How to Assess the Stage of Fibrosis in Chronic Hepatitis C

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MECHANISMS OF PROGRESSION

Fibrosis and cirrhosis are a result of excess accumulation of extracellular matrix (ECM) molecules (collagens, noncollagenous glycoproteins, glycosaminoglycans, proteoglycans and of elastin [1]. Extensive perisinusoidal fibrosis has marked effects on liver function, due to the blockade of nutrient and metabolite exchange between hepatocytes and the circulation (Figure 1) and the liver is further impaired by the formation of novel intrahepatic vessels via porto-portal and porto-central collaterals that shunt the blood away from hepatocytes. The imbalance of two dynamic processes, fibrogenesis and fibrolysis leads to fibrosis. Activated hepatic stellate cells and myofibroblasts stimulate fibrogenesis by producing most ECM molecules, downregulating the expression of certain matrix metalloproteinases (MMPs), and increasing synthesis of physiological and tissue MMP inhibitors (TIMPs) [1-3] (Figure 2). Even advanced liver fibrosis and cirrhosis are reversible when the causes of fibrogenesis such as viral infection or biliary obstruction, are removed and the liver is given time to recover [4-11]. Furthermore a growing number of gene polymorphisms may either protect against or enhance the development of hepatic fibrosis (Table 1) [12-20]. In addition to the known external factors and the histological and serological markers of fibrosis and its development, these genetic polymorphisms

may provide individual risk profiles for the development of severe fibrosis.

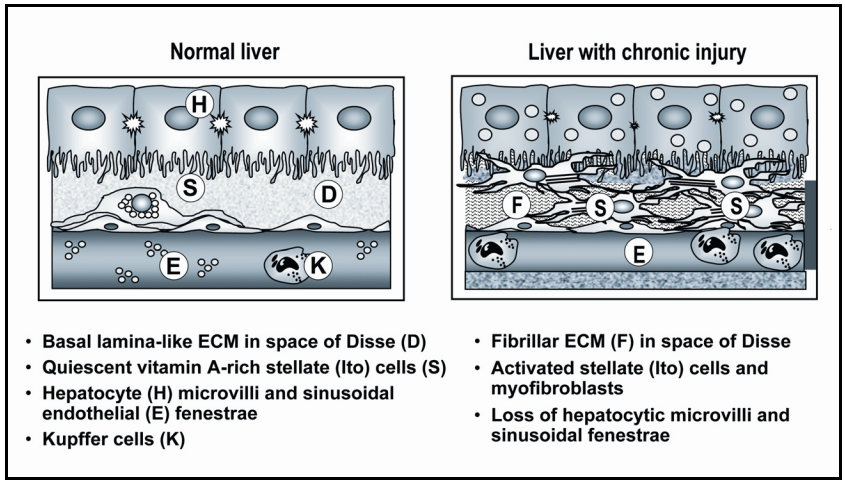

Figure 1: Capillarization of the sinusoids. Illustration of the major cell biological events that determine functionally relevant fibrosis [modified from a sketch kindly provided by Dr. M. Pinzani, Florence, Italy].

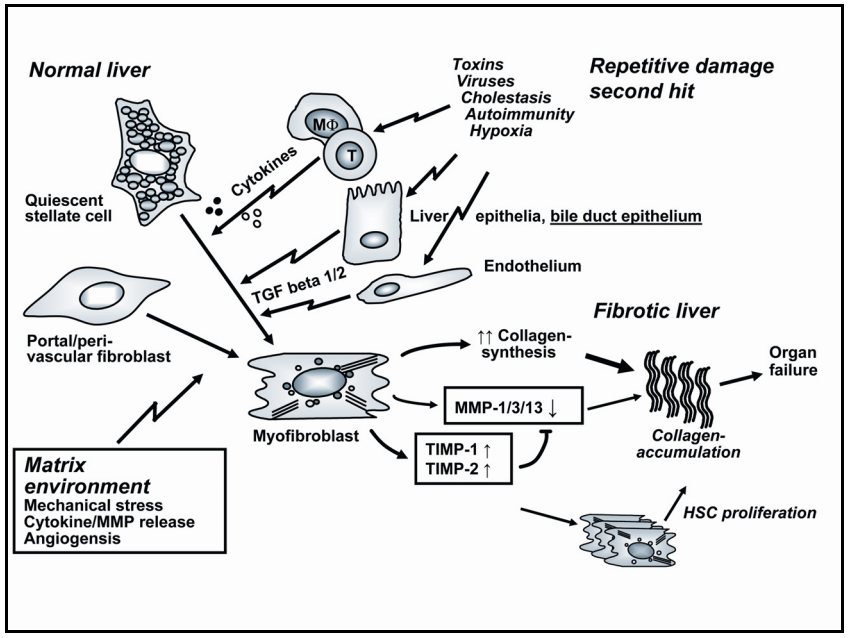

Figure 2: Initiation and maintenance of fibrogenesis. With continuous injury, primarily to hepatocytes or bile duct epithelia, and / or mechanical stress the normally quiescent hepatic stellate cells and portal/perivenular fibroblasts undergo activation and transdifferentiation to myofibroblasts. These myofibroblasts produce excessive amounts of collagens, downregulate certain MMPs and show an enhanced expression of the physiological inhibitors of the MMPs (TIMP-1 and -2). TIMP-1 can also promote myofibroblast proliferation and inhibit their apoptosis.

