Review

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Fibrosis and alcohol-related liver disease

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Key point

While the extent and type of fibrosis is a key prognostic indicator in ALD, there is currently no universally accepting staging system for assessing the extent of fibrosis.

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Summary

Histological fibrosis stage is one of the most important prognostic factors in compensated and decompensated alcohol-related liver disease (ALD). Morphological assessment of fibrosis is useful for patient stratification, enabling individualised management, and for evaluation of treatment effects in clinical studies. In contrast to most chronic liver diseases where fibrosis is portal-based, fatty liver disease (FLD) of alcoholic or non-alcoholic aetiology (NAFLD) is associated with a centrilobular pattern of injury which leads to perivenular fibrosis and/or pericellular fibrosis. Progression of FLD drives expansive pericellular fibrosis, linking vascular structures and paving the way for the development of cirrhosis. At the cirrhotic stage, ongoing tissue damage leads to increasing fibrosis severity due to parenchymal loss and proliferation of fibrous scars. Histologic fibrosis staging systems have been devised, based on topography and the extent of fibrosis, for most chronic liver diseases. The utility of histological staging is reflected in different risks associated with individual fibrosis stages which cannot be reliably distinguished by non-invasive fibrosis assessment. In contrast to NAFLD, ALD-specific staging systems that enable the standardised prognostication required for clinical management and trials are lacking. Although morphological similarities between NAFLD and ALD exist, differences in clinical and histological features may substantially limit the utility of established NAFLD-specific staging systems for prognostication in ALD. This review summarises morphological features of fibrosis in ALD and compares them to other chronic liver diseases, particularly NAFLD. ALD-related fibrosis is examined in the context of pathogenetic mechanisms of fibrosis progression, regression and clinical settings that need to be considered in future prognostically relevant ALD staging approaches.

Introduction

In chronic liver disease, fibrosis develops as the result of an imbalance between the new deposition of extracellular matrix (ECM) and its resorption, representing the wound healing response of the liver to repeated injury.[1] The accumulation of ECM in the fibrotic liver is quantitatively, qualitatively and topographically abnormal. The end-result is the development of cirrhosis, a diffuse morphology that is characterised by excess fibrous tissue circumscribing parenchymal nodules, which consist of regenerating hepatocytes, and by alterations in hepatic vascular architecture.[2] Hepatic fibrosis was initially thought to be irreversible but now, based on evidence from successful treatment of chronic liver disorders that resulted in fibrosis regression, it is widely seen as a dynamic process with potential for resolution.[1] Histologically assessed hepatic fibrosis is one of the most important predictors of prognosis in chronic liver disease, independent of aetiology.[3] In metabolic syndrome-associated (non-alcoholic) fatty liver disease (NAFLD), individual histological fibrosis stages are associated with distinct outcomes,[4,5] with advanced fibrosis emerging as the most important prognostic indicator in longitudinal studies.[6] Meanwhile in both compensated and decompensated alcohol-related liver disease (ALD)[7] fibrosis is also a strong prognostic factor.[8,9]

Over the years, several staging systems have been developed for the histological evaluation of hepatic fibrosis in chronic liver disease, including NAFLD, and although they are still considered the "gold standard" for fibrosis assessment, it is accepted that they are not able to quantify fibrosis as a dynamic process at the tissue level and cannot accurately substage cirrhosis.[3,10] Despite the abundance of staging systems for fibrosis, currently there is no universally accepted staging system for assessing the extent of fibrosis in ALD.

This review aims to summarise what is known about hepatic fibrosis in the context of ALD, focussing on its pathogenesis, morphological patterns and types, differences from NAFLD-related fibrosis, evaluation of extent and, lesions of prognostic significance, which could provide the basis for the development of a bespoke staging system.

Natural history and prognostic impact of fibrosis in alcohol-related liver disease

Naturally, development of ALD is dependent on alcohol consumption,[11] as illustrated by a direct relationship between the quantity of alcohol consumed and ALD risk.[12,13] The risk of developing alcohol-related cirrhosis is increased with chronic alcohol consumption of 12–24 g/day compared to

not drinking.[13] In the Dionysos study[12] the probability of developing ALD increased from a relative risk of 7.1 for individuals who consume 50 g/day to >26 for those who drunk 100 g/day. Prolonged heavy alcohol abuse, which can be defined as >60 g/day, results in the development of fatty liver in most individuals,[14] an estimated 10–35% of whom progress to alcohol-related steatohepatitis (ASH), which is defined as evidence of hepatocellular injury in the form of hepatocellular ballooning and lobular inflammation on histology.[15] ASH has a negative prognostic impact, driving hepatic fibrogenesis[16] and resulting in destruction of the lobular architecture and development of cirrhosis in an additional 8–20% of cases.[17] Fibrosis progression may be accompanied by episodes of alcoholic hepatitis, a clinical syndrome characterised by recent onset of jaundice and/or ascites and high short-term mortality of 20–50% within 3 months[18] or it may evolve with few or no clinical symptoms in the setting of ongoing alcohol abuse.

However, these numbers also imply that the majority of drinkers will not develop severe liver disease with fibrosis. Why only a minority will be affected by disease progression is currently not known in detail or precisely predictable on an individual basis. Several factors including genetic susceptibility (see below), female gender, Hispanic ethnicity, obesity, hepatic siderosis on histology, nicotine abuse, and other concomitant liver diseases, in particular chronic viral hepatitis are known accelerators of disease progression and may also impact on fibrogenesis on an individual level.[19,20] Genome wide association studies identified phenotypes of the patatin-like phospholipase domain containing 3 (*PNPLA3*), the transmembrane 6 superfamily member 2 (*TM6SF2*) and membrane bound O-acyltransferase domain containing 7 (*MBOAT7)* genes as risk factors for alcohol-related cirrhosis.[21] Interestingly a combination of genotypes of the *ADH1B*, *MnSOD* and *GSTM1* genes implicated in alcohol metabolism have recently been described as independent predictors of ALD in 2 large cohorts of Indian patients.[22] The utility of genetic markers to identify populations at risk ofALD remains to be studied.

