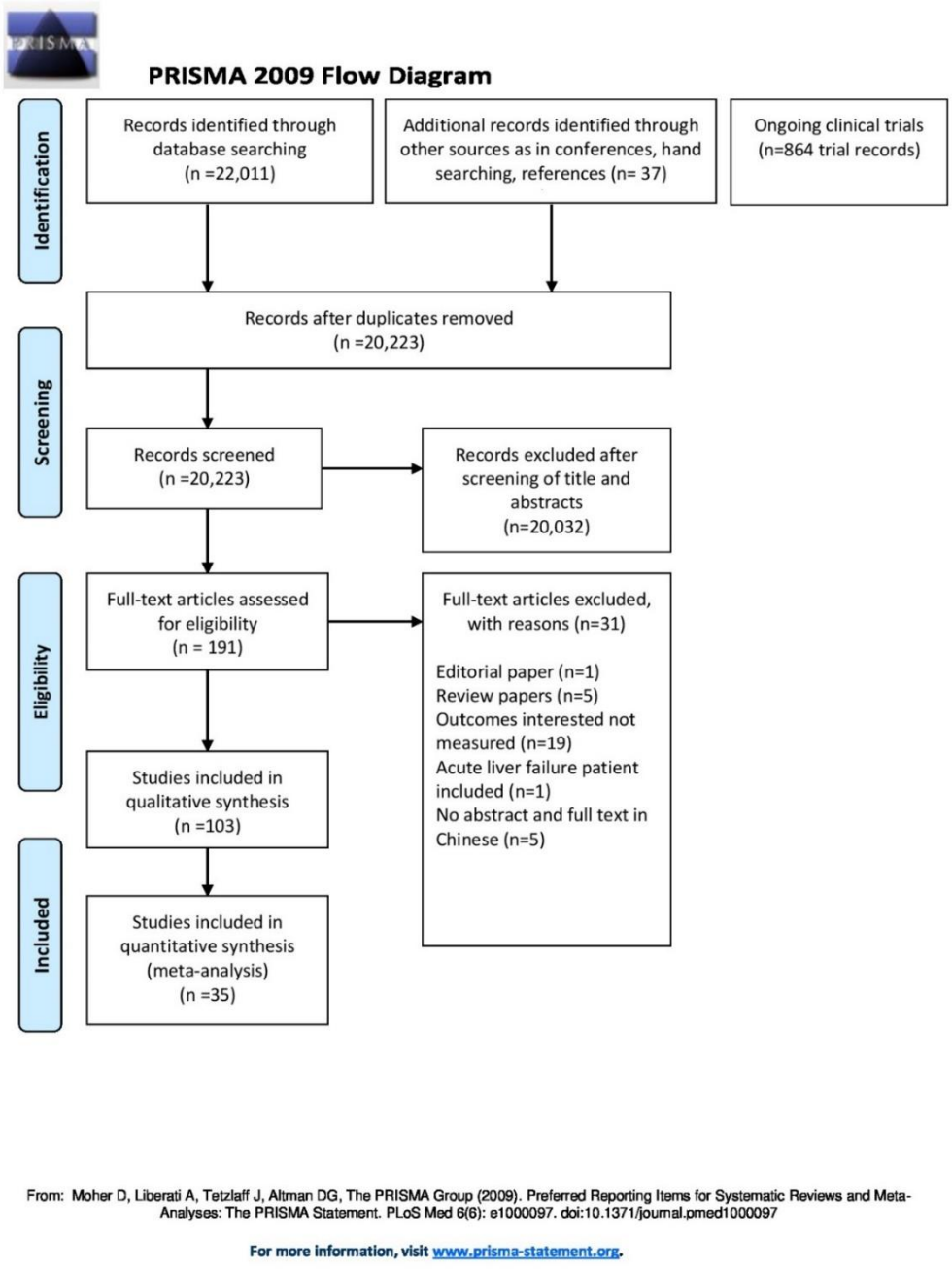


Figure 22: Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow chart of the systematic review

7.4 (ii): Data extraction and management

I extracted the data from the included studies was performed using a standardised data extraction form (appendix 6) and checked independently by a second reviewer for all the studies (88). Disagreements will be resolved through discussion or referral to a third reviewer (88). For each study, the data required on (but not limited to) the following will be sought:

1. Study characteristics: authors, geographical origin, year of publication, study design (to include bias/confounding minimisation), years and duration of recruitment, number of arms, sample size, duration of follow up.

2. Participant characteristics: enrolment criteria, age, sex, number of participants, diagnosis and disease manifestations.

3. Intervention and comparator details: sample size for each treatment arm, dose and type of interventions/comparator (HSC, MSC, unsorted stem cell or GCSF therapy alone), type of treatment received before or during therapy and the duration of treatment.

4. Results: outcomes measured, time points, method of assessment, completeness of follow-up, statistical methods employed, findings, effect sizes and associated uncertainty.

There was likely to be a limited number of RCT on this topic and therefore as mentioned previously all observational evidence was obtained, whether controlled or uncontrolled, to gain an overview of existing observational evidence (88). However, the uncontrolled observational studies were only analysed where primary outcomes did not report in the controlled studies, or, where uncontrolled studies had longer follow-up for these outcomes (88). To facilitate this decision making and to be efficient, data from controlled studies was extracted first and data from uncontrolled studies would be extracted when needed (88).

7.5: Assessment of risk of bias of included studies

Data was extracted to allow quality assessment of the included studies (88). Study quality was assessed using tools specific to a given study design. The risk of bias tool from the Cochrane Handbook was used for RCTs [91]. For non-RCT studies, the domains in the risk of bias tool for RCTs was used as a minimum assessment (accepting that the studies are not randomised) (88). Regarding RCT and non-RCT, the risk of bias assessment was documented as "high risk", "low risk" or "unclear risk".

For controlled observational studies, the guidelines outlined in Chapter 13 of the Cochrane Handbook was used (88) and in this manuscript, the selected studies were assessed using Newcastle- Ottawa Scale (98).

7.6: Analysis

Initially, the plan was to perform a narrative synthesis of evidence. The analysis was planned based on the type of cells (HSC, MSC, BMSC or GCSF), by population (CLD or ACLF) and by outcomes interested. Subgroup analysis was considered to investigate based on the source of stem cells (allogeneic and autologous) or the route of administration (central or peripheral infusion) if feasible to extract the data. Analysis methods was guided by the considerations outlined in the Cochrane Handbook (99). Meta-analytic methods will be employed where appropriate, to combine data for each population, comparison, outcome combination across the same, or very similar time points.

Data integration and analysis were performed using review manager (RevMan Cochrane Collaboration) version 5.3 software. Subgroup analysis will be considered where appropriate based on the type of stem cells, the source of cells and the route of administration. Meta-analysis was performed where appropriate, to combine data for each population, comparison, outcomes across the same or similar time points. Results for dichotomous data were expressed as odds ratio (OR) with 95% confidence interval (CI). OR of each study were combined to give

a pooled OR. Mean and standard deviations were extracted from continuous data and measured as mean difference (MD) with 95% CI. Standard mean difference (SMD) of each study was combined to give a pooled SMD. The significance of pooled estimates was assessed with Z tests in in which p-value of <0.05 was considered statistically significant. The heterogeneity between studies were assessed with Chis square based Q test and I^2 statistic. Chi-square tests ($p<0.1$) and $I^2>50\%$ was considered to represent significant statistical heterogeneity. If $P\geq0.1$ and $I^2 \leq 50\%$, fixed effects models was used but otherwise, random effects model was used.

7.7: Reporting of data

The review and its findings will be reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis guidelines (PRISMA) (100).

8: RESULTS OF SYSTEMATIC REVIEW AND META-ANALYSIS

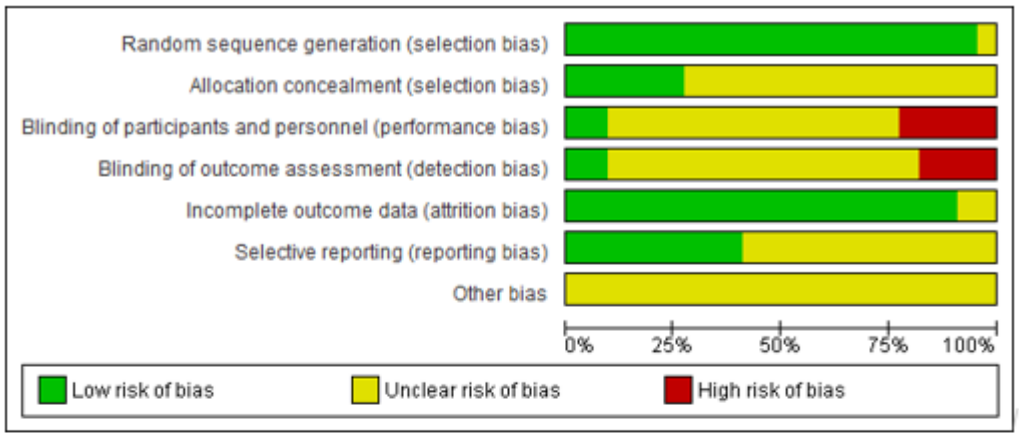
8.1: Search outcomes

Using the search criteria listed in appendix 3, searches were done up to week 3 of September 2017. A total of 22,011 records were identified through database searching with 1,581 records from Medline, 201 records from Medline in Process and 20,229 records from Embase. An additional 37 records were identified through other sources such as conferences, hand searching and references. After removing duplicates (n=1,825), 20,223 records remained. After screening titles and abstracts of those records, a further 20,032 were excluded. Finally, 191 full text articles were assessed for eligibility using the pre-set checklist (appendix 6). Among them, a further 31 were excluded and the reasons for exclusion were shown in Figure 22 and Appendix 4. Finally, 160 articles with 103 studies were included in qualitative studies and 35 studies were eligible for quantitative analysis (Figure 22).

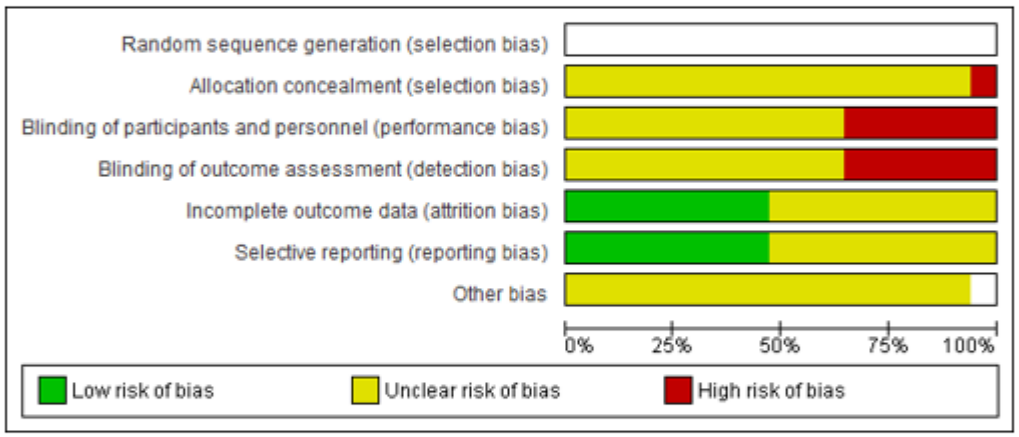
8.2: Quality assessment of the included studies

The quality assessment of RCT and non-RCT studies were performed using Cochrane risk of bias assessment tool. For controlled studies, Newcastle-Ottawa scale was used for methodological assessment. The risk of bias for RCT and non-RCT studies for CLD and ACLF were presented as RevMan's risk of bias graph (Figure 23 A-C). For controlled studies, the risk of bias assessment was documented in Table 13.

A: Randomised controlled trials (Chronic liver failure)



B: Non-randomised controlled trials (Chronic liver failure)



C: Randomised controlled trials (Acute on chronic liver failure)

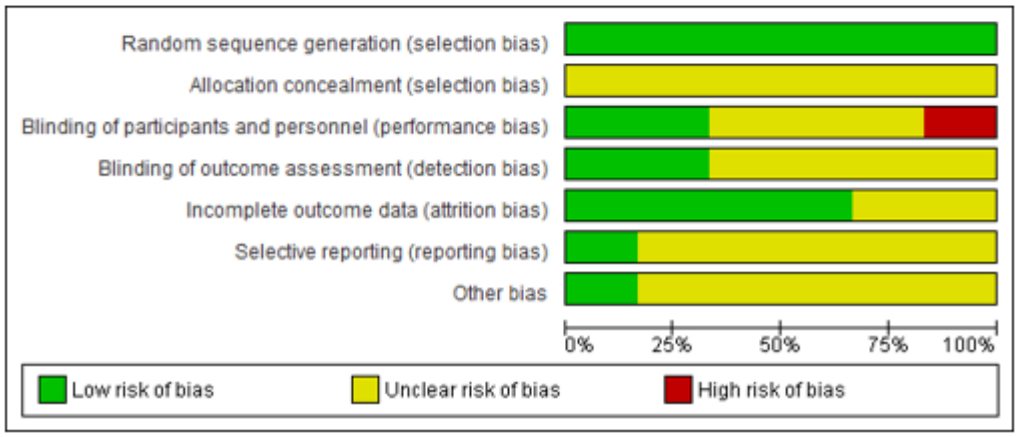


Figure 23: Risk of bias assessment graphs using Cochrane risk of bias assessments tool: A: Randomised controlled trial for Chronic liver failure, B: Non-randomised controlled trial for Chronic liver failure, C: Randomised controlled trial for Acute on chronic liver failure.

Table 13: Risk of assessments for controlled studies (both chronic liver failure and acute-on-chronic liver failure) using Newcastle-Ottwa Scale:

A summary table (* equals 1 score)

Chronic liver failure									
		Selection				**Comparability**	**Outcome**		
		Representativeness of the exposed cohort	**Selection of the non-exposed cohort**	**Ascertainment of exposure**	**Outcome of interest not present at start of study**	**Comparability of cohorts on basis of design or analysis**	**Assessment of outcome**	**Follow-up long enough for outcomes to occur**	**Adequacy of follow-up**
Peng L 2011 (101)	Paper	*	*	*	*	**	*	*	*
Peng L 2011 (102)	Conference	*	*	not mentioned	*	*	*	*	*
Shi M 2010 (103)	Conference	*	*	not mentioned	*	*	*	*	*
Shi M 2017 (104)	Conference	*	*	*	*	*	*	*	*
Shi M 2016(105)	Conference	*	*	*	*	*	*	*	*
Salama H 2012(106)	Paper	*	*	*	*	**	*	*	*
Zhu J 2013(107)	Conference	*	*	*	*	*	*	*	*
Iwamoto 2012(108)	Paper	*	*	*	*	*	*	*	*

Chen J 2015 #(109)	Paper	Unable to comment	Unable to comment	Unable to comment	Unable to comment	Unable to comment	Unable to comment	Unable to comment	Unable to comment
Wei Z 2016(110)	Conference	*	*	*	*	*	*	*	*
Gaia S 2013(111)	Paper	*	*	*	*	**	*	*	*
Gaia S 2011(112)	Conference	*	*	not mentioned	*	*	*	*	*
Gaia 2006 (113)	Paper	*	*	*	*	*	*	*	*
Lin S 2017(114)	Conference	*	*	*	*	*	*	*	*
Acute on chronic liver failure									
Shi M 2012(115)	Paper	*	*	*	*	**	*	*	*
Weng WZ 2013(116)	Conference	*	*	*	*	*	*	*	*

Article in Chinese language

8.3: Results of outcomes

8.3 (i): Chronic liver disease (CLD)

Study characteristics

There were 22 randomised controlled studies (56, 82, 86, 96, 97, 117-133), 17 non-randomised controlled studies (61, 134-149), 14 controlled studies (101, 104, 106-111, 113, 114, 150-153) and 50 studies with no controls (53, 60, 85, 154-200). The studies were conducted in these countries: Austria (n=2), Brazil (n=1), China (n=47), Egypt (n=10), Greece (n=1), India (n=8), Iran (n=4), Italy (n=6), Japan (n=10), Korea (n=1), Malaysia (n=1), Republic of Belarus (n=1), South Korea (n=2), Spain (n=1), Sweden (n=1), Switzerland (n=2), Turkey (n=1) and United Kingdom (n=4). Among those studies, 84 were full paper publications and 19 were conference abstracts. The types of stem cells were as followed: BMSC (n=28), BMSC with GCSF (n=4), GCSF (n=7), both HSC and MSC infusion (n=1), HSC without GCSF (n=2), HSC with GCSF (n=21), MSC without GCSF (n=27), MSC with GCSF (n=1), other cell types (n=6), SCT (n=1), Peripheral blood stem cell (PBSC) without GCSF (n=1) and PBSC with GCSF (n=4). Cells were extracted from adipose tissue (n=1), bone marrow (n=51), fetal liver cells (n=2), GCSF with no cell collection (n=7), GCSF with peripheral blood collection (n=24), umbilical cord (n=12) and no data available (n=6). The cells were delivered by portal vein followed by peripheral infusion (n=1), hepatic artery (n=44), hepatic artery or portal vein (n=8), hepatic or splenic artery (n=1), intrahepatic (n=3) or intra-splenic (n=2), intra-parenchymal (n=1), peripheral vein (n=20), portal vein (n=6), no cells delivery involved (n=7) and no data available (n=12).

The details of each included study were shown in Table 14.

Table 14: Summary of the included studies for chronic liver disease

Author name	Country	Types of publication	Causes of liver diseases	Type of stem cells	Autologous / Allogenic	Type of control therapy	Extraction of cells	Ways of cell delivery	No of cells	No of pts (Stem cell, n=)	No of pts (control, n=)	Follow up (months)
Chronic liver disease												
Randomised controlled trial (RCT)												
Mohamadnejad M 2013 (56)	Iran	Paper	Mixed aetiologies	BM-MSC	Autologous	SMT	Bone marrow	Peripheral vein	median 195 million cells	15	12	12
Suk K 2016 (128)	South Korea	Paper	Alcohol	BM-MSC	Autologous	SMT	Bone marrow	Hepatic artery (HA)	$5x10^7$ cells	18 (1xinjection MSC), 19 (2xinjection MSC)	18	12
Xu L 2014 (129)	China	Paper	Hepatitis B	BM-MSC	Autologous	SMT	Bone marrow	Hepatic artery	Average cell count: 8.45+/-3.28x10*8 per patient	27 (20 analysed)	29 (19 analysed)	12
Amer M 2011 (117)	Egypt	Paper	Hepatitis C	BM-MSC	Autologous	SMT	Bone marrow	Intra-hepatic and intra-splenic	5 million cells	20	20	6
Zeng Z * 2015(132)	China	Paper	Decompensated, not specified	UC-MSC	Allogenic	SMT	Umbilical cord	not mentioned	not mentioned	13	19	12

Author name	Country	Types of publication	Causes of liver diseases	Type of stem cells	Autologous / Allogenic	Type of control therapy	Extraction of cells	Ways of cell delivery	No of cells	No of pts (Stem cell, n=)	No of pts (control, n=)	Follow up (months)
Zhang Z 2012 (133)	China	Paper	Hepatitis B	UC-MSC	Allogenic	Saline injection as control	Umbilical cord	Peripheral vein	0.5x10*6 cells/kg	30	15	12
Zekri A 2015 (131)	Egypt	Paper	Hepatitis C	GCSF and CD133/CD34 and MSC	Autologous	SMT	Bone marrow	Portal vein followed by peripheral vein	1x10*6 cells/kg	30 (1 injection), 30 (2 injections)	30	12
Salama H 2014 (125)	Egypt	Paper	Hepatitis C	GCSF and MSC	Autologous	SMT	Bone marrow	Peripheral vein	1x10*6 cells/kg	20	20	6
Newsome 2017 (82)	United Kingdom	Paper	Mixed aetiologies	GCSF or GCSF and CD133+ cells	Autologous	SMT	Peripheral blood collection	Peripheral vein	0.2x10*6 cells/kg	26 (GCSF), 28 (GCSF+CD133)	27	12
Raju B 2014 (124)	India	Conference	Decompensated, not specified	GCSF and CD34+ cells	Autologous	SMT + IV albumin for 3 days	Peripheral blood collection	Hepatic artery	not mentioned	20	20	12
Salama H 2010 (126)	Egypt	Paper	Hepatitis C	GCSF and CD133/CD34+ cells	Autologous	SMT	Bone marrow	Portal vein (PV)	0.5x10*8 cells as a single bolus	90	50	6
Nikeghbalian, S 2011 (123)	Iran	Paper	Mixed aetiologies	CD133 group in comparison to MNC group	Autologous	CD133+ vs MNC cells group	Bone marrow	Portal vein	mean MNC: 295+/-17.6ml mean CD133:	3 (CD133), 3 (MNC)	0	24

Author name	Country	Types of publication	Causes of liver diseases	Type of stem cells	Autologous / Allogenic	Type of control therapy	Extraction of cells	Ways of cell delivery	No of cells	No of pts (Stem cell, n=)	No of pts (control, n=)	Follow up (months)
									245.7+/-17.8ml			
Mohamadnejad M 2016 (96)	Iran	Paper	Mixed aetiologies	BM-CD133+ cells or BM-MNC	Autologous	SMT	Bone marrow	Portal vein	mean MNC: 125+/-21ml for 1st one, 126+/-12ml for 2nd one mean CD133: 125+/-16ml for 1st one, 120+/-14ml for 2nd one	4 in CD133, 8 in MNC	6	12
Huang M 2014 (119)	China	Paper	Hepatitis B	TIPS and BMSC	Autologous	TIPS alone as control	Bone marrow	Hepatic artery	Mean: 2.65+/-1.20x10*9 cells	5	5	12
Lyra A 2010 (122)	Austria	Paper	Mixed aetiologies	mononuclear enriched BMC	Autologous	SMT	Bone marrow	Hepatic artery	Mean: 3.78x10*8 (+/-2.69x10*8)	15	15	12
Fu N* 2010 (150)	China	Paper	Not mentioned	BMSC+/-octreotide	Autologous	SMT	not mentioned	Hepatic artery	not mentioned	14	19	1
Yu S 2017 (130)	Korea	Paper	Hepatitis C and Alcohol	GCSF and peripheral blood	Autologous	SMT	Peripheral blood collection	Portal branch puncture (right)	1.67x10*9-2x10*10/50 ml	3 (GCSF), 3 (GCSF	3	6

Author name	Country	Types of publication	Causes of liver diseases	Type of stem cells	Autologous / Allogenic	Type of control therapy	Extraction of cells	Ways of cell delivery	No of cells	No of pts (Stem cell, n=)	No of pts (control, n=)	Follow up (months)
				monocytes (PBMC)						+PBMC)		
Spahr L 2013 (127)	Switzerland	Paper	Alcohol	GCSF and BM-MNC	Autologous	SMT	Bone marrow	Hepatic artery	Mean: 0.47+/-0.15x10*8/kg	28	30	3
Liu L 2014 (121)	China	Paper	Hepatitis B	GCSF and BMSC	Autologous	SMT	Bone marrow	Hepatic artery (right)	3.2 to 1.6x10*10/ml	40	37	1
Kedariset ty C 2015 (120)	India	Paper	Mixed aetiologies	GCSF and darpopoietin or placebo	Autologous	SMT	No collection	No cell delivered externally	Not applicable	29	26	12
Prajapati R. 2017 (86)	India	Paper	Mixed aetiologies	GCSF	Autologous	SMT	No collection	No cell delivered externally	Not applicable	126	127	6
Spahr L 2008 (128)	Switzerland	Paper	Alcohol	GCSF	Autologous	SMT	No collection	No cell delivered externally	Not applicable	13	11	1
Non-Randomised controlled trials												
El-Ansary M. 2012 (139)	Egypt	Paper	Hepatitis C	BM-MSC	Autologous	SMT	Bone marrow	Peripheral vein	1 million cells/kg	15	10	6
Ouyang S* 2013 (144)	China	Paper	Hepatitis B	BM-MSC	Autologous	SMT	Bone marrow	Hepatic artery	not mentioned	33	34	6

Author name	Country	Types of publication	Causes of liver diseases	Type of stem cells	Autologous / Allogenic	Type of control therapy	Extraction of cells	Ways of cell delivery	No of cells	No of pts (Stem cell, n=)	No of pts (control, n=)	Follow up (months)
Zhang S* 2017 (147)	China	Paper	Decompensated, not specified	UC-MSC	Allogenic	SMT	umbilical cord	not mentioned	1.4-2.3x10*6 cells/kg 9amml), injection done every 15 days for 3 times	25	25	6
Wang P 2011 (146)	China	Conference	Hepatitis B	MSC (co-cultured with peripheral blood mononuclear cells)	Allogenic	SMT	not mentioned	Hepatic artery	5x10*6 cells per injection	6	6	72 hours
Nakamura T 2014 (143)	Japan	Paper	Mixed aetiologies	GCSF and CD34+ cells	Autologous	SMT	Peripheral blood collection	Hepatic artery	3 doses: 5x10*5, 1x10*6, 2x10*6 cells/kg	10	7	24
Cai T 2015 (135)	China	Paper	Hepatitis B	GCSF and CD34+ cells	Autologous	SMT	Peripheral blood collection	Hepatic artery	2-4x10*7 cells	23	28	12
Deng Q 2015 (137)	China	Paper	Hepatitis B	GCSF and CD34+ cells	Autologous	SMT	Peripheral blood collection	Hepatic artery	2 to 4 x 10*7 cells	33	35	12
Deng Q * 2015 (138)	China	Paper	Hepatitis B	GCSF and CD34+ cells	Autologous	SMT	Peripheral blood collection	Peripheral vein	not mentioned	33	35	12

Author name	Country	Types of publication	Causes of liver diseases	Type of stem cells	Autologous / Allogenic	Type of control therapy	Extraction of cells	Ways of cell delivery	No of cells	No of pts (Stem cell, n=)	No of pts (control, n=)	Follow up (months)
Huang X 2012 (141)	China	Conference	Decompensated, not specified	GCSF and CD34+ cells (PV vs HA)	Autologous	cells through HA vs cell through PV	Peripheral blood collection	Hepatic artery or portal vein	not mentioned	no data yet (total number 80)	no data yet	12
Sharma M 2015 (61)	India	Paper	Decompensated, not specified	GCSF and CD34+ cells	Autologous	SMT	Peripheral blood collection	Hepatic artery	not mentioned	23	22	3
Bai Y 2014 (134)	China	Paper	Hepatitis B	BM-MNC	Autologous	SMT	Bone marrow	Hepatic artery	10ml of cell suspension/ hour	32	15	24
Zhao D 2015 (149)	China	Conference	Decompensated, not specified	BMSC	Autologous	SMT	Bone marrow	Hepatic artery	not mentioned	22	22	24
Saito T 2011 (145)	Japan	Paper	Alcohol	BMSC	Autologous	SMT	Bone marrow	Peripheral vein	Mean: 8.0+/- 7.3x10*9 cells	5	5	12
Zhang ZQ* 2012 (148)	China	Paper	Decompensated, not specified	BMSC	Autologous	SMT	Bone marrow	not mentioned	not mentioned	27	31	12
Cao H 2017 (136)	China	Conference	Hepatitis B	BMSC	Autologous	SMT	Bone marrow	Hepatic artery or portal vein	not mentioned	15	30	12, followed by 60
Han Y 2008 (140)	China	Paper	Hepatitis B	GCSF and peripheral blood monocytes	Autologous	GCSF as control	Peripheral blood collection	Hepatic artery	10*7-10*8 cells/kg	20	20	6

Author name	Country	Types of publication	Causes of liver diseases	Type of stem cells	Autologous / Allogenic	Type of control therapy	Extraction of cells	Ways of cell delivery	No of cells	No of pts (Stem cell, n=)	No of pts (control, n=)	Follow up (months)
Liao X 2013 (142)	China	Paper	Hepatitis C	BM derived liver stem cells	Autologous	normal saline infusion with splenectomy/peri-oesophago-gastric devascularization	Bone marrow	Hepatic artery	not mentioned	6	6	3
Controlled studies												
Peng L 2011 (101, 102)	China	Paper	Hepatitis B	BM-MSC	Autologous	SMT	Bone marrow	Hepatic artery	3.4 to 3.8x10*8 cells	53	105	12
Shi M 2017 (104)	China	Conference	Hepatitis B	UC-MSC	Allogenic	Saline as control	umbilical cord	not mentioned	0.5-1.0x10*6/kg, 1-2 times with 4-week interval	122	120	85
Chen J* 2015 (109)	China	Paper	Decompensated, not specified	UC-MSC	Allogenic	SMT	umbilical cord	Peripheral vein	2x10*7 cells (4 x with interval of 5 to 7 days)	Not mentioned	Not mentioned	3
Salama H 2012 (106)	Egypt	Paper	Hepatitis B	GCSF and CD34+ cells	Autologous	SMT	Peripheral blood collection	Hepatic artery or portal vein	1 billion expanded cells	50	50	12

Author name	Country	Types of publication	Causes of liver diseases	Type of stem cells	Autologous / Allogenic	Type of control therapy	Extraction of cells	Ways of cell delivery	No of cells	No of pts (Stem cell, n=)	No of pts (control, n=)	Follow up (months)
Huang X 2014 (151)	China	Paper	Mixed aetiologies	GCSF and CD34+ cells (PV vs HA)	Autologous	cells through hepatic artery vs cell through portal vein	Peripheral blood collection	Hepatic artery or portal vein	range was 1.13x10*6-2.45x10*6 cells/kg	36 (PV), 44 (HA)	0	12
Zhu J 2013 (107)	China	Conference	Hepatitis B	Peripheral blood MNC	Autologous	SMT	Peripheral blood collection	not mentioned	not mentioned	251	275	72
Iwamoto T 2012 (108)	Japan	Paper	Decompensated, not specified	splenectomy+ ABMI vs ABMI	Autologous	splenectomy without cell therapy as control	Bone marrow	not mentioned	not mentioned	9	3	6
Zheng L 2013 (152)	China	Paper	Hepatitis B	GCSF and BMSC	Autologous	healthy controls	Peripheral blood collection	Hepatic artery	10*7-10*8/kg in 60ml	42	0	12
Wei Z 2016 (110)	China	Conference	Decompensated, not specified	BMSC	Autologous	matched control (age, gender, ALT, Alb, Bil, PT)	Bone marrow	Hepatic artery	not mentioned	22	22	6
Zhu K* 2007 (153)	China	Paper	Decompensated, not specified	BMSC	Autologous	cells through HA vs cell through PV	Bone marrow	Hepatic artery or portal vein	not mentioned	36 (31 HA, 5 PV)	0	3

