INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) consists of a broad spectrum of liver lesions ranging from fatty liver (FL), through nonalcoholic steatohepatitis (NASH), up to cirrhosis. The incidence of nonalcoholic fatty liver disease has increased in recent years due to the high rate of obesity in developed countries. Although the dogma "simple steatosis - benign prognosis, nonalcoholic steatohepatitis - severe evolution" still stands for many hepatologists, plenty of data underline the unsuspected evolution, i.e., that some patients may progress from fatty liver towards cirrhosis and hepatocellular carcinoma (HCC). NAFLD is the hepatic manifestation of the metabolic syndrome. In certain metabolic circumstances, isolated hepatic steatosis is not necessarily a benign disorder associated with cardiovascular risk and evolution towards severe liver diseases including HCC. We tried to shed some light on this problem, taking into account its major health impact and the variegated and sometimes unpredictable evolution of NAFLD.

Key words: liver diseases, nonalcoholic fatty liver disease

IS FATTY LIVER ALWAYS BENIGN AND SHOULD NOT CONSEQUENTLY BE TREATED?

Nonalcoholic fatty liver disease (NAFLD) consists of a broad spectrum of chronic liver diseases ranging, from simple hepatic steatosis or fatty liver (FL) or not definitely nonalcoholic steatohepatitis (NASH), up to cirrhosis. The incidence of nonalcoholic fatty liver disease has increased in recent years due to the high rate of obesity in developed countries. Although the dogma "simple steatosis - benign prognosis, nonalcoholic steatohepatitis - severe evolution" still stands for many hepatologists, plenty of data underline the unsuspected evolution, i.e., that some patients may progress from fatty liver towards cirrhosis and hepatocellular carcinoma (HCC). NAFLD is the hepatic manifestation of the metabolic syndrome. In certain metabolic circumstances, isolated hepatic steatosis is not necessarily a benign disorder associated with cardiovascular risk and evolution towards severe liver diseases including HCC. We tried to shed some light on this problem, taking into account its major health impact and the variegated and sometimes unpredictable evolution of NAFLD.

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INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) comprises a large spectrum of chronic liver diseases ranging, from simple hepatic steatosis or fatty liver (FL) or not definitely nonalcoholic steatohepatitis (NASH), through NASH, up to cirrhosis post-NASH (the previously named cryptogenic cirrhosis). Originally, the term NASH was first introduced by Ludwig and co-workers in 1980, when they described the histological findings that occurred in obese, diabetic patients (mainly women) who denied alcohol use (1). These modifications were highly similar to those of alcoholic hepatitis but the prognosis was somehow better. The prevalence of NAFLD is highly variable depending on the studied population and it is considered a further manifestation of the metabolic syndrome (MS) (2). Unclassified NAFLD is described as equally affecting the adult and pediatric populations, both males and females (3). Recent data have reported variations in the prevalence of NAFLD among racial and ethnic groups (4). These variations might be related to the inherent biological differences between them (5, 6). We can affirm that the proportion of patients is increasing and we are witnessing a sort of epidemic. Although the incidence of unclassified NAFLD and, specifically, of NASH has grown along with the incidence of obesity, little is known about their impact on overall health. In the last decade a lot of studies addressed this issue, resulting into conflicting and questionable data. These studies can be divided into two main categories: some looking for the evidence of histological progression using serial biopsies and others examining the clinical evolution of patients with the generic diagnosis of NAFLD. We looked into several series of patients trying to figure out if the dogma, i.e. FL is benign and NASH progresses towards liver cirrhosis and hepatocarcinoma (HCC), still stands and where we can find evidence to support it or not.

