

Nonalcoholic fatty liver disease – an etiological approach

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Abstract: Nonalcoholic fatty liver disease (NAFLD) is defined as the presence of fat in the liver (hepatic steatosis) either on imaging or on liver histology only after the exclusion of secondary causes of fat accumulation in the liver (e.g. high alcohol drinking, drugs and other medical ailments). Considering the fact that there are many causes of hepatic steatosis, the term NAFLD is reserved for the liver disease that is predominantly associated with obesity and metabolic syndrome. The presence of inflammation and cell injury defines steatohepatitis (NASH) which has the potential to evolve into cirrhosis and hepatocarcinoma, being, therefore, the stage of NAFLD most amenable to treatment. Among the treatments available, the most important are: weight loss, vitamin E and, last but not least, probiotics.

INTRODUCTION

Excessive alcohol consumption must be excluded (>21 drinks per week in men and >14 drinks per week in women over a 2-year period before the baseline liver biopsy).

Insulin resistance (figure 1) is, therefore, central to the development of NAFLD, as it is central to metabolic syndrome (MS). The Adult Treatment Panel III defines MS as the presence of three or more of the following features:

1. Waist circumference greater than 102 cm in men or greater than 88 cm in women,
2. Triglyceride level greater than or equal to 150 mg/dL,
3. High-density lipoprotein cholesterol level less than 40 mg/dL in men and less than 50 mg/dL in women,
4. Systolic blood pressure greater than or equal to 130 mm Hg or a diastolic pressure greater than or equal to 85 mm Hg, and
5. Fasting plasma glucose level greater than or equal

to 110 mg/dL.

Patients with features of MS are at high risk for NAFLD.

The gold standard for the diagnosis of NAFLD is hepatic biopsy which further characterizes the ailment deriving two stages of histological evolution: nonalcoholic fatty liver (NAFL) (this may be the plain hepatic steatosis) and nonalcoholic steatohepatitis (NASH).

NAFL is defined as hepatic steatosis with no evidence of hepatocellular injury in the form of hepatocyte ballooning. NASH is defined as the presence of hepatic steatosis and inflammation with hepatocyte injury (ballooning) with or without fibrosis. Although NAFL may be proportionally more common than NASH,

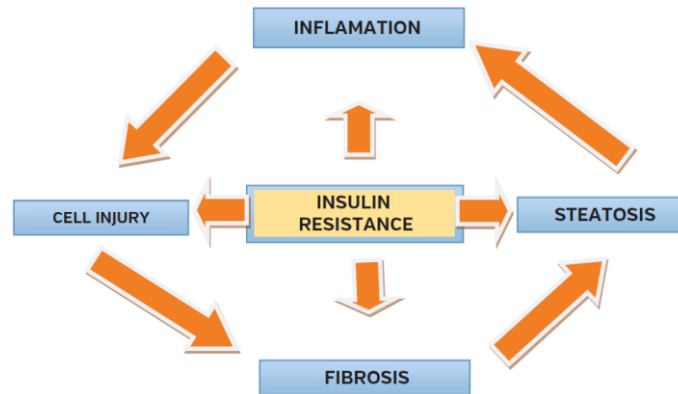
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only patients with NASH have the potential to progress to cirrhosis. The presence of the

characteristic ballooning injury is considered to be the key to the diagnosis.

Figure 1: Insulin resistance – central to metabolic syndrome



Ballooning injury results in enlarged vacuolated cells, classically containing Mallory-Denk bodies, which are eosinophilic cytoplasmic inclusions near the nucleus. The most important injury may be identified in zone 3 (around the central venule of the hepatic lobule) and this pattern of distribution is also characteristic of NAFLD.

The cardinal histologic feature of NAFLD is the presence of an excessive accumulation of triacylglycerols (TAG) and in hepatocytes. The presence of obesity and insulin resistance lead to an increased hepatic free fatty acid (FFA) flux creating an environment appropriate for the development of NAFLD/NASH.

The resultant net increase in hepatic FFA is hepatotoxic unless it is converted to nontoxic intracellular triglyceride (TG). When the synthesis of TG is impaired, the level of FFA in the liver is increased with subsequent augmentation of hepatic fatty acid oxidation resulting in the overproduction of reactive oxygen species (ROS) also known as free radicals causing hepatocellular injury.

Based on this biochemical knowledge, a two-hit hypothesis for the pathogenesis of NASH has been proposed. The first hit involves the accumulation of excess triglyceride and particularly FFA in hepatocytes. The second hit is the generation of toxic reactive oxygen species with the production of hepatic injury and inflammation as a consequence of FFA oxidation which ultimately leads

to the initiation and progression of fibrosis [1].

