Annals of Hepatology
The Official Journal of the Mexican Association of Hepatology, the Latin-American Association for Study of the Liver and the Canadian Association for the Study of the Liver

OPINIONS

March-April, Vol. 16 No. 2, 2017: 182-184

Perspectives on NASH Histology: Cellular Ballooning

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ABSTRACT

Interpretation of liver biopsy in NAFLD can be challenging to distinguish histological NASH from non-NASH fatty liver – a broad dichotomy which carries significant prognostic and therapeutic implications and underlies the utility of many non-invasive tests. There is usually a reasonable degree of inter-observer agreement for some key parameters like steatosis, inflammation and fibrosis staging. However, the assessment of cellular ballooning can be a stumbling block even for experienced observers. Below, we recount some aspects of the history of histological definitions in NASH and propose specific methods to more objectively identify cellular ballooning in routine biopsy assessments.

Disease histopathology constitutes a pillar of modern therapeutic medicine. It also provides the essential foundation for 'non-invasive' disease assessments which have become increasingly popular in various liver diseases including (perhaps especially so) the assessment of NASH or 'non-alcoholic steatohepatitis'. Together with Laboratory analysis and Radiological imaging, the histopathology of a potentially severe condition can tell us what we are up against and whether we need to intervene or not. Ultimately, histopathology defines the significance of a condition: how bad is it? Or… is it not bad at all? Should we undertake therapy or should we step back? This depends on the underlying histopathological diagnosis, the associated prognosis and the balance of risk and benefit of a given intervention. So, where does the histological grounding of NAFLD and NASH stand in 2017 in this regard?

Early studies in this field established the existence of a form of non-alcohol related chronic hepatitis characterized by fatty infiltration of the liver with inflammation, cellular injury evident by inflammation, cellular ballooning and fibrosis.[1] The histology was indistinguishable by conventional light microscopy from alcohol-related liver injury.[2] The condition could evolve over years to a form of cirrhosis with loss of its primordial histological hallmarks and, in the absence of an antecedent diagnosis, could only be classified as 'cryptogenic' cirrhosis.[3-5] However, these associations left something of a conundrum between the previously described 'benign' fatty liver with no significant long-term sequela[6] and another form that worsens to cirrhosis, decompensated liver disease and sometimes to hepatocellular cancer.

Art McCullough, *et al.* recognized that some forms of nonalcoholic fatty liver disease (NAFLD) were indeed fairly benign over time but others were more clinically significant – so-called 'Little NASH' and 'Big NASH'. They proposed four classes of 'NAFLD' - Class 1 and 2: steatosis alone or steatosis with only histological inflammation and classes 3 and 4 which were characterized as having either cellular ballooning or some degree of fibrosis.[7] These latter two groups were subsequently identified as what we know today as NASH. While use of the four histological classes has largely fallen away, this dichotomy of histological findings within the umbrella term 'NAFLD' – NASH versus non-NASH fatty liver (or what we refer to as 'NNFL') has endured over the years and carries prognostic significance.[8,9] It is notable that, although usually considered to be long term stable conditions, transition of NNFL to NASH over time has been reported.[10]

So where are the problem areas in histological interpretation of NASH or NNFL? Variation in fibrosis staging due to sampling error is well known but can be

Manuscript received: January 31, 2017. **Manuscript accepted:** January 31, 2017.

DOI:10.5604/16652681.1231550.

minimized with core lengths of at least 2 cm.[11] Less well known is the existence of variation in the identification of hepatocellular ballooning. Hepatocellular ballooning or 'balloon degeneration' in NASH is defined as rounded hepatocyte enlargement > 1.5 – 2 times the normal diameter with loss of the usual polygonal shape of the cell and usually containing pale staining cytoplasm, variably sized cytoplasmic vacuoles, and frequently Mallory Denk bodies. Using specialized stains, it is now known that these cells have significant destruction of the keratin cytoskeleton ('keratin empty cells'),[12] activated sonic hedgehog signaling[13] and accumulation of small to medium sized fat droplets with oxidized phospholipids and altered perilipin expression as well as dilation of the endoplasmic reticulum.[14] These characteristic cells have also been dubbed the 'undead' cells which are a maladapted source of noxious substances that promote their survival but amplify the local injury.[15]

Although there is little debate when these cells are abundant and markedly enlarged, it can be more challenging when there is a less striking presence on routine H&E staining resulting in a degree of observer dependent subjectivity.[16] This situation likely explains at least some of the inter-observer variation in NASH biopsy scoring since hepatocellular ballooning accounts for a significant portion of scoring systems like the NAFLD Activity Score (NAS)[17,18] and the Steatosis, Activity, and Fibrosis score (SAF).[19] This problem can also introduce variability in study results too where balloon scores serve as a target of treatment.[20]

A degree of uncertainty is inherent in many aspects of Medical Science and it seems to always grow as one parses an issue into ever more granular aspects and thus requires attention to minimize doubt. Distinguishing NASH from non-NASH fatty liver carries significant prognostic and therapeutic implications. Although the histological diagnosis of NASH (present or not present) isn't dependent on any single parameter,[21] detection of ballooning provides a more confident diagnosis. While not widely adopted, we suggest that the incorporation of stains such as keratin or sonic hedgehog into routine liver biopsy processing is warranted to more objectively and consistently identify NASH-related ballooning and more confidently establish the prognosis.

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