Histological fibrosis stage has emerged as one of the most important factors for prognosis in the clinical settings of compensated and decompensated ALD.[8,9] In early stage ALD, fatty liver collagen deposition around terminal hepatic veins called perivenular fibrosis (PVF) has been described as one of the earliest fibrotic changes.[23] However, this is not undisputed as similar changes may also occur in early stages of other chronic liver diseases.[24] PVF seems to correlate with the amount and duration of alcohol consumption[25] and may represent an important predictor of disease progression with development of fibrosis and cirrhosis.[26,27] Furthermore, a recent study evaluating the prognostic impact of clinical, biochemical and histological factors on long-term survival in a cohort of 60 patients with early/compensated ALD identified septal fibrosis and cirrhosis on histology as the only independent predictors of liver-related mortality. Importantly, approximately half of the patients in this cohort already had at least septal fibrosis or were cirrhotic[9] (Fig. 1).

Substages of cirrhosis severity may be important to consider for outcome prediction in compensated ALD.[28] In liver biopsies taken from patients with ALD or viral hepatitis, semiquantitative histological subclassification of cirrhosis severity and quantitative measurement of collagen content (collagen proportionate area, CPA) revealed that CPA, but none of the semiquantitative cirrhosis staging systems evaluated, was useful for prediction of hepatic decompensation. Furthermore, only CPA and model of end-stage liver disease emerged as independent predictors of decompensation.[29]

Patients with decompensated ALD are at high short-term (30 days) mortality risk.[18] Most of these patients are cirrhotic and develop the clinical syndrome of alcoholic hepatitis, which is often a consequence of severe alcohol abuse but can also be caused by biliary obstruction, gastrointestinal bleeding events, diffuse hepatocellular carcinoma (HCC), drug-induced liver injury and sepsis,[30] the last of which is also the most frequent cause of death.[31] In a prospective study including patients with ALD and acute-on-chronic liver failure 10% did not have cirrhosis on histology. While approximately 46% of the cirrhotic patients died within 48 h of admission all of the non-cirrhotic patients survived.[31] In another study in patients with clinical alcoholic hepatitis and histological steatohepatitis (ASH), bilirubinostasis as well as septal fibrosis or cirrhosis were identified as independent adverse predictors of 90-day mortality.[32] Furthermore, in the older studies on prognostic factors for patients with ALD histological cirrhosis was identified as an unfavourable predictor of long-term mortality especially if associated with ASH.[33,34] This indicates that histological confirmation of severe (septal) fibrosis or cirrhotic stages in a liver biopsy is crucial for prognosis prediction,[35] because hepatic inflammation and/or cholestasis associated with severe ASH are known to substantially bias non-invasive fibrosis assessments.[36]

Notably, not only the degree but also the type of fibrosis seems to play an important prognostic role. In a few recent studies severe perisinusoidal and pericellular fibrosis (PCF; please see later for more detailed description of this type of fibrosis) was associated with better outcomes in patients with decompensated ALD and in those with clinical alcoholic hepatitis and ASH.[9,32] PCF, a parenchymal feature triggered by lobular inflammation and ballooning,[37] is devoid of the elastic fibres which are characteristic of mature septal fibrosis.[38] PCF may represent an earlier fibrosis stage than dense septal fibrosis which could help to explain these initially surprising results.

Key point

Histological assessment of fibrosis in ALD may be improved by accurately substaging cirrhosis and incorporating a quantitative measurement of collagen content.

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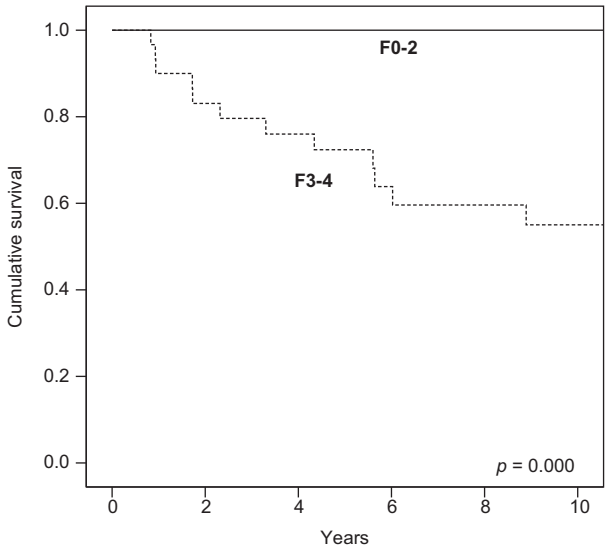

Fig. 1. Kaplan-Meier plots of survival probability of patients with early/compensated alcohol-related liver disease with respect to fibrosis stage. Survival of patients staged F3–4 is significantly worse than for patients with stage F0–2 (p <0.001 by log-rank test) (reproduced with permission from[9]).

Key point

The typical morphology of alcohol-related liver injury is characterised by macrovesicular steatosis, lobular inflammation, hepatocellular ballooning and eventually necrosis in the central portions of hepatic lobules.

Cirrhosis caused by ALD confers a medium to high risk of HCC, which is more pronounced for women and increases with age as well as quantity and duration of alcohol abuse.[39] Detection of advanced fibrosis is therefore pivotal for efficient patient management. However, most patients with ALD present at an advanced clinical stage. It has been speculated that low awareness of alcohol-related health risks among patients and physicians may be at least partly responsible for the higher prevalence of severe fibrosis or cirrhosis at initial presentation in individuals with early/compensated ALD[8] compared to NAFLD.[40]

Other investigations found no evidence for an association between histological fibrosis stage and outcome in patients with ALD.[41,42] The discrepancies may be due to differences in study design. In one of the studies only patients with severe fibrosis or cirrhosis were included and the amount of PCF, substages of cirrhosis or clinical status with respect to compensated or decompensated disease were not considered.