Hence, steatosis is mandatory for the diagnosis of NAFLD but alcohol consumption and chronic hepatitis C should be taken into account as two of the most important alternative causes amenable to different treatments.

The diagnosis of NAFLD requires the following:

- (1) Hepatic steatosis according to imaging or histology
 - (2) No significant alcohol consumption
 - (3) No competing etiologies for hepatic steatosis (table 1)
- and
- (4) No coexisting causes for chronic liver disease.

NAFLD is the most common cause of abnormal liver chemistry, so other causes, like those in table 1, should be ruled out. The majority of patients with NAFLD are asymptomatic. The most frequently encountered symptoms are: vague right upper quadrant dull ache or discomfort. Hepatomegaly is the most common physical finding. Other clinical symptoms and physical findings are, also, nonspecific: general malaise, abdominal discomfort, nausea.

Celiac disease always should be ruled out in suspected individuals considering the fact that this disease is often underdiagnosed and seldom to be taken into account as a differential diagnosis of hypertransaminasemia.

Table 1. Conditions associated with the risk of hepatic steatosis

STEATOSIS
<ul style="list-style-type: none"> • Insulin resistance • Obesity • Type 2 diabetes mellitus • Dyslipidemia • Hypertension • Sedentary lifestyle • Corticosteroids • Estrogens • Amiodarone • Antiretroviral medications • Obesity surgery (e.g., jejunioileal bypass) • Rapid weight loss • Carbohydrate excess (e.g., diet and total parenteral nutrition) • Chronic hepatitis C virus, mainly genotype 3 • Hypothyroidism • Polycystic ovarian syndrome

Once NAFLD is diagnosed, the next step is to determine the severity as it is necessary to establish the prognosis. Clinical examinations and laboratory and imaging studies in combination lack the sensitivity and specificity for distinguishing NAFL from NASH and for determining the presence and stage of fibrosis, which is the most important determinant for the severity and progression of disease.

Circulating levels of cytokeratin 18 fragments have been investigated extensively as novel biomarkers for the presence of steatohepatitis in patients with NAFLD, but this testing is not routinely recommended. Other noninvasive tests are emerging; however, these are not yet ready for prime time. [1,2, 3]

Liver biopsy still remains the most reliable approach for identifying the presence of steatohepatitis and fibrosis in patients with NAFLD.

The recommendations for liver biopsy areas follows (figure 2):

1. Patients at increased risk for steatohepatitis and advanced fibrosis according to the presence of features of MS and possibly the NAFLD fibrosis score.
2. Patients with suspected NAFLD for whom competing etiologies of hepatic steatosis and coexisting chronic liver diseases cannot be excluded without liver biopsy.

There is a general consensus that patients with NAFL

have a very slow progression (if any). On the other hand, patients with NASH can exhibit histological progression and can develop fibrosis (37%-41%) and cirrhosis (Approximately 5%) [3]. Importantly, hepatic cancer can occur in NASH in the absence of cirrhosis. This is why every effort should be made to identify patients with NASH as they are the ones to progress to more severe forms of disease. The presence of NASH can be associated with higher liver-specific mortality in comparison with the general population. Cardiovascular ailments associated with NASH (as metabolic syndrome) contribute significantly to mortality and morbidity. Patients with NAFLD are also at an increased risk for hepatocellular carcinoma, but this risk is likely limited to those with advanced fibrosis and cirrhosis (1%-42%) [2]. Furthermore, a comparison of the natural history of NASH cirrhosis with hepatitis C cirrhosis reveals that patients with NASH cirrhosis have a significantly lower risk of hepatocellular carcinoma. [2]

