Clinical evidence for the regression of liver fibrosis

Elizabeth L. Ellis, Derek A. Mann*

Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne NE2 4HH, UK

Summary

Fibrosis is a common pathological process for the majority of liver diseases which in a significant minority of patients leads to end-stage cirrhosis and/or hepatocellular carcinoma. Data emerging from small rodent models of chronic liver disease have demonstrated that fibrotic extracellular matrix can be remodelled and near-normal hepatic architecture regenerated upon cessation of injury. Moreover, regression of liver fibrosis in these model systems can be stimulated with drugs that target the activities of fibrogenic hepatic stellate cells. These findings are exciting as they suggest that established fibrosis is susceptible to regression and possibly even reversion. Alongside these experimental studies is a growing body of clinical data that suggest regression of fibrosis may also occur in liver disease patients for whom an effective treatment is available for their underlying liver injury. This paper provides an up-to-date review of the currently available clinical data and also considers technical caveats that highlight the need for caution in establishing a new dogma that human liver fibrosis is reversible.

© 2012 European Association for the Study of the Liver. Published by Elsevier B.V. All rights reserved.

Introduction

The burden of chronic liver disease is rising in the UK and worldwide. Whilst viral hepatitis remains the leading cause of liver transplantation globally, the prevalence of non-alcoholic fatty liver disease (NAFLD) has escalated over the last decade and is increasingly being recognised as a cause of liver cirrhosis and hepatocellular carcinoma (HCC) [1,2]. A common pathological feature of chronic liver disease is fibrosis which results from unregulated wound-healing and is characterised by the progressive replacement of functional hepatic tissue with highly cross-linked collagen I/III-rich extracellular matrix. Fibrosis perturbs both the normal architecture and functions of the liver especially in the end-stage of cirrhosis. Fibrosis is also considered a pre-cancerous state that provides microenvironments in which primary tumours may develop. The dogma prevailing in the literature until recently was that fibrosis is irreversible and the best hope therapeutically would be to halt progression. However, there is now mounting clinical evidence that liver fibrosis can regress in a variety of liver diseases, observed either on cessation of the cause of liver injury or treatment of the underlying disease. Significant advances in our understanding of the pathogenesis of liver fibrosis have enabled the identification of potential therapeutic targets but as yet, there are no licensed anti-fibrotic therapies [3]. If fibrosis is genuinely a reversible state then the scene is set for clinical trials that determine the ability of anti-fibrotics to promote fibrosis regression.

Definition of fibrosis and cirrhosis

Fibrosis is a consequence of almost all chronic liver diseases predominantly arising from viral, alcohol-induced, autoimmune, and metabolic aetiologies. It describes the result of a dysregulated wound healing response driven by iterative injury and resulting in the balance of extracellular matrix turnover favouring net deposition. Iterative injury is vital in perpetuating this response. The progressive accumulation of matrix ultimately leads to the development of cirrhosis in a proportion of patients with associated important clinical sequelae.

Cirrhosis is historically a morphological definition describing an abnormal liver architecture encompassing fibrous bands surrounding regenerative nodules [4]. It is important to highlight that fibrosis and cirrhosis, whilst sometimes used interchangeably, are clinically distinct entities. Fibrosis, per se, in a pre-cirrhotic liver, is arguably of little clinical consequence as the hepatic reserve has not been significantly compromised at this stage. One caveat however, is that whilst the increased risk of HCC is associated with liver cirrhosis of all aetiologies, it has been recognised that there is an increased risk of HCC in pre-cirrhotic patients in some liver diseases. Indeed, in the context of chronic hepatitis B, up to 40% of HCC cases occur in pre-cirrhotic patients whilst data from the HALT-C trial indicate that approximately 17% of pre-cirrhotic patients with chronic hepatitis C develop HCC [5,6]. The definition of cirrhosis should incorporate at least three other important factors: firstly, disruption to the vasculature which contributes to the development of portal hypertension, secondly, alteration in hepatic function which may ultimately lead to decompensated liver disease, and thirdly, increased risk of neoplastic transformation, a phenomenon relevant to cirrhosis of all aetiologies. These factors therefore translate into important clinical outcomes leading to liver-related morbidity and mortality. It has become increasingly apparent that the development of liver fibrosis is a dynamic process with bidirectionality. Whilst
Review

effective removal of the causative agent can result in fibrosis regression, dual hepatic pathologies such as HIV/hepatitis C co-infection can lead to an accelerated fibrosis progression [7,8].

