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LIVER FIBROSIS: Pathophysiology and Clinical Implications

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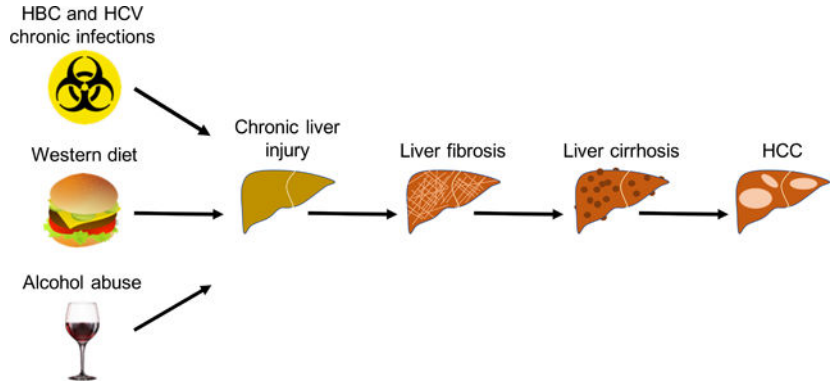
Abstract

Liver fibrosis is a clinically significant finding that has major impacts on patient morbidity and mortality. The mechanism of fibrosis involves many different cellular pathways, but the major cell type involved appears to be hepatic stellate cells. Many liver diseases, including Hepatitis B, C and fatty liver disease cause ongoing hepatocellular damage leading to liver fibrosis. No matter the cause of liver disease, liver related mortality increases exponentially with increasing fibrosis. The progression to cirrhosis brings more dramatic mortality and higher incidence of hepatocellular carcinoma. Fibrosis can also affect outcomes following liver transplantation in adult and pediatric patients and require retransplantation. Drugs exist to treat Hepatitis B and Hepatitis C that reverse fibrosis in patients with those viral diseases, but there are currently no therapies to directly treat liver fibrosis. Several mouse models of chronic liver diseases have been successfully reversed using novel drug targets with current therapies focusing mostly on prevention of myofibroblast activation. Further research in these areas could lead to development of drugs to treat fibrosis, which will have invaluable impact on patient survival.

Graphical Abstract



1. Introduction:

Liver fibrosis is a clinically significant finding that has major impact on patients' morbidity and mortality. Many liver diseases, including Hepatitis B, C and fatty liver diseases,

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cause ongoing hepatocellular damage leading to liver fibrosis. Fibrosis is the single histopathologic feature with the greatest impact on mortality (Stal, 2015). It eventually leads to cirrhosis, which is plagued by other complications including hepatocellular carcinoma and liver failure, leaving liver transplant as the only therapy. New medications to treat hepatitis C may halt the progression of fibrosis, but currently no definitive treatment for fibrosis exists.

Liver fibrosis involves multiple cellular mechanisms. Current models for liver fibrosis show an injury driven response from the hepatic cells which release C-C motif chemokine ligand 2 (CCL2), also known as monocyte chemoattractant protein 1 (MCP1), and TGF-ß1 promoting inflammation and activation of hepatic stellate cells (HSCs) into collagen producing myofibroblasts. Myofibroblasts have been shown as a key mechanism in the development of liver fibrosis and are the primary target of antifibrotic therapy (Kisseleva & Brenner, 2011). Research has been mainly focused on developing strategies to understand the molecular mechanisms behind liver fibrosis so that effective treatments for prevention and progression can be developed. Our review emphasizes the pathophysiology, cellular mechanisms clinical significance of fibrosis and its effect on healthcare, and explores opportunities for the development of treatments.

2. Pathophysiology of liver fibrosis

Liver fibrosis is caused by chronic liver injury of two different etiologies: hepatotoxic and cholestatic injuries. Hepatotoxic injury is triggered by cellular injury from outside factors including : hepatitis B and C (HBV and HCV) viral infections, alcoholic (ASH) and non-alcoholic (NASH) steatohepatitis (Bataller & Brenner, 2005). Cholestatic injury, which is characterized by reduced or obstructed bile flow in the liver, is caused by primary (and secondary) disease including: primary biliary cholangitis (PBC), primary sclerosing cholangitis (PSC) and biliary atresia (Bataller & Brenner, 2005). Liver fibrosis results in accumulation of extracellular matrix (ECM) proteins, mostly collagens Type I and Type III, followed by formation of fibrous scar, which can ultimately compromise normal liver function (Figure 1). Regardless of the etiology, liver fibrosis is characterized by common molecular mechanisms such as hepatocyte death, chronic inflammation with cytokine release, activation of HSCs and disruption of the epithelial or endothelial barrier (Dhar, Baglieri, Kisseleva, & Brenner, 2020). Therefore, hepatic fibrogenesis is a complex process requiring cellular and extracellular signaling. In the next section we describe the different cell types and pathways involved in the pathophysiology of liver fibrosis.