NONALCOHOLIC FATTY LIVER DISEASE AND CARDIOVASCULAR RISK

An important point regarding the prognosis of NAFLD is related to the cardiovascular (CV) risk. The classic belief based on anecdotal reports or small studies on heterogeneous patients was that there the chronic liver disease (CLD) has a "protective" CV effect due to a reduced arterial pressure, an improved lipid profile or altered coagulation. In some ways or others, this is true for the more advanced forms of CLD, but for the NAFLD there is growing evidence linking it to a major CV risk. To address this point, Soderberg and co-workers compared the mortality rates in 256 Swedish patients who underwent liver biopsy from 1980 to 1984, due to elevated liver enzymes, to the mortality rates in the general Swedish population (7). The mean age of the subjects was 45 years. The liver biopsies were scored for NAFLD (comprehending FL) and NASH and the causes of death were determined from the national Cause of Death Registry. During the 28 years follow-up period, 113 patients in the overall study group (44%) died of any cause. Of the 113 total deaths, 37 were caused by CV diseases and 16 by liver diseases. Interestingly, among the 113 deceased, 47 deaths were reported in subjects diagnosed with NAFLD (41% of the 113 patients) (7). The overall survival rate was reduced in the subjects with NASH, whereas simple hepatic steatosis or FL with mild or mild-moderate fibrosis, but without severe fibrosis, was not
associated with an increase in the mortality risk compared to the general population. Patients with the generic diagnosis of NAFLD were 69% more likely to die than the general Swedish population (standardized mortality ratio 1.69; 95% CI 1.24 to 2.25). Patients with NASH were at 86% higher risk (95% CI 1.19 to 2.76; p=0.007). Patients with NASH had a lower risk of death than those with alcoholic liver disease or chronic viral hepatitis but a higher risk than those suffering from autoimmune and other metabolic liver diseases. In this study, CV diseases and extra hepatic malignancies were the first and second causes of death among the NASH patients. Death from liver-related causes was more frequent compared to the general population, with four subjects with NASH who died of complications of cirrhosis, and five who died of HCC. Similarly to other studies, the long-term mortality of those with certain NASH was not significantly different from those with uncertain NASH (7). The association between the histological severity of NAFLD and CV risks remains poorly understood, with conflicting data, especially from older studies. To assess this relationship, a large, well-characterized cohort of 83 consecutive patients with biopsy-proven NAFLD were analyzed (8). The patients were subsequently divided into three groups: normal biopsy (n=11) simple steatosis or FL (n=36), and NASH (n=36). CV risk markers included: triglyceride/high-density lipoprotein (HDL), total cholesterol/HDL, and low-density lipoprotein/HDL ratios. All lipid ratios were found to be significantly associated with the entire spectrum of NAFLD (p<0.05) after adjustment for age and gender. More importantly, there was a stepwise, statistically significant increase in lipid ratios from patients with normal biopsies to patients with simple hepatic steatosis or FL to those with NASH (p<0.05). A positive correlation was found between the lipid ratios and NAFLD activity score (NAS) as well as the individual histological features of the NAS (steatosis, inflammation, and ballooning), the strongest correlation being the one to NAS (8). This study showed that the histological severity of liver injury and inflammation is strongly associated with an increased CV risk and an atherogenic lipid profile (8).

In a study of 38 patients with biopsy-proven NASH, Furuta et al. found an interesting association between liver fibrosis and several indicators of atherosclerosis such as: ankle-brachial index, pulse wave velocity measurement and carotid ultrasonography (9). Most studies focused on the association between the NAFLD and CV diseases suggesting that similar inflammatory mediators produce both liver and vascular lesions. The pro-inflammatory state that induces the progression from simple hepatic steatosis or FL to NASH can also induce pro-atherogenic effects (9-11).

**NONALCOHOLIC FATTY LIVER DISEASE AND PROGRESSION TO END STAGE OF LIVER DISEASE AND HEPATOCELLULAR CARCINOMA**

As repeatedly mentioned, NAFLD comprises a large spectrum of histological diseases and because NASH can lead to cirrhosis-post NASH, the development of HCC may be part of the progressive nature of this condition.

In one series of 42 Australian patients with NASH, HCC developed after 4 years of follow-up in only one cirrhotic patient (2%), (12). There are two case reports on the development of HCC, 6 and 10 years after the initial diagnosis of NASH and cirrhosis, respectively (13, 14).