Assessment of fibrosis

Assessment of liver fibrosis through histological examination, with tissue obtained through percutaneous or transjugular liver biopsy, remains the current reference standard for quantifying fibrosis but, as such, is imperfect. Fibrosis is scored using a non-linear semi-quantitative scoring system, namely the METAVIR or Ishak scoring system, assigning between 5 or 7 stages, respectively. The difference in the degree of fibrosis between early stages of these scoring systems is significantly less than that observed between the later stages of these scales [9]. Cirrhosis is represented by stage 4 on the METAVIR scoring system or stage 6/7 on the Ishak scale. There is, however, a great deal of variation within this classification with respect to cirrhosis, such as the thickness of fibrous septa and nodule size. As a result, Laennac sub-classified cirrhosis into three separate grades based on the above features and this sub-classification appears to correlate with clinical stage and degree of portal hypertension, as measured by the hepatic portal venous gradient (HVPG) [10]. Nagula et al. as well as Garcia-Tsao et al. have also highlighted the need to incorporate clinical, haemodynamic and biological features when developing a new sub-classification of cirrhosis [11,12].

Given that a typical liver biopsy represents a mere 1/50,000th of the liver, it is unsurprising that sampling error can give rise to significant variation in results. The size of the biopsy specimen has also been shown to be important in the interpretation of the fibrosis stage. The smaller the sample, the more the fibrosis stage is likely to be underestimated [13]. One study showed that Tru-cut biopsies taken laparoscopically, in duplicate, from right and left lobes of the liver, in patients with chronic hepatitis C differed in histological assessment as either stage 3 or 4 disease in 14.5% of cases [14]. In a similar study, which included patients with differing aetiologies, this discrepancy increased to 23.5%. Of note, all samples met criteria of adequate size [15].

Alternatives to the liver biopsy include more attractive non-invasive approaches including transient elastography and serum marker panels, incorporating combinations of markers of matrix turnover and/or markers of liver function. Other imaging modalities have also gained interest including specialised magnetic resonance techniques and an ultrasound based technology, acoustic radiation force impulse (ARFI) which was found to be comparable or superior to serum markers and transient elastography in distinguishing moderate fibrosis and cirrhosis [16,17]. Each of these methods is associated with strengths and weaknesses and performance is variable dependent on the aetiology of the liver disease [18,19]. No single method can provide the same information as histological examination but combining non-invasive modalities can differentiate between mild and significant fibrosis and potentially avoid unnecessary liver biopsy in a sub-group of patients [20].

Whilst these methods have provided some impressive results in the analysis of cross-sectional data, there remains a paucity of longitudinal studies to validate their use in disease monitoring or assessment of potential anti-fibrotic therapies. This is in part hampered by the ethics of performing serial liver biopsies, which at present, is really the only means of validating the use of such markers longitudinally. Unfortunately, neither of these methods can provide the same information as a liver biopsy, but if validated, could provide a very useful adjunct for disease monitoring. Given that liver histology is a surrogate measure of clinical outcome, liver specific outcomes could be used as a reference to assess markers [21].

Key Points

- There is increasing clinical evidence for the regression of liver fibrosis in a variety of liver diseases associated with improved clinical outcomes
- Histological evaluation through liver biopsy remains the reference standard for assessing liver fibrosis but is impractical for repeated measures; combining non-invasive technologies, in conjunction with hard clinical outcomes, will be important in longitudinal evaluation of future treatment trials
- There is a real need for standardised reporting methods to aid interpretation of anticipated anti-fibrotic therapy trials

The implications of utilising a less than perfect reference standard impose a real limitation on developing new technologies. Potentially, an alternative diagnostic test may be more accurate than liver biopsy in correctly distinguishing disease severity but this would never be realised using the current evaluation. Indeed, Mehta et al. have demonstrated that the measurement error of liver biopsy itself can significantly impact on the observed diagnostic performance of a surrogate marker as measured by area under the ROC curve (AUROC), potentially leading to rejection of a perfect surrogate marker [22].

Defining fibrosis regression

The interpretation of studies addressing the regression of fibrosis relies heavily not only on defining how we measure changes in fibrosis, but also how we analyse the resultant longitudinal data. Histological findings with respect to regression of fibrosis are often reported as a percentage of patients with an improved METAVIR score, usually perceived as a —1 or —2 decrease in score, although some studies also include those with an unchanged score in the improved category. Other studies report a mean fibrosis score for a subgroup of patients at each timepoint and look for a statistically significant difference in mean scores. The statistical validity of reporting longitudinal data in this manner is flawed on two counts: firstly, the METAVIR score is a semiquantitative scoring system and not a linear scale rendering a mean METAVIR fibrosis score an unsound concept and secondly, comparing mean fibrosis scores at different time points loses the changes that occur at an individual level. With the advent of serum marker panels and other non-invasive methods, repeated measures for individual cases become feasible rendering the latter problem more significant. One alternative approach, particularly useful when analysing longitudinal continuous data such as serum marker scores, is to report changes in fibrosis as a summary measure for each individual, for example, by measuring the area under the curve for each case using trapezoidal integration [23].

Defining progression and regression of fibrosis is imperative to the assessment and development of potential anti-fibrotic therapies and for the evolution and translation of our increased
understanding of the pathogenesis of liver fibrosis to targeted therapies.

