Non-Alcoholic Fatty Liver Disease: What We Need to Know

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ABSTRACT

Nonalcoholic fatty liver disease (NAFLD) represents the accumulation of lipids in the hepatocytes, lipids represented by triglycerides (most frequently), cholesterol, phospholipids, and sphingolipids. NAFLD is rapidly becoming one of the most important causes of chronic liver disease, liver cirrhosis and liver transplantation in developed countries. It can present as simple steatosis, which is usually a benign condition, to steatohepatitis, which may lead to advanced fibrosis and cirrhosis. Although there are many risk factors for the development of fatty liver disease, a central role is played by metabolic disorders: obesity, diabetes mellitus and dyslipidemia. The common mechanism in the development of NAFLD is represented by the accumulation of triglycerides in the liver. The accumulation of lipids in the liver is secondary to an increased dietary intake of lipids and fatty acids or to a decrease of fatty acid beta-oxidation, and to their increased synthesis in the hepatic mitochondria. The therapeutic approach to patients with NAFLD depends on the type of disease. Patients with simple steatosis only require surveillance of the liver disease and treatment of comorbidities (hypolipemians, antihypertensive medication, antidiabetics). On the other hand, life-style changes, weight loss and pharmacological treatment are recommended in patients with steatohepatitis. Ursodeoxycholic acid, vitamin E and antidiabetic agents have been shown to improve biological and histological parameters in these patients. Many drugs (such as elafibranor, a dual agonist of the PPAR α/δ receptor) are currently being investigated in clinical trials for the treatment of patients with NASH, and are expected to be on the market in the near future. In conclusion, as the incidence and prevalence of NAFLD increase, it is important to identify the patients at risk of developing advanced fibrosis and cirrhosis.

Key words: non-alcoholic fatty liver disease, steatosis, steatohepatitis, hepatic inflammation, fibrosis, cirrhosis.

Nonalcoholic fatty liver disease (NAFLD) represents the accumulation of lipids in the hepatocytes, lipids represented by triglycerides (most frequently), cholesterol, phospholipids, and sphingolipids. NAFLD, a term introduced in 1986, is defined as the sum of the hepatic steatotic changes (lobular hepatitis), and the absence of alcohol consumption. NAFLD is characterized by the presence of fatty infiltration of the liver (over 5% of liver weight), involving >5% of hepatocytes.

Liver steatosis may determine a variety of conditions, from accumulation of fat with moderate inflammation (classes 1 and 2) to severe steatohepatitis

Received: 06.03.2019

Accepted: 04.04.2019

(classes 3 and 4) (NASH-nonalcoholic steatohepatitis), that can progress to liver cirrhosis.

Simple steatosis is a type of fatty infiltration, with no or minimal inflammation, and without fibrosis. On the other hand, NASH (nonalcoholic steatohepatitis) represents NAFLD with inflammation and fibrosis, which usually has periportal distribution, and which may progress to cirrhosis.

Epidemiology

In Western populations, the prevalence of NASH is 7-9%, and in Japan 1.2%, while alcoholic steatosis is 10-15 times more frequent. Most cases of NAFLD appear in the 5th-6th decade, and are two times more frequent in men (1).

Etiology

Obesity

Obesity is frequently associated with liver steatosis, 69% to 100% of NAFLD patients being obese; NAFLD also occurs in obese subjects who have undergone bariatric surgery (2). Obesity is associated with inhibition of fatty acid oxidation and with hyperinsulinism, and is subsequently complicated by liver fibrosis. In subjects with BMI>30, liver steatosis occurs in 85% of cases, liver fibrosis in 30% of cases, and liver cirrhosis in 1% (3).

Diabetes mellitus

Type 1 diabetes mellitus is associated with liver steatosis in 20% of subjects with controlled diabetes, while 50% of deceased diabetics presented liver steatosis on biopsy. For type 2 diabetes mellitus, liver steatosis is described in 75% of those with obesity and dyslipidemia (4).

