

## **PATHOLOGICAL FEATURES OF NASH**

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### **1. ABSTRACT**

Nonalcoholic fatty liver disease (NAFLD) is a term currently applied to accumulation of triglycerides in the liver which is observed microscopically as “macrovesicular” steatosis. The term applies to the full range of liver parenchymal injury from “simple steatosis” to “steatohepatitis” with or without fibrosis. This article discusses the full histopathologic spectrum of NAFLD. Additionally, the summary includes an historical overview as well as contemporary thoughts on the significance of the process(es). While largely focused on adult NAFLD, the manuscript also summarizes what has been reported to date for NAFLD in the pediatric population.

### **2. INTRODUCTION**

Nonalcoholic fatty liver disease (NAFLD) is an increasingly recognized medical problem throughout the Western world and in Asia, in adults and in children. NAFLD may be the most common cause of chronic liver disease, with an estimated prevalence of up to 24% in the general population (1-5). Recent analyses from population screening in the United States have shown that NAFLD may be the underlying explanation for 80% of elevated liver tests in adults (6-8), far exceeding the prevalence of hepatitis C (9). In addition, NAFLD is no longer considered a simple “benign” process, but rather a spectrum of liver injury that spans simple steatosis to steatohepatitis with progressive injury including fibrosis, cirrhosis, liver failure and hepatocellular carcinoma (10-12).

Long associated with obesity, NAFLD is now closely associated with features of the cluster of disorders known as Metabolic Syndrome which includes central obesity, impaired glucose tolerance or type II diabetes, arterial hypertension and dyslipidemia; some authors have concluded that NAFLD is the hepatic manifestation of the Metabolic Syndrome (13-15). Certainly, the association with insulin resistance has been shown in careful clinical assessments (15-17) and in recent treatment trials specifically targeted improvement in insulin sensitivity (18-21).

Other reported clinical associations with the histologic features of nonalcoholic steatohepatitis (NASH) include nutritional disorders (rapid weight loss or malnutrition, as well as obesity), certain drugs and toxins, obesity (bariatric) surgery, pancreato-biliary diversions, bacterial contamination of the small bowel, and some inherited metabolic conditions (1,4,22). Interestingly, studies have shown that NASH may be diagnosed in biopsies of patients who are neither overweight (23,24), nor have elevated liver tests(25).

The diagnosis is derived from a combination of clinical and histopathologic information; the lack of “significant” alcohol use (commonly stated as <20g/d for women, <40g/day for men) can only be established clinically, while the presence of the lesions of NAFLD and NASH, discussed below, can only reliably be detected by microscopic evaluation. Various imaging tests may detect

**Table 1.** Pathological findings in NASH Modified from reference 29

Necessarily present	Usually present; but not necessary for diagnosis	May be present but not necessary for diagnosis	Unusual for NASH; consider other causes of liver disease
Steatosis, macrovesicular > microvesicular; most commonly present in acinar zone 3  Minimum quantity not known but at least > 5%	Zone 3 perisinusoidal/pericellular fibrosis; eventually is component of central-portal bridging fibrosis	Mallory's hyaline in Zone 3 hepatocytes; typically poorly-formed, may require antibodies to ubiquitin, p62 or CKs 8,18 to detect	Purely or predominantly microvesicular steatosis  Very little macrosteatosis in a nonzonal distribution or zone 1 predominant
Mixed, mild lobular inflammation; scattered polymorphonuclear leukocytes as well as mononuclear cells	Zone 1 hepatocellular glycogenated nuclei	1+ granular periportal (zone 1) hepatocellular iron or mild panacinar sinusoidal lining cell iron by Prussian Blue	Portal inflammation greater than lobular inflammation; lymphoid aggregates; numerous plasma cells
Hepatocellular ballooning; most frequent near steatotic liver cells, typically in zone 3	Lipogranulomas in the lobules; of varying, but usually small size	Megamitochondria in hepatocytes	Sclerosing hyaline necrosis; veno-occlusive lesions; perivenular fibrosis, phlebosclerosis
	Occasional acidophil bodies; occasional PAS-d Kupffer cells		Portal/periportal fibrosis in absence of, or markedly greater than zone 3 perisinusoidal fibrosis; portal-based bridging fibrosis
	Fat cysts		Lobular disarray and marked inflammation; endophlebitis; confluent or bridging necrosis  Acute or chronic cholestasis; bile duct lesions, bile duct loss, or ductular proliferation (reaction)
			PAS-d globules in hepatocytes