Clinical evidence of regression of fibrosis

A landmark paper by Perez-Tamayo in 1979 described evidence in both animal models and human disease for reversal of fibrosis and cirrhosis [24]. Subsequently there have been a plethora of studies in a range of liver diseases providing further support. Clinical evidence for the regression of fibrosis can be sub-divided into histological regression achieved through treatment of the primary disease versus deceleration of the rate of fibrosis progression in the context of accelerated fibrosers, as seen in recurrent hepatitis C post-transplantation, HIV–hepatitis C co-infection and patients with dual hepatic pathologies.

There is growing clinical evidence that early to moderate fibrosis can regress and possibly even resolve in a number of liver diseases. It is difficult to believe from a clinical perspective that established cirrhosis may resolve to a pre-cirrhotic state. There are however, a number of studies reporting evidence of such reversal. This evidence is predominantly based on changes in histological stage, subject to sampling error and interpretation and should be interpreted with caution. The limitations of liver biopsy render dissecting true regression from sampling error a challenge. Clinical outcomes of such patients may be more reliable as a determinant of regression of disease and indeed, histological assessment is a surrogate marker for clinical outcome.

This leads us to ask: Does an improved clinical outcome equate with histological regression? It is conceivable that stasis or failure of disease progression, driven by removing the causative agent or treating the underlying aetiology, may in fact be associated with an improved outcome. Indeed, a number of studies have reported a reduced risk of neoplastic transformation in treated chronic hepatitis C [25–28].

Is there any evidence of cirrhosis regression?

Regression of cirrhosis is still a debated topic. Reports of apparent cirrhosis regression are few in number and mostly not correlated with clinical outcomes. Wanless et al. presented serial biopsies from a patient with hepatitis B following treatment with lamivudine [29]. Histology revealed apparent disease regression. The results from one patient alone of course do not rule out the possibility of sampling error. In addition, 52 explant cirrhotic livers removed at transplantation were examined for features of regression of cirrhosis. Unfortunately, the findings were not correlated with clinical outcomes.

Serpaggi examined histological evidence for regression of cirrhosis following disease-specific therapy in a range of liver diseases including HCV, HBV and autoimmune cirrhosis [30]. Interestingly, 14/113 patients (12.4%) demonstrated post-treatment regression of their disease, a frequency consistent with the sampling error observed in Regev’s study [14]. All 14 patients repeat biopsies were reported as stage F1 or F2, that is, consistent with regression by more than one stage of fibrosis. According to Regev’s study, the frequency of a scenario where a biopsy may be reported as F3–F4 in one lobe and F0–F2 in the other lobe was still 9.7%. Serpaggi’s study therefore unfortunately does not dispel all doubt that the apparent histological improvement is an accurate reflection of change in fibrosis.

In this section, we will discuss the evidence for fibrosis regression focusing on histological evidence. In addition to the limitations of liver biopsy previously discussed including variation of results with specimen size, there are perhaps other caveats to be considered in the context of hepatitis C. Two studies examining the natural history of CHC demonstrated a spontaneous decrease in fibrosis score by two stages in up to 14% of patients which may again be a reflection of sampling error [31,32].

A number of studies have performed serial liver biopsies on patients treated for CHC. In many of these studies, post-treatment biopsies were performed at a relatively short time interval following end of treatment, often between 6 months and 1 year. Whilst improvements in inflammatory scores were often quite marked, perhaps unsurprisingly, changes in fibrosis scores were less frequently observed. The largest of these studies was reported by Poynard et al. who retrospectively pooled individual data from four randomised CHC treatment trials including 10 different treatment regimes of interferon, pegylated interferon and ribavirin. Three thousand and ten patients included in this study had paired pre-treatment and post-treatment liver biopsies with a mean time between biopsies of 20 months. Results were reported as percentage of patients with either a 1 stage increase or decrease in fibrosis as well as fibrosis progression rate per year [33]. The overall histological response was an improvement in fibrosis stage in 20% of patients, no change in 65% and an increase in fibrosis stage in 15%. All patients had a decrease in fibrosis progression rate post-treatment, irrespective of treatment regime, but with statistically significant lower progression rates observed in responders compared to non-responders (p <0.001). An increase in fibrosis was also less frequently observed in those patients who attained SVR (7% compared to 17% of relapsers and 21% of non-responders).

Given that the development of fibrosis can progress over many years, intuitively it would seem more appropriate to re-examine liver histology to assess for fibrosis regression at a greater time interval following end of treatment. Indeed, there are a number of studies which have achieved this and correlated results with clinical outcomes (Table 1). The limitations of the majority of these studies which have reassessed histology greater than 18 months post-treatment are that reporting methods vary between studies which hamper interpretation. Most of these studies were also performed over a decade ago when interferon monotherapy was standard treatment. The largest and most recent of these studies by George et al. enrolled 150 patients who had all attained SVR following treatment for CHC with...
Table 1. Histological, virological, and clinical evidence for the regression of liver fibrosis in patients treated for chronic hepatitis C. Cohort studies of treated chronic hepatitis C patients with long term follow up biopsies (minimum 18 months after end of treatment).

