Fibrosis and Disease Progression in Hepatitis C

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The progression of fibrosis in chronic hepatitis C determines the ultimate prognosis and thus the need and urgency of therapy. Fibrogenesis is a complex dynamic process, which is mediated by necroinflammation and activation of stellate cells. The liver biopsy remains the gold standard to assess fibrosis. Scoring systems allow a semiquantitative assessment and are useful for cross-sectional and cohort studies and in treatment trials. The rate at which fibrosis progresses varies markedly between patients. The major factors known to be associated with fibrosis progression are older age at infection, male gender, and excessive alcohol consumption. Viral load and genotype do not seem to influence significantly the progression rate. Progression of fibrosis is more rapid in immunocompromised patients. Hepatic steatosis, obesity, and diabetes may also contribute to more rapid progression of fibrosis. There are no tests that reliably predict the rate of progression of fibrosis in an individual patient. High serum alanine aminotransferase (ALT) levels are associated with a higher risk of fibrosis progression, and worsening of fibrosis is uncommon in patients with persistently normal serum aminotransferase levels. Serum markers for fibrosis are not reliable and need to be improved and validated. Liver biopsy provides the most accurate information on the stage of fibrosis and grade of necroinflammation, both of which have prognostic significance. Repeating the liver biopsy, 3 to 5 years after an initial biopsy is the most accurate means of assessing the progression of fibrosis. (HEPATOLOGY 2002;36:S47-S56.)

The typical histological features of chronic hepatitis C are variable degrees of hepatocellular necrosis and inflammation (referred to as the activity or grade of disease) and fibrosis (referred to as stage of disease). While the activity of the liver disease can fluctuate, worsening and improving over time, the stage of fibrosis is believed to be progressive and largely irreversible. Importantly, it is the progression of fibrosis that ultimately leads to architectural distortion of the liver and cirrhosis. For these reasons, the rate of progression of fibrosis is the defining feature of the natural history of chronic hepatitis C.

In chronic hepatitis C, the rate at which fibrosis progresses varies markedly from person to person and may vary over time. In some individuals, the rate of fibrosis is rapid so that cirrhosis eventually develops and, with it, the major complications of hepatitis C: end-stage liver disease, portal hypertension, and hepatocellular carcinoma. In other patients, fibrosis does not develop or progresses so slowly that after decades of infection, little or no fibrosis is found on liver biopsy. Such patients are unlikely to suffer the long-term complications of chronic hepatitis C. For these reasons, assessment of the stage and rapidity of progression of fibrosis are helpful in determining the prognosis and the need of therapy in the individual patient. Factors associated with fibrosis progression in hepatitis C are not well defined and the role of the accompanying necroinflammatory activity is still controversial.

Abbreviations: HCV, hepatitis C virus; TGF-β, transforming growth factor-β; HAI, histology activity index; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HIV, human immunodeficiency virus.

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Fibrogenesis

Chronic infection with hepatitis C virus (HCV) typically induces injury and inflammation of the liver, which appear to be responsible for the associated fibrogenesis. Fibrogenesis is a dynamic process characterized by the synthesis of constituents of the extracellular matrix, which is a complex mixture of glycoproteins (collagen, elastin, fibronectin, laminin) and proteoglycans organized in a tridimensional network.[1] Fibrogenesis is a non-specific mechanism, which lasts as long as injury persists in the liver and is believed to help limit the extension of the

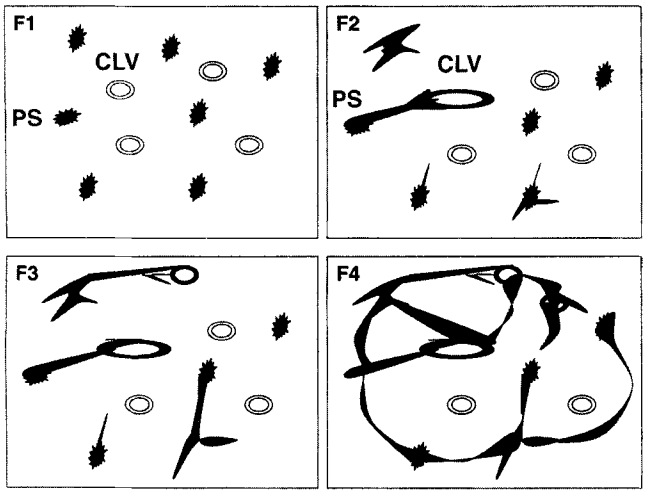

Fig. 1. Progression of fibrosis from periportal fibrosis to cirrhosis according to the Metavir scoring system.[9] F1, portal and periportal fibrosis only; F2, periportal fibrosis with few septa; F3, septal fibrosis and bridging without cirrhosis; F4, cirrhosis. PS, portal areas; CLV, centrolobular veins. (Reprinted with permission.[63])

inflammatory reaction. Fibrosis, therefore, is a physiologic mechanism, which is at first beneficial, but which can become pathological if the viral infection and chronic hepatocellular injury persist.

