Immunohistochemical Analysis in Steatohepatitis

Does It Have a Role in Diagnosis and Management?

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Steatohepatitis, in particular nonalcoholic steatohepatitis (NASH), is one of the most common diagnoses made in routine liver biopsy practice, yet there remain gaps in our knowledge. Among these are basic questions, including the minimal histologic criteria for the distinction of steatosis from steatohepatitis, criteria for the prediction of outcome, and features allowing determination of cause or origin. The latter is an issue because steatohepatitis encompasses a wide spectrum of causes that fall into the general categories of toxic agents (alcohol and various medications) and metabolic disease, including most commonly the metabolic syndrome,[1] although other metabolic diseases (eg, Wilson disease or tyrosinemia) technically might be included as well. These 3 aspects of steatohepatitis—diagnosis, prognosis, and cause or origin—are not independent because the criteria used for diagnosis may portend the prognosis and cause, and, conversely, different etiologic agents will determine the histologic features and prognosis, the latter perhaps independent of histologic features.

So what is the problem with diagnosis? NASH originally was defined by the clinical observation that liver disease with a histologic picture similar to alcoholic liver disease was occurring in patients who did not consume alcohol.[2] Although somewhat controversial in its early days, epidemiologic considerations, including the association with obesity, diabetes, or both, soon persuaded most that NASH was a real entity. Given that realization, clarification of the histologic features of NASH vs alcoholic steatohepatitis (ASH) should have been possible because we could define the disease by epidemiologic rather than histologic features (because we were no longer constrained by the original definition of NASH having features similar to those of ASH). Defining a disease as "similar" to alcoholic liver disease always was limited by the presupposition that there was only 1 histologic appearance to alcoholic liver disease, which is far from true. Alcohol causes a wide range of histologic changes, ranging from simple macrovesicular steatosis to microvesicular steatosis in the form of acute foamy degeneration.[3,4] So the histologic definition of "looking like alcoholic liver disease" has been problematic from the start.

The upshot of this is that the histologic description of NASH has included a wide range of features, and, in the defining article by Ludwig et al,[2] simple fatty change without inflammation was included as a form of NASH. There is no question that the definition of steatohepatitis starts with fatty change as a necessary feature. Most current definitions of steatohepatitis require more than just fatty change, however, based on a study of a relatively small number of patients that often is quoted to demonstrate that the disease in patients with fatty change alone (or fatty change with inflammation) does not progress to cirrhosis, whereas in patients with ballooning degeneration, Mallory hyaline, and/or pericellular fibrosis, the disease progresses in a considerable number of cases.[5]

Observational experience demonstrates that this rigid dichotomy is not absolutely true, but rather represents a relative truth and can be explained by viewing the results as indicating that we are looking at sequential steps of an ongoing process, which would fit the 2-hit hypothesis commonly invoked for steatohepatitis.[6] This theory suggests that fatty change itself (steatosis) is relatively innocuous but primes the liver for a second hit, leading to more significant hepatocellular damage and, eventually, fibrosis (steatohepatitis). Livers with fatty change alone are livers waiting for the second hit, which may or may not come, whereas livers with

more significant damage have already encountered their second hit and, therefore, are on a faster track to fibrosis.

Understanding this stepwise progression from steatosis and steatohepatitis is important in understanding this disease: the stepwise progression predicts that intervention at the stage of steatosis alone could prevent the second hit from occurring and that some livers demonstrating only steatosis in a current biopsy specimen eventually will have a second hit, and, therefore, disease will progress, despite inferences that this does not happen.[7] Nevertheless, as a practical matter, livers with ballooning degeneration, Mallory hyaline, and/or fibrosis have demonstrated that they have had the second hit and, therefore, are more deserving of more intense follow-up. For this reason, the concept of diagnosing steatohepatitis only after these changes are present is a useful and practical one, as long as a diagnosis of steatosis alone carries a disclaimer that the absence of features of steatohepatitis does not guarantee that steatohepatitis will not occur in the future in that patient.

The wide spectrum of histologic patterns seen with steatohepatitis often is not appreciated when trying to distinguish different causes. For example, amiodarone is a medication often reported as causing "pseudoalcoholic liver disease." However, my experience with the toxic effects of amiodarone demonstrates that in many cases there is only mild fatty change, with extensive and well-formed Mallory hyaline that seems out of proportion to the degree of steatosis. Marked cholestasis also is common, again out of proportion to the other changes. These cases usually look quite different from alcoholic liver disease or NASH, although descriptively they might sound quite similar.

A similar situation exists in the more common problem of distinguishing ASH from NASH related to the metabolic syndrome. Several articles have highlighted histologic difference, including differing types of inflammatory infiltrate, different character to the Mallory hyaline, and other more unique changes like abundant glycogenated nuclei in NASH and more prominent central venous changes with endophlebitis in severe cases of ASH.[7,8] Despite this, the differences sometimes are subtle or matters of degree, and the article in this issue of the *Journal* by Sanderson and Smyrk[9] applies a novel approach to distinguishing these 2 causes of steatohepatitis in a more direct manner.

