Practical management of the increasing burden of non-alcoholic fatty liver disease

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Abstract

Obesity-induced liver disease (non-alcoholic fatty liver disease (NAFLD)) describes a spectrum from steatosis through steatohepatitis to cirrhosis. Its prevalence is rising in tandem with societal rates of obesity which through consequent insulin resistance and fat deposition in hepatocytes lead to hepatocyte death and attempts at repair, which if persistent, lead to activation of liver fibrogenic cells. NAFLD, which may also progress to primary liver cancer, is now the most common cause of chronic liver disease in affluent countries. There is currently no single accurate diagnostic test besides a liver biopsy. The decision to consider a liver biopsy will be informed by the presence of insulin resistance determined by comparatively easy-to-measure factors together with other putative markers of progression such as hypertension. If a liver biopsy is performed, patients with steatosis with no evidence of inflammation may be less aggressively managed while those with steatohepatitis, since they have a faster trajectory to cirrhosis, should be managed more robustly. Besides lifestyle changes and increased aerobic exercise other strategies include considering referral to centres with ongoing clinical trials. Emerging treatments include α 1 adrenoceptors antagonists, angiotensin receptor blockers, glitazones and vitamin E.

Introduction

Non-alcoholic fatty liver disease (NAFLD), now the leading cause of liver dysfunction in developed countries, defines the spectrum from steatosis to cirrhosis and hepatocellular carcinoma.¹ The predominant cause of NAFLD is obesity and its pathogenesis involves insulin resistance and increased oxidant stress¹ with consequent activation of matrix producing cells and propagation of fibrogenesis. NAFLD cannot be diagnosed with a single test.¹ Given its association with obesity, the

initial treatment is diet and increased physical activity. Emerging treatments include glitazones, vitamin E, angiotensin receptor blockers and α 1 adrenoceptor antagonists.¹

Definition

NAFLD was first described as a clinical entity by Ludwig *et al*.² The term implicitly acknowledges that the characteristics of NAFLD resemble those of alcoholic fatty liver but without a history of immoderate alcohol use. An alcohol consumption of <140 g for men and <70 g per week for women has been accepted as 'little or no alcohol' by some authorities and in recent times NAFLD has been defined as the hepatic manifestation of the metabolic syndrome.^{2,3}

NAFLD is a spectrum of liver disease ranging from hepatosteatorosis (fatty liver), to non-alcoholic steatohepatitis (NASH) (fat with inflammation), through to fibrosis and, potentially, cirrhosis and hepatocellular carcinoma.^{4,5} Patients with NAFLD who become cirrhotic usually exhibit a reduction in their degree of steatosis.⁵ The terms NASH and NAFLD are often wrongly used as synonymous. More correctly, NASH is a more severe stage of NAFLD.

Prevalence

The population prevalence of NAFLD has been estimated at 7–35%, using alanine transaminase (ALT) level, ultrasound scanning or magnetic resonance spectroscopy (MRS), as diagnostic techniques, of which MRS is most sensitive for detecting liver fat.^{6–10} NAFLD prevalence is around 15% in Asian¹¹ and 20–30% in Western adults.^{10,12} The Dionysos study, reported 25% of adult Italians with NAFLD, of whom 55% had normal transaminase levels, suggesting that use of liver enzymes

alone as a diagnostic marker of NAFLD underestimates the true population prevalence.¹³ Estimates of NASH prevalence are around 2.5%,^{14–16} making NASH more widespread than chronic hepatitis B and C, alcoholic liver disease and other metabolic liver diseases. The rising rates of obesity world wide^{17–18} parallel rising rates of NAFLD and thus it is projected that within the next decade a greater number of patients will receive a liver transplant for NAFLD than for end-stage chronic hepatitis C.¹⁹