Abstinence is a cornerstone in the clinical management of patients with ALD. Abstaining from alcohol has been shown to improve histological features of liver disease, including the severity of fibrosis in many but not all patients,[43,44] as well as outcome at all stages of ALD.[9,44–48] Abstinence may also substantially decrease the risk of HCC.[49] However, some studies reported that abstinence may be less beneficial in women.[44,50]

Alcohol as a co-factor for fibrosis progression in chronic liver disease of variable aetiology

Large amounts of alcohol (>30 g/day) negatively affect the outcome of most patients with liver disease.[51] In hepatitis C virus-related liver disease, even low to moderate amounts of alcohol are related to progression of fibrosis and increased liver tissue injury.[51,52] Similarly, in hepatitis B virus (HBV)-related chronic liver disease, use of alcohol can increase the risk of fibrosis progression, as well as the frequency of HCC development on a background of HBV-related cirrhosis.[52]

Alcohol in large amounts increases the risk of progression in NAFLD-related fibrosis, while low or moderate alcohol use has been related to fibrosis progression in patients with the metabolic syndrome.[53,54] In contrast, liver biopsies from patients with known NAFLD and low to moderate life-time use of alcohol showed less advanced fibrosis stages compared to abstainers.[55]

In autoimmune liver diseases, due to their rarity, there are no robust data on the effect of alcohol use on fibrosis progression. In primary sclerosing cholangitis, a study from Sweden showed no correlation between alcohol use and transient elastography values.[56] In hereditary haemochromatosis, heavy alcohol consumption has been proven to increase the risk of fibrosis progression in several studies[57] but no data exist on the effect of low to moderate alcohol use on liver fibrosis progression in these patients.[51]

Pathogenesis and morphological features of fibrosis progression or regression in alcohol-related liver disease

In typical cases of ALD the morphological evidence of liver injury – macrovesicular steatosis, lobular inflammation, hepatocellular ballooning and necrosis – is most pronounced in the central portions of the hepatic lobules.[15,58] These features can be present in various combinations. However, ASH characterised by a combination of each, steatosis, lobular inflammation and hepatocellular ballooning is considered the most potent driver of fibrosis.[16] The zonal predominance of lesions is at least partially caused by the configuration of ethanol metabolising enzyme systems, in particular cytochromes such as CYP2E1 which are more abundant in centrilobular than periportal hepatocytes. The products of alcohol metabolism are implicated in the generation of acetaldehyde, increased oxygen demand and relative hypoxia, mitochondrial damage, disturbances of lipid metabolism and oxidative stress,[59,60] as well as mediating hepatocellular injury represented by the morphological features of hepatocellular ballooning[61,62] and/or death.[59,60]

Ballooned and dying hepatocytes and hepatic progenitor cells have been shown to produce hedgehog ligands which induce hedgehog-responsive genes including GLI family zinc finger 2 (Gli2), alpha-smooth muscle actin (αSMA), and vimentin in adjacent stromal cells in a paracrine fashion.[37,63] Hedgehog ligands also mediate deposition of ECM around ballooned hepatocytes, presumably giving rise to the particular PCF type

characteristic of ASH and non-alcoholic steatohepatitis (NASH). Furthermore, profibrogenic hedgehog ligands can also be released from myofibroblasts and reactive ductular cells of an injured liver.[64] Hepatocellular death releases damage-associated molecular patterns, which in combination with necrotic debris and oxidative stress generated from ethanol metabolism induce the activation of Kupffer cells and hepatic inflammation via innate and adaptive immune responses. This also results from an increase of pathogen-associated molecular patterns, a possible consequence of an alcohol-related compromise of the gut mucosal barrier (“leaky gut“) and translocation of bacteria, lipopolysaccharide and bacterial DNA to the liver via the portal blood stream.[8,30]

Thus, hedgehog ligands from ballooned or dying hepatocytes and hepatic progenitor cells, chemokines and growth factors from activated Kupffer cells, lipopolysaccharide from leaky gut, and acetaldehyde are all important factors implicated in the activation of hepatic stellate cells (HSCs) and their transdifferentiation to αSMA-expressing, ECM-producing myofibroblasts. Activated HSCs are considered key players in the pathogenesis of liver fibrosis in animal models and humans.[65,66] The ECM deposits in the space of Disse and around individual and small groups of hepatocytes in PCF consist mostly of fibrillar collagen types I, III and IV, fibronectin, hyaluronan, and proteoglycans which also provide a reservoir for cytokines and chemokines, as well as growth factors, further contributing to ECM accumulation (reviewed in[67]) (Fig. 2).

Liver fibrosis is assessed at the tissue level, using specific histochemical stains for collagens which highlight different types and quantities of abnormally deposited collagen, as well as collagen normally present in the liver. In FLD, collagen fibres may be detected even by routine haematoxylin & eosin staining as amorphous pink bands along the sinusoids or in septa (Fig. 3A). The special stains for collagen include: Masson trichrome (collagens stain blue), which is the most widely used in routine practice (Fig. 3B); van Gieson (collagens stain red), which has the added benefit that the increasing intensity of the red stain reflects a longer duration of collagen deposition and in chronic hepatitis helps differentiate between recent collapse and pre-existing older fibrosis; chromotrope aniline blue (collagen stains dark blue), which also highlights Mallory-Denk bodies (blue or red) and megamitochondria (red) (Fig. 3C); Picrosirius red with or without fast green for contrast (collagens stain red, while fast green stains non-collagenous proteins), which is the most accurate for morphometric image analysis (Fig. 3D); Sweet’s reticulin (collagen type III stains black).[68]

Early stage ALD fibrosis may progress to cirrhosis in the absence of ASH in some cases with alcohol-related steatosis and perivenular fibrosis. The mechanisms of fibrogenesis in this setting are poorly characterised. ECM deposits and myofibroblasts contribute to the thickening of the terminal hepatic venule (THV) wall (Fig. 4A,B).[69,70] However, ASH can be associated with a more severe centrilobular fibrosis which may be accompanied by fibro-obliterative changes to the THV and perivenular hepatocyte necrosis with Mallory-Denk bodies (older term: Mallory’s hyaline), a lesion referred to as sclerosing hyaline necrosis[71] (Fig. 4C,D). If detected without any necroinflammatory changes this lesion is considered indicative of prior disease activity (steatohepatitis in remission), depending on the clinical setting.[26] Portal hypertension can develop in the absence of cirrhosis in ALD cases with sclerosing hyaline necrosis and fibro-obliteration of the THV.

