CLINICAL PRACTICE

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Nonalcoholic fatty liverdisease – an etiologicalapproach

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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 beexcluded (>21 drinks per week in menand >14 drinks per week in women overa 2-year period before the baseline liverbiopsy).

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

1. Waist circumference greater than 102 cm inmen or greater than 88 cm in women,
2. Triglyceride level greater than or equal to 150mg/dL,
3. High-density lipoprotein cholesterol level lessthan 40 mg/dL in men and less than 50 mg/dLin women,
4. Systolic blood pressure greater than or equalto 130 mm Hg or a diastolic pressure greaterthan 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 forNAFLD.

The gold standard for the diagnosis of NAFLDis hepatic biopsy which further characterizesthe ailment deriving two stages of histologicalevolution: nonalcoholic fatty liver (NAFL) (thismay be the plain hepatic steatosis) and nonalcoholicsteatohepatitis (NASH).

NAFL is defined as hepaticsteatosis with no evidence of hepatocellular injuryin the form of hepatocyte ballooning. NASH isdefined as the presence of hepatic steatosis andinflammation with hepatocyte injury (ballooning)with or without fibrosis. Although NAFL may beproportionally more common than NASH,

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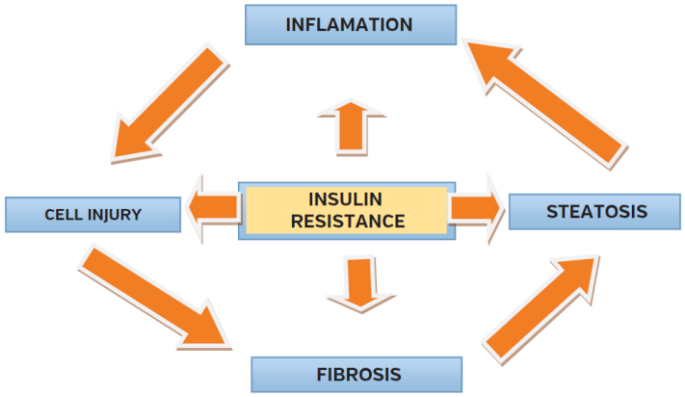
onlypatients with NASH have the potential to progressto cirrhosis. The presence of the characteristicballooning injury is considered to be the key tothe diagnosis.

Figure 1: Insulin resistance – central to metabolic syndrome



Ballooning injury results in enlargedvacuolated cells, classically containing Mallory-Denk bodies, which are eosinophilic cytoplasmicinclusions near the nucleus. The most importantinjury may be identified in zone 3 (around thecentral venule of the hepatic lobule) and this patternof distribution is also characteristic of NAFLD.

Thecardinal histologic feature of NAFLD is the presenceof an excessive accumulation of triacylglycerols(TAG) and in hepatocytes. The presence of obesityand insulin resistance lead to an increased hepaticfreefatty acid (FFA) flux creating an environmentappropriate for the development of NAFLD/NASH.

The resultant net increase in hepatic FFAis hepatotoxic unless it is converted to nontoxicintracellular triglyceride (TG). When the synthesisof TG is impaired, the level of FFA in the liver isincreased with subsequent augmentation of hepaticfatty acid oxidation resulting in the overproductionof reactive oxygen species (ROS) also known as freeradicals causing hepatocellular injury.

Based on this biochemical knowledge, a two-hithypothesis for the pathogenesis of NASH has beenproposed. The first hit involves the accumulationof excess triglyceride and particularly FFA inhepatocytes. The second hit is the generation oftoxic reactive oxygen species with the production ofhepatic injury and inflammation as a consequenceof FFA oxidation which ultimately leads to theinitiation and progression of fibrosis [1].

Hence, steatosis is mandatory for the diagnosisof NAFLD but alcohol consumption and chronichepatitis C should be taken into account as two ofthe most important alternative causes amenable todifferent 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 abnormalliver chemistry, so other causes, like those in table 1,should be ruled out. The majority of patients withNAFLD are asymptomatic. The most frequentlyencountered symptoms are: vague right upperquadrant dull ache or discomfort. Hepatomegaly isthe most common physical finding. Other clinicalsymptoms and physical findings are, also, nonspecific:general malaise, abdominal discomfort, nausea.

Celiac disease always should be ruled out insuspected individuals considering the fact that thisdisease is often underdiagnosed and seldom to betaken into account as a differential diagnosis ofhipertransaminasemia.