Fibrosis is characterized by the deposition of collagen and other extracellular matrix proteins and their organization in complex polymers, which are insoluble and induce loss of the liver architecture.[2] In all forms of chronic hepatitis, including chronic hepatitis C, active fibrosis begins around the portal areas (periportal or zone 1 fibrosis) and gradually extends out into the lobules towards the central veins (zone 3) with septa formation and then bridging fibrosis[3-5] (Fig. 1). The final stage of fibrosis constitutes cirrhosis with extensive fibrosis linking portal and central areas and nodular regeneration of the liver parenchymal.

Collagen and matrix proteins that constitute fibrosis are largely produced by activated hepatic stellate cells.[6,7] The stellate cells are activated from a quiescent lipocyte phenotype to a fibroblastic phenotype. The activation occurs in 2 phases: initially activation of stellate cells by cytokines, chemokines, and other signalling molecules induced by the inflammatory process, followed by transformation of the stellate cells into a myofibroblastic phenotype, in which the cell can proliferate, attract leukocytes, and produce extracellular collagen and matrix proteins.

The steps in stellate cell activation and transformation have been demonstrated to occur in chronic hepatitis C.[8] The major fibrogenic cytokine, transforming growth factor (TGF)-β, is increased in expression in the liver in patients with chronic hepatitis C, and mRNA levels for this cytokine are also increased.[9,10] Serum levels of TGF-β are present in increased amounts in patients with chronic hepatitis C,[9,10] and levels of connective tissue growth factor, which are associated with the expression of TGF-β, are also markedly increased, serum levels correlating with the degree of liver fibrosis.[11]

During the fibrogenesis process, a basal membrane appears separating the hepatocytes from sinusoidal blood and perturbing the exchange of nutrients between blood and hepatocytes, a process known as capillarization of the sinusoids. Also, the accumulation of the fibrosis components in the extracellular matrix is responsible for the storage of very reactive molecules (growth factors and cytokines) in an inactive form, which may be activated in certain circumstances.[11] In addition, during the progression of fibrosis, there is a quantitative increase of the extracellular matrix, but also qualitative changes in repartition of each different component. Indeed, liver fibrosis is characterized by the transformation from normal extracellular matrix (basal membrane) into a reticulated and dense matrix (fibrillar type), which is much more resistant to enzymatic degradation.

Assessment of Stage of Fibrosis

Liver biopsy remains the gold standard for assessment of hepatic fibrosis. Several systems for scoring liver fibrosis have been proposed, each based on visual assessment of collagen staining of liver biopsy samples. The more frequently used systems are the histology activity index (HAI: Knodell score),[12] the Ishak modification of the HAI score,[13] and the Metavir score.[14]

The HAI system scores necroinflammatory activity from 0 to 18 assessing periportal necrosis and inflammation (0 to 10), lobular necrosis and inflammation (0 to 4), and portal inflammation (0 to 4).[12] Fibrosis in the HAI system is staged as 0, 1, 3, or 4, with 1 indicating portal fibrosis only, 3 indicating bridging fibrosis, and 4 cirrhosis. The discontinuous scale was used initially to allow for clear separation of mild (1+) from extensive (3+) fibrosis, which was believed to have important prognostic value. The HAI system is simple and has been widely used, particularly in the large multicenter, registration trials of interferon and ribavirin combination therapy of chronic hepatitis C. However, the intra- and interobserver reproducibility of the HAI is only fair, and the lack of a score for stages between mild (1+) and severe (3+) fibrosis is problematic. In addition, the discontinuous scale complicates statistical analysis in clinical trials.

Ishak et al.[13] have proposed a modification of the HAI scoring system, which uses similar scores for necroinflammatory changes (activity: 0 to 18), but scores fibrosis on a scale from 0 to 6 with no missed numbers. Scores of 1 and

2 indicate portal fibrosis, 3 and 4 bridging fibrosis, 5 incomplete or early cirrhosis, and 6 established cirrhosis. The Ishak scale provides better discrimination in assessing small changes in fibrosis, permitting a better assessment of progression of disease, and possible effects of therapy. The intra- and interobserver variability of the Ishak scoring system has yet to be carefully defined.

The Metavir system scores both necroinflammatory changes on a 4-point scale of 0 to 3 and fibrosis on a 5-point scale from 0 to 4, no numbers being skipped (Fig. 1).[14] The Metavir system has been carefully validated and shows good intra- and interobserver reproducibility. This system is commonly used in Europe.

The scoring systems for hepatic fibrosis have been extremely helpful in natural history studies and clinical trials of therapy of hepatitis C. However, all of these systems have important limitations. Hepatic fibrosis may not be homogenous throughout the liver, and the liver specimen obtained by the needle biopsy may not accurately reflect the overall average degree of fibrosis. The reliability of the assessment of fibrosis stage increases with the size of the liver sample. The sample size is critical, a minimum length of 10 mm being essential. Regardless of biopsy length, however, fibrosis may be underestimated and cirrhosis missed in some patients. In addition, scoring systems are artificial and based on visual assessment. Fibrosis may not progress linearly in the same manner as the scoring systems: thus, progression from stage 1 to stage 2 may be far more important and require a longer period than progression from stage 3 to stage 4 (or vice versa). Thus, nonparametric analysis is needed in assessing differences in fibrosis scores in clinical studies.