Gender (protection by high dose estrogens)
Pro/antioxidative enzyme polymorphisms (MnSOD, GSTP1, CYP2D6), e.g., in hemochromatosis
Immune system (profibrogenic Th2 vs. Th1 response)
Single nucleotide-polymorphisms (IL-1beta, IF-gamma, MCP-1, TNF-alpha, Factor V Leiden, MMP-3, TGF beta 1, DQB1*0503)
Genetically determined comorbidities: HFE mutations, metabolic syndrome (NASH)
Regulation of regeneration and apoptosis

Table 1: Genetic predisposition for hepatic fibrosis [12-20].
CYP2D6, cytochrome P450 2D6; GSTP1, glutathione S-transferase P1 [Stickel et al. unpublished data]; MnSOD, manganese superoxide dismutase [Oesterreicher et al. unpublished data]; NASH, non-alcoholic steatohepatitis.

IS THERE A GOLD STANDARD OF LIVER FIBROSIS?

Sequential histological grading of inflammation and particularly staging of fibrosis are still considered the gold standard to assess progression. However, certain studies have demonstrated sampling errors not only in patients with liver diseases with a high degree of intrahepatic heterogeneity such as biliary fibrosis, but also in patients with alcoholic or hepatitis C virus (HCV)-induced fibrosis and inflammation. Thus, when the well accepted, easy to use, 4 stage METAVIR score is used to stage fibrosis [21], roughly one third of the scores differed by at least one stage in the same patient when biopsies from the left and right liver lobes were compared [22]. Similar results were obtained when laparoscopic assessment of cirrhosis vs. non-cirrhosis (which is questionable as a gold standard) was matched to histological findings [23] (Table 2 and 3). Similar results were obtained for the grading of inflammation. This discrepancy was confirmed and systematically investigated in a recent study using the overall scoring of large surgical liver specimens from

patients with chronic hepatitis C as a gold standard. Results of this study showed that small, virtual biopsies derived from these large sections were correctly categorized in only 65% vs. 75% of cases when the biopsies were 15mm and 25mm long [24]. Moreover, a further increase in length from 25-45mm did not significantly increase accuracy. Therefore, although it is indispensable for many reasons, liver biopsy cannot be considered the ultimate gold standard for the assessment of stage and grade and thus the progression of fibrosis. This uncertainty complicates the search for non-invasive (serological) markers of the progression of fibrosis.

Homogeneity of staging & grading in chronic hepatitis C. HCV, laparoscopic biopsy of right and left liver n=124, METAVIR score

Difference	n	%
≥1 stage	41/124	33.1
≥2 stages	3/124	2.4
≥1 grade	30/124	24.2
≥2 grade	2/124	1.6
cirrhosis vs. stage 3	18/124	14.5

Table 2: Sampling error in chronic hepatitis C [22].

Laparoscopy vs. Histology Retrospective, 1992-1994, 434 consecutive patients. HCV 52%, HBV 8%, FL 8%, PBC 4%, AIH 3%, others 25%			
	Laparoscopy	**Histology**	**Error**
Cirrhosis	169	115	32%
No cirrhosis	265	263	0.8%
Detection of cirrhosis (gold standard laparoscopy)			
Sensitivity of biopsy		68%	
Specificity of biopsy		0.8	

Table 3: Sampling error in chronic liver diseases [23].

