Assessment of Fibrosis in Liver Biopsy. Progression and Regression
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Fibrosis is part of the liver alterations in most chronic liver diseases (CLD). The pattern of fibrosis appears to be related to the pathogenetic mechanism of disease, explaining the relative diagnostic usefulness of various patterns of fibrosis for the liver histopathologist. Semiquantitative evaluation of fibrosis is also an important issue supporting the central role of the biopsy since this feature is a major predictor for the prognosis of most CLD. Also, clinical and experimental evidences support the possible occurrence of fibrosis regression in appropriate conditions. Therefore liver biopsy remains an essential tool in the management of patients with chronic liver diseases.

Liver biopsy for evaluation of liver fibrosis: limits and precautions

Liver biopsy is the reference tool for evaluation of fibrosis. However, liver biopsy has some drawbacks that must be acknowledged. Because liver biopsy involves only a very tiny part of the whole organ, there is a risk that this part might be irrelevant for evaluation of fibrosis in the whole liver due to heterogeneity in its distribution. Extensive literature has shown that increasing the length of liver biopsy decreases the risk of sampling error. Except for cirrhosis, for which micro-fragments may be sufficient, a 25 mm long biopsy is considered an optimal specimen for accurate evaluation, though 15 mm is considered sufficient in most studies.

Observer variation is another potential limit related to the discordance between pathologists in biopsy interpretation. The use of histopathological scoring systems for evaluation of liver fibrosis (staging) increases reproducibility and several studies have shown that the different scoring systems help to provide highly consistent fibrosis evaluation especially when used by experienced liver pathologists. In this regard, specific fibrous tissue stains are also of great help. Histological studies on liver fibrosis have mainly been performed with classical ‘connective tissue stains’ such as silver impregnation techniques for the demonstration of ‘reticulin fibres’ but Masson's trichrome or Sirius red appears also of great value in everyday practice. Thus, although liver biopsy has its limitations, appropriate precautions may reduce the flaws inherent to this method.

Digital image analysis (morphometry) provides objective quantitative results similar to but more accurate than those determined by semi-quantitative scoring methods. With this approach the relative area of fibrosis is measured. Falling costs and improvements in electronic and computer technology, alongside have enabled the implementation on this technology in many specialized centers.

Patterns of liver fibrosis progression

Evaluation of liver fibrosis should be regarded in 2 ways. In the context of a known CLD, assessing the amount of fibrosis may help for the patient management, choice of treatment options and prediction of prognosis. The other scenario is when the etiology is not known. Extent and distribution of hepatic fibrosis may realize different patterns, the recognition of whom being helpful, even when not diagnostic, in suggesting etiological possibilities. The main pattern of fibrosis will be described shortly.

- Portal and periportal fibrosis. In this pattern, excess connective tissue forms within the portal tracts, which consequently become densely staining and expanded into the adjacent parenchyma. It may occur in virtually any condition associated with persistent portal inflammation, including chronic hepatitis, steatohepatitis, biliary obstruction, inflammatory cholangiopathy and various systemic
diseases. If the trigger persists, then fibrous tissue occupies the periportal region and may extend into the neighboring parenchyma, sometimes in association with varying degrees of bile ductular proliferation. Periportal fibrosis can represent the first stage in the evolution to bridging fibrosis, and it therefore often connotes a progressive process.

Portal fibrosis may also be accompanied by sclerosis or obliteration of the portal vein branches. This occurs to a minor extent in many portal inflammatory conditions. At its most, it constitutes a distinguishing feature of hepatoportal sclerosis. In this condition, the larger portal tracts are most conspicuously affected, and recanalized venous thrombi or aberrant proliferated vessels may be noted.

- **Pericellular fibrosis.** This pattern is characterized by connective tissue fibers that extend along the sinusoids to surround single or small groups of hepatocytes. On connective tissue stains, pericellular fibrosis has a chickenwire appearance, and, because of its distribution, it is also referred to as perisinusoidal fibrosis. The most prominent cause of pericellular fibrosis is steatohepatitis. Initially arising in the centrilobular region, the fibrosis is accompanied in active disease by swollen hepatocytes, an inflammatory infiltrate with neutrophils and fatty change. In diabetes, pericellular fibrosis spreads across entire lobules, producing a pattern of diffuse intralobular fibrosis (diabetic hepatopathy).

- **Perivenular fibrosis.** In this pattern, the centrilobular zone is chiefly affected with deposition of connective tissue in the vicinity of the central vein. The extent of the deposition varies from minor vein wall thickening to marked scarring of the centrilobular region. When significant, perivenular fibrosis is generally accompanied by pericellular fibrosis of varying degree.