Table 1. Conditions associated with the risk of hepatic steatosis

STEATOSIS
• Insulin resistance
• Obesity
• Type 2 diabetes mellitus
• Dyslipidemia
• Hypertension
• Sedentary lifestyle
• Corticosteroids
• Estrogens
• Amiodarone
• Antiretroviral medications
• Obesity surgery (e.g., jejunoilealbypass)
• Rapid weight loss
• Carbohydrate excess (e.g., diet andtotal parenteral nutrition)
• Chronic hepatitis C virus, mainlygenotype 3
• Hypothyroidism
• Polycystic ovarian syndrome

Once NAFLD is diagnosed, the next step is todetermine the severity as it is necessary to establish theprognosis. Clinical examinations and laboratory andimaging studies in combination lack the sensitivityand specificity for distinguishing NAFL from NASHand for determining the presence and stage offibrosis, which is the most important determinant forthe severity and progression of disease.

Circulating levels of cytokeratin 18 fragmentshave been investigated extensively as novelbiomarkers for the presence of steatohepatitisin patients with NAFLD, but this testing is notroutinely recommended. Other noninvasive testsare emerging; however, these are not yet ready forprime time. [1,2, 3]

Liver biopsy still remains the most reliable approachfor identifying the presence of steatohepatitisand fibrosis in patients with NAFLD.

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

1. Patients at increased risk for steatohepatitisand advanced fibrosis according to the presenceof features of MS and possibly the NAFLD fibrosisscore.
2. Patients with suspected NAFLD for whomcompeting etiologies of hepatic steatosis andcoexisting chronic liver diseases cannot be excludedwithout liver biopsy.

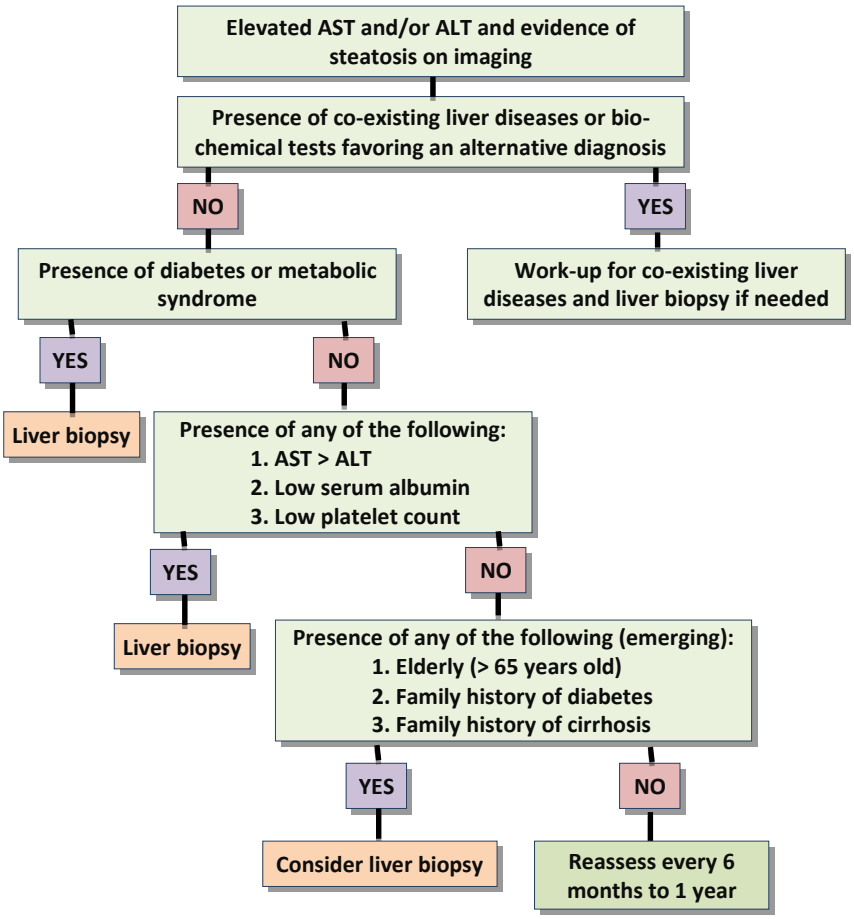
There is a general consensus that patients withNAFL have a very slow progression (if any). Onthe other hand, patients with NASH can exhibithistological progression and can develop fibrosis(37%-41%) and cirrhosis (Approximately 5%) [3].Importantly, hepatic cancer can occur in NASH inthe absence of cirrhosis. This is why every effortshould be made to identify patients with NASH asthey are the ones to progress to more severe formsof disease. The presence of NASH can be associatedwith higher liver-specific mortality in comparisonwith the general population. Cardiovascularailments associated with NASH (as metabolicsyndrome) contribute significantly to mortalityand morbidity. Patients with NAFLD are also atincreased risk for hepatocellular carcinoma, butthis risk is likely limited to those with advancedfibrosis and cirrhosis (1%-42%) [2]. Furthermore, acomparison of the natural history of NASH cirrhosiswith hepatitis C cirrhosis reveals that patients withNASH cirrhosis have a significantly lower risk ofhepatocellular carcinoma. [2]