2.1 Cell types in liver fibrosis

Hepatic Stellate Cells—HSCs are the main cell type involved in liver fibrosis. In normal livers HSCs are quiescent, reside in the space of Disse, store vitamin A in lipid droplets and they serve as liver pericytes (Kisseleva et al., 2012; Troeger et al., 2012). However, in response to continuous liver injury HSCs reduce the expression of genes such as glial fibrillar acidic protein (GFAP) and peroxisome proliferator-activated receptor gamma (PPARγ), lose lipid droplets and activate into myofibroblasts. Myofibroblasts start expressing fibrogenic genes such alpha-smooth muscle actin (α-SMA) and Collagen Type

I. They proliferate and migrate to the liver injury site where they secrete ECM (Kisseleva & Brenner, 2008). Myofibroblasts also release vascular endothelial growth factor (VEGF) which directly promotes HSC proliferation (Kukla, 2013). Interestingly, studies performed in patients and experimental fibrosis models have shown that fibrosis can be reversed when the injury is removed (Iredale et al., 1998; Issa et al., 2003). In particular myofibroblasts can undergo apoptosis or inactivation once the causative etiology is cleared, leading to fibrosis regression (Kisseleva et al., 2012). Several mechanisms have been suggested to trigger HSC apoptosis. Following reduction of fibrogenic signals, HSCs increase expression of death-receptor mediated genes like Fas receptor or TNF receptor 1 (TNFR1), they upregulate pro-apoptotic proteins (p53, Bax, caspase 9) and downregulate anti-apoptotic factors (Bcl-2) (Iredale, 2001). Alternatively, interferon-γ (IFN-γ)-activated natural killer (NK) cells have also been involved in resolution of liver fibrosis by eliminating HSCs (Gao, Radaeva, & Park, 2009). Moreover, recent studies showed that besides senescence and apoptosis, myofibroblasts can also revert to an inactive phenotype during liver fibrosis regression (Kisseleva et al., 2012; Troeger et al., 2012). Interestingly these inactivated HSCs are more responsive to fibrogenic stimuli than quiescent HSCs

Several studies have intensively investigated the cellular origin of myofibroblasts. Following carbon tetrachloride (CCl_4) induced liver fibrosis, HSCs have been seen as the main source of myofibroblasts. However, in early cholestatic injury, myofibroblast origin is from portal fibroblasts (Iwaisako et al., 2014). Portal fibroblasts localize underneath the bile duct epithelium and, similar to HSCs, they activate into α-SMA- and Collagen Type I-expressing myofibroblasts in response to biliary injury (Desmoulière et al., 1997; Dranoff & Wells, 2010). Bone marrow-derived cells such as fibrocytes and mesenchymal stem cells (MSCs) have also been suggested to originate myofibroblasts (Kisseleva et al., 2006; Scholten et al., 2011). There is no evidence however that parenchymal cells like hepatocytes can contribute to the myofibroblast population (Taura et al., 2010).

Hepatocytes—Following liver injury, hepatocytes start producing several fibrogenic factors including osteopontin, NADPH oxidase 4 (NOX4), TAZ, Indian Hedgehog and Notch (Lan, Kisseleva, & Brenner, 2015; Wang et al., 2016; Xie et al., 2013; Zhu et al., 2018). Moreover, exosomes containing micro RNAs (miRNAs) that activate HSCs can also be released by injured hepatocytes (Lee et al., 2017). Nonetheless, hepatocyte-derived fibrogenic factors cannot lead to liver fibrosis in absence of chronic inflammation.

Inflammatory cells and cytokines—Inflammation caused by acute liver injury is considered to be beneficial in supporting hepatic regeneration. However, chronic inflammation is detrimental and has a critical role in the pathogenesis of liver fibrosis. In vitro and in vivo studies have demonstrated that inflammatory cells like neutrophils, Kupffer cells (the resident macrophages of the liver) bone marrow-derived monocytes and Th17 cells can promote HSC activation by secreting cytokines and growth factors (Seki & Schwabe, 2015). Liver macrophages, including Kupffer cells, are the main source of transforming growth factor-β (TGF-β), which plays a key role in liver fibrogenesis (Dooley & ten Dijke, 2012). TGF-β binds to its receptor in HSCs, promoting their activation into myofibroblasts and the synthesis of Collagen Type I and III. Therefore, blocking TGF-β

or its genetic deletion has been shown to reduce liver fibrosis (de Gouville et al., 2005; Hellerbrand, Stefanovic, Giordano, Burchardt, & Brenner, 1999). Th17 cells secrete IL-17, which is also a profibrogenic cytokine, and the disruption of IL-17 signaling attenuates development of liver fibrosis (Meng et al., 2012). Th17 cells also produce also IL-22. Interestingly, some studies have shown that IL-22 protects from liver injury and reduces liver fibrosis (Feng et al., 2012; Ki et al., 2010; Kong et al., 2012; Radaeva, Sun, Pan, Hong, & Gao, 2004), however other reports have suggested that IL-22 is profibrogenic and its pharmacological inhibition ameliorates liver fibrosis (Fabre et al., 2018). Another fibrogenic cytokine is CCL2 which is secreted by macrophages in response to liver injury (Marra et al., 1998). CCL2 promotes activation of HSCs by recruiting monocyte-derived macrophages in the liver (Karlmark et al., 2009). Platelet-derived growth factor (PDGF) is released by macrophages and is widely considered a powerful mitogen for HSCs. The PDGF signaling pathway has been shown to be crucial to HSC activation and the development of liver fibrosis (Borkham-Kamphorst et al., 2015; Czochra et al., 2006). During liver injury neutrophils and activated Kupffer cells also release also reactive oxygen species (ROS), which promote HSC activation (Luangmonkong et al., 2018). Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX) is responsible for ROS production. Mice lacking the NOX1 and NOX4 isoforms show reduced liver inflammation, reduced HSC activation and fibrosis (Lan et al., 2015). Similar results were obtained by pharmacological inhibition of NOX1 and NOX4 in mouse models of liver injury (Aoyama et al., 2012; Bettaieb et al., 2015; Jiang et al., 2012). Interestingly, macrophages not only promote liver fibrosis, but can also support fibrosis regression. Macrophages promote myofibroblast apoptosis (Ramachandran et al., 2012) and phagocytize the apoptotic cells. Furthermore, they secrete matrix metalloproteinases like MMP9, MMP12 and MMP13 which degrade ECM, a crucial process in fibrosis resolution (Popov et al., 2010; Uchinami, Seki, Brenner, & D'Armiento, 2006). Increased activity of MMPs downregulate the expression of tissue inhibitor of metalloproteinases (TIMP1). TIMP1 is a promoter of HSC activation and survival (Parsons et al., 2004).