The inter- and intraobserver reproducibility in the scoring systems is variable and therefore, in most clinical studies, all biopsies are read by the same pathologist or team of pathologists under code, with paired liver biopsies being read in a blinded manner without knowledge of the chronology of the biopsies or treatment received. Paired biopsies can also be ranked under code as to worsening, no change, or improvement. In large studies, the problem of sampling error is overcome by the numbers of biopsies that are assessed: thus the number that appears to improve due to sampling error should be counterbalanced by the number that appears to worsen.

Progression of Fibrosis

Limitations of Studies on Progression of Fibrosis. Although there have been many studies of fibrosis in chronic hepatitis C, most have had shortcomings that limit their applicability and reliability. Most studies have been cross-sectional and based on a single liver biopsy. A smaller number of studies have been longitudinal and based on patients who have had 2 liver biopsies. There have been few prospective studies of histological changes in chronic hepatitis C, and the few that have been performed were limited in size. In addition, almost all studies have been performed on the highly selected patients that are seen at referral centers.

Cross-Sectional Studies. The major advantage of cross-sectional studies is the ability to include large numbers of patients, including virtually all patients who had a liver biopsy on whom information is available regarding the time of onset of infection. The major shortcomings of cross-sectional studies are that they rely on mathematical modelling to define the rate of progression of fibrosis and are based on an assumption that the progression of fibrosis is linear over time. In addition, the duration of infection is estimated based on a clinical history of time of exposure or onset of injection drug use. The time of onset derived from clinical history may not be reliable; the date of transfusion for instance may not be accurately reported, and studies of injection drug users usually assume that onset of infection occurs within the first year of drug use, which may not be accurate. In addition, sporadic cases in which the time of onset of infection is unknown are excluded in these analyses, which limits the applicability of the results.

Longitudinal Studies. The major advantage of longitudinal studies of patients with 2 liver biopsies is that the rate of progression of fibrosis can be clearly demonstrated between the 2 time points of the biopsies. The disadvantage of this approach, however, is that patients who have had 2 liver biopsies are usually a selected group of patients who are being observed carefully because of concerns about the progression of disease, but for one reason or other have not been treated. Thus, this group may not be representative of the overall rate of progression of hepatitis C. Furthermore, retrospective studies require 2 liver biopsies, which may exclude patients who have developed end-stage liver disease, in whom a second liver biopsy is not needed or is contraindicated because of coagulopathy. Finally, sampling error in assessment of fibrosis may cause problems, with patients appearing to have worsening of fibrosis. The effects of sampling error can be partially corrected by assuming that worsening of fibrosis will be counterbalanced by improvement in fibrosis if entirely due to sampling error. Thus, an overall proportion of cases with improvements in fibrosis can be subtracted from the proportion with worsening to give an overall proportion with worsening. This correction, however, is rarely used. Furthermore, in assessing specific factors associated with worsening fibrosis, one cannot tell which individual has actual worsening and which only apparent worsening due to sampling error, so that factors associated

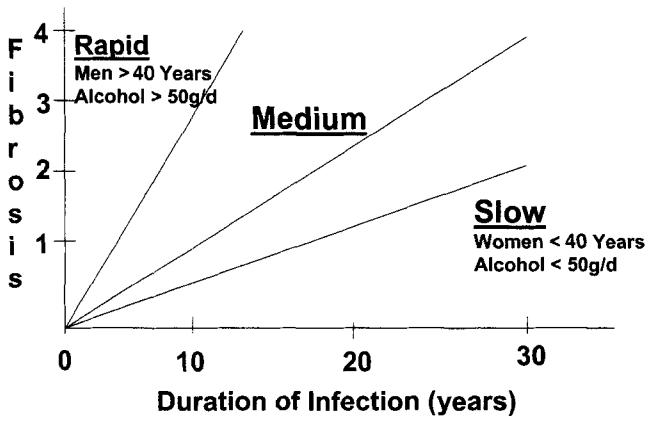

Fig. 2. Three different rates of progression of fibrosis (from stage 0 to 4) by duration of infection. A rapid rate might be seen in men infected after the age of 40 years who drink more than 50 g alcohol daily. A slow rate might be seen in women infected before the age of 40 years who are non-drinkers or drink less than 50 g alcohol daily. (Reprinted with permission from Elsevier Science [*The Lancet*, 1997, vol 349, pp 825-832].[15])

with worsening will be confounded by factors associated with sampling error.