Out of 44 patients with cryptogenic cirrhosis and HCC from an Italian registry, 23 patients were actively followed and analyzed in a case-control study. The study looked into the patients' characteristics and their evolution (15). Those patients were matched with patients having HCC arising in liver cirrhosis secondary to alcohol or viral hepatitis. These cases were compared with controls regarding the body mass index (BMI - current and pre-cirrhosis), MS, type 2 diabetes mellitus (T2D) and presence of hypertriglyceridemia. The overall prevalence of HCC arising in cryptogenic cirrhosis was of 6.9%, lower than the prevalence of HCC arising in alcoholic or viral cirrhosis but higher than that for HCC arising in primary biliary cirrhosis (15). In the univariate analysis T2D, dyslipidemia, and higher fasting levels of glucose, cholesterol, and triglycerides were more frequently found in patients with cryptogenic cirrhosis and HCC. Insulin resistance (IR) was also clearly associated with HCC secondary to cryptogenic cirrhosis. A multivariate analysis showed that hypertriglyceridemia, T2D, and normal alanine aminotransferase were independently associated with cryptogenic cirrhosis or cirrhosis-post NASH and HCC. The authors concluded that HCC is part of the clinical spectrum of liver diseases in NAFLD and it should be considered in the natural history of progressive NAFLD (Fig. 1).

The studies of Marrero (16) and Ratziu (17) documented a range of 18% to 27% incidence of HCC in cryptogenic cirrhosis in obese patients (but not in lean patients with cryptogenic cirrhosis). In both studies cryptogenic cirrhosis was attributed to unclassified NAFLD based on clinical parameters of MS. Both groups of study noted that the incidence of HCC in NAFLD-related cirrhosis (cirrhosis post-NASH) is similar to the incidence of HCC in HCV-related cirrhosis. These data contrast with Hui's study on 23 patients with cirrhosis post-NASH in which after 10 years of follow-up no cases of HCC occurred (18).

More interestingly, a study that evaluated 162 patients with HCC, noted that a significant number of non-cirrhotic NAFLD patients developed HCC, without the crucial stage of cirrhosis.

![Fig. 1. Natural history of NAFLD.](image-url)
(19). Even more, the life expectancy of NASH patients with HCC is generally thought to be poor because of their associated co-morbidity, i.e., MS that often limits therapeutic choices (including orthotopic liver transplantation) (20).

These results are conflicting and they must be confirmed by future prospective studies. Nevertheless, a more aggressive screening program for HCC must be offered to patients with cirrhosis-post NASH (8).

The lack of impact on the mortality caused by simple hepatic steatosis or FL was initially observed in Danish study (21). This study found a liver-related mortality rate of less than 1% among patients with bland steatosis during an average follow-up of 17 years. Quite similar data were found in a cohort study (22), even though the follow-up period was shorter (ten years). Surprisingly, in these studies the mortality rate from other causes than liver failure (mainly CV and malignancy) was not different in patients with the generic diagnosis of NAFLD compared to normal population. Would this finding forecast a "protective" CV effect of NAFLD? Clinical investigators faced a paradox or a series of misunderstandings since the studied population was extremely heterogeneous.

Another community based study found that the overall death rate in 420 patients with NASH was higher than expected after a mean follow-up of seven years. This study was a registry study, avoiding the bias of referred patients, and it included possibly all the not deeply clarified NAFLD patients in that population. In this study none of the 10 patients with histological proven simple hepatic steatosis developed cirrhosis or died from a liver-related cause. Authors reported 53 deaths in their cohorts and described liver-related illnesses as the third most common cause of death (13%) after malignancy (28%) and ischemic heart diseases (25%). The liver-related mortality, in the 49 patients with histological proven NASH was 8%. However, these liver-related deaths appeared in 8 out of 49 NASH patients who had proven cirrhosis at the beginning of the study (5 out of 7 liver-related deaths occurred in these 8 patients). If this group would be excluded from the final analysis, then only 2 patients died of a liver-related illness, which accounts for 4.1% of causes of deaths and 0.5% of all the deaths in the cohort. After eliminating the patients with cirrhosis on initial presentation, the liver-related death would have ranked the 13th place as the leading cause of death in the general population (23). So, we can infer that the outcome of patients suffering from confirmed cirrhosis at both baseline and during follow-up period, is generally poor, with a third of patients dying from a liver related cause and very few patients developing HCC. The increased overall mortality in the unclassified NAFLD patients from this study (23) was inconsistent with the normal mortality ratios reported in the Danish and Italian studies even though this higher rate is partially attributable to the presence of a major liver cirrhosis. Surprisingly, some independent risk factors for NAFLD, like obesity and MS, showed no correlation with the mortality, although in the unclassified NAFLD population studied they reached nearly 70%. These are well-known risk factors for CV diseases and also for malignancy even though we would have expected a higher mortality or complications in this group of patients. The strength of this study was to consider the entire spectrum of NAFLD as one disease and to try to study the natural history as a whole. These data are similar to previous consistent studies concerning the natural history of cirrhosis post-NASH (24). As its prevalence and its recognition are growing, cirrhosis post-NASH is an increasingly common indication for liver transplantation. However, NASH may recur post transplantation, as documented in multiple case reports (25-27).