Ongoing alcohol abuse and ASH set the stage for the extension of PCF from centrilobular areas into the lobular parenchyma (Fig. 5A–C) eventually resulting in a panlobular involvement. More often PCF adopts a septal configuration linking THVs and portal tracts (Fig. 5D) as well as adjacent THVs (Fig. 5E). Loss of hepatocytes from areas with septal PCF results in condensation of PCF and the formation of septa consisting of fibrous tissue devoid of hepatocytes[72] (Fig. 5F) with crosslinked fibrillar collagen, elastic fibres and deposition of clusterin.[38] Elastic fibres are hallmarks of mature scar-like fibrosis, resembling the septa of chronic viral hepatitis.[73]

Mechanisms involved in the maturation of fibrosis in ALD have not been described in detail. Alcohol is an inhibitor of anti-fibrotic mechanisms, one of which is inhibition of natural killer-cell-mediated interferon-gamma-induced death of activated HSCs.[74,75] Activated Kupffer cells, hepatic inflammation-associated profibrogenic activity as well as integrin-,[76] collagen-1-,[77] and discoidin domain receptor-mediated[78] prolonged survival of activated HSCs may be important factors. Furthermore, activated HSCs produce tissue inhibitors of metalloproteinases (TIMPs) which downregulate the activity of ECM degrading metalloproteinases (MMPs).[67,73] Ongoing hepatic injury and inflammation pave the way for the disruption of the lobular architecture and the development of cirrhosis (Fig. 2) characterised by mostly small parenchymal nodules (<3 mm in diameter) surrounded by fibrous septa. Persisting fibrogenesis in cirrhosis is reflected in morphological stages of increasing severity. In end-stage ALD, large areas of parenchymal extinction may predominate, reflecting the effect of secondary vascular changes (see later)[69] (Fig. 6A–D). Alcohol-related cirrhosis may become macronodular, especially in abstinent patients, with regenerative nodules of variable diameter that may measure up to several centimetres. While in other patients mixed cirrhosis, with a combination of small and larger nodules, may develop.