Prospective Studies. Prospective studies of the natural history of hepatitis C with timed liver biopsies in a wide array of patients who are not treated would be the ideal approach to define rates of fibrosis in this disease. However, such prospective studies are difficult to organize and conduct. Because there are effective therapies for hepatitis C, it is not reasonable or ethical to follow patients with severe disease without therapy. Patients willing to enroll in a natural history study in which they do not receive treatment are not likely to be representative of hepatitis C overall. In addition, in most prospective studies, the time between 2 liver biopsies is usually relatively short (3 to 8 years) in relationship to the natural duration of the disease (20 to 50 years). Nevertheless, prospective studies have been conducted, but largely on patients with mild disease or normal serum aminotransferase levels.

Rates of Progression of Fibrosis

Estimates From Cross-Sectional Studies. Cross-sectional studies using mathematical modelling performed on large numbers of patients with a single liver biopsy suggest that the average (median) rate of progression of fibrosis in chronic hepatitis C is 0.13 Metavir fibrosis points per year.[15] Based on this rate, the average patient would develop cirrhosis within 30 years (Fig. 2). Importantly, rates of progression of fibrosis were not normally distributed, and greatly different estimated rates of progression were found in different patient groups. Thus, the rate of progression was higher for men than women (0.15 vs. 0.11 fibrosis units per year), in older than younger persons (0.33 if infected over the age of 50 vs. 0.09 if below the age of 20 years), and in heavy alcohol drinkers than in non-drinkers (0.17 vs. 0.12). As a consequence, the estimated average time to development of cirrhosis ranged considerably, from as short as 13 years in men infected with hepatitis C after 40 years of age who drink more than 50 g alcohol daily, to as long as 42 years in women infected under the age of 40 who are non-drinkers. These estimates were based on the assumption that the progression of fibrosis is linear, which may not be the case. For instance, the time required to progress from stage 1 to 2 may be far longer than the time required to progress from stage 3 to 4. Moreover, fibrosis progression may occur intermittently and may accelerate with age (particularly after the age of 50). Finally, fibrosis may remain stable for decades and not progress. Indeed, in some patients fibrosis may regress spontaneously.

In a more recent study with mathematical modelling based on liver biopsies from 2,213 patients, Poynard et al.[16] proposed a new model for fibrosis progression in chronic hepatitis C, in which the fibrosis progression occurs at different rates in 4 consecutive phases of infection with acceleration after 50 years of age. During the first phase representing the initial 10 years of infection, there is little if any progression unless infection occurs after the age of 50 or human immunodeficiency virus (HIV) infection is present. During the second phase, lasting approximately 15 years, there is slow, but steady, progression in fibrosis. Fibrosis progression is intermediate in rate during the next 10 years of the third phase. Finally, in the last phase, there is rapid progression of fibrosis (Fig. 3). In this model, the mean delay to development of cirrhosis is approximately 40 years, unless infection occurs at an older age.

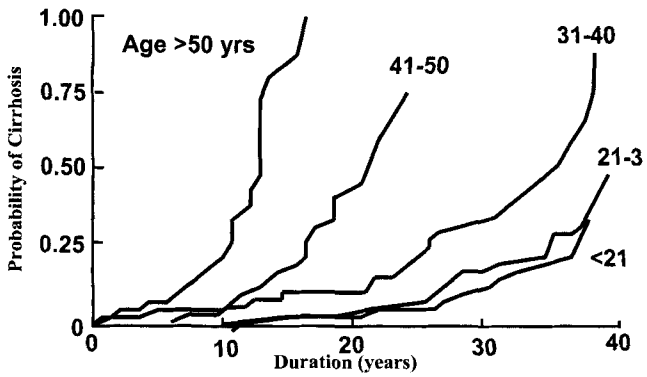

Fig. 3. Probability of developing cirrhosis (Metavir fibrosis stage 4) by duration of infection in 5 cohorts of patients based on age of onset of infection, <21 years, 21 to 30 years, 31 to 40 years, 41-50 years, or greater than 50 years of age. (Reprinted with permission from Elsevier Science, Copyright 2001.[16])

Table 1. Progression of Fibrosis in Three Series of Patients With Chronic Hepatitis C With Two Liver Biopsies

Feature	Marcellin et al.[17]	Ghany et al.[18]	Alberti et al.[19]
Number of patients	110	123	105
Mean delay between the 2 biopsies (yrs)	3.2	3.6	8.3
Proportion of patients with progression	32%	39%	59%
Progression of 1 point	30%	30%	31%
Progression of 2 points	2%	9%	15%
Progression of 3 points	0%	0%	13%

This model has major implications for the prognosis of chronic hepatitis C. The model implies that progression of fibrosis is linear in each phase, but accelerates from phase to phase, increasing exponentially with age and time after onset of infection. According to this prediction, a patient might have minimal or no fibrosis after 2 decades of infection, but with subsequent decades and after the age of 50, fibrosis may develop rapidly. This model should be interpreted with caution, because it was based on a cross-sectional analysis of a selected population of patients: patients from 16 referral centers and from randomized controlled trials of antiviral therapy. In addition, only a small proportion of patients had an estimated duration of the disease of more than 20 years when the accelerated course was predicted to occur. Finally, a minority of patients had bridging fibrosis (11%) or cirrhosis (9%), the outcome that is predicted to occur eventually in the majority of patients.