In an updates Mayo Clinic series of 16 patients, who were transplanted for cirrhosis post-NASH, post transplant FL, NASH and cirrhosis occurred in 60%, 33%, and 12.5% of the recipients, respectively (28, 29).

In a preliminary study Selzner et al. (30) presented the outcome following liver transplantation for cirrhosis post-NASH in 106 patients of whom 56 underwent at least one follow-up liver biopsy. Biopsy-proven NASH was observed in 12% of the cases. All patients had T2D and a BMI higher than 30. These studies confirm that, after transplantation, initial unclassified NAFLD, comprehending simple hepatic steatosis or FL, can progress to advanced fibrosis/cirrhosis in some patients.

The analysis of patients with 1) cirrhosis, 2) NASH, 3) simple hepatic steatosis, or 4) unclassified NAFLD, separately should give more clear results on the natural history, mortality and prognosis and could provide less questionable data on the prognostic factors. A proof to this is given by Matteoni et al. (31). They analyzed all liver biopsy specimens with fat accumulation from 1979 to 1987. The biopsies were assessed for the presence of inflammation, ballooning degeneration, Mallory hyaline, fibrosis, iron deposits and HCV-RNA. The outcomes were cirrhosis, mortality, and liver-related mortality (31). In the 132 patients for whom complete data were available, FL (type 1) did not differ from the other three types combined, with respect to gender, race, age, or obesity. Cirrhosis was more common in the other types combined (22%) than in FL alone (4%; p< 0.001). Most of the liver-related deaths occurred in cirrhotic patients. The authors conclude that the outcome of cirrhosis and liver-related death is not uniform across the spectrum of NAFLD, with a poor occurrence in patients with ballooning degeneration and Mallory hyaline or fibrosis (31).

The main studies regarding long-term prognosis of NAFLD are summarized in Table 1.

What makes the disease take an aggressive course? Are we supposed to predict the prognosis on the basis of other factors different from the epidemiological ones? In fact, the main

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Diagnosis</th>
<th>Average follow-up (years)</th>
<th>Number of patients</th>
<th>Cirrhosis prevalence (%)</th>
<th>Liver-related deaths (%)</th>
<th>Overall mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tell, 1995 (32)</td>
<td>FL</td>
<td>9.6</td>
<td>40</td>
<td>0</td>
<td>0</td>
<td>14 (35)</td>
</tr>
<tr>
<td>Dam-Larsen, 2004 (21)</td>
<td>FL</td>
<td>16.7</td>
<td>109</td>
<td>1</td>
<td>0.9</td>
<td>27 (24.8)</td>
</tr>
<tr>
<td>Matteoni, 1999 (31)</td>
<td>NAFLD</td>
<td>8.3</td>
<td>98</td>
<td>20</td>
<td>9</td>
<td>48 (49)</td>
</tr>
<tr>
<td>Adams, 2005 (23)</td>
<td>NAFLD</td>
<td>7.6</td>
<td>420</td>
<td>5</td>
<td>1.7</td>
<td>53 (12.6)</td>
</tr>
<tr>
<td>Ekstedt, 2006 (33)</td>
<td>NAFLD</td>
<td>13.7</td>
<td>129</td>
<td>7.8</td>
<td>1.6</td>
<td>26 (20.2)</td>
</tr>
<tr>
<td>Lee, 1989 (34)</td>
<td>NASH</td>
<td>3.8</td>
<td>39</td>
<td>16.3</td>
<td>3</td>
<td>10 (26)</td>
</tr>
<tr>
<td>Powell, 1990 (35)</td>
<td>NASH</td>
<td>4.5</td>
<td>42</td>
<td>7</td>
<td>2</td>
<td>5 (2)</td>
</tr>
<tr>
<td>Hui, 2003 (19)</td>
<td>Cirrhotic-stage NASH</td>
<td>5.0</td>
<td>23</td>
<td>100</td>
<td>5</td>
<td>6 (26)</td>
</tr>
<tr>
<td>Hashimoto, 2005 (36)</td>
<td>NASH with septal fibrosis or cirrhosis</td>
<td>3.7</td>
<td>89</td>
<td>48</td>
<td>6</td>
<td>8 (9)</td>
</tr>
<tr>
<td>Sanyal, 2006 (37)</td>
<td>Cirrhotic-stage NASH</td>
<td>10</td>
<td>152</td>
<td>100</td>
<td>14.5</td>
<td>29 (19.1)</td>
</tr>
</tbody>
</table>
limitations of these studies are a relatively short-term follow-up and a degree of selection bias of patients undergoing biopsy (usually patients with a more severe disease undergoing the procedure and obviously resulting in a worse prognosis).