The article by Sanderson and Smyrk[9] attempts to distinguish NASH from ASH by using immunohistochemical analysis, making use of the known relationship between insulin resistance and NASH. Liver biopsy specimens were stained for insulin receptors (IRs) and for protein tyrosine phosphatase 1B (PTP1B), a protein that acts as a negative regulator of IR expression. By using the concept that cases of obesity-related NASH might have decreased IR and increased PTP1B expression vs more normal IR expression and lower levels of PTP1B expression in alcoholic liver disease, cases were categorized immunohistochemically into NASH and ASH and compared with the clinical diagnosis made in each case.

In general, their hypothesis was validated; there was reasonably good correlation between immunohistochemical results and the clinical diagnosis; however, the correlation was not perfect, with a number of clinical ASH cases staining as NASH (16/53 [30%]) and a number of clinical NASH cases staining as ASH (23/188 [12.2]%). As the authors discuss, there is nothing exclusive about the diagnoses of NASH and ASH. Having a NASH-like staining pattern in the ASH group might be explained by a combination of obesity-related insulin resistance in a group of patients with alcohol-related liver injury, the NASH-like staining pattern being the more specific of the 2 patterns (ie, the "ASH" pattern is, in reality, a normal staining pattern of liver tissue, not a specific pattern related to alcohol use). To support this contention, the authors point out that the body mass index of patients with NASH-staining clinically diagnosed ASH was slightly higher than in the clinically diagnosed ASH group as a whole (28.4 vs 27.2 kg/m^2), although the difference is small. Explaining the lack of NASH-like staining in the clinical NASH group is a bit more difficult, unless the clinical diagnosis of NASH was simply incorrect or the underlying hypothesis about the relationship of IR expression to NASH is incomplete and patients with NASH might have different mechanisms of disease causation. These findings also could result from insensitivity of the method in patients with early disease, as suggested by the authors. The data presented do not allow us to make these distinctions.

The authors suggest that their staining results might be used to provide evidence for supporting one or the other cause of steatohepatitis in selected cases. Given the considerable number of cases that did not fit the profile expected, this is unlikely to find general acceptance unless immunohistochemical analysis can be demonstrated to be more specific than the clinical diagnoses used to establish the method. This is particularly true when using this technique to suggest that a case represents ASH rather than NASH because the pattern of expression in ASH is nonspecific.

Although the rationale for the decreased expression of IR in NASH has strong support, the specificity of decreased immunohistochemical expression of IR is unknown and requires more study. It is possible that some of the medications that cause NASH do so by inhibition of IR. There is evidence that other inflammatory conditions such as hepatitis C might have some effect on insulin resistance, which could, in theory, lead to down-regulation of IR receptors.[10] Therefore, evaluation of a larger set of steatohepatitis cases, including cases associated with medications or other metabolic diseases, would be necessary before assuming that decreased IR and increased PTP1B expression always indicate metabolic syndrome–associated NASH.

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Perhaps a more promising use for this technology would be to diagnose NASH as a cause of cirrhosis. It is estimated that at least 30% of cases of cryptogenic cirrhosis are secondary to NASH, although this often is difficult to prove because the histologic hallmarks of NASH often disappear after the development of cirrhosis, leaving a diagnosis based on exclusion of other known causes of cirrhosis, combined with a clinical picture of diabetes, obesity, or the metabolic syndrome.[7,11] If we could use immunohistochemical analysis for IR to bolster the likelihood of NASH causing cirrhosis, we perhaps would be more comfortable with the diagnosis. The authors confirmed this staining pattern of disease in patients with cirrhosis due to NASH; however, there continue to be false-positive and false-negative results in a significant proportion of cases (21% for clinically diagnosed ASH and 14% for clinically diagnosed NASH). In addition, the diagnosis of cryptogenic cirrhosis invokes a much larger differential diagnosis than does the distinction of NASH from ASH. Therefore, before concluding that this method would be useful to diagnose NASH-associated cirrhosis in the absence of steatohepatitis, one would need to see this method applied to a large number of cases of cirrhosis of differing causes. As noted, hepatitis C can be associated with insulin resistance, as can cirrhosis in general, although in neither case has the presence of IR on hepatocytes been evaluated directly by immunohistochemical analysis.[10,12]

Aside from these diagnostic considerations, there is a list of potentially very useful applications for this technology that might not require the degree of specificity needed for diagnosis. There are several promising therapeutic trials in NASH with medications such as metformin and the thiazolidinediones, which modulate insulin resistance.[13-15] If these drugs are confirmed to be clinically effective, the use of immunohistochemical analysis to support a diagnosis of NASH might become very important. Confirmation might avoid using these medications for patients unlikely to benefit from them. Use of this staining also might be very useful as part of these therapeutic trials. It may be that changes in the degree of IR or PTP1B expression might have some predictive value for the efficacy of therapy in these patients or for predicting which therapies might work in which patients. In theory, this staining might predict which patients with metabolic syndrome and steatosis without steatohepatitis might be at greater risk of the second hit, allowing treatment before chronic liver damage begins.

Finally, aside from the diagnostic issues raised, does this staining tell us anything useful about the pathogenesis of NASH? As noted by the authors, the answer probably is "not at the current time," because much of the change in the liver related to insulin resistance seems secondary to insulin resistance in peripheral tissues, and this staining pattern could simply represent an epiphenomenon. However, this article represents an important first step looking at the potential for applying practical molecular methods to the diagnosis and management of this very common and important condition.

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