Author name	Country	Types of publication	Causes of liver diseases	Type of stem cells	Autologous / Allogenic	Type of control therapy	Extraction of cells	Ways of cell delivery	No of cells	No of pts (Stem cell, n=)	No of pts (control, n=)	Follow up (months)
Fu N* 2010 (150)	China	Paper	Mixed aetiologies	BMSC	Autologous	SMT	not mentioned	Hepatic artery	not mentioned	12	0	2
Lin S 2017 (114)	China	Conference	Decompensated, not specified	SCT	Autologous	control	not mentioned	not mentioned	not mentioned	27	132	60
Gaia S 2013 (111)	Italy	Paper	Mixed aetiologies	GCSF	Autologous	SMT	No collection	No cell delivered externally	Not applicable	15	15	12
Gaia S 2006 (113)	Italy	Paper	Mixed aetiologies	GCSF	Autologous	healthy volunteer stem cell donors	No collection	No cell delivered externally	Not applicable	8	40 (healthy volunteers)	6 days
Non-controlled studies												
Fu Q 2013 (160, 201)	China	Conference	Viral (Hep B and C)	UC-MSC	Allogenic	no control	umbilical cord	Peripheral vein	3 does: 5x10*7, 1x10*8, 2x1088 cells	20	0	13
Wang L 2013 (192)	China	Paper	UDCA-resistant PBC	UC-MSC	Allogenic	no control	umbilical cord	Peripheral vein	0.5x10*6 cells/kg	7	0	12
Xue H 2015 (194)	China	Paper	Mixed aetiologies	UC-MSC	Allogenic	no control	umbilical cord	Hepatic artery	3x10*7 cells in 15ml Saline	50	0	12
Chin S 2014 (156)	Malaysia	Conference	Mixed aetiologies	UC-MSC	Allogenic	no control	umbilical cord	Peripheral vein	not mentioned	5	0	3

Author name	Country	Types of publication	Causes of liver diseases	Type of stem cells	Autologous / Allogenic	Type of control therapy	Extraction of cells	Ways of cell delivery	No of cells	No of pts (Stem cell, n=)	No of pts (control, n=)	Follow up (months)
Lin H* 2012 (176)	China	Paper	Hepatitis B	UC-MSC	Allogenic	not mentioned	umbilical cord	Peripheral vein	not mentioned	Not mentioned	Not mentioned	not mentioned
Zhou B* 2012 (199)	China	Paper	Decompensated, not specified	UC-MSC	Allogenic	no control	umbilical cord	Hepatic artery	not mentioned	60	0	not mentioned
Mohamadnejad M 2007 (182)	Iran	Paper	Mixed aetiologies	BM-MSC	Autologous	no control	Bone marrow	Peripheral vein	volume of 20ml, mean 31.7x10*6 cells (range 10.2-60x10*6)	4	0	12
Wang L 2014 (191)	China	Paper	UDCA-resistant PBC	BM-MSC	Allogenic	no control	umbilical cord	not mentioned	not mentioned	10	0	12
Kantarcioglu M. 2013 (166)	Turkey	Conference	Mixed aetiologies	BM-MSC	Autologous	no control	Bone marrow	Peripheral vein	1x10*6 cells/kg	12	0	6
Amin M 2013 (154)	Egypt	Paper	Hepatitis C	BM-MSC	Autologous	no control	Bone marrow	Intra-splenic	10x10*6 cells	20	0	6
El-Ansary M. 2012 (139)	Egypt	Paper	Hepatitis C	BM-MSC	Autologous	no control	Bone marrow	Intra-splenic vs peripheral infusion	10 million cells	6 (intra-splenic) and 6 (peripheral)	0	6

Author name	Country	Types of publication	Causes of liver diseases	Type of stem cells	Autologous / Allogenic	Type of control therapy	Extraction of cells	Ways of cell delivery	No of cells	No of pts (Stem cell, n=)	No of pts (control, n=)	Follow up (months)
Kharaziha P 2009 (170)	Sweden	Paper	Mixed aetiologies	BM-MSC	Autologous	no control	Bone marrow	Portal vein	30 to 50 million MSCs	8	0	6
Jang Y 2014 (165)	South Korea	Paper	Alcohol	BM-MSC	Autologous	no control	Bone marrow	Hepatic artery (right)	5x10*7 in 10ml Saline-injected at week 4 and 8	12	0	3
Kim M 2012 (202)	South Korea	Conference	Alcohol	BM-MSC	Autologous	no control	Bone marrow	Hepatic artery (right)	5x10*6 cells/ml (injected 2x- at 4 and 8 weeks)	11	0	3
Yannaki E 2006 (196)	Greece	Paper	Alcohol	GCSF and CD34+ cells	Autologous	no control	Peripheral blood collection	Peripheral vein	Pt1: 4x1086/kg (1 mobilisation); Pt 2: 2.31x10*6/kg (3 mobilisations)	2	0	30
Levicar N 2008 (174)	France	Paper	Mixed aetiologies	GCSF and CD34+ cells	Autologous	no control	Peripheral blood collection	Hepatic artery (n=2) or portal vein (n=3)	1x10*6 to 2x10*8 cells as a single bolus	5	0	12 to 18

Author name	Country	Types of publication	Causes of liver diseases	Type of stem cells	Autologous / Allogenic	Type of control therapy	Extraction of cells	Ways of cell delivery	No of cells	No of pts (Stem cell, n=)	No of pts (control, n=)	Follow up (months)
Salama H 2010 (187)	Egypt	Paper	Mixed aetiologies	GCSF and CD34+ cells	Autologous	no control	Peripheral blood collection	hepatic artery or portal vein	1 billion expanded cells	48	0	12
Yao Y* 2014 (197)	China	Paper	Decompensated, not specified	GCSF and CD34+ cells	Autologous	no control	Peripheral blood collection	not mentioned	not mentioned	100	0	12
Andreone P 2015 (85)	Italy	Paper	Mixed aetiologies	GCSF and CD133+ cells	Autologous	no control	Peripheral blood collection	Hepatic artery	4 cohorts (from 5x10*4 to 1x10*6 kg)	17	0	12
Khan A 2008 (167)	India	Paper	Decompensated, not specified	GCSF and CD34+ cells	Autologous	no control	Bone marrow	Hepatic artery	0.1x 10*8 cells in all patients	4	0	6
Mohamadnejad M 2007 (60)	Iran	Paper	Mixed aetiologies	GCSF and CD34+ cells	Autologous	no control	Bone marrow	Hepatic artery	mean 3.13x10*8 cells	4	0	6
Gordon M 2006 (53)	United Kingdom	Paper	Mixed aetiologies	GCSF and CD34+ cells	Autologous	no control	Peripheral blood collection	Portal vein	Between 1x10*6 and 2x10*8 cells	5	0	2
Margini C 2014 (180, 203)	Italy	Conference	Decompensated liver cirrhosis, not specified	GCSF and CD133+ cells	Autologous	no control	Peripheral blood collection	Hepatic artery	5x10*4-1x10*6/kg	12	0	3
Pai M 2008 (183)	United Kingdom	Paper	Alcohol	GCSF and CD34+ cells	Autologous	no control	Peripheral blood collection	Hepatic artery	mean volume 135 ml	9	0	3

Author name	Country	Types of publication	Causes of liver diseases	Type of stem cells	Autologous / Allogenic	Type of control therapy	Extraction of cells	Ways of cell delivery	No of cells	No of pts (Stem cell, n=)	No of pts (control, n=)	Follow up (months)
Kim JK 2017 (171)	Korea	Paper	Mixed aetiologies	BMSC infusion	Autologous	no control	Bone marrow	Peripheral vein	mean: 0.925x10*8 cells/kg	19	0	12, followed by 60
Zhang B 2013 (198)	China	Conference	Decompensated, not specified	BM-MNC	Autologous	no control	Bone marrow	not mentioned	not mentioned	32	0	24
Sakaida I# 2011 (186)	Japan	Paper	Decompensated, not specified	BMSC	Autologous	no control	Bone marrow	Peripheral vein	5.20+/- 0.63 x 10*9	19	0	12
Couto B 2011 (157)	Brazil	Paper	Mixed aetiologies	BM-MNC	Autologous	no control	Bone marrow	Hepatic artery	2.0-15x 10*8 cells	8	0	12
Huang M*2013 (164)	China	Paper	Hepatitis B	TIPS and BMSC	Autologous	no control	Bone marrow	not mentioned	not mentioned	5	0	12
Lukashyk S 2014 (178)	Republic of Belarus	Paper	Hepatitis C	BM-stromal cells	Autologous	no control	Bone marrow	Intra-parenchymal	5ml, 1x10*6/kg	6	0	6
Mizunaga, Y. 2012 (181)	Japan	Paper	Viral (Hep B and C)	BMSC	Autologous	no control	not mentioned	not mentioned	not mentioned	19	0	6
Terai S 2006 (189)	Japan	Paper	Mixed aetiologies	BM-MNC	Autologous	no control	Bone marrow	Peripheral vein	5.2+/- 0.63x10*9 cells	9	0	6
Lyra A 2007 (179)	Austria	Paper	Mixed aetiologies	mononuclear enriched BMSC	Autologous	no control	Bone marrow	Hepatic artery	100 million cells	10	0	4

Author name	Country	Types of publication	Causes of liver diseases	Type of stem cells	Autologous / Allogenic	Type of control therapy	Extraction of cells	Ways of cell delivery	No of cells	No of pts (Stem cell, n=)	No of pts (control, n=)	Follow up (months)
Guo X* 2011 (162)	China	Paper	Decompensated, not specified	BM-MNC	Autologous	no control	Bone marrow	Hepatic artery	not mentioned	20	0	3
Li N* 2010 (175)	China	Paper	Decompensated, not specified	BMSC	Autologous	no control	Bone marrow	Hepatic artery (n=7) or portal vein (n=20)	not mentioned	27	0	3
Pan X* 2008 (184)	China	Paper	Decompensated, not specified	BMSC	Autologous	no control	Bone marrow	Hepatic or splenic artery	not mentioned * in abstract	24	0	3
Wang Z* 2010 (193)	China	Paper	Not mentioned	BMSC	Autologous	no control	Bone marrow	Into the liver	not mentioned	6	0	3
He J 2010 (163)	China	Conference	Decompensated, not specified	BMSC	Autologous	no control	Bone marrow	Hepatic artery	not mentioned	39 (32 decompensated cirrhosis, 7 chronic liver failures)	0	2
Takami T 2011 (188)	Japan	Paper	Decompensated, not specified	BM-MNC	Autologous	no control	Bone marrow	Peripheral vein	not mentioned	24	Not mentioned	not mentioned

Author name	Country	Types of publication	Causes of liver diseases	Type of stem cells	Autologous / Allogenic	Type of control therapy	Extraction of cells	Ways of cell delivery	No of cells	No of pts (Stem cell, n=)	No of pts (control, n=)	Follow up (months)
Wang J* 2007 (190)	China	Paper	Decompensated, not specified	BMSC	Autologous	no control	Bone marrow	Hepatic artery	not mentioned	25	Not mentioned	not mentioned
Yan L 2007 (195)	China	Paper	Hepatitis B	GCSF and peripheral blood monocytes	Autologous	no control	Peripheral blood collection	Hepatic artery	10*7-10*8/kg	2	0	12
Gao Y 2016 (161)	China	Conference	Mixed aetiologies	GCSF and BM-MNC	Autologous	no control	Bone marrow	Hepatic artery	not mentioned	16	0	6
Zhu Y* 2013 (200)	China	Paper	Mixed aetiologies	GCSF and peripheral blood mononuclear cells	Autologous	no control	Peripheral blood collection	Hepatic artery	not mentioned	4	0	6
Khan 2010/ 2011 (169, 204)	India	Paper	Mixed aetiologies	human fetal liver derived stem cells	Allogenic	no control	Fetal liver	Hepatic artery	80x10*6 (1ml/min)	25 (into 4 groups)	0	6
Cardinale V 2014 (155)	Italy	Paper	Hepatitis C	human biliary tree stem/progenitor cells	Allogenic	no control	Fetal liver	Hepatic artery	1 million cells per ml (overall) 1st patient-42 million cells 2nd patient-60 million cells	2	0	12

Author name	Country	Types of publication	Causes of liver diseases	Type of stem cells	Autologous / Allogenic	Type of control therapy	Extraction of cells	Ways of cell delivery	No of cells	No of pts (Stem cell, n=)	No of pts (control, n=)	Follow up (months)
D'Avola D 2016 (158)	Spain	Paper	Mixed aetiologies	BM-Endothelial Progenitor cells	Autologous	no control	Bone marrow	Hepatic artery	max at 100x10*6 in 50ml// ranging from 8.45x10*6 to 450x10*6 cells	11	0	12
Khan A 2009 (168)	India	Conference	Crigler-Najjar syndrome and biliary atresia	human hepatic stem cells	not mentioned	no control	not mentioned	Hepatic artery	not mentioned	5	0	not mentioned/ clinical data is for only 1 month
Sakai Y 2017 (185)	Japan	Paper	Mixed aetiologies	Adipose tissue derived stromal/stem cells (ADRC)	Autologous	no control	Adipose tissue	Hepatic artery	3.3-6.6x10*5 cells/kg	4	0	6
Lorenzini S 2008 (177)	United Kingdom	Paper	Mixed aetiologies	GCSF	Autologous	no control	No collection	No cell delivered externally	Not applicable	18	0	7 days
Kumar A 2014 (173)	India	Conference	Mixed aetiologies	GCSF	Autologous	no control	No collection	No cell delivered externally	Not applicable	62	0	not mentioned

BM: Bone marrow, MSC: Mesenchymal stem cell, SMT: Standard medical therapy, UC: Umbilical cord, GCSF: Granulocyte colony stimulating factor, IV: Intravenous, MNC: Mononuclear cell, TIPS: Transjugular intrahepatic porto-systemic shunt, BMSC: Bone marrow stem cell, BMC:

Bone marrow cell, ABMI: Autologous bone marrow infusion, HA: Hepatic artery, PV: Portal vein, UDCA: Ursodeoxycholic acid, PBC: Primary biliary cholangitis, ALT: Alanine transaminase, Bil: Bilirubin, ALT: Alanine transaminase, PT: Prothrombin time, Alb: Albumin

8.3.1: CLD and granulocyte colony stimulating factor (GCSF) therapy

Study characteristics

There were 5 RCT studies (82, 86, 120, 128, 130), 2 controlled (111, 113) and 2 uncontrolled studies (173, 177) that examined the effect of GCSF therapy in CLD patients. The longest duration of follow up was 12 months in 2 of the RCT studies (82, 120) and 1 controlled study (111) and therefore, only RCT studies are included in both quantitative and qualitative analysis. The doses of GCSF varied between the studies as noted in Table 15.

Table 15: GCSF (Granulocyte colony stimulating factor) dose variations noted in the included studies for chronic liver disease

Study ID	**GCSF details**
Randomised controlled trial	
Newsome P 2017 **(82)**	15 ug/kg/day for 5 days
Yu S 2017 **(130)**	5 ug/kg/day for 3 days
Kedarisetty C 2015 **(120)**	5 ug/kg/day for 5 days and then every 3rd day for 12 total doses
Prajapati R 2017 **(86)**	5 ug/kg twice a day for 5 days
Spahr L 2008 **(128)**	10ug/kg/day for 5 days
Controlled studies	
Gaia S 2013 **(111)**	5 ug/kg twice a day for 3 days, repeated every 3 months for 4 courses
Gaia S 2006 **(113)**	5 ug/kg twice a day for 3 days
Non-controlled studies	
Lorenzini S 2008 **(177)**	G1:2ug/kg/day G2:4ug/kg/day G3:6.6ug/kg/day G4:10ug/kg/day G5:15 ug/kg/day
Kumar A 2014**(173)**	300 ug twice a day for 5 days

Primary outcomes

Overall patient survival: Overall patient survival showed an improvement towards GCSF treated group in both 6 months and 12 months duration. The analysis showed that GCSF group significantly less mortality than SMT group at 6 months (OR=0.45, 95% CI: 0.26-0.78, P=0.005) but no effect at 12 months (Figure 24).

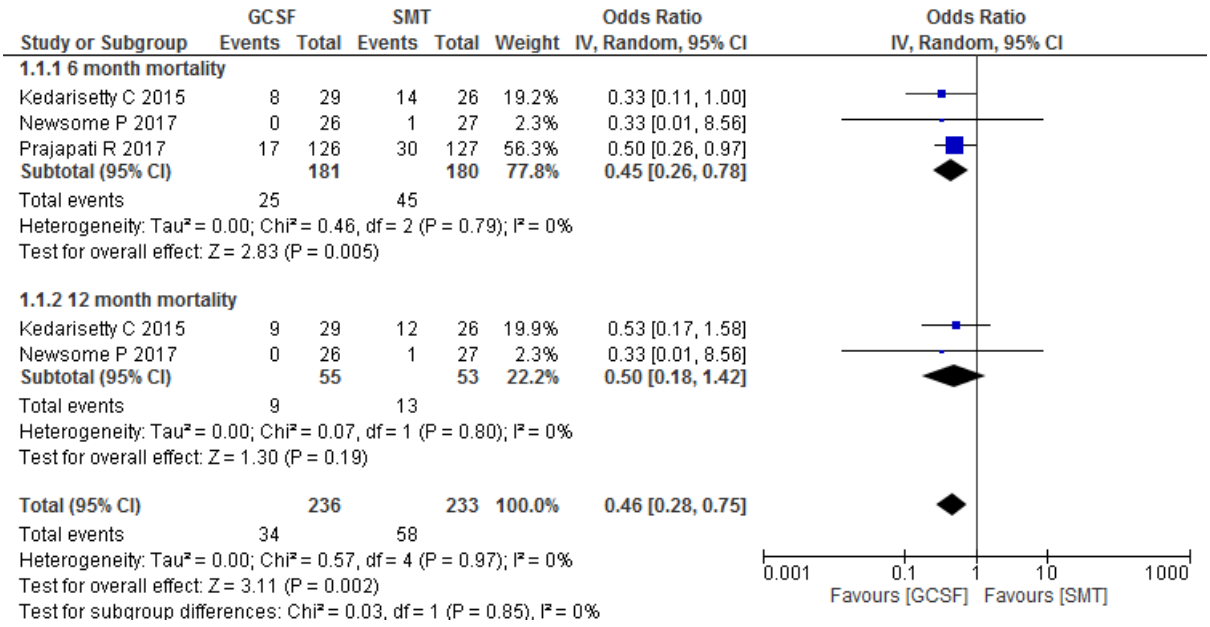


Figure 24: Overall patient survival in patients with chronic liver disease who had GCSF (Granulocyte colony stimulating factor) compared to standard medical therapy (SMT) at 6 months and 12 months of follow up

Liver transplant free survival: Three studies (82, 86, 120) examined the effect of liver transplant free survival. There was no improvement in LT free survival in GCSF compared to the SMT group at 6 months (OR=2.23, 95% CI: 0.61-8.17, p=0.23) and at 12 months (OR=0.13, 95% CI: 0.001-2.58) (Figure 25).

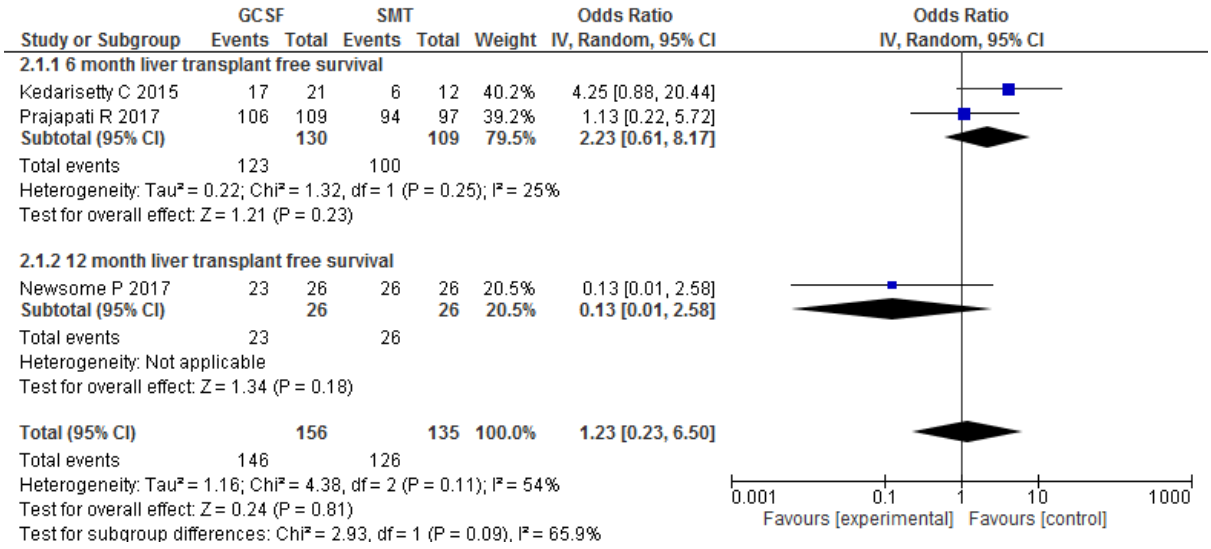


Figure 25: Liver transplant free survival for patients with chronic liver disease who had GCSF (Granulocyte colony stimulating factor) compared to standard medical therapy (SMT) at 6 months and 12 months of follow up

MELD: In Newsome et al (82), there was no difference in MELD score between GCSF and SMT groups at days 30, 60, 90 and 360. Yu et al study (130) also mentioned that there was no statistical difference in MELD between two groups over 6 months FU. Kedarisetty et al study (120) showed that at 12 months, GCSF group had significantly improved MELD reduction by 40.4% compared to 33% in SMT group (p=0.03) (120). Spahr et al study (128) showed that at 1 month, MELD reduction was better in SMT when compared to baseline but not in GCSF group.

QoL: Only one study (82) had data for quality of life (Chronic Liver Disease Questionnaire score) and there was no difference in overall score between GCSF and SMT groups at day 90 [median (IQR): GCSF: -0.1 (-0.4 to 0.3) vs SMT: 0.2 (-0.1 to 0.6)].

Adverse events to intervention: All studies showed good safety profile with GCSF therapy. There were no events of splenic rupture. The details of the safety profile were shown in Table 16.

Table 16: Safety profile of GCSF (Granulocyte colony stimulating factor) therapies in included studies of chronic liver disease

Study	GCSF group (Safety profile)
Newsome P 2017 **(82)**	No serious events noted
Yu S 2017 **(130)**	Well tolerated None had splenic rupture
Kedarisetty C 2015 **(120)**	Malaise in 37%, generalised discomfort 23% after GCSF injection None had portal vein thrombosis
Prajapati R 2017 **(86)**	Safe and none had significant adverse effects. Some patients had nausea, vomiting and body ache resolved with symptomatic treatment.
Spahr L 2008 **(128)**	Well tolerated. No spleen tenderness. 3 patients complained of transient mild lower back pain- reversible upon stopping GCSF

Secondary outcomes

Liver function tests: Only one study (82) had biochemical and coagulation blood tests (AST, ALT, Bilirubin, Albumin, INR) data and there was no improvement in GCSF group at 1 month and 3 months compared to baseline which was similar to SMT group.

Child Pugh Score: One study (128) showed significant improvement ($p<0.05$) of CPS 1 week after GCSF therapy. Two studies (120, 128) showed significant improvement ($p<0.05$) of CPS in GCSF group at 1-month FU but not in another study (82). At 3 months FU, 2 studies (86, 120) showed significant improvement ($P<0.05$) of CPS in cell therapy group but not in one study (82). At 6 months FU, 2 studies (86, 120) showed significant improvement ($p<0.05$) of CPS in GCSF group.

Liver decompensation events: In two studies (82, 130), liver cancers were not detected during the study period in GCSF cohort but in another study (120) one patient from the GCSF group developed a small hepatocellular cancer (HCC) by 12 months of follow up. In addition, 3 patients developed hepato-renal syndrome (HRS) in the GCSF group compared to 3 patients from the SMT group (120). The number of patients who developed spontaneous bacterial peritonitis (SBP) was similar between GCSF and SMT groups as per studies from Kedarisetty et al (120) and Spahr et al (128). Sepsis rate was significantly lower in GCSF group vs SMT group (6.9% vs 38.5%, p=0.005) at 12 months of FU in Kedarisetty et al study (120). Three studies (82, 120, 130) mentioned that no one from GCSF group had hepatic encephalopathy (HE) during the study follow up. In Newsome et al study (82), 1 patient developed ascites at 2 months and 3 months respectively compared to 10 patients at baseline in GCSF group. In Kedarisetty et al study (120), the percentage of patients requiring large volume paracentesis in GCSF group was significantly reduced from baseline to 1 month, 3 months and 6 months compared to SMT but there was no difference at 9 and 12 months between GCSF and SMT groups.

8.3.2: CLD and mesenchymal stem cell therapy (MSC)

Study characteristics

There were 8 RCT studies (56, 97, 117, 125, 129, 131-133), 4 non-RCT studies (139, 144, 146, 147), 3 controlled studies (102, 104, 109) and 14 uncontrolled studies (154, 156, 159, 165, 166, 170, 172, 176, 182, 191, 192, 194, 199, 201). RCT studies had the longest duration of follow-up and they were included in quantitative analysis.

In RCT, 4 studies examined the effect of allogenic BM-MSC (56, 97, 117, 129) and 2 studies examined the effect of allogenic UC-MSC (132, 133). In a study by Zekri A et al (131), the study group received autologous haematopoietic stem cells initially followed by mesenchymal

stromal cells and they are included in both the analysis of HSC and MSC when deemed appropriate. In Salama H et al study (125), the treatment group received autologous MSC with GCSF. The ways that MSC were given in these studies include: hepatic artery (97, 129), portal vein (131), peripheral vein (56, 125, 133), intrasplenic/intrahepatic (131) and unclear route (132).

Primary outcomes

Overall patient survival: One study showed that three patients from MSC group died of liver failure compared to none in SMT group (56) but in another study (131), 5 patients died in each group from decompensated liver disease during the 12-month FU period. For 6 month FU data, one study showed that 5 patients died in SMT group with decompensated liver disease but no one died in MSC group (125). Overall, at 12 months, there was no difference in survival between two groups (OR=1.24, 95% CI: 0.09-16.42, p=0.87) (Figure 26).

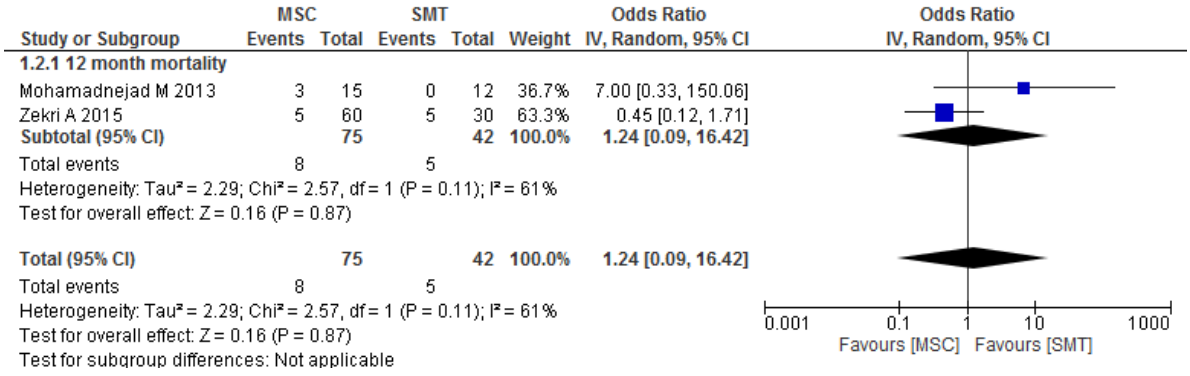


Figure 26: Overall patient survival in chronic liver disease patients who received mesenchymal stem cell (MSC) therapy compared to standard medical therapy (SMT) at 12 months of follow up

LT free survival: There were no data available on liver transplant free survival with MSC therapy.

MELD: In a study by Xu L et al (129), MELD improved significantly in MSC group over 24 weeks duration compared to baseline as well as to SMT group. In another study (132), it mentioned that MELD was improved in both MSC and SMT groups but the difference between them was not significant at 3, 6 and 13 months duration. A study by Zhang et al (133), there was a significant improvement of MELD in MSC group from week 2 to 48 as well in in control group from week 8 to 48 and when compared MSC to control group, the significant difference was only noted at week 48 only. Salama H et al (125) noted that there was a significant difference in MELD score between MSC and SMT groups from week 2 till end the of the study at 6 months' time. In few studies (56, 117, 131), data were able to combine to review the effect of MSC in 3, 6 and 12 months. MELD was significantly improved in MSC treated group at 3 months (MD -2.39, 95% CI: -3.07, -1.71, p=<0.00001) and 6 months (MD -2.97, 95% CI: -3.71, -2.23, p=<0.00001) but not at 12 months (MD -1.17, 95% CI: -2.91, 0.57, p=<0.19) (Figure 27).

