Histopathological Evaluation of Non-Alcoholic Fatty Liver Disease: Mini-Review of Literature

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in the industrialized world and will become one of the most important challenges to public health in the coming decades due to its hepatic and extrahepatic complications[1].

NAFLD is a result of metabolic syndrome in the liver, and its pathological processes include steatosis to steatohepatitis[2], which can progress to fibrosis, cirrhosis and hepatic carcinoma[3,4,5]. It is characterized by accumulation of fat in hepatocytes greater than 5% in hepatic tissue, in the absence of significant consumption of alcohol, drugs and viral hepatopathy[6]. It is associated with obesity, type 2 diabetes and dyslipidemia[7].

Insulin resistance and excessive accumulation of lipids are strongly associated with non-alcoholic fatty liver disease (NAFLD), which represents the hepatic manifestation of a systemic deficiency of the insulin network[8]. This pathogenesis is multifactorial and includes alterations in lipid metabolism, with aberrant accumulation of triglycerides, mitochondrial dysfunction, inflammation and oxidative stress[9].

Hepatic Steatosis

Non-alcoholic steatosis is the most common form of chronic liver disease and is characterized by accumulation of fat in hepatocytes[10].

The arbitrary threshold for treating steatosis as pathological is the presence of lipid droplets in at least 5% of hepatocytes. Steatosis can be classified into: macrovesicular, composed of large vacuoles that move the nucleus to the periphery of the cell; mid vesicular composed of small and large vacuoles; and microvesicular steatosis composed of innumerable small vacuoles with foamy appearance[11,12].
MATERIALS AND METHODS

In order to deepen the theme of the study, a review of the literature was carried out in order to answer the following question: Which scientific productions deal with the criteria of histological evaluation of the liver?

The literature search occurred in November and December 2017 in the databases PUBMED and MEDLINE.

For the selection of the sample, the criteria proposed by Moher et al. (2009) were divided into stages: identification, screening, eligibility and inclusion. Figure 1 shows the flowchart of the whole process of identification and selection of articles.

In the identification and screening, the adopted inclusion criteria were: be available at the electronic address, free of charge in full and be disclosed in English, Portuguese or Spanish. Thus, dissertations, theses, reports, news, letters to the editor and scientific articles were not available in full online and those that were repeated in the databases. The following keywords or descriptors were used in the Health Sciences Descriptors of the Virtual Health Library: fatty liver, histology, fibrosis.

For the eligibility of publications, each title and abstract was read exhaustively to confirm whether they addressed the guiding question of this research and whether it would meet the inclusion and exclusion criteria established. Then, it happened to the stage of inclusion of the articles. The selection of studies is shown in Table 1.

HISTOLOGICAL EVALUATION

Currently, liver biopsy, through histopathological evaluation, is still the gold standard for the diagnosis of NAFLD and the evaluation of its progression[14,15].

Histologic evaluation remains the only accurate means of assessing the degree of steatosis, necroinflammatory lesions, fibrosis lesions and non-alcoholic steatohepatitis (NASH), and serves as the primary means of distinguishing NASH from a “simple” steatosis, or steatosis with inflammation[16].

Brunt et al. (1999) proposed a semiquantitative evaluation system for the unique lesions recognized for NASH. The proposed system was based on the concept that the histological diagnosis of NASH is formed by a set of features rather than any individual characteristic. In this system the extent of steatosis can be classified according to the percentage of steatotic hepatocytes: mild, 0-33%; moderate, 33-66%; and severe, > 66%[17]. However, it was developed for NASH and was not developed to encompass the entire spectrum of NAFLD as defined by Matteoni et al. (1999).

From 2002, the NASH Clinical Research Network proposed to develop and validate a histological evaluation system, characterized by NAFLD Activity Score (NAS), which would cover the spectrum of NAFLD and could be applied to pediatric NAFLD, and this would allow the evaluation of changes with therapy. The histological characteristics were grouped into five broad categories: steatosis, inflammation, hepatocellular lesion, fibrosis and various characteristics. The evaluation system was divided into 4 grades, classified as 0 > 5%, 1-5% - 33%, 2-> 33% -66% and 3-> 66%[19]. This system was based on and further perfected Brunt et al’s (1999) classification proposal.

Several semiquantitative histological scoring systems were proposed to diagnose and classify NAFLD. Each one has certain advantages and some limitations. However, the recent scoring system - Steatosis, Activity, Fibrosis (SAF) algorithm proposed by Bedossa et al. (2012) based on the findings of the American Association for the Study of Liver Disease scored the same histological characteristics but with steatosis classified separately from the activity score (balloonization and lobular inflammation).

