The Liver as a Target

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ASH and NASH

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Abstract
Non-alcoholic steatohepatitis (NASH) and alcoholic steatohepatitis (ASH) have a similar pathogenesis and histopathology but a different etiology and epidemiology. NASH and ASH are advanced stages of non-alcoholic fatty liver disease (NAFLD) and alcoholic fatty liver disease (AFLD). NAFLD is characterized by excessive fat accumulation in the liver (steatosis), without any other evident causes of chronic liver diseases (viral, autoimmune, genetic, etc.), and with an alcohol consumption ≤20–30 g/day. On the contrary, AFLD is defined as the presence of steatosis and alcohol consumption >20–30 g/day. The most common phenotypic manifestations of primary NAFLD/NASH are overweight/obesity, visceral adiposity, type 2 diabetes, hypertriglyceridemia and hypertension. The prevalence of NAFLD in the general population in Western countries is estimated to be 25–30%. The prevalence and incidence of NASH and ASH are not known because of the impossibility of performing liver biopsy in the general population. Up to 90% of alcoholics have fatty liver, and 5–15% of these subjects will develop cirrhosis over 20 years. The risk of cirrhosis increases to 30–40% in those who continue to drink alcohol. About 10–35% of alcoholics exhibit changes on liver biopsy consistent with alcoholic hepatitis. Natural histories of NASH and ASH are not completely defined, even if patients with NASH have a reduced life expectancy due to liver-related death and cardiovascular diseases. The best treatment of AFLD/ASH is to stop drinking, and the most effective first-line therapeutic option for NAFLD/NASH is non-pharmacologic lifestyle interventions through a multidisciplinary approach including weight loss, dietary changes, physical exercise, and cognitive-behavior therapy.

Introduction

Non-alcoholic steatohepatitis (NASH) and alcoholic steatohepatitis (ASH) have a similar pathogenesis and histopathology but a different etiology and epidemiology [1, 2]. NASH and ASH are the advanced stages of non-alcoholic fatty liver disease (NAFLD) and alcoholic fatty liver disease (AFLD) respectively, but the conditions causing the progression of uncomplicated liver steatosis to NASH or ASH are presently unknown. NAFLD/NASH and AFLD/ASH are increasingly relevant public health issues, first of all because of their close association with the worldwide epidemics of diabetes and obesity. They are common chronic liver diseases (CLD) and are expected to affect substantially the healthcare expenditure in

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forthcoming years [3]. Here we will review NAFLD/NASH and AFLD/ASH focusing on the association between lifestyle and liver disease.

Definition, Epidemiology and Natural History

Both NAFLD and AFLD are characterized by excessive fat accumulation in the liver, i.e. liver steatosis. When steatosis coexists with cell injury and inflammation (steatohepatitis), the disease is named NASH or ASH. Primary NAFLD/NASH is associated with insulin resistance (IR) and its metabolic manifestations. Secondary NAFLD/NASH, which is rare in adults, is not associated with IR but is caused by a number of medical or surgical conditions and drug toxicity. The operational definition of NAFLD/NASH requires the exclusion of other causes of liver disease (viral, autoimmune, genetic, etc.) and an alcohol intake $\leq$20–30 g/day. This amount is based on epidemiological studies showing that alcohol-induced steatosis occurs above this threshold [4, 5]. Owing to its increasing prevalence and strong association with the metabolic syndrome [6], it is now recognized that NAFLD/NASH can occur in association with other CLD [2], and that in some circumstances (chronic hepatitis C [7], hemochromatosis [8], alcoholic liver disease [9]) this association can increase liver damage [10].