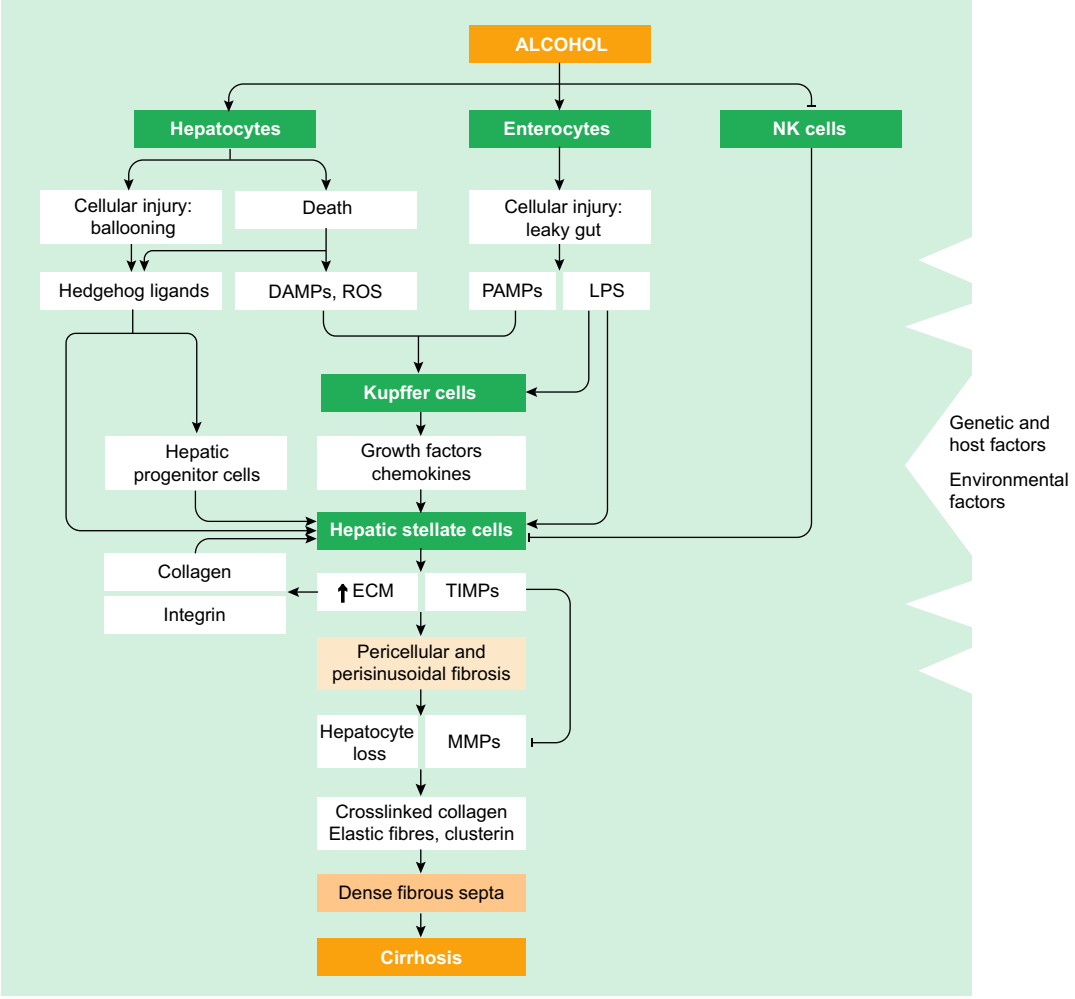

Fig. 2. Pathogenesis and maturation of fibrosis in alcohol-related liver disease. Alcohol or acetaldehyde–related hepatocellular injury (ballooning) trigger the release of hedgehog ligands. These induce hedgehog-responsive genes and activation of HSCs in a paracrine fashion, promoting the deposition of ECM along sinusoids and around ballooned hepatocytes, giving rise to PCF. Profibrogenic hedgehog ligands can also be released from dying hepatocytes, hepatic progenitor cells and a few other cell types of the injured liver. Other important pathways of HSC activation include the activation of Kupffer cells and the secretion of growth factors and chemokines via the release of DAMPs from dead hepatocytes and PAMPs and LPS from alcohol-related compromised gut (“leaky gut”). Furthermore, fibrogenesis is fuelled by components of the ECM, like collagen-1 and integrin, contributing to the survival of activated HSC, whereas alcohol abrogates NK cell-mediated elimination of activated HSC. TIMPs produced by activated HSC inhibit the fibrolytic activity of MMPs and together with hepatocyte loss from areas of septal PCF contribute to maturation of fibrosis with condensed collagen and accumulation of elastic fibres as well as clusterin. This results in the formation of a dense fibrous septa that resembles septa in chronic viral hepatitis. Ongoing fibrogenesis and loss of parenchyma contribute to destruction of the lobular architecture resulting in cirrhosis. ALD, alcohol-related liver disease; DAMPs, damage-associated molecular patterns; ECM, extracellular matrix; HSCs, hepatic stellate cells; LPS, lipopolysaccharides; MMPs, matrix metalloproteinases; NK, natural killer; PAMPs, pathogen-associated molecular patterns; PCF, perisinusoidal and pericellular fibrosis; ROS, reactive oxygen species; TIMPs, tissue inhibitors of metalloproteinases.

Key point

It is now known that fibrosis is reversible. There are similarities between progressed and regressed fibrosis that could allow for regression to be assessed with commonly used staging systems.

Studies in animals and humans indicate that liver fibrosis can be considered a dynamic bidirectional process of progression and regression. The cornerstone of fibrosis regression is elimination of the cause of chronic liver injury, in this case alcohol abuse, which is associated with regression of hepatocellular ballooning and reduction of profibrogenic hedgehog ligands, decrease in TIMPs and concomitant increase in MMP-mediated fibrolytic activity, as well as apoptosis and inactivation of HSCs (reviewed in[79,80]). The activity of hedgehog ligands is further inhibited by hedgehog interacting protein produced by quiescent HSCs.[81] Complete resolution of fibrosis with restoration of lobular architecture is possible at pre-cirrhotic stages, and fibrosis may even regress from cirrhosis to pre-cirrhotic stages in patients with chronic hepatitis after successful treatment with antivirals.[80,82,83] Paucicellular scar tissue with crosslinked fibres of severe fibrosis or cirrhosis seems to be more resistant to degradation than ECM deposits of earlier fibrosis stages and PCF devoid of elastic fibres and thus may represent a major obstacle to fibrosis regression.[73,77,79] Unfortunately, to date there are only very few longitudinal studies characterising morphological changes and/or molecular pathways associated with fibrosis regression in patients with ALD in a setting of prolonged abstinence. One of the early studies investigated histological features of ALD in 26 patients (14 men and 12 women), comparing morphological findings in relation to drinking habits in base-line and follow-up biopsies obtained after a period of 1–3 years. The majority of drinkers

showed increased severity of ASH or progression to cirrhosis in the follow-up biopsy whereas most abstainers had decreased ASH severity. However, some female abstainers progressed to cirrhosis. This indicates that fibrosis progression can also occur in some individuals despite abstinence[43,44] which may be influenced by environmental and/or host-related factors. Data from studies in NAFLD, which exhibits broad morphological overlap with ALD, seem to suggest that features of pro- and regressed fibrosis share similarities and that regressed fibrosis may be assessed by commonly used staging systems.[84]

Fibrosis types in chronic liver disease of variable aetiology including fatty liver disease

In chronic hepatitis of viral or autoimmune aetiology, as well as in some cases of drug-induced aetiology (*i.e.* methotrexate-related liver injury), in chronic cholestatic diseases (primary biliary cholangitis, primary sclerosing cholangitis, long-standing biliary obstruction) and in some rare metabolic diseases, such as haemochromatosis, the pattern of fibrosis is portal-based with early disease presenting with portal/periportal fibrosis, with or without short fibrous septa, progressing to advanced disease with bridging fibrosis, and finally cirrhosis.[69] As described in more detail later, in adult non-cirrhotic ASH or NASH, fibrosis first develops in centrilobular areas as PCF adjoining the THV, a pattern which is different to the portal-based fibrosis of other forms of chronic liver disease. The PCF pattern of fibrosis is very characteristic, but not specific, for fFLD as it can also be observed in venous outflow obstruction[69,85] or certain drug/toxin-induced hepatitides (*i.e.* amiodarone, highly active antiretroviral therapy, certain chemotherapeutic agents, hypervitaminosis A *etc.*). With progression of FLD, the perivenular changes may occur together with portal and periportal fibrosis, although isolated portal fibrosis has been reported in both ALD and NAFLD. Bridging fibrosis develops in advanced disease and results in a predominantly micronodular cirrhosis.[85] In cirrhosis of alcoholic aetiology, the hepatocellular nodules are very small, possibly because of the inhibitory effect of alcohol on liver cell regeneration.[86]

Other characteristic fibrotic lesions in alcohol and metabolic syndrome-related fatty liver disease