PAS-d: Periodic acid Schiff stain after diastase predigestion; CK: cytokeratin

fat accumulation in the liver, but below 30%, none have proven to be reliable(26) and liver biopsy remains the “gold standard” (27). The minimal histologic criterion is the presence of steatosis, i.e. the accumulation of triglycerides within hepatocytes; the lower limit of steatosis necessary for the diagnosis is often noted at >5%, but this is not a well-established figure (28,29). Liver biopsy also serves other purposes not attainable by imaging studies: to establish severity of activity, severity of fibrosis and architectural remodeling, establish the possible concurrence with other liver diseases, and offer alternative diagnoses in cases that are not otherwise diagnostic or suggestive of NAFLD or NASH (27,30-32).

## 3. PATHOLOGIC FEATURES OF NAFLD AND NASH

### 3.1. Historical Overview

Although the current “epidemic” of obesity has brought NAFLD and NASH to the forefront in liver disease and the larger medical and lay communities, pathologists and clinicians specialized in liver disease historically have noted the lesions and clinical associations we discuss today. For instance, Connor described fatty infiltration and cirrhosis in diabetics in 1938 (33) and Westwater et al (34) and Thaler et al (35,36) discussed steatosis in obesity in 1958, 1962, and 1975, respectively. In fact, prior to 1980 when Ludwig et al published the seminal series credited for establishing NASH as an entity (37), there were several series in the literature that identified the hepatic lesions that resembled those of alcoholic liver disease, and the relationship of steatosis with obesity, diabetes and the potential for progression to cirrhosis; several examples are listed (38-44).

Although the terms “NASH” and “NAFLD” had been utilized in prior literature, Ludwig et al are credited with establishing the acronym “NASH” in their careful study of 20 biopsies with features of alcoholic hepatitis

from patients with well-documented lack of alcohol use (37). Matteoni et al are credited with expanding the concept of fatty liver disease to include steatosis without necroinflammation or fibrosis in the study that proposed the 4 classifications of fatty liver disease based on differing rates of cirrhosis and death in 98 patients (45). It is upon the foundations established by the early studies, and the work of Ludwig et al, and Matteoni et al, that current work has developed.

### 3.2. Adult NAFLD and NASH

We have chosen to discuss the lesions of adult and pediatric NAFLD and NASH separately, as much more work has been done and therefore more is understood and agreed upon in the former. Interestingly, almost 25 years after the Mayo Clinic study, there remain ongoing discussions amongst pathologists as to minimum necessary criteria for diagnosis. This topic was recently highlighted by a published survey of 10 international hepatopathologists who have published in the field (27); specific areas of agreement and discussion will be highlighted. Younossi et al noted several years ago that the differences in histologic criteria utilized in different studies may be a source of discordance in results (46); unfortunately, that concept remains valid today.

It is generally agreed upon that a constellation of lesions is required; included in the constellation are steatosis, inflammation (lobular and variably portal), and hepatocellular injury (commonly ballooning); common, but not “required” is perisinusoidal fibrosis (Table 1). In **noncirrhotic** steatohepatitis (both alcoholic and nonalcoholic) the lesions are usually concentrated in the centrilobular area, zone 3 of the hepatic acinus. Each of the histological lesions of steatohepatitis have each been associated with specific mechanisms speculated in pathogenesis, as reviewed (47,48).

#### 3.2.1. Steatosis

Hepatocellular steatosis in NAFLD is more commonly macrovesicular, large droplet fat that appears as

a single or several areas of clearing within affected hepatocytes. Typically, the involved hepatocyte's nucleus is displaced eccentrically by the fat droplet. Clusters of hepatocytes with small droplet steatosis or true microvesicular steatosis may be observed, but never as a majority. The large droplet steatosis represents aberrations of lipid metabolism (excess delivery, altered intracellular metabolism, possibly increased synthesis, and abnormal export mechanisms), whereas abundant microvesicular steatosis results from defective beta-oxidation, such as in mitochondrial cytopathies (Reye's syndrome, fatty liver of pregnancy) and disorders of urea metabolism. As has been noted, the latter group of disorders are characterized by rapid progression to liver failure, whereas disorders of macrovesicular steatosis are more commonly slowly progressive.