NONALCOHOLIC FATTY LIVER DISEASE AND HISTOLOGICAL PROGNOSTIC FACTORS

Though most studies consider simple hepatic steatosis or FL a benign condition and NASH a progressive one, the basic mechanisms are identical, and therefore additional factors are likely to account for the difference. Whatever the form of the disease within its large spectrum, the initial mechanism, i.e. the "first hit", is the same: insulin resistance (IR), while the different histological aspects are the consequence of the secondary events, or "second/multiple hits" that are contextually or successively determined (38).

An analysis of the data from all long-term mortality studies published to date suggests that the presence and severity of fibrosis dictate both overall and liver-related mortality in NAFLD regardless of the pathologists’ determination of NASH or uncertain NASH.

Hepatic inflammation may not be as important as a prognostic factor (39). The exact evaluation of risk factors for fibrosis in the generic diagnosis of NAFLD helps target resources and treatments in order to reduce the development of cirrhosis. In another meta-analysis, longitudinal histological data were compared in order to characterize the natural history of fibrosis progression, and secondly, to identify the predictive factors for progression to advanced fibrosis (stage 3 or greater) in NASH patients (40).

Ten studies were selected comprising 221 patients. Subjects had a histological diagnosis compatible with NASH on their initial biopsy. They received no intervention of proven histological benefit, and underwent two liver biopsies with at least an interval of one year between them. Thirty seven percent of the patients had progressive fibrosis over a mean follow-up interval of 5.3 years (40). The proportional hazards regression analysis demonstrated that age and inflammation on initial biopsy (any inflammation, HR=2.5, p=0.001; grade 1, HR=2.5, p=0.001; grade 2, HR=2.4, p=0.003) are independent predictors of progression to advanced fibrosis. Other traditional parameters (e.g. obesity, T2D, and arterial hypertension) were not statistically significant predictors contrarily to the findings of other studies. In contrast with a previous study (39), the presence of inflammation on the initial biopsy and age are independent predictors of progression to advanced fibrosis in patients with NASH (40).

Then, what are the risk factors that can induce the progression from simple hepatic steatosis or FL to NASH and even cirrhosis? And what triggers a florid inflammation taking to progression from simple hepatic steatosis or FL to NASH and even cirrhosis (41)?

A further support of the fact that simple hepatic steatosis or FL is not by definition benign results from the study of Tarantino et al. (42). They demonstrated similar serum levels of the transforming growth factor-β1 both in patients with FL and in those with NASH suggesting that these entities, apparently separate (at least histologically) and with different evolution and prognosis, share the same pathogenic mechanisms. Thus, they conclude that the "benignity" of FL could be accepted but it is difficult to maintain as some patients with FL may theoretically progress to NASH and end stage of liver disease (42).

There is also a methodological limitation in the studies on the natural history of the NAFLD since two stages of the same disease are followed-up as separate entities and not as two separate moments of the same entity. The studies on unclassified NAFLD progression are largely limited due to the presence of patients with NASH, the more severe subgroup of NAFLD.