The prevalence of AFLD/ASH and NAFLD/NASH varies among populations in relation to drinking and lifestyle habits. Up to 90% of alcoholics have fatty liver (FL) at ultrasound and 5–15% of them will develop ASH and cirrhosis over 20 years [11]. From 10 to 35% of alcoholics exhibit changes on liver biopsy consistent with alcoholic hepatitis. If alcohol is suspended, 10% of these patients will reverse completely the clinical and histological picture. The risk of cirrhosis is 30–40% higher in those who continue to drink alcohol. One epidemiological study has estimated that for every liter increase in *per capita* alcohol consumption and independently from the type of beverage, there is a 14% increase in the risk of cirrhosis in men and one of 8% in women [11]. Simple FL is usually asymptomatic and may reverse after 4–6 weeks of abstinence from alcohol [12]. Progression to fibrosis and cirrhosis may occur however in 5–15% of patients despite abstinence from alcohol [13, 14]. In one study, persistent alcohol intake >40 g/day increased the risk of fibrosis or cirrhosis of 30–40% [15].

The prevalence of AFLD/NAFLD in the general population, as assessed by ultrasonography, is 20–30% in Europe [16] and in the Middle East [17] and 15% in the Far East [18, 19]. It is of interest that the prevalence of NAFLD is similar (16%) in selected populations made by normal weight subjects without metabolic risk factors [5]. A similar prevalence (15–25%) had been reported in the past by autoptic studies [20, 21]. A surprisingly high prevalence of histologically diagnosed NAFLD has been reported in apparently healthy liver donors (12–18% in Europe [22, 23] and 27–38% in the USA [24, 25]). With sensitive imaging techniques such as magnetic resonance spectroscopy (MRS), 34% of US adults appear to have NAFLD [26]. Nearly 40% of newly identified cases of CLD in the USA are attributable to NAFLD [27]. Recent studies performed in tertiary-care centers have shown a high prevalence of histologically diagnosed NASH among patients with NAFLD: 43–55% in patients with elevated serum aminotransferase levels [28, 29], 49% in morbidly obese patients [30, 31], and 67% in a subset of patients with incident CLD [27]. The incidence of primary NAFLD in Italy was estimated to be 2/100/year [32] while a Japanese study in a more selected population reported 10/100/year [33]. In comparison, NASH secondary to tamoxifen use has an estimated incidence of 0.2/100 women/year [34].

Older age, male gender and Hispanic ethnicity are risk factors for NAFLD [26, 27, 35–38]. Having a family member with NAFLD also puts at greater risk for the disease, independently from age and BMI [39, 40]. In the general population, NAFLD/NASH is most commonly associated with IR and its phenotypic manifestations (obesity, visceral adiposity, type 2 diabetes, hypertriglyceridemia and arterial hypertension [6, 41, 42]). A causal association has been suggested by longitudinal studies showing a chronological association between the progression of the metabolic syndrome and the occurrence of NAFLD [43, 44].

The natural history of ASH and NASH is not completely known. ASH is the most frequent organ damage in chronic alcoholics and the annual death rate attributable to alcohol-induced end-stage liver disease exceeds that of car accidents. While simple steatosis is not associated with excess mortality in long-term follow-up studies [45, 46], patients with either ASH or NASH have a reduced life expectancy due to liver disease and, for NASH, cardiovascular disease [47].

Pathogenesis

The pathogenesis of ASH is a complex process that is not substantially different from the pathogenesis of NASH, and that involves several mechanisms at different

