LEADING ARTICLE

Non-alcoholic steatohepatitis (NASH): where are we now and where are we going?

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Although non-alcoholic steatohepatitis (NASH) was considered relatively uncommon prior to the middle of the last decade, over the past three years there has been an explosion of studies on various aspects of NASH with one study reporting that after hepatitis C, NASH was the most common diagnosis in patients presenting largely with persistent abnormalities of liver function tests. The field of NASH has come a long way in a relatively short space of time. This article considers advances in knowledge that have arisen as a result of these studies and highlights areas for further work.

Prior to the middle of the last decade, non-alcoholic steatohepatitis (NASH) was widely considered to be a relatively uncommon condition, occurring almost exclusively in obese females, often associated with non-insulin dependent diabetes mellitus (NIDDM), and with a relatively benign prognosis. Not surprisingly therefore very little attention was paid to the condition in terms of either basic or clinical research and no clinical trials of treatment were reported. From around 1994 however these perceptions were challenged by the publication of several series of patients drawn from unselected clinical practice rather than from "at risk" groups. A study of "liver" referrals to an urban hospital based practice reported that, after hepatitis C, NASH was the most common diagnosis in patients presenting largely with persistent abnormalities of standard liver function tests.[1] It became clear that NASH has an equal sex distribution and that many, perhaps even the majority of patients are neither obese nor diabetic.[2 3] Perhaps of most concern however was a review of studies reported up to 1998 highlighting the fact that fibrosis or even cirrhosis was present in between 15% and 50% of patients on their index biopsy, suggesting that at least some individuals with NASH develop progressive liver disease.[4] As well as drawing attention to the potential importance of NASH, this and other reviews and reports drew attention to the considerable gaps in our knowledge on a variety of critical issues, including prevalence, natural history, indications for liver biopsy, pathogenesis, and treatment strategies. Accordingly, over the past three years there has been an explosion of studies in the literature on various aspects of NASH as well as NIH and FALK sponsored symposia in North America and Europe, respectively. This article considers advances in knowledge that have arisen as a result of these studies/symposia and highlights areas where further work is required.

There have been no recent studies on the prevalence of NASH in unselected populations and few in relevant high risk groups. Certainly a plethora of case series of NASH have been reported over the past three years but whether this indicates a true increase in prevalence or simply an increased awareness of the disorder is unclear. However, as another disease of "affluence"[5]—part of the insulin resistance/obesity constellation known as metabolic syndrome X[6]—an increase in prevalence seems likely in view of the present epidemic of obesity. Perhaps most worryingly, recent studies showing that obesity is increasing in both children[7] and adolescents suggest that the prevalence of NASH is likely to go on increasing for the foreseeable future.

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With respect to the prevalence of NASH in "at risk" groups, a recent study in 105 unselected, consecutive, severely obese patients undergoing laparoscopic obesity surgery[8] reported a 25% incidence of NASH, similar to that observed in previous studies.[9] What is urgently required are prevalence studies in the much larger populations of mild/moderately obese individuals and patients with NIDDM. Until this information is available it will remain difficult to convince physicians managing these patients that NASH has great clinical significance.

There has been only one detailed natural history study of NASH published to date.[10] This study retrospectively determined the histological and/or clinical outcome of 98 patients with the whole range of non-alcoholic fatty liver disease (NAFLD) from simple steatosis through NASH to fatty cirrhosis. After a median eight year follow up, 25% of individuals with evidence of hepatocyte necrosis with or without Mallory's hyaline or fibrosis, either already had cirrhosis on index biopsy or progressed to cirrhosis. This compared

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Abbreviations: ALT, alanine transaminase; AST aspartate transaminase; BMI, body mass index; FFA, free fatty acids; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; NIDDM, non-insulin dependent diabetes mellitus; ROS, reactive oxygen species.

with only 3.4% of patients with simple steatosis with or without non-specific inflammatory changes. Furthermore, 11% of those with hepatocyte necrosis had a liver related cause of death compared with less than 2% of those with fat and non-specific inflammation. Almost 80% of those developing cirrhosis and all but one of those dying from a liver related cause had fibrosis on index biopsy. The observation that NAFLD patients without NASH have a benign prognosis confirmed results from a previous study confined to patients with non-alcoholic fatty liver only.[11] Further evidence that NAFLD/NASH can progress to cirrhosis in some patients has come from two studies that have examined the frequency of risk factors for NASH (obesity and diabetes) in patients with cryptogenic cirrhosis compared with patients with cirrhosis of known aetiology.[12 13] Both studies reported a high prevalence of both risk factors and suggested that NASH may account for most, if not all, cases of cryptogenic cirrhosis. Prospective natural history studies are now urgently required to determine both the frequency of progression to cirrhosis/liver related death and factors predicting progression.

Perhaps the commonest question faced by a clinician managing a patient presenting with possible NAFLD/NASH is whether or not to perform a liver biopsy. This question is particularly pertinent given recent evidence that NAFLD is by far the most likely histological diagnosis in the increasing number of patients presenting to liver clinics with a persistently raised transaminase or other abnormalities of standard liver blood tests.[14 15] If we accept the limited natural history data that among patients with NAFLD only those with NASH and/or fibrosis can progress to cirrhosis, then the principal aim of investigation is to identify these patients as they will require monitoring and possible intervention/treatment (see below). Clearly, all patients with mild elevations of transaminases cannot be subjected to liver biopsy with its associated morbidity and mortality. Fortunately, the results of three recent studies in different patient groups have identified several clinical and laboratory features that predict the presence of NASH and/or fibrosis.

Two studies have been performed in obese patients. The first included 93 mildly obese patients (body mass index (BMI) >25 kg/m^2) being investigated for abnormal liver blood tests.[16] Age >50 years, BMI >28 kg/m^2, alanine transaminase (ALT) more than twice normal, and serum triglycerides >1.7 mmol/l were independent predictors of septal fibrosis. No separate analysis was performed for predictors of NASH but 96% of those with septal fibrosis had NASH compared with only 41% of those without septal fibrosis, suggesting that similar factors were predictive of both lesions. Diabetes and impaired glucose tolerance were significantly associated with fibrosis in univariate but not multivariate analyses.

> "Independent predictors of NASH and advanced pericellular fibrosis were an ALT greater than normal, hypertension, and either insulin resistance index (NASH) or fasting C peptide (fibrosis)"

The second study was performed in 105 severely obese patients undergoing obesity surgery.[8] Independent predictors of NASH and advanced pericellular fibrosis were an ALT greater than normal, hypertension, and either insulin resistance index (NASH) or fasting C peptide (fibrosis). Half of the patients with NASH had overt NIDDM compared with only 6% of those without NASH and the waist/hip ratio (an indicator of central obesity) was significantly higher in the NASH patients. Mean age was similar in those with (44±11 years) and without (40±11 years) NASH. A further study in 144 patients selected on the basis of biopsy proven NASH reported that age >45 years, obesity (BMