Definition and natural history of metabolic steatosis: histology and cellular aspects

V. Paradis*, P. Bedossa

Pathology Department, Beaujon hospital Clichy, 110, bd Général Leclerc, 92118 Clichy cedex, France; & Inserm U773 Paris

Abstract

In patients with diabetes and metabolic syndrome, liver changes may be observed on histology that are characterized as non-alcoholic fatty liver disease (NAFLD). The NAFLD spectrum covers a variety of histological features, including steatosis, necroinflammation and fibrosis. Although steatosis usually follows a benign course, steatohepatitis is prone to progress to fibrosis and cirrhosis. Establishing the degree of severity of liver lesions, the main endpoint of the disease, can identify patients at risk of disease progression. This may be achieved by liver biopsy. For that purpose, a scoring system for both activity (grade) and fibrosis (stage) is available with good reproducibility. In addition to the commonly seen histopathological patterns of lesions, additional changes are reported in patients with diabetes, including glycogenic hepatopathy and hepatic hepatosclerosis.

© 2008 Elsevier Masson SAS. All rights reserved.

Keywords: Steatosis; Steatohepatitis; Fibrosis; Diabetes; Metabolic syndrome; Review.

1. Introduction

In patients with diabetes and metabolic syndrome, the liver may display damages typical of the spectrum of non-alcoholic fatty liver disease (NAFLD). Given the significant increase in patients with features of the metabolic syndrome, the growing prevalence of NAFLD is expected [1]. Although liver disease is most often benign, it is nevertheless the third most common cause of death in patients with NAFLD, following cardiovascular diseases and malignancy [2,3]. The NAFLD spectrum covers a variety of histological features, including steatosis, fibrosis and necroinflammation. Although steatosis is a benign condition that usually does not progress to more severe liver disease, steatohepatitis (NASH) is a risk factor for the development of cirrhosis, end-stage liver failure and hepatocellular carcinoma [4,5]. The main objective of this review is to describe the pathological appearances of NAFLD and the specific features associated with diabetes.

*Corresponding author.

E-mail Address: vparadis@teaser.fr

© 2008 Elsevier Masson SAS. Tous droits réservés.
2. Liver pathology in NAFLD

2.1. Basic pathological features

Steatosis is defined as triglyceride accumulation in hepatocytes, and a minimum excess overload of at least 5-10% of hepatocytes is considered significant steatosis [6]. In NAFLD, steatosis is usually macrovesicular and most often located in the centrolobular area [7] (Fig. 1 and 2). Hepatocyte ballooning, a feature denoting cellular injury, is characterized by enlarged, swollen hepatocytes with or without Mallory’s hyaline in the cytoplasm (Fig. 3) [6]. Balloon cells are often closely associated with steatotic hepatocytes in the perivenular areas in perisinusoidal fibrosis. Lobular inflammation is usually mild, typically composed of mixed inflammatory cells, including mononuclear and polymorphonuclear leukocytes. Portal inflammation may be present, but with no specific characteristics, and mainly in obese pediatric populations [7]. As the disease progresses, liver fibrosis may occur. Indeed, natural history studies suggest that fibrosis progression occurs in approximately 35% of patients over 3-6 years, and up to 12% of patients will progress to cirrhosis over 8-10 years [8,9]. The characteristic pattern of fibrosis that distinguishes steatohepatitis from other forms of chronic liver disease is the initial deposition of extracellular matrix in the perisinusoidal area of lobule zone 3 (Fig. 4). In addition, periportal fibrosis with the formation of fibrous septa, leading to bridging fibrosis and cirrhosis, may eventually develop (Fig. 5). Finally, additional features may be reported in the context of NAFLD, including megamitochondria, granular iron pigmentation within hepatocytes and glycogenated nuclei (Fig. 6).

2.2. Histological scoring system of NAFLD

One major goal of the pathological analysis of patients with NAFLD is accurate evaluation of the extent of liver damage. To address this issue, histological scores of grading and staging have been developed. A system for a semi-
quantitative type of evaluation, initially proposed by Brunt et al. in 1999, was based on the idea that the histological diagnosis of NASH relies on a constellation of features rather than on any one feature [10]. Such an approach was recently refined to provide a semi-quantitative feature-based scoring system for NAFLD for both pediatric and adult populations [11]. In this scoring system, histological features are grouped into five categories: steatosis; inflammation; hepatocellular injury; fibrosis; and miscellaneous features (Table 1). More

![Fig. 4. Presence of moderate perisinusoidal fibrosis in the centrolobular area (sirius red stain).](image1)

![Fig. 5. Presence of portal fibrosis with few septa. Note the presence of marked steatosis (trichrome stain).](image2)

![Fig. 6. Presence of glycogenated nuclei in hepatocytes. Note the presence of steatosis (hematoxylin & eosin stain).](image3)

**Table 1**

Semi-quantitative scores for basic features according to the histological scoring system for non-alcoholic fatty liver disease by Kleiner et al. [11].