Liver sinusoidal endothelial cells—In healthy livers liver sinusoidal endothelial cells (LSECs) are crucial for transport of nutrients, recruitment of lymphocytes from the blood stream and secretion of cytokines and growth factors (Asahara et al., 1999). Uninjured LSECs are differentiated, they have fenestrations and have been shown to maintain HSCs in a quiescent state (Deleve, Wang, & Guo, 2008; Xie et al., 2012). Production of nitric oxide (NO) by endothelial NO synthase (eNOS) is important in maintaining a physiological LSECs phenotype, in preventing HSCs activation and in promoting reversal of activated HSCs to quiescence (DeLeve, Wang, Hu, McCuskey, & McCuskey, 2004). However, in chronic liver injury LSECs undergo capillarization, which consists in loss of fenestration and reduction in eNOS activity and NO synthesis (Iwakiri, Grisham, & Shah, 2008). Therefore, in the injured liver LSECs lack of the gatekeeper function that promotes HSCs quiescence. Moreover, following disruption of the endothelium, LSECs will secrete profibrogenic cytokines like TGF-β1, PDGF, interleukins, tumor necrosis factor α (TNFα), and VEGF, which will recruit inflammatory cells to the site of injury and activate HSCs (DeLeve, 2015). Interestingly, VEGF has a biphasic nature; it induces fibrosis-associated angiogenesis (Baeck et al., 2012) and also promotes fibrosis resolution

by regulating vascular permeability, monocyte migration and scar-associated macrophages function (Deleve et al., 2008; L. Yang et al., 2014). As described above, NO produced by LSECs is important in preventing HSCs activation. Furthermore, it has also been shown that NO promotes apoptosis of VEGF-activated HSCs during fibrosis reversal (Langer et al., 2008). NO-dependent apoptosis occurs through a mechanism which involves mitochondria, generation of ROS and is independent of caspase activation.

Chronic liver damage of different etiologies causes injured hepatocytes and LSEC to release proinflammatory and profibrogenic factors, which lead to chronic liver inflammation followed by activation of quiescent HSCs into Collagen Type I and III-producing myofibroblasts. As a result, fibrous scar accumulates into the liver impairing its normal functions. (See text for details).

2.2 Animal models of fibrotic liver diseases

The use of animal models is of key importance to understand the fibrotic process and to develop novel antifibrotic therapies. In the following section we describe the main features of current rodent models of liver injuries.

Toxic models of liver fibrosis—Administration of carbon tetrachloride (CCl_4) in mice or rats is the most commonly used model of toxin-induced experimental liver fibrosis. In mice generally 0.5 to 2 mL/Kg body weight of CCl_4 is administered either intraperitoneally or by oral gavage two to three times per week (Liedtke et al., 2013). CCl_4 treatment causes activation of HSCs followed by deposition of ECM and development of highly reproducible liver fibrosis after 4 to 6 weeks from the first CCl_4 injection. Cytochrome P450 2E1 (CYP2E1) in centrilobular hepatocytes metabolizes CCl_4 to generate toxic trichloromethyl (CCl_3) radicals, which promote liver necrosis (Slater, Cheeseman, & Ingold, 1985). Interestingly, withdrawal of CCl_4 treatment results in full regression of liver fibrosis (Iredale et al., 1998; Kisseleva et al., 2012)

Thioacetamide (TAA) is another model of experimental liver fibrosis in rodents. TAA can be either administered either intraperitoneally (150–200 mg/Kg body weight) three times a week (Ding & Zhuo, 2013) or orally by adding TAA (200 mg/L) to the drinking water. In rats TAA treatment causes fibrosis and cirrhosis between 12–16 weeks, in mice between 16–24 weeks (Reif et al., 2004; Salguero Palacios et al., 2008). TAA affects both zone 1 and zone 3 hepatocytes causing portal-portal and portal-central fibrosis, which eventually recapitulates a state similar to human cirrhosis (Kang et al., 2008; Li, Benjamin, & Alexander, 2002). Moreover, unlike CCl_4-induced fibrosis which regresses rapidly, fibrosis lasts for more than 2 months after cessation of TAA treatment (Reif et al., 2004). Similarly to CCl_4, TAA is also bioactivated by CYP2E1 in the liver, giving rise to *S,S*-dioxide, which is most likely the agent causing hepatotoxicity (Hajovsky et al., 2012).

Administration of dimethylnitrosamine (DMN) is less commonly used as fibrosis model (Jenkins et al., 1985). 10 mg/Kg body weight of DMN is administered intraperitoneally twice a week. DMN induces activation of HSCs and Kupffer cells with liver fibrosis development within 4 weeks (Kitamura et al., 2002). A disadvantage of using DMN is its carcinogenic properties, which may complicate interpretation of the results.

Models of biliary fibrosis—Cholestatic liver diseases like PBC and PSC are characterized by injury of the biliary epithelium and bile duct, which can cause liver fibrosis, cirrhosis and end-stage liver disease. Several animal models are available to mimic cholestatic liver injuries (Delire, Stärkel, & Leclercq, 2015).