metabolic levels. These mechanisms include increased fat synthesis, increased fat mobilization, defective export of fat from the liver, and decreased fat breakdown [48]. The 'two hits' theory of NASH suggests that oxidative stress and cytokines lead to the development of necroinflammation and ultimately fibrosis and cirrhosis [49]. However, this hypothesis has been challenged by recent data suggesting that mechanisms that can drive disease progression can also induce steatosis. Oxidative stress [50], selected gut bacteria and some cytokines [48] can induce steatosis as well as necroinflammation and fibrosis. Free fatty acid can stimulate hepatocyte apoptosis [51] and endoplasmic stress can lead to steatosis, oxidative stress and apoptosis [52]. Because these mechanisms are important also in obesity and IR, they may be the first hits leading to an increased hepatic flux of free fatty acid, oxidative stress, and cytokine activity that result in steatosis and progressive liver damage in susceptible individuals. Some of the novel findings in the pathogenesis of ethanol-induced liver damage involve the down-regulation of peroxisome proliferator-activated receptor α (PPAR-alpha) and the up-regulation of lipogenic enzymes through the induction of sterol regulatory element-binding protein (SREBP) [51]. A promising line of research involves the adenosine 5′-monophosphate-activated protein kinase, which controls the key metabolites (malonyl coenzyme A and long-chain acyl-coenzyme A) responsible for the balance between fat synthesis and fat degradation [53]. The effect of an excessive dietary intake of n–6 and n–3 polyunsaturated fatty acids (PUFA) remains controversial [54]. The association of alcoholic liver disease with circulating autoantibodies, hypergammaglobulinemia, antibodies to unique hepatic proteins, and cytotoxic lymphocytes reacting against autologous hepatocytes, strongly suggests an altered immune regulation with loss of immunotolerance [55]. There are several immune processes recognizing self-proteins that are modified by alcohol metabolites. In the past, ALD was attributed to dietary deficiencies, but experimental and clinical studies have established that alcohol hepatotoxicity is produced by oxidative stress mostly through the microsomal cytochrome P4502E1 (CYP2E1) and by immune responses against self-proteins [56]. Steatosis should therefore be considered only a part of the liver early 'adaptive' response to stress rather than a first hit for disease progression. Instead, the attention should be focused on the mechanisms responsible for cellular injury and fibrosis, which may be similar for ASH and NASH. Identified mediators of fibrosis include hepatocyte factors arising as a direct result of steatosis or and apoptosis such as reactive oxygen species and cytokines, Kupffer cells, T cells, hepatocytes, stellate cells and other cells responding to hepatocyte injury and gut-derived bacterial products [53]. IR and hyperglycemia may induce fibrosis directly or by up-regulating the synthesis of connective tissue or by generating advanced glycation end-products [57, 58]. Extrahepatic contributions to liver fibrosis come from the gut, a source of profibrogenic bacterial products such as lipopolysaccharide, and visceral adipose tissue as a source of profibrogenic adipcytokines as leptin, renin-angiotensinogen and norepinephrine [59]. Equally important may be the lower secretion of adiponectin in obesity, an antisteatotic, anti-inflammatory and antifibrotic adipocytokine [60]. At least some of the postulated anti-NASH effects of adiponectin may be mediated by the activation AMP-kinase, which is also a target for some antidiabetic drugs such as metformin and glitazones [61] (fig. 1).

Diagnosis

The diagnosis of alcoholic liver disease is based on a combination of features, including a past and present history of significant alcohol intake (≥20–30 g/day), clinical evidence of liver disease, and supporting laboratory abnormalities [62]. Different biomarkers of alcohol intake have been evaluated in various settings, including large population surveys. However, the low sensitivity and/or specificity of these tests prevents reliance on any single biomarker [63]. Among these markers, the most commonly employed are γ-glutamyl transpeptidase [64, 65], mean corpuscular volume, serum transaminases (AST/ALT ratio >3) [66, 67] and carbohydrate-deficient transferrin [68]. Tests able to assess the presence of simple steatosis would be useful if they had a higher sensitivity than conventional imaging. Tests that quantify steatosis might also be clinically useful to monitor changes induced by therapy and to predict metabolic complications of liver disease. The available tests cannot be easily compared for their diagnostic performance as they have been validated against different standards: ultrasonography [69], liver biopsy [70] or magnetic resonance imaging (MRI) [71]. The fatty liver index (FLI) [69] and the lipid accumulation product (LAP) [72] predict liver steatosis in the general population and may be useful for large-scale screening in place of ultrasonography. The SteatoTest [70] and the NAFLD score [71] have higher sensitivity than ultrasonography and may be used to quantify steatosis. Only the SteatoTest and the FLI have been independently validated [47, 69, 70, 72, 73]. Also available are non-invasive tests

