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INSIGHTS INTO NON-ALCOHOLIC FATTY LIVER DISEASE AND NON-ALCOHOLIC STEATOHEPATITIS

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Abstract

Non-alcoholic fatty liver disease (NAFLD) constitutes a common pathological condition of the liver, the prevalence of which is currently increasing in western countries. NAFLD is frequently diagnosed in males and its incidence is higher in individuals with type 2 diabetes mellitus and obesity. Hence, the disease is considered to be the hepatic manifestation of the metabolic syndrome. A multitude of interconnected risk factors have been described over the years – genetic, hormonal and nutritional, which play important roles in the development of NAFLD. Insulin resistance is considered to be the central pathophysiological condition that promotes the disease in diabetic patients, whereas dyslipidemia and cardiovascular comorbidities (arterial hypertension, ischaemic heart disease) are frequently associated conditions. Although there are currently numerous pathophysiological mechanisms involved in NAFLD that are still unknown or poorly understood, there has been some advancements concerning the pathogenesis of the disease and its progression towards its severe form, known as non-alcoholic steatohepatitis (NASH). In the absence of a clear diagnosis and carefully controlled treatment, NAFLD/NASH may evolve towards liver cirrhosis, liver failure or hepatocellular carcinoma. However, the disease may also generate systemic effects, including the development of chronic kidney disease (CKD). The diagnosis of NAFLD/NASH is based both on its clinical manifestations, revealed by a carefully conducted patient history and physical examination of the patient, and on other investigations; histopathological findings upon liver biopsy, liver ultrasonography and the use of transient elastography (or FibroScan) are some of the most important investigations in NAFLD/NASH. The understanding of the most important risk factors and pathogenic mechanisms of the disease is fundamental for the elaboration of the most efficient treatment, to prevent chronic liver disease or the development of hepatocellular carcinoma.

Keywords: *non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, metabolic syndrome, liver cirrhosis*

Rezumat

Ficatul gras non-alcoolic (NAFLD) constituie o condiție patologică comună a ficatului, a cărei prevalență este, la ora actuală, în creștere în țările occidentale. NAFLD este frecvent diagnosticat la sexul masculin, iar incidența sa este crescută la indivizii cu diabet zaharat de tip 2 și obezitate. Prin urmare, boala este considerată manifestarea la nivel hepatic a sindromului



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metabolic. S-a descris o multitudine de factori de risc în cursul ultimilor ani - genetici, hormonali și nutriționali, aceștia având roluri importante în dezvoltarea NAFLD.
Rezistența la insulină este considerată condiția fiziopatologică centrală care determină boala la pacienții diabetici, în timp ce dislipidemia și comorbiditățile cardiovasculare (hipertensiunea arterială, cardiopatia ischemică) reprezintă condiții frecvent asociate. Deși sunt numeroase mecanisme fiziopatologice implicate în NAFLD care rămân încă necunoscute sau incomplet înțelese, s-au înregistrat anumite progrese în ceea ce privește patogenia bolii și evoluția spre forma sa mai severă, cunoscută ca steatohepatită non-alcoolică (NASH).
În absența unui diagnostic clar și a unui tratament atent condus, NAFLD/NASH poate evolua spre ciroză hepatică, insuficiență hepatocelulară sau carcinom hepatocelular. Cu toate acestea, boala poate genera și efecte sistemice, inclusiv dezvoltarea bolii renale cronice (BRC). Diagnosticul NAFLD/NASH se bazează atât pe manifestările clinice, evidențiate printr-o anamneză atent efectuată și pe examenul fizic al pacientului, cât și pe alte investigații; aspectele histopatologice date de biopsia hepatică, ultrasonografia hepatică și elastografia (sau FibroScan) reprezintă câteva dintre cele mai importante investigații în cadrul NAFLD/NASH. Înțelegerea factorilor de risc și a mecanismelor patogenice ale bolii este fundamentală pentru elaborarea celui mai eficient tratament, astfel încât să se prevină evoluția spre boala cronică de ficat sau dezvoltarea carcinomului hepatocelular.
Cuvinte cheie: *ficatul gras non-alcoolic, steatohepatita non-alcoolică, sindrom metabolic, ciroză hepatică*

Introduction

Non-alcoholic fatty liver disease (NAFLD) is defined as a pathological condition of the liver, characterized by heavy cytoplasmic accumulation of fatty compounds in the hepatocytes, in the absence of alcohol consumption[1]. Thus, this condition is fundamentally different than the alcohol-associated form, known as alcoholic fatty liver disease (AFLD), in which the prolonged alcohol consumption plays the central etiological role in developing the build-up of lipids within the hepatocytes.
NAFLD is most frequently associated with at least one of the components of the metabolic syndrome, type II diabetes mellitus, with peripheral insulin resistance, and/or obesity, and represents the direct cause of a more severe form of the disease, characterized by

hepatic tissue necroinflammation and defined by non-alcoholic steatohepatitis (NASH)[2], associated with hepatocyte necrosis and immune cell infiltration of the hepatic parenchyma, as seen in optical microscopy. In the absence of treatment, NASH may evolve towards the development of liver cirrhosis (hepatic fibrosis, hepatocyte necrosis and the formation of regenerative nodules) or hepatocellular carcinoma[3]. Over the last approximately 20 years, NASH has been recognized as one of the main causes of cryptogenic liver cirrhosis[3], an entity that is unrelated to viral hepatitis, prolonged alcohol consumption or the effects of various hepatotoxic drugs.