Risk factors

Risk factors associated with NAFLD are mainly features of the (dys)metabolic syndrome: obesity, type 2 diabetes, insulin resistance, hypertension and dyslipidaemia. Male sex and increased waist circumference are also well-established risk factors.^{20–22} NAFLD is primarily associated with increased intra-abdominal fat mass. However, NAFLD can occur in lean subjects and in patients with forms of lipodystrophy.^{14–15–23} NAFLD has been shown to be clustered within families^{24–25} and patterns of interethnic variation have also been documented.¹² It has, moreover, been recently shown that maternal obesity by offspring programming during neonatal and immediate perinatal development can increase susceptibility to NAFLD in adulthood.²⁶

Natural history of NAFLD

The natural history of NAFLD is imprecisely defined. Current publications examining NAFLD manifestation can be divided into two main categories: cohort studies using patients diagnosed ultrasonographically with investigation of their clinical outcomes and sequential biopsy studies investigating histological progression.

An observational study reported that 40 patients with steatosis, over a median follow-up period of 11 years, did not progress to NASH or cirrhosis.²⁷ A subsequent study with 132 patients diagnosed with NAFLD and a mean follow-up period of 8 years found that cirrhosis was more common with ballooning degeneration, Mallory hyaline inclusions and/or fibrosis than pure steatosis alone.²⁴

The most recent study from two Scandinavian hospitals with a mean follow-up period of 14 years demonstrated a low risk of progression to cirrhosis from steatosis alone. However, around 20% of patients with NASH developed cirrhosis over the 14-year follow-up period. It was found that patients more often died from cardiovascular and liver-related comorbidities. Furthermore, 5.4% of patients with NASH at inception of the study developed end-stage liver disease including hepatocellular carcinoma.²⁵

In a sequential biopsy study with a mean interval between biopsies of 3.2 ± 3.0 years, fibrosis progression occurred in 37% while 34% remained stable with regression occurring in 29%. Additionally, comorbid cardiovascular disease and diabetes increased the risk of fibrosis progression and mortality after exclusion of cirrhotic patients.²⁸ It appears that NAFLD disease

progression is largely dependent on comorbid insulin resistance as shown by a large prospective cohort study involving around 1 million patients. This study demonstrated an increased risk of hepatocellular carcinoma in patients with type 2 diabetes and NAFLD.²⁹

Clinical features

Most patients with NAFLD are asymptomatic in the early stages of the disease. However, symptoms, when they occur, can include right upper quadrant discomfort and general fatigue. Hepatomegaly and right upper quadrant tenderness may be present on examination.³⁰

Diagnosis

No single diagnostic test reliably detects NAFLD. The diagnosis of NAFLD is presently one largely of exclusion. Its presence is confirmed in the absence of focal liver lesions, positive serology for hepatitis A, B, C and other hepatophilic viruses or aberrant autoimmune, $\alpha 1$ antitrypsin, caeruloplasmin and ferritin profiles.¹ Its definitive diagnosis is dependent on identification of hepatic triglyceride accumulation at the tissue level in an obese, insulin-resistant and dyslipidaemic patient with little or no alcohol history (figures 1–5).

The majority of patients with NAFLD have abnormal liver function tests. However, elevated transaminase and γ -glutamyltransferase levels lack sensitivity and specificity for NAFLD. It has been reported that ALT levels may be normal in the presence of radiological evidence of hepatosteatosis.¹² Furthermore, aberrant liver enzymes may normalise with disease progression. The aspartate transaminase (AST) to ALT ratio is usually <1 , unlike alcoholic liver disease where it is >1 . However, reversal of the AST/ALT ratio to >1 implies advanced fibrosis in NAFLD.¹

Serum albumin and bilirubin remain within physiological ranges in patients with NAFLD. However, these levels become perturbed with disease progression. A low-level inflammatory state exists in obesity and NAFLD and is reflected by increased ferritin levels in the presence of normal iron indices. The increased serum ferritin in this pathophysiological setting is believed to be an acute phase reactant.^{5–31–32} Raised antismooth muscle and antinuclear antibodies, seen in up to 25% of patients with NAFLD, are indicative of more severe inflammation and injury.³³