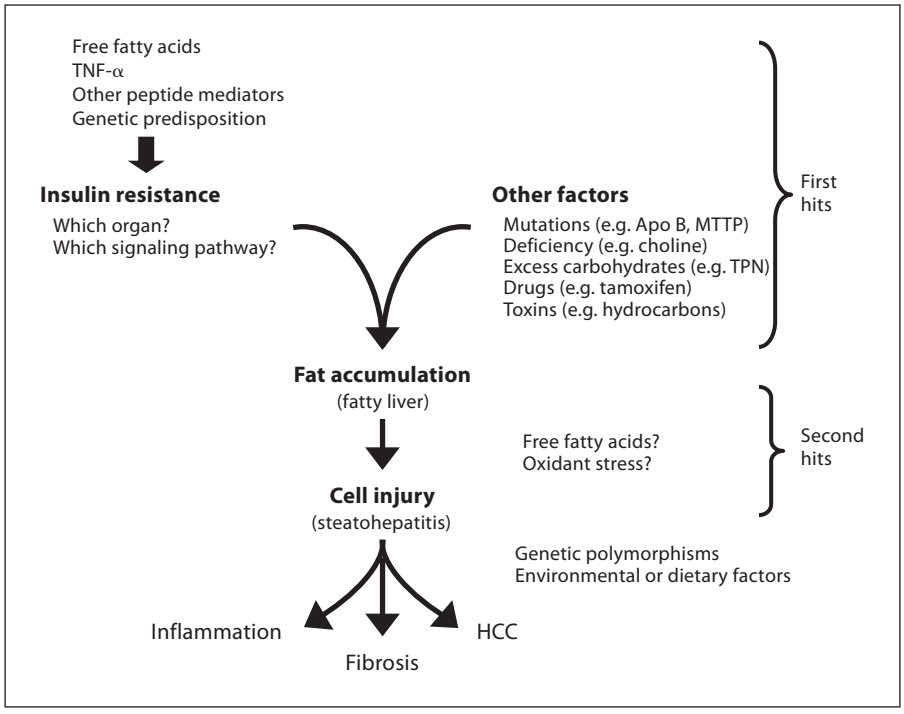

Fig. 1. Mechanisms of fat-induced inflammation and fibrosis in ASH and NASH.

that predict fibrosis (FibroTest [74], ELF panel [75] and FibroMeter [76]) and simpler clinical scores [77–79]. Most of these tests can be used to distinguish between advanced and minimal/no fibrosis but a few offer a proper staging of fibrosis [74, 76]. Since IR is strictly associated with the presence of NAFLD/NASH, direct measurement of IR or surrogate markers is useful for clinical or research purposes. In this respect, waist circumference, FLI and LAP are strongly associated with IR [69, 72, 80]. The Homeostasis Model Assessment (HOMA) [81] and the Quantitative Insulin Sensitivity Check Index (QUICKI) [82] are the most commonly employed surrogate indexes of IR or IS. Other more sophisticated methods are based on the dynamics of glucose and insulin in response to a glucose tolerance test [83, 84]. Insulin sensitivity on lipid metabolism may be assessed in the fasting state using the ratio between triglycerides and HDL cholesterol [85].

Imaging techniques, such as ultrasonography, computed tomography (CT) and MRI, detect steatosis in 20–30% of patients from the general population [86] but offer no information on inflammation or fibrosis [87]. Despite lower sensitivity and specificity as compared to CT, ultrasonography is an acceptable first-line procedure to diagnose AFLD and NAFLD in clinical practice. Quantification of steatosis by ultrasonography is reliable only if performed by a single and skilled operator [88]. MRI and MRS quantify steatosis reliably and but are limited by standardization problems and the high cost [89, 90]. Other techniques for quantifying fibrosis, such as diffusion-weighted imaging or magnetic resonance elastography, are promising but still experimental. In selected patients with NAFLD, the measurement of liver stiffness by transient elastography has a diagnostic performance for fibrosis close to that for hepatitis C [91, 92]. However, the available cut-offs for fibrosis have not been extensively cross-validated and steatosis and inflammation can increase liver stiffness [93, 94]. Body mass index (BMI) is a major predictor of the failure of transient elastography (25% >30 kg/m^2, 41% >35 kg/m^2 [95]). Moreover, the usual values of liver stiffness in non-obese healthy individuals without the metabolic syndrome can be as high as 8 kPa [95], and increased liver stiffness can be seen without fibrosis in different conditions [96–98]. New probes for obese individuals are currently being tested. None of the available non-invasive blood or imaging tests can distinguish simple steatosis from NASH or ASH. The diagnosis of steatohepatitis allows to identify patients at risk for fibrosis progression and justifies more intensive lifestyle counseling and the use of pharmacological treatments [99]. Two serum markers, the NASH test [100] and the serum CK18 level [101], have been validated in large multicentric studies, while another one, the NASH diag-