Few literature findings emphasize the evaluation of scores and their relevance in the diagnosis of hepatic steatosis by the SAF method. With this, the objective of this study was to evaluate the new diagnostic method of SAF hepatic steatosis by means of a review in the literature.

SAF HISTOPATHOLOGICAL SCORES

NAFLD is defined by the presence of steatosis in > 5% of hepatocytes; and NASH, by the presence, in addition, of balloonized hepatocytes of any degree and lobular inflammatory infiltrate of any amount[20]. Therefore, steatosis was used as input criteria to the algorithm weighted by hepatocellular balloonization and lobular inflammation.

The SAF score (steatosis, activity, fibrosis) is the usual method of evaluation in liver histological lesions. The steatosis score (S) was evaluated as the amount of lipid droplet, however without apparent foamy microvesicles, from 0 to 3 (S0: < 5%, S1: 5% -33%, light, S2: 34-66%, moderate; S3: > 67%). The degree of activity (A, from 0 to 4) was assessed by the presence of unweighted balloonized hepatocytes (0-2) and lobular inflammation (0-2). Cases with A0 (A = 0) had no activity, A1 (A = 1), mild activity, A2 (A = 2), moderate activity, A3 (A = 3) severe activity. The fibrosis stage (F) was evaluated using the NAS-CRN score; stage 0 (F0) (none); stage 1 (F1): 1a or 1b perisinusoidal zone 3 or 1c of portal fibrosis, stage 2 (F2): perisinusoidal and periportal fibrosis without bridge, stage 3 (F3): bridge fibrosis and stage 4 (F4): cirrhosis[21].

RESULTS AND DISCUSSION

Twelve articles were analyzed in this study. The articles were found in the Medline (02) and Pubmed (10) databases. Regarding the periodical and language, all were published in international magazines with English language.

As for the histopathological evaluation of the studies, one classified according to the percentage of steatotic hepatocytes (Brunt et al., 1999), eight articles performed the diagnosis of NAFLD according to the NAS score (Kleiner et al., 2005; Vajro et al. The results are shown in Table 1 and Table 2 for the results obtained by Dowman et al and the use of the SAF score (Bedossa et al, 2012, Singh et al,
The histopathological interpretation of the liver biopsy was described using the SAF score (steatosis, activity, fibrosis). The SAF score is the usual method in the liver, where the degree of steatosis was defined on sections. In 2005, the Pathology Committee of the NASH Clinical Research Network proposed to develop and validate a histological assessment system, characterized by the NAFLD Activity Score (NAS). Although this score is easy to understand and clearly separates the three lesions, it is a weighted sum of steatosis score, lobular inflammation and hepatocellular ballooning, but it does not include a parameter of fibrosis in NAS.

NAS without the inclusion of fibrosis may lose great reliability in the distinction of liver diseases. Based on the findings of the American Association for the Study of Liver Disease, Bedossa et al. (2012) proposed the histological algorithm of NASH SAF, which classifies active and chronic lesions separately, but together as SAF scores. They increased uncertainty about the inclusion of steatosis in scores because its harmful effect is not proven, further asserting that this leads to the discrepancy between the NAS and NASH diagnosis. To date, the NAFLD score remains controversial, with the need to assess the utility and limitations of current systems. Histopathological descriptions and feature-based diagnosis are arguably the most important, but the usefulness of scoring in clinical trials, comparative studies, and ease of understanding of these outcomes, for both pathologists and clinicians, can not be underestimated.

In addition, the fibrosis stage was independently associated with general long-term mortality, liver transplantation and liver-related events. The use of NAS without the inclusion of fibrosis would underestimate the presence of significant liver disease and has also been reported in other studies. These results suggest the need to include fibrosis in the total score or modify the final diagnosis.

Therefore, by describing the main histopathological characteristics in a practical way, the SAF score allows an easy comparison between biopsies and becomes able to deal with more precise diagnosis in any case.

CONCLUSION

Thus, the scoring system that uses the algorithm as the SAF score can provide practical tools for pathologists that would simplify the understanding of hepatic lesions by hepatologists.

Table 1 Selection of research articles in the PubMed and Medline databases, according to established inclusion criteria.