Ultrasonography is a simple, cost-effective and non-invasive technique used to detect hepatosteatosis. It has a sensitivity of 66–100% for a fat content $>33\%$. Ultrasonography permits grading of steatosis into three main broad categories; mild, moderate and severe. However, quantitative information on fat content is limited using this imaging technique.^{34–35} CT and MRI have similar sensitivity and specificity to ultrasonography, although are clearly more expensive.^{35–36} MRS is the most sensitive imaging modality and detects hepatosteatosis at around 5%.⁶

Transient elastography (fibroscan) is an alternative ultrasound imaging modality. It provides information on liver stiffness, which correlates with hepatic fibrosis. However, it is technically difficult to use in obese patients.³⁷ Furthermore, the published reference ranges for cirrhosis and fibrosis using fibroscan have been largely derived using patients with viral hepatitis, thus lowering its accuracy for detecting fibrosis in NAFLD.³⁷ It is important to note that none of the aforementioned imaging techniques permit differentiation between pure steatosis and steatohepatitis.³⁵

Liver biopsy although invasive is the preferred diagnostic technique. Skelly *et al*³⁸ reported that, of 354 patients being investigated for abnormal liver function tests with negative liver serology, 34% required revision of diagnosis after biopsy. However, a needle biopsy represents only around 1/50 000 of total liver mass and histological lesions are unevenly distributed throughout the hepatic parenchyma.^{39–40} Thus, liver biopsies can misrepresent underlying disease.

Macrovesicular steatosis is the hallmark histological lesion present in NAFLD. Fat accumulation starts in zone 3 but may spread depending on severity to encompass the entire acinus. The extent of hepatosteatosis is broadly graded into three categories; 0–33%, 33–66% and >66% with >5% considered as NAFLD.⁴¹ Haematoxylin and eosin staining illustrate acute or chronic inflammatory cell infiltrates and Mallory's hyaline. Pericellular fibrosis may also be evident on connective tissue staining (figures 1–3).¹

Histopathological features of NASH include the presence of lobular inflammation, hepatocellular ballooning, lytic necrosis and/or apoptotic bodies, predominately in zone 3. Moreover, portal inflammation and dispersed lobular lipogranulomas and microgranulomas may be present.⁴¹

The NAFLD Activity Score is a semiquantitative scoring system developed by the NIH NASH Clinical Research Network for the grading and staging of histological lesions, applicable to both adult and paediatric NAFLD. It is based on the presence of steatosis, hepatocellular ballooning and lobular inflammation, and provides a composite score. A score >5 is classified as NASH and <3 as not NASH.^{42–44} Fibrosis is scored separately with a score of 4 equating to cirrhosis.⁴³

Pathogenesis

The pathogenesis of NAFLD is poorly understood. Obesity in parallel with insulin resistance is thought to be crucial for the development of NAFLD. The association between fat accumulation and hepatic injury, until recently, was explained using the 'two-hit hypothesis'. This hypothesis proposed that the increased supply of free fatty acids (FFAs) due to obesity and enhanced de novo lipogenesis from extrahepatic insulin resistance led to hepatic fat accumulation—the 'first hit'. Subsequently, the liver becomes sensitised to 'second hits' such as oxidative, cytokine-mediated

and endoplasmic reticulum-mediated stress. Hepatic inflammation and injury as seen in NAFLD ensue.⁴⁵ Excess fat deposition within the liver was also thought to arise from impaired FFA oxidation due to hyperinsulinaemia and mitochondrial damage, reduced export of triacylglycerol (TAG) as very-low-density lipoproteins and increased esterification of FFAs to TAGs.⁴⁶ Emerging evidence suggests that TAGs may not be as villainous as previously portrayed since inhibition of TAG synthesis has been shown to increase hepatic injury.^{47–48}

However, studies have demonstrated increased lipid peroxidation secondary to accumulation of hepatic triglycerides.⁴⁹ Consequently, profibrogenic and proinflammatory cytokine pathways, with adiponectin and tumour necrosis factor α being key mediators, are activated.^{50–52} Animal models of NASH

NAFLD referral algorithm

Figure 1 An accurate history of alcohol intake is a mandatory first step in the diagnosis of non-alcoholic fatty liver disease and the decision to refer to a specialist centre. ALT, alanine aminotransferase; ALP, alkaline phosphatase; GGT, γ -glutamyl transferase; LFTs, liver function tests.