The presence of fibrosis is becoming a classical and validated prognostic factor. But, is fat deposition in hepatocytes or steatosis itself as benign as it is believed? The relationship between the severity and zonal location of steatosis and the presence of steatohepatitis and various histological features that define NASH was studied by Chalasani and co-workers (43). They looked at the relationship between the severity and zonal location of steatosis and the lobular inflammation, presence of ballooning, Mallory bodies, fibrosis score, and definite steatohepatitis. Increasing levels of steatosis severity were positively associated with lobular inflammation (p<0.0001), zone 3 fibrosis (p<0.001), and definite steatohepatitis (p<0.02), but were unrelated to ballooning, Mallory bodies, or advanced fibrosis (43). As compared to zone 3 steatosis, panacinar steatosis was more often associated with ballooning, Mallory bodies, and advanced fibrosis. Although they concluded that deeper studies are needed to confirm this observation and to explore its significance, their histological study proved that patients with severe steatosis are more likely to have steatohepatitis and consequently a worse prognosis (43). Anyway, we should rely on various prognostic factors (clinical aspects, laboratory data, instrumental findings, histological or imaging features).

An up-to-date study based on electron microscopic findings of FL and NASH showed that ultrastructural characteristics are similar, which suggests that simple hepatic steatosis or FL may also have the potential to progress to fibrosis and cirrhosis like NASH (44). Moreover, another group found that patients with simple hepatic steatosis or FL may still develop NASH and fibrosis progression, testified by paired repeated liver biopsies at three years (45). Studying the mechanisms of progression of the initial form of NAFLD could be a satisfactory approach to further clarify this aspect. It is stated that starvation strongly affects the metabolism of lipids (46), but it is not clear if these processes are subsequently accompanied by fibrosis of the liver tissue and how long it develops. It could be of some interest to verify how much time is required for simple steatosis development depending on the restriction stage, and whether it merges into other NAFLD more severe nosologic units.

The objective of a recent work was to study the mechanism of liver parenchyma development under the influence of diet restriction in a chicken animal model of NAFLD. The slices of liver parenchyma samples, obtained at 35 days (advanced age)
by necropsy, were stained by hematoxylin-eosin and by Sirius red kit for collagen type I and reticulin visualization. Microvesicular liver steatosis was observed. Hepatocyte diameter was significantly influenced by sex, and by the experimental group (restriction and control). An increase in the histologic grade of steatosis was associated with inflammatory changes, with an increase of the hepatocyte diameter and with an increasing proportion of interstitial tissue to the liver parenchyma. These results show that prolonged restriction is associated with the development of fibrosis of the liver tissue and could support the hypothesis that simple hepatic steatosis or FL can develop into a first step to fibrosis and then towards NASH/cirrhosis (47).

To assess whether isolated steatosis was considered a benign condition with no or a minimal rate of progression, in contrast to NASH which can progress to cirrhosis, Ratziu et al. reported on a series of six patients with a biopsy-proven isolated steatosis followed-up by performing a second biopsy five years after. All but one of the initial biopsy samples were adequate, i.e., longer than 1.5 cm. At follow-up, inflammation and ballooning were detected in all patients and mild fibrosis in three out of five. All patients had at least one feature of MS at baseline. As expected, NAFLD was an early manifestation of the MS (2). Most interesting, progression to NASH occurred independently of the elevation of aminotransferases. Five patients showed further metabolic risks during the follow-up: increase in body mass index, triglyceride levels, HOMA or presence of arterial hypertension. Only one patient did not exhibit progression, even though he was still exposed to metabolic risks factors at the end of the follow-up. This report demonstrated that isolated steatosis was not necessarily a benign, non-progressive condition. Ratziu et al. concluded that if metabolic risk factors persist or deteriorate during the follow-up period and/or non-invasive markers suggest a disease progression, a control liver biopsy should be considered (48).

A further confirmation comes from a recent study on 50 NAFLD patients, well characterized by histology. This study showed that the hepatic venous pressure gradient (HVPG) was normal in 27 patients (54%), borderline (5 mmHg) in 9 patients (18%) and elevated in 14 patients (28%). Nine of these latter patients had fibrosis score 0. The degree of steatosis and not the score of fibrosis was the only independent predictor of the presence of portal hypertension (49).

CHALLENGES FOR THE FUTURE

The pathogenesis of NASH is multifactorial. IR and its subsequent inflammatory cascade is associated with the progression of initial NAFLD towards higher risk fibrotic states and appears to also play a significant role in the carcinogenesis (50). A different point of view emphasizes that circulating concentrations of inflammatory cytokines from the adipose tissue are the most important factor in causing and maintaining the IR (51). Growth factors and hormone imbalances are also fundamental in the development of NAFLD (52).