The most common model of obstructive cholestatic injury in rodents is surgical bile duct ligation (BDL) in which the common extrahepatic bile duct is ligated. Fibrosis is generally observed after 21–28 days (Scholten et al., 2010).

Another model is phospholipid transporter multi-drug resistant protein 2 (MDR2) deficient mice. MDR2 is encoded by the *Abcb4* gene. *Abcd4* knock out mice (*Abcb4*$^{-/-}$) are not able to secrete phospholipid into bile, resulting in increased concentration of free bile acids. This damages hepatocytes and cholangiocytes, resulting in inflammatory cholangitis, portal inflammation and periductal fibrosis(Mauad et al., 1994). In this model biliary fibrosis appears shortly after birth and progresses to end-stage disease in 3–6 months.

Cholestatic liver injury can also be induced by modified diets in mice. A diet supplemented with 0.1% 3,5-diethoxycarbonyl-1,4 dihydrocollidine (DDC) for 8 weeks causes biliary liver fibrosis similar to human PSC (Fickert et al., 2007).

A diet supplemented with low doses (0.025%) of α-naphthylisothiocynate (ANIT) is another xenobiotic model of biliary injury (ELIAKIM, EISNER, & UNGAR, 1959). ANIT is toxic to hepatocytes and bile duct cells. Mice subjected to this diet will develop periportal inflammation and fibrosis with mild hepatocellular injury (Sullivan, Weinreb, Violette, & Luyendyk, 2010).

Models of alcohol-induced fibrosis—Although currently there are no animal models able to mimic all features of alcoholic liver disease (ALD), several animal models for ALD have been generated (Mathews, Xu, Wang, Bertola, & Gao, 2014). In the Lieber-De Carli model (Iseri, Lieber, & Gottlieb, 1966), animals are fed solely with an alcohol-containing isocalorically controlled liquid diet in which 36% of calories comes from alcohol. After 4–12 weeks of feeding, animals develop mild liver steatosis and inflammation, which recapitulates chronic drinking patterns seen in humans. However, no fibrosis is observed in this model (Bala et al., 2012). Another drawback is the natural aversion of animals to alcohol consumption. The Tsukamoto-French model (Tsukamoto et al., 1985) overcomes these issues and obtains prolonged high blood alcohol levels (Rouach et al., 1997). In this model animals are infused with alcohol by an intragastric canula and develop steatosis, inflammation and necrosis in 2–4 weeks, and fibrosis in 6–8 weeks (Iimuro, Ikejima, Rose, Bradford, & Thurman, 1996). However, the surgical procedure of insertion and maintenance of the intragastric canula requires intensive medical care limiting the use of this model (Ueno et al., 2012). Additional models were developed combining liquid diets containing different concentrations of alcohol (1–5%) together with intraperitoneal injections of CCl_4 (Chiang et al., 2013; Jeong, Park, & Gao, 2008). Activation of HSCs and severe fibrosis was observed in these models after 5–8 weeks of feeding. Interestingly, these studies demonstrated that chronic administration of alcohol exacerbates CCl_4-induced liver fibrosis.

Models of NASH-induced liver fibrosis—Human NASH is characterized by fatty liver, ballooning hepatocytes, inflammation and fibrosis (Rinella, 2015). To date no animal models can fully recapitulate the histology and pathophysiology of human NASH. Nonetheless, several dietary and genetic animal models have been described (Kucera & Cervinkova, 2014).

The methionine- and choline-deficient diet (MCDD) lack 2 key factors in the production and secretion of very low density protein (VLDL) from the liver (Ghoshal, 1995). Shortly after the start of the feeding, animals develop steatohepatitis and by 7–10 weeks perisinusoidal fibrosis (Ip, Farrell, Hall, Robertson, & Leclercq, 2004). However, unlike human NASH, MCDD diet causes weight loss and insulin hypersensitivity, which need to be taken into account in interpretation of the results (Rinella & Green, 2004).

Unlike MCDD, the choline-deficient, L-amino acid-defined diet (CDAA) contains low levels of methionine. The CDAA diet mechanism of action is similar to MCDD diet, however it causes moderate pericellular fibrosis. Animals chronically fed with CDAA may also develop HCC (Kodama et al., 2009).

In high fat diets (HFD), animals receive 30–60% of calories from fat. As a consequence they will gain weight, acquire insulin resistance and develop hepatic steatosis (Riordan & Nadeau, 2014). Diets that contain high glucose and fructose levels have also been developed, as well as diets enriched in both lipids and carbohydrates (cafeteria diet, atherogenic diet, western diet) (Kucera & Cervinkova, 2014).

Several genetic animal models of NASH have also been generated (Larter & Yeh, 2008). Among these, one of the most used is the *ob/ob* (ob=obese) mouse (Lindström, 2007). These mice are deficient of leptin, which is a hormone produced by the adipose tissue. The mice are hyperphagic, become obese and develop hyperglycemia, hyperinsulemia and hepatic steatosis. However, these mice require additional stimulation, such as MCDD or HFD diets to develop steatohepatitis (Brix et al., 2002).

Another genetic mouse model that recapitulates the progression of NASH in patients is the Mup-uPA mouse (Nakagawa et al., 2014). This mouse expresses urokinase-type plasminogen activator (uPA) under the control of major urinary protein (MUP) promoter. Overexpression of the transgene makes hepatocytes sensitive to injury. When these mice are fed with HFD for 8 months they will develop steatosis, steatohepatitis and eventually HCC.