Despite their histological similarities, non-cirrhotic ALD and NAFLD may be differentiated at the tissue level based on certain features that have been described in ALD but have not yet been described in NAFLD, such as sclerosing hyaline necrosis, fibro-obliterative and inflammatory lesions of the outflow veins, alcoholic foamy degeneration, and acute cholestasis. PVF is also more common in ALD than in NAFLD. In addition, diffuse PCF is more common in ALD, even at the cirrhotic stage while it is rather unusual in NAFLD. In cirrhosis of alcoholic aetiology, as mentioned, large areas of parenchymal extinction with very thick septa are more evident than in NAFLD. Qualitative differences in collagen composition have been described with collagen type I predominating in NAFLD-associated fibrosis, whereas collagen type III is more frequent in ALD.[87]

Phlebosclerosis, which refers to perivenular fibrosis obliterating THV lumens, is very frequent in alcoholic hepatitis and cirrhosis. The degree of portal hypertension in these cases is analogous to the extent of phlebosclerosis and veno-occlusion. In contrast, veno-occlusive lesions, characterised by intimal proliferation, thickening of the vascular wall and gradual obliteration of the vascular lumen by fibrosis have been reported in only 10% of liver biopsies from patients with alcoholic hepatitis and cirrhosis[88] and are thought to be responsible for the atrophy of hepatic parenchyma and functional impairment observed in advanced ALD.

Fibrosis staging systems in NAFLD and shortcomings of their application in ALD

The use of standardised systems for histopathological scoring ensures that observers apply similar criteria for fibrosis staging. This increases reproducibility, provides a useful method for

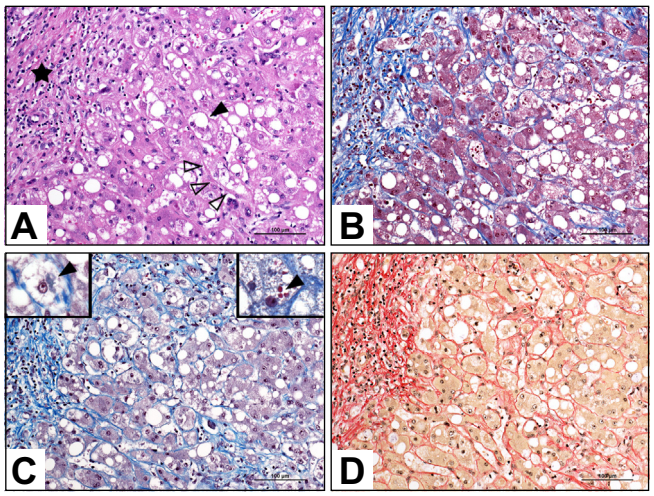

Fig. 3. Collagen deposits in alcohol-related liver disease in different histochemical stains. (A) Collagen fibres appear as pink amorphous structures in fibrous septa (marked by asterisks) or along sinusoids by haematoxylin & eosin stain (marked by white arrow heads) in the case of alcohol-related cirrhosis with steatohepatitis. A ballooned hepatocyte is indicated by black arrowheads. However, sensitivity of haematoxylin & eosin staining is lower than of special histochemical stains for detection of collagen. (B) The Masson trichrome stains collagen in blue. It is the most widely used connective tissue stain in practice. (C) Chromotrope aniline blue stains collagen in dark blue. Chromotrope aniline blue not only highlights collagen but is also useful for the detection of Mallory-Denk bodies (indicated by arrowhead in the left inset) and megamitochondria (indicated by arrowhead in the right inset), the latter has been identified as a beneficial prognostic factor in some studies of alcohol-related liver disease.[32] (D) Picrosirius red stains collagen in red. Picrosirius red is the most accurate for fibrosis detection and thus often used for digital image analysis (morphometry)-based evaluation of fibrosis.

Key point

Despite a number of histological similarities, individual cases of ALD can be differentiated from NAFLD by the presence of certain specific features, including sclerosing hyaline necrosis, fibro-obliterative and inflammatory lesions of the outflow veins, alcoholic foamy degeneration, and acute cholestasis.

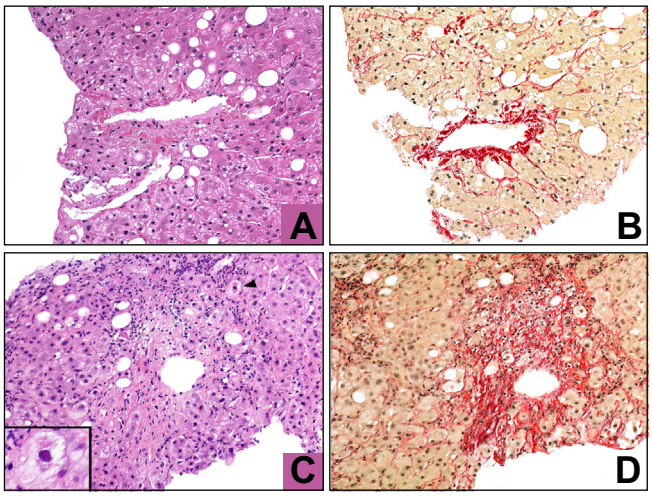

Fig. 4. Histological features of perivenular fibrosis and sclerosing hyaline necrosis in alcohol-related liver disease. (A) Centrilobular area in a case of alcohol-related steatosis (haematoxylin & eosin). Some hepatocytes contain large lipid vesicles, but ballooned hepatocytes and inflammatory infiltrates are absent. The terminal hepatic venule has a rim of pink collagen fibres, which (B) are stained red by Picrosirius red stain while the parenchyma is stained yellow. In addition, the Picrosirius red stain reveals mild pericellular and sinusoidal fibrosis not readily evident in the respective haematoxylin & eosin stain. (C) Sclerosing hyaline necrosis in a case of alcohol-related steatohepatitis. The terminal hepatic venule surrounded by fibrous tissue stained pink on haematoxylin & eosin stain contains inflammatory infiltrates and a few ballooned hepatocytes with cytoplasmic Mallory-Denk bodies (indicated by arrowhead; enlargement shown in the inset). (D) Collagen of the centrilobular area is marked in red by Picrosirius red stain.