Steatosis in NAFLD and NASH is evaluated in a semiquantitative fashion microscopically; a common method follows the acinar architecture of the liver parenchyma and reports steatosis' involvement in terms of thirds: <33%, 33-66%, >66% (49,50). A recently proposed modification includes the minimal amount of steatosis:<5% as grade 0 and 5-33% as grade 1 (51). While studies have shown correlation of steatosis grade with CT determination of hepatic steatosis (as determined by liver:spleen ratio) (52), a recent abstract reports lack of correlation of microscopic assessment of steatosis with biochemically-determined triglycerides (53).

As with all the lesions of NASH, steatosis may or may not persist in cirrhosis. The determination of which cases will be involved and the actual course of "disappearance" of lesions is not known, but the fact has been well-established in biopsy series (54,55). These observations, and careful clinical studies of cirrhotic patients have led to current speculation that many cases of cryptogenic cirrhosis are actually "burned-out" NASH (56,57).

### 3.2.2. Hepatocellular injury

Hepatocellular ballooning refers to enlarged, swollen hepatocytes with cytoplasmic rarefaction. In noncirrhotic cases, these are commonly located in acinar zone 3 admixed with steatotic hepatocytes and are often identified in areas of perisinusoidal fibrosis (27,29,30,58). Ballooned hepatocytes have historically been considered to be manifestations of microtubular disruption in severe cell injury preceding lytic necrosis (28). A recent ultrastructural analysis has shown that at least some contain small droplet steatosis, and the investigators consider this as an adaptive change (59).

Apoptosis is another form of hepatocellular injury; acidophil bodies are occasionally found in NASH. Recent studies have shown markers for apoptosis in NASH and have found them to be more common than in alcoholic hepatitis (60,61). Feldstein et al have further speculated that apoptosis is a manifestation of lipotoxicity that leads to apoptosis via the TNFalpha pathway and correlates with disease severity (60).

Large regions of necrosis, such as confluent or bridging necrosis, are very uncommon in NASH, although some cases have been reported (62,63).

### 3.2.3. Inflammation

Both lobular and portal inflammation may occur in NASH. Steatosis without inflammation (Matteoni NAFLD type 1) or steatosis with only mild inflammation but no ballooning (Matteoni, type 2) are considered relatively "nonprogressive" forms of NAFLD (45). In the Matteoni study, cirrhosis was noted in 21-28% of subjects with types 3 and 4 respectively, compared with 4% and none in types 1 and 2, respectively (45). Lobular inflammation is commonly mixed: lymphocytes, other mononuclear cells, occasional polymorphonuclear leukocytes, and lipogranulomas. Portal inflammation, on the other hand, is lymphocytic; the presence of polymorphonuclear leukocytes in portal and periportal regions is suggestive of alcoholic liver disease, or another process altogether (30,58,64).

Portal inflammation is either absent or mild in NASH; when portal inflammation is disproportionate to the lobular inflammation, there is concern of a concurrent process, such as hepatitis C (30). Recently, one group has noted that portal inflammation greater than lobular inflammation was identified as a histologic finding of resolution in treated NASH (18).

Lipogranulomas may or may not be present. They may consist of a single hepatocyte rimmed by mononuclear cells and an eosinophil. When large, lipogranulomas may elicit fibrosis and this is not to be confused with the perisinusoidal fibrosis of NASH (30). Microgranulomas and single, PAS-diastase-positive pigmented Kupffer cells may also be noted scattered in the lobules; these foci represent prior necroinflammatory activity.

### 3.2.4. Fibrosis

The characteristic pattern of initial deposition of collagen of NASH distinguishes this process from the patterns of fibrosis in early stages in chronic hepatitis of viral and autoimmune origins, or chronic cholestatic liver diseases. The latter and last are characterized by portal-based fibrosis, whereas in steatohepatitis, due to either alcoholic or the various nonalcoholic etiologies, the earliest deposition is in the pericentral, zone 3 perisinusoidal spaces (28,37,58,62). The pattern has been described variably as "chickenwire" and "pericellular", as the collagen fibers are present in a manner that resembles the lattice of a chickenwire fence. The collagen may be dense, and easily noted by routine staining, or may be quite delicate and require special stains for collagen, such as Masson's trichrome, to appreciate. With progression, the periportal regions may show fibrosis; bridging fibrosis may evolve between the vascular structures and with progression, cirrhosis. At some stage in the progression, the perisinusoidal fibrosis may become incorporated and no longer be appreciated as a discrete entity. Some investigators "require" the presence of zone 3

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perisinusoidal fibrosis for the diagnosis of NASH, whereas others recognize it as common but not necessary (27).