3. Clinical aspects of liver fibrosis.

3.1 Stages and classification of liver fibrosis

Although several noninvasive methods exist to diagnose hepatic fibrosis, such as MRI fibroscans, they are not precise enough for the staging of fibrosis. These can be used as a screening process to determine the presence of hepatic fibrosis, but the gold standard for diagnosis and staging remains the liver biopsy. The actual staging of fibrosis has several classification systems and the most common clinically used scores are illustrated in Table 1. The Knodell score was proposed in 1981 and names three stages of fibrosis (Knodell et al.,

1981). The favored Batts-Ludwig and Scheuer Stages divide fibrosis into 4 stages (Locke, Therneau, Ludwig, Dickson, & Lindor, 1996; Scheuer, 1991). In the Ishak scale instead fibrosis stages range from 0–6 (Everhart et al., 2010; Ishak et al., 1995; Pinzani & Luong, 2018).

3.2 Significance of Fibrosis Stage

3.2.1 Clinical Classification of Liver Disease—The severity of clinical liver disease is classified by several different systems, including the Child Pugh Score and the Model for End Stage Liver Disease (MELD) score. Each of these predicts mortality with cirrhosis (Brown et al., 2002; Kamath et al., 2001). The Childs Pugh Score was initially developed to measure operative mortality in cirrhotic patients. It is calculated with a mix of laboratory and clinical factors including ascites and encephalopathy. Patients have a score from 5–15 and a classification (A, B, or C). The most compensated cirrhotic patient would be 5A, and the most decompensated 15C. MELD score is calculated using a patient's laboratory values (creatinine, bilirubin, INR, and sodium) and correlates strongly with 3-months mortality. MELD score is used for the liver transplant waitlist to prioritize transplanting the patients with highest mortality.

3.2.2 Mortality in fibrosis—Epidemiological studies have demonstrated that staging of fibrosis has significant clinical implications. Increased fibrosis stage is associated with higher all-cause mortality when compared to patients without fibrosis, and even stage 1 fibrosis. Different disease etiologies of liver fibrosis may affect the degree of mortality as well. However, no matter the cause of liver disease, liver related mortality increases exponentially with increasing fibrosis (Dulai et al., 2017; Ekstedt et al., 2015). In alcoholic liver disease severe fibrosis, prior to developing cirrhosis, has a major impact on 10-year mortality (Lackner et al., 2017). In non-alcoholic fatty liver disease (NAFLD) higher stages of fibrosis are proven to be the most important predictor of mortality (Angulo et al., 2015; Ekstedt et al., 2015; Lackner et al., 2017; Lackner & Tiniakos, 2019) In one NAFLD study, the ten-year transplant free survival rate with stage 4 fibrosis (and Childs Pugh A6) was shown to be only 17%. In contrast, ten-year transplant free survival in stage 4 fibrosis with Child Pugh A5 was 74%, and in stage 3 fibrosis was 94% (Vilar-Gomez et al., 2018).

3.3 Cirrhosis

With the progression of fibrosis to cirrhosis comes more dramatic liver related mortality (Vilar-Gomez et al., 2018). In 2017 chronic liver disease and cirrhosis were the 11th leading cause of death in the United States (Melonie Heron, June 24, 2019).

An Italian study evaluating the effects of the degree of decompensation in 494 patients over 3 years showed higher mortality with increasing decompensation. Patients with compensated cirrhosis had a 5-year mortality of 1.5% while patients with two decompensating events had an 88% 5-year mortality. These results were shown to be independent from Child Pugh score or MELD score (D'Amico et al., 2014). Examining the natural history of cirrhosis in Sweden in 1,317 patients over a 10-year period, the 10-year incidence of decompensation was found to be 89%. 75% of those patients died over the study period, most commonly

due to liver failure and complications of cirrhosis (Nilsson, Anderson, Sargenti, Lindgren, & Prytz, 2019).

Rates of decompensation may vary by etiology of disease, with 10-year decompensation rates of 89% in alcoholic cirrhosis, 58% in hepatitis C and 75% in cryptogenic cirrhosis. One study that followed patients from four countries (Australia, USA, UK, and Italy) found a higher rate of liver related complications, including HCC, in HCV cirrhosis than in NAFLD cirrhosis (Bhala et al., 2011). Alcoholic liver disease may also progress to cirrhosis, and complications, including HCC, are faster than in non-alcoholic fatty liver disease (Shoreibah et al., 2016). Patients with cirrhosis, especially decompensated patients, have high rates or hospital readmissions and contribute to higher healthcare costs (Sempokuya et al., 2019). In a study involving Veterans Health Administration patients, annual costs of care increased as patients progressed from non-advanced fibrosis to advanced fibrosis, HCC, and liver transplant (Gidwani-Marszowski et al., 2019).