<table>
<thead>
<tr>
<th>Study, [Ref.]</th>
<th>n</th>
<th>Virologic response at study entry</th>
<th>Genotype</th>
<th>n (%)</th>
<th>Fibrosis stage at index biopsy</th>
<th>Timing of repeat biopsy (mean)</th>
<th>Treatment regime</th>
<th>Length of follow-up (mean)</th>
<th>Histological response on repeat biopsy</th>
<th>Virologic response (end of study)</th>
<th>Clinical response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tsubota (1997), [94]</td>
<td>93</td>
<td>All SVR</td>
<td>GT 1: 42%</td>
<td>93</td>
<td>Mean Scheuer fibrosis score (combined grps A, B and C): 2.3 ± 0.4</td>
<td>15.2 ± 6.7 mo after EOT</td>
<td>Standard IFN-α course</td>
<td>53.6 ± 14.0 mo</td>
<td>Mean Scheuer fibrosis score: 1.5 ± 0.7 (vs. pretreatment score 2.3 (p &lt; 0.0001))</td>
<td>For Grp C where post-treatment biopsies were taken ≥2 yr after EOT the decrease in fibrosis stage was also significant p = 0.0024</td>
<td>No reported virologic relapses during follow-up</td>
</tr>
<tr>
<td>RT study</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Improved histology in 94% of patients (decrease ≥2 points on Knodell score)</td>
<td>1 patient had definite relapse</td>
<td>Deaths: n = 1 (from peritoneal carcinomatosis related to colon cancer) No patients developed HCC or decompensated liver disease</td>
</tr>
<tr>
<td>Marcellin (1997), [55]</td>
<td>80</td>
<td>All SVR</td>
<td>GT 1: 33%</td>
<td>69</td>
<td>Cirrhosis: n = 5</td>
<td>2.2 ± 1.3 yr after EOT in 48/69 patients</td>
<td>IFN-α (different regimes according to treatment trial)</td>
<td>4 yr</td>
<td>Improved histology in 94% of patients (decrease ≥2 points on Knodell score)</td>
<td>NB: Total Knodell score - activity as well as fibrosis</td>
<td>No patients with definite relapse</td>
</tr>
<tr>
<td>P cohort study</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Deaths: n = 1 (from recurrent liver cancer post OLT)</td>
</tr>
<tr>
<td>Marcellin (1997), [55]</td>
<td>80</td>
<td>All SVR</td>
<td>GT 1: 33%</td>
<td>69</td>
<td>Cirrhosis: n = 5</td>
<td>2.2 ± 1.3 yr after EOT in 48/69 patients</td>
<td>IFN-α (different regimes according to treatment trial)</td>
<td>4 yr</td>
<td>Improved histology in 94% of patients (decrease ≥2 points on Knodell score)</td>
<td>NB: Total Knodell score - activity as well as fibrosis</td>
<td>No patients with definite relapse</td>
</tr>
<tr>
<td>P cohort study</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Deaths: n = 1 (from recurrent liver cancer post OLT)</td>
</tr>
<tr>
<td>Reichard (1999), [78]</td>
<td>26</td>
<td>All SVR</td>
<td>GT 1: 41%</td>
<td>23</td>
<td>Scheuer 0-3: n = 22</td>
<td>5 ± 1.8 yr after EOT</td>
<td>IFN-α course (duration of course varied between trials)</td>
<td>5.4 ± 1.6 yr after EOT</td>
<td>Mean fibrosis score post-treatment = 1.0 (vs. pre-treatment score 1.9 (p = 0.0008))</td>
<td>All 4 cirrhotic patients had a decrease in fibrosis stage on post-treatment biopsy</td>
<td>2/26 had a late virological relapse &gt;2 yr after EOT</td>
</tr>
<tr>
<td>George (2008), [29]</td>
<td>150</td>
<td>All SVR</td>
<td>GT 1: n = 75 (53%)</td>
<td>60</td>
<td>Scheuer stage 1: n = 27</td>
<td>4 yr after EOT</td>
<td>IFN-α2b + RBV: n = 146</td>
<td>39/49 (80%) had a decrease in fibrosis stage</td>
<td>No patients with definite relapse</td>
<td>HCC development: n = 2 (both cirrhotic pre-treatment)</td>
<td></td>
</tr>
<tr>
<td>P cohort study</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No patients developed decompensated liver disease</td>
</tr>
<tr>
<td>Toccaceli (2008) [93]</td>
<td>112</td>
<td>Sustained responder*: n = 87</td>
<td>GT 1: Sustained responder grp 55%; Relapsers 80%</td>
<td>112</td>
<td>Mean Knodell fibrosis score: Sustained responder Grp (n = 87): 1.2 ± 1.1 Relapsers (n = 25): 1.6 ± 1.2</td>
<td>2.5 ± 1.2 yr after EOT (range 12-76 mo) in sustained responder grp 2 ± 0.7 yr after EOT (range 12-31.4 mo) in relapers</td>
<td>Standard IFN-α course</td>
<td>3 yr minimum</td>
<td>Mean Knodell fibrosis score: Sustained responder grp 29/66 (44%) of sustained responder grp with abnormal index fibrosis score had decreased fibrosis score post-treatment. 37 (56%) had an unchanged score. None had increased score 3/21 relapers with abnormal index fibrosis score had decreased score after treatment, 15 had unchanged score and 3 had increased score (p &lt; 0.001 vs. SVR grp)</td>
<td>No late virologic relapses in sustained responder grp</td>
<td>No liver-related deaths, decompensated cirrhosis or HCC occurred</td>
</tr>
<tr>
<td>RT multi-centre study</td>
<td>Relapers: n = 25</td>
<td>Relapsers 80%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Sustained responder = patients with persistently normal ALT and negative serum HCV RNA levels at EOT and during following 12 months. RT, retrospective; P, prospective, RCT, randomised controlled trial; n.r., not reported.
*With paired liver biopsies.
*With blinded analysis.
follow up for 5 years monitoring histological, virological, biochemical and clinical outcomes [34]. One hundred and forty-six patients had a pre-treatment biopsy and 60 patients had a post-treatment biopsy performed at a mean of 4 years after end of treatment (EOT); 48 of these patients had paired pre- and post-treatment biopsies available for re-scoring by a pathologist blinded to clinical information. Patients (40/49) had decreased fibrosis scores on repeat biopsy. Interestingly, two patients with cirrhosis pre-treatment developed HCC during follow-up and one patient died from recurrent liver cancer post-OLT. This highlights that treated patients with SVR are still at risk of HCC development. Other studies also support the association between SVR and improved clinical outcomes including a decrease in liver related death and decompensated disease [35,36]. Mallet et al. describe a cohort of 96 patients with CHC cirrhosis who underwent repeat liver biopsy following interferon-based treatment [37]. The subgroup who attained SVR had significantly fewer liver-related deaths and events compared to non-responders. Moreover, 18 patients were reported to have regression of cirrhosis on repeat biopsy performed at a median of 17 months post-EOT and of those, 17 had attained SVR. In this subgroup, there were no reported liver-related deaths or events. Further evidence supporting improved clinical outcomes secondary to virologic response include a 12 year follow up study of 218 patients with compensated cirrhosis which showed that patients attaining SVR did not develop de novo esophageal varices compared to 22 of the 69 untreated subjects [38]. In addition, a study by Roberts et al. demonstrated that treatment of compensated CHC cirrhosis with a standard pegylated interferon and ribavirin regime may invoke a significant reduction in hepatic venous pressure gradient in sustained responders compared to non-responders [39].