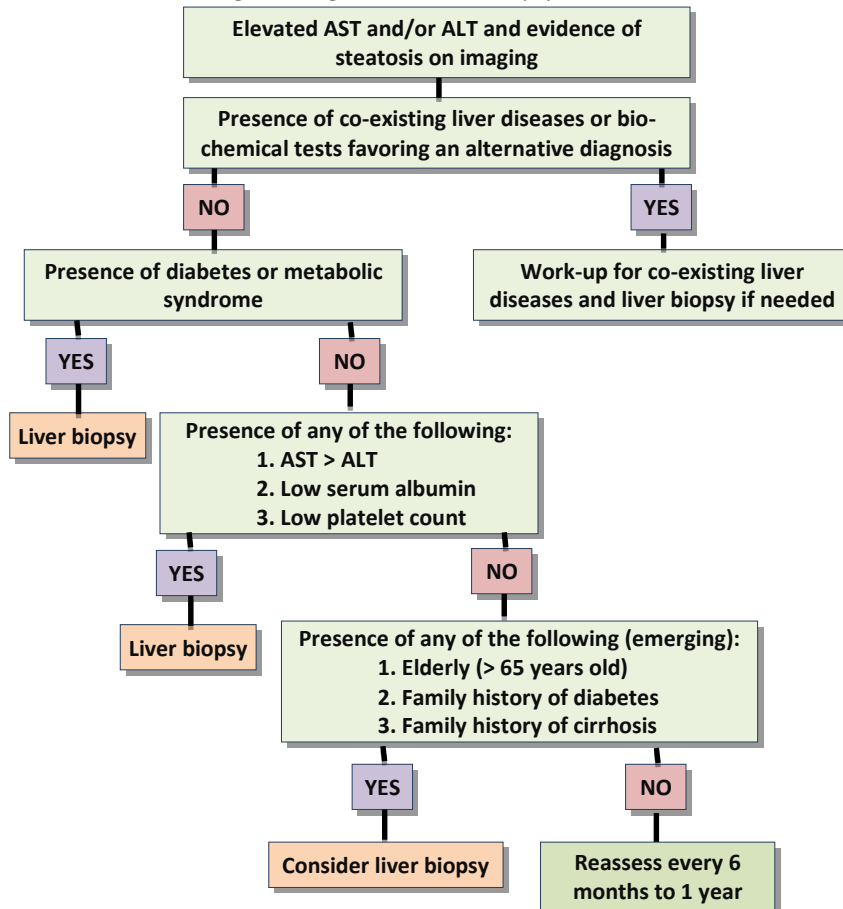
NAFLD is typically characterized by a hepatocellular pattern of liver-related enzymes with mild elevations (1-2 times the upper limit of normal) in serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Up to 50% of NAFLD patients have normal liver biochemistry. Therefore, several biomarkers may aid in the diagnosis. The diagnosis of NASH without a liver biopsy remains the most significant clinical challenge in the evaluation of a patient with hepatic steatosis. Several biomarkers may distinguish between simple steatosis and NASH. Some of the inflammatory markers include serum C-reactive protein, interleukin-6, ferritin, hyaluronic acid (HA), tumor necrosis factor α , leptin, adiponectin, and resistin.

Apoptosis plays a key role in the pathogenesis of NASH. Among the markers of apoptosis, plasma cytokeratin 18 (CK-18) is emerging as one of the promising biomarkers for the noninvasive detection of NASH. Since oxidative stress also plays an important role in the pathogenesis of NASH, several biomarkers of oxidative stress have been investigated. Among these, oxidized low-density lipoprotein, thiobarbituric acid reacting substances, superoxide dismutase, and glutathione

peroxidase dismutase have been examined. The NASH test combined 13 variables [age, sex, height, weight, and serum levels of triglycerides (TGs), cholesterol, a2-macroglobulin, apolipoprotein A1,

haptoglobin, gamma-glutamyl transpeptidase (GGT), ALT, AST, and total bilirubin] to achieve positive predictive value, and negative predictive value of 66%, and 81%, respectively. [2, 3, 4]

Figure 2: Algorithm for liver biopsy in NAFLD



Two of the most promising tests for diagnosing advanced fibrosis in NAFLD are the European Liver Fibrosis (ELF) score and the NAFLD fibrosis score. The ELF score includes HA, tissue inhibitor of metalloproteinase 1 (TIMP1), aminoterminal peptide of procollagen 3, and age. The NAFLD fibrosis score is helpful in the clinical setting because it uses routinely available variables in the clinical setting, including age, BMI, hyperglycemia, platelet count, serum albumin, and AST/ALT ratio. We use routinely available models or markers that increase the pretest likelihood of finding more advanced liver disease on liver biopsy. These tests can aid in clinical decision making for patients with NAFLD.

Some of these markers are a high AST/ALT ratio,

high AST/platelet ratio, low albumin levels, and low platelet levels. [2, 3]

TREATMENT LANDMARKS

Among patients suffering from NAFLD (more than 50% of them being asymptomatic) treatment is mandatory only in NASH patients because only those have the potential to evolve into more severe diseases (cirrhosis, hepatocarcinoma) [5].

Because NASH is linked to excess body weight and resulting insulin resistance, diet and lifestyle measures are the recommended first-line therapy.