IR may be an important factor in accumulation of hepatocellular fat, whereas genetic predisposition, apoptosis, adenosine triphosphate depletion, elevated dietary fructose intake, altered intestinal motility, serotonin degradation by monoamine oxidase A as an important source of reactive oxygen species, mitochondrial dysfunction, impaired innate immunity regulation and endoplasmic reticulum (ER) stress may be important causes of hepatocellular injuries in the steatotic liver.

Recently, several researchers have focused their attention on the ER stress activation, which has been reported in models of hepatic steatosis in rodents. The ER stress activation, observed in the adipose tissue of obese humans (53), could have metabolic consequences and participate in the deposition of fat in the liver. The ER stress activation could directly induce an insulin-resistant state in adipocytes. Indeed, it has been shown that the activation of the ER stress sensor kinase/endonuclease inositol-requiring protein 1 (IRE1), branch of the unfolded protein response (UPR), could stimulate c-Jun amino-terminal kinase (JNKs) (54), which, by phosphorylating insulin substrate receptor 1 on serine residues, is a key player in the development of the IR (55). The IRE1/-box binding protein 1, branch of the ER-stress signaling pathway, has been recently shown to regulate and be regulated by innate immune signaling pathways in both the presence and absence of ER-stress (56). Disruption of ER homeostasis has been observed in the liver and adipose tissue of humans with NAFLD and/or obesity. Very importantly, the signaling pathways activated by the disruption of ER homeostasis, the UPR, has been linked to inflammation and apoptosis, lipid biosynthesis, insulin action, all of these being involved in the formation/evolution of NAFLD. Indeed, the ER is a crucial organelle for the cellular homeostasis, in which the synthesis and the post-translational modifications of membrane and secreted proteins take place, as well as the synthesis of lipids and cholesterol for membranes formation. However, the ER quality control system can be compromised under a variety of conditions such as accumulation of unfolded protein, alteration of calcium homeostasis or disruption of the redox state. UPR activates JNKs (54, 57).

On the other hand, the contribution of adipose tissue to metabolic homeostasis has become a focus of interest. The adipose tissue secretes free fatty acids (FFAs) and hormones, known as adipokines, and thus it seems to play a major role in the development of NAFLD. Indeed, toxic FFAs can activate the intrinsic apoptosis pathway in hepatocytes via-c-jNK. JNK activates the proapoptotic protein Bim, resulting in Bax activation (58) and enhanced apoptosis, termed ‘lipoapoptosis’. Obviously, the death of apoptotic cells is a prominent feature in NASH. The chicken-egg dilemma of IR being the cause or effect of inflammation is presently unsolved. Anyway, it is high time researchers thought of studying anti-inflammatory drugs such as aspirin, anti IL-6 receptors, immune-modulators (motor- or calcineurin inhibitors), (59) and anti-JNKs in well-conceived trials to try to minimize the high impact of the spectrum of NAFLD, similar different expressions of the same disease, on the whole population. Anyway, the mechanisms are complex and very difficult to decode.

Recently the common adiponutrin (PNPLA3) polymorphism p.1148M has been identified as a genetic determinant of severe forms of non-alcoholic fatty liver disease and alcoholic liver disease (60). This is a confirmation of the complexity of the metabolic pathways involved in NAFLD and is a direction of further studies.

To complicate the puzzle a simple measure as the administration of melatonin can lead to significant improvement of the mean plasma ALT, AST and GGT levels among patients with NAFLD probably through a hepatic cell protection from oxidative damage by a variety of mechanisms (61).

CONCLUSIONS

Can we identify patients at risk of progression towards more severe forms of NAFLD? Is the biopsy the only reliable predictor we can count on? How many times and at what intervals of follow-up should this invasive procedure be used to establish the evolution of NAFLD? When are we supposed to start treating NAFLD patients? Extremely difficult questions to be answered. Anyway, we have tried to shed some light on this problem, taking into account its major health impact and the variate and sometimes unpredictable evolution of NAFLD.
Until researchers do not clarify the exact steps towards the evolution of the various forms of NAFLD, the dogma: "simple" steatosis-benign prognosis, cirrhosis-severe disease tends to somehow vacillate. Each case of NAFLD, mainly FL, should be regarded cautiously and should be separately judged and eventually treated in the light that NAFLD is not an exclusive hepatic disease (62).

Conflict of interests: None declared.

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