NAFLD is typically characterized by ahepatocellular pattern of liver-related enzymeswith mild elevations (1-2 times the upper limit ofnormal) in serum alanine aminotransferase (ALT)and aspartate amino-transferase (AST). Up to 50%of NAFLD patients have normal liver biochemistry.Therefore, several biomarkers may aid in thediagnosis. The diagnosis of NASH without a liverbiopsy remains the most significant clinical challengein the evaluation of a patient with hepatic steatosis.Several biomarkers may distinguish between simplesteatosis and NASH. Some of the inflammatorymarkers include serum C-reactive protein,interleukin-6, ferritin, hyaluronic acid (HA), tumornecrosis factor a, leptin, adiponectin, and resistin.

Apoptosis plays a key role in the pathogenesis ofNASH. Among the markers of apoptosis, plasmacytokeratin 18 (CK-18) is emerging as one ofthe promising biomarkers for the noninvasivedetection of NASH. Since oxidative stress also playsan important role in the pathogenesis of NASH,several biomarkers of oxidative stress have beeninvestigated. Among these, oxidized low-densitylipoprotein, thiobarbituric acidreacting substances,superoxide dismutase, and glutathione

peroxidasedismutase have been examined. The NASH testcombined 13 variables [age, sex, height, weight, andserum levels of triglycerides (TGs), cholesterol, a2-macroglobulin, apolipoprotein A1, haptoglobin,gammaglutamyl transpeptidase (GGT), ALT, AST,and total bilirubin] to achieve positive predictivevalue, and negative predictive value of 66%, and81%, respectively. [2, 3, 4]

Figure 2: Algorithm for liver biopsy in NAFLD



Two of the most promising tests for diagnosingadvanced fibrosis in NAFLD are the EuropeanLiver Fibrosis (ELF) score and the NAFLD fibrosisscore. The ELF score includes HA, tissue inhibitorof metalloproteinase 1 (TIMP1), aminoterminalpeptide of procollagen 3, and age. The NAFLDfibrosis score is helpful in the clinical setting becauseit uses routinely available variables in the clinicalsetting, including age, BMI, hyperglycemia, plateletcount, serum albumin, and AST/ALT ratio. We useroutinely available models or markers that increasethe pretest likelihood of finding more advancedliver disease on liver biopsy. These tests can aid inclinical decision making for patients with NAFLD.

Some of these markers are a high AST/ALT ratio, ahigh AST/platelet ratio, low albumin levels, and lowplatelet levels. [2, 3]

TREATMENT LANDMARKS

Among patients suffering from NAFLD (morethan 50% of them being asymptomatic) treatmentis mandatory only in NASH patients because onlythose have the potential to evolve into more severediseases (cirrhosis, hepatocarcinoma) [5].

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

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

patientswith NAFLD is a weight loss of 5% to 10% of totalbody weight over a 6- to 12-month period [6]. Thosemeasures may improve insulin sensitivity, increaseadiponectin expression, lipid profiles, and liverbiochemistry. The improvement in liver enzymedoes not always correlate with improvement inhepatic histology, unfortunately. As it is alreadydemonstrated, weight loss by dietary changesmay be beneficial even without physical exercise;although physical exercise may lead to furtherimprovement in insulin sensitivity. The initiationof increased physical activity must be the first stepto the treatment of NAFLD; vigorous exercise andresistance training are more helpful than aerobicexercises. The intensity may be more important thanthe duration or total volume of exercise. [7,8, 9]