Etiology and pathophysiology

NAFLD is considered to be the hepatic manifestation of the metabolic syndrome, its pathophysiological mechanisms being determined by a complex interrelationship of numerous risk factors, such as genetic, hormonal and nutritional factors[1]. Hence, NAFLD has no one etiological condition or causative factor, but a complex association of risk factors, which must be considered in clinical practice. Furthermore, being frequently associated with type II diabetes mellitus, NAFLD is a direct effect of peripheral insulin resistance[4].

Even though over the years numerous studies covered the pathophysiology of NAFLD/NASH, there is a multitude of mechanisms that remain unknown or poorly understood.

1. Genetic factors

Studies have shown there are a multitude of genes related to the pathogenesis of NAFLD, such as PNPLA-3, TM6SF-2, FTO, LIPA and HMOX-1, which are associated not only with the modulation of carbohydrate and lipid intermediate metabolism but also being involved in increased oxidative stress and the activity of numerous cytokines [1]. One study from 2016, published in the World Journal of Gastroenterology, suggested important pathogenic roles of the PNPLA-3 gene, which codes for the PNPLA-3 protein (Patatin-like phospholipase domain-containing protein 3), also known as adiponutrin, a 481-amino-acid functional protein. Under physiological conditions, adiponutrin, being strongly expressed within the hepatocytes, operates as a hydrolase, catalysing the hydrolysis of triglycerides into fatty acids and glycerol, and also as an acetyl-CoA-independent transacylase.

These two physiological activities of adiponutrin suggest its double metabolic roles, as a catabolic lipase, which catalyses the breakdown of triglycerides into fatty acids and glycerol, and also as an anabolic lipogenic enzyme[6].

Following the mutation of the PNPLA-3 gene, expressed as the rs738409 variant, the biochemical structure of adiponutrin is modified; this particular molecular modification resides in the change of isoleucine to methionine at residue 148[6]. Once this molecular alteration is expressed, the catabolic function of adiponutrin is also altered, which blocks its capacity to catalyse the breakdown of triglycerides within the hepatocytes. Thus, the mutation of PNPLA-3 determines the triglycerides accumulation within the hepatocytes and the development of steatosis or NAFLD [6]. According to the same study, another important pathogenic aspect of PNPLA-3 gene mutation is that it is also associated with steatohepatitis and hepatic fibrosis.

A second important gene involved in the pathogenesis of NAFLD, and NASH is TM6SF2



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(Transmembrane 6 superfamily 2), its mutation being related to a more severe form of hepatic steatosis and fibrosis[6], but also with dyslipidemia and cardiovascular disease risk (atherosclerosis).

2. Hormonal factors

Hormones play important pathogenic roles in NAFLD. Insulin, glucagon, several intestinal hormones, such as ghrelin, glucagon-like peptide-1 (GLP-1), and adipokines (adiponectin) are some of the most studied biological factors[1]. Insulin has been proved to play the most important pathogenic role in NAFLD, since insulin resistance is considered to be one of the most important pathophysiological factors.

Insulin resistance characterizes type II diabetes[1]. Obesity has also been linked to developing insulin resistance; one of the presumed pathogenic mechanisms is the infiltration of visceral adipose tissue by macrophages and high local secretion of proinflammatory cytokines, such as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), which, according to some studies, promote insulin resistance, thereby causing type II diabetes[7]. Insulin is known to be not only a hypoglycemic biological factor but also an anabolic hormone, stimulating lipogenesis in the adipose tissue. However, in type II diabetes, insulin has no lipogenic effect, which explains the increased intensity of lipolysis. The intensified hydrolysis of triglyceride molecules within the adipose tissue increases the flux of free fatty acids in the bloodstream, these circulating molecules being transported within the hepatocytes, in which they are either stored as triglycerides or lipid cytoplasmic inclusions or they are converted to very-low-density lipoproteins (VLDL)[1], which will be subsequently secreted by the hepatocytes in the systemic circulation. Furthermore, the high levels of VLDL in the plasma are hydrolyzed by lipoprotein-lipase (LPL) in triglycerides and low-density lipoproteins (LDL), which increases the risk of developing hypertriglyceridemia and atheroma plaques (atherosclerosis) in different arterial territories (coronary arteries, internal carotid arteries etc).