**Hepatitis B**

Chronic hepatitis B (CHB) is a significant worldwide problem with up to 25% of patients developing HCC. Standard treatments include interferon-alpha (IFN), pegylated interferon PEG-IFN alpha 2a and nucleos(t)ide analogues (NUCs) [40]. A number of studies have shown that HBV DNA suppression is associated with biochemical and histological response and importantly, there is evidence that these surrogate markers correlate with improved long term clinical outcomes.

Interferon has been used in the treatment of CHB since the 1980s and typically approximately 33% of patients will attain biochemical and virological response following a finite treatment course [41]. Interferon therapy has been shown to reduce fibrosis progression in HBeAg-positive patients, with a greater response seen in those who sustain HBeAg seroconversion, as well as in HBeAg negative patients with sustained virological response [42–45]. In addition to a decrease in fibrosis progression, clinical outcomes also improve. The long term clinical response to interferon treatment has been recently addressed by a meta-analysis evaluating the effects of interferon treatment in 975 patients versus 1147 untreated controls from 11 studies with a 6 year mean follow up. Interferon treatment was found to decrease the risk of hepatic events and cirrhotic complications with the greatest benefit seen in sustained responders [46].

Long term therapy with nucleoside analogues has also been shown to improve liver fibrosis and disease progression. A recent study evaluating the long term benefits of entecavir in the treatment of CHB in nucleoside naive patients found a reduction in liver fibrosis by at least 1 point on the Ishak fibrosis score in 88% of the 57 patients from the 293 enrolled with serial biopsies treated with entecavir for 6 years [47]. This was associated with both a virological and biochemical response although long term clinical outcomes were not reported with respect to incidence of HCC. Similarly, histological improvement including fibrosis regression has been seen following long-term treatment with lamivudine or adefovir but resistance mutations are more common with these agents [48,49].

**NAFLD**

Non-alcoholic fatty liver disease (NAFLD) encompasses a spectrum of disease including simple steatosis, steatohepatitis, liver fibrosis and cirrhosis. There are currently no licensed therapies for NAFLD and management strategies are based on targeting risk factors and detecting patients with significant fibrosis and cirrhosis. Whilst there have been a number of studies assessing the potential benefits of various pharmacological agents in NAFLD, the majority of these studies have been relatively small scale with short follow-up and have not been designed to specifically address effects on liver fibrosis [50].