- **Bridging fibrosis.** Also referred to as septal fibrosis, this pattern designates the presence of connective tissue septa that extend across lobules and connect portal tracts and central veins in various arrangements. It represents an extension of periportal or perivenular fibrosis and, as a marker of progressive disease, is an important indicator of prognosis. Fibrous septa exhibit a variety of shapes and sizes, ranging from slender, well-defined bands to broad irregular collagenous zones that sometimes encompass entire lobules. Based on the vascular structures involved, bridging fibrosis can be separated into portal-portal, portal-central, and central-central types. Although this classification is conceptually valuable, the nature of a fibrous bridge can be difficult to determine in biopsy specimens, especially in advanced cases with mature fibrosis.

- **Cirrhosis.** Cirrhosis is defined as a diffuse process characterized by annular fibrosis and a conversion of normal architecture into structurally abnormal nodules. Cirrhosis is associated with important vascular changes: the development of intrahepatic porto-hepatic vascular shunts. Regenerative nodules may form between the fibrous septa but they are not necessary for the histological diagnosis of cirrhosis. Regeneration of hepatocytes may develop but is a late phenomenon. Several classifications of cirrhosis have been proposed based on morphology, pathogenesis, or clinical features. A widely employed classification relies on macroscopic appearance and, based on the diameter of the parenchymal nodules, divides cirrhosis into three categories: micronodular cirrhosis, macronodular cirrhosis, and mixed cirrhosis. More useful is the grading of cirrhosis severity that matches more or less the different clinical stages:
  - Incomplete septal cirrhosis is the milder form. It is characterized by the presence of very slender septa radiating from enlarged fields toward the center of the lobule. This feature is also encountered in regressing cirrhosis
  - Early cirrhosis is formed by thin fibrous septa with dissecting nodules. Regenerative nodules are usually absent.
  - Advanced cirrhosis consists in wide scars of parenchymal extinction containing clusters of regenerative hepatocytes. Large scars may contain large portal fields recognizable with a stain for elastic fibers.
These different patterns are mirrored at best by the recently developed Laennec scoring system which subdivided cirrhosis into 3 groups (4a, 4b, 4c), taking into account both thickness of fibrous septa and size of liver cell nodules.

**Pattern of fibrosis/cirrhosis regression**

It is now well-accepted that fibrosis and even a subset of cirrhosis cases can regress following successful treatment of the underlying disease. The landmark paper by Perez-Tamayo in 1979 first described evidence in both animal models and human disease for reversal of fibrosis and cirrhosis. Thereafter, Wanless et al presented histologic evidences from serial biopsies of patients that showed apparent shift from fully developed cirrhosis to incomplete septal cirrhosis. The most fascinating demonstration of the reversibility of fibrosis/cirrhosis came from studies analyzing large cohorts of patients with HCV or HBV effectively treated with antiviral regimens. In a number of these studies, patients have been followed with serial liver biopsies performed with sufficient time interval. After viral suppression or eradication, most patients display fibrosis regression while the regression of cirrhosis was observed in 50-60% of patients with baseline diagnosis of cirrhosis.

Liver biopsy has also a role in this context. Indeed, cirrhosis regression is associated with a net decrease of fibrous tissue amount that can be easily shown by morphometric measurement of fibrosis area. Among representative feature is the major thinning of fibrous septa that may be reduced to a few collagen fibers. Associated with septa thinning is the vanishing of shunting neovessels. Indeed, thin septa of regressing cirrhosis usually lack vessels or contain only few residual capillaries that may not function as shunting vessels anymore. Thereafter, septa become incomplete (incomplete septal cirrhosis) or may totally disappear. Regression of fibrosis is associated with a partial or full restoration of the lobular organization. Portal tracts and central veins are present, some portal tract containing an incomplete portal triad. Nodular organization may persist despite disappearance of fibrous septa leading to a pattern that resembles vaguely to nodular regenerative hyperplasia. Whether perisinusoidal fibrosis and sinusoidal capillarization may regress with a reversion of endothelial capillary to a sinusoidal phenotype is not clear. Indeed, recent studies suggest that this pattern may regress more inconsistently. By contrast, ductular proliferation, a feature associated with fibrosis progression, quickly disappears in regressing cirrhosis. In addition, while metabolic zonation is generally lost in cirrhosis, reversion is associated with restitution of normal lobular enzymatic zonation.

**References**

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