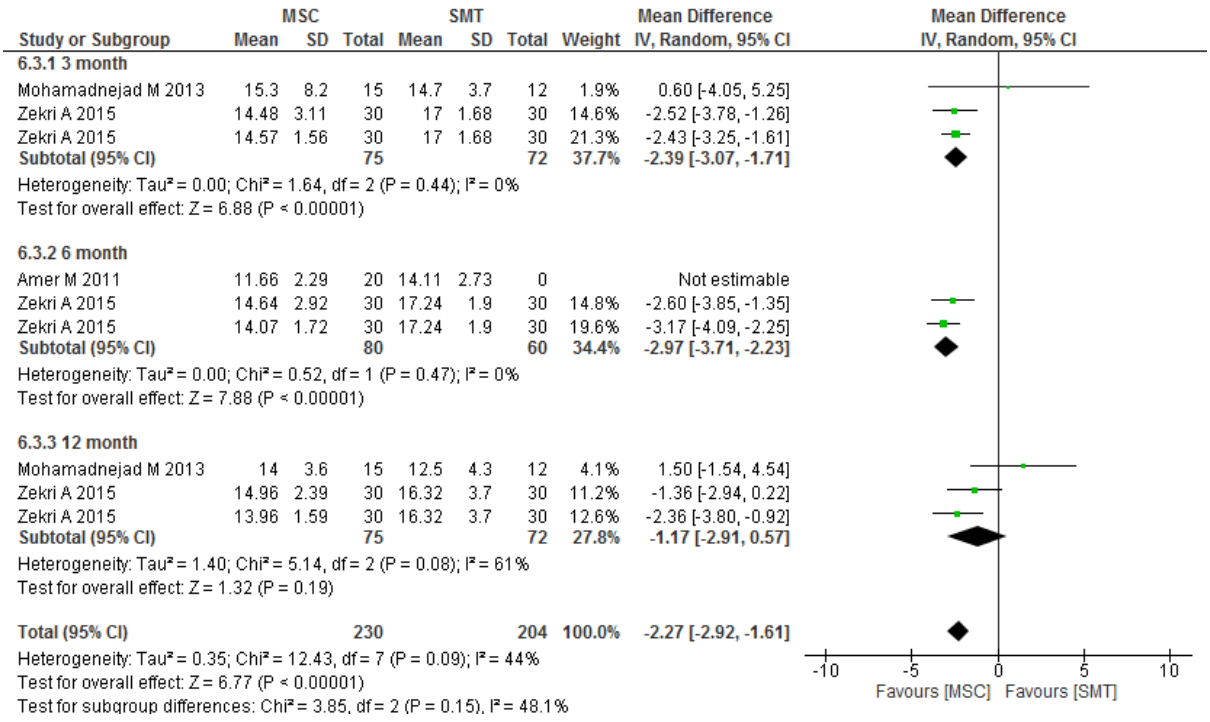


Figure 27: Model for End Stage Liver Diseases (MELD) score changes in chronic liver disease patients who had mesenchymal stem cell therapy (MSC) compared to standard medical therapy (SMT) at 3 months, 6 months and 12 months of follow up

QoL: There were 2 studies (117, 132) that had outcomes for quality of life. One study (117) showed that there was an improvement in fatigue score after 2 weeks with the maximum effect observed at 1 month, which was maintained for 6 months as well as a significant improvement in performance scale from 2 weeks up to 6 months. The other study (132) showed that there was a significant improvement in QoL at 1 month although the effect was not maintained at 3 or 12 months. From these two studies, it appeared that MSC therapy seemed to have a short term beneficial effect on QoL.

Adverse events from intervention: Fever is a very common adverse event noted with MSC therapy (97, 117, 129, 131-133) and in most cases, fever tended to subside 12 hours post therapy. No other serious adverse events were recorded in any of the studies. From the analysis, 19 patients in total had fever in MSC group compared to none in SMT group (OR=9.87, 95% CI: 2.17, 44.92, p=0.003) (Figure 28).

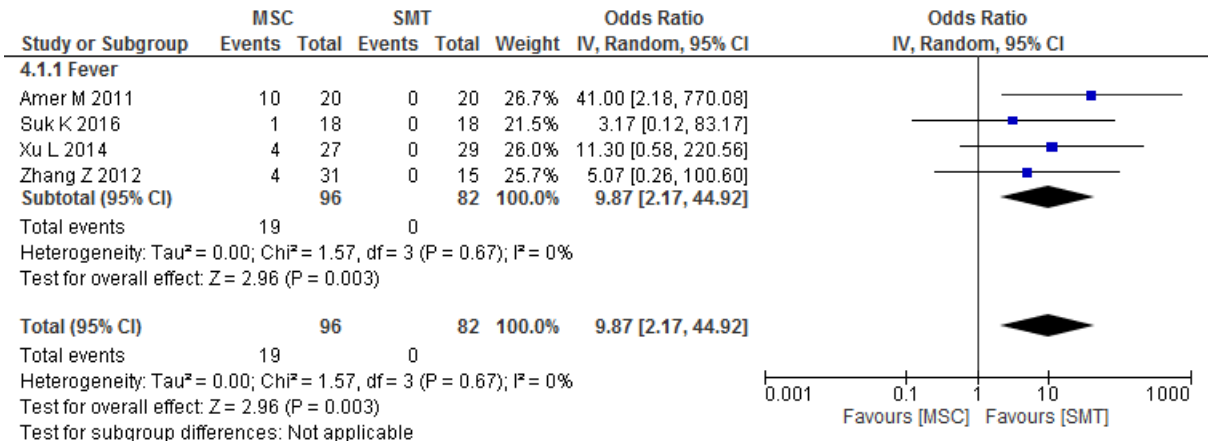


Figure 28: Incidence of fever seen in chronic liver disease patients who had mesenchymal stem cell therapy (MSC) compared to standard medical therapy (SMT)

Secondary outcomes

Liver function tests: A study by Mohamadnejad et al (56) mentioned that serum transaminases level between MSC and SMT group did not differ at 12 months of duration. In Xu L et al (129), ALT improved significantly at week 1, 2, 4, 8, 12 and 24 weeks in both MSC and SMT group compared to baseline ($p<0.05$) although there was no data comparing MELD between MSC and SMT at each time-point. Amer et al study (117) did not show any difference in liver enzymes in both MSC and SMT group at 6 months duration. Zheng et al study (132) mentioned that liver function improved in both MSC and SMT groups at 1, 3 and 12 months but there was no difference between the groups. Salama et al paper (125) mentioned that ALT fold improved significantly in MSC group at 6 months compared to SMT group ($p=0.029$), but not at other time points (2 weeks, 1 and 3 months). Salama et al paper (125) showed that AST fold change was only significant at the 3 month time-point in MSC compared to control ($p=0.48$) but not at week 2, 1 month and 6 months. In Suk et al paper (97), ALT and AST improved in both MSC and SMT group at 12 months compared to baseline but there was no difference between the two groups.

Xu et al study (129) mentioned that bilirubin improved significantly in MSC group from week 1 to week 24 when compared to baseline but was only significant at 24 weeks when compared to SMT group ($p<0.05$). Zhang et al study (133) mentioned that bilirubin was improved significantly in both MSC and SMT group from weeks 4 to 48 compared to baseline but only significant at week 48 when MSC were compared to SMT ($p<0.05$). In Amer et al study (117), there was no difference in bilirubin between MSC and SMT groups at 6 months. From Zekri (131), Suk (97) and Salama (125) studies, bilirubin data were combined to perform analysis at 2 weeks, 1, 3, 6 and 12 months. From the analysis, bilirubin was significantly improved in MSC group at 6 months only (MD: -13.5, 95% CI: -26.16, -0.83, $p<0.04$) but not at 1 week, 1 month, 3 months and 12 months (Figure 29).

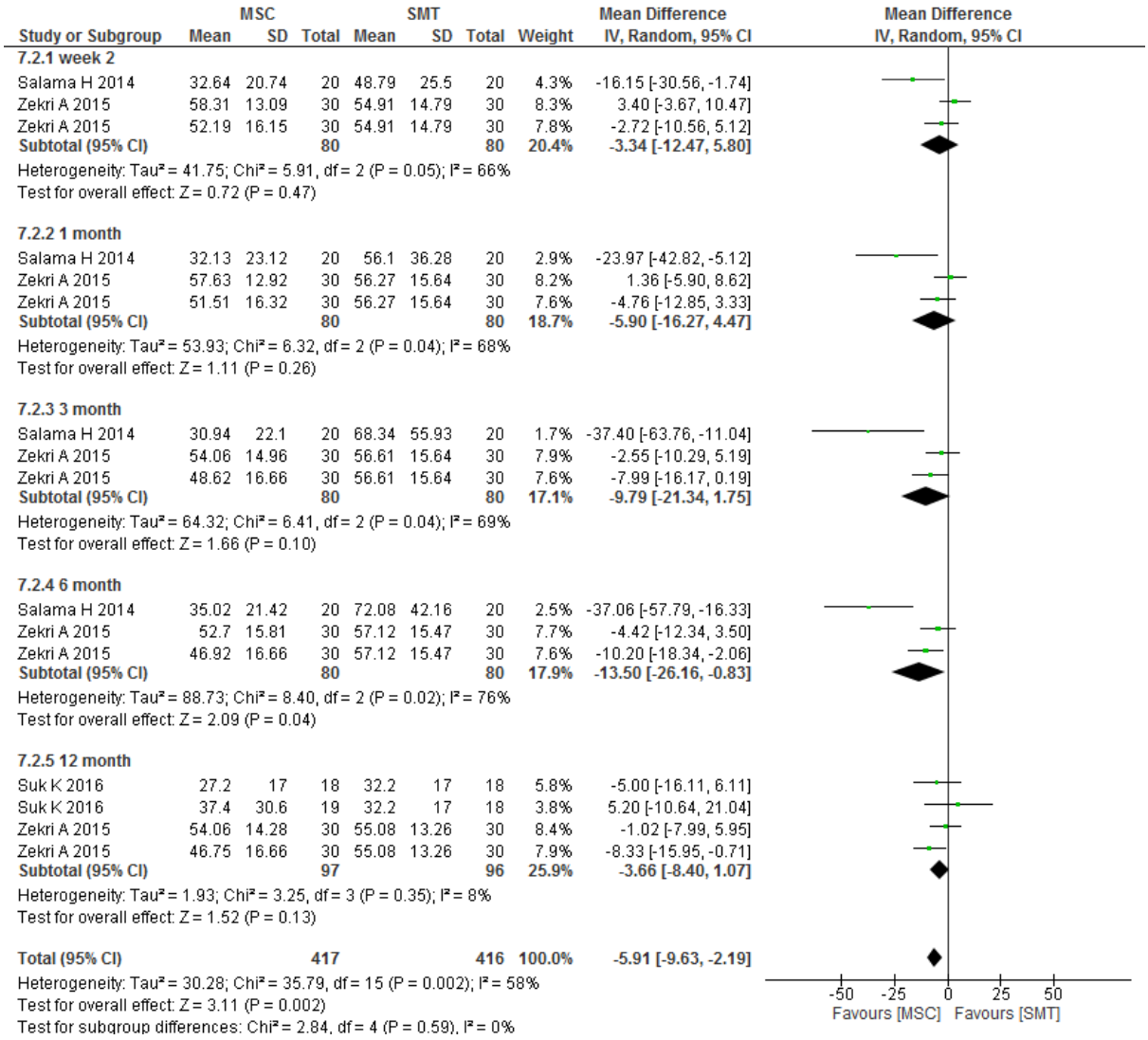


Figure 29: Bilirubin changes seen in chronic liver disease patients who had mesenchymal stem cell therapy (MSC) compared to standard medical therapy (SMT) at week 2, 1 month, 3 months, 6 months and 12 months of follow up

Xu et al study (129) showed that albumin improved in both MSC and SMT groups at 2, 4, 8, 12 and 24 weeks compared to baseline and when compared MSC to SMT groups, albumin improved significantly at week 2 (p=0.039) and week 24 (p=0.030). In Amer et al study (117), albumin improved significantly (p<0.05) from week 2 which was maintained over the study period of 6 months. In Zhang Z study (133), albumin improved significantly in both MSC and SMT group from weeks 2 to weeks 36 when compared to baseline but there was no difference

between MSC and SMT at each time point. Two studies (125, 131) were able to combine the data for 2 weeks, 1, 3 and 6 months. Three studies (56, 97, 131) had 12 months albumin data and combined for analysis. It was shown that albumin improved more significantly in the SMT group at 1 month (MD: 2.40, 95% CI: 0.35-4.46, p=0.02), 3 months (MD: 4.00, 95% CI: 1.90-6.10, p=0.0002) and 6 months (MD: 5.35, 95% CI: 2.74-7.96, p<0.0001) compared to MSC group but not at 12 months (MD: 0.78, 95% CI: -7.50, 9.06, p=0.85) (Figure 30).

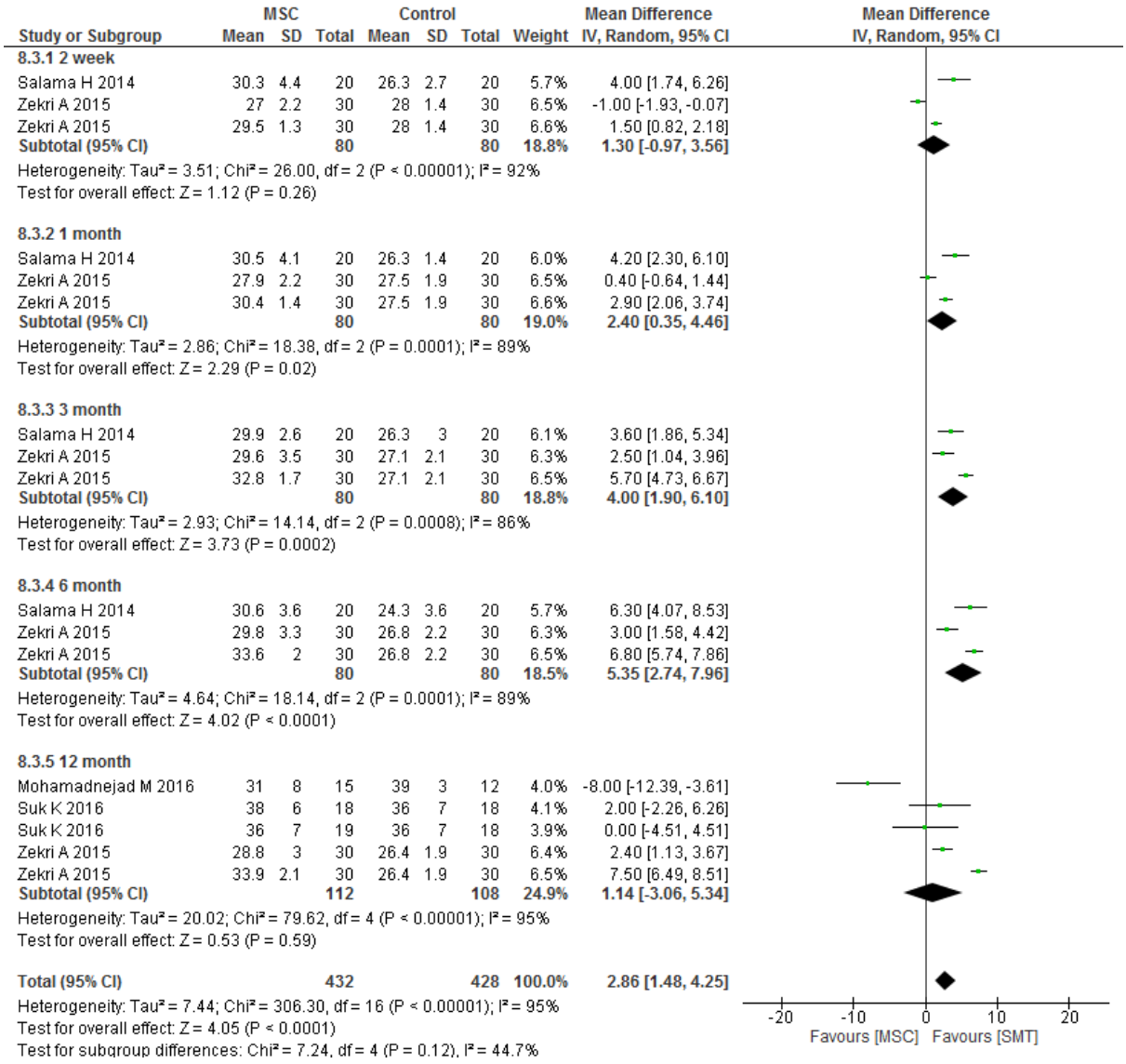


Figure 30: Albumin changes seen in chronic liver disease patients who had mesenchymal stem cell therapy (MSC) compared to standard medical therapy (SMT) at 2 weeks, 1 month, 3 months, 6 months and 12 months of follow up

Suk et al study (97) data for ALP and GGT measurement pre-and post-therapy and for ALP showed that the MSC group had a greater reduction in ALP compared to SMT group (mean difference of -20 vs -3). However, there was no difference in GGT level between MSC and SMT groups at 12 months. Mohamadnejad et al study (56) mentioned that there was no difference in INR between MSC and SMT group at 12 months of FU and Amer et al study (117) showed similar outcomes in 6 months FU. Zheng et al study (132) mentioned that PT improved in both MSC and SMT groups at 1 months, 3 months and 12 months period but there was no difference between the two groups. In Zhang et al study (133), PT activity was improved significantly from weeks 2 to weeks 48 compared to baseline in both MSC and SMT group but there was no difference between them at any time point. In 3 studies (97, 125, 131), the data were able to be combined for analysis at different time points. INR improved significantly in MSC group at 3 months (MD: -0.26, 95% CI: -0.33, -0.19, $p<0.00001$), 6 months (MD: -0.28, 95% CI: -0.36, -0.21, $p<0.00001$) and 12 months (MD: -0.16, 95% CI: -0.29, -0.02, $p<0.03$) (Figure 31).

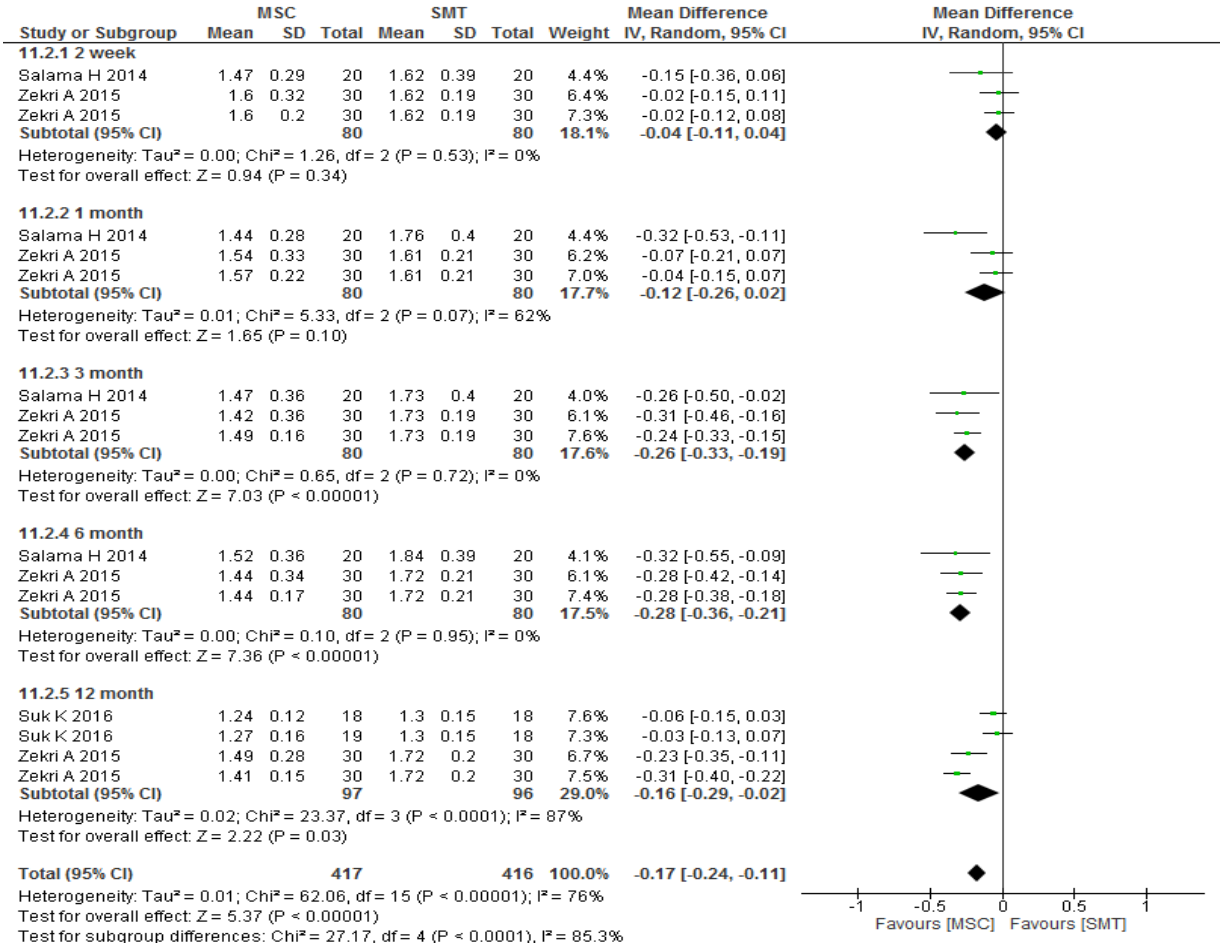


Figure 31: International normalised ratio (INR) changes seen in chronic liver disease patients who had mesenchymal stem cell therapy (MSC) compared to standard medical therapy (SMT) at 2 weeks, 1 month, 3 months, 6 months and 12 months of follow up

Child Pugh Score: Amer et al study (117) showed that Child Pugh score (CPS) improved in MSC group from 2 weeks till end of the study at 6 months but deteriorated in SMT group. In Zheng et al study (132), CPS improved in both MSC and SMT groups but there was no difference between the two groups ($p>0.05$). Salam et al study (125) mentioned that at the end of 6 months FU, 20% of MSC treated group had an improvement in CPS compared with baseline but not in the SMT group. Zekri et al (131) study showed that 40% had an improvement in their Child Pugh grade compared with the baseline in MSC group and the improvement occurred from 6 months onwards. Two studies (56, 97) had data for 12 months

FU that was able to be combined for analysis and it showed that CPS improved significantly with MSC therapy at 12 months (MD: -0.87, 95% CI: -1.55, -0.20, p=0.01) (Figure 32).

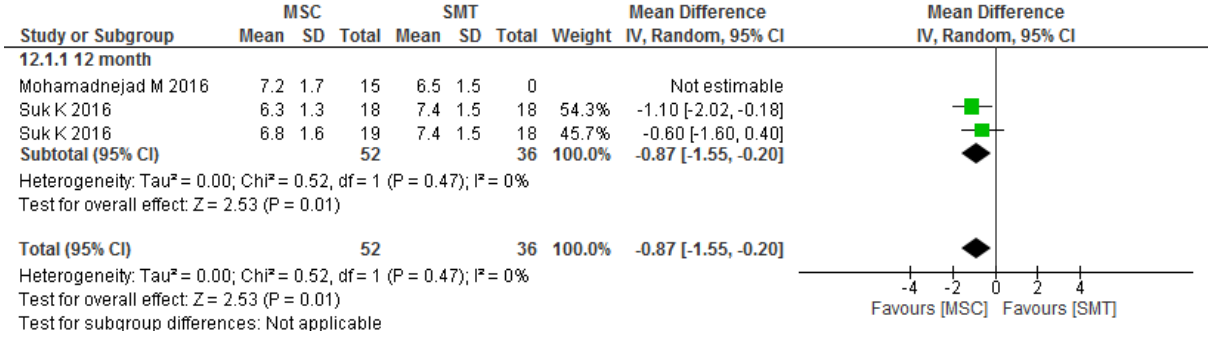


Figure 32: Child Pugh score changes seen in chronic liver disease patients who had mesenchymal stem cell therapy (MSC) compared to standard medical therapy (SMT) at 12 months of follow up

Liver decompensation: Three studies (97, 131, 133) mentioned that there were no events of hepatocellular carcinoma occurring during the 12-month FU in MSC treated group. However, one study (131) mentioned that a focal lesion was found in the liver after 12 months of FU (1 patient in MSC group compared to 2 patients in SMT). No events of sepsis were mentioned in any of the studies. There is only one study that gave MSC via the portal vein and the incidence of portal vein thrombosis was 3.3% (1 patient) in the MSC treated group compared to 6.7% (2 patients) in SMT group (131). There were 4 studies (117, 125, 131, 133) that mentioned HE events although 2 studies (117, 133) reported that there was no significance between MSC and SMT group. However, two other studies (125, 131) showed that patients who received MSC had fewer episodes of HE at 3 months (OR: 0.26, 95% CI: 0.06- 1.21, p=0.09) and 6 months (OR=0.27, 95% CI: 0.07-1.04, p=0.06) post-treatment although they did not reach statistical significance (Figure 33).

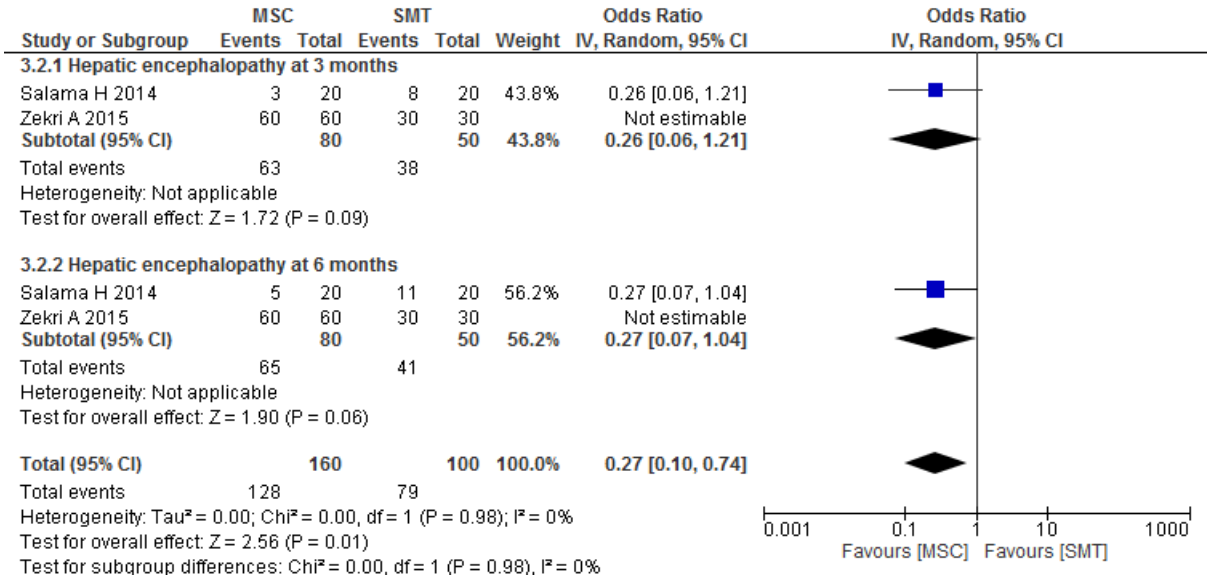


Figure 33: Hepatic encephalopathy events in chronic liver disease patients who had mesenchymal stem cell therapy (MSC) compared to standard medical therapy (SMT) at 3 months and 6 months of follow up

Three studies (125, 131, 133) reported the event of gastrointestinal (GI) bleeding and in one study (133), there was no difference in the incidence of GI bleeding between MSC and SMT groups during 12 month of FU, whereas two other studies (125, 131)showed an improvement of GI bleeding in the MSC group. A second study (131) showed that the event of GI bleeding was 10% in the MSC group compared to 16.7% in SMT group at 12 months of FU. The other study (125) reported that there was a significant improvement in event of GI bleed in MSC group (100% at baseline to 0% at 3 months and 6 months) compared to SMT group (90% at baseline to 15% at 3 months and 16.7% at 6 months). Overall, there seemed to be a reduction of GI bleeding in MSC group compared to SMT group at 6 and 12 months.

Four studies (117, 125, 131, 133) reported the development of ascites in MSC treated cohorts. These studies showed trends towards ascites improvement in MSC treated group compared to SMT group. The details of studies that reported the event of ascites were documented in Table 17.

Table 17: Events of ascites seen in chronic liver disease patients who had mesenchymal stem cell therapy (MSC) at 6 and 12 months of follow up

Study ID	FU (months)	Results (MSC group)
Amer M 2011 **(117)**	6	Ascites improved from 2 weeks (p=0.001),1 month, 2 months and 4 months. Not significant at 6 months.
Salama H 2014 **(125)**	6	25% showed an improvement of the grade of ascites, as assessed by abdominal ultrasound when compared with the baseline.
Zhang Z 2012 **(133)**	12	Ascites reduced at weeks 1, 2, 12, 36, 48. The rate of reduction is higher that SMT group.
Zekri A 2015 **(131)**	12	40% showed improvement in degree of ascites. 60% showed no change in degree of ascites. None had deterioration in the degree of ascites at the end of FU.

8.3.3: CLD and haematopoietic stem cell therapy (HSC)

Study characteristics

There were 6 RCT studies (82, 96, 123, 124, 126, 131), 6 non-RCT studies (61, 135, 137, 138, 141, 143), 2 controlled studies (106, 141) and 10 non-controlled studies for HSC therapies (53, 85, 96, 167, 174, 180, 183, 187, 196, 197). All RCT studies were included in the final quantitative and qualitative analysis. There was a non-RCT study that was presented as a conference abstract (205) with 5-year outcome data and therefore, included in the analysis due to longer duration of FU.

In five studies (82, 124, 126, 131, 143), subcutaneous GCSF injection was used to mobilise cells from the one marrow and the dose of GCSF varied in each study. In 2 studies (126, 131)

cells were aspirated directly from the bone marrow after giving GCSF but in the other 3 studies (82, 124, 143) they were collected from peripheral blood after GCSF injection. In two studies, the dose of GCSF was 300 ug/day for 5 days (126, 131) but in other studies, the investigator had used 15 ug/kg/day for 5 days (82) or 10 ug/kg/day for 5 days respectively (143). In other two RCT studies (96, 123) GCSF was not used and HSC was collected directly from the bone marrow.

Two studies (126, 131) used both CD133+/CD34+ cells, other 3 studies used CD133+ cells only (82, 96, 123) and 2 other studies (124, 143) used CD34+ cells only.