NAFLD referral algorithm

Figure 2 An enhanced liver screen will include ultrasound scanning to exclude focal liver lesions plus autoimmune liver serology, viral hepatitis and metabolic liver disease screen.

NAFLD referral algorithm

Figure 3 If the enhanced liver screen is unrevealing and a diagnosis of non-alcoholic fatty liver disease (NAFLD) is being considered, a pragmatic approach may be to wait for about 3 months during which lifestyle changes are started. ALT, alanine aminotransferase; BMI, body mass index; LFTs, liver function tests.

NAFLD referral algorithm

Refer to Liver Clinic or consider liver biopsy if:
 Insulin resistance as evidenced by T2DM
 Raised HOMA score
 Waist circumference >100 cm as a surrogate for ↑HOMA⁹³
 Hypertension

HOMA=Homeostatic assessment method
 HOMA=Fasting insx Fasting glucose/22.5
 HOMA>3.99=Insulin resistance

Figure 4 When to refer to a specialist liver clinic or consider a liver biopsy.

NAFLD referral algorithm

Liver biopsy

Figure 5 If a liver biopsy confirms non-alcoholic fatty liver disease, patients with steatosis may be managed less aggressively than those with non-alcoholic steatohepatitis (NASH) who are at increased risk of progression to cirrhosis and therefore need focused management including referral to centres with ongoing clinical trials.

have demonstrated that an adiponectin deficiency increases susceptibility to hepatocellular carcinoma development.⁵⁰

Additionally, increased oxidation of FFAs by peroxisomes, the microsomal oxidising system in hepatocytes

and mitochondria, all contribute to direct liver injury by upregulation of cytokine gene transcription.^{50 52 53} Other stressors include intestinal bacteria and hepatic iron since studies have demonstrated increased liver injury in NAFLD in the presence of raised hepatic iron.⁵⁴

The effect of diet on NAFLD pathogenesis has recently been investigated. Rapidly absorbed carbohydrates—that is, those with a high glycaemic index, were shown to promote hyperinsulinaemia and hepatic TAG accumulation compared with slowly absorbed carbohydrates.^{55–57} Rapidly absorbed carbohydrate consumption has also been shown to decrease β oxidation and increase hepatic de novo lipogenesis,⁵⁸ inevitably increasing the risk of hepatosteatosis.^{55 59} Common therapeutic agents may also induce hepatosteatosis. Examples include non-steroidal anti-inflammatory drugs, tetracycline, amiodarone, sodium valporate and antiviral drugs such as zidovudine and interferon. Fat accumulation in this context arises from inhibition of the mitochondrial β oxidation of fatty acids.⁶⁰

The endocannabinoid system has also been shown to modulate steatosis and fibrogenesis in NAFLD. Activation of the cannabinoid type 1 receptor in animal models stimulates fatty acid synthesis and hepatic fat accumulation via the transcription factor *SREBP-1c*.⁶⁰ Cannabinoid type 2 receptor expression has also been found to be upregulated in patients with NAFLD,⁶¹ although their actions are predominantly anti-fibrotic.⁶²

It has also been proposed that the sympathetic nervous system and the related renin–angiotensin system both enhance fibrogenesis in NAFLD such that animal models of NASH lacking a functional sympathetic nervous system either through pharmacological or genetic manipulation or reduced angiotensin-1 signalling are poorly fibrogenic.^{63–65}

Treatment

No single therapeutic intervention is currently available. Since NAFLD is largely the consequence of obesity, malnutrition and sedentary behaviour, initial treatment centres on lifestyle modifications. Exercise improves biochemical and histological parameters by reducing visceral fat, enhancing insulin sensitivity and lipid oxidation.⁶⁶ However, the risk of disease progression and cardiovascular comorbidity may warrant pharmacological intervention.