The possibility of fibrosis arising only from the portal areas and not involving zone 3 in morbidly obese patients is an area of debate (65). One study in morbidly obese patients documented perisinusoidal fibrosis but only analyzed the portal-based fibrosis for features of prognosis (66), while another in a similar patient population required the zone 3 lesions present for diagnosis and prognosis, and documented the relationship to features of metabolic syndrome with these criteria (67). More experience will certainly be gained with this unique group of subjects with fatty liver disease.

Investigations of hepatic stellate cell activation in NASH have shown varying results in terms of relationship to steatosis, steatohepatitis and fibrosis visible by light microscopy (68,69).

### 3.2.5. Other histologic lesions of NASH

Mallory's hyaline, megamitochondria and glycogenated nuclei are lesions that are not uncommonly described in NASH, yet none are considered "required" for the diagnosis (27). An important reason to discuss them rests in the fact that their presence raises differential diagnostic possibilities that should be considered in histologic evaluation (30).

Mallory's hyaline (MH) is characterized by dense perinuclear intracytoplasmic inclusions, usually in ballooned hepatocytes in zone 3; this finding may be seen in alcoholic or nonalcoholic steatohepatitis, but in the former, MH may additionally be noted in shrunken, acidophilic hepatocytes and may actually require special stains to identify. As MH is chemotactic, the affected hepatocytes may be surrounded by polymorphonuclear leukocytes, a lesion referred to as "satellitosis". MH are noted most readily in more severe cases (50) and were identified in the study of Matteoni et al as a feature of the "progressive" forms of NAFLD (45). It has been noted by several investigators, however, that when MH are numerous, the process is more likely of alcoholic than nonalcoholic origin (28,30,62).

MH, however, is not an absolute diagnostic feature of NASH; MH are noted in disorders of copper metabolism including Wilson disease, copper toxicosis, chronic cholestatic disorders and some drugs (amiodarone, as example). In chronic cholestasis and in amiodarone effect, the MH are found in the periportal, zone 1 hepatocytes, in contrast to the zone 3 localization in steatohepatitis (28-30,58). Interestingly, MH may also occur in focal nodular hyperplasias, hepatocellular adenomas and hepatocellular carcinomas.

In contrast to the early concepts that MH represented a passive collapse of intracytoplasmic filaments, work in the past several years has shown that the formation of MH is a metabolically-active process for formation of a "sequestosome" for cytoprotection (70). Immunohistochemical assays available for identification of

MH (ubiquitin, p62 as examples) have resulted from detailed analysis of the pathophysiology (71,72).

Megamitochondria, noted by light microscopy as eosinophilic intracytoplasmic round or needle-shaped inclusions, are present in many cases of NASH; the exact frequency of occurrence in all of NAFLD is not known. Aberrations in mitochondrial beta oxidation are speculated as significant causes of production of reactive oxygen species and subsequent oxidative stress in NASH (47), hence the possible association of functional and structural abnormalities have been examined (73,73). Two groups have studied mitochondria by light and electron microscopy and have noted dysmorphic changes including crystalline inclusions, loss of cristae and multilamellar membranes (74-76). A recent report documented random distribution throughout the lobules, and concluded the abnormalities likely represent an adaptive response to oxidative stress rather than the result of cell injury (77). Megamitochondria are not known to affect assessments of histologic "severity"; similarly altered mitochondria may be noted in other liver diseases, such as alcoholic liver disease, acute fatty liver of pregnancy, and Wilson disease.

Glycogenated nuclei are noted as vacuolated nuclei in hepatocytes. In NASH, they are most commonly found in zone 1, but may be seen in clusters in a nonzonal distribution. These nuclear changes are noted in diabetes of all etiologies (78), in Wilson disease, and in pediatric livers; in NAFLD and NASH they are of uncertain significance beyond the possible association with diabetes (37). The lesion is more often noted in NASH than in alcoholic steatohepatitis (58,64,79).

Iron deposition in hepatocytes and/or in the reticulo-endothelial cells of the liver (sinusoidal lining cells, portal macrophages and endothelium of larger vascular structures) in uncomplicated NASH is mild, if present at all (27,29,30,45,50,52). The finding has been reported in 15-55% of the cases for which iron was specifically evaluated. Results of studies to find associations of abnormal iron indices, iron genetics or abnormal iron deposition in tissue have given conflicting results. One study reported an association of increased hepatocellular iron by staining with increased portal fibrosis (80), and some have speculated that insulin resistance and aberrant iron metabolism are related metabolic disorders (81-86). Studies of genetics have been inconclusive (87-90).