Primary outcomes

Overall patient survival: Three studies (82, 126, 131) had 12-month mortality data and one study (205) had 5-year mortality data. From the meta-analysis, it seemed that patients who had HSC therapy did not have improved survival outcomes compared to the control group at 12 months (OR=0.34, 95 % CI: 0.07, 1.58, p=0.17) and 5 years (OR=0.32, 95 % CI: 0.10, 1.07, p=0.35) (Figure 34).

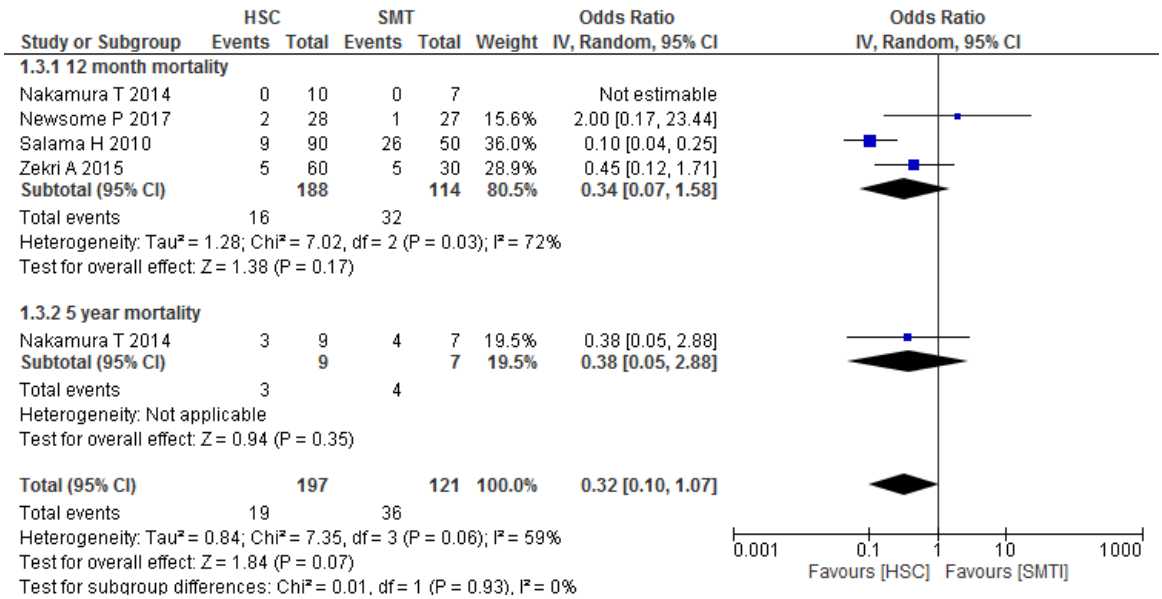


Figure 34: Overall patient survival in chronic liver disease patients who had haematopoietic stem cell therapy (HSC) compared to standard medical therapy (SMT) at 12 months and 5 years of follow up

LT free survival: Three studies (82, 96, 124) mentioned 12-month LT-free survival data which showed that patients who received HSC therapy were no less likely to receive LT compared to SMT group at 12 months (OR: 0.26, 95% CI: 0.05, 1.41, p=0.12) (Figure 35).

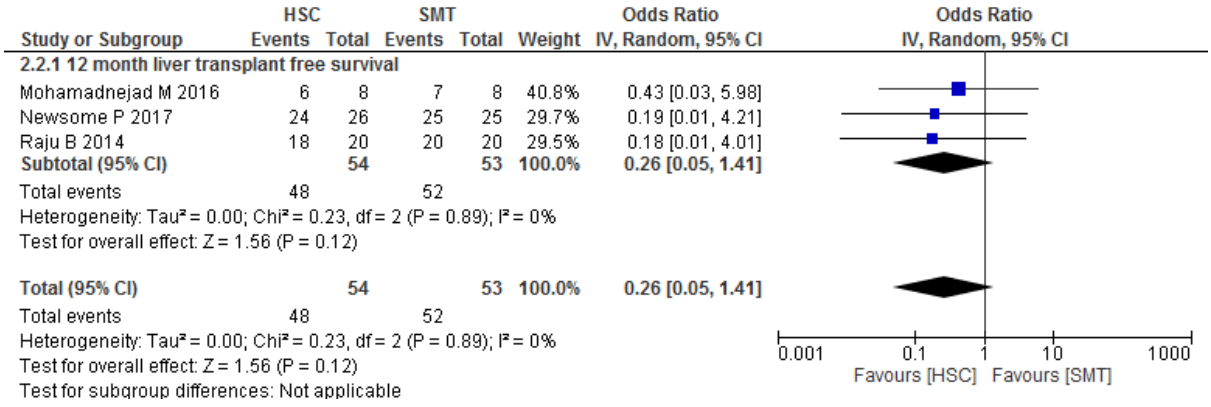


Figure 35: Liver transplant free survival in chronic liver disease patients who had haematopoietic stem cell therapy (HSC) compared to standard medical therapy (SMT) at 12 months of follow up

MELD: In three studies (96, 131, 143), data were able to be combined and there was a significant improvement of MELD seen in the HSC treated group at 3 months (MD: -2.12, 95% CI: -3.3, -0.93, p=0.0005) but this was not seen at 6 months compared to SMT group (MD: -0.54, 95% CI: -3.53, 2.44, p=0.72) (Figure 36). In Newsome et al (82), there was no difference in MELD score at baseline, days 90 and days 180 in both HSC and SMT treated groups. One study (205) had MELD data up to 5 years in HSC group and the mean difference of MELD from baseline were -2.6 at 1 year, -2.2 at 3 years and -0.2 at 5 years.

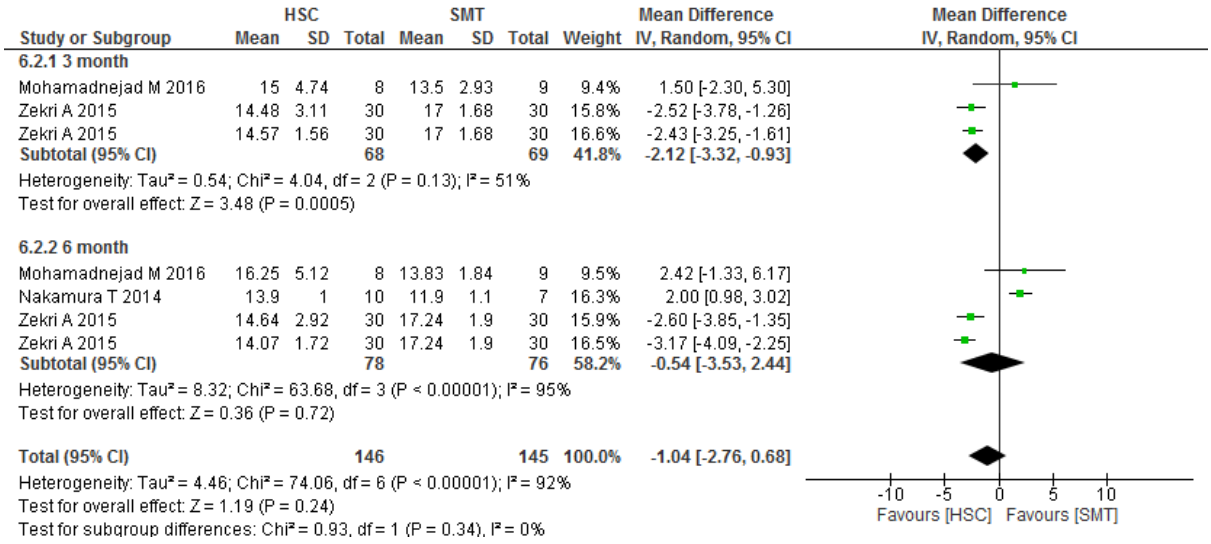


Figure 36: Model for End Stage Liver Disease (MELD) changes in chronic liver disease patients who had haematopoietic stem cell therapy (HSC) compared to standard medical therapy (SMT) at 3 months and 6 months of follow up

QoL: Three studies (82, 126, 143) mentioned the impact of HSC therapy on patients' QoL. Newsome et al (82) showed similar change in QoL in both HSC and SMT groups when compared at baseline and day 90. However, Nakamura et al mentioned that HSC therapy improved all sections of QoL especially in social role functioning at 24 weeks (143). In Salama et al (126), the performance scale was improved in HSC treated group compared to SMT group (at 6 months: 75% of HSC group had score 0 compared to 5% in SMT group).

Adverse events to intervention: Most of the adverse events are related to GCSF and the common events recorded were low grade fever (123, 126, 131, 143) and bone pain (126, 131, 143). There were no complications reported with stem cell infusion in any of the studies. In 4 studies (96, 123, 126, 131), the cells were given via portal vein and according to analysis, the incidence of portal vein thrombosis was similar between HSC and SMT group (OR=0.96, 95% CI: 0.13, 7.31, p=0.97) (Figure 37).

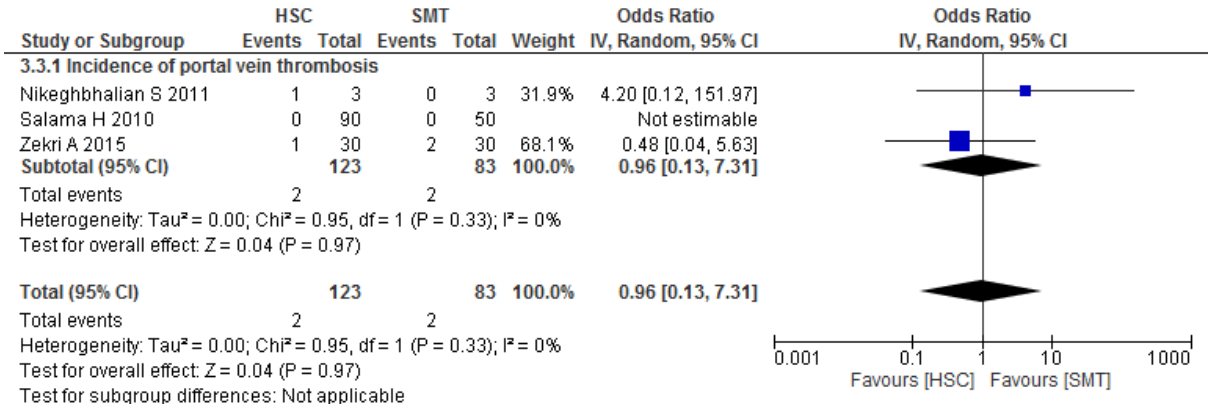
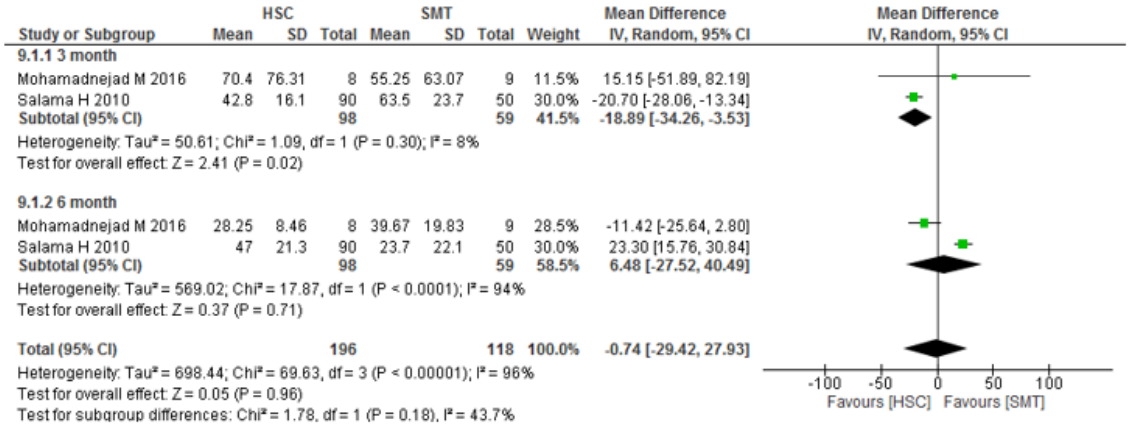


Figure 37: Incidence of portal vein thrombosis in chronic liver disease patients who had haematopoietic stem cell therapy (HSC) compared to standard medical therapy (SMT)

Secondary outcomes

Liver function tests: In a study by Newsome et al (82), both ALT and AST improved at 1 and 3 months compared to baseline in both HSC and SMT groups although there was no difference between the two groups at any time point. Two studies (96, 126) had ALT and AST data for 3 months and 6 months. For ALT, there was an improvement at 3 months for HSC group (MD: -18.89, 95% CI: -34.26, -3.53, p=0.02) although that was not seen at 6 months (MD: 6.48, 95% CI: -27.52, 40.49, p=0.71) (Figure 38a). For AST, the improvement was not seen either at 3 months (MD: 7.52, 95% CI: -46.22, 61.29, p=0.78) or 6 months (MD: -8.51, 95% CI: -17.25, 0.24, p=0.06) post-HSC therapy (Figure 38b).

A: Alanine aminotransferase (ALT) changes



B: Aspartate aminotransferase (AST) changes

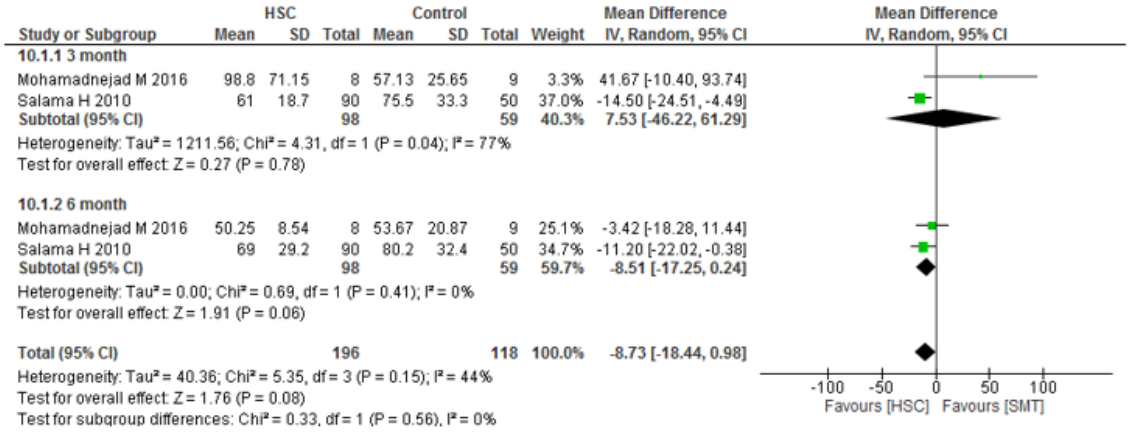


Figure 38: Liver enzyme changes (ALT: Alanine transaminase and AST: Aspartate transaminase) seen in chronic liver disease patients who had haematopoietic stem cell therapy (HSC) compared to standard medical therapy (SMT) at 3 months and 6 months of follow up

Two studies (126, 131) had bilirubin data at 1 and 2 months and for 3 and 6 months. There were 3 studies (96, 126, 131) able to be combined for meta-analysis. Bilirubin level was improved significantly in HSC group at 6 months (MD: -12.70, 95% CI: -23.78, -1.62, p=0.02) but not at 1 month (MD: -1.24, 95% CI: -5.9, 3.42, p=0.60), 2 months (MD: -5.23, 95% CI: -10.74, 0.28, p=0.06) or 3 months (MD: -7.79, 95% CI: -16.5, 0.92, p=0.08) (Figure 39). Zekri et al study (131) had 9 months and 12 months data for bilirubin but did not show any difference between HSC and SMT groups. Newsome et al (82) had bilirubin data for days 90 and 180 but

there was no difference between the two groups. Nakamura T study (143) had bilirubin data up to 6 months and did not show any difference between the two groups.

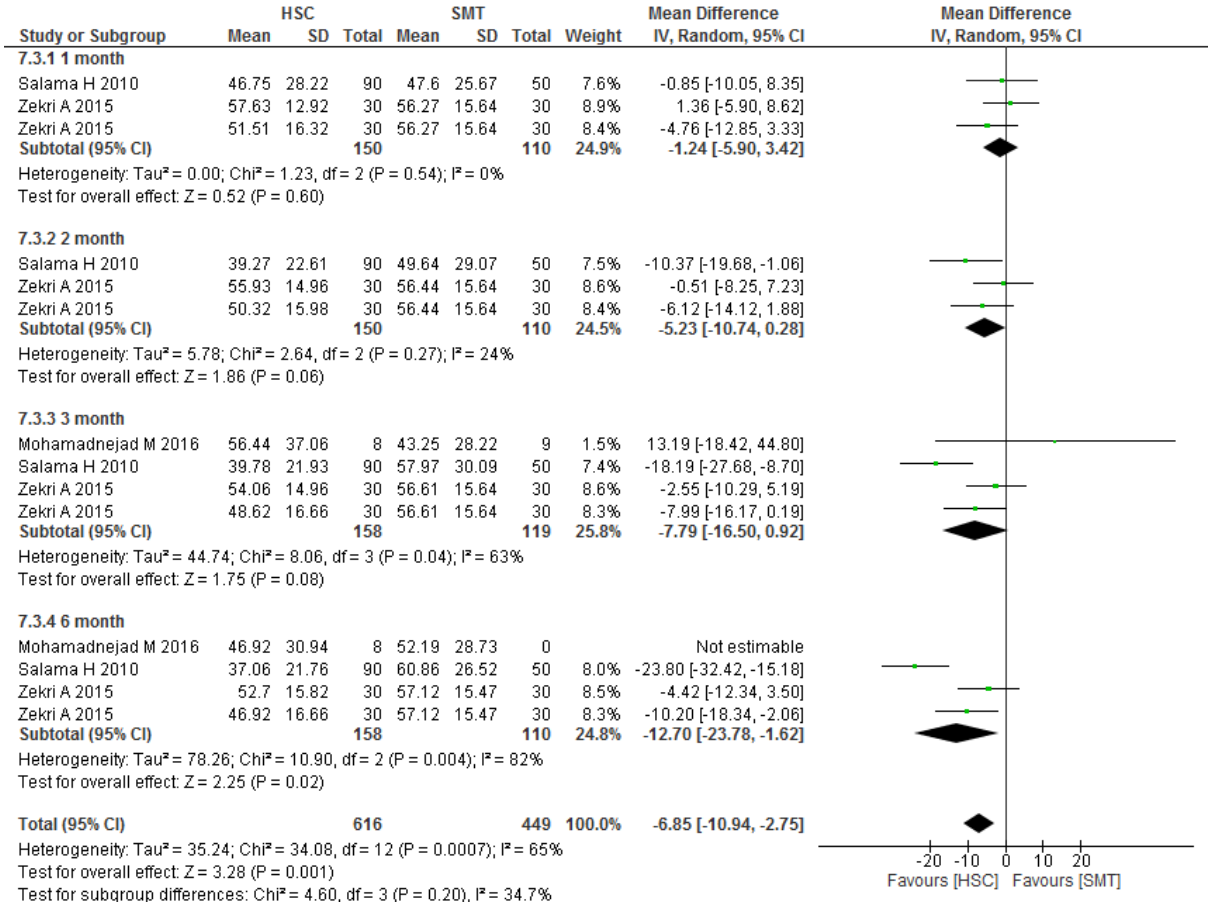


Figure 39: Bilirubin changes seen in chronic liver disease patients who had haematopoietic stem cell therapy (HSC) compared to standard medical therapy (SMT) at 1 month, 2 months, 3 months and 6 months of follow up

A study by Nakamura et al (143) showed that serum albumin level at 24 weeks was not changed in either HSC or SMT treated groups compared to baseline. Another study (82) mentioned that there was no difference in albumin level in days 30 and 90 in both HSC and SMT group. In Raju et al (124), 93% of patients showed improvement in albumin during 12 months of FU. In 3 studies that was combined for data analysis (96, 126, 131), albumin level was significantly improved in SMT group at 3 months (MD: 3.25, 95% CI: 1.00, 5.51, p=0.005) but became similar between the two groups at 6 months (MD: 3.14, 95% CI: -0.63, 6.92, p=0.10) (Figure

40). Overall, albumin was improved better in SMT group compared to HSC group (OR= 3.28, 95% CI: 1.26, 5.31, p=0.001).

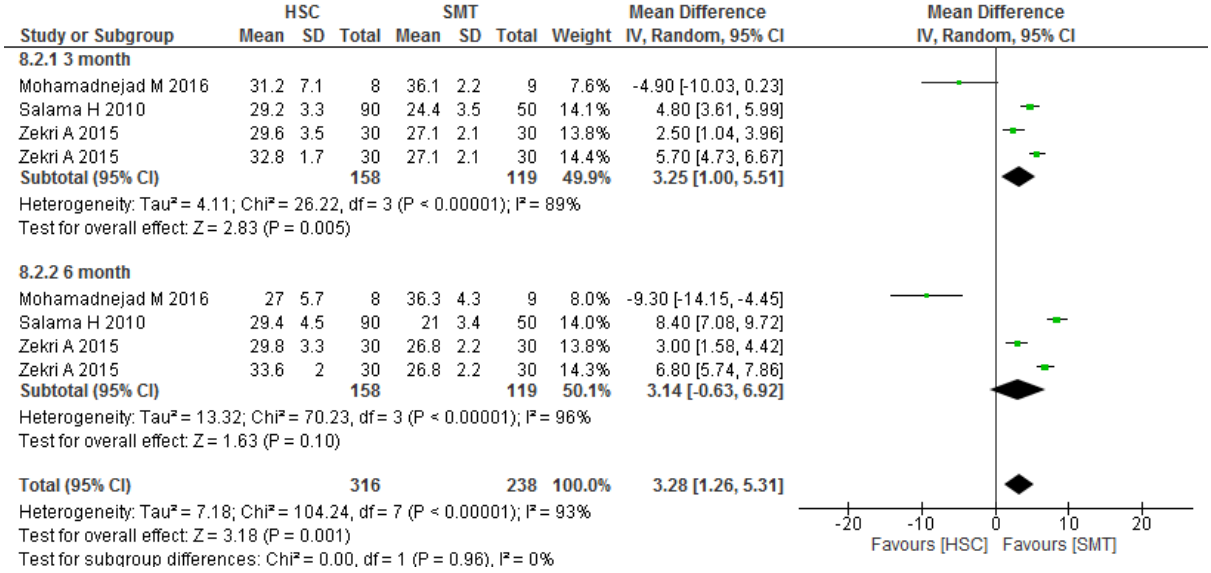


Figure 40: Albumin changes seen in chronic liver disease patients who had haematopoietic stem cell therapy (HSC) compared to standard medical therapy (SMT) at 3 months and 6 months of follow up

A study by Raju B et al (124) mentioned that 68% of patients had improvement in INR during 12 months FU but in another study by Newsome et al (82), there was no difference in change between HSC and SMT group at days 30 and 90 compared to baseline. In Salama et al (126), prothrombin concentration (PC) percentage was measured and HSC group had significantly improved PC% at 1, 2, 3 and 6 months after HSC therapy. Another study (143) did not show any change in INR between HSC and SMT treated groups. In two studies (96, 131), INR seemed to improve at 3 months with HSC therapy (MD: -0.24, 95% CI: -0.34, -0.14, p<0.00001) but not at 6 months post-treatment (MD: -0.17, 95% CI: -0.38, 0.04, p=0.12) (Figure 41).

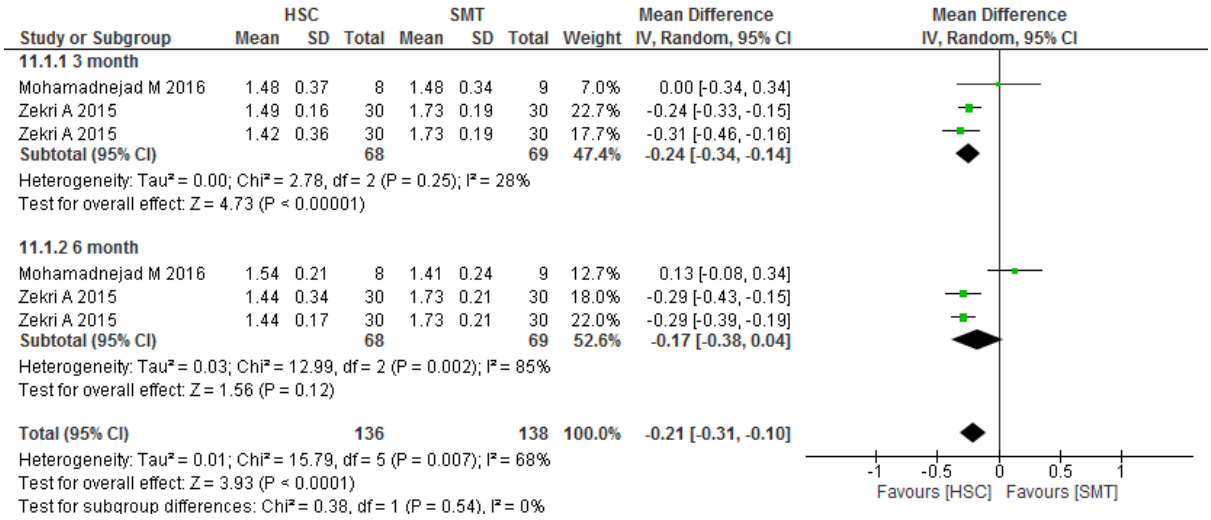


Figure 41: International normalised ratio (INR) changes seen in chronic liver disease patients who had haematopoietic stem cell therapy (HSC) compared to standard medical therapy (SMT) at 3 months and 6 months of follow up

Child Pugh Score: Four studies (82, 126, 131, 143) reported Child Pugh Score (CPS) but due to variation in reporting, the data were not able to be combined for meta-analysis. In Zekri et al (131), 40% of patients that received 2 injections of HSC had an improvement in CPS compared to baseline ($p<0.05$) during 12 months FU and the maximum benefit was noted from 6 months onwards after therapy. Another study (126) also mentioned that the maximum improvement of CPS occurred after 6 months. In Newsome et al (82), there was difference in CPS at days 30 and 90 in both HSC and SMT groups. In Nakamura et al (143), HSC treated group had maintenance and/or improvement in CPS compared to control during 24 months of FU although it did not reach statistical significance.

Liver decompensation events: There were no cases of malignancies reported in any of the studies within 12 months (82, 126, 131) as well as 24 months of FU (143). Nakamura et al (205) reported the incidence of HCC within 5-year FU and showed similar rates between HSC and SMT groups (44.4% vs 42.9%). The incidence of variceal bleeds was similar between HSC and SMT group in one study (131) although in another study, there was an improvement

of GI bleed in HSC group over SMT group over 6 months FU (126) . Hepatic encephalopathy incidence was similar between the HSC and SMT groups in one study over 12 month FU (131). However, study by Salama et al (126) showed improvement of HE in HSC treated group compared to SMT over 6 months FU. Overall, it appeared that ascites improved with HSC therapy. One study showed that there was a 40% improvement of ascites in HSC treated group at the end of 12 month FU (131). In another study (124), there was less frequency of paracentesis and required lower dose of diuretic use to control ascites in HSC group and in third study (126), it showed that the maximum improvement of ascites was found after 6 months in 63.6% of HSC treated groups.

8.3.4: CLD and Bone marrow stem cell (BMSC)

Study characteristics

Any studies with unspecific stem cells types but received directly from the bone marrow as well as peripheral blood stem cells that had been mobilised with GCSF from bone marrow were included. There were 6 RCT studies (119, 121, 122, 127, 130, 150), 6 non-RCT studies (134, 136, 140, 145, 148, 149), 5 controlled studies (107, 108, 110, 150, 152) and 20 non-controlled studies (153, 157, 161-163, 171, 175, 178, 179, 181, 184, 186, 188-190, 193, 195, 198, 200, 206). RCT studies had 12 months duration of FU (119, 122) but in 2 non-RCT studies (134, 149) that had longer duration of FU at 24 months and they were also included in final quantitative and qualitative analysis.

In three studies (121, 127, 130), GCSF was used to mobilise stem cells and the dose varied in each study: 5 micrograms per kg per day for 3 days (130), 10 ug/kg/day for 5 days (127) and 100 ug for one day (121). Cells were obtained directly from bone marrow in 6 studies (119, 121, 122, 127, 134, 149), from peripheral blood in one study (130) and unknown in the other

study (150). Hepatic artery was used to deliver the cells in 7 studies (119, 121, 122, 127, 134, 149, 150) and portal vein was used in the other study (130).

Primary outcomes

Overall patient survival: In a study by Lyra et al (122), 3 patients from the BMSC group died compared to 2 patients from the SMT group during 12 months FU (20% vs 13.3%) and in another study, 2 patients from BMSC died compared to 4 patients from SMT group during 3 months FU (7% vs 13.3%).

LT free survival: In one study (122) a patient from the BMSC group underwent liver transplantation 45 days after randomisation due to progressive liver disease but no data were available on SMT group.

MELD: Three studies (122, 127, 130) reported the outcome of MELD and in 2 studies (122, 127) the data were able to be combined in the first 3 months post-therapy. From these studies, despite the improvement in MELD score in BMSC, the difference was similar when compared to the control group at 1 month (MD: 0.33, 95% CI: -1.43, 2.08, p=0.71), 2 months (MD: 0.21, 95% CI: -1.22, 1.63, p=0.77) and 3 months (MD: 0.41, 95% CI: -1.75, 2.56, p=0.71) (Figure 42). Yu et al (130) mentioned that there was no difference in MELD between BMSC and SMT group. Lyra et al reported (122) MELD outcomes in 1, 2, 3, 6 and 12 months and in this study MELD score remained stable in the treated patients with mean relative changes (RC) from -2% at 2 months to +6% at 12 months compared to control group with mean RC +6% at 2 months to +18% at 12 months. Despite these changes, when comparing the slope between the BMSC and control group at 3 months and 12 months there was no statistical significance. In a study from Spahr et al (127), 68% of patients from BMSC achieved a decrease of more than 3 points in MELD score at 3 months compared to 81% in control group (p= 0.43).