3. Nutritional factors

The majority of patients with NAFLD are either overweight or obese, with a body mass index (BMI) ≥30 kg/m^2, many epidemiological studies suggesting that the risk of developing the disease is directly proportional to the increased weight of the individual[1]. Regarding obesity, this pathological condition, even though it has a multifactorial etiology (genetic factors and environmental factors)[7], is correlated with high intake of dietary sugars (fructose) and fats (high cholesterol and saturated fatty acids diet),

but also with increased ingestion of animal proteins[8], over a long period of time.

Recent studies suggest important pathophysiological implications of the intestinal microbiota. According to these studies, dysbiosis, which defines the alteration of the intestinal bacterial environment, is involved in the progression of NAFLD to NASH and liver fibrosis, through complex mechanisms.

Dysbiosis is associated with increased intestinal permeability, which results in endotoxemia, through the increased serum levels of lipopolysaccharides (LPS) of Gram-negative bacteria and increased metabolic bacterial products, such as short-chain fatty acids, choline, trimethylamine-N-oxide, and endogenous ethanol, which, via the portal venous system, translocate in the liver and activate the Kupffer cells and Ito cells, promoting inflammation and insulin resistance[8]. Furthermore, it must be noted that westernized foods, which are high in sugar and fats, can negatively influence the intestinal bacterial environment, causing dysbiosis, intestinal inflammation and increased intestinal permeability, which determines the translocation of numerous bacterial metabolic products and LPS to the liver via the portal vein[8].

4. Pathophysiological evolution

The classic pathogenic theory of NAFLD is the ”two-hit” hypothesis, in which the different grades of liver steatosis, induced by the accumulation of lipids within the hepatocytes, represent the ”first hit”, and steatohepatitis, determined by the inflammation of the hepatic tissue, secondary to high oxidative stress and the effects of proinflammatory cytokines (IL-6, TNF-α), defines the ”second hit”[1]. During the “first-hit”, because of the insulin resistance and the increased intensity of lipolysis in the adipose tissue, lipids accumulate within the hepatocytes, which determines different grades of liver steatosis (grade I, grade II or grade III). Liver steatosis may be seen on ultrasonography.

During the” second-hit”, there is an increased oxidative stress in the hepatocytes, which become functionally vulnerable, but also increased secretion of proinflammatory cytokines; dysbiosis is associated with elevated serum LPS (endotoxin of Gram-negative bacteria) and, along with IL-6, TNF-α and other proinflammatory cytokines, contributes to the progression of liver inflammation, through the activation of Kupffer cells and stellate cells from the perisinusoidal space (Disse). The” second-hit” describes the progression of NAFLD to NASH[1]. This theory, however, is now considered to be obsolete.

Recent studies from 2020 and 2021 suggest a “multiple hit” theory, which better explains the pathophysiology of the disease[8, 9]. According to Lujan P. et colab. and Rada P. et colab., in comparison with the classic “two-hit theory”, the pathogenesis of NAFLD/NASH is governed by insulin resistance associated with lipotoxicity and other metabolic disturbances[9].

The lesions in the liver begin with high influx of fatty compounds within the hepatocytes, in which they are transformed into lipid droplets, thereby gradually generating liver steatosis. This condition is determined by several mechanisms: free fatty acids influx within the hepatocytes, decreased intensity of β-oxidation of fatty acids in the mitochondria and increased hepatic lipogenesis [8]. Because insulin resistance is also present, steatosis is aggravated, as lipolysis in the adipose tissue intensifies and elevates the levels of free fatty acids in the plasma. Furthermore, the affected hepatocytes have a reduced transport of



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triglycerides in the form of VLDL, which also accumulate in the serum, generating hypertriglyceridemia, but also elevated LDL levels.

Lipotoxicity occurs when the hepatocytes' metabolism is severely altered by the massive fatty acid influx within the cytoplasm. Studies have shown that the high influx of saturated fatty acids, such as palmitate and stearate, which cannot be esterified (thereby neutralizing their hepatotoxic effects), promotes endoplasmic reticulum stress and hepatocyte injury[9].

Regarding the increased oxidative stress in NAFLD, this phenomenon is directly associated with the high influx of fatty acids in the hepatocytes and defines the increased intracellular levels of reactive oxygen species (ROS), the mitochondria being the most important sources of these chemical elements[9].

Because of this lipotoxic state, the processes of β-oxidation of fatty acids and Krebs' cycle are altered, thereby enhancing electron overflow and the formation of superoxide (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl (HO^-), which are highly ROS[10] and having negative effects on numerous intracellular structures.

Once formed, ROS react with polyunsaturated fatty acids, resulting in the formation of highly-reactive hepatotoxic aldehydes, such as 4-hydroxy-nonenal (4-HNE) and malondialdehyde (MDA)[9]. These molecules aggravate hepatocyte injury, but they can also diffuse in the extracellular matrix surrounding the hepatocytes, which worsens tissue damage. It has been shown in experimental models that both 4-HNE and MDA are highly increased in patients with NASH, compared with patients with NAFLD[10].