## 4. SEMIQUANTITATIVE ASSESSMENTS IN NAFLD AND NASH

Semiquantitative assessments for chronic viral (and autoimmune, metabolic and drug-induced) liver disease, such as "Scheuer" (91), "Knodel" (92), "METAVIR" (93,94), "Ishak" (95) were developed in order to standardize evaluations of liver biopsies primarily for treatment trials; with time, these methods have evolved into methods utilized in daily practice and serve as a means of reasonably reproducible assessment of ongoing injury (activity grade) and fibrosis (stage) (96). In 1999, a

**Table 2.** Proposed grading and staging of NASH. Modified from reference 50

Grading				
Grade	Steatosis	Ballooning	Lobular Inflammation	Portal Inflammation
Grade 1, Mild	Predominantly macrovesicular; any amount	Occasionally observed; zone 3 hepatocytes	Scattered, mild acute (polymorphs) and occasional chronic (mononuclear)	None or mild
Grade 2, Moderate	Usually mixed; any amount	Obvious; present in zone 3	Polymorphs may be noted associated with ballooned hepatocytes, pericellular fibrosis; mild chronic inflammation may be seen	Mild to moderate
Grade 3, Severe	Usually mixed; typically >66% and panacinar	Marked; zone 3	As above	As above
Steatosis: graded as 1 = 0-33%, 2 = 33%-66%, 3 = >66%. Ballooning: zonal location noted and severity (mild or marked) recorded Lobular Inflammation: 0 – 3 based on observations of foci per 20x field: 1 = 1-2 foci, 2 = up to 4 foci, 3 = > 4 foci. In addition, cell types (acute or chronic) and location are noted. Portal inflammation: 0-3, 1 = mild, 2 = moderate, 3 = severe				
Staging				
Stage	Zone 3 Perisinusoidal Fibrosis	Periportal Fibrosis	Bridging Fibrosis	Cirrhosis
Stage 1	+, focal or extensive	No	No	No
Stage 2	As above	Present, focal or extensive	No	No
Stage 3	May or may not be present	May or may not be present	Present, focal or extensive	No
Stage 4	As above	As above	As above	Yes
Stage 1: Zone 3 perivenular, perisinusoidal/ pericellular fibrosis, focal or extensive Stage 2: As above with focal or extensive periportal fibrosis Stage 3: Bridging fibrosis, focal or extensive Stage 4: Cirrhosis				

proposal to do the same for NASH was published; this proposal (the “Brunt” system) was based on the concept that the lesions of NASH are sufficiently different from those of chronic hepatitis as to warrant a unique system of evaluation (50). The system reflects the concept that NASH is a diagnosis based on a constellation of lesions; while steatosis, ballooning and inflammation (lobular and portal) are the factors to be evaluated for grade, it is the latter two that influence progression of grade. Marked steatosis, of itself, is not considered a marker of severity. Fibrosis scoring is based on the above-described progression from zone 3 perisinusoidal fibrosis to cirrhosis. (Table 2) The system has been widely used, but has not been formally tested in a prospective fashion.

In the past year, the Pathology Committee of the National Institute for Digestive Diseases, Diabetes and Kidney-sponsored NASH Clinical Research Cooperative did a prospective validation study of a modification of the “Brunt” scoring system that showed good interobserver and intraobserver correlations for each of the features, and found the lesions most associated with the diagnosis of “NASH” to be steatosis, lobular inflammation and ballooning for an activity score. The fibrosis score modification includes separation of stage 1 into dense or delicate, and offers a choice for “portal only” in order to evaluate the presence of this lesion (Table 3). The system is broad enough to evaluate for the spectrum of NAFLD, and for pediatric NAFLD, as discussed below. The system has been presented as an abstract, and is formally under review for publication (51).

## 5. PEDIATRIC NAFLD AND NASH

It is increasingly recognized that the full clinical and histologic spectrum of NAFLD and NASH may be present in children. The subject has been recently reviewed (97,98). While there are striking pathogenic similarities with adults, there have been differences in histologic changes in the cases published to date. The primary

differences include the abundance of steatosis, apparent lack of ballooning and MH, and potential absence of zone 3 perisinusoidal fibrosis with preponderance of portal fibrosis (27). It is not clear, at this stage, if the differences are true across all population groups, nor in all age groups, as several investigators have noted cases of pediatric NASH with histologic findings similar to adults (personal observations and personal communications). This is an area that will continue to evolve with increasing numbers of biopsies and large studies including the broad range in ages of “pediatrics”. What is agreed upon, however, is the potential for cirrhosis is as real in children as in adults (97,99,100).