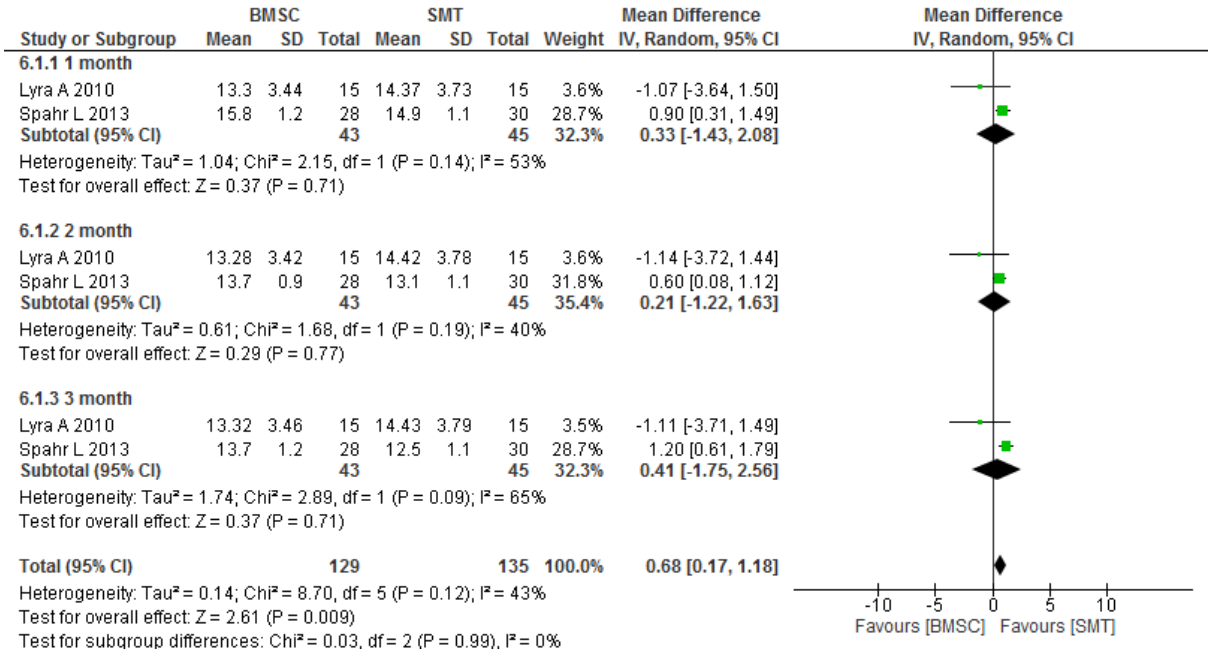


Figure 42: Model for End Stage Liver Disease (MELD) changes seen in chronic liver disease patient who had bone marrow stem cell (BMSC) therapy compared to standard medical therapy (SMT) at 1 month, 2 months and 3 months of follow up

QoL: Not many studies had reported QoL data except one study that mentioned degree of fatigue before and after treatment. In that study (121), more patients in BMSC group had improvement in fatigue score with 21 patients having less symptoms of fatigue in the BMSC group compared to 7 patients in the SMT group (52.5% vs 18.9%).

Adverse events to intervention: In 3 studies (119, 149, 150) no serious complications were reported in patients that received BMSC. GCSF therapy was well tolerated and only one patient experienced low grade fever (121). There were no reports of splenic rupture recorded due to GCSF as well as no event of PVT in patients who received cell therapy via the portal vein (130). Mild pain at the puncture site and haematoma at puncture site was noted in patients who received cells via the hepatic artery (122).

Secondary outcomes

Liver function tests: A study showed that there were no changes in ALT over 12 months FU (119). In another study (121), there was an improvement in both ALT and AST at 4 weeks in both BMSC and control compared to baseline as well as BMSC compared to SMT (BMSC: ALT baseline 75.1 ± 19.8 vs 4 weeks 35.2 ± 12.5 vs Control: ALT baseline 72.3 ± 21.5 vs 4 weeks 52.7 ± 13.4). Zhao et al (149) reported that AST improved in cell therapy group at both 6 months (p=0.034) and 12 months (p=0.05).

Regarding bilirubin, there was no improvement in bilirubin level at 52 weeks in BMSC group (baseline: 50.4 +/- 6.0 vs at 52 weeks: 41.52 +/- 8.55) (119). The data were able to be combined for 1, 3, 6 and 12 months duration in some studies (121, 122, 134) and presented in meat-analysis format. There was a significant improvement of bilirubin in BMSC groups at 1 month (MD: -8.73, 95% CI: -13.31, -4.15, p=0.0002), 6 months (MD: -10.56, 95% CI: -19.81, -1.30, p=0.03) and 12 months (MD: -9.49, 95% CI: -17.44, -1.54, p=0.02) (Figure 43).

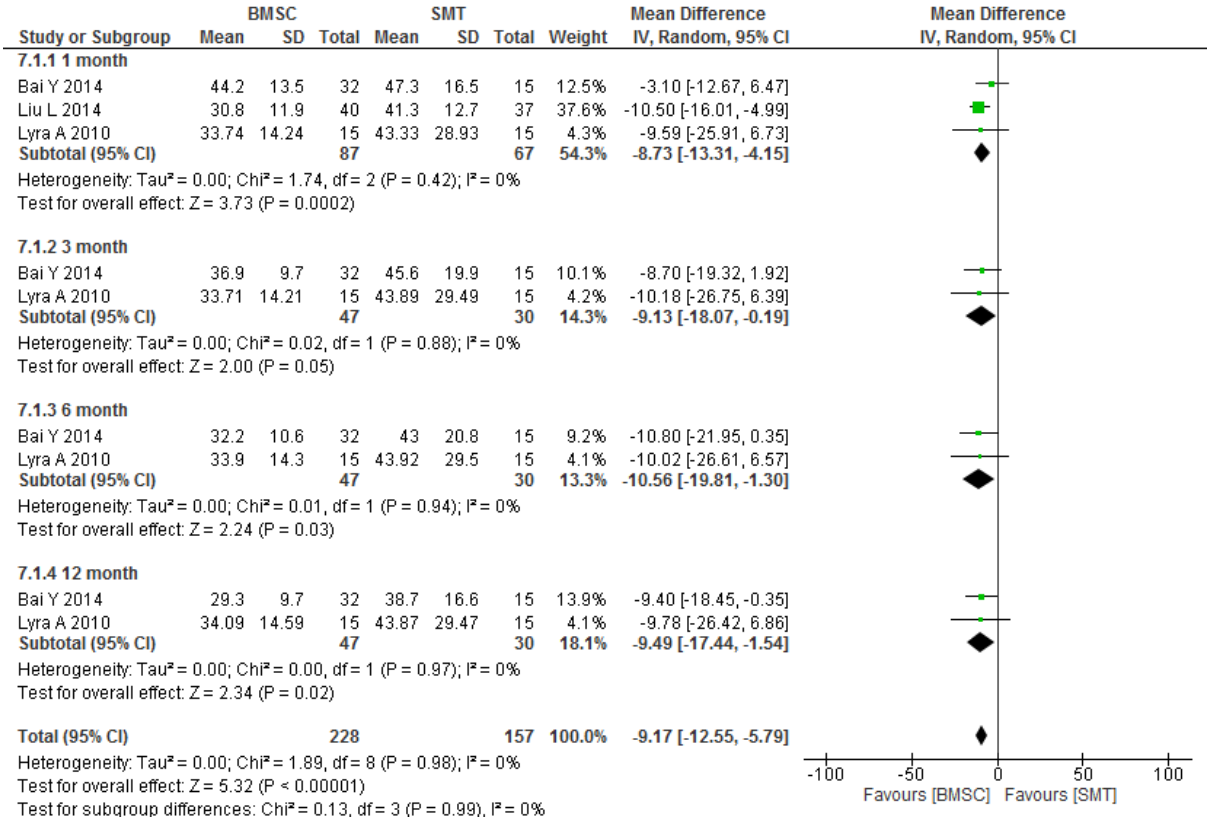


Figure 43: Bilirubin changes seen in chronic liver disease patient who had bone marrow stem cell (BMSC) therapy compared to standard medical therapy (SMT) at 1 month, 3 months and 6 months of follow up

One study found that albumin level improved at 4 weeks and 52 weeks with BMSC therapy compared to baseline, but no data was available to compare with SMT (119). In another study, albumin level improvement significantly at 6, 12 and 24 months post cell therapy (p=0.046, 0.033, 0.024) respectively (149). From the studies (121, 122, 134), BMSC therapy did not improve albumin at either 1 month (MD: 0.56, 95% CI: -2.21, 3.33, p=0.69), 3 months (MD: 0.11, 95% CI: -6.58, 6.80, p=0.97), 6 months (MD: 0.21, 95% CI: -6.79, 7.22, p=0.95) or 12 months (MD: 0.78, 95% CI: -7.50, 9.06, p=0.85) (Figure 44).

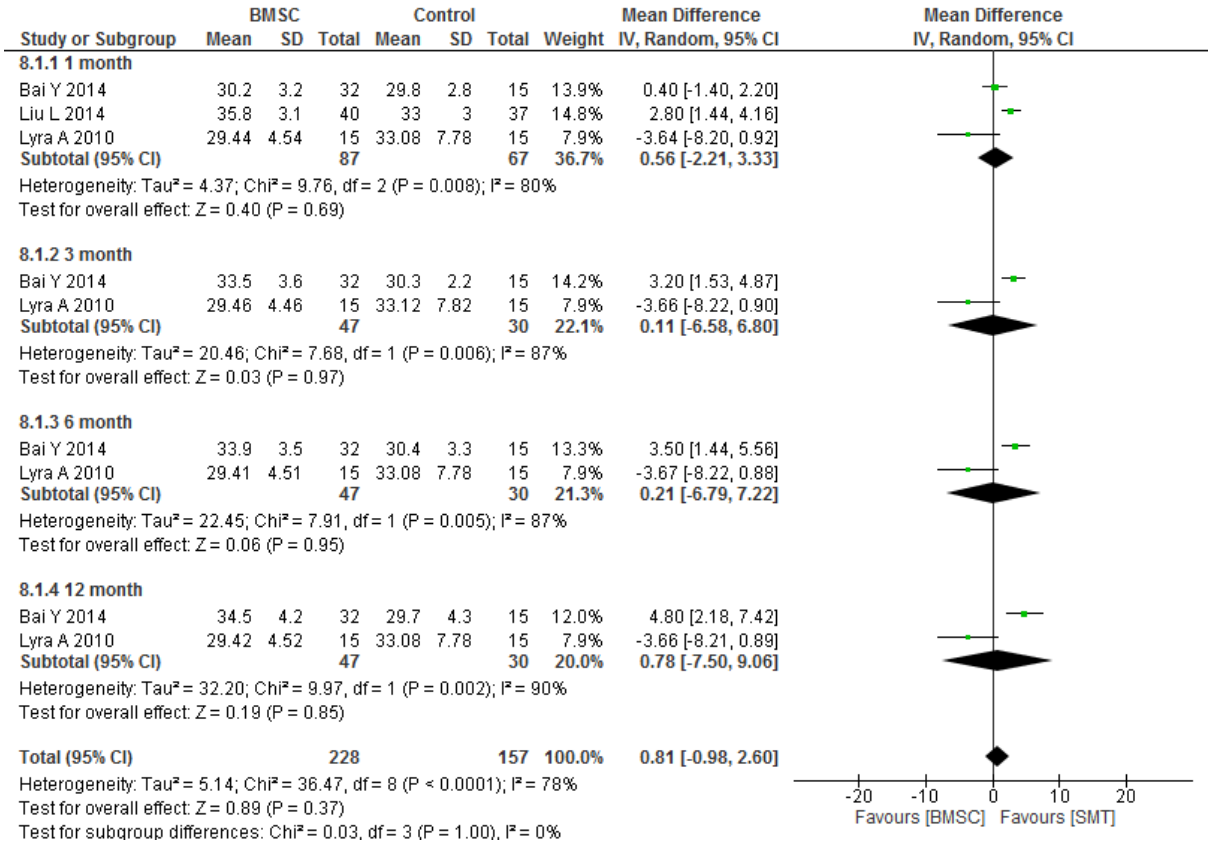


Figure 44: Albumin changes seen in chronic liver disease patient who had bone marrow stem cell (BMSC) therapy compared to standard medical therapy (SMT) at 1 month, 3 months, 6 months and 12 months of follow up

There was no difference in INR between BMSC and SMT group at 1, 2, 3, 6, 12 and 24 month in one study (122) but in another study (134), there was an improvement of PT at 3 months (p=0.046), 6 months post therapy (p=0.019) but not at 12 or 24 months.

Child Pugh Score: Three studies (119, 122, 149) had outcomes for Child Pugh Score. Huang et al mentioned that there was a reduction in CPS in both BMSC and control group (mean reduction of -0.8 in BMSC vs -2.8 in SMT group at 52 weeks) and in SMT group, there was a statistical difference in CPS compared to baseline ($p<0.05$) but not in the BMSC group. In Lyra A et al (122), CPS improved in the first 3 months in BMSC group compared to controls with mean RC of -8% in BMSC vs +5% in control group (p=0.017) but not maintained in 6 months or 12 months data. In another study (149), there was a significant improvement in CPS at 6, 12 and 24 months (p=0.001, 0.046, 0.003) respectively.

Liver decompensation events: Only one study reported that there was no event of HRS or HE in BMSC treated group (130) and 3 studies reported no event of hepatocellular carcinoma in the cell therapy group (122, 127, 130). There were no events of GI bleeding reported. (119, 130). Three studies reported that there was an improvement in ascites within 4 weeks in BMSC treated groups compared to SMT (119, 121, 150). Rates of infection reported were similar between the BMSC and SMT treated groups (127, 130, 134).

8.3 (ii): Acute-on-Chronic liver failure (ACLF)

Study characteristics

There were 6 RCT studies (57, 69, 207-210), 2 controlled studies (115, 116) and 2 non-controlled studies (211, 212). The longest duration of follow-up from RCT study was 12 months but it was a conference abstract study and only had limited information (209). Therefore, a controlled study with 12 to 18 months follow up was included in the analysis

(115). Other controlled and non-controlled studies had similar follow up duration to RCT studies and hence, were not included in the quantitative analysis.

Finally, six RCT studies (57, 69, 207-210) and one controlled study (115) were reviewed for both primary and secondary outcomes. In RCT, 2 studies examined the effect of BM-MSC (57, 207) and 4 studies looked at the effect of GCSF (69, 208-210, 213). Khanam et al 2014 (213) shared the same patient population cohort with Garg V et al (210) and therefore, results from Khanam et al paper were combined with Garg et al. In patients who received MSC therapy, they received them through peripheral vein and all had hepatitis B related liver disease. A controlled study examined the effect of UC-MSC (115) and for quantitative analysis, both BM-MSC and UC-MSC were grouped together. In studies with GCSF therapy, the dose varied from each study and they were given subcutaneously as follows: 5 ug/kg/day for 6 days (208), 5 ug/kg/day for 5 days and every 3rd day until day 26 (209), 5ug/kg twice daily for 5 days (69) and 5 ug/kg once daily for 5 days and every 3rd day for total of 12 doses (210). The details of the included studies were documented in Table 18 and appendix 8.

Table 18: Summary of the included studies (Acute-on-chronic liver failure)

Study	Country	Type of studies	Gender (male, n=) cell group/ SMT	Underlying liver condition	Cell types	Allogenic or Autologous	Control	Source of stem cells	Stem cell delivery route	Amount of stem cells given	Cell group (n=)/ SMT (n=)	FU (months)
Randomised controlled trial (RCT)												
Lin B 2017 (57)	China	Paper	53/ 51	Hepatitis B	BM-MSC	Allogenic	SMT	Bone marrow	Peripheral vein	1.0 to 10x10*5 cells/kg	56 /54	6
Chen J 2013; Lin B 2013 (207, 214)	China	Conference	No data	Hepatitis B	BM-MSC	Allogenic	SMT	Bone marrow	Peripheral vein	MSC1: 1x10*5/kg MSC2: 1x10*6/kg	Not mentioned (total number is 28)	6
Duan X 2013; Duan X 2013 (208, 215)	China	Paper; Conference	22/ 22	Hepatitis B	GCSF	Autologous	SMT	No collection	No cell delivered externally	Not applicable	27/ 28	3
Engelmann, C. 2015 (209)	Germany	Conference	No data	not specified	GCSF	Autologous	SMT	No collection	No cell delivered externally	Not applicable	No data yet (Trial due to start in 2015)	12
Singh V 2014; Singh V 2013 (69, 216)	India	Paper; Conference	23/ 23	Alcohol	GCSF	Autologous	SMT	No collection	No cell delivered externally	Not applicable	23/ 23	3

Study	Country	Type of studies	Gender (male, n=) cell group/ SMT	Underlying liver condition	Cell types	Allogenic or Autologous	Control	Source of stem cells	Stem cell delivery route	Amount of stem cells given	Cell group (n=)/ SMT (n=)	FU (months)
Garg V 2012; Garg V 2010; Khanam A 2014 (210, 213, 217)	India	Paper; Conference ; Paper	20/ 21	Mixed aetiologies	GCSF	Autologous	SMT + Saline injection as control	No collection	No cell delivered externally	Not applicable	23/ 24	2
Controlled studies												
Shi M 2012 (115)	China	Paper	20/ 15	Hepatitis B	UC-MSC	Allogenic	Saline injection as control	Umbilical cord	Peripheral vein	~0.5x10*6 per kg// given 3x at 4 weeks interval	24/ 19	12 to 18
Weng W 2013 (116)	China	Conference	No data	Hepatitis B	BM-MSC	Allogenic	SMT // control (matched for age, sex, biochemical indexes)	Bone marrow	not mentioned	1x10*5-6/kg for 4 weeks (time of 1st dose different)	MSC rise (12)/ SMT rise (12) MSC plateau/ SMT plateau (33)	3
Non-controlled studies												
Park C 2013 (212)	South Korea	Paper	2/ 0	Mixed aetiologies	BM-MNC	Autologous	no control	Bone marrow	hepatic artery	pt 1: 26.25 (x10*6/kg) pt 2: 4.1 (x10*6/kg) pt 3: 12.4 (x10*6/kg)	5/0	4

Study	Country	Type of studies	Gender (male, n=) cell group/ SMT	Underlying liver condition	Cell types	Allogenic or Autologous	Control	Source of stem cells	Stem cell delivery route	Amount of stem cells given	Cell group (n=)/ SMT (n=)	FU (months)
										pt 4: 8.84 (x10*6/kg) pt 5: 6.82 (x10*6/kg)		
He H 2015 (211)	China	Conference	No data	Hepatitis B	UC-MSC+ plasma exchange	Allogenic	no control	umbilical cord	Peripheral vein	1x10*5 cells/kg once a week, 4 times	5/0	3

BM: Bone marrow, MSC: Mesenchymal stem cell, GCSF: Granulocyte colony stimulating factor, SMT: Standard medical therapy, UC: Umbilical cord, MNC: Mononuclear cell

Primary outcomes

Overall patient mortality: Two studies (57, 115) with MSC therapy had survival outcome data although their study design and FU duration were dissimilar and hence they were unable to be combined. In Lin et al study (57), the survival rate in MSC group was 73.2% compared to 55.6% in SMT group for 6 months FU. In Shi M 2012 study (115), the survival rate in MSC group was 87.5% compared to 89.5% in SMT at 18 months FU.

Three RCT studies with GCSF therapy had patient survival outcome data during 3 months of FU and hence, the data were combined (69, 208, 210). The analyses showed that patients with ACLF who received GCSF therapy tended to have significantly improved survival outcome at 3 months (OR=0.16, 95% CI: 0.08, 0.34, p<0.00001) (Figure 45). The causes of death in each study were shown in Table 19. Most patients died from sepsis and multi-organ failure.

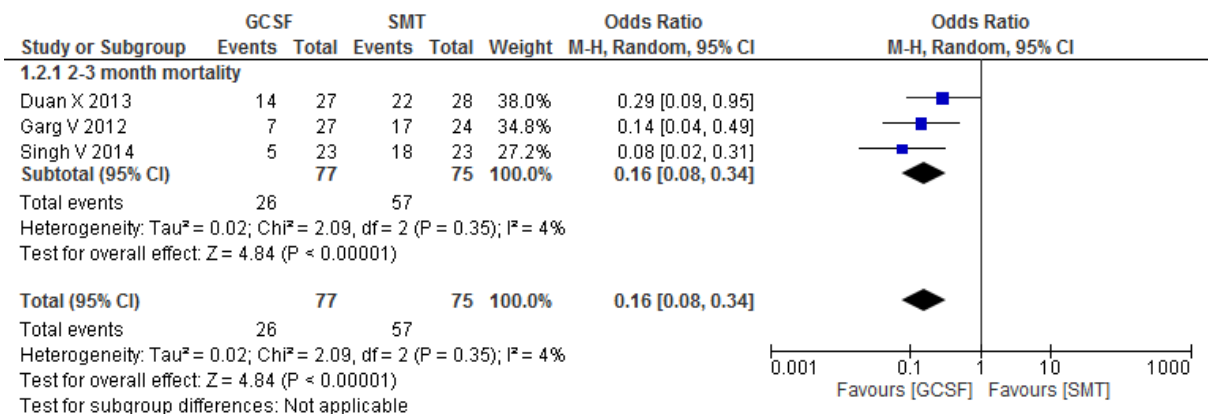


Figure 45: Overall patient survival of patients with acute on chronic liver failure (ACLF) who had granulocyte colony stimulating factor (GCSF) compared to standard medical therapy (SMT) at 3 months of follow up

Table 19: Causes of death reported in patients with acute on chronic liver failure (ACLF) who had granulocyte colony stimulating factor (GCSF)

Study ID	Study design	Cell therapy	Complications			
			Total death in cell group	Cell group	Total death in standard therapy	Standard medical therapy group
Lin B 2017**(57)**	RCT	BM-MSC	N=15	Severe infection (n=18) Hepatic coma (n=7) HRS (n=10) GI bleed (n=2) MOF (n=20)	N=24	Severe infection (n=9) Hepatic coma (n=4) HRS (n=5) GI bleed (n=1) MOF (n=10)
Duan X 2013**(208)**	RCT	GCSF	N=14	Hepatic encephalopathy (n=4) GI bleed (n=5) HRS (n=2) Sepsis (n=3)	N=22	Hepatic encephalopathy (n=6) GI bleed (n=3) HRS (n=6) Sepsis (n=7)
Singh V 2014**(69)**	RCT	GCSF	N=5	MOF (n=1) Infection from pneumonia/SBP (n=2) SBP, sepsis and renal failure (n=2)	N=18	GI bleed, pneumonia, sepsis and HRS (n=3) Pneumonia, sepsis and HRS (n=5) Urine infection and renal failure (n=3) Septic shock (n=7)
Garg V 2012 **(210)**	RCT	GCSF	N=7	Liver failure (n=2) MOF (n=3) GI bleed (n=2)	N=17	Liver failure (n=1) MOF (n=12) GI bleed (n=2) Intracranial bleed (n=1)

Liver transplant free survival: None of the studies reviewed the effect of stem cell on liver transplant free survival.

MELD: Three studies of MSC (57, 115, 207) and GCSF (69, 208, 210) therapies reported MELD data. However, the data were not suitable to combine for meta-analysis due to differences in statistical method and follow up time frame. Therefore, the data were presented in Table 20. In studies with MSC therapy, one study (57) showed improvement of MELD in week 1 and 2 after therapy and other studies (115, 207) showed improvement from week 4 to week 12.

In GCSF groups, there is improvement of MELD within 4 weeks in 2 studies (69, 208). In GCSF treated patients, one study showed improvement of MELD at week 1 and 2 (208) and 2 studies (69, 208) showed improvement of MELD at week 4.

Table 20: Model of end stage liver disease (MELD) changes seen in acute on chronic liver failure (ACLF) who had granulocyte colony stimulating factor (GCSF) at baseline, 1 week, 2

weeks, 3 weeks, 1 month, 3 months and 6 months of follow up when compared to standard medical therapy (SMT)

Study ID	**Cell therapy**	**Statistics**	**MELD**							
			Baseline	**Week 1**	**Week 2**	**Week 3**	**1 month**	**2 months**	**3 months**	**6 months**
Lin B 2017 (57)	MSC	*Median and range*	24.8 (22.4-28.3)	24.1 (21.5-27.5) **p=0.01**	24.1 (20.1-26.4) **p=0.02**	24.3 (20.3-27.6)	23 (18.2-27.7)	19.1 (12-24.1)	14.7 (9.7-20.4)	11 (7.4-13.9)
	SMT		25.2 (22.8-27.8)	26.6 (23.1-30.4)	25.8 (22.2-29.1)	24.5 (20.8-30.2)	22.8 (19.6-29)	18.4 (14.3-26.4)	14.8 (11.5-19.6)	11.3 (6.9-14.8)
Chen J 2013 (207)	MSC vs SMT	*Not mentioned*				**p=0.024**	**p=0.018**	**p=0.045**		
Shi M 2012 (115)	MSC vs SMT	*Not mentioned*	p=0.099				**p= 0.039**	**p=0.042**	**p=0.040**	
Duan X 2013 (208)	GCSF	*Mean +/- SD*	25.11 +/- 3.3	24.4 +/- 3.9 **P=0.004**	23.7 +/- 5.8, **p<0.001**		23.3 +/- 6.9, **p<0.001**			
	SMT		26.3 +/- 4.12	27.6 +/- 4.1	28.4 +/- 4.5		29.8 +/- 5.7			
Singh V 2014 (69)	GCSF	*Median*	27	24			**p=0.05**		p=0.078	
	SMT		30	29						
Garg V 2012 (210)	GCSF	*Median (range)*	29 (21-40)				21 (18–40)	16 (10–20)		
	SMT		31.5 (20-40)				34 (23–40)	38 (21-48)		

Quality of life (QoL): None of the studies reported QoL in ACLF patients who received cell therapies.

Adverse events to intervention: In patients who received MSC therapy, one study (57) mentioned that 15 patients from MSC group had fever compared to 12 patients in control group within first 4 weeks of infusion which had increased significantly in 5 to 24 weeks post infusion period (n=10 in MSC group vs n=1 in SMT group, p=0.02). This study also showed that 8 patients had rash in MSC group compared to 7 patients in control group as well as 7 patients in each group had diarrhoea. Another study from Shi et al also mentioned that two patients who received MSC infusion developed fever within 2 to 6 hours of infusion and resolved within 12 hours of infusions but none in the control group developed fever (115).

Three studies (69, 208, 210) reported the adverse events secondary to GCSF injection and among these 3 studies, low grade fever (n=9), headache (n=7), nausea (n=4), bone pain (n=3), rash (n=1) and herpes zoster infection (n=1) were found in patients who received GCSF therapy.

Secondary outcomes

Liver function tests: The data were expressed differently in each study which made it not possible to combine the data together for analysis. In the MSC treated group, there was an significant improvement of ALT during week 1 (57, 207) and week 2 (207) compared to baseline although in another study (115), there was an significant improvement in week 8 and 48 weeks post-MSC therapy compared to SMT group. There was no improvement of bilirubin level in MSC treated group in one study within 24 weeks FU (57) but in another study, it showed significant improvement in the MSC group compared to SMT group at week 36 and 48 (115). Albumin level was significantly improved 1 week after MSC therapy in one study (57) but in another study, it showed improvement in MSC group at week 12, 24, 36 and 48 weeks compared to SMT group (115). INR improved significantly in week 2 and 4 after MSC therapy (207) but in another study, it improved at week 1, 2, 4, 36 and 48 weeks after MSC therapy compared to SMT group (115).

In GCSF treated groups, no data were available for ALT and albumin changes. One study showed that bilirubin was improved significantly compared to baseline in GCSF treated group in week 4 and 8 post therapy (210). One study (210) documented the change in INR within 8 weeks of GCSF therapy but there was no difference noted with therapy.

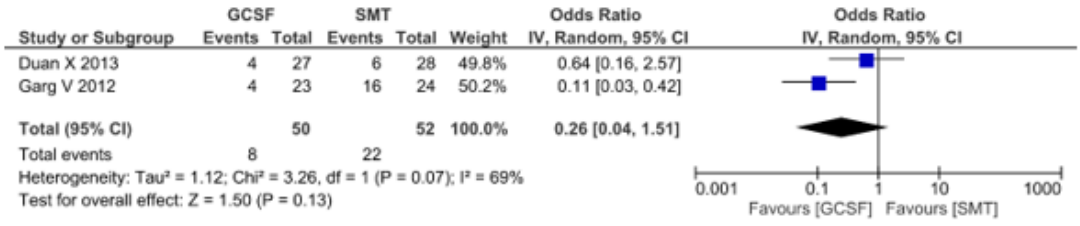
Child Pugh Score: None of the MSC studies had outcomes for Child Pugh score.

One study of GCSF showed a significant improvement of CPS in GCSF group compared to control group (208). In Singh et al paper (69), there was a significant reduction in median delta change percentage in CPS at 1, 2 and 3 months post GCSF ($p<0.05$). Another study from Garg et al (210) showed that there was a significant median change CPS improvement at days 7, 30 and 60 in GCSF treated group.

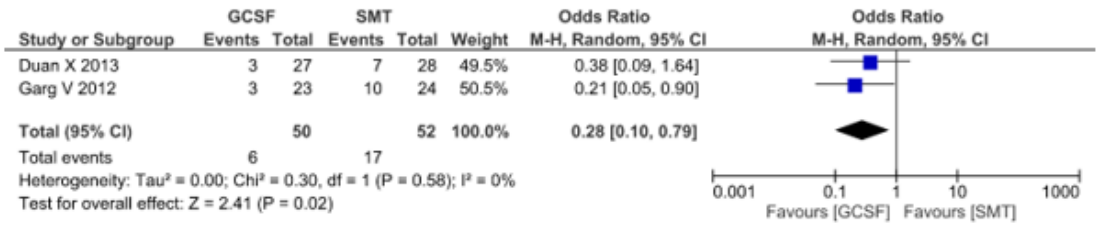
Events of liver decompensation: For MSC treated group, only one study (57) mentioned liver decompensation events and it showed that patients who had MSC therapy experienced less liver decompensation events compared to the control group: MSC group had hepatic encephalopathy (n=8), gastrointestinal bleed (n=1), HRS (n=6) and infection (n=14) compared to control group whom had hepatic encephalopathy (n=14), gastrointestinal bleed (n=3), HRS (n=12) and infection (n= 24). No one developed hepatocellular carcinoma in that study.