nostics test [102], was studied only in small series. CK-18 is a promising marker but its diagnostic performance may be suboptimal if used alone [102] and it is also affected by the amount of fibrosis [101].

Histopathology

Liver biopsy is the 'gold standard' for the diagnosis of NASH and ASH as it is the only reliable means by which to evaluate inflammation and fibrosis. The presence of steatosis is a prerequisite for the diagnosis of AFLD and NAFLD, with the exception of the cirrhotic stage, where it can be absent. Steatosis is defined by a hepatocyte content of fat ≥5%. Simple steatosis or steatosis with lobular inflammation but without hepatocellular injury do not qualify as NASH because they have a more favorable outcome [103]. When ASH or NASH is present, inflammatory infiltrates of mixed cells can be detected in the hepatocytes and portal spaces. In the case of NASH, the liver infiltrates are mainly centrilobular (zone 3 of the acinus). Other findings are hepatocyte ballooning and Mallory bodies [104]. Histology cannot reliably differentiate ASH from NASH, even if cytoplasmic clarification and hepatocyte ballooning, with or without acidophil bodies or spotty necrosis are considered now a cardinal feature for the diagnosis of NASH [105]. There is no widely accepted grading system for NASH. The NASH score is the unweighted sum of steatosis, ballooning and lobular inflammation [106] and was designed primarily to assess treatment-induced changes. It can be used for grading purposes, but it should not be used for the diagnosis of NASH [106]. All the available grading and staging systems have not been sufficiently validated for use by general pathologists. As for most CLD, fibrosis may be present or not and therefore it is not part of the NASH/ASH definition. Perisinusoidal fibrosis is a characteristic feature of NASH and current staging systems incorporate both perisinusoidal and portal fibrosis [106]. Fibrosis is believed to start in the perivenular area in ASH and is proportional to the amount of alcohol [107, 108]. It occurs in 40–60% of those patients who have drunk >40–80 g/day of alcohol for 25 years and is an independent risk factor for the progression to fibrosis or cirrhosis [109]. Progression of alcoholic liver disease culminates in the development of cirrhosis, which is usually micronodular [110]. A distinct histological pattern has been reported for two specific populations with NASH. In children, NASH is characterized by portal inflammation and fibrosis, azonal steatosis and infrequent ballooning or perisinusoidal fibrosis [111]. In bariatric surgery patients, NASH is characterized by isolated portal fibrosis and azonal steatosis [105, 112, 113]. As in other CLD, sampling variability is a limitation of liver biopsy in AFLD/NAFLD [114] and has been shown to affect the diagnosis and staging of ASH/NASH [114–116]. By analogy with other CLD, a bioptic fragment of liver of a minimum of 15 and preferably 25 mm is desirable [115].