3.4 Hepatocellular Carcinoma Development

Fibrosis and cirrhosis are also associated with the development of hepatocellular carcinoma (HCC) (White, Thrift, Kanwal, Davila, & El-Serag, 2017). HCC can be treated with transplant if the tumors are confined to the liver and meet size criteria. The most common criteria are called the Milan criteria (Mazzaferro et al., 1996). The Milan criteria were developed after studying the size of HCC at transplant that would result in acceptable survival and low recurrence rates (Table 2). HCC is a common cause of death in cirrhotic patients, and the major cause of liver related death in compensated cirrhotic patients (Fattovich, Stroffolini, Zagni, & Donato, 2004). In the Swedish cohort outlined above, 15% of deaths in cirrhotic patients over the 10-year period were due to HCC (Nilsson et al., 2019). Although, patients with cirrhosis are more likely to develop HCC than non-cirrhotic, or patients with stage 1 or 2 fibrosis, the incidence increases as the stage of fibrosis increases (Danielsson Borssen et al., 2015; Fattovich et al., 2004; Gronbaek, Vilstrup, & Jepsen, 2014). The rate of HCC development varies in different studies and by etiology of liver disease. A study evaluating 634 Swedish patients with autoimmune hepatitis (AIH) found 4% of cirrhotic patients developed HCC with an incidence rate of 0.3% (Danielsson Borssen et al., 2015). Another study in Danish patients with AIH showed a 10-year cumulative risk of 0.7% of HCC (Gronbaek et al., 2014). The highest rates of HCC development appear to be with hereditary hemochromatosis (5-year incidence of 21%), hepatitis B infection (5-year risk of 10–15%), and HCV cirrhosis. In HCV cirrhosis the 5-year risk is as high as 17% in Western countries and even up to 30% in Japan (Fattovich et al., 2004). When evaluating the effects of treating hepatitis C virus with new direct acting antiviral medications on HCC incidence, patients with cirrhosis and treatment failure had higher rates of HCC than patients without cirrhosis and those who obtained sustained virologic response (SVR) (Ioannou, Green, & Berry, 2017). Other studies have also shown significantly decreased rates of HCC in patients with fibrosis and cirrhosis who have obtained SVR (Beste et al., 2017; Pinero et al., 2019).

3.5 Liver Transplant and Fibrosis

3.5.1 Liver Transplantation in Adults—Recurrent fibrosis after liver transplant has shown to be a significant indicator of chronic failure of the graft. The most common causes of late graft failure after transplant are recurrence of primary disease, de novo disease (autoimmune hepatitis or NAFLD) and chronic rejection, all which lead to fibrosis and cirrhosis (Berumen & Hemming, 2017; Kitchens, Yeh, & Markmann, 2014). In HCV disease recurrence post-transplant, fibrosis has been found to progress faster than pre-transplant (Berenguer et al., 2000). Nonalcoholic steatohepatitis also has a high rate of recurrence post-transplant, but a low incidence of fibrosis development, with only 5% and 10% of patients developing bridging fibrosis or cirrhosis after 5 and 10 years, respectively (Yalamanchili, Saadeh, Klintmalm, Jennings, & Davis, 2010).

3.5.2 Pediatric Liver Transplantation—Pediatric liver disease has also seen an increase in the last several years, mainly with a higher incidence of nonalcoholic fatty liver disease. This is linked to an increase in metabolic syndrome and obesity seen in children. NAFLD in these children is linked with a higher mortality rate than age matched children and can lead to the need for transplant at a young age (Feldstein et al., 2009; Hadzic, Baumann, McKiernan, McLin, & Nobili, 2017). Biliary atresia remains the most common indication for liver transplant in children ("Scientific Registry of Transplant Recipients. 2018 Annual Data Report,").

In pediatric allograft recipients, there is an association with acute cellular rejection and donor specific antibody (DSA) formation with the development of fibrosis in the transplanted liver. Patients who developed antibodies demonstrated significantly higher stages of fibrosis when compared to patients who had episodes of rejection without DSA formation, regardless of other factors, including initial primary disease (Tokodai et al., 2018). Some pediatric liver transplant patients with chronic hepatitis had progression over several years to fibrosis, and some subsequently to cirrhosis. HSCs also appear to be involved in the process, with higher levels of α-SMA secreted by HSCs correlating with higher stages of fibrosis in pediatric liver transplant recipient biopsies (Varma et al., 2017). Fibrosis can occur in a periportal pattern, perisinusoidal pattern, or perivenular pattern. The mechanism of each is not completely understood, but immunologic factors seem to have a significant role(Kelly et al., 2016; Markiewicz-Kijewska et al., 2015). One study looking at 47 liver biopsies of children post-transplant at 1 and 5 years showed some specific associations with the pathologic fibrosis patterns. Portal fibrosis was linked to biliary complications post-transplant, sinusoidal fibrosis was associated with prior rejection episodes, and centrilobular fibrosis was associated with immunologic mismatch (related to DSA) (Baas et al., 2017) .

3.6 Treatment and management of fibrosis

3.6.1 Existing therapies—Removal of the underlying etiological agent in liver disease may halt or improve liver fibrosis. This likely results from decreasing hepatocyte injury, which in turn decreases inflammation, and subsequent activation of hepatic stellate cells to become scar-producing myofibroblasts (Bataller & Brenner, 2005; Kisseleva & Brenner, 2011; Manka, Zeller, & Syn, 2019). Phlebotomy and iron chelation with subsequent

depletion of iron overload may cause the regression of liver fibrosis in hereditary hemochromatosis (Falize et al., 2006). Copper chelating agents can improve or stabilize liver disease in patients with liver disease and cirrhosis from Wilson's disease (Merle, Schaefer, Ferenci, & Stremmel, 2007). Specific therapies exist for specific liver diseases, including direct acting antivirals for hepatitis C virus, which decrease progression to cirrhosis and rates of HCC in HCV (Ioannou et al., 2017; Pinero et al., 2019) Specific drugs treating chronic hepatitis B have resulted in the regression of fibrosis and cirrhosis (Marcellin et al., 2013). Ceasing alcohol consumption in patients with alcoholic liver disease can improve outcomes and prevent progression to cirrhosis. Weight reduction either by bariatric surgery or by diet has been shown to improve inflammation and fibrosis in NASH (Promrat et al., 2010).