IMAGING TECHNIQUES

At present imaging techniques lack the sensitivity and specificity necessary for the assessment of the stage of fibrosis in patients with chronic liver diseases. Structural, non-homogenous findings at ultrasound are not associated with the stage of fibrosis, and liver echogenicity can only be used for the detection or exclusion of moderate to extensive fatty infiltration [25]. Although the hepatic artery resistance index as measured by Doppler ultrasound was slightly higher in severe than in mild fibrosis, and no correlation was found with histological inflammation, necrosis or portal flow velocity, the method lacks sensitivity [26]. A slightly better differentiation between slight and severe fibrosis is found with magnetic resonance (MR)-techniques, such as superparamagnetic iron oxide-enhanced MR, which shows hypersignal intensities with a reticular pattern in most patients with advanced fibrosis (METAVIR F2-4), while the signal from non-fibrotic areas where more Kupffer cells are present is decreased [27]. The fibroscan, an interesting new technique using both ultrasound and low-frequency (50Hz) elastic waves whose propagation velocity are directly related to elasticity, was evaluated to

quantify liver fibrosis in 106 patients with chronic hepatitis C. The areas under the (ROC) curves were 0.88 and 0.99 for the diagnosis of significant fibrosis or cirrhosis (METAVIR F2-4 and F4) [28]. Further prospective studies are needed to determine whether this technique can be used to detect changes in the stage of fibrosis in individual patients, e.g. during antifibrotic therapy.

SEROLOGICAL MARKERS OF PROGRESSION

Several studies have been performed with combinations of known serum markers of synthetic, metabolic or excretory liver functions, to derive an algorithm that predicts the histological severity (stage and grade) of chronic liver diseases. These algorithms were retrospectively determined and prospectively validated. Examples are the fibroscore, using alpha 2-macroglobulin, haptoglobin, gamma glutamyl transferase (GGT), gamma-globulin and bilirubin [29-31], and another score using platelet count, GGT, age and cholesterol [32] in patients with chronic hepatitis C (Table 4 and 5). Although these scores can be used instead of liver biopsy in a certain number of patients when a decision to treat or not must be made, they do not appear to be suitable for scientific studies requiring greater accuracy and an assessment of the dynamics of fibrogenesis and fibrolysis. Thus, when making a treatment decision, simple indicators may suffice. For example a single increase in alanine aminotransferase (ALT) during a 6 month observation period in patients with chronic hepatitis C indicated ≥stage 1 fibrosis allowing treatment to begin. These results occurred in 90% of patients (Table 6) [33]. Other indices are the PGA (prothrombin time, GGT, apolipoprotein A with or without alpha 2-macroglobulin) which has been validated in patients with alcoholic liver disease (Table 7) [34, 35].

Non-connective tissue markers as predictors of relevant liver fibrosis in hepatitis C (Fibroscore)
205 retrospective, 134 prospective patients with hepatitis C
METAVIR F0-1 vs. F2-4
5/11 serum markers predictive: alpha-2 macroglobulin haptoglobin gamma-globulin GGT bilirubin
Index 0-0.1: 100% negative predictive of F2-4 (12%)
Index 0.6-1.0: 90% positive predictive of F2-4 (34%)
Index 0.1-0.6: no assignment possible (54%)

Table 4: Diagnostic value of the fibroscore to predict fibrosis stage in patients with chronic hepatitis C [29].

Score to predict absent/little fibrosis (F0-1) in hepatitis C		
351 retrospective, 125 prospective patients with hepatitis C		
METAVIR F0-1 vs. F2-4		
Score: 7.811-3.131 ln (platelet count) + 0.781 ln (GGT) + 3.647 ln (age) – 0.014 (cholesterol)		
Score <4.2	**Stage 0-1**	**Stage 2-4**
Estimation	120/266	5/125
Validation	47/92	2/49
Score >6.9	**Stage 0-1**	**Stage 2-4**
Estimation	10/47	37/85
Validation	5/15	10/33
Score <4.2: sensitivity 51%, NPV 96%		
Score >6.9: sensitivity 30%, PPV 66%		

Table 5: Alternative index for prediction of fibrosis in patients with chronic hepatitis C [32].

Prediction of absent/little fibrosis (F0-1) by ALT					
864 retrospective patients with hepatitis C					
METAVIR F0-1 vs. F2-4					
ALT normal vs. ALT elevated during 6 months					
Stage	**0**	**1**	**2**	**3**	**4**
ALT normal	34.8	51.5	12.1	0	1.5
ALT elevated	0.8	23.7	50.5	17	8
ALT persistently normal (n=66): 65% ≥F1, 26% >A1F1					
ALT elevated: 99% ≥F1, 88% >A1F1					
Cut-off ALT >2.25 ULN: clear indication for treatment					
All patients with elevated ALT can be treated					
Biopsy only for patients with normal ALT					

Table 6: ALT as a predictor of relevant fibrosis or inflammation in patients with chronic hepatitis C [33].

PGA- or PGAA-index and alcoholic liver disease
Patients with alcoholic liver disease: n=333 retrospective, n=291 prospective
METAVIR F0-1 vs. F4
Serum markers: prothrombin time gamma GT apolipoprotein A
Index 0-2: 100% neg. pred. for F3/F4, 83% pos. pred. for F0/F1
Index 9-12:0% neg. pred. for F0/F1, 86% pos. pred. for F3/F4
Correct classification of 65% of patients (Poynard et al. 1991 [34])
PGAA-Index (incl. alpha-2 macroglobulin n=316 prospective):
Correct classification of 70% of patients (Naveau et al. 1994 [35])

Table 7: PGAA and PGA indices to predict the severity of alcoholic liver fibrosis [34-35].