For studies with GCSF therapy, one study (69) showed that there was a significant reduction of ascites in GCSF group compared to control group (p=0.047) at 3 months FU. Two studies (208, 210) reported the event of hepatic encephalopathy (HE), sepsis and hepatorenal syndrome (HRS) and all patients who received GCSF had significantly less episodes of sepsis (OR=0.28, 95% CI: 0.10, 0.79, p=0.02) and HRS (OR=0.14, 95% CI: 0.04, 0.47, p=0.001) compared to control group over 2-3 months FU but not in event of HE (OR=0.26, 95% CI: 0.004, 1.51, p=0.13) or GI bleeding (OR=1.53, 95% CI: 0.45, 5.24, p=0.50) (Figure 46a-46d) .

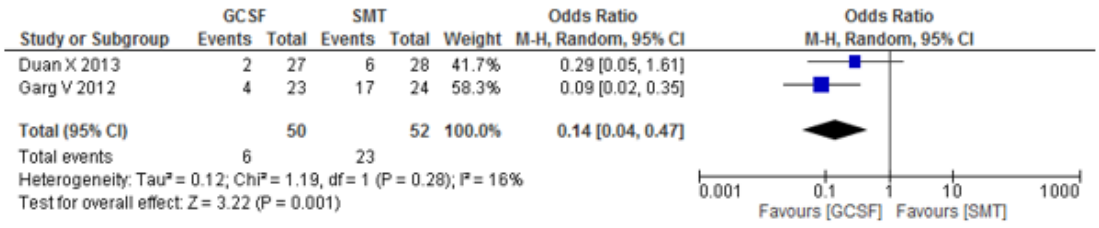
A: Hepatic encephalopathy in ACLF (granulocyte colony stimulating factor vs standard medical therapy)



B: Sepsis seen in ACLF (granulocyte colony stimulating factor vs standard medical therapy)



C: Hepatorenal syndrome in ACLF (granulocyte colony stimulating factor vs standard medical therapy)



D: Gastrointestinal bleeding in ACLF (granulocyte colony stimulating factor vs standard medical therapy)

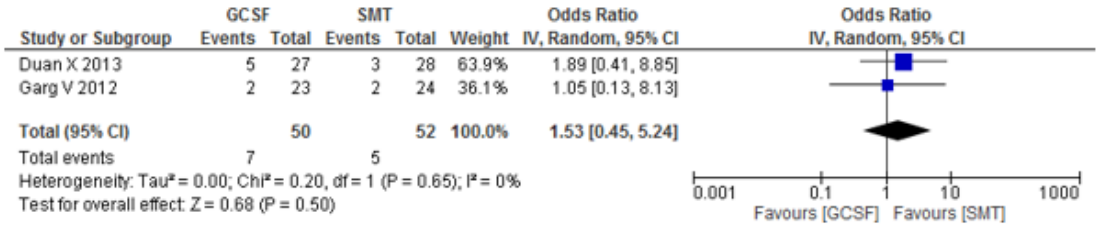


Figure 46 (A-D): Hepatic decompensation events in patients with acute on chronic liver failure who had granulocyte colony stimulating factor (GCSF) compared to standard medical therapy (SMT): A: Hepatic encephalopathy event, B: Sepsis, C: Hepatorenal syndrome, D: Gastrointestinal bleeding

8.4: Subgroup analysis

Subgroup analysis were planned based on cell administration route (central vs peripheral) or the source of stem cell (autologous vs allogenic) for each population (CLD and ACLF) and each cell types (as in MSC, HSC, BMSC and GCSF). However, the analyses were not performed due to smaller number of studies when grouping them together with significant heterogeneity among studies.

8.5: Discussion

The current systematic review and meta-analysis aimed to analyse the clinical effectiveness of stem cell therapies in patients with CLD and ACLF. The stem cells of interest were HSC, MSC,

BMSC and GCSF and the outcomes interested were mortality of patients, clinical improvements assessed by QoL, MELD, CPS, biochemical blood tests as well as the safety profile of cell therapies.

8.5 (i): Chronic liver disease

This systematic review showed that GCSF therapy significantly improved mortality at 3 for ACLF and 6 months for CLD post therapy although in our clinical study (82), GCSF did not improve survival of patients compared to either SMT or HSC group. There was no difference in survival found between MSC and SMT group for 12 months FU duration. HSC did not improve survival at 12 months and at 5 years FU when compared to SMT. Moreover, no other cell therapies seemed to improve overall patient survival compared to SMT. Regarding GCSF, HSC and BMSC treated groups, there were no significant differences in LT survival rate when compared to SMT group. There were no data available for LT survival in MSC treated group.

There were significant variations in the reporting of MELD. MELD improved at 6 months in one study (120) but not in 2 other studies (82, 130) when treated with GCSF. MELD significantly improved in MSC treated group at 3 and 6 months but not in 12 months. MELD improved in HSC treated group at 6 months in the systematic review but not in longer term follow up at 12 months or even at 5 years. In our clinical study (82), MELD was not improved in any of the study group. In patients who had BMSC treatment, MELD did not improve either in short term (3 months) or long term (12 months) follow up period. From these studies, all cell therapies (GCSF, MSC and HSC) MELD seemed to improve at shorter term (up to 6 months) but not in longer term.

QoL data was sparse amongst studies. Newsome et al (82) measured QoL in GCSF treated group but there was no significant improvement at 3 months when compared with SMT. Studies from MSC (117) and HSC (126, 143) showed improvement in fatigue and performance score at 6 months. BMSC treated group also had improvement in fatigue score at 4 weeks post treatment (121). All cell therapies had a good safety profile with no significant event of portal vein thrombosis compared to SMT. Fever is a common side effects seen in patients that had treatment with GCSF or MSC therapies.

Child Pugh score was improved significantly with GCSF therapy up to 6 months post treatment, but we did not find improvement of CPS in our clinical study (82). There was an improvement of CPS at 6 and 12 months for MSC treated group. No difference in improvement was noted for HSC treated group either at 12 or 124 months of FU.

Liver cancer incidence did not increase with cell therapies (GCSF, MSC, HSC or BMSC). Events of HRS were similar between cell treated groups (GCSF or BMSC) and SMT group. SBP was similar between GCSF and SMT. Sepsis rate was lower in GCSF group, but no difference was found in BMSC group compared to SMT. There was no difference in event of HE between cell therapies (GCSF, MSC, HSC and BMSC) and SMT groups. Ascites seemed to improve in all cell therapies (GCSF, MSC, HSC and BMSC) between 6 and 12 months duration. There seemed to be reduction of GI bleed in either MSC, HSC of BMSC treated groups. In patients that received stem cells through portal vein, the incidence of portal vein thrombosis was similar between stem cell and SMT groups.

8.5 (ii): Acute on chronic liver failure

Similar survival outcome was found between MSC and SMT group. However, GCSF therapy significantly improved survival at 3 months compared to SMT. No studies reported LT free survival. There was an improvement towards MELD in both MSC and GCSF treated group compared to SMT group in short term FU (up to 4 weeks) but unable to combine data for analysis. No QoL data available from any of the studies.

Fever was common in both MSC and GCSF treated cohorts, but no other serious events were documented. There was no significant change in ALT, bilirubin for MSC treated group but albumin and INR showed some improvement at 48 weeks FU. There were no data available for albumin and ALT in GCSF treated groups, but bilirubin improved at 8 weeks post therapy, but they were not significant compared to SMT. MSC treated group had no data for CPS and in GCSF, two studies (69, 210) showed improvement of CPS at 3 months. MSC treated groups had lesser events of liver decompensation compared to SMT. GCSF improved ascites at 3 months with less patients developed sepsis, HRS but no difference was found in GI bleed event. No HCC was documented in cell therapy group.

8.5 (iii): Strength of the study

There were many strengths in this study. This is the only study so far with most recent literature searches up to 2017 with the largest numbers of included randomised controlled trials. Prior to starting this review, a well thought out protocol was written in regard to population, interventions, outcomes and study that we were interested to review. The plan on how to handle the data and how to analyse were clearly thought out and written in the protocol paper before starting. The eligibility criteria for the study population was clear in that we excluded patients who had liver surgery or had underlying liver cancer.

8.5 (iv): Limitations of the study

There were several limitations. Although we aim to include non-English studies, due to time constraint, we were unable to translate non-English papers. However, for RCT, there were only 2 papers in Chinese language that did not include in analysis and it is unlikely to make significant difference in the outcomes of the studies. Although the searches were done as comprehensive as possible, it might not be enough to cover all related references. To remove irrelevant articles, one reviewer screened all the titles and abstracts and to ensure consistency, another reviewer checked a proportion (minimum 50% of all articles) independently. This way of screening articles was a limitation of the study due to this project being unfunded.

The analyses were performed according to cell types, types of liver disease and study design although there was still significant heterogeneity in the aetiologies of liver diseases. We were unable to do subgroup analysis on type of liver disease or route of stem cell administration due to limited number of studies as well as heterogeneity across the studies. Regarding performing meta-analysis, there was variations in the statistical methods that data was presented and the time frame that the data was collected which makes the direct comparison difficult. That leads to inherent weakness in combing heterogeneous data sets at time although we tried to limit this variation by combining similar cell types and study design using the random effect model although it might not still eliminate the effect of heterogeneity.

Due to limitations with availability of data, the source of the stem cells was not differentiated (as in bone marrow-MSC vs umbilical cord-MSC). Although sub-group analyses were planned, the number of studies when breaking down into each category became small that any meaningful outcomes could not derived from the analyses.

From these studies, we were unable to report on the optimal dose and duration of GCSF therapy due to significant variations across studies. With that, the benefits of the therapy may be sub-optimal in some studies with lesser dose or duration of treatment.

9. OVERALL DISCUSSION

Incidence and the mortality associated with chronic liver disease is rising and the only curative modality is liver transplantation. Due to increased demand but stable organ donation rate, some patients die while waiting on the list for organ. Stem cell therapies had been promising and over the past decade, various types of stem cells in various form of liver diseases had been studied either in animal or human. Most animal studies showed improvement of liver fibrosis after stem cell therapy (218-220).

As part of the thesis, I worked on the previous animal project completed by one the researcher in our unit. The CCl_4 chronic liver injury mice model was given repeated infusion of HSC cells. I worked on the response of oval cell reaction by staining liver tissue with pan-CK and Sox-9 staining. Pan-CK staining was significant higher in the livers of KSL treated mice although Sox-9 staining was similar between KSL treated and control mice. The probable reason for this difference is that Sox-9+ cells likely only contribute a minor response towards hepatic regeneration process. This is supported by the study from Tarlow et al which showed Sox9+ ductal proliferation makes only a minor contribution to parenchymal regeneration in liver injuries (81). KSL treated mice had significantly higher levels of MMP-9 and 12 compared to control which potentially increased the process of breaking down fibrosis in ECM remodelling. For qPCR analysis, MMP-9 was significantly higher in KSL treated mice but not for other MMPs (2,8,12 and 13). Individual macrophage markers (iNOS and Arg-1) were similar between the two groups although the Arg-1/NOS-2 ratio was significantly lower in KSL treated mice indicating a change in the fibrotic milieu. Due to the benefit of repeated stem cell infusions in animal mode, we proceeded to a phase II clinical trial. It was a multi-central open label randomised study and we recruited 81 patients in total. They were randomised to 3 groups: standard care, GCSF only and GCSF and stem cell (CD133+) infusion group. The trial primary outcome was change of delta MELD in day 90 from day 1 (at randomisation). There was no significant improvement of MELD in all 3 groups. All the other secondary outcomes were not significant either. Our study finding contrasted with many clinical trials (96, 110, 114, 221) that had been performed and published with improvement in function of the liver. Therefore, a systematic review was conducted to analyse whether stem cell therapies was effective treatment in patients with liver diseases. We examined all types of stem cells (HSC, MSC, BMSC and GCSF) in patients with CLD or ACLF. The systematic review showed that GCSF therapy significantly improved mortality at 3 months for ACLF patients and 6 months for CLD patients although we did not find similar finding in our clinical trial (82). There was no

difference in LT free survival between any stem cell therapies and standard medical therapy groups. MELD improved significantly in MSC treated groups at 3 and 6 months but not with any other cells. Albumin improved in MSC group up to 6 months and for HSC, the improvement is only up to 3 months. Bilirubin improved at 6 months for all cell types (HSC, MSC, BMSC) except GCSF group. INR was significantly improved in MSC treated group at 3, 6 and 12 months but only improved at 3 months for HSC group.

In our trial (82), neither GCSF nor HSC infusion improve overall survival, MELD score, CPS, QoL or clinical blood parameters compared to SMT. For GCSF, our study was dissimilar to systematic review because in systematic review, GCSF improved patient survival at 6 months compared to SMT but not in longer term at 12 months. More studies are required to understand the effect of GCSF therapy in patients with liver diseases. For HSC infusion, neither mortality, MELD or liver enzymes were improved in both our trial and in systematic review.

10: CONCLUSION

Stem cells showed degree of improvement in biochemical markers, MELD and CPS only for shorter duration (<6 months). We needed well designed, adequately powered, robust randomised studies with longer duration of follow up (more than 12 months) and large number of patients. We will need to have studies with more robust clinical outcomes to examine the clinical efficacy of stem cell therapy. I do not think that change sin MELD or Fibroscan may not be a good clinical end outcome. We need to have significant changes in these findings to interpret meaningfully in clinical settings. We may need to consider other means to determine the progression of fibrosis such as spleen stiffness measurement or performing hepatic venous pressure gradient (HVPG) measurement. We also need to perform *in-vivo* tracking of stem cells to examine the clinical effectiveness of stem cell therapies in patients with liver diseases.

In summary, stem cells therapy in liver cirrhosis is still an emerging filed and need better understanding on pathogenesis of liver fibrosis and how stem cells can improve the fibrosis. Further robust randomised clinical trials are before stem cells can be used in daily clinical practice to manage patients with liver diseases.

11: APPENDICES

Appendix 1: Chronic liver disease questionnaire (CLDQ)

General	**Patient trial number** **Date of questionnaire** **Assessment: Day 1 (screening)** **Day 90 (visit 5)** **Day 180 (visit 6)** **Day 360 (visit 7)**
This questionnaire is designed to find out how you have been feeling during the last two weeks. You will be asked about your symptoms related to your liver disease, how you have been affected in doing activities, and how your mood has been. Please complete all of the questions and select only one response for each question.	
The answer should be selected from one of the following choices for any given questions.	1. All of the time 2. Most of the time 3. A good bit of the time 4. Some of the time 5. A little of the time 6. Hardly any of the time 7. None of the time
	How much of the time during the last 2 weeks...??
Question 1	Have you been troubled by a feeling of abdominal bloating?
Question 2	Have you been tired or fatigued during the last two weeks?
Question 3	Have you experienced bodily pain?
Question 4	Have you felt sleepy during the day?
Question 5	Have you experienced abdominal pain?
Question 6	Has shortness of breath been a problem for you in your daily activities?
Question 7	Have you not been able to eat as much as you would like?
Question 8	Have you been bothered by having decreased strength?
Question 9	Have you had trouble lifting or carrying heavy objects?
Question 10	Have you felt anxious?
Question 11	Have you felt a decreased level of energy?
Question 12	Have you felt unhappy?

Question 13	Have you felt drowsy?
Question 14	Have you been bothered by a limitation of your diet?
Question 15	Have you been irritable?
Question 16	Have you had difficulty sleeping at night?
Question 17	Have you been troubled by a feeling of abdominal discomfort?
Question 18	Have you been worried about the impact of your liver disease has on your family?
Question 19	Have you had mood swings?
Question 20	Have you been unable to fall asleep at night?
Question 21	Have you had muscle cramps?
Question 22	Have you been worried that your symptoms will develop into major problems?
Question 23	Have you had a dry mouth?
Question 24	Have you felt depressed?
Question 25	Have you been worried about your condition getting worse?
Question 26	Have you had problems concentrating?
Question 27	Have you been troubled by itching?
Question 28	Have you been worried about never feeling any better?
Question 29	Have you been concerned about the availability of a liver if you need a liver transplant?

Appendix 2: Trial schedule

	Screening	Treatment					Follow up	
	Visit 1 (screening)[1,2]	Visit 2a-2e[1] (1st- 5th day of treatment)[7]	Visit 2f (5th and 6th day of treatment)[7]	Visit 3 (day 30)	Visit 4 (day 60)	Visit 5 (day 90)	Visit 6 (day 180)	Visit 7 (day 360)
Informed consent	X							
Clinical assessment[2]	X	X	X	X	X	X	X	X
Vital signs[3]	X	X	X	X	X	X	X	X
Screening blood tests[4]	X							
ECG	X							
Standard blood tests[5]	X	X	X	X	X	X	X	X
Mandatory microbiology[7]	X							
Abdominal USS	X		X				X	
Fibroscan	X					X	X	X
ELF panel	X			X	X	X	X	X
CLDQ	X					X	X	X
G-CSF administration		Group 2 Group 3						
Leukapharesis			Group 3					
Blood test for circulating CD34+		Group 2 Group 3	Group 2 Group 3					
Blood test for circulating CD133+		Group 2 Group 3	Group 2 Group 3					
CD133+ cell infusion			Group 3	Group 3	Group 3			
Adverse effects[6]	X	X	X	X	X	X	X	X
Clinical events[6]		X	X	X	X	X	X	X
Concomitant medication	X	X	X	X	X	X	X	X

(1) For patients in groups 1 and 2 visits 1 and visit 2a should be combined into 1 day where possible. For patients in group 3, timing of visit 2a will depend on scheduling of leukapheresis. (2) All screening tests must be completed less than 7 days prior to randomisation and treatment and must start less than 7 days following randomisation. Day of randomisation will be considered as day 1 for scheduling purposes. (3) Clinical assessment consists of complete history and examination at screening and focused history and relevant examination at subsequent visits. (4) Vital signs to include heart rate, blood pressure, temperature and weight. (5) Screening blood tests as detailed in section 5. (6) Standard blood

tests consist of full blood count, urea and electrolytes, liver function tests, magnesium and alanine aminotransferase (ALT) international normalised ratio (INR). (7) Adverse effects and clinical events will be monitored continuously until completion of follow-up. Serious adverse events (SAE's) will be reported from the date of consent. All adverse events experienced by patients will be recorded irrespective of the causality (see section 7). (8) Mandatory microbiological testing must be performed between 7 and 30 days prior to leukapheresis—HBV, HCV, HIV, human T-lymphotropic virus 1 and 2 (HTLV-1, HTLV-2) and syphilis. (9) The first re-infusion of CD133+ (group 3 patients only) will occur on one occasion only between days 6 and 10. The timing will be determined by the timing of each patient's leukapheresis. CD133+ cell isolation and the local hospital arrangements (72)

Appendix 3: Search strategy used in MEDLINE, MEDLINE in Process, EMBASE

1. exp Acute-On-Chronic Liver Failure/or exp Liver Diseases/or exp Liver Regeneration/or exp End Stage Liver Disease/or exp Liver Cirrhosis/

2. liver disease$. ti, ab.

3. liver cirrhosis. ti, ab.

4. acute-on-chronic liver failure. ti, ab.

5. liver regenerat$. ti, ab.

6. exp Hematopoietic Stem Cells/or exp Stem Cells/or exp Stem Cell Transplantation/or exp Bone Marrow/

7. exp Mesenchymal Stromal Cells/

8. stem cell$. ti, ab.

9. exp Granulocyte Colony-Stimulating Factor/

10. granulocyte colony stimulating factor. ti, ab

11. mesenchymal stromal cell. ti, ab.

12. 1 or 2 or 3 or 4 or 5

13. 6 or 7 or 8 or 9 or 10 or 11

14. 12 and 13

15. limit 14 to (humans and yr=”1990-Current”)

Appendix 4: Details of excluded studies of the systematic trial

Name of author	Year of publication	Types	Reason for exclusion
Alzoubi	2013	Paper	Editorial paper
Anand	2014	Conference abstract	Outcomes interested not measured
Anand	2016	Conference abstract	Outcomes interested not measured
Annonymous	2012	Paper	Review paper
Annonymous	2014	Paper	Review paper
Bica	2012	Conference abstract	Outcomes interested not measured
Bihari	2016	Paper	Outcomes interested not measured
Brodosi	2012	Conference abstract	Outcomes interested not measured
Burganova	2015	Conference abstract	Outcomes interested not measured
Burganova	2015	Conference abstract	Outcomes interested not measured
Burganova	2017	Conference abstract	Outcomes interested not measured
Cardinale	2014	Paper	Outcomes interested not measured
Cardinale	2014	Paper	Outcomes interested not measured
Catani	2012	Conference abstract	Outcomes interested not measured
Chen HQ	2010	Paper	No abstract and full text in Chinese
Engelmann	2015	Conference abstract	Outcomes interested not measured
Engelmann	2016	Paper	Acute liver failure patients included
Kantarcioglu	2013	Conference abstract	Outcomes interested not measured
Kantarcioglu	2015	Conference abstract	Outcomes interested not measured
Kim JK	2015	Conference abstract	Outcomes interested not measured
Liu LY	2010	Paper	Outcomes interested not measured
Peng	2011	Paper	Outcomes interested not measured
Pan XN	2014	Paper	Systematic review paper
Qi X	2015	Paper	Review paper
Qin AC	2010	Paper	Outcomes interested not measured
Terai	2012	Paper	Review paper
Yao P	2005	Paper	No abstract and full text in Chinese
Yang H Z	2013	Paper	No abstract and full text in Chinese
Yang H	2015	Paper	No abstract and full text in Chinese
Yu SJ	2016	Paper	No abstract and full text in Chinese
Zheng L	2013	Paper	Outcomes interested not measured

Appendix 5: **Preferred Reporting Items for Systematic Reviews and Meta-Analyses (*PRISMA) flow chart of existed systematic review**

			Liu Z 2016 (90)	**Yang Q 2016 (95)**	**Chavez -Tapia 2015 (64)**	**Kim G 2015 (89)**	**Ma X-R 2015 (91)**	**Qi X 2015 (93)**	**Wang K 2015 (94)**	**Moore J 2014 [44]**	**Pan X-N 2014 (92)**
Section/topic	#	Checklist item	page #	page #	page #	page #	page #	page #	page #	page #	page #
TITLE											
Title	1	Identify the report as a systematic review, meta-analysis, or both.	499	90	631	1405	1	166	147	673	14051
ABSTRACT											
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	499	90	631	1405	1	166	147	673	14051
INTRODUCTION											
Rationale	3	Describe the rationale for the review in the context of what is already known.	500	90	NR	1405	2	NR	148	674	14052
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	500	90	632	1406	2	167	148	674-675	14052
METHODS											

			Liu Z 2016 (90)	**Yang Q 2016 (95)**	**Chavez -Tapia 2015 (64)**	**Kim G 2015 (89)**	**Ma X-R 2015 (91)**	**Qi X 2015 (93)**	**Wang K 2015 (94)**	**Moore J 2014 [44]**	**Pan X-N 2014 (92)**
Section/topic	#	Checklist item	page #	page #	page #	page #	page #	page #	page #	page #	page #
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	NR	NR	NR	NR	NR	NR	NR	NR	NR
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	NR	NR	632-635	1406	2	167-168	148-149	674-675	14052
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	500	90-91	632,635	1406	2	167-168	149	674-675	14052
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	NR	NR	633-634	NR	2	178-179	NR	674	NR
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	500-501	90-91	635	1406	2	168	148-149	675	14052

			Liu Z 2016 (90)	**Yang Q 2016 (95)**	**Chavez -Tapia 2015 (64)**	**Kim G 2015 (89)**	**Ma X-R 2015 (91)**	**Qi X 2015 (93)**	**Wang K 2015 (94)**	**Moore J 2014 [44]**	**Pan X-N 2014 (92)**
Section/topic	#	Checklist item	page #	page #	page #	page #	page #	page #	page #	page #	page #
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	500	91	635, Figure 1	1406, Figure 1	2	NR	149	675	14052
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	NR	NR	NR	1406	2	NR	148-149	NR	NR
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	NR	91	636-637	1406-1408, Figure 2	NR	167, Supp table 2 and 3	149	675-677, Table 1	NR
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	500	91	635	1406	2	167-168	149	675	14052
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	500	91	635	1406	2	167-168	149	675	14052-14055

			Liu Z 2016 (90)	**Yang Q 2016 (95)**	**Chavez -Tapia 2015 (64)**	**Kim G 2015 (89)**	**Ma X-R 2015 (91)**	**Qi X 2015 (93)**	**Wang K 2015 (94)**	**Moore J 2014 [44]**	**Pan X-N 2014 (92)**
Section/topic	#	Checklist item	page #	page #	page #	page #	page #	page #	page #	page #	page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	505	91	636-637	1407-1408, Figure 2	NR	168, Supp table 2 and 3	149-150, Figure 2	675-677, Table 1	NR
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	503-505	NR	NR	NR	2, 5-7	167-168, Supp table 25	NR	NR	NR
RESULTS											
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	500-501	90-91, Figure 1	635-637, Figure 1, Table 1 and 2	1406-1408, Figure 1	2, Figure 1	168	149-150, Figure 1	675-676, 678-680, Table 2	14052-14053, Figure 1
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	500-501, Table 1	91-93, Table 1	635-637, Table 1 and 2	1406-1407, Table 1	2-3, Table 1	169-171	149-152	678-680, Table 2	14052-14053, Table 1
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	NR	NR	NR	1406-1408, Figure 1	NR	168, Supp table 2 and 3	149-150, Figure 2	675-677, Table 1	NR

			Liu Z 2016 (90)	**Yang Q 2016 (95)**	**Chavez -Tapia 2015 (64)**	**Kim G 2015 (89)**	**Ma X-R 2015 (91)**	**Qi X 2015 (93)**	**Wang K 2015 (94)**	**Moore J 2014 [44]**	**Pan X-N 2014 (92)**
Section/topic	#	Checklist item	page #	page #	page #	page #	page #	page #	page #	page #	page #
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	502-503, Table 2	93-94	636-639	1408-1413	2-7	168, 171, 175-177	149-156	675-683	14052-14055
Synthesis of results	21	Present the main results of the review. If meta-analyses are done, include for each meta-analysis, confidence intervals and measures of consistency.	502-505, Table 2	93-94	637-639	1408-1413	NR	168-177, Supp table 16-24	149-156	675-683	14052-14055
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	NR	NR	NR	1407-1408, Figure 2	2-7	168, Supp table 2 and 3	149-150, Figure 2	675-677, Table 1	NR
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	503-505	NR	NR	NR	2-7	167-168, Supp table 16-25	153-155	NR	NR
DISCUSSION											
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to	NR	93-95	637-639	1413-1414	7-8	177-178	149-157	675-683	14052-14056

			Liu Z 2016 (90)	Yang Q 2016 (95)	Chavez -Tapia 2015 (64)	Kim G 2015 (89)	Ma X-R 2015 (91)	Qi X 2015 (93)	Wang K 2015 (94)	Moore J 2014 [44]	Pan X-N 2014 (92)
Section/topic	#	Checklist item	page #	page #	page #	page #	page #	page #	page #	page #	page #
		key groups (e.g., healthcare providers, users, and policy makers).									
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	506	95	639	NR	8	178	156	680	14056
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	506	95	NR	NR	8	178	157	NR	NR
FUNDING											
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	506	NR	NR	NR	NR	NR	NR	NR	NR

Appendix 6: Data collection proforma

Study Reference: ……

Reviewer: ……

Inclusion criteria full text:

Include: YES □ NO □ UNSURE □

Type of study:

……
……

1. Population: YES □ NO □ UNSURE □
i. Adult patients (>18 yrs) □ ii. Liver cirrhosis/fibrosis (chronic liver disease/damage/End stage liver disease) – any cause □ iii. Acute on chronic liver failure/disease – as defined by the author □

2. Intervention: YES □ NO □ UNSURE □
To include any dose, duration and route of administration 1) Bone marrow derived stem cells (with or without GCSF) 1a: Haematopoietic stem cells □ 1b: Mesenchymal stem cells □ 1c: Mononuclear cells □ 1d: other type, name…… 1e: GCSF given or not? (Y/N) Y □ N □ 2) Non-bone marrow derived cells or allogenic from another source (if yes) Type of stem cells……. □ 3) Granulocyte colony stimulating factor (GCSF) only □
Please state dose, duration and route of administration ……

3. Comparison:	Yes □	No □	Unsure □
Standard therapy	Yes □	No □	
No standard therapy	Yes □	No □	
4. Outcomes:	Yes □	No □	Unsure □
Minimum FU of 4 weeks	Yes □	No □	

Total Duration of FU

……

i)	Liver function tests	Yes □	No □
ii)	Model for End stage liver disease	Yes □	No □
iii)	Child Pugh Score	Yes □	No □
iv)	Survival	Yes □	No □
v)	Quality of life	Yes □	No □

Exclusion criteria full text: (exclude if any of it is yes)

i.	Children	Yes □	No □
ii.	Acute liver failure	Yes □	No □
iii.	Cancer of any form (as unsure how stem cell therapy affects cancer OR how cancer affects stem cell therapy)	Yes □	No □
iv.	Any article that does not state cirrhosis/fibrosis/CHRONIC liver failure e.g. in patients with acute alcoholic hepatitis, where they did not have any evidence of underlying liver fibrosis or cirrhosis.	Yes □	No □
v.	Follow up less than 4 weeks	Yes □	No □
vi.	Cellular morphology/histology outcomes ONLY	Yes □	No □

Case report Yes □ No □

Appendix 7: Published studies

Appendix 7.1: Animal data published manuscript

Gastroenterology 2017;153:233–248

BASIC AND TRANSLATIONAL—LIVER

Sphingosine-1-Phosphate Prevents Egress of Hematopoietic Stem Cells From Liver to Reduce Fibrosis

Andrew King,[1] Diarmaid D. Houlihan,[1] Dean Kavanagh,[2] Debashis Haldar,[1] Nguyet Luu,[1] Andrew Owen,[1] Shankar Suresh,[1] Nwe Ni Than,[1] Gary Reynolds,[1] Jasmine Penny,[1] Henry Sumption,[1] Prakash Ramachandran,[3] Neil C. Henderson,[3] Neena Kalia,[2] Jon Frampton,[4] David H. Adams,[1] and Philip N. Newsome[1]

[1]Birmingham Liver Biomedical Research Unit, National Institute for Health Research, Centre for Liver Research, [2]Centre for Cardiovascular Sciences, [4]School Institute of Immunology and Immunotherapy, College of Medical and Dental Sciences, University of Birmingham, Birmingham, United Kingdom; [3]Medical Research Council Centre for Inflammation Research, University of Edinburgh, Edinburgh, United Kingdom

BACKGROUND & AIMS: There is growing interest in the use of bone marrow cells to treat liver fibrosis, however, little is known about their antifibrotic efficacy or the identity of their effector cell(s). Sphingosine-1-phosphate (S1P) mediates egress of immune cells from the lymphoid organs into the lymphatic vessels; we investigated its role in the response of hematopoietic stem cells (HSCs) to liver fibrosis in mice. **METHODS:** Purified (c-kit+/sca1+/lin-) HSCs were infused repeatedly into mice undergoing fibrotic liver injury. Chronic liver injury was induced in BoyJ mice by injection of carbon tetrachloride (CCl_4) or placement on a methionine-choline–deficient diet. Some mice were irradiated and given transplants of bone marrow cells from C57BL6 mice, with or without the S1P antagonist FTY720; we then studied HSC mobilization and localization. Migration of HSC lines was quantified in Transwell assays. Levels of S1P in liver, bone marrow, and lymph fluid were measured using an enzyme-linked immunosorbent assay. Liver tissues were collected and analyzed by immunohistochemical quantitative polymerase chain reaction and sphingosine kinase activity assays. We performed quantitative polymerase chain reaction analyses of the expression of sphingosine kinase 1 and 2, sphingosine-1-phosphate lyase 1, and sphingosine-1-phosphate phosphatase 1 in normal human liver and cirrhotic liver from patients with alcohol-related liver disease (n = 6). **RESULTS:** Infusions of HSCs into mice with liver injury reduced liver scarring based on picrosirius red staining (49.7% reduction in mice given HSCs vs control mice; $P < .001$), and hepatic hydroxyproline content (328 mg/g in mice given HSCs vs 428 mg/g in control mice; $P < .01$). HSC infusion also reduced hepatic expression of α-smooth muscle actin (0.19 ± 0.07-fold compared with controls; $P < .0001$) and collagen type I α 1 chain (0.29 ± 0.17-fold compared with controls; $P < .0001$). These antifibrotic effects were maintained with infusion of lymphoid progenitors that lack myeloid potential and were associated with increased numbers of recipient neutrophils and macrophages in liver. In studies of HSC cell lines, we found HSCs to recruit monocytes, and this process to require C-C motif chemokine receptor 2. In fibrotic liver tissue from mice and patients, hepatic S1P levels increased owing to increased hepatic sphingosine kinase-1 expression, which contributed to a reduced liver:lymph S1P gradient and limited HSC egress from the liver. Mice given the S1P antagonist (FTY720) with HSCs had increased hepatic retention of HSCs (1697 ± 247 cells in mice given FTY720 vs 982 ± 110 cells in controls; $P < .05$), and further reductions in fibrosis. **CONCLUSIONS:** In studies of mice with chronic liver injury, we showed the antifibrotic effects of repeated infusions of purified HSCs. We found that HSCs promote recruitment of endogenous macrophages and neutrophils. Strategies to reduce S1P signaling and increase retention of HSCs in the liver could increase their antifibrotic activities and be developed for treatment of patients with liver fibrosis.