Estimates From Longitudinal Studies. There have been 3 recent studies (1 prospective[17] and 2 retrospective[18,19]) of untreated patients with chronic hepatitis C who underwent 2 liver biopsies 3 to 8 years apart (Table 1).[17-19] In these studies, 32% to 59% of patients showed a progression of fibrosis. In up to 24% of patients, there was an apparent improvement in fibrosis. In 2 studies in which liver biopsies were 3 years apart, patients who showed progression mostly had only a 1-point worsening of fibrosis; a 2-point increase occurred in 2% to 9%, and no patient had a 3-point or more increase. In the study with 2 liver biopsies with a longer intervening period (averaging 8.3 years), 31% of patients had a 1-point, 15% a 2-point, and 13% a 3-point increase in fibrosis scores.[19] These 3 studies showed similar average rates of progression of fibrosis that were consistent with the original reports from Poynard et al.[15] that predicted the development of cirrhosis in the average patient after 30 to 40 years of infection. It should be stressed, however, that these studies were performed in referral centers, and the patients who were studied may not have been representative of the average patient with chronic hepatitis C.

Factors Associated With Fibrosis Progression

Most studies of liver histology and fibrosis have analyzed factors that correlated with the degree of fibrosis and estimated rate of fibrosis progression. Factors identified in most studies included age, age at infection, male sex, and history of heavy alcohol use. Immune deficiency, such as due to HIV infection or inherited conditions of the immune system, has also been identified as correlating with more rapid progression of fibrosis in chronic hepatitis C. In recent reports, steatosis on liver biopsy, obesity, and diabetes have also been associated with more rapid progression of fibrosis.

Age. Age at onset of infection has consistently been found to be a major factor influencing the rate of progression of fibrosis in hepatitis C. Thus, studies of posttransfusion hepatitis in which most patients are over the age of 40 at the time of onset of infection have indicated that at least 20% of patients develop cirrhosis during the first 15 to 20 years of HCV infection.[20-22] In contrast, in studies of young women infected as a result of exposure to HCV-contamined Rh immune globulin, less than 5% develop cirrhosis within the first 15 to 20 years of infection.[23,24]

In the analyses of fibrosis progression by Poynard et al.,[15,16] the rate of progression of fibrosis was correlated directly with age of onset of infection. In univariate analyses, cirrhosis developed within 20 years in only 2% of patients infected before the age of 20, in 6% infected between 21 and 30 years, 10% infected between 31 and 40 years, 37% infected between 41 and 50 years, and 63% infected over the age of 50 years. In the hazard function model, virtually all patients infected after 40 years of age develop cirrhosis within 16 years (Fig. 3).[16] Interestingly, in this model, the rate of progression of fibrosis accelerates after 50 years of age, regardless of the duration of infection up to that time. This model with a progressive acceleration of fibrosis progression needs to be validated in cohort studies.

The mechanisms responsible for the influence of age on fibrosis progression are not known, but might include immune factors, increased fibrogenesis, or decreased fibrolysis.

Gender. Most studies of hepatic fibrosis have reported that male sex is significantly associated with progression of fibrosis.[15,16,23-25] Two long-term retrospective-prospective studies of hepatitis C in women who received HCV-contaminated Rh immune globulin also suggested an important role of gender in progression of fibrosis. In these 2 studies, only 2% and 0.4% of women had cirrhosis on histological evaluation 17 and 20 years after exposure.[23,24] The mechanisms by which sex affects fibrosis

progression are unknown. Other confounding factors, such as age of onset of infection, lower levels of alcohol intake, and lower body mass index (BMI) may also help to explain the slower development of fibrosis in women than men with hepatitis C.

Alcohol. In almost all studies, a high consumption of alcohol (more than 50 g/d) has been found to be associated with higher fibrosis stage.[15,16,26-28] The effects of a lower level of alcohol consumption, between 10 and 40 g/d, have not been clearly defined. In univariate analysis, patients who drank moderate amounts of alcohol (<50 g daily) had a slightly higher estimated rate of fibrosis progression (0.143) than non-drinkers (0.125), but the difference was not statistically significant and was confounded by other features, such as gender, body weight, and age. Alcohol, which by itself can cause liver disease and fibrosis, may worsen fibrosis in hepatitis C at amounts that are not injurious in non-infected persons, but the amount of alcohol beyond which the progression of fibrosis is increased in hepatitis C is unknown. Because of the negative influence of high alcohol consumption, abstinence or minimal consumption are usually recommended in patients with chronic hepatitis C.