Optimal treatment begins with weight loss and physical exercise. A tangible target for

patients with NAFLD is a weight loss of 5% to 10% of total body weight over a 6- to 12-month period [6]. Those measures may improve insulin sensitivity, increase adiponectin expression, lipid profiles, and liver biochemistry. The improvement in liver enzymes does not always correlate with improvement in hepatic histology, unfortunately. As it is already demonstrated, weight loss by dietary changes may be beneficial even without physical exercise; although physical exercise may lead to further improvement in insulin sensitivity. The initiation of increased physical activity must be the first step to the treatment of NAFLD; vigorous exercise and resistance training are more helpful than aerobic exercises. The intensity may be more important than the duration or total volume of exercise. [7,8, 9]

Current guidelines do not recommend the use of hepatic pharmacological therapy in patients with steatosis alone. Instead, patients with NASH and significant liver disease (bridging fibrosis) are good candidates for this type of therapy. According to the clinical practice guidelines of the American Association for the Study of Liver Diseases, the first choice of therapy is vitamin E (preferably 800 IU/day). A 2-year treatment in the PIVENS trial (800 IU/day) reversed steatohepatitis and improved all histological features of NASH (except fibrosis) in comparison with a placebo. This beneficial effect of vitamin E was not associated with an improvement in insulin sensitivity. Recent studies and meta-analysis showed increased mortality, a risk of hemorrhagic stroke, and a risk of prostate in long term vitamin E treatments. [10, 11]

Animal studies have shown that omega-3 polyunsaturated fatty acids promote insulin sensitivity, reduce intrahepatic triglyceride content, and ameliorate steatohepatitis. [10, 11]

The drugs to increase insulin sensitivity (glitazone, metformin) may be indicated as a treatment alternative in NASH [16]. Ursodesoxycholic acid and pentoxifylin, which may benefit marginally. [6]

Probiotics and NAFLD

The newest topic in treatment of NAFLD is that of the involvement of gut microbiota in the pathogenesis of liver steatosis and inflammation.

Intestinal microbiota plays an important role in health and disease. The gut-liver axis involves an interaction between bacterial components like lipopolysaccharide and hepatic receptors (Toll-like receptors). Our gut has approximately 100 trillion (10¹⁴) microbes, which make up approximately 1 to 2 kilograms of our weight. Gut microbiota perform diverse immunologic, digestive, and metabolic functions. [11]

Changes in microbiota may be involved in various disease pathogenesis (nonalcoholic fatty liver disease (NAFLD), hepatic encephalopathy, alcohol-related liver disease, and hepatocellular carcinoma). Gut microbiota may cause NAFLD by luminal ethanol production by metabolism of carbohydrates, causing an increased intestinal permeability ("leaky gut") just like in alcohol-associated steatohepatitis (ASH). [12, 13]

In 2009 Miele was the first author to provide evidence of increased intestinal permeability in patients suffering from NAFLD and this fact was associated with increased prevalence of small bowel bacterial overgrowth (SIBO) in those patients [14]. The increased permeability appears to play an important role in the pathogenesis of NAFLD. Loguercio demonstrated in 2005 that probiotics may improve NAFLD histology and biochemistry [15]. In October 2013 Yan-Yan Ma et al published a meta-analysis in World Journal of Gastroenterology to conclude that the treatment with probiotics and prebiotics may definitely benefit patients with NASH [11].

Probiotics can inhibit the proliferation of harmful bacteria, reduce SIBO, restore gastrointestinal barrier function and modulate the immune system, all of which contribute to the improvement of NAFLD. This meta-analysis showed that probiotics significantly reduced ALT, AST, T-chol, TNF- α and insulin resistance, which are all related to the process and consequences of NAFLD. Regular consumption of probiotics reduced, also,

cholesterol levels which is part of metabolic disturbances in NAFLD patients. [11]

A high fat diet that induces obesity, insulin resistance and hepatic steatosis also leads to hepatic NKT cell depletion. The hepatic NKT cell is the key mediator of HF diet-induced metabolic abnormalities. Moreover, recently, Cani and colleagues reported that a high-fat diet increases plasma lipopolysaccharide (LPS) level, which also contributes to the pathogenesis of insulin resistance and increased liver triglyceride content. It is possible that this bacterial endotoxemia caused by high fat diet reduces intrahepatic NKT cells and leads to worsened or amplified insulin resistance. The ability of probiotics to restore hepatic NKT cells and improve HF diet-induced insulin resistance and fatty liver are novel findings and intriguing. [12, 13]

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CONCLUSIONS

NAFLD/NASH is a prevalent health problem in general population in close proximity with the same increased rate of obesity, diabetes mellitus and metabolic syndrome to which it is pathogenically related. Proper management of insulin resistance by diet, weight loss and physical exercise may provide the patients with strong tools to fight the disease.

The increasing evidence of the role of gut microbiota in disease pathogenesis and the role of probiotics in decreasing hepatic steatosis and inflammation points firmly towards new and handy solutions in the nearest future.

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