Keywords: Mouse Model; CCR2; Sphingolipid; Immune Cell Localization.

The incidence of chronic liver disease is increasing worldwide[1] and is characterized by the progression of liver injury from hepatic fibrosis to cirrhosis, resulting in death from liver failure, complications of portal hypertension, or hepatocellular carcinoma.[2] At present, liver transplantation remains the only curative treatment for end-stage liver disease but is limited by the availability of donor organs and the risks of lifelong immunosuppression.[3–5] The development and resolution of hepatic fibrosis is recognized as a bidirectional process, with resolution of fibrosis mediated through degradation of hepatic collagen[6] and apoptosis of activated hepatic myofibroblasts.[7]

Abbreviations used in this paper: α-SMA, α-smooth muscle actin; APC, allophycocyanin; BM, bone marrow; CCL, chemokine (C-C motif) ligand; CFSE, carboxyfluorescein succinimidyl ester; CLP, common lymphoid progenitors; CMP, common myeloid progenitors; DiR, 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindotricarbocyanine iodide; HPC-7, hematopoietic progenitor cell line; HSC, hematopoietic stem cell; KSL, c-kit$^+$ sca-1$^+$ and lineage^{neg}; MMP, matrix metalloproteinase; PSR, picrosirius red; SGPL1, sphingosine-1-phosphate lyase; SGPP1, sphingosine-1-phosphate phosphatase; S1P, sphingosine 1-phosphate; SphK1, sphingosine kinase 1.

Most current article

0016-5085
http://dx.doi.org/10.1053/j.gastro.2017.03.022

Appendix 7.2: Clinical Trial published manuscript

Granulocyte colony-stimulating factor and autologous CD133-positive stem-cell therapy in liver cirrhosis (REALISTIC): an open-label, randomised, controlled phase 2 trial

Philip Neil Newsome, Richard Fox, Andrew L King, Darren Barton, Nwe-Ni Than, Joanna Moore, Christopher Corbett, Sarah Townsend, James Thomas, Kathy Guo, Diana Hull, Heather A Beard, Jacqui Thompson, Anne Atkinson, Carol Bienek, Neil McGowan, Neil Guha, John Campbell, Dan Hollyman, Deborah Stocken, Christina Yap, Stuart John Forbes

Summary

Background Results of small-scale studies have suggested that stem-cell therapy is safe and effective in patients with liver cirrhosis, but no adequately powered randomised controlled trials have been done. We assessed the safety and efficacy of granulocyte colony-stimulating factor (G-CSF) and haemopoietic stem-cell infusions in patients with liver cirrhosis.

Methods This multicentre, open-label, randomised, controlled phase 2 trial was done in three UK hospitals and recruited patients with compensated liver cirrhosis and MELD scores of 11·0–15·5. Patients were randomly assigned (1:1:1) to receive standard care (control), treatment with subcutaneous G-CSF (lenograstim) 15 μg/kg for 5 days, or treatment with G-CSF for 5 days followed by leukapheresis and intravenous infusion of three doses of CD133-positive haemopoietic stem cells ($0·2\times10^6$ cells per kg per infusion). Randomisation was done by Cancer Research UK Clinical Trials Unit staff with a minimisation algorithm that stratified by trial site and cause of liver disease. The coprimary outcomes were improvement in severity of liver disease (change in MELD) at 3 months and the trend of change in MELD score over time. Analyses were done in the modified intention-to-treat population, which included all patients who received at least one day of treatment. Safety was assessed on the basis of the treatment received. This trial was registered at Current Controlled Trials on Nov 18, 2009; ISRCTN, number 91288089; and the European Clinical Trials Database, number 2009-010335-41.

Findings Between May 18, 2010, and Feb 26, 2015, 27 patients were randomly assigned to the standard care, 26 to the G-CSF group, and 28 to the G-CSF plus stem-cell infusion group. Median change in MELD from day 0 to 90 was −0·5 (IQR −1·5 to 1·1) in the standard care group, −0·5 (−1·7 to 0·5) in the G-CSF group, and −0·5 (−1·3 to 1·0) in the G-CSF plus stem-cell infusion group. We found no evidence of differences between the treatment groups and control group in the trends of MELD change over time (p=0·55 for the G-CSF group *vs* standard care and p=0·75 for the G-CSF plus stem-cell infusion group *vs* standard care). Serious adverse events were more frequent the in G-CSF and stem-cell infusion group (12 [43%] patients) than in the G-CSF (three [11%] patients) and standard care (three [12%] patients) groups. The most common serious adverse events were ascites (two patients in the G-CSF group and two patients in the G-CSF plus stem-cell infusion group, one of whom was admitted to hospital with ascites twice), sepsis (four patients in the G-CSF plus stem-cell infusion group), and encephalopathy (three patients in the G-CSF plus stem-cell infusion group, one of whom was admitted to hospital with encephalopathy twice). Three patients died, including one in the standard care group (variceal bleed) and two in the G-CSF and stem-cell infusion group (one myocardial infarction and one progressive liver disease).

Interpretation G-CSF with or without haemopoietic stem-cell infusion did not improve liver dysfunction or fibrosis and might be associated with increased frequency of adverse events compared with standard care.

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Lancet Gastroenterol Hepatol 2017

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See Online/Comment http://dx.doi.org/10.1016/S2468-1253(17)30359-X

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Introduction

Chronic liver disease is a common cause of death globally, the incidence of which is rising due to a combination of alcohol consumption, obesity, and viral hepatitis.[1,2] Although the primary causes of injury, such as alcohol or viruses can be removed or treated, patients with cirrhosis often still have progression to liver decompensation leading to death.[3] For such patients, the only proven treatment is liver transplantation, but access to this approach is limited globally by the shortage of donors, sequelae of long-term immunosuppression, and high lifelong medical costs.

Promising preclinical data have suggested that injections of bone-marrow-derived cells can reduce hepatic fibrosis and stimulate liver regeneration, thereby improving the synthetic function of the liver,[4–6]

Appendix 7.3: Systematic review protocol published manuscript

Than et al. Systematic Reviews (2016) 5:100
DOI 10.1186/s13643-016-0277-6

Systematic Reviews

PROTOCOL Open Access

Clinical effectiveness of cell therapies in patients with chronic liver disease and acute-on-chronic liver failure: a systematic review protocol

Nwe Ni Than[1], Claire L. Tomlinson[2], Debashis Haldar[1], Andrew L. King[1], David Moore[3] and Philip N. Newsome[1*]

Abstract

Background: Chronic liver disease (CLD) is a major health burden worldwide. Liver cirrhosis, a form of CLD is the fifth most common cause of death in the UK. Acute-on-chronic liver failure (ACLF) is the result of an acute insult superimposed on patients with liver cirrhosis as a result of precipitating events such as infection or bleeding. ACLF has a high associated mortality as a result of multi-organ failure. The only effective treatment for CLD is liver transplantation, but the treatment is limited by shortage of donor organs. As a result, alternative treatments such as cell therapies have been studied in patients with liver diseases. This study will systematically review the evidence on clinical effectiveness of cell therapies in patients.

Methods: All types of study design that investigate the effectiveness of cell therapies (haematopoietic, mesenchymal and unsorted cell types) of autologous or allogeneic origin and/or the use of granulocyte colony-stimulating factor in patients with CLD including ACLF will be included (except case reports). Both autologous and allogenic cell types will be included. The primary outcomes of interest are survival, model for end-stage liver disease score, quality of life and adverse events. Secondary outcomes include liver function tests, Child-Pugh score and events of liver decompensation. A literature search will be conducted in the following databases: MEDLINE, MEDLINE in Process, EMBASE and Cochrane Library (CENTRAL, CDSR, DARE, HTA databases). Trial registers will be searched for ongoing trials, as will conference proceedings. Reference lists of relevant articles and systematic reviews will be screened. Randomised controlled trial (RCT) evidence is likely to be scant; therefore, controlled trials and concurrently controlled observational studies will be primarily analysed and uncontrolled observational studies will be analysed where primary outcomes are not reported in the control studies or where uncontrolled studies have longer follow-up. Initial screening of studies will be carried by one reviewer with a proportion checked by another reviewer. Full-text selection will be performed by two reviewers independently against the pre-defined selection criteria. The data collection and the risk of bias assessment will be completed by one reviewer and counter checked by another reviewer for all selected studies. Where appropriate, data will be meta-analysed for each study design, therapy and outcome. Data specifically on ACLF will be treated as a subgroup.

Discussion: This systematic review will identify the available evidence on the effectiveness of cell therapies in patients with CLD and in ACLF subgroup. The findings will aid decision-making by clinicians and health service leaders.

Systematic review registration: PROSPERO CRD42016016104

Keywords: Chronic liver disease, Acute on chronic liver failure, Model for end-stage liver disease, Survival, Quality of life, Cell therapy, Granulocyte colony-stimulating factor

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Full list of author information is available at the end of the article

Appendix 8: Study design details of included randomised and non-randomised controlled trials

Study ID	Age of patients	Causes of liver diseases	Type of stem cells	statistical analysis	Unit data for blood tests	Recruitment dates	Inclusion criteria	Exclusion criteria	Details of standard therapy	Primary endpoint	Secondary endpoint
Randomised controlled trials (Chronic liver disease)											
Mohamadnejad M 2013	18-65	Cryptogenic, Primary biliary cholangitis (PBC), Hepatitis B virus (HBV), Hepatitis C virus (HCV), Autoimmune hepatitis (AIH)	BM-MSC	mean+/-SD, SPSS 11.5, p<0.05 considered statistically significant	Alb (g/dL), INR, AST (U/L), ALT (U/L)	July 2007 to August 2010	Decompensated cirrhosis, age 18 to 65 yr. olds	active HE, refractory ascites, active AIH, Cr>1.5 mg/dl, active HCV in HCV related cirrhosis, +ve HBV in HBV related cirrhosis, PV or HV thrombosis, variceal bleeding during last 2 months, use of alcohol or hepatotoxic drugs in 3 months before, co-morbidity as in cardiac or malignancy, not willing to participate	Not mentioned	absolute change in MELD score, CPS, LFTs, Liver volume between MSC and placebo at 12 months FU	Not mentioned

Study ID	Age of patients	Causes of liver diseases	Type of stem cells	statistical analysis	Unit data for blood tests	Recruitment dates	Inclusion criteria	Exclusion criteria	Details of standard therapy	Primary endpoint	Secondary endpoint
Suk K 2016	20-70	Alcohol related liver disease (ALRD)	BM-MSC	mean +/- SD, $p<0.05$ is significant, SPSS 13	GGT, ALP, AST and ALT (IU/L), Alb (g/dl), Bil (mg/dl). INR	Jan 2013 to Nov 2015	bx proven alcoholic cirrhosis included, off alcohol for at least 6 months before, age 20-70, CP B-C	age >70, viral hepatitis (A, B, C, E), HIV, Child A, MELD >20, severe disease, sepsis, high dose steroid or antibiotics, liver tumour, liver cancer or pregnancy	Not mentioned	improvement in fibrosis quantification based on picrosirius red staining. (Lannec fibrosis score)	liver function test, Child-Pugh score, and MELD score
Xu L 2014	18-60	HBV	BM-MSC	SPSS 13.0, mean +/- SEM	ALT (U/L), total bil (umol/L), Alb (g/L)	March 2012 to December 2012	18-60 years of age; hepatitis BsAg of all HBV patients were seropositive for more than 6 months; HBV-DNA levels >2x10*3 IU/ml	pregnant or nursing females; co-infection with HIV or HCV or HDV; serious bacterial infection; evidence of other causes of liver cirrhosis (e.g. alcohol/ PBC/ drug induced/ inherited metabolic disease); anti-viral or	Entecavir (0.5 mg/day) or ETC+BM-MSC	Absolute changes in MELD score, improvement in LFTs during the 24 weeks of FU	serum levels of HBV-DNA, Creatinine, side effects, complications

Study ID	Age of patients	Causes of liver diseases	Type of stem cells	statistical analysis	Unit data for blood tests	Recruitment dates	Inclusion criteria	Exclusion criteria	Details of standard therapy	Primary endpoint	Secondary endpoint
							9range 1.63x10*4-7.8x10*7 IU/ml); cirrhosis was diagnosed by USS, CT or MRI	immunomodulatory therapy within 6 months before the study; liver cancer or other malignancies; other vital organ dysfunctions (heart failure/ renal); severe complications of liver cirrhosis (HE, variceal bleeding); Cr>150 umol/L; platelets <30x10*9/L; PT activity <20%			
Amer M 2011 **(117)**	46-60	HCV	BM-MSC	NM	Alb (mg/dl),	Oct 2008 to June 2009	Age 46-60 years; liver cirrhosis with child Pugh C; chronic HCV infection; agree to consent;	malignancies or on chemotherapy; autoimmune disorders; other causes of chronic hepatitis such as hepatitis B; with fulminant hepatitis; active bleeding or	Close monitoring in ICU; IV fluids: Nutritional supplements (NG for ICU case); Zinc supplementation for	Therapeutic benefit of injection of autologous bone marrow stems cells in	Not mentioned

Study ID	Age of patients	Causes of liver diseases	Type of stem cells	statistical analysis	Unit data for blood tests	Recruitment dates	Inclusion criteria	Exclusion criteria	Details of standard therapy	Primary endpoint	Secondary endpoint
							serum albumin level >2.5mg/dl; PT concentration < 60%, MELD score <25	platelet <50,000/cmm; with concomitant renal failure; improving on medical treatment for hepatitis C; refuse to sign a consent	appetite; Vitamin D supplementation; regular exercise; management of pruritus with cholestyramine and antihistamine; ascites with diuretic, paracentesis and shunts; management of portal hypertension with beta blockers, banding; avoidance of medications metabolised by the liver	patients with liver failure due to chronic HCV infection	

Study ID	Age of patients	Causes of liver diseases	Type of stem cells	statistical analysis	Unit data for blood tests	Recruitment dates	Inclusion criteria	Exclusion criteria	Details of standard therapy	Primary endpoint	Secondary endpoint
Zeng Z 2015	not mentioned	decompensated, not specified	UC-MSC	Not mentioned	Not mentioned	Not mentioned	decompensated cirrhosis	Not mentioned	Not mentioned	Not mentioned	Not mentioned
Zhang Z 2012	median age 47/48	HBV	UC-MSC	SPSS 13, $p<0.05$ was considered significant	ALT (IU/L), Alb (g/L), Total bil (umol/L), PT (%), Ascites (mm)	Not mentioned	All patients had a history of chronic HBV infection, with ultrasound evidence of liver cirrhosis with ascites and/or portal hypertension, and/or low serum albumin (ALB) and/or high total bilirubin (TBIL),	moderate to severe HE, variceal bleeding, ascites, recent infection or dialysis 2 months before enrolment, presence of severe co-morbidities, presence of neoplasm. Extrahepatic biliary disease, hepatic, portal or splenic vein thrombosis on doppler USS, active substance misuse, lack of family support, unable to sign consent	standard therapy	safety and efficacy of UC-MSC infusion in patients with HBV cirrhosis	Not mentioned

Study ID	Age of patients	Causes of liver diseases	Type of stem cells	statistical analysis	Unit data for blood tests	Recruitment dates	Inclusion criteria	Exclusion criteria	Details of standard therapy	Primary endpoint	Secondary endpoint
							and/or a prolonged international normalized ratio (INR).				
Zekri A 2015	>40	HCV	GCSF and CD133/ CD34 &MSC	SPSS version 12.0, mean +/- SD for quantitative variables. qualitative data were expressed as frequency (number) and percent.	Bil (mg/dl), ALB (g/dl), ALT and AST (U/L)	May 2010 to May 2012	age range from 20 to 60 years, evidence of chronic liver insufficiency (decreased s-albumin and/or increased bilirubin and/or increased INR, Child–Pugh scores B and C and (MELD)	aged younger than 20 or older than 60 years; were pregnant or lactating women; had recent and/or recurrent upper gastrointestinal bleeding or spontaneous bacterial peritonitis (SBP) within 1 month before the procedure or hepatocellular carcinoma (HCC); were patients with portal vein thrombosis (PVT) on Doppler	ribavirin and amantadine sulphate. For ascites-diuretics+/ paracentesis/ /varices-band ligation+/- sclerotherapy	Not mentioned	Not mentioned

Study ID	Age of patients	Causes of liver diseases	Type of stem cells	statistical analysis	Unit data for blood tests	Recruitment dates	Inclusion criteria	Exclusion criteria	Details of standard therapy	Primary endpoint	Secondary endpoint
				P<0.05 is SS			scores >14) and who cannot receive a liver transplant owing to organ shortage and/or high cost of liver transplantation in Egypt. All had a World Health Organization (WHO) performance score ≤2 and were able to give written informed consent.	ultrasonography or severe co-morbid diseases (e.g. renal or cardiac disease); had evidence of human immunodeficiency virus or other life-threatening infection; were unable to give written consent; had a history of hypersensitivity to granulocyte colony-stimulating factor (G-CSF); or were included in any other clinical trial within the previous 6 months.			

Study ID	Age of patients	Causes of liver diseases	Type of stem cells	statistical analysis	Unit data for blood tests	Recruitment dates	Inclusion criteria	Exclusion criteria	Details of standard therapy	Primary endpoint	Secondary endpoint
							All patients were post-HCV infection with viral load ranging from 3690 to 954,473 IU (mean = 523,764 IU). Some patients received interferon/ribavirin with no response (16 patients were previously non-responders in group I, five patients in group II				

Study ID	Age of patients	Causes of liver diseases	Type of stem cells	statistical analysis	Unit data for blood tests	Recruitment dates	Inclusion criteria	Exclusion criteria	Details of standard therapy	Primary endpoint	Secondary endpoint
							and six patients in the control group), and others did not receive previous interferon therapy.				
Salama H 2014	20-60	HCV	GCSF and MSC	SPSS 13.2 mean ± SD for quantitative variables, whereas qualitative data were expressed as frequency (number) and	Alb, PT and concertation in %,	June 2010 to October 2011	age 20 to 60 years, evidence of chronic liver insufficiency (decreased albumin +/- increased bilirubin +/- increased INR), unlikely to receive a liver transplant,	<20 or >60 yr. of age; pregnant or lactating, recurrent of recent upper GI bleeding or SBP within 1 month before procedure, HCC, HIV or other life threating infection, unable to give a consent, hx of hypersensitivity to GCSF treatment, or included in other trial within previous month	regular liver supportive treatment	Not mentioned	Not mentioned

Study ID	Age of patients	Causes of liver diseases	Type of stem cells	statistical analysis	Unit data for blood tests	Recruitment dates	Inclusion criteria	Exclusion criteria	Details of standard therapy	Primary endpoint	Secondary endpoint
				percentage. P<0.05 is SS			WHO performance status of <2, able to give consent. None of the received interferon or other therapy within 6 months before cell transplantation or during the follow-up				
Newsome P 2017	Median 52-56	ALD (12/12/14), HCV (4/3/3), NAFLD (5/3/5), PBC (5/7/3), CC (0/1/2), Mixed (1/0/1)-	GCSF or GCSF and CD133+ cells	data are n (%) or median (IQR). all analyses were done in the	Bil (umol/L), Alb (g/L), ALT/AST? ALP/GGT (U/L),	May 18, 2010 to Feb 6, 2015	aged 18–75 years with predominantly compensated cirrhosis (most causes were	alcohol >21 units per week for men, 14 units for women, any alcohol consumption within 6 months for ALD cirrhosis, no changes in	All HCV patients had previous antiviral treatment-none had cleared of the virus. Standard	co-primary outcome 1) change in MELD at 90 days from baseline	Secondary outcome measures were liver stiffness (Fibroscan),

Study ID	Age of patients	Causes of liver diseases	Type of stem cells	statistical analysis	Unit data for blood tests	Recruitment dates	Inclusion criteria	Exclusion criteria	Details of standard therapy	Primary endpoint	Secondary endpoint
		Control/GCSF/Stem cell		modified intention-to-treat (mITT) population. mITT population: who received 1 day of GCSF+ 1-day infusion. Pre-protocol population: who received 5 days of GCSF plus 3 infusions each.	INR, FS (kPa)		allowed except for autoimmune hepatitis) and a MELD score of 11·0–15·5	degree of ascites within 3 months, HE event or admission in 3 months, portal HTN bleeding within 3 months, HCC hx, presence of dysplastic nodules, previous LT, hx of pulmonary infiltrates or pneumonia.	management.	and trend of treatment activity established by incorporating MELD score measured at baseline and days 30, 60, and 90. 2) make better use of data collected and detect difference before 90 days	enhanced liver fibrosis (ELF) test, Chronic Liver Disease Questionnaire (CLDQ) scores, individual components of liver function (bilirubin, albumin, INR, and creatinine, UK End-Stage

Study ID	Age of patients	Causes of liver diseases	Type of stem cells	statistical analysis	Unit data for blood tests	Recruitment dates	Inclusion criteria	Exclusion criteria	Details of standard therapy	Primary endpoint	Secondary endpoint
				All control patients are included in mITT and pre-protocol populations. Stata version 14 for all analyses.							Liver Disease (UKELD) score, circulating peripheral blood HSCs, long-term MELD and UKELD (to day 360), clinical events, and transplant-free survival.

Study ID	Age of patients	Causes of liver diseases	Type of stem cells	statistical analysis	Unit data for blood tests	Recruitment dates	Inclusion criteria	Exclusion criteria	Details of standard therapy	Primary endpoint	Secondary endpoint
Raju B 2014	not mentioned	decompensated, not specified	GCSF and CD34+ cells	Not mentioned	Not mentioned	Not mentioned	liver cirrhosis who are unlikely to receive LT	Not mentioned	regular treatment	Not mentioned	Not mentioned
Salama H 2010	20-60	HCV	GCSF and CD133/ CD34+ cells	SPSS 12.0 mean+/- SD for quantitative variables, qualitative data were expressed by frequency (number) and %, p <0.05 were SS.	Bil (mg %), Alb (gm/dl), PC (%), AST (U/L), ALT (U/L)	June 2008 to May 2009	age 20-60 years, evidence of chronic liver insufficiency (decreased serum albumin +/- increased bilirubin +/- INR), unlikely to received liver transplant, WHO performance score of <2, able to	<20 or >60 years of age, pregnant or lactating, recurrent GI bleeding, HCC or SBP, evidence of HIV infection, life threatening infection, unable to give consent, hypersensitivity to GCSF, involved in other clinical trial within previous month of recruitment	standard therapy	To access the utility of stem cells infusion as a possible therapeutic modality in patients with ESLD.	Not mentioned

Study ID	Age of patients	Causes of liver diseases	Type of stem cells	statistical analysis	Unit data for blood tests	Recruitment dates	Inclusion criteria	Exclusion criteria	Details of standard therapy	Primary endpoint	Secondary endpoint
							give consent				
Nikeghbalian S 2011	18-75	CD133 (AIH/CCx2), MNC (CCx2, Haemochormatosis)	CD133 group in comparison to MNC group	Not mentioned	Alb (g/L), PT (s), INR, Bil (mg/dl), AST and ALT (IU/ml),	Not mentioned	18-75 yr., cirrhosis, ab LFT between 2x to 2x ULN	organ failure other than liver, any degree of HE, refractory ascites, HCC or other malignancies, extra hepatic biliary disease, HIV, HBV or HCV infection, active or chronic thrombosis of portal or hepatic veins, active variceal bleeding, SBP or any sepsis, active AIH (IgG >2x ULN)	Not applicable (compared between CD 133 and MNC)	Not mentioned	Not mentioned
Mohamadnejad M 2016	median age 48	CC (3/7/5), PBC (1/0/1), AIH (5/3/1),	BM-CD133+ cells or	expressed as mean+/-	Alb (g/dl), Bil	March 2010 to	cirrhotic patients	The exclusion criteria included grade III or IV	Not mentioned	changes in MELD at 3 and 6	mortality and develop

Study ID	Age of patients	Causes of liver diseases	Type of stem cells	statistical analysis	Unit data for blood tests	Recruitment dates	Inclusion criteria	Exclusion criteria	Details of standard therapy	Primary endpoint	Secondary endpoint
		NASH (0/0/1)-Control/MNC/CD133+	BM-MNC	SD, median and range or frequency and percentage as appropriate. SPSS version 19. p<0.05 is SS	(mg/dl), AST and ALT (U/L), INR, mean +/- SD	June 2012	(Child B or C)	hepatic encephalopathy during the 6 months before study entry; refractory ascites; elevated serum transaminases (three times the normal values); active autoimmune hepatitis, manifested as serum g-globulin more than twice the normal limit; serum creatinine of more than 1.5 mg/dl; positive HIV antibody; positive hepatitis C virus RNA quantitative polymerase chain reaction; hepatitis B virus DNA level of more than 200		months after infusion	ment of a poor outcome (LT requirement/ Development of HCC/ Death) during the 12 months of follow-up

Study ID	Age of patients	Causes of liver diseases	Type of stem cells	statistical analysis	Unit data for blood tests	Recruitment dates	Inclusion criteria	Exclusion criteria	Details of standard therapy	Primary endpoint	Secondary endpoint
								IU/ml; primary sclerosing cholangitis; hepatocellular carcinoma (HCC); active infectious disease; grade 3 oesophageal varices; a positive history of oesophageal variceal bleeding 1 month before enrolment; portal and/or hepatic vein thrombosis diagnosed by Doppler ultrasonography; comorbid conditions that included cardiovascular, pulmonary, neurologic, and nephrological problems;			

Study ID	Age of patients	Causes of liver diseases	Type of stem cells	statistical analysis	Unit data for blood tests	Recruitment dates	Inclusion criteria	Exclusion criteria	Details of standard therapy	Primary endpoint	Secondary endpoint
								malignancy; substance abuse; alcohol consumption; and/or a hepatotoxic medication prescription at least3monthsbefore enrolment.			
Huang M 2014	20-70	HBV	TIPS and BMSC	SPSS 17.0, data are mean +/- SD, $p<0.05$ was considered significant	Alb (g/L), ALT (IU/L), TB (umol/L), PT (s)	Sept 2011 to July 2012	age 20-70 yr., decompensated liver cirrhosis, abnormal Alb +/- bil +/- PT, CPS>/7, no viable HCC on CT	HE, variceal bleeding within 2 months, evidence of extrahepatic biliary disease, severe cardiac insufficiency or coagulation disorders.	TIPS, LMWH given SC every 12hour for 5 to 7 days, warfarin (2.5-7.5mg/day) for 6 months-keep INR of 2.0, diuretic (furosemide 160mg/day and spironolacto	safety of the combined treatment of TIPS and BMC	liver functions and portal hypertension at 52 weeks after surgery including CPS, Alb, ALT, Bil, PT