Weight loss, preferentially achieved through lifestyle modification, is often the first-line management strategy in this patient group. Weight loss is often associated with beneficial effects on multiple components of the metabolic syndrome. Histological improvements have also been observed, particularly with respect to steatosis, but evidence of fibrosis regression is less convincing [51–56]. A recent randomised controlled trial assessing the effect of weight loss by lifestyle intervention on non-alcoholic steatohepatitis (NASH) over a 48 week period in 31 overweight patients demonstrated significant improvements in the NASH histological activity score following an average weight loss of 9.3% but failed to show a significant change in fibrosis [51].

With respect to surgical intervention, there are currently no random controlled trials (RCTs) evaluating bariatric surgery versus lifestyle modification or placebo. Dixon et al. published a case series of 36 patients with NAFLD (23 patients with NASH) who had paired liver biopsies before and after weight loss following laparoscopic gastric band placement [57]. Index biopsies were obtained at the time of surgery with follow up biopsies taken either laparoscopically or percutaneously at a mean of 26 months following the first biopsy. A mean weight loss of 34 kg was achieved with mean BMI decreasing from 47 to 34. Of the 23 patients with NASH on index biopsy, 19 patients had histological remission of NASH following weight loss. With respect to fibrosis, there were 10 patients with stage 3 fibrosis on index biopsy and all but 1 of these patients had a decrease in fibrosis stage, including seven patients with complete fibrosis regression to stage 0 on repeat biopsy. However, surgical intervention may not always be beneficial as reports of extreme weight loss following bariatric surgery have been associated with liver-related morbidity. From 21 cohort studies evaluated in a recent Cochrane review there were reports of histological deterioration following bariatric surgery including increased fibrosis [58].

**Alcoholic liver disease**

Evidence for regression of fibrosis in alcoholic liver disease is limited. Alcoholic liver disease is the leading cause of liver transplantation in the UK yet there are surprisingly few studies examining...
Results from RCTs assessing the effects of pharmacological therapy on alcoholic fibrosis and cirrhosis have been disappointing. A Cochrane Intervention Review assessing the effect of colchicine for alcoholic and non-alcoholic liver fibrosis and cirrhosis from 15 randomised controlled trials concluded there was no statistically significant improvement in any significant clinical outcome, including liver histology, assessed from the results of four RCTs [60]. It should be noted that only one of these RCTs included patients solely with alcoholic liver disease, one RCT assessed patients with hepatitis B and the remaining RCTs included mixed aetiologies.

What has been addressed to a limited degree, however, is the effect of abstinence on clinical outcome. One of the earliest studies to demonstrate an increased survival in patients with alcoholic cirrhosis following abstinence was described by Powell [61]. This study examined 283 cases of histologically proven ‘Laennec’s cirrhosis’ or micronodular cirrhosis between 1951 and 1963. Survival analyses showed a statistically significant difference between abstainers (63% 5 year survival) and those who continued to drink (40.5% 5 year survival) (p <0.001). Surprisingly, not all subsequent studies have supported abstinence as a factor influencing prognosis. Verrill et al. reported that the benefits of abstinence may not be realised immediately following abstention and postulate that a number of studies failing to identify an association between abstinence and improved prognosis may be due in part to a relatively short follow up [62]. In their study, Verrill et al. followed 100 patients with alcoholic cirrhosis for 7 years from baseline histological assessment and found abstinence, assessed 1 month post-biopsy, was associated with a significant improvement in long-term survival [62]. Abstinence at 1 month post-biopsy was found to be an excellent predictor of long term abstinence with 98% of patients remaining abstinent at 5 years. They found that the benefits of abstinence were realised after longer follow-up with statistically significant difference in 5 year survival rates between abstainers (75% survival) versus drinkers (50% survival) (p <0.002). Surprisingly, patients with milder rather than severe cirrhosis as graded by the Laennec grading system had a worse survival rate. With respect to other clinical outcomes, available evidence suggests that abstinence does not guard against HCC development and that HCC can occur in pre-cirrhotic patients [63–65].

One alternative prognostic measure of outcome is the assessment of hepatic venous pressure gradient. Vorobioff et al. assessed 30 patients with alcoholic cirrhosis and portal hypertension with no previous history of gastrointestinal haemorrhage over a 42 month period [66]. All patients were abstinent from alcohol for a minimum of 4 weeks at the start of the study but nine patients subsequently failed to abstain when assessed at the first follow-up. Repeated portal pressure measurements were taken and frequency of variceal haemorrhage and hepatic mortality recorded. Although limited by its small sample size, abstinence in this study was associated with a marked improvement in Child Pugh’s score and a significant decrease in portal pressure which correlated with a reduction or disappearance of oesophageal varices, decreased risk of variceal haemorrhage and increased survival rate.