3.6.2 Future therapies—Although there are currently no therapies to directly treat liver fibrosis, such therapies are under development. Fibrosis in several mouse models of chronic liver disease has been successfully reversed using novel drug targets (Ellis & Mann, 2012). A common theme of anti-fibrotic drugs is blocking the activation of hepatic stellate cells to become myofibroblasts. Such targets include the transforming growth factor ß (TGF ß) pathway, which is the main fibrogenic pathway. Other targets include the platelet-derived growth factor (PDGF) pathway, which is the major mitogen for activated HSCs and the CB1 cannabinoid receptor, which functions as a co-activator of HSCs. Drugs are now being developed particularly in NASH for use in humans and several Phase III trials are underway (Alkhouri, Lawitz, & Noureddin, 2019; Bataller & Gao, 2015). Interestingly, a recent work has shown that inhibiting the synthesis of hyaluronan (HA), which is a component of ECM and a marker of cirrhosis, by using 4-methylumbelliferone reduced HSC activation and progression of liver fibrosis in mice (Y. M. Yang et al., 2019). This suggests that HA inhibition may be a novel anti-fibrotic therapeutic target.

Another antifibrotic therapeutic approach consists of reducing inflammation to avoid HSCs activation. Anti-inflammatory agents like corticosteroids have been used for treating autoimmune hepatitis, however they have several adverse effects in long-term treatment (Bansal, Nagórniewicz, & Prakash, 2016). Pentoxifylline (PTX) inhibits the production of TNFα, therefore blocking therefore the release of inflammatory cytokines (Vircheva et al., 2010). NASH patients treated with PTX show reduction in liver fibrosis (Zein et al., 2011). Although this study demonstrated that PTX lacks adverse effects, correlation between PTX and TNFα downregulation was not proved.

Cenicriviroc (CVC) blocks the inflammatory cytokine receptors CCR2 and CCR5 which could potentially ameliorate liver fibrosis (Seki, De Minicis, et al., 2009; Seki, de Minicis, et al., 2009). CVC has been shown to be safe (Lefebvre et al., 2016) and it is currently under evaluation for the treatment of NASH.

Galectin-3 (Gal-3) is a β-galactosidase binding lectin and a powerful activator of macrophages. Gal-3 deficiency has been shown to reduce susceptibility to CCl_4-induced fibrosis in mice (Henderson et al., 2006). Gal-3 inhibition by using the inhibitor GR-MD-02 (galactoarabino-rhamnogalacturonate) was demonstrated to be successful in reducing liver

fibrosis in rats (Traber et al., 2013). Clinical trials demonstrated that GR-MD-02 was safe in NASH patients and ameliorated liver fibrosis and inflammation(Harrison et al., 2016).

Inflammation can also be reduced by inhibition of ROS generation and oxidative stress. Therefore, several antioxidants like vitamin E, phosphatidylcholine, silymarin and N-acetylcysteine (NAC) have been tested with encouraging results as antifibrotic drugs (Sanyal, 2010).

A different category of antifibrotic therapies focus on inhibiting the formation of scar tissue. A key component of the scar tissue deposition process is lysyl oxidase-like-2 (LOXL2), which is involved in Collagen Type I cross-linking(Barry-Hamilton et al., 2010). Since LOXL2 is a copper-dependent enzyme, copper-binding ligands like β-aminopropionitrile and D-penicillamine have been studied as LOXL2 inhibitors (Jung, Kim, Seo, Kim, & Kim, 2003; Rodriguez et al., 2010; Vadasz et al., 2005). However, more successful strategies are the use of monoclonal anti-LOXL2 antibodies. AB0023 is a murine monoclonal antibody that has been shown to reduce cytokines release and HSCs activation (Barry-Hamilton et al., 2010). Simtuzumab (GS-6624) which is in clinical trials for liver fibrosis, is a monoclonal antibody targeting human LOXL2. GS-6624 is well tolerated in NASH patients and appears to be a promising antifibrotic drug alone or in combination with selonsertib (GS-4997) (Schuppan & Kim, 2013).

4. Conclusions

Considering the significant impact on survival with the progression of fibrosis to cirrhosis, early detection of disease at the fibrosis stage is important in order to enact interventions to block the progression of fibrosis. There is an increasing incidence of non-alcoholic steatohepatitis in the population with an estimated 25% of the world's population with NAFLD, and with over 4 million people assumed to have NAFLD associated fibrosis in the US in 2010 (Kabbany et al., 2017; Younossi et al., 2016). The increase in NAFLD is also likely contributing to a trend in increased diagnosis of HCC in the United States. Alcoholic liver disease related fibrosis (stage 2 and 3) has been increasing over the last several years, now with an increase in alcoholic cirrhosis related deaths, particularly in younger patients aged 25 to 34 years (Tapper & Parikh, 2018; Wong, Dang, Ladhani, Singal, & Wong, 2019). The continued growing number of patients worldwide with liver disease will mean that therapies for fibrosis and liver disease will become even more necessary.

No specific fibrosis drug treatment currently exists in practice, but the ability to stop and potentially reverse fibrosis progression in other interventions (weight loss, alcohol cessation, HCV treatment, etc) give hope that fibrosis can be easily treated in the future. With the success of direct acting antivirals in HCV, and antivirals in hepatitis B there is proof of success in reversal and treatment of fibrosis with removal of the insult. There are also diseases, like alpha-1-antitrypsin deficiency, that cause liver disease and have no interventions to reduce fibrosis, ultimately leading to cirrhosis and the need for liver transplant. HSCs are activated by a chronic cycle of cell death and regeneration in alpha-1-antitrysin deficiency (Teckman & Blomenkamp, 2017). Current antifibrotic research is focusing on specific pathways, including blocking the activation of HSCs into

myofibroblasts. Targeted drug therapies are currently being developed with many focusing on NASH diseases. If we are able to prevent progression and development of fibrosis, the impact on healthcare resources and patients' survival would be invaluable. The need for liver transplant could be significantly reduced and countless lives could be saved.