comparing the extent of injury in sequential liver biopsies, and may also be used as research tools. Semiquantitative staging systems have been used by pathologists to assess the extent of fibrosis and architectural parenchymal alterations for more than 20 years in chronic viral hepatitis[89–92] and more recently in chronic biliary diseases.[93–95] These systems evaluate the extent of portal-based fibrosis which is one of the hallmarks of chronic viral hepatitis and are also suitable for staging chronic hepatitis of other aetiologies where portal-based injury predominates.[69]

However, these systems are not appropriate for application in FLD of alcoholic or non-alcoholic aetiology because liver tissue injury and fibrotic changes are centrally located in steatohepatitis. Therefore, addressing the need for a specific staging system that considers this fundamental feature, a 5-tiered staging system (stages 0–4) for adult NAFLD was developed in 1999 by Brunt *et al.*[96] The Brunt scoring system provided separate methods to grade histological activity and stage fibrosis and standardised the reporting of fibrosis progression in adult NAFLD (from early sinusoidal and/or pericellular fibrosis present only in zone 3 [stage 1] to the additional presence of portal/periportal fibrosis [stage 2] progressing to bridging fibrosis (stage 3), and finally cirrhosis [stage 4]). In 2005, the NIDDK sponsored NASH Clinical Research Network (CRN) published a new scoring system applicable for both adult and paediatric NAFLD.[97] In essence, the newly proposed staging was based on the Brunt system but in order to provide more granularity in stage 1 and to address the isolated portal fibrosis frequently seen in paediatric and bariatric cases, stage 1 was further subdivided into 1a for delicate zone 3 sinusoidal fibrosis, 1b for dense zone 3 sinusoidal fibrosis, and for non-bridging, isolated portal fibrosis. In 2012, the SAF scoring system for NAFLD was developed by the European Fatty Liver Inhibition of Progression (FLIP) consortium[98] based on biopsies of bariatric patients and was validated in 2014 in patients with metabolic syndrome-associated NAFLD.[99] In the SAF system, an acronym for steatosis (S), activity (A), and fibrosis (F), fibrosis is evaluated using a 5-tier scale based on the NASH CRN staging system and is considered significant fibrosis if F ≥2.[98] Independent of scoring system, fibrosis staging has been shown to be the strongest prognostic factor in NAFLD.[100–102] In addition, fibrosis severity, as semi-quantitatively assessed using 5-tier systems, appears to determine cause-specific mortality in advanced NAFLD; liver-related mortality predominates in patients with NAFLD cirrhosis, while mortality results predominantly from non-hepatic cancers and vascular events in patients with bridging fibrosis.[5]

Despite their robustness in NAFLD, 5-tiered staging systems are not sufficiently accurate to assess the advanced fibrosis stages (stages 3 and 4) that are most strongly related to liver-related outcomes. Recently, in an effort to increase the accuracy of fibrosis staging in NAFLD, the EU-funded Elucidating Pathways of Steatohepatitis (EPoS) histopathological consortium proposed a linear, expanded 7-tiered staging system (stages 0–6) that more accurately mirrors the different stages of liver fibrosis during NAFLD progression.[103] Compared to NASH CRN, in the EPoS staging system stages 1a, b and c were grouped together, stage 2 included periportal or central-based fibrosis with further expansion into zone 2 or a combination of portal- and central-based fibrosis, while NASH CRN stages 3 and stage 4 were each further expanded into 2 stages. The new NAFLD staging system proved to be highly reproducible among 9 expert liver pathologists using both glass and digital slides (kappa score 0.84 and 0.80, respectively) and is currently being evaluated in relation to patient outcomes.[103]

In ALD, the use of histological scoring is very limited and there is no official fibrosis staging system that has been developed by consensus and validated for routine applicability and inter/intra-observer variability.[69,104] In 2006, Yip and Burt proposed a detailed semiquantitative scoring system for the assessment of disease severity in ALD, including a 7-tiered staging system[15] but this has not been further evaluated or validated nor applied in routine practice (Table 1).

Since NAFLD and ALD share a similar pattern of tissue injury and zonal/lobular distribution of key diagnostic histological features, with respect to zone 3/centrilobular fibrotic changes and PCF in

early stages, and central-portal septa and micronodular cirrhosis in later stages,[105,106] some authors have proposed[106,107] that existing NAFLD staging systems may be adapted to assess the extent of fibrosis in ALD. However, there are certain morphological aspects of fibrosis and clinical characteristics that differ between typical ALD and NAFLD cohorts and these may limit the applicability of NAFLD staging systems for patients with ALD. Compared to NAFLD, presumably because of delayed clinical diagnosis,[8,40] the rate of severe fibrosis or cirrhosis and clinical decompensation at initial diagnosis is higher in patients with ALD. Approximately 70% of patients in a tertiary care centre were cirrhotic and had hepatic decompensation at initial diagnosis.[9] In contrast, in a recent study on the natural history of NAFLD only 13% of patients were cirrhotic and none of them had decompensated liver disease in a comparable clinical setting.[108] Furthermore, by the same token, it may be hypothesised that the presentation of a relatively high number of ALD patients with advanced disease is associated with a relatively high prevalence of severe cirrhosis, with extensive septal fibrosis and PCF and sparse residual parenchyma. In most histological semiquantitative systems used for staging of NAFLD, cirrhosis is classified as a single stage essentially reflecting an architectural change corresponding to the destruction of the lobular organisation regardless of the extent of septal fibrosis or PCF. Subclassification of cirrhosis into several stages of severity based on the extent of fibrosis and amount of residual parenchyma may carry important prognostic information. Results from recent studies in patients with cirrhosis mostly of non-alcoholic origin indicated that subclassification of cirrhosis into 3 stages of severity based on the width of fibrous septa and the size of parenchymal nodules, as proposed by the Laennec staging system, correlates with clinical cirrhosis stages and portal hypertension severity,[109,110] as well as with the development of liver-related complications.[111]

Morphological staging in ALD is pivotal for accurate prediction of prognosis, meaningful stratification of patients for individualised management as well as reliable assessment of treatment effects in clinical studies.[3] Therefore, all known prognostically relevant features of ALD-related fibrosis besides the definition of architectural derangement, including PVF, extent of PCF and substages of cirrhosis severity must be considered when developing ALD-specific fibrosis staging systems. In addition, morphometrical analysis of fibrosis by CPA providing linear quantification of liver tissue collagen may be a very useful approach for clinical studies, enabling the exact measurement of changes in total collagen content[10,29,112,113] as well as, in the case of FLD, assessment of changes in PCF and septal fibrosis.