Autoimmune liver disease

Evidence for fibrosis regression in autoimmune liver disease is limited predominantly to small scale case series and case reports. The largest study to date by Czaja et al. retrospectively examined 325 histological specimens from 87 patients treated with one of two regimens: dual therapy with prednisolone and azathioprine or higher dose prednisolone as a single agent [67]. Following the index biopsy, indications for repeat liver biopsy were treatment failure or following remission, prior to discontinuation of therapy. Ishak fibrosis scores decreased by 1–6 points in 46 patients (53%) over 57± months follow up and 30 of these patients had a decrease in score of at least 2 points. Fibrosis scores remained unchanged in 23 patients over 62±12 month follow up. Improvements in fibrosis score were commonly observed where patients had an improvement in histological activity indices. With respect to cirrhosis, 14 patients had histological cirrhosis on index biopsy whereas only 10 patients were reported as having cirrhosis at the end of follow up.

Other reports of cirrhosis regression include a case report of a 42 year old female with autoimmune hepatitis and cirrhosis on open-liver biopsy who was treated with prednisolone and azathioprine. She underwent laparotomy with wedge liver biopsy 14 years later with apparent complete resolution of cirrhosis [68]. Dufour et al. also reported 8 patients with autoimmune hepatitis and either cirrhosis or extensive fibrosis on index biopsy who responded to medical therapy with apparent reduction in fibrosis scores on repeat biopsy [69]. The mean fibrosis score decreased from 3.3 to 0.8 at a median biopsy interval of 47 months. Again these results must be interpreted in the context of the limitations of liver biopsy.

Primary biliary cirrhosis (PBC)

The only approved medical treatment for PBC is ursodeoxycholic acid (UDCA) [70]. Unfortunately, many of the trials evaluating UDCA were poorly designed. A Cochrane Review evaluating 16 RCTs of UDCA versus placebo identified almost half of these trials had a high risk of bias and concluded that UDCA did not significantly improve liver histology and had no demonstrable effect on improving mortality [70].

The role of immunosuppressive agents in PBC remains controversial. A number of studies evaluating methotrexate have had conflicting results and raised concerns that methotrexate may worsen mortality [71–73]. The largest RCT to date (PUMPS trial) was terminated prematurely due to futility [74]. Kaplan conducted a prospective case study describing 5 out of 19 pre-cirrhotic patients who achieved disease remission following low dose methotrexate for a minimum of 6 years [75]. Two of the five patients’ fibrosis score decreased by 2 points (4 point scale) and the remaining three patients' scores decreased by 1 point. More recently Kaplan et al. described a much larger case series of 91 PBC patients who failed treatment with UDCA and were subsequently treated with 6 months of colchicine followed by methotrexate if alkaline phosphatase levels failed to fall [76]. Patients were on combination therapy with the three agents for a mean of 2.2 years and underwent a minimum of three liver biopsies. Whilst the response to methotrexate was heterogeneous, the results suggested that 80% of patients either partially or completely responded to treatment. Mean METAIVR fibrosis scores significantly decreased from 3 to 2 with a mean interval between
third and fourth biopsies of 3.5 years. It should be emphasised however that the study was not a randomised controlled trial and its design is a major limitation with respect to data interpretation.

Hereditary haemochromatosis

Case reports identifying regression of fibrosis and even cirrhosis following venesection in patients with hereditary haemochromatosis date back to the 1960s [77]. The largest study to date reported by Niederau followed a cohort of 251 patients with haemochromatosis over a 14 year period [78]. All patients had index biopsies and 185 patients had one or more repeat biopsies following iron depletion. Fibrosis was graded using a scoring system described by Loreal et al. and Deugnier et al. with four stages from 0 which includes septal fibrosis to 3 which includes cirrhosis [79,80]. Forty-two (23%) patients (10 stage 1, 20 stage 2 and 12 stage 3) had a decrease in fibrosis stage and only two patients had an increased fibrosis stage on repeat biopsy. The patients were recruited from two hospitals in Germany with some variation in biopsy technique between centres; the majority of biopsies were undertaken using ultrasound guidance in one centre compared with the majority performed laparoscopically in the second hospital. As well as an improvement after treatment in both nonspecific symptoms and biochemical parameters, namely ALT, subgroup analyses to assess the effect of iron removal demonstrated that the prognosis of patients receiving less than 80 phlebotomies to achieve iron depletion was significantly better than those patients requiring >80 phlebotomies to completely remove iron. Survival was also diminished in patients who could not be depleted of iron after >80 phlebotomies. 21/251 patients, all with cirrhosis, developed HCC and interestingly, 17 of these patients had documented iron depletion. This again highlights that removal of the causative agent of liver disease is not always protective against development of HCC.