Measuring circulating metabolites of the ECM appears be a more straightforward approach to assess fibrogenesis and fibrolysis, especially in studies on the inhibition or reversal of liver fibrosis (Figure 3) [36-38]. However, serum levels of these markers are influenced by their excretion via the kidney or in bile, and by their uptake by endothelial cells, especially by liver sinusoidal endothelial cells. In addition, other organs with a high ECM turnover can contribute to these serum levels. Cross-sectional studies suggest a significant, but insufficient predictive value of single ECM markers for the stage of fibrosis [39-41]. Meanwhile the cross-sectional evaluation of the European liver fibrosis consortium (ELF) study using 10 automatized ECM parameters in more than 1000 patients with various chronic liver diseases provided algorithms of 3-4 ECM markers with a better predictive value than an assessment by an independent expert pathologist who was not trained as well as two reference pathologists [42]. As in other studies correlating histology

with noninvasive markers, the problem of validation for bioptical sampling errors remains (see chapter above) which introduces an error of one stage (METAVIR scale) in 25% of biopsies; this is expected to increase when liver diseases other than chronic hepatitis C are included (as in the ELF study). The results of the two-year follow-up arm of the ELF study have not yet been published.

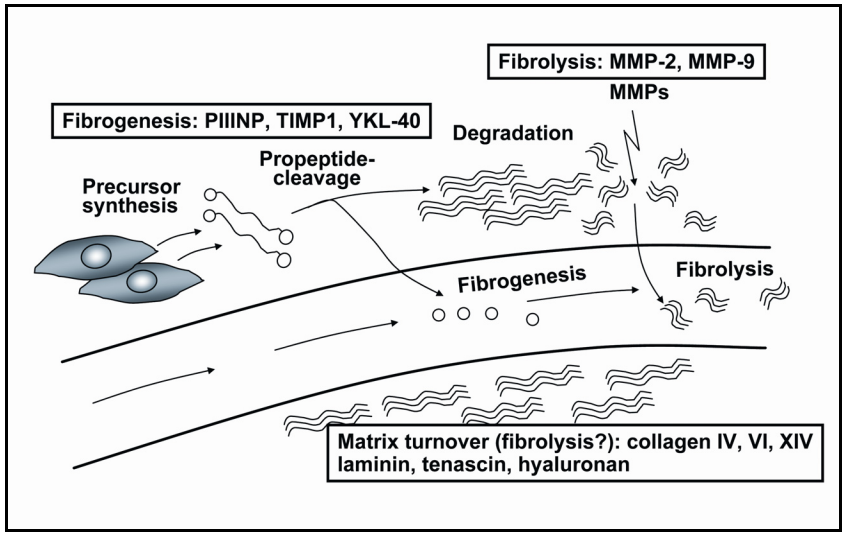

Figure 3: Circulating matrix proteins related to fibrogenesis and fibrolysis. Procollagen precursors released by fibrogenic cells are processed by procollagen peptidases. Only removal of the bulky propeptides allows the formation of collagen fibrils in the extracellular space. Thus circulating propeptide levels should reflect de novo synthesis and deposition of collagen, i.e. fibrogenesis. On the other hand, action of MMPs is expected to generate fragments of already deposited matrix proteins the levels of which should reflect matrix dissolution, i.e. fibrolysis. Most other molecules appear to rather represent an accelerated matrix turnover.
The two large multicenter studies that evaluate the predictive value of circulating matrix markers as predictors of fibrosis stage are mentioned (ELF: patients with all chronic liver diseases; Prometheus: patients with chronic hepatitis C). The ELF study also assesses the predictive value as to fibrosis progression.

A more direct approach to validate the true serum markers of fibrogenesis and fibrolysis, which is nevertheless equally prone to sampling errors, is the use of real time quantification of fibrosis-relevant mRNA expression from liver biopsies compared to serum fibrosis markers. In a study of 50 patients with various types of liver disease, we found a fairly good correlation between liver procollagen I or TIMP-1 expression and serum levels of the aminoterminal procollagen type III peptide or TIMP-1 (data not shown). These results need to be confirmed in larger studies. The availability of serum markers of hepatic fibrogenesis (or fibrolysis) will provide a quick and frequent assessment of the antifibrotic potential of drugs in patients with progressive liver disease. If these reliable serological tests can be combined with drugs that inhibit or revert fibrosis [43] the desire to revert fibrosis or even cirrhosis may be fulfilled.

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