One of the most important pathophysiological phenomena associated with NAFLD/NASH is lipoapoptosis, which, according to Ibrahim S. et. colab., defines the programmed hepatic cell death induced by the heavy accumulation of fatty compounds in the hepatocytes[11]. Lipoapoptosis, generated by the fatty acid-induced endoplasmic reticulum and mitochondrial oxidative stress, through the activation of both intrinsic and extrinsic pathways, results in the release of numerous cytokines, chemokines and other intracellular molecules, which then activate the nearby Kupffer cells, Ito cells, but also the endothelial cells.

The activation of these hepatic cellular elements is proinflammatory in nature and is strongly involved in the recruitment of other circulating immune cells, such as monocytes/macrophages and lymphocytes[12, 13], which aggravates NASH. It must be noted that the persistence of liver inflammation (NASH) gradually leads to excessive collagen deposition and scarring, which ultimately induces hepatic fibrosis and liver cirrhosis[14],

through the development of regenerative nodules. When NASH has been developed, Kupffer cells, because of their heavily expressed proinflammatory phenotype, secrete large quantities of chemokines and proinflammatory cytokines, such as IL-1β, IL-6 and TNF-α, which not only exacerbates tissue damage, but also stimulates the further recruitment of circulating immune cells, which aggravates NASH. Also, there is a high expression of inducible-nitric-oxid-synthase (iNOS) within the Kupffer cells[15,16].

Ito cells, also known as hepatic stellate cells or lipocytes, located in the perisinusoidal space of Disse, have important pathogenic contributions in the progression of NAFLD/NASH. Upon exposure to the heavy influx of fatty compounds, Ito cells manifest a fibrogenic response, which gradually determines the development of fibrosis in the perisinusoidal space, which further alters the hepatocyte function, mainly by blocking the bidirectional flow of substances between the cells and the capillary blood, with subsequent cell necrosis, but also have an enhanced proinflammatory reaction, through secretion of chemokines, proinflammatory cytokines, promoting further tissue damage and recruitment of circulating immune cells[17, 18].

The profibrogenic and proinflammatory phenotype of Ito cells has important pathophysiological implications not only in NAFLD/NASH, but also in the pathogenesis of liver cirrhosis, in which they can biologically convert into myofibroblasts[19], which suggests the high risk of development of liver cirrhosis in patients with NASH.

As liver inflammation and fibrosis persist, along with hepatocyte death, the function of the liver declines, which, in time, generates hepatic failure and alteration of other systemic functions, including kidney function.

Although there are several pathogenic mechanisms that link NASH to chronic kidney disease (CKD), one important theory suggests that the increased levels of ROS, proinflammatory cytokines (IL-1β, IL-6, TNF-α), along with TGF-β and plasminogen-activator inhibitor-1 (PAI-1), all of these being released by hepatocytes, Kupffer cells and Ito cell into the bloodstream during NASH, may play important pathophysiological roles in the development of CKD[20].

Another pathophysiological mechanism that explains the link between NASH and CKD has been proposed by Ix and Sharma[21]; according to their theory, there is a particular link between the liver, adipose tissue and kidneys, this link being provided by fetuin-A, which is a major protein carrier of fatty acids in the plasma, secreted by the hepatic tissue, and adiponectin, considered to be both a hormone and an anti-inflammatory cytokine, secreted by the adipose tissue and being involved in glucose level regulation and fatty acid breakdown. In obesity and type II diabetes, as in NAFLD, adiponectin has lower serum levels.

The lower levels of adiponectin in the hepatic tissue and kidneys reduce the activation of 5'-AMP-Activated-Protein-Kinase (AMPK), which alters hepatocyte and glomerular podocyte functions, leading not only to proinflammatory and fibrogenic cascades, but also to end-stage liver disease (decompensated liver cirrhosis) and end-stage renal disease (kidney failure). This theory suggests a pathophysiological connection between NAFLD/NASH and CKD.