Study ID	Age of patients	Causes of liver diseases	Type of stem cells	statistical analysis	Unit data for blood tests	Recruitment dates	Inclusion criteria	Exclusion criteria	Details of standard therapy	Primary endpoint	Secondary endpoint
									ne 400mg day)		
Lyra A 2010	18-74	Stem cell group: CC, ALD, HCV, Haemochromatosis, PBC// Control: ALD, HCV, Budd Chiari	mononuclear enriched BMC	mean, SD, STATA statistical software	Bil (mg/dl), Alb (g/dl), INR, PT, mean +/- SD	Jan 2006 to April 2006	age 18-74, chronic liver disease, no liver tumours, neg for HIV, no other malignancy except non-melanoma cancer, no hx of heart failure, plt >300, INR, /2.4, Cr </2.5 mg/dl, absence of PVT, no participation in other trials, non-	unable to subject to bone marrow aspiration or active HE or active sepsis	HBV pt. - one had lamivudine and the other had adefovir. // Only one patient from each group was on a regular therapeutic paracentesis program with albumin replacement every 4–8 weeks, on average, before the study was initiated.	Not mentioned	Not mentioned

Study ID	Age of patients	Causes of liver diseases	Type of stem cells	statistical analysis	Unit data for blood tests	Recruitment dates	Inclusion criteria	Exclusion criteria	Details of standard therapy	Primary endpoint	Secondary endpoint
							lactating or pregnant woman				
Fu N 2011	42-75	? Chinese paper	BMSC+/- octreotide	Not mentioned	Alb (g/L),	Not mentioned	cirrhotic patients with refractory ascites	Not mentioned	conventional therapy with octreotide	Not mentioned	Not mentioned
Yu S 2017	20-79	HCV (5), ALD (4)	GCSF and peripheral blood monocytes	median and range, SPSS 19, p<0.05 was considered significant	Bil (mg/dl), Bil (g/L), PT(INR),	July 2012 to January 2013	age 20-79, decompensated cirrhosis with CPS 8 or 9, no radiological evidence of HCC for more than 2 years after treatment.	age<20 or >80, hep B sAg+ve, not abstained for alcohol for at least 6 months, active status of HCC, hx of haemochromatosis and/or AIH, pregnant or lactating woman, Haemoglobin <7.5-8 or WCC<15 or neutrophil <500 or plt count <50, Cr	antiviral therapies: PEGylated interferon and ribavirin, paracentesis with albumin	Not mentioned	Not mentioned

Study ID	Age of patients	Causes of liver diseases	Type of stem cells	statistical analysis	Unit data for blood tests	Recruitment dates	Inclusion criteria	Exclusion criteria	Details of standard therapy	Primary endpoint	Secondary endpoint
								>1.5x ULN or Cr clearance <60ml/min, malignancy, GI bleeding within 3 months or hx of SBP, presence of PVT, presence of acute infections			
Spahr L 2013	mean age 54 yr.	ALD	GCSF and BM-MNC	mean/SD or median/ range. P<0.05 was significance. SPSS 10.0	Bil (umol/L), INR, Alb (gr/L)	February 2008 to March 2011	clinical decompensation manifested by ascites +/-jaundice in active drinkers (>/80g/day of alcohol); liver bx performed within 7 days of admission; age 18 to 75 yrs. old: MELD	pregnancy; HBV, HCV, HIV; documented HCC; biliary tract obstruction; liver bx showing causes other than ALD; complete PV thrombosis; hyper-sensitivity to GCSF; severe coagulopathy (platelets <50, INR >1.5); serum Cr >150 umol/l; any ongoing infection; recent (,10 days) GI	vitamin B supplements, calorie intake, support to help with alcohol abstinence, no pharmacological intervention to help with abstinence, prednisolone 4 weeks course of 40mg/day in	>/ 3 points decrease in MELD score at 3 months	Safety and evolution of parameters associated with liver regeneration and inflammation, including serum cytokines and liver

Study ID	Age of patients	Causes of liver diseases	Type of stem cells	statistical analysis	Unit data for blood tests	Recruitment dates	Inclusion criteria	Exclusion criteria	Details of standard therapy	Primary endpoint	Secondary endpoint
							<26; able to give informed consent	bleeding, estimated survival <6 months and clinically over HE	severe ASH (Maddrey score >/32)		tissue studies
Liu L 2014	>18	HBV	GCSF and BMSC	SPSS 13 mean +/- SD, p<0.05 was considered significant/*P < 0.05 for comparison of the two groups before and after treatment. # P < 0.05 for comparison of	AST and ALT (U/L), ALB (g/L), Total bil (uM).	April 2009 to October 2010	liver cirrhosis	Not mentioned	general symptomatic and supportive treatment, hep B-lamivudine and adefovir	Not mentioned	Not mentioned

Study ID	Age of patients	Causes of liver diseases	Type of stem cells	statistical analysis	Unit data for blood tests	Recruitment dates	Inclusion criteria	Exclusion criteria	Details of standard therapy	Primary endpoint	Secondary endpoint
				groups A and B after treatment.							
Kedariset ty C 2015	median 44-46	ALD (73/54), HBV (3/8), HCV (7/4), CC (17/34) = Stem cell/Control numbers	GCSF and darpopoietin or placebo	median and IQR or number (percentage), SPSS 15.0/ CTP and MELD as delta % change	Bil (mg/dl), Alb (g/dL), ALT and AST (IU/L),	May 2011 to June 2012	Patients who were adequately treated for infection and became culture negative were also screened and, if found eligible, were enrolled in the study	Patients aged younger than 18 years or older than 65 years, with evidence of alcoholic hepatitis or active alcohol abuse with last intake 1 month, with suspected autoimmune hepatitis (antinuclear antibody/ASMA-positive in titres 1:80 and/ or IgG 1.5 times upper limit of normal), hepatocellular carcinoma (HCC), any focus of sepsis as proven by	Standard medical therapy comprising albumin, diuretics, nutritional rehabilitation, b-blockers and treatment based on aetiology, such as antivirals for hepatitis B, were continued in both groups. Patients with alcoholic liver disease	Survival at 12 months	survival at 6 months; reduction in liver disease severity scores; reduction in need for large-volume paracentesis (LVP); development of new onset complications,

Study ID	Age of patients	Causes of liver diseases	Type of stem cells	statistical analysis	Unit data for blood tests	Recruitment dates	Inclusion criteria	Exclusion criteria	Details of standard therapy	Primary endpoint	Secondary endpoint
								culture positivity or presence of spontaneous bacterial peritonitis, multi-organ dysfunction, grade 3 or 4 hepatic encephalopathy (as per West Haven criteria), human immunodeficiency virus seropositivity, pregnancy, uncontrolled hypertension, coronary artery disease, planned for liver transplantation and those refusing to participate in the study were excluded.	were abstinent from alcohol throughout the study in both groups. These patients had undergone periodic psychosocial counselling in follow-up visits.		such as acute kidney injury, sepsis, and variceal bleed; change in AFP levels at 1 month; hemodynamic improvement at 1 month; histological evidence of hepatic regeneration; and safety of treatment.

Study ID	Age of patients	Causes of liver diseases	Type of stem cells	statistical analysis	Unit data for blood tests	Recruitment dates	Inclusion criteria	Exclusion criteria	Details of standard therapy	Primary endpoint	Secondary endpoint
Prajapati R 2017	18-75	ALD (49/43), CC 961/65), Viral (16/16), miscellaneous (0/3)- stem cell/control	GCSF	SPSS version 16, Continuous variables were presented as median (range), whereas categorical variables were expressed as frequencies and percentages. P<0.05 is SS	Bil (mg/dl), Alb (mg. dl), INR,	June 2014 and August 2015	(1) Age: 18–75 years. (2) Patients with decompensated cirrhosis with CPS of at least 6 and 13 or less. Decompensation was defined as the occurrence of any of the following events: ascites, encephalopathy, variceal bleeding, jaundice, or	HCC or any other malignancy/ uncontrolled sepsis/severe cardio or pulmonary disease/G3 or 4 HE, active variceal bleeding, HRS/HIV+/Pregnancy/Refusal to participate/previous known hypersensitivity to GCSF	antivirals, abstinence from alcohol, nutrition, diuretics, β-blockers, selective intestinal decontamination, and other supportive measures depending on clinical status and requirement.	improvement in survival at 6 months	improvement in clinical outcome according to CP score.

Study ID	Age of patients	Causes of liver diseases	Type of stem cells	statistical analysis	Unit data for blood tests	Recruitment dates	Inclusion criteria	Exclusion criteria	Details of standard therapy	Primary endpoint	Secondary endpoint
							hepatorenal syndrome (HRS). (3) Patients who were listed for transplantation, but for whom liver transplantation was not feasible soon because of financial reasons or unavailability of donors (in India, most transplants are living donor liver transplantations that are self-funded).				

Study ID	Age of patients	Causes of liver diseases	Type of stem cells	statistical analysis	Unit data for blood tests	Recruitment dates	Inclusion criteria	Exclusion criteria	Details of standard therapy	Primary endpoint	Secondary endpoint
Spahr L 2008 **(128)**	mean age 54 yr.	ALD	GCSF	StatView 5.0 program, median and range, p<0.05 significant	Not mentioned	September 2005 to August 2006	recent heavy alcohol intake (>80 g/day), biopsy-proven ASH, Maddrey score>/ 20 and</ 70, leukocytes </ 15 G/L, age 18 to 70 years, and ability to give informed written consent	platelets <20 G/L; international normalized ratio >1.9; known hypersensitivity to filgrastim; creatinine >150 mol/L; infection or haemorrhage within the last 10 days; documented hepatocellular carcinoma; hepatitis B, C, or human immunodeficiency virus seropositivity; and pregnancy.	Standard medical therapy included a 28-day course of prednisone, 40 mg daily in case of severe ASH.	Not mentioned	Not mentioned
Non-randomised controlled trials (Chronic liver disease)											

Study ID	Age of patients	Causes of liver diseases	Type of stem cells	statistical analysis	Unit data for blood tests	Recruitment dates	Inclusion criteria	Exclusion criteria	Details of standard therapy	Primary endpoint	Secondary endpoint
El-Ansary M 2012	39-60	HCV	BM-MSC	SPSS 15, numerical data as mean and SD or median and range as appropriate. Qualitative data as frequency and %, p<0.05 is SS	Alb (g/dl), ALT and AST (U/L), ALP (U/L), Bil (mg./dl), GGT (U/L),	Not mentioned	advanced liver cirrhosis following HCV G4, Child C, MELD>12	Not mentioned	PEGylated IFN and ribavirin	Not mentioned	Not mentioned
Ouyang S 2013	not mentioned	HBV	BM-MSC	Not mentioned	Not mentioned	Not mentioned	hepatitis B cirrhosis-decompensated	Not mentioned	oral administration of nucleoside analogues antiviral treatment and SMT	Not mentioned	Not mentioned

Study ID	Age of patients	Causes of liver diseases	Type of stem cells	statistical analysis	Unit data for blood tests	Recruitment dates	Inclusion criteria	Exclusion criteria	Details of standard therapy	Primary endpoint	Secondary endpoint
Zhang S 2017	not mentioned	decompensated, not specified	UC-MSC	Not mentioned	Not mentioned	Not mentioned	chronic liver failure	Not mentioned	basic medical comprehensive treatment	Not mentioned	Not mentioned
Wang P 2011	?	HBV	MSC (co-cultured with peripheral blood mononuclear cells)	Not mentioned	Not mentioned	Not mentioned	liver failure patients with hepatitis B	Not mentioned	Not mentioned	Not mentioned	Not mentioned
Nakamura T 2014	20-75	HBV, HCV, ALD	GCSF and CD34+ cells	All data are expressed as mean +/- standard error, $p<0.05$ is SS. SPSS 14 version	Alb (g/dl), Bil (mg/dl), PT-INR	Jan 2009 to Dec 2017	age 20–75 years; decompensated LC; serum albumin less than 3.0 g/dL; total bilirubin less than 5.0 mg/dL; absence of	creatinine more than 2.0 mg/dL; prothrombin time and international normalized ratio (PT-INR) more than 2.5; liver tumours (including HCC) or other cancer; recent recurrent gastrointestinal bleeding or	Not mentioned	to assess the safety of dose enhanced autologous CD34+ cell infusion.	improvement in liver functions and symptoms

Study ID	Age of patients	Causes of liver diseases	Type of stem cells	statistical analysis	Unit data for blood tests	Recruitment dates	Inclusion criteria	Exclusion criteria	Details of standard therapy	Primary endpoint	Secondary endpoint
							viable HCC on computed tomography, or magnetic resonance imaging or a duplex Doppler ultrasound (US); not a candidate for liver transplant; if a woman of child-bearing age, use of appropriate contraception; life expectancy of at least 24 weeks; and the ability to	spontaneous bacterial peritonitis; or portal vein thrombosis.			

Study ID	Age of patients	Causes of liver diseases	Type of stem cells	statistical analysis	Unit data for blood tests	Recruitment dates	Inclusion criteria	Exclusion criteria	Details of standard therapy	Primary endpoint	Secondary endpoint
							give informed consent				
Cai T 2015	18-65	HBV	GCSF and CD34+ cells	SPSS 13, $p<0.05$ is considered significant, mean +/- SD	Bil (umol/L), AST and ALT (IU/L), Alb (g/L), PTA (%),	July 2011 to April 2012	hep B induced decompensated liver cirrhosis, age 18 to 65 yrs. old., Child B or C, HBV DNA <1.0x10*3, no liver tumours or the use of albumin, plasma or other blood products 1 month	if they had end-stage liver cirrhosis with severe complications, unstable vital signs, severe infections at other sites, or heart, lung, or kidney failure.	conventional medical treatment including anti-HBV nucleoside analogue, liver protection, jaundice treatment and diuretic administration.	Not mentioned	Not mentioned

Study ID	Age of patients	Causes of liver diseases	Type of stem cells	statistical analysis	Unit data for blood tests	Recruitment dates	Inclusion criteria	Exclusion criteria	Details of standard therapy	Primary endpoint	Secondary endpoint
							before enrolment.				
Deng Q 2015	18-65	HBV	GCSF and CD34+ cells	SPSS 13 mean+/- standard error of the mean (SEM), p<0.05 was considered significant	Total bil (umol/L), Alb (g/L), PTA (%),	July 2011 to December 2013	1) age of 18 – 65 years; 2) treatment for more than six months with nucleoside analogue anti-HBV therapy, so that HBV DNA levels were< 10*3 IU/mL; and 3) no treatment with plasma, albumin, or	1) cirrhosis caused by factors other than HBV (including hepatitis C/D virus infection, autoimmune liver disease, fatty liver, alcoholic liver disease, and genetic metabolic liver disease); 2) tumour; 3) primary or secondary renal disease or receiving renal replacement therapy; 4) severe cardiopulmonary disease or hemodynamic instability; 5)	received conventional medical treatment with nucleoside analogues administered in the form of entecavir (0.5 mg od). All patients had received antiviral therapy for more than six months before they were enrolled in the study. The patients	Not mentioned	Not mentioned

Study ID	Age of patients	Causes of liver diseases	Type of stem cells	statistical analysis	Unit data for blood tests	Recruitment dates	Inclusion criteria	Exclusion criteria	Details of standard therapy	Primary endpoint	Secondary endpoint
							other blood components for one month prior to enrolling in the study	severe infection; 6) active bleeding; 7) coma; or 8) blood diseases.	were also given silymarin and polyene phosphatidylcholine during the study to ensure liver protection. Thus, they received symptomatic and supportive treatment; however, no plasma, albumin, or other blood products were used during this period.		
Deng Q 2015*	18-65	HBV	GCSF and	using t-test, non-	Not mentioned	Not mentioned	HBV related decompens	Not mentioned	conventional therapy	Not mentioned	Not mentioned

Study ID	Age of patients	Causes of liver diseases	Type of stem cells	statistical analysis	Unit data for blood tests	Recruitment dates	Inclusion criteria	Exclusion criteria	Details of standard therapy	Primary endpoint	Secondary endpoint
			CD34+ cells	parametric test and chi square test			ated cirrhosis				
Huang X 2012	?	Decompensated, not specified	GCSF and CD34+ cells (PV vs HA)	Not mentioned	Not mentioned	Not mentioned	end stage liver disease	Not mentioned	Not mentioned	Not mentioned	Not mentioned
Sharma M 2015	18-70	decompensated, MELD>14	GCSF and CD34+ cells	mean and SD or median and range if appropriate. P<0.05 is SS, graph pad software 2014	Bil (mg/dl), AST and ALT (IU/ml), Alb (mg/dl), INR	July 2012-June 2013	Patients aged between 18-70 years, with clinically diagnosed hepatic cirrhosis, having a MELD score of >14 and unwilling for immediate liver	Patients with liver tumours or history of any other malignancy, active infections including human immunodeficiency virus (HIV), hepatitis B virus, hepatitis C virus, severe cardiac and pulmonary co-morbidities unrelated to cirrhosis, recent gastrointestinal bleed, acute	ascites management with diet/diuretics	serum albumin level, ALT, AST, Cr, MELD	transplant free survival at 1 month and 3 months

Study ID	Age of patients	Causes of liver diseases	Type of stem cells	statistical analysis	Unit data for blood tests	Recruitment dates	Inclusion criteria	Exclusion criteria	Details of standard therapy	Primary endpoint	Secondary endpoint
							transplantation, with life expectancy of at least 3 months (based on MELD score) and ability to give informed consent were included in the study group.	kidney injury or hepatorenal syndrome, portal vein thrombosis, pregnancy, lactation, and inability to give informed consent were excluded from the study.			
Bai Y 2014	mean 46	HBV	BM-MNC	SPSS 17, $p<0.05$ is SS. Mean +/- SD	ALT and AST (IU/L), Alb (g/L), Total bil (mmol/L), PT	March 2009 to March 2011	decompensated liver cirrhosis, able to get written informed consent, ultrasonographic evidence of	combined heart and lung function abnormality, blood system diseases, acquired immunodeficiency disease, malignant tumour, acute or chronic thrombosis of the	conventional medical regime included drugs for anti-HBV virus, liver cell protection, transaminase	Not mentioned	Not mentioned

Study ID	Age of patients	Causes of liver diseases	Type of stem cells	statistical analysis	Unit data for blood tests	Recruitment dates	Inclusion criteria	Exclusion criteria	Details of standard therapy	Primary endpoint	Secondary endpoint
					(s), PTA (%)		liver cirrhosis with ascites, portal hypertension, low serum albumin (ALB), high total bilirubin (TBIL), prolonged prothrombin time (PT), normal alpha fetoprotein level, and no hepatocellular carcinoma on hepatic artery	hepatic vein or portal vein, a history of severe infection, refractory ascites, and moderate to severe hepatic encephalopathy or variceal bleeding during the last two months before enrolment	and jaundice reducing drugs		

Study ID	Age of patients	Causes of liver diseases	Type of stem cells	statistical analysis	Unit data for blood tests	Recruitment dates	Inclusion criteria	Exclusion criteria	Details of standard therapy	Primary endpoint	Secondary endpoint
							angiographic imaging.				
Zhao D 2015	not mentioned	decompensated, not specified	BMSC	SPSS 13, x+/-SD, p<0.05 had a significant difference	Not mentioned	April 2009 to April 2011	Decompensated liver cirrhosis	Not mentioned	Not mentioned	Not mentioned	Not mentioned
Saito T 2011	mean age 64	ALD	BMSC	p<0.05 is considered significant. Data: mean +/- standard error. SPSS 15	Alb (g/dL), Total bil (mg/dL), PT (%),	Not mentioned	advance cirrhosis due to ALD, neg for hep B and C, abstinence for >24 weeks before entering into the	NM//control patients: who were not given autologous BM infusion (ABMi) but matched for age, sex, medication, various biochemical parameters	Not mentioned	safety and feasibility of ABMi therapy for ALC/ serum parameters of liver function (Alb,	Not mentioned

Study ID	Age of patients	Causes of liver diseases	Type of stem cells	statistical analysis	Unit data for blood tests	Recruitment dates	Inclusion criteria	Exclusion criteria	Details of standard therapy	Primary endpoint	Secondary endpoint
							study. Plt count >50, bil <3mg/dL, absence of liver cancer			PT), CPS, degree of liver cirrhosis with type IV collagen 7S domain	
Zhang ZQ 2012	mentioned, not sure	decompensated, not specified	BMSC	Not mentioned	Fibroscan kpa	Not mentioned	decompensated cirrhosis	Not mentioned	routine medical treatment	Not mentioned	Not mentioned
Cao H 2017	not mentioned	HBV	BMSC	Not mentioned	Not mentioned	Not mentioned	decompensated liver cirrhosis (HBV related)	Not mentioned	comprehensive therapy	Not mentioned	Not mentioned
Han Y 2008	20-70	HBV	GCSF and peripheral blood monocytes	continuous variables: mean and SD for ND, median and	Alb (g/L), AST and ALT (IU/L), Total bil	recruitment started in 2005	age 20-70, decompensated cirrhosis, abnormal serum Alb +/- Bil +/- PT,	age<20 or >70, liver cancer or other tumours, recurrent GI bleeding or SBP, HRS, PVT, acute infection, severe cardiac	patient did not receive antiviral therapies such as IFN, lamivudine and ribavirin	Endpoint: safety, different time points of LFs (at 1,2,3,4 week and 2,3,4,5,6 months)	

Study ID	Age of patients	Causes of liver diseases	Type of stem cells	statistical analysis	Unit data for blood tests	Recruitment dates	Inclusion criteria	Exclusion criteria	Details of standard therapy	Primary endpoint	Secondary endpoint
				quartile for asymmetric variables. P<0.05 is SS, SPSS 13	(umol/L), PT		Haemoglobin >/7g/L, Plt >300, CPS?.7, ascites controlled to a moderate degree, no hx of HE, SBP or bleeding in the last month.	insufficiency and coagulation disorders.			
Liao X 2013	not mentioned	HCV	BM derived liver stem cells	Not mentioned	Not mentioned	Not mentioned	hepatic cirrhosis and portal hypertension who required splenectomy with peri esophagogastric devascularization	Not mentioned	Not mentioned	Not mentioned	Not mentioned

Study ID	Age of patients	Causes of liver diseases	Type of stem cells	statistical analysis	Unit data for blood tests	Recruitment dates	Inclusion criteria	Exclusion criteria	Details of standard therapy	Primary endpoint	Secondary endpoint
Randomised controlled trials (Acute on chronic liver failure)											
Lin B 2017	ACLF	HBV	BM-MSC	mean+/-SD, or median and range as appropriate. SPSS 17 software. p<0.05 is SS	ALT (U/L), Alb (g/L), Bil (umol/L), INR	From Oct 11 to April 2013	ACLF manifesting as jaundice-bil >/10x ULN, coagulopathy (INR>/1.5 or PT<40%), complicated within 4 weeks by ascites +/- HE, diagnosed or undiagnosed CLD, +ve HBsAg for more than 6 months, MELD	serious complications in the previous 2 months (bleeding, infection/sepsis), superinfection with other hepatitis viruses, concomitant AIH, organ dysfunction-renal not due to liver disease or malignancies, pregnancy and lactation, liver tumour or regenerative nodules, bioartificial liver support therapy or previous LT	nutritional supplementation; administration of human serum albumin (10 g/day until serum albumin was 35 g/L), fresh frozen plasma (200-400 mL/ day until the INR was <1.5), entecavir (0.5 mg/day; S-adenosylmethionine (1.0 g/day) and appropriate treatment for complication	survival time and status of patient after allogenic BM-MSC infusions.	incidence of adverse reactions (fever, rash, diarrhoea), LFTs-ALT, ALB, Bik, INR and MELD, incidence of serious complications such as infection, HE, HRS, GI bleeding and

Study ID	Age of patients	Causes of liver diseases	Type of stem cells	statistical analysis	Unit data for blood tests	Recruitment dates	Inclusion criteria	Exclusion criteria	Details of standard therapy	Primary endpoint	Secondary endpoint
							ranging 17-30, age 16-60		s such as infections (including of the respiratory tract, urinary tract, biliary tract, and digestive tract and spontaneous peritonitis), encephalopathy, gastrointestinal bleeding, and hepatorenal syndrome [HRS]).		causes of death.
Chen J 2013	ACLF	HBV	BM-MSC	Not mentioned	Not mentioned	Not mentioned	HBV induced ACLF	Not mentioned	Not mentioned	safety and efficacy	Not mentioned

Study ID	Age of patients	Causes of liver diseases	Type of stem cells	statistical analysis	Unit data for blood tests	Recruitment dates	Inclusion criteria	Exclusion criteria	Details of standard therapy	Primary endpoint	Secondary endpoint
Duan X 2013	ACLF	HBV	GCSF	SPSS 12 mean+/-SD (quantitative), qualitative data (frequency or %), P<0.05 is SS	ALT and AST (U/L), Bil (umol/L), INR, Alb (g/L)	June 2009 to May 2011	1) ACLF 2) Hepatitis B sAg in the serum for at least 6 months 3) HBV DNA >/1x10*4 copies/ml 4) flare of hepatitis (ALT >5xULN) 5) age 18 to 65 years	1) co-infection with A, C, D, E, EBV, CMV or HIV 2) previous course of antiviral, immunomodulator or cytotoxic or immunosuppressive therapy within last 12 months 3) Decompensated LD prior to enrolment 4) HCC 5) co-existing other liver disease 6) any evidence of sepsis 7) malignant jaundice 8) prolonged PT due to blood system disease	Entecavir 0.5 mg/day and SMT include reduced glutathione, glycyrrhizin, ademetionine, polyene phosphatidylcholine, alprostadil, human serum albumin on the day of admission	changes in liver function and survival rate	Not mentioned
Engelmann C 2015	ACLF	not mentioned	GCSF	Not mentioned	Not mentioned	Due to start in 2015 (ongoing)	Age >18, ACLF diagnosis as per CANONIC study	Not mentioned	Not mentioned	transplant free survival up to 90 days	overall survival, complications of ACLF, rates of

Study ID	Age of patients	Causes of liver diseases	Type of stem cells	statistical analysis	Unit data for blood tests	Recruitment dates	Inclusion criteria	Exclusion criteria	Details of standard therapy	Primary endpoint	Secondary endpoint
							criteria, able to give written consent				infections, liver function, duration of hospital stay
Singh V 2015	ACLF	Alcoholic hepatitis/ ALD cirrhosis	GCSF	all statistical tests were two sided and were performed at a significance level of 0.05. no power calculation was performed	Bil (mg/dl), ALT, ALP (IU/L), Alb (g/dl), INR, PT (s)	July 2010 to June 2012	age 18-75, average alcohol intake of >100g/day during the 3 months before enrolment.	presence of HCC, portal vein thrombosis, refusal to participate, previous treatment with steroids, significant co-morbidities, HRS, grade 3 or 4 HE, upper GI bleeding within the preceding 10 days, uncontrolled bacterial infection, HIV infection, HBV, HCV, AIH, HH, Wilson's, A1ATD, pregnancy or previous known	pentoxyphylline 400mg TDS, normal hospital nutrition of 1800-2000 kcal/day, diuretics, sodium restriction, albumin for ascites, FFP for coagulopathy, antibiotics	survival at 90 days after recruitment and commencement of treatment	1) mobilisation of CD34+ cells in peripheral point 2) clinical scores-MELD, CTP, safety of GCSF in alcoholic hepatitis

Study ID	Age of patients	Causes of liver diseases	Type of stem cells	statistical analysis	Unit data for blood tests	Recruitment dates	Inclusion criteria	Exclusion criteria	Details of standard therapy	Primary endpoint	Secondary endpoint
								hypersensitivity to GCSF			
Garg V 2012 **(210)**	ACLF	Acute event (Alc hepatitis, reactivation of HBV, anti-TB therapy, HEV, Cryptogenic) on background of CLD (ALD, HBV, CC, Wilson's disease)	GCSF	descriptive statistics - median (range) or no (%). Survival - log rank tests, SPSS 15	Bil (mg/dl), ALT (IU/L), Alb (g/dl), INR	December 2008 to August 2010	ACLF as defined by APASL (bil >/5 mg/dl, INR >/1.5, complicated within 4 weeks by ascites +/- HE in patient with previous diagnosed CLD	age<12, >75. HCC or portal vein thrombosis, refusal to participate, any concurrent sepsis, MOF, grade 3-4 HE, HIV+, pregnancy, previous hypersensitivity to GCSF	lactulose, bowel wash, albumin, FFP, terlipressin, antibiotics, mechanical ventilation, vasopressors, renal replacement therapy. HBV-tenofovir, Alcoholic hepatitis-pentoxyphylline.	survival at day 60	1) improvement in CTP and SOFA scores, 2) development of new onset HE, HRS and sepsis

Study ID	Age of patients	Causes of liver diseases	Type of stem cells	statistical analysis	Unit data for blood tests	Recruitment dates	Inclusion criteria	Exclusion criteria	Details of standard therapy	Primary endpoint	Secondary endpoint
Khanam A (same pt. cohort as Garg V)	ACLF	ALD (17/12), HBV (4/7), CC (2/4), Wilson (0/1)-Stem cell/Control	GCSF	median with range or mean with SD. Non-parametric Mann-Whitney U test was used to calculate P values. Significance if p<0.05.	Bil (mg/dl), Alb (g/dl), ALT (U/L)	December 2008 to August 2010	ACLF, Grade 1 or 2 HE, with or without HRS	HCC, portal vein thrombosis, cardiovascular co-morbidities, grade 3 or 4 HE, sepsis, con-infection with HIV, pregnancy, refusal to participate, previous allergy to GSCF, age <12 or >75 excluded	lactulose, bowel wash, IV albumin, FFP, antibiotics, terlipressin for HRS, Tenofovir for HBV, Pentoxyphylline for alcoholic hepatitis	Not mentioned	Not mentioned

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