## 6. PERSPECTIVES

Newer observations in NAFLD and NASH are arising from recent therapeutic trials in well-characterized subjects with NASH. While not consistently done until recently, therapeutic trials now require biopsy evaluation at entry and in post-intervention follow-up (101). From these studies, lesions that “disappear” with treatment and lesions that may be manifestations of “resolution” are being documented. Most of the recent studies with intervention by weight loss or drug intervention have shown clinical efficacy and resolution of steatosis and lobular inflammation (18,19,102). One study of 30 subjects with follow-up biopsies in 22, showed a shift in the ratio of lobular:portal inflammation to favor the latter in resolution; in addition, there was an apparent qualitative change in dense perisinusoidal fibrosis to delicate perisinusoidal fibrosis in successfully treated subjects (18). A recent study showed lack of efficacy of ursodeoxycholic acid, but because the study design included a placebo group, the study demonstrated “spontaneous” improvement in some cases (103). This finding has also been observed in other clinical trials (18), in which there was an apparent improvement in histologic findings from the time of identification of the patient by biopsy, to the protocol enrollment biopsy for the study. These types of findings

**Table 3.** NASH Clinical Research Network (NIH) Histologic Scoring system for NAFLD and NASH. Reference 51

Activity score				
Steatosis	Lobular Inflammation	Ballooned hepatocytes	Total Score	
0-3	0-3	0-2	0-8	
Steatosis: 0: <5%; 1:5-33%; 2:>33-66%; 3:>66%, Lobular inflammation: 0: none; 1:<2; 2:2-4; 3:>4 foci/20x, Ballooned hepatocytes: 0: none; 1: few; 2: many/prominent				
Fibrosis. Modified "Brunt" criteria, reference 50				
Score	Zone 3 perisinusoidal fibrosis	Portal/periportal Fibrosis	Bridging Fibrosis	Cirrhosis
0	None	None	None	None
1a	Delicate, requires trichrome stain	No	No	No
1b	Dense	No	No	No
1c	Not present	Present	No	No
2	As above (1a,1b)	Present	No	No
3	May or may not be present	May or may not be present	Present	No
4	As above	As above	As above	Yes
0: None, 1: 1a Mild (Delicate) Zone 3 Perisinusoidal Fibrosis, 1b Moderate (Dense) Zone 3 Perisinusoidal Fibrosis, 1c: Portal Fibrosis Only, 2: 1a/B With Periportal Fibrosis, 3: Bridging Fibrosis, Focal Or Extensive, 4: Cirrhosis Modified from reference 51				

are of great value as the field is progressing and our understanding of this process broadens to include the possibility of improvement without specific intervention.

Another area of growing interest is that of the co-existence of NAFLD and NASH with other forms of chronic liver disease. This has been well-documented in a variety of biopsy-proven and serologically proven diseases (104-106). The association with hepatitis C infection is an area of exponential growth in the past 5 years, as both hepatitis C and NAFLD are common problems. Steatosis is a common histologic feature in both. The overlap (presence of steatosis) may be due to coincidence of two common processes, or may actually be due to virally-mediated mechanisms, as several recent studies have shown (106-118).

A third area of growing interest is progression to hepatocellular carcinoma, as discussed, and the putative role of hepatic progenitor cells in NASH regeneration and malignancy (119,120).

Finally, several clinico-pathologic systems have been proposed to predict fibrosis, or to predict which patients would "benefit" from biopsy; some methods have been carefully developed in morbidly obese subjects (66,121), and some are more widely applicable to the broader population (49,122). It is postulated that with refinements in clinical predictors, such as a recently proposed system to include adiponectin levels (123), and increased sensitivity of radiologic imaging tests, in combination with increased understandings of possible genetic markers (47,124,125) and use of technology such as microarray analysis (126), the necessity for liver biopsy may gradually become less. In the meantime, the ongoing value of liver biopsy includes not only confirmation of diagnosis, but also exclusion (or evaluation) of other liver disease (31), and careful analysis of clinical correlations (52).

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