Liver cirrhosis or liver failure are not the only complications of NAFLD/NASH. Hepatocellular carcinoma has been taken into consideration as well. Although the pathophysiology of NAFLD/NASH-related hepatocellular carcinoma is not clearly understood, several

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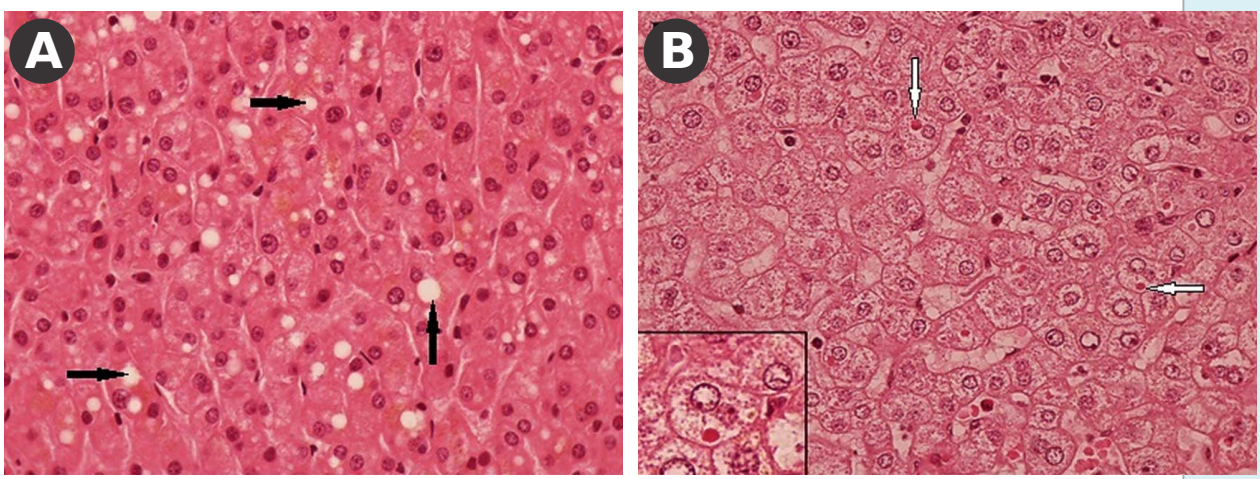


Figure 1. *Liver histology, hematoxylin and eosin staining. Simple steatosis (A), with fat droplets in the cytoplasm of hepatocytes (black arrows) and NASH (B), with presence of Mallory-Denk bodies in ballooned hepatocytes (white arrows). Adapted after Mariana Verdelho Machado, Duarte M. S. Ferreira, Rui E. Castro, Ana Rita Silvestre, Teresinha Evanghelista, João Coutinho, Fátima Carepa, Adília Costa, Cecília M. P. Rodrigues, Helena Cortez-Pinto. Liver and Muscle in Morbid Obesity: The interplay of Fatty Liver and Insulin Resistance*[39]

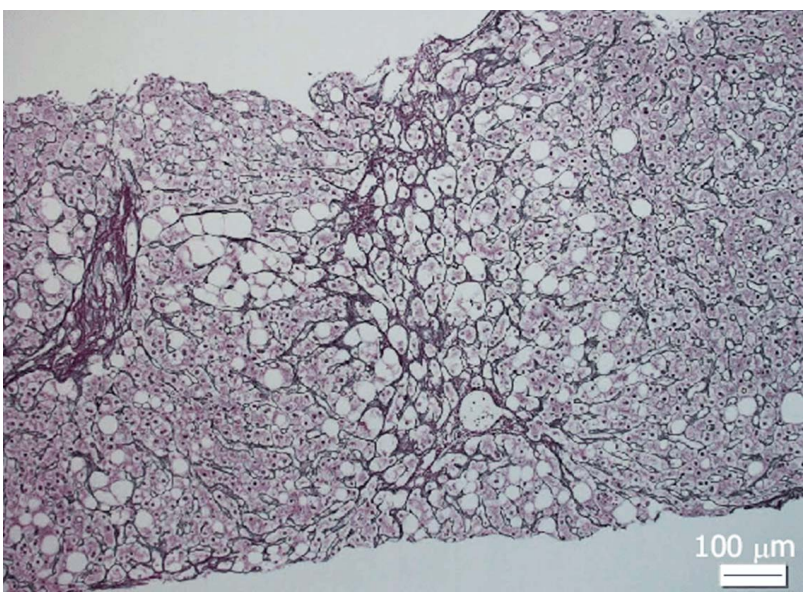


Figure 2. *"Chicken wire" fibrosis, reticulin staining. Fibrosis in NASH typically begins in zone 3, but progresses towards bridging fibrosis and cirrhosis as the disease develops.*
Adapted after Takahashi Y, Fukusato T. Histopathology of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. World J Gastroenterol 2014[28]

carcinogenic mechanisms have been incriminated: increased release of fatty acids, the persistent activity of multiple proinflammatory cytokines - associated with a radically decreased activity of anti-inflammatory factors, the most important being adiponectin[22].

As mentioned above, all of these biological disturbances lead to hepatocyte apoptosis (lipoapoptosis), fibrosis and compensatory proliferation of viable hepatocytes, which generate the cirrhosis-related regenerative nodules. In the absence of rapid treatment, this ultimately leads to carcinogenesis of the liver[23]. Furthermore, some studies show the development of hepatocellular carcinoma in some patients without NAFLD/NASH-related liver cirrhosis, who have a shorter survival time, a more advanced hepatic tumor at the time of the diagnosis and a lower probability of liver transplantation[24].