Besides its direct effects on fibrogenesis, excess alcohol intake may have other adverse effects on the course of hepatitis C. Thus, alcohol may affect immune responses to HCV[29,30] and may cause increases in HCV RNA levels in serum and liver, an effect that has been reported by some investigators,[27,31] but not all.[32]

Immune Status. Immune status probably has a major affect on the natural history of hepatitis C and the development of cirrhosis. Several studies have shown that hepatitis C is more likely to progress to cirrhosis, and that the rate of fibrosis progression is greater in HIV-coinfected patients. In a study from Spain, the mean estimated time to development of cirrhosis was 7 years in HIV-positive and 23 years in HIV-negative injection drug users with hepatitis C ($P < .01$).[33] In a study from France, the HAI score was significantly higher among 80 HIV-positive patients compared with 80 HIV-negative injection drug users (matched for age, gender, and duration of disease) (7.5 ± 2.9 vs. 6.5 ± 2.2, $P = .01$).[34] During a mean follow-up of 52 ± 30 months, the incidence of cirrhosis was significantly higher among HIV-positive than -negative patients ($P = .04$) (Fig. 4).

In immunosuppressed liver transplant recipients, cirrhosis of the graft occurs in up to 30% of patients within 5 years of transplantation, and the estimated mean time to development of cirrhosis is only 12 years.[35,36] Most strikingly, fibrosis progression appears to be more rapid in patients who have undergone transplantation for hepatitis C in recent years compared with the initial studies of 5 to

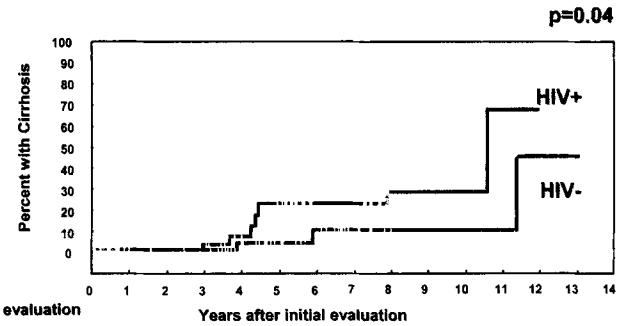

Fig. 4. Proportion of patients with cirrhosis on liver biopsy by duration after initial evaluation and presence or absence of HIV infection. (Reprinted with permission.[34])

10 years ago. The recent increase in rate of fibrosis progression is unexplained, but may be due to an increase in the average liver donor age.[37] Other factors associated with fibrosis progression after liver transplantation were a history of acute hepatitis and the types of immunosuppressive therapies.[37]

Viral Factors. In retrospective and cross-sectional studies, virological factors, such as serum HCV RNA levels and HCV genotype, have not been associated with the rate of progression of fibrosis in chronic hepatitis C.[15,16,18,19]

Most cross-sectional studies have reported the absence of correlation between serum HCV RNA levels and the activity or grade of liver disease.[38] Interestingly, patients with chronic HCV infection who have normal serum alanine aminotransferase (ALT) levels and nearly normal liver histology may have high serum HCV RNA levels.[39] However, serum HCV RNA levels are an indirect reflection of intrahepatic HCV replication, and the correlation between fibrosis and intrahepatic levels of HCV RNA has not been adequately investigated and should be included in prospective studies.[40]

The influence of viral genotype in the pathogenesis of the liver disease is not completely resolved. In several early studies, HCV genotype 1b was found to be associated with a more severe liver disease, including a higher frequency of cirrhosis and hepatocellular carcinoma.[41] However, many of these studies did not control for important confounding factors, such as age, source, and duration of infection. Genotype 1b is more common among older than younger patients and has been commonly linked to spread by blood transfusion. In studies with adjustment for these variables, the association between genotype 1b and a more severe liver disease has not been found.[15,38] Interestingly, the distribution of genotypes is not different in patients with chronic hepatitis C and normal serum ALT levels, as compared with those with increased serum levels.[42] Further studies are needed to better determine the possible role of genotype in the outcome of HCV-related liver disease.

Several studies have shown a modest association between a high quasispecies heterogeneity and more severe liver injury in chronic hepatitis C.[43] In one study, quasispecies heterogeneity was less in patients with normal ALT levels compared with those with elevated levels.[44] Quasispecies heterogeneity can also be confounded by features of gender, age, and duration of disease. Further studies are needed on the significance of quasispecies heterogeneity in the natural history of hepatitis C and its association with hepatic fibrosis.

Other Factors Associated With Fibrosis. The factors, which are clearly associated with the progression of fibrosis, such as age, gender, alcohol intake, and immune status account for only a part of the variability in progression of fibrosis in chronic hepatitis C. Other factors, which have been suggested to be important, include genetic, racial/ethnic, and metabolic. The role of heterozygote mutations of HFE gene is controversial.[45] The influence of overweight has been emphasized recently; it is believed that steatosis related to overweight is responsible for the more rapid progression of fibrosis.[46-48] In addition, diabetes has been shown to be associated with fibrosis.[49]

Predicting Progression of Fibrosis

Most analyses of factors that correlate with fibrosis have been performed in cross-sectional studies. The factors, therefore, are associated with what is found on liver biopsy histology performed at the time. The ability of these factors to predict future worsening of fibrosis has not been verified. Indeed, it is assumed, but not proven, that the estimated rate of fibrosis based on readings from an initial biopsy is likely to predict future progression of fibrosis. However, the validity of these predictions has not been confirmed in prospective studies. Indeed, in the longitudinal studies, factors that correlate best with future progression of fibrosis have been age, serum ALT elevations at the time of initial liver biopsy or during follow up, and degree of disease activity or grade on the initial biopsy.