Controlled weight reduction of 5–10% of initial body mass improves or normalises liver enzymes, reduces hepatosteatosis, inflammation and fibrosis.⁶⁷ Weight loss surgery (bariatric surgery) in a cohort of patients with NASH has also been shown to improve hepatosteatosis, necroinflammatory changes and fibrosis. Similarly, a recent prospective study reported improvement of ballooning and steatosis after bariatric surgery at 1 and 5 years, in a cohort of patients with

NASH.^{68 69} Rapid weight loss has, however, been correlated with accelerated disease progression in patients with NAFLD.⁷⁰

Until recently anti-obesity pharmacotherapy included orlistat, sibutramine and the cannabinoid type 1 receptor antagonist, rimonabant. Unfortunately, only orlistat now remains since the withdrawal of rimonabant in 2009 and sibutramine early in 2010. Orlistat is a gastrointestinal lipase inhibitor and as such reduces the absorption of fatty acids. It is thought to be most efficacious as an adjunct to dietary intervention.⁷¹

Data from animal studies have shown that insulin sensitising agents, such as metformin, reduce circulating glucose concentrations by decreasing gluconeogenesis and glycogenolysis in the liver and increasing glucose uptake into skeletal muscle. Additionally, metformin may act on the AMP activated protein kinase pathway.⁷² Finally, metformin has been shown to reduce the extent of hepatosteatosis and inflammation in a small group of patients with NAFLD.⁷³

Thiazolidinediones have also been investigated as treatment options for NAFLD. Caldwell *et al*⁷⁴ reported that troglitazone (now withdrawn) improved liver enzyme levels and hepatosteatosis, an effect subsequently corroborated in small-scale studies using rosiglitazone and pioglitazone.^{75 76} The recently published PIVENS trial,⁷⁷ a large-scale trial with 247 patients, compared the effects of pioglitazone, vitamin E and placebo in non-diabetic patients with NASH over a 96-week period. Vitamin E, compared with placebo, was associated with a significant rate of improvement in NASH (43% vs 19%, $p=0.001$). Pioglitazone compared with placebo also induced an improvement in NASH but at a lower level than vitamin E (34% vs 19%). Serum ALT and AST were also reduced with both vitamin E and pioglitazone, as compared with placebo ($p<0.001$ for both comparisons). Disappointingly, there was no improvement in fibrosis scores with either vitamin E or pioglitazone. Importantly though, subjects who received pioglitazone gained more weight than those who received vitamin E or placebo.

Other antioxidants, besides vitamin E, and hepato-protectants such as ursodeoxycholic acid, s-adenosylmethionine, betaine and pentoxifylline may also have a therapeutic role in NAFLD. Ursodeoxycholic acid has been shown to have limited efficacy for normalising aminotransferase levels, even at high concentrations, unless given with vitamin E.⁷⁸

Finally, other emerging treatments in NAFLD include angiotensin receptor blockers and $\alpha 1$ adrenoceptor antagonists, which reduce hepatic fibrogenesis and enhance liver regeneration in animal models of NASH.^{65 79}

Summary

The term NAFLD encompasses a spectrum of histological features from steatosis to cirrhosis. It is now

the most common cause of chronic liver disease world wide, rising in tandem with obesity and type 2 diabetes. Insulin resistance and oxidative stress are important in disease progression. There is no single diagnostic test for NAFLD, although emerging modalities with greater sensitivity and specificity may aid future diagnosis, staging and management.

Currently, there are no approved treatments for NAFLD. Treatment strategies mostly start with lifestyle modifications and may then progress to pharmacotherapy with insulin sensitizers, cholesterol lowering agents, anti-obesity agents and anti-oxidants such as vitamin E. Therapeutic trials for drugs such as adrenoceptor antagonists and angiotensin 1 receptor blockers are planned and appear to be promising following convincing findings in animal studies.

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