Current guidelines do not recommend theuse of hepaticopharmacological therapy in patientswith steatosis alone. Instead, patients with NASHand significant liver disease (bridging fibrosis) aregood candidates for this type of therapy. Accordingto the clinical practice guidelines of the AmericanAssociation for the Study of Liver Diseases, the firstchoice of therapy is vitamin E (preferably 800 IU/day). A 2-year treatment in the PIVENS trial (800IU/day) reversed steatohepatitis and improved allhistological features of NASH (except fibrosis) incomparison with a placebo. This beneficial effect ofvitamin E was not associated with an improvement ininsulin sensitivity. Recent studies and meta-analysesshowed increased mortality, a risk of hemorrhagicstroke, and a risk of prostate in long term vitamin Etreatments. [10, 11]

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

The drugs to increase insulin sensitivity (glitazone,metformin) may be indicated as a treatmentalternative in NASH 16. Ursodesoxicholic acid andpentoxifilin, which may benefit marginally. [6]

Probiotics and NAFLD

The newest topic in treatment of NAFLD isthat of the involvement of gut microbiota in thepathogenesis of liver steatosis and inflammation.

Intestinal microbiota plays an important rolein health and disease. The gut-liver axis involvesan interaction between bacterial components likelipopolysaccha-ride and hepatic receptors (Toll-likereceptors). Our gut has approximately 100 trillion(1014) microbes, which make up approximately 1 to2 kilograms of our weight. Gut microbiota performdiverse immunologic, digestive, and metabolicfunctions. [11]

Changes in microbiota may be involved invarious disease pathogenesis (nonalcoholic fattyliver disease (NAFLD), hepatic encephalopathy,alcohol-related liver disease, and hepatocellularcarcinoma). Gut microbiota may cause NAFLDby luminal ethanol production by metabolizationof carbohydrates, cau-sing an increased intestinalpermeability (“leaky gut”) just like in alcoholassociated steatohepatitis (ASH). [12, 13]

In 2009 Miele was the first author to provideevidence of increased intestinal permeability inpatients suffering from NAFLD and this fact wasassociated with increased prevalence of small bowelbacterial overgrowth (SIBO) in those patients [14]. Theincreased permeability appears to play an impor-tantrole in the pathogenesis of NAFLD. Loguerciodemonstrated in 2005 that probiotics may improveNAFLD histology and biochemistry [15]. In October2013 Yan-Yan Ma et al published a meta-analysisin World Journal of Gastroenterology to concludethat the treatment with probiotics and prebioticsmay definitely benefit patients with NASH [11].

Probiotics can inhibit the proliferation of harmfulbacteria, reduce SIBO, restore gastrointestinalbarrier function and modulate the immune system,all of which contribute to the improvement ofNAFLD. This meta-analysis showed that probioticssignificantly reduced ALT, AST, T-chol, TNF-αand insulin resis-tance, which are all related to theprocess and con-sequences of NAFLD. Regularconsumption of probi-otics reduced, also,

cholesterollevels which is part of metabolic disturbances inNAFLD patients. [11]

A high fat diet that induces obesity, insulinresistance and hepatic steatosis also leads tohepatic NKT cell depletion. The hepatic NKT cellis the key mediator of HF diet-induced metabolicabnormalities. Moreover, recently, Cani andcolleagues reported that a high-fat diet increasesplasma lipopolysaccharide (LPS) level, which alsocontributes to the pathogenesis of insulin resistanceand increased liver triglyceride content. It is possiblethat this bacterial endotoxinemia caused by highfat diet reduces intrahepatic NKT cells and leadsto worsened or amplified insulin resistance. Theability of probiotics to restore hepatic NKT cellsand improve HF diet-induced insulin resistance andfatty liver are novel findings and intriguing. [12, 13]

These data suggest that strategies designedto down regulate inflammatory mediators withprobiotics have promising potential in patients withNAFLD.

CONCLUSIONS

NAFLD/NASH is a prevalent health problemin general population in close proximity with thesame increased rate of obesity, diabetus mellitus andmetabolic syndrome to which it is pathogenicallyrelated. Proper management of insulin resistance bydiet, weight loss and physical exercise may providethe patients with strong tools to fight the disease.

The increasing evidence of the role of gut microbiotain disease pathogenesis and the role of probioticsin decreasing hepatic steatosis and inflammationpoints firmly towards new and handy solutions inthe nearest future.

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