Serum Aminotransferase Levels. In cross-sectional studies, serum ALT and aspartate aminotransferase (AST) levels have correlated weakly with disease activity (necroinflammatory scores on liver biopsy) and little or not at all with hepatic fibrosis. In longitudinal studies, however, ALT levels at the time of an initial biopsy have correlated with future worsening of fibrosis (Table 2). In a study from the National Institutes of Health, patients with serum ALT levels above 5 times the upper limit of normal were the only group that had a worsening of fibrosis during an average follow-up of 3 years.[18] In a study from France, patients with a mean serum ALT level above twice the upper limit of the normal range had a higher risk of progression of fibrosis on follow-up liver biopsy 3 years later.[17] These findings support the clinical usefulness of monitoring ALT levels in assessing disease progression. However, the validity of this approach and the level above which the ALT elevations are predictive of more rapid progression require further delineation.

Table 2. Characteristics Associated With Progression of Fibrosis in Three Series of Patients With Chronic Hepatitis C

Feature	Marcellin et al.[17]	Ghany et al.[18]	Alberti et al.[19]
Age	NS	NS	0.02
Gender	NS	NS	NS
Alcohol	NS	NS	0.5
ALT	0.04	0.001	0.001
Necroinflammatory activity	NS	0.01	0.02
Fibrosis	*	0.0001	*

Abbreviation: NS, not significant.
*No or mild fibrosis in 100% on first liver biopsy.

Patients with persistently normal ALT levels documented on several occasions usually have mild degrees of hepatitis disease activity and either no or minimal fibrosis.[42] Among 87 patients with chronic hepatitis C with persistently normal ALT levels, Ishak fibrosis scores were 0 or 1 in 76% of patients, and no patient had cirrhosis or severe bridging fibrosis.[50] The rate of progression of fibrosis was estimated to be 0.05 Metavir points per year in patients with normal ALT levels compared with 0.13 points per year in those with elevated ALT levels ($P < .001$).[51] Cirrhosis has been found in some patients with normal ALT levels, but this appears to be rare, occurring in less than 1% in published series. Furthermore, patients with cirrhosis and normal ALT levels will often have abnormalities of other liver tests, such as AST, alkaline phosphatase, bilirubin or albumin, or low platelet count or abnormalities of liver ultrasonography. In these individuals, cirrhosis may have been due to previous episodes of active disease (and increased ALT levels) or to other factors that can cause cirrhosis, such as hemochromatosis or alcohol.[51]

There have been several prospective studies of liver histology in patients with normal serum ALT levels. In a study from Italy, none of 37 patients had a worsening of hepatic fibrosis on follow-up liver biopsy 5 years later.[52] In a study from France, there were no significant changes in the Ishak fibrosis scores among 24 patients who had a second liver biopsy 3 to 5 years later.[50] On the other hand, in both of these studies, a proportion of patients (approximately 5% per year) developed abnormal ALT levels during follow-up.[50,52] Thus, patients may have a change in disease activity over time and the lack of fibrosis progression during one period may not predict future lack of progression of disease.

Serum Markers of Fibrosis. Serum biochemical tests do not reliably predict the stage of fibrosis. Non-specific tests are used in clinical practice, such as serum AST/ALT ratio,[53,54] γ globulin and γ glutamyl transpeptidase levels, platelet count,[54] or ferritin levels. Scores based on combinations of several biochemical tests have been assessed to predict liver fibrosis.[55] Many studies have assessed the correlation between liver fibrosis serum markers of fibrosis, such as type III procollagen peptide,[56] type IV collagen 7S domain,[56] hyaluronic acid,[57] laminin,[58] TGF-β,[59] and metalloproteinases or metalloproteinase inhibitors.[59,60] Serum hyaluronic acid seems the best of these serum markers. None of these markers have been used in longitudinal studies to assess progression of fibrosis prospectively.

Currently available serum markers of fibrosis are not reliable, particularly in discriminating between mild and moderate degrees of fibrosis, and these tests do not have a good predictive value when used in an individual patient. Further studies are needed to determine and validate more reliable serum markers of fibrosis.

Necroinflammatory Activity on Liver Biopsy. The association between fibrosis progression and the necroinflammatory activity scores on liver biopsy is controversial. On a single liver biopsy, there is little or no correlation between severity of the necroinflammatory activity and degree of fibrosis.[15,16] However, necroinflammatory activity is a dynamic process in chronic hepatitis C and may fluctuate over time. The activity score likely reflects the severity of necrosis and inflammation at a given point. In some cross-sectional studies and in most longitudinal studies, the degree of necroinflammatory activity has been associated with the subsequent progression of fibrosis[18,19,61] (Table 2). There is reason to believe that the necroinflammatory process is implicated in the fibrogenesis process, because the stellate cells are activated around the necroinflammatory lesions.[62] Thus, severe degrees of inflammatory activity predict worsening of hepatic fibrosis and constitute an indication for therapy independent of the current level of fibrosis.