Treatment

Pharmacologic treatment for ASH is usually employed in acute hepatitis and to promote alcohol abstinence in alcoholics. For NASH, the main use of drugs is for the correction of concurrent metabolic disorders (statins, antihypertensive agents, antidiabetic drugs, etc.). Pharmacologic treatment of alcoholic liver disease should be based on the stage of the disease and the aims of treatment [117]. As for NASH, there are no approved medications and all the drugs in use are experimental. For these reasons, we will not consider any kind of pharmacological treatment [for recent reviews, see 118, 119] but we will focus on lifestyle and behavioral intervention. FL is the early stage of alcoholic liver disease and is usually reversible with abstinence [120]. Abstinence is the most important therapeutic intervention for patients with alcoholic liver disease [121] and has been shown to improve clinical and histological outcomes and survival [122–124]. Continued alcohol ingestion results in an increased risk of bleeding from portal hypertension and decreases survival [125]. Protein-energy malnutrition is common and is a strong predictor of survival in chronic alcoholics. They have also deficiencies of some vitamins (folate, thiamine, pyridoxine and vitamin A), with corresponding clinical pictures of anemia, altered mentation and night blindness. The causes of malnutrition in these patients are multiple and include anorexia, abnormal digestion of macronutrients, abnormal absorption of several micronutrients, increased skeletal and visceral protein catabolism, and altered lipid metabolism [126]. An above-normal intake of proteins (1.5 g/kg body weight/day) and energy (40 kcal/kg body weight/day) is to be used in the presence of intermittent acute illness or exacerbations of the underlying disease [127]. Nutritional therapy provided either enterally or parenterally improves malnutrition and may improve survival. Micronutrient deficiencies require specific supplementation. Psychotherapy is often essential to achieve abstinence and better manage liver transplantation in selected individuals.

Conversely, the most effective first-line therapeutic option for NAFLD/NASH patients are lifestyle changes obtained by means of a multidisciplinary approach including weight loss, dietary changes, physical exercise, and cognitive behavior therapy [128]. These changes should be implemented on a long-term basis, in all patients with NAFLD/NASH, regardless of the severity of liver disease. The minimal amount of weight loss for improving NASH has not been determined. A modest weight loss results in a significant reduction in liver fat despite minimal reduction in body fat [129, 130]. A 5–10% weight loss can suffice for aminotransferase normalization [131, 132]. A small study has shown that a weight loss of 9% improves steatosis and has a modest effect on inflammation but no effect on fibrosis [133]. A comparison of four dietary regimens has shown that weight loss is similar regardless of the macronutrient profile [134] and it is possible that any type of diet may be beneficial as long as the patient adheres to it. However, only 15% of NAFLD patients lost more than 10% of their weight and most regained weight [134]. Behavioral therapy should be implemented whenever the required resources, which are considerable, are available [135]. Regardless of weight loss, consumption of high fructose corn syrup and industrial *trans*-fats (present in many processed foods) is associated with the development of NAFLD, IR and hepatic inflammation [136–139], and soft drinks and certain dietary constituents should be kept to a minimum or avoided. Finally, a low dietary n–3/n–6 PUFA ratio has been reported for NASH patients [140–142] and experimental data suggest that n–3 PUFA supplementation may lead to both metabolic and histological improvement [143–146]. Patients with NAFLD/NASH engage in less than half the amount of exercise performed by age- and sex-matched controls [73] and only 20–33% of them meet current recommendations for physical activity [147]. Reasons for not exercising include fatigue [148], reduced cardiorespiratory fitness [147, 149], weight-related arthritis, cardiovascular disease and psychological reasons [150]. Physical activity is inversely associated with intrahepatic fat [151], insulin sensitivity [152] and abdominal fat [153]. In obese individuals, short-term (4-week) aerobic exercise reduces hepatic and visceral fat even without a change in body weight or dietary intake [154]. Longer-term (3-month) exercise improves cardiorespiratory fitness, IR and liver enzymes independent of weight loss [155]. Physical activity targets obtained from diabetes prevention trials could be applied to adult patients with NAFLD/NASH: at least 150 min/week of moderate activity (brisk walking) and at least 75 min/week of intensive activity (jogging), in addition to muscle-strengthening activities twice a week. However, individualized counseling is preferable whenever possible and even limited physical activity is better than none. Limiting sedentarity is equally important [156]. Complications of alcoholic and non-alcoholic cirrhosis such as encephalopathy and portal hypertension are treated in the usual way but with particular attention to organ disease triggered by alcohol [157].

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