Dyslipidemia

Dyslipidemia (hypertriglyceridemia and hypercholesterolemia) is associated with liver steatosis in 20-80% of cases.

Jejuno-ileal by-pass

Jejuno-ileal by-pass, performed for the treatment of obesity, is complicated by liver steatosis 3-6 months after the intervention in 70% of subjects, while 26% associate various degrees of fibrosis; this technique has been abandoned.

Parenteral nutrition

Parenteral nutrition may determine liver steatosis by increasing the intake of carbohydrates and lipids (5); it is accompanied by an increase of aminotransferase, alkaline phosphatase and bilirubin levels. Hepatic lesions are initially perilobular and centrolobular, and liver steatosis is reversible in this case (6).

Protein-caloric malnutrition

Protein-caloric malnutrition is complicated by liver steatosis due to a severe caloric deficit, accompanied by defects of triglyceride excretion in the plasma, while lipids accumulate in the liver under the form of triglycerides.

Inflammatory bowel

Inflammatory bowel disease is frequently accompanied by liver steatosis (45% of those with ulcerative colitis).

Cushing disease

Cushing disease is associated with macrovesicular steatosis. Hypertyroidism determines centrolobular steatosis, while hypothyroidism is associated with moderate liver steatosis.

Corticosteroids

Corticosteroids may determine the development of liver steatosis, which is not always dose-dependent. Toxic or drug-induced hepatitis can be accompanied by steatosis associated with hepatocellular necrosis.

Genetic conditions

Genetic conditions can also be accompanied by liver steatosis: hyperlipemia, abetalipoproteinemia, type I glycogenosis, galactosemia, fructosemia, tyrosinemia, Wilson's disease, and may all progress to liver cirrhosis.

Congenital metabolic anomalies

Wolman disease (storage of esterified cholesterol), Farber disease (storage of ceramide), Nieman-Pick disease (storage of sphingomyelin), Tay-Sachs disease (storage of ganglioside), Gaucher disease (storage of glucocerebroside) – are all associated with liver steatosis.

Rare causes of liver steatosis can be cardiogenic shock, heart failure, regeneration after liver resection, systemic lupus erythematosus (secondary to corticotherapy), tuberculosis, radiotherapy, porphyria cutanea tarda, antitrypsin deficit.

Physiopathology

Non-alcoholic fatty liver disease can occur secondary to defects in the synthesis and hepatic metabolism of lipids. NAFLD and NASH appear in various conditions: obesity, protein-caloric malnutrition, diabetes mellitus, corticotherapy; in all of these conditions, the common mechanism is represented by the accumulation of triglycerides in the liver (7). The accumulation of lipids in the liver is secondary to an increased dietary intake of lipids and fatty acids or to a decrease of fatty acid beta-oxidation, and to their increased synthesis in the hepatic mitochondria, as well as to a decreased synthesis or impairment of cholesterol secretion from VLDL. There is also a dysfunction of hepatic macrophages, which increases the susceptibility of hepatocytes to endotoxins. Increased synthesis of fatty acids and triglycerides occurs in obese patients. In type 1 diabetes mellitus, increased synthesis of triglycerides occurs secondary to insulin deficiency, with peripheral lipolysis and an increase in fatty acid influx, the increased glucagon secretion inhibiting lipoprotein synthesis. In type 2 diabetes mellitus, increased triglyceride levels are secondary to the excessive intake and endogenous fatty acids in the liver (8).