Histopathology

Even though non-invasive methods show promise in the diagnosis of NAFLD/NASH, liver biopsy remains the golden standard now[25, 26]. Since the differentiation of NAFLD from alcoholic steatosis through biopsy can be difficult or almost impossible, alcohol consumption should be excluded by the clinician for a diagnosis of NAFLD to be made[27].

The most important pathological changes that can be observed through optical microscopy are steatosis, inflammation, signs of cellular damage and fibrosis. Mallory-Denk bodies (MDBs) and megamitochondria may also be present[27,28].

1. Steatosis

The most important finding in NAFLD is steatosis[27, 28]. Conventionally, only steatosis involving 5% or more of the liver can be considered clinically relevant[29].

Accumulation of lipid droplets within the hepatocytes can be both macrovesicular and microvesicular[27, 28]. Microvesicular steatosis was reported to appear in 10% of liver biopsies and was linked to more severe forms of the disease[30]. Later studies reported a higher prevalence of microvesicular steatosis (30%)[31]. Steatosis is most commonly found in zone 3[27, 28] - the hepatic lobular region surrounding the central vein, which is the most exposed to liver injury, being the furthest from the portal triad (lobular arteriole of the proper hepatic artery, bile duct, lobular venule of the portal vein), especially when mild, although it can also be located in the pan-acinar area in more advanced NAFLD cases, where it has been associated with an increase in ballooned hepatocytes, Mallory-Denk bodies and higher levels of fibrosis[32].

2. Inflammation

Inflammation is one of the main pathophysiological mechanisms that lead to fibrosis in NASH, the other one being hepatocyte injury. Both intralobular and portal inflammation may be present, however the intralobular distribution is more common[27,28].

Lobular inflammation is usually comprised of a mixed cellular infiltrate, containing mainly lymphocytes and Kupffer cells, which can form aggregates also known as microgranulomas[27, 28]. Eosinophils and neutrophils may also be present, but not consistently. Lipogranulomas have been associated with steatosis and could be a marker of prior steatosis in burnout NASH[33].

Portal inflammation is less frequently observed in NAFLD. Histologically, the infiltrate contains mostly lymphocytes[28]. There is a positive correlation between greater than mild chronic portal inflammation and the amount of



steatosis, its location, the presence of ballooned hepatocytes and fibrosis[34].

3. Cellular damage

Histologically, cellular damage can be reflected by the presence of ballooned hepatocytes and apoptotic cells.

Hepatocellular ballooning is believed to be caused by molecular alterations in the structure of the proteins that form the cytoskeleton of the hepatocytes. Under the optic microscope, hepatocellular ballooning is characterized by the presence of enlarged hepatocytes, with rarefied cytoplasm and with optional presence of MDBs[27, 28]. Satellitosis is defined as the presence of ballooned hepatocytes, surrounded by neutrophils[27, 28], their presence suggesting an active inflammatory process. Apoptotic cells, also known as acidophil bodies, are described as eosinophilic, rounded, with the possible presence of nuclear fragments[28], but do not hold much diagnostic value. There have been studies that show death receptors pertaining to the TNF-receptor superfamily hold a major role in hepatocyte apoptosis[35]. Stress caused by lipotoxicity is another key factor involved in hepatocellular damage, including apoptosis[36].

4. Fibrosis

Fibrosis in NASH and in alcoholic steatohepatitis follows a characteristic pattern, known as "chicken wire" fibrosis[27, 28]. Fibrosis begins in zone 3, but progresses towards bridging fibrosis and cirrhosis, involving the perisinusoidal spaces [27]. In later stages, periportal fibrosis may also be present[27, 28]. Burn-out NASH is characterized by a decrease in steatosis and inflammation in the liver, which can mislead towards a diagnosis of fibrosis or cirrhosis of different etiology. In this case, the presence of hepatocellular ballooning, MDBs and megamitochondria might indicate prior steatosis[37].

5. Mallory-Denk bodies (MDBs)

Mallory-Denk bodies are eosinophilic aggregates situated in the cytoplasm of ballooned hepatocytes, containing several types of misfolded proteins[27, 28, 38]. Although common, MDBs are not specific to NASH, appearing more frequently in alcoholic liver disease. Their presence is also associated with other diseases of the liver, as well as with a reaction to drugs[27,28,38].

6. Other findings

Other findings include megamitochondria and iron deposits. Megamitochondria are round or irregular in shape, eosinophilic, situated in the cytoplasm of hepatocytes[27, 28], but their presence is not specific to NAFLD/NASH. Iron deposits can be situated both in hepatocytes and in the reticuloendothelial system[27, 28], but there have been conflicting results regarding the importance of their localization[28].