<table>
<thead>
<tr>
<th>Basic features</th>
<th>Definition</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steatosis</td>
<td>&lt;5%</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>5-33%</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>&gt;33-66%</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>&gt;66%</td>
<td>3</td>
</tr>
<tr>
<td>Lobular inflammation</td>
<td>No foci</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2 foci/200 × field</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>2-4 foci/200 × field</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>&gt;4 foci/200 × field</td>
<td>3</td>
</tr>
<tr>
<td>Hepatocellular ballooning</td>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Few balloon cells</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Many cells/prominent ballooning</td>
<td>2</td>
</tr>
</tbody>
</table>
important, it has been demonstrated that agreement between pathologists in adult cases show reasonable concordance with the main categories of pathological features, including steatosis, fibrosis and ballooning injury, with weighted kappa values over 0.5.

In addition, a NAFLD activity score (NAS), which includes features of active injury, has been defined as the unweighted sum of the scores for steatosis (0-3), lobular inflammation (0-3) and ballooning (0-2). According to this scale, cases with scores ≥5 are diagnosed as NASH, and scores <3 are diagnosed as not NASH. It has been clearly emphasized that the NAS is not intended to be used as a diagnostic tool, but rather to provide a uniform tool for assessing disease severity and, ideally, in clinical trials [11].

As with viral chronic hepatitis, fibrosis is separately assessed by a 5-stage scale—ranging from no fibrosis to cirrhosis—that pays particular attention to the evaluation of the intensity of perisinusoidal fibrosis [11-13]. A description of fibrosis stages according to Kleiner et al. is presented in Table 2.

Table 2: Definition of fibrosis stages according to the histological scoring system for non-alcoholic fatty liver disease by Kleiner et al. [11].

<table>
<thead>
<tr>
<th>Fibrosis</th>
<th>Definition</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Perisinusoidal or periportal</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Mild, zone 3, perisinusoidal</td>
<td>1A</td>
<td></td>
</tr>
<tr>
<td>Moderate, zone 3, perisinusoidal</td>
<td>1B</td>
<td></td>
</tr>
<tr>
<td>Portal/perportal</td>
<td>1C</td>
<td></td>
</tr>
<tr>
<td>Perisinusoidal, portal/peripoportal</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Bridging fibrosis</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Cirrhosis</td>
<td></td>
<td>4</td>
</tr>
</tbody>
</table>

2.3. Specific pathological aspects in diabetes

Many of the most severe complications of diabetes are the result of diabetic microangiopathy, defined as thickening of the capillary basement membranes of various tissues and organs. Hepatic abnormalities associated with diabetes have long been recognized, including NAFLD. More recently, additional histological findings have been described in patients with diabetes. Among them, hepatic hepatosclerosis, characterized by dense perisinusoidal fibrosis, has been reported in liver biopsies performed in diabetic patients for evaluation of abnormal liver test results [14]. Interestingly, perisinusoidal fibrosis was not associated with steatosis or necroinflammatory activity, but was associated with hyaline thickening of the small hepatic artery branches (Fig. 3). Glycogenic hepatothapathy, characterized by marked glycogen accumulation leading to pale, swollen hepatocytes, was initially described in the context of Mauriac syndrome [15]. A pathological review of 14 liver biopsies from patients with poorly controlled type 1 diabetes demonstrated abundant cytoplasmic glycogen deposits in hepatocytes, no or mild fatty change and no or minimal necroinflammation. Such a morphological pattern clearly differs from steatohepatitis and may be reversed following adequate control of hyperglycemia [16].

3. Liver biopsy: the gold standard so far

NAFLD is defined as a clinicopathological entity that requires liver biopsy for diagnostic confirmation and estimation of disease severity. Indeed, no diagnostic laboratory test or imaging study has yet performed well enough to replace biopsy. Imaging procedures fail to detect either mild steatosis (<33%) or necroinflammation as well as biopsy does [17]. However, in addition to the potential variability in observer reproducibility and sampling errors, pathologists recognize that sample size, technique for obtaining the biopsy and the method of processing are all important considerations in liver biopsies [18,19]. Regarding sampling variability between the left and right lobes of the liver, except for necroinflammation, minimal variability was found for steatosis, NAS or fibrosis in a series of morbidly obese patients [20].

4. Pathogenesis of NAFLD

It is clear that, in patients with metabolic syndrome and diabetes, several molecular mechanisms and inflammatory mediators are involved in the development of steatosis, steatohepatitis and fibrosis. Among them, insulin resistance may play a major role in the blockade of hepatic insulin-receptor signaling through activation of different molecules, such as protein kinase C, an inhibitor of kappa B kinase. Superimposed necroinflammatory injury involves additional mechanisms, including oxidative stress, release of endotoxins, and other cytokines and chemokines. Finally, as with other forms of chronic liver disease, the production and accumulation of extracellular matrix by fibrocompetent cells, including portal fibroblasts and hepatic stellate cells, require mobilization of profibrogenic molecules such as connective tissue growth factor and TGF-β [18].

5. Conclusion

NAFLD, which represents the manifestation of metabolic syndrome in the liver, covers a wide spectrum of morphological changes, from steatosis to fibrosis and cirrhosis. Although liver biopsy comes with several drawbacks, it remains the best tool for evaluating, grading and staging the disease so far. In addition to features associated with metabolic syndrome, specific changes related to diabetes have also been described.
**Conflicts of interest:** The authors have none to declare.

**References**