The development of fibrogenesis has not been completely elucidated. It seems that lipidic peroxidation generates toxic intermediate products, which in turn induce an inflammatory response in the hepatocytes (9). Hepatic infiltration with fatty acids determines an increased production of fibroblasts. Ito cells (cells in which lipids are stored), located in the subendothelial Disse space, are activated; this represents the earliest event in the development of fibrosis under the action of lipidic peroxides, with subsequent proliferation of lipocytes, with the initiation of the hepatic fibrogenetic cascade. Fatty acids accumulate in the liver in obese patients with hyperinsulinism, insulin inhibiting the oxidation of free fatty acids, with subsequent increase of toxic fatty acid levels in the liver. The accumulation of free fatty acids in the hepatocytes determines mitochondrial ballooning, with an increase in the fragility and permeability of membranes. Lipid accumulation initially takes place in the endoplasmic reticulum, under the form of small globules; these globules merge and pass the barrier of the endoplasmic reticulum, with the development of large drops, and thus the transition from microvesicular to macrovesicular steatosis occurs.

Histology

Liver biopsy reveals the presence of lipids in the

hepatocytes. The quantification of steatosis is based on the number of hepatocytes involved: <30%: minimal hepatic steatosis, 30-60%: moderate steatosis, >60%: massive steatosis. The distribution of steatosis (periportal, centrilobular) can be determined histologically, as well as the associated histological lesions – inflammation, fibrosis, Mallory bodies, inclusions, cirrhosis. Steatosis may be macrovesicular or microvesicular. NASH indicates a more severe liver disease and a poorer prognosis than simple steatosis. In simple steatosis, inflammation and fibrosis are absent. In patients with NASH, biopsy reveals inflammatory infiltrate with neutrophils and lymphocytes in the perivenular areas, focal necrosis and perivenular or diffuse macrovesicular lipid deposits. Moreover, peripheral venous fibrosis and Mallory bodies may appear.

According to the histological aspect, NAFLD can be classified as follows (10):

- Class 1: simple steatosis (no inflammation, no fibrosis);
- Class 2: steatosis with lobular inflammation, with the absence of fibrosis and of ballooned cells;
- Class 3: steatosis, inflammation and fibrosis of various degrees;
- Class 4: steatosis, inflammation, fibrosis, ballooned cells and Mallory hyaline. Steatosis can be macrovesicular with distended hepatocytes, with a lipidic vacuole of 1-10 microns, or microvesicular, with lipids in small drops of under 1 micron which surround the nucleus.

In patients with nonalcoholic steatohepatitis (NASH), the following classification of fibrosis has been proposed (11):

- Stage 1: zone 3 perilobular vein, sinusoids or pericellular fibrosis;
- Stage 2: zone 3 sinusoidal fibrosis or zone 1, periportal fibrosis;
- Stage 3: bridging fibrosis between zone 3 and zone 1;
- Stage 4: regeneration nodules which indicate cirrhosis.

Clinical and paraclinical aspects

The clinical picture in NAFLD varies from the absence of symptoms (48-100%) to fatigue in 73% of cases, right upper quadrant pain (50%), or hepatomegaly. The presence of palmar erythema suggests the diagnosis of cirrhosis. Ulcerations of the members may occur, more frequently in obese patients who associate NASH. Acanthosis nigricans can be present in children

with NAFLD. Family history of fatty liver or cryptogenic cirrhosis is present in 20-25% of cases.

Laboratory

Increased levels of aminotransferases, usually $< 2 \times$ ULN, of GGT and of alkaline phosphatase may occur during the evolution of NAFLD. Antinuclear antibodies may appear in 30% of patients with NASH, increased serum iron levels in 20-60% of cases, while a decreased IgG/IgA ratio may be associated with severe fibrosis. Weber-Christian disease (nodular panniculitis), present especially in the extremities, can be associated with NASH and is accompanied by disorders of the lipidic metabolism, increased aminotransferase levels, jaundice, while Mallory bodies appear on histology.

Abdominal ultrasound

Abdominal ultrasound: may detect liver steatosis, the ultrasonographic signs being represented by increased hepatic echogenicity, the presence of posterior attenuation, liver with normal or increased dimensions. It is difficult to distinguish on ultrasound between liver fibrosis and fatty infiltration, or between focal areas of fatty infiltration and hypoechoic areas, the discriminatory power of detection being 30%. Central obesity can also be evaluated on ultrasound (12).