Clinical manifestations

Patients with NAFLD are, most frequently, asymptomatic [1], but they may be known with type II diabetes and/or obesity, which should suggest a liver affection as well. In other situations, the patient may have arterial hypertension, other vascular diseases, sleep apnea or, in the case of females, polycystic ovary syndrome (PCOS)[1].

It must be noted that moderate liver steatosis does not express itself through clinical manifestations[40]. If the patient does exhibit symptoms upon presentation, they are generally mild and non-specific, most of these being defined by fatigability and vague discomfort in the right hypochondriac region[1]. Jaundice is usually absent in patients with NAFLD, but it may be present in patients with steatohepatitis.

During the physical examination, palpation of the abdomen may reveal steatosis-related hepatomegaly[1]; the inferior margin of the liver is sharp and with firm consistency[40].

Upon ultrasound examination, the patient may be diagnosed with different grades of hepatic steatosis (grade I, grade II or grade III), relative to its severity. In some situations, the clinical manifestations may rapidly evolve towards hepatic decompensation, which suggests subacute steatohepatitis[1].

Diagnosis

While NAFLD can be diagnosed by imaging studies such as ultrasound, computed tomography (CT) or magnetic resonance imaging (MRI), the presence of NASH still requires liver biopsy to identify the presence and location of its features such as inflammation, hepatocyte ballooning, Mallory-Denk bodies and early fibrosis[41]. This implies that the liver biopsy currently represents the diagnostic gold standard for NAFLD[42], since it allows the exclusion of other causes of liver damage and the estimation of the severity of the disease.

Moreover, taking into consideration that NAFLD is a diagnosis of exclusion, a thorough history of the patient is essential and should exclude alcohol-related liver pathology and highlight NAFLD risk factors, such as family history and/or comorbidities: type II diabetes mellitus, obesity, dyslipidemia, cardiovascular diseases, the most important being arterial hypertension.

Serum markers play important roles in the diagnosis. Many patients may have elevated serum transaminases (ALT and/or AST) at a screening test, usually values at least 2-3 times the upper limit of the normal values. It should be noted that patients with advanced forms of chronic liver disease, such as decompensated liver cirrhosis, may have normal transaminase levels. In this situation, the decrease in platelet count may also occur, as a result of the installation of hypersplenism in the context of portal hypertension.

The fatty liver index (FLI) is a complex algorithm, based on four simple and routine parameters, such as body mass index (BMI), waist circumference, triglycerides and gamma-GT, which lead to the prediction of the presence of a fatty liver with an accuracy of 84%[43]. The index varies from 0 to 100, and a FLI <30 should exclude the presence of NAFLD, while a FLI ≥60 confirms the clinical suspicion.

This score is one of the efficient indexes, identifying patients at high risk for NAFLD, who should be examined through ultrasonography (US), and intensification of lifestyle counseling; successively, it was used as a good prognostic marker in predicting increased intima-media thickness, coronary heart disease risk and reduced insulin sensitivity[44], as well as 9-year incident diabetes[45] and early



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atherosclerosis[46]. **Imaging methods** play an important role in the diagnosis and evaluation of patients with NAFLD. One of these investigations is **abdominal ultrasound**, which can detect the diffuse accumulation of fat in the liver. A special imaging technique is **transient elastography** (TE), which allows the quantification of steatosis by the controlled attenuation parameter (CAP). However, this method has documented conflicting results, mostly due to the fact that TE is difficult to perform in case of obesity, since subcutaneous fatty tissue attenuates elastic share wave, reducing success rate and diagnostic performance[47].

Computed tomography is an imaging method that detects fatty infiltration of the liver with a sensitivity of 82% and a specificity of 100%[48].

Magnetic resonance imaging (MRI) permits not only the evaluation of liver steatosis but also a comprehensive study of the other abdominal organs. So even if it represents an accurate tool for the detection and dynamic quantification of liver steatosis, it is expensive and not widely available.

Treatment

The main purpose of treatment in NAFLD is the reduction of steatosis and the prevention of the progression of liver disease by countering multiple pathological risk factors involved in NAFLD, the most important being obesity, type II diabetes or metabolic syndrome[49]. The main therapeutic strategies in NAFLD are presented as follows.

1. Weight loss, dietary modification and changes in lifestyle

Although there is no specific diet dedicated to NAFLD, multiple studies show dietary modification, plays a key role in the treatment of NAFLD. This is particularly relevant, since a carbohydrate-rich diet, especially with high fructose, is the major cause of obesity, insulin resistance and NAFLD development[50]. Studies have shown that the Mediterranean diet, which is rich in monounsaturated and omega-3 fatty acids, should be recommended to patients, whereas saturated fats and omega-6 fatty acids should be avoided[51].

Hence, the fact that the primary therapy for most patients with NAFLD should be weight loss must be taken into consideration. These measures are applied to people who are overweight (BMI >25 kg/m^2) or obese (BMI >30 kg/m^2), since weight loss may lead to improvement in liver biochemical tests, liver histology, serum insulin levels and the quality of life in patients with NAFLD[52,53,54].