<table>
<thead>
<tr>
<th>Articles/Source</th>
<th>Medline</th>
<th>Pubmed</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Found</td>
<td>678</td>
<td>432</td>
<td>1110</td>
</tr>
<tr>
<td>Articles not related to the theme</td>
<td>621</td>
<td>412</td>
<td>1033</td>
</tr>
<tr>
<td>Repeated</td>
<td>35</td>
<td>12</td>
<td>47</td>
</tr>
<tr>
<td>Unavailable</td>
<td>20</td>
<td>5</td>
<td>25</td>
</tr>
<tr>
<td>Selected</td>
<td>2</td>
<td>10</td>
<td>12</td>
</tr>
</tbody>
</table>

Table 2 Characteristics of studies included in this study.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Title</th>
<th>Year of publication</th>
<th>Histological evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vajro et al. [16]</td>
<td>Diagnosis of nonalcoholic fatty liver disease in children and adolescents: position paper of the ESPGHAN Hepatology committee</td>
<td>2012</td>
<td>The recommendation of the use for histological evaluation comes from the association of several risk factors, combined with the NAFLD activity score (NAS).</td>
</tr>
<tr>
<td>Brunt et al. [17]</td>
<td>Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions</td>
<td>1999</td>
<td>With the inclusion of an extension to steatosis, it was classified according to the percentage of steatotic hepatocytes.</td>
</tr>
<tr>
<td>Kleiner et al. [19]</td>
<td>Design and validation of a histological scoring system for nonalcoholic fatty liver disease</td>
<td>2005</td>
<td>The NASH Clinical Research Network proposed to develop and validate a histological assessment system, characterized by the NAFLD Activity Score (NAS).</td>
</tr>
<tr>
<td>Bedossa et al. [20]</td>
<td>Histological algorithm and scoring system for evaluation of liver lesions in morbidly obese patients</td>
<td>2012</td>
<td>Based on the results of the American Association for the Study of Liver Disease scored the same histological characteristics but with steatosis classified separately from the score of the activity.</td>
</tr>
<tr>
<td>Singh et al. [22]</td>
<td>Fibrosis progression in nonalcoholic fatty liver vs nonalcoholic steatohepatitis: a systematic review and meta-analysis of paired-biopsy studies</td>
<td>2015</td>
<td>The SAF score (steatosis, activity, fibrosis) is the usual method of evaluation in liver histological lesions.</td>
</tr>
<tr>
<td>Kishida et al. [23]</td>
<td>Development of a novel mouse model of hepatocellular carcinoma with nonalcoholic steatohepatitis using a high-fat, choline-deficient diet and intraperitoneal injection of diethylnitrosamine</td>
<td>2016</td>
<td>Variables were blindly scored by two experienced hepatopathologists using a modified scoring system adapted from the NAFLD activity score (NAS).</td>
</tr>
<tr>
<td>McPherson et al. [24]</td>
<td>Evidence of NAFLD progression from steatosis to fibrosing-steratohepatitis using paired biopsies: implications for prognosis and clinical management.</td>
<td>2015</td>
<td>The NAFLD activity score (NAS).</td>
</tr>
<tr>
<td>Auberval et al. [25]</td>
<td>Metabolic and oxidative stress markers in Wistar rats after 2 months on a high-fat diet</td>
<td>2014</td>
<td>In the liver, the degree of steatosis was defined on sections according to Kleiner et al.</td>
</tr>
<tr>
<td>Mohamed et al. [26]</td>
<td>Circulating adipokines in children with nonalcoholic fatty liver disease: possible noninvasive diagnostic markers</td>
<td>2017</td>
<td>The histopathological interpretation of the liver biopsy was determined according to the NAFLD activity score (NAS).</td>
</tr>
<tr>
<td>Bedossa [27]</td>
<td>Pathology of non-alcoholic fatty liver disease.</td>
<td>2017</td>
<td>Describing histopathological lesions in NAFLD using the SAF.</td>
</tr>
<tr>
<td>Zhang et al. [28]</td>
<td>Effects of telmisartan on improving leptin resistance and inhibiting hepatic fibrosis in rats with non-alcoholic fatty liver disease</td>
<td>2017</td>
<td>The sections were then routinely analyzed for NAFLD activity score (NAS) and fibrosis score (FS) using the modified Chevallier semi-quantitative scoring system.</td>
</tr>
<tr>
<td>Dowman et al. [29]</td>
<td>Development of hepatocellular carcinoma in a murine model of nonalcoholic steatohepatitis induced by use of a high-fat/fructose diet and sedentary lifestyle.</td>
<td>2014</td>
<td>Each section was allocated a NAFLD activity score (NAS) through blinded assessment according to the Kleiner scoring system.</td>
</tr>
</tbody>
</table>
REFERENCES


30. Santiago-Rolón A. A Comparison of Brunt Criteria, the Non Alcoholic Fatty Liver Disease Activity Score (NAS) & a Proposed NAS-including fibrosis as Valid Diagnostic Scores for NAS. *Puerto Rico health sciences journal* 2015; 34: 189. [PMID: PMC4720965]

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