The most recent study addressing reversibility of liver fibrosis assessed histological outcome following venesection in 36 cases of C282Y homozygotes with documented F3 or F4 fibrosis on index biopsy [81]. All biopsy specimens were a minimum of 10 mm with six portal tracts with regression of fibrosis defined as a decrease of 2 points on the METAVIR score. Sixty-nine percent of patients with F3 fibrosis on index biopsy were reported as attaining histological regression compared to 35% of patients with F4 staging on initial biopsy. Whilst 63% is a respectable percentage of patients to achieve regression and is likely to supersede the proportion of patients inaccurately staged due to sampling error, the study is limited by its small sample size. Other limitations of this study include a recruitment bias, variation in biopsy size and lack of correlation of histology with hard clinical outcomes.

Wilson’s disease

Wilson’s disease is rare affecting approximately 1 in 30,000 in many populations [82]. To date there have been seven case series examining serial liver histology since 1975 [83–89]. These studies are heterogeneous with respect to treatment regimes and patient populations, some focussing solely on paediatric patients. The most recent study by Cope-Yokoyama et al. reported serial histology on a group of 12 patients with Wilson’s disease who had received either zinc and/or penicillamine treatment with mean follow up of 5 ± 3 years [83]. On index biopsy there were no cirrhotic patients; seven patients had stage 0 fibrosis, three had stage 1 and two had stage 2 fibrosis. Half of these patients showed worsening of histology on repeat biopsy and half had improved fibrosis or stable fibrosis. There was no correlation between the type of treatment received and histological response.

Cirrhosis regression remains controversial. A case report by Falkmer et al. describes apparent cirrhosis reversal in a 10 year old girl following treatment of Wilson’s disease with penicillamine for 2 years [90]. Whilst sampling error must always be considered when interpreting results, in this particular case 3 surgical liver biopsy specimens were taken both before and after treatment with unified results in both groups. The liver was visualised on both occasions at laparatomy and was reported as moderately enlarged with a finely nodular surface as well as evidence of ascites (2 l drained) pre-treatment whilst on repeat laparatomy, the liver was only slightly enlarged with a smooth surface. Pre-treatment histology confirmed a nodular cirrhosis whilst post-treatment biopsies showed no fibrosis and near-normalisation of the parenchyma. The apparent histological improvement correlated with both a biochemical and a clinical improvement, the latter evidenced by resolution of ascites and peripheral oedema, normalisation of an electroencephalograph and resolution of ocular pathology.

Anti-fibrotic therapies

As our understanding of the pathogenesis of liver fibrosis increases, a number of novel targeted approaches to treat liver fibrosis are being explored [91–93]. Targeted approaches include firstly, molecular targets paramount to liver fibrogenesis and/or fibrolysis pathways such as anti-TIMP-1 and anti-PDGF-β receptor blocking antibodies and secondly, targeted drug delivery to key fibrogenic cells within the liver such as hepatic stellate cell-targeted drug delivery through vitamin A-modified liposomes [94].

Whilst the majority of novel targeted approaches to treat liver fibrosis are still experimental, there are a number of clinical studies in progress focussing predominantly on repositioned agents already licensed for other clinical indications. These include angiotensin II receptor blockers whose antifibrogenic properties have been characterised in animal models. Unfortunately, results from a recent large-scale RCT evaluating angiotensin blocking agents over a 3.5 year period in patients with chronic hepatitis C have not been as promising as hoped [95].

What remains uncertain is whether anti-fibrotic therapy per se will result in positive clinical outcomes. There remain many unanswered questions: Would anti-fibrotic therapy decrease the risk of neoplastic transformation in those with advanced fibrosis/cirrhosis? Conversely, could anti-fibrotic therapy actually increase the risk of neoplastic disease? As discussed in Friedman’s recent review, there is as yet there no proof of concept trial demonstrating the positive clinical effects of specifically targeting and decreasing liver fibrosis in man [96].

Conclusions

There is a growing portfolio of published work suggestive that liver fibrosis can regress in all chronic liver diseases, regardless
of aetiology, on removal of the causative agent or treatment of the disease. However, limitations of liver biopsy including sampling error and interpretation of results subject to intra- and inter-observer variation mean that distinguishing real changes in fibrosis longitudinally is a challenge. The most convincing evidence for the regression of liver fibrosis derives from large-scale studies of antiviral therapies for the treatment of chronic hepatitis C. Long-term follow up studies indicate that regression of liver fibrosis is associated with improved clinical outcomes so strengthening the perceived histological regression as a real phenomenon. Cirrhosis regression however remains a controversial topic and evidence is limited mainly to individual cases subject to the limitation of liver biopsy.

Defining universal parameters for the assessment of liver fibrosis is a funnel-neck to our development of anti-fibrotic therapies. Longitudinal assessment using a combination of liver biopsy with non-invasive testing and clearly defined clinical end-points should aid interpretation of results. There is a real need for universal standardised reporting methods to aid interpretation and comparison of potential anti-fibrotic therapies. As yet, there is still no proof of concept trial confirming that anti-fibrotic therapy will result in positive clinical outcomes. Indeed, it is paramount that potential therapies targeting matrix degradation and liver regeneration do not increase the risk of neoplastic transformation.

Conflict of interest

The authors declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

References