Native computed tomography

Native computed tomography remains the optimal imaging technique for evaluating fatty liver disease. The sensitivity and specificity for diagnosing fatty liver vary between 84-99%. Abdominal CT scan can evaluate visceral fat at the L4-L5 level, adjusted by age and gender.

Magnetic resonance imaging

Magnetic resonance imaging is less accurate in evaluating fatty liver. A better technique may be MRS (magnetic resonance spectroscopy), which may also provide information regarding other metabolic parameters, such as adenosine triphosphate (ATP) and lipidic peroxidase.

New markers for evaluating NAFLD

Leptin

Leptin is a circulating protein, coded by chromosome 7q31, and produced by white adipose tissue (13). Leptin has an effect on insulin and on the activated gamma receptor of peroxisomes, and its level increases in cirrhosis. The role of leptin is to modulate the

sensation of hunger, acting at the hypothalamic level. Obesity is associated with increased leptin levels, which is involved in the appearance of histological injuries in NAFLD, determining inflammation. Activated hepatic stellate cells can produce leptin, which promotes histological injury.

TNF-alpha

The increase of TNF-alpha levels has been incriminated in the development of NASH. In animal models, obesity per se increases liver susceptibility to endotoxins and to cytokine-mediated injury: TNF-alpha induces mitochondrial uncoupling of proteins in the liver (14). Transforming factor beta and IL-6 are involved as fibrosis mediators in NASH. It is possible that abnormal cytokines appear secondary to lipidic peroxidation in NASH and not as a primary stimulus, while stimulation of stellate cells by cytokines can accelerate the development of collagen deposits, lipidic peroxidation being the trigger of hepatic injuries or of cell death.

Adipocytokines (15)

The increase of extrahepatic fatty deposits may play a role in the progression of NAFLD; these deposits are not just a way of storing energy, but also secrete physioactive substances: adiponectin, TNF-alpha (16), plasminogen activator inhibitor, adipon and resistin. The adiponectin levels decrease in obese and in diabetic patients, playing an essential role in the development of insulin resistance. However, studies are necessary to elucidate the role of these mediators in the pathogenesis of NASH.

The prognosis of non-alcoholic steatohepatitis

Patients with NASH frequently associate other comorbidities, which influence survival and progression of liver disease. 5 and 10-year survival in NASH varies between 59-67%, the main causes of death being cardiovascular disease (19.5%) or cirrhosis (6.5%). The number of cases of cirrhosis which lead to death increases when fatty infiltration is associated with more severe histological markers (fibrosis, ballooned cells, Mallory bodies). Simple steatosis or steatosis with minimal inflammation are relatively stable pathological conditions. Predictive factors of severity are: age > 40 -50 years, female gender, degree of obesity or of steatosis, arterial hypertension, diabetes or increased insulin resistance, increased ALT, AST and GGT levels, AST/ALT ratio > 1 , hypertriglyceridemia, and increased IgA levels.

Studies on biopsy series have shown that 20% of patients with NASH on the initial biopsy progress to cirrhosis within 5 years, developing fibrosis, and many of the cases of cryptogenetic cirrhosis are actually due to the silent progression of NASH.

Treatment of NAFLD

There is no solid proof to clearly demonstrate the effects of a milder or a more aggressive therapy in NAFLD. Patients with simple steatosis require surveillance of the liver disease and treatment of comorbidities (hypolipemiant, antihypertensive medication, antidiabetics), as well as monitoring for potential hepatotoxic effects. For patients with moderate inflammation, without fibrosis, who have a good prognosis, surveillance and less aggressive therapies are recommended (5).

Physical exercise, diet and weight loss

Physical exercise, diet and weight loss are the most frequent recommendations. A weight loss of 1.6 kg/week and lifestyle changes prevent evolution towards diabetes in obese patients. Reduction of intake of fats and polyunsaturated fatty acids (fish oil, omega 3 acids) are recommended, which also reduce cardiovascular risk, by modifying lipidic peroxidations and insulin resistance.