Fibrosis. The stage of fibrosis on the initial biopsy was associated with progression of fibrosis in some studies.[10,18,61] This observation is consistent with the hypothesis that fibrosis may accelerate with duration of infection and/or that fibrosis by itself might enhance the fibrogenesis process. However, estimates of the rate of fibrosis progression from a single liver biopsy have not always been predictive of subsequent progression or worsening of fibrosis in longitudinal studies.

Steatosis. Recently, the association between steatosis and the stage of fibrosis has been emphasized. In a cross-sectional study of 148 patients with chronic hepatitis C, the amount of steatosis was independently associated with the stage of fibrosis ($P < .03$).[48] At issue was whether the steatosis per se contributed to worsening of fibrosis. Steatosis on liver biopsy correlated significantly with obesity as measured by BMI ($P < .0001$), and obesity, insulin resistance, or diabetes may have been the factors most responsible for worsening of liver disease. In another cross-sectional study of 211 patients, there was a significant association between the grade of steatosis and the stage of fibrosis ($P < .001$) and the grade of necroinflammation ($P < .007$).[46] The grade of steatosis was associated with the BMI in patients with genotype 1 ($P < .001$), but was independent of obesity in patients with HCV genotype 3 infection ($P < .01$). The mechanisms responsible for the association between steatosis and fibrosis are unknown. Some patients may have non-alcoholic steatohepatitis due to obesity and metabolic disorders in addition to having chronic hepatitis C and the concurrence of the two diseases may be synergistic in causing hepatic fibrosis. Alternatively, steatosis may itself worsen the fibrogenic stimuli that accompany chronic hepatitis C. Finally, steatosis may be the consequence of more severe cell injury and necroinflammation in chronic hepatitis C (especially in patients infected with HCV genotype 3) and thus may be the effect of the more severe disease activity, rather than the direct cause of the worsening fibrosis. Direct cytopathogenic effects of the virus (especially genotype 3) causing steatosis cannot be ruled out. At present, these hypotheses are all equally possible.

Summary

In the majority of patients with chronic hepatitis C, the progression of fibrosis is insidious and slow. Only a minority of patients has rapid progression of fibrosis with early development of cirrhosis. Current knowledge on the factors that are responsible and that correlate with more rapid progression of fibrosis is limited, because it is based largely on cross-sectional studies using mathematical modeling and on a few, small scale prospective or retrospective longitudinal studies of changes in hepatic fibrosis. Carefully conducted prospective studies on untreated patients, mainly patients with mild disease in whom therapy is not indicated, are needed to validate the current hypothesis on the progression of fibrosis.

The mechanisms and factors associated with progression of fibrosis are poorly understood. Older age, male gender, excessive alcohol consumption, overweight, and immune deficiency are associated with more rapid progression of fibrosis. Therefore, counseling of patients should include abstinence from alcohol or minimal, occasional consumption and dietary measures to reduce overweight and metabolic disorders.

The progression of fibrosis is difficult to predict in the individual patient particularly based on assessment at one point in time. Serial serum ALT levels, grade of activity, and stage of fibrosis are the main predictors of progression of fibrosis. However, the overall predictive value of these characteristics is relatively weak and the progression of fibrosis is difficult to predict in the individual patient. The liver biopsy remains the best method to assess fibrosis and is valuable in determining prognosis and aiding in the decision for or against therapy. In untreated patients, regular ALT measurements are useful, and repeat liver biopsy is the only reliable means of assessing the progression of fibrosis and is commonly recommended every 3 to 5 years in untreated patients. A second liver biopsy can distinguish patients with rapidly progressive fibrosis, but may also merely indicate that the initial biopsy underestimated the degree of fibrosis. Overall, the risk of progression of fibrosis of more than 1 point in a 3- to 5-year period is low. In patients with factors associated with a higher risk of progression, such as age above 50 years, excessive alcohol consumption, or high serum ALT levels, liver biopsy may be recommended more frequently (every 2 to 3 years); in contrast, in the younger patient with no other risk factor, the liver biopsies may be performed less frequently (every 5 to 10 years).

Future Research Needs

Prospective studies of the natural history of chronic hepatitis C would be helpful in defining the course of fibrosis in this disease, but are unlikely to be conducted in view of the rapidly evolving advances in therapy of this disease. However, prospective analyses of sequential liver biopsies from patients with mild chronic hepatitis C or who choose not to undergo therapy or who fail to respond to treatment are helpful. Most important in this regard would be the development and verification of accuracy of noninvasive measures of liver fibrosis. Serum fibrosis markers and combinations of several serum and blood tests to assess stage of hepatitis C need to be further refined and analyzed.

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