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Abbreviations:

NAFLD	non-alcoholic fatty liver disease
NASH	non-alcoholic steatohepatitis
HCC	hepatocellular carcinoma
MELD	Model for End Stage Liver Disease
HCV	Hepatitis C virus

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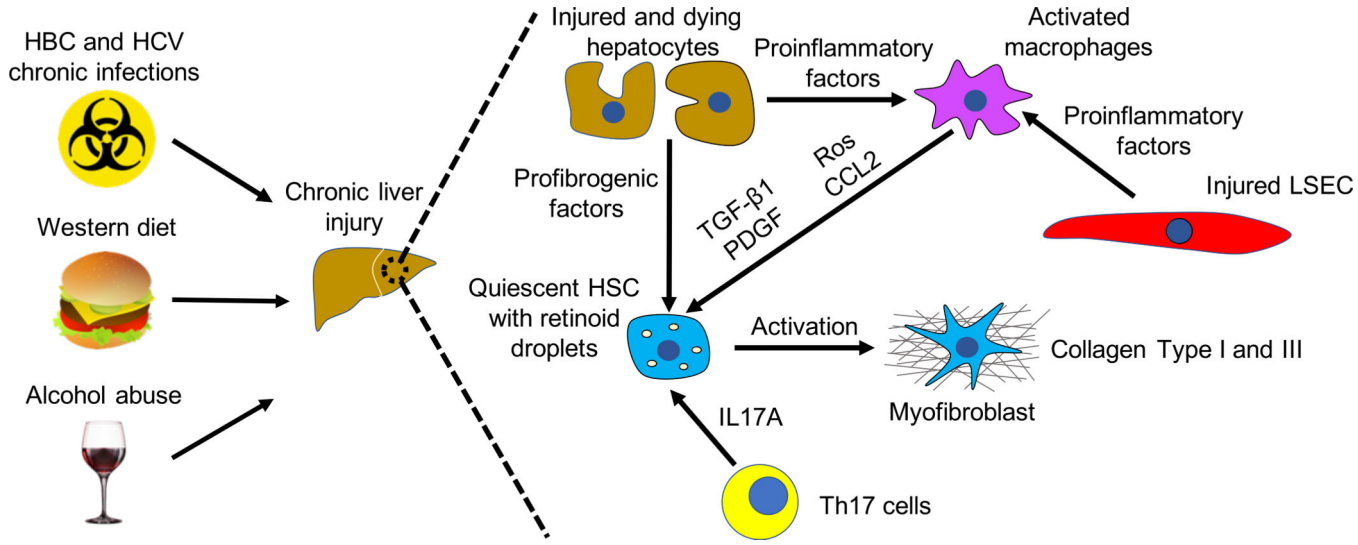

Figure. 1. Pathophysiology of liver fibrosis.

Table 1.

Basic liver fibrosis classifications

GENERAL CLASS	KNODELL	BATTS-LUDWIG	SCHEUER	ISHAK
NO FIBROSIS	0 - NO FIBROSIS	0 - NORMAL CONNECTIVE TISSUE	0 - NO FIBROSIS	0 - NO FIBROSIS
EARLY FIBROSIS	FIBROUS PORTAL EXPANSION	1 - FIBROUS PORTAL EXPANSION	1 - ENLARGED, FIBROTIC PORTAL TRACTS	1 - FIBROUS EXPANSION OF SOME PORTAL AREAS WITH OR WITHOUT SHORT FIBROUS SEPTA
				2 - FIBROUS EXPANSION OF MOST PORTAL AREAS WITH OR WITHOUT SHORT FIBROUS SEPTA
PERIPORTAL FIBROSIS	BRIDGING FIBROSIS (PORTAL-PORTAL OR PORTAL-CENTRAL LINKAGE)	2 - PERIPORTAL FIBROSIS +/− PORTAL-PORTAL SEPTA	2 - PERIPORTAL FIBROSIS OR PORTAL-PORTAL SEPTA, INTACT ARCHITECTURE	3 - FIBROUS EXPANSION OF MOST PORTAL AREAS WITH OCCASIONAL PORTAL TO PORTAL BRIDGING
				4 - FIBROUS EXPANSION OF PORTAL AREAS WITH MARKED PORTAL-PORTAL AND PORTAL-CENTRAL BRIDGING
LATE BRIDGING FIBROSIS		3 - BRIDGING FIBROSIS BUT NO OBVIOUS CIRRHOSIS	3 - FIBROSIS WITH ARCHITECTURAL DISTORTION	5 - MARKED BRIDGING WITH OCCASIONAL NODULES (INCOMPLETE CIRRHOSIS)
CIRRHOSIS	CIRRHOSIS	4 - REGENERATIVE NODULES ENCIRCLED BY FIBROUS SEPTA	4 - PROBABLY OR DEFINITE CIRRHOSIS	6 - CIRRHOSIS, PROBABLY OR DEFINITE

Table 2.

Milan criteria for liver transplantation

MILAN CRITERIA FOR LIVER TRANSPLANTATION and HEPATOCELLULAR CARCINOMA
- One single lesion less than 5 cm
- 3 lesions or less with none exceeding 3 centimeters
- No extrahepatic involvement
- No evidence of vascular invasion
- HCC diagnosed radiologically
- Biopsy only obtained if radiologic diagnosis can’t be made