The recommended physical exercises are walking and jogging. Severe diets, with under 500 Kcal/day, are not recommended, because they induce focal necrosis and hepatocellular degeneration. The most frequent recommendations are diets with 35 Kcal/kg/day and physical activity 6 hours/week, for obtaining a weight loss of 500 grams/week, over a period of 6 months. It has been noticed that weight loss is accompanied by normalization of hepatic enzyme levels and the improvement of the ultrasonographic aspect of the liver. Supplements for losing weight (orlistat, tetrahydrolipstatin) decrease absorption of fats by inhibiting lipase. The malabsorption of liposoluble vitamins, vitamin E, should be evaluated and corrected. The surgical methods for weight loss, gastric by-pass Roux-in Y, is the most frequent choice for many patients. Studies on obese patients who underwent gastroplasty and sustained weight loss have shown the decrease of parameters that define the metabolic syndrome, the levels of glycemia, insulin, fibrinogen, triglycerides, uric acid, and ALT (17). In patients in whom biopsy was performed post-procedure, improvement of steatosis was observed, with a slight increase of inflammation and without significant changes of fibrosis.

Cytoprotective agents and ursodeoxycholic acid (UDCA)(18)

UDCA confers stability to the mitochondrial membrane. The dose of 13-15 mg/kg/day decreases ALT levels, but does not have a clear effect on AST, on inflammation or on fibrosis. Taurin may be administered in children with NAFLD, as triacetyluridine (19).

Hypolipemiant agents

Fibrates are used for hypertriglyceridemia, decreasing the levels of free fatty acids in the liver, of VLDL levels, and having a beneficial effect on altered LDL metabolism (16). Clofibrates, in doses of 2 gr/day for 12 months, are administered to patients with NASH and hypertriglyceridemia. Gemfibrosil, in 4-week cycles, decreases liver enzyme levels, while atorvastatin provides benefits on biochemical and histological changes in patients with NASH and hyperlipemia.

Antidiabetic agents

Insulin resistance is present in patients with NASH and hyperinsulinism or diabetes mellitus. Thiazolidinediones (20,21) are associated with weight increase, but with a decrease of central adiposity, increased expression of glucose transporters and growth of mitochondria. They also decrease cytokine levels, inhibit NO-synthetase production, and block fatty acids. In NASH patients, troglitazone, in 4-6 month cycles, normalized liver enzyme levels and decreased inflammation in liver tissue, proven by liver biopsy (22). Metformin determines a reduction of steatosis and improves the histologic score of NASH by stimulating aerob metabolism and increasing lactate levels in pre-adipocytes (23,24).

Antioxidants and nutritive supplements

Vitamin E has a beneficial effect by inhibiting lipidic peroxidation, TNF-alpha and the genes that code collagen expression. The recommended dose of vitamin E is of 400-1200 units/day. N-acetyl cistein, which at the hepatic level is converted to glutathion, improves hepatic enzyme levels. The recommended dose is of 1 gr/day, for 3 months. Silimarin has been proven to decrease the expression of CYP3A4 and decreases mitochondrial respiration in hepatocyte cultures.

Experimental drugs

Many drugs are currently being investigated in clinical trials for the treatment of patients with NASH. Of these drugs, elafibranor is one of the most

advanced, currently being tested in phase III trials. Elafibranor, a dual agonist of the PPAR α/δ receptor, has been shown to improve liver histology in NASH patients, as well as improve the cardio-metabolic profile of these patients (25).

Liver transplantation

Liver transplantation is reserved for patients with advanced NASH, and may be encumbered by complications due to comorbidities: obesity, diabetes, hyperlipemia. Recurrence of NAFLD and NASH in the graft is possible after transplantation. Steroids may induce steatosis, and cyclosporine has an effect at the mitochondrial level.

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