Despite the difficulties associated with the study of physical exercise, there have been well-designed studies showing a beneficial effect of physical activity regardless of changes in body weight. Regarding aerobic

exercise, there was a study in which a significant reduction in hepatic steatosis was found in 48 subjects divided into 4 different exercise routines (control, low volume-high intensity, high volume-low intensity, low volume-low intensity); the effect was independent of changes in body weight[55]. Studies developed on endurance exercise have shown similar results[56].

2. Pharmacological treatment

For insulin sensitizers, the first treatment is thiazolidinediones (TZDs), which are linked to the nuclear transcription factor - gamma peroxisome proliferator activated receptor (PPAR)-γ, improving hepatic and peripheral insulin sensitivity[57]. First generation TZDs (troglitazone), despite showing improvement in steatohepatitis, had to be abandoned due to the hepatotoxic effect[58]. However, it opened the way for second generation TZDs (rosiglitazone and pioglitazone), which, in comparison to the first generation, don't induce hepatotoxic effects and showed improvement in insulin resistance, hepatic steatosis and aminotransferases levels[59,60].

The second pharmacological agent is metformin, a hypoglycemic drug used for the treatment of type 2 diabetes. It causes great improvement on the liver and on peripheral insulin resistance, by decreasing hepatic gluconeogenesis, lipogenesis and glucose intestinal absorption, despite having gastrointestinal side effects[61].

Because NAFLD is associated with metabolic syndrome (which includes obesity and type II diabetes), the use of lipid lowering agents could have positive results. However, not all of these pharmacological agents have beneficial effects; studies have shown clofibrate did not exert any positive modifications on liver tests or histological scores[62], whereas gemfibrozil showed clear improvement, especially in reducing ALT serum levels in patients with NAFLD[63].

Vitamin E and vitamin C decrease oxidative stress and thus have been evaluated in patients with NASH. Even though there have been a multitude of clinical trials, one of the conclusions was that vitamin E was associated with an improvement in liver test functions and reduction in oxidative stress markers, but significantly less with histological grading of the disease[64,65].

Glucagon-like peptide-1 (GLP-1) agonists are a class of medications utilized in the treatment of type II diabetes and obesity. Their mechanism of action consists of lowering blood glucose levels by decreasing glucagon secretion, delaying gastric depletion and stimulating β-pancreatic insular cells to increase insulin secretion. Furthermore, these agonists have a central appetite suppressive effect and promote weight loss, which are favorable outcomes for obese patients with NAFLD/NASH[66]. In an obese mouse model, GLP-1 has been shown to improve insulin sensitivity and reduce hepatic steatosis[67].

A clinical study elaborated in 2020 involved the use of emricasan on 318 patients (5 mg to 50 mg of emricasan). Emricasan is an anti-caspase pharmacological agent, which operates by inhibiting apoptosis in NASH. However, the results of this study showed no improvement in liver damage and in the evolution towards liver cirrhosis, although emricasan did reduce the levels of alanin-aminotransferase in patients. Emricasan was associated with worsening of hepatocyte ballooning and hepatic fibrosis, which suggests the injured hepatocytes had an alternative molecular pathway of programmed cell death[68]. Thus, further clinical studies should be conducted soon regarding anti-apoptotic therapeutic strategies on patients diagnosed with NASH, since such



pharmacological agents could prove to be efficient in the prevention of hepatic tissue damage through apoptosis and the development of liver fibrosis.

Conclusions

NAFLD/NASH, one of the most important causes of cryptogenic liver cirrhosis, currently constitutes a spectrum of liver diseases, ranging from simple liver steatosis to liver inflammation and fibrosis, which can evolve not only towards the development of decompensated liver cirrhosis or liver failure, but also of hepatocellular carcinoma and other systemic diseases, including CKD. In order to prevent the progression of this pathological condition, a thorough clinical examination of the patient is required and, since NAFLD is most frequently associated with type II diabetes and obesity, it is essential to focus on these two comorbidities. Other pathological conditions may be present as well, such as dyslipidemia and arterial hypertension.

Understanding the etiology and pathophysiology of NAFLD/NASH, as well as of type II diabetes and obesity, is proving to be essential for elaborating the most efficient treatment.

Furthermore, there is currently an increase in the number of individuals with metabolic risk factors, such as obesity, type II diabetes mellitus and dyslipidemia, which could explain the increasing prevalence of NAFLD over the years, both in Eastern and in Western countries, all of these entities being associated with a generally high mortality rate. Hence, the identification of these individuals, especially with simple liver steatosis or NAFLD, and a carefully controlled treatment, mainly focused on type II diabetes and/or obesity, may well facilitate the decrease in the number of patients with NASH and non-alcoholic non-viral (cryptogenic) liver cirrhosis and the increase in the quality of the patients' life.

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