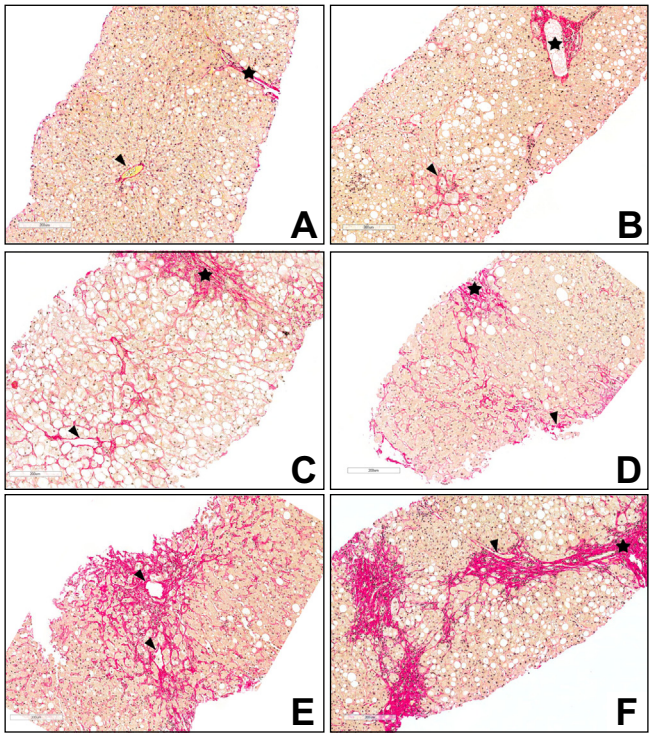

Fig. 5. Pre-cirrhotic stages of fibrosis detected by Picrosirius red stain in alcohol-related liver disease. Portal tracts and THVs are indicated by asterisks or arrowheads, respectively. (A) Normal lobular architecture. Portal tract with vascular structures and THVs are separated by non-fibrotic parenchyma. Some hepatocytes show macrovesicular fatty change. (B) Collagen fibres around centrilobular hepatocytes are seen in early stages of alcohol-related liver disease. Regular size portal tract at the periphery of the lobule. (C) In more advanced disease portal tracts become enlarged by fibrous tissue and in addition perisinusoidal and pericellular collagen deposition (PCF) is found in the parenchyma. (D) In progressed disease PCF may form septa (septal PCF) linking vascular structures. In this case a septal PCF links a THV and the portal tract, while in (E) a septal PCF links 2 THVs. (F) Further accumulation of collagen and loss of hepatocytes from areas of septal PCF leads to the condensation of collagen and formation of dense septa resembling septa in chronic hepatitis. PCF, perisinusoidal and pericellular fibrosis; THV, terminal hepatic venules.

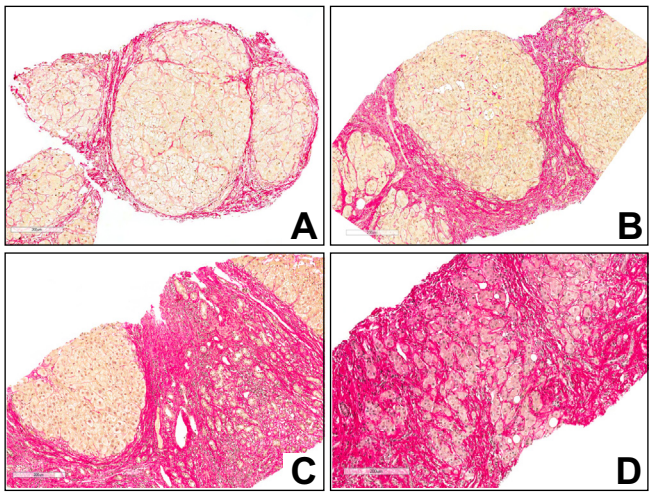

Fig. 6. Stages of fibrosis in alcohol-related cirrhosis. (A) Regenerative parenchymal nodules are surrounded by thin fibrous septa in cirrhosis of mild severity. Progressive disease with further parenchymal loss and proliferation of fibrosis is associated with the formation of (B) broad and (C) very broad septa. (D) In addition, the parenchyma of regenerative nodules can be further dissected by sinusoidal and pericellular fibrosis.

Table 1. Proposed 7-tier (0–6) fibrosis staging system for ALD.[15]

Stage	Criteria*
0	No fibrosis
1	Mild zone 3 fibrosis (pericellular and perivenular fibrosis: focal not all zones 3)
2	Marked zone 3 fibrosis (most/all zones 3)
3	Fibrous linkage between hepatic veins with septa
4	Fibrous linkage between hepatic veins and portal tracts with septa
5	Incomplete cirrhosis
6	Probable or definite cirrhosis

* Isolated portal fibrosis recorded: present/absent.

Conclusion

Histological assessment of fibrosis is one of the most important prognostic indicators in chronic liver disease, reflecting different disease risks which cannot be reliably determined by non-invasive assessment. In contrast to NAFLD, ALD-specific staging systems for standardised prognostication are lacking. Although morphological similarities between NAFLD and ALD exist, differences in clinical and histological features may substantially limit the utility of established NAFLD-specific staging systems for prognostication in ALD.

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Conflict of interest

C.L. receives compensation and grant money from Galmed Pharmaceuticals. D.T. reports consultancy for Intercept Pharmaceuticals and Allergan and Educational grants from Histoindex Pte. Ltd.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

Both authors contributed equally to the manuscript.

Supplementary data

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Author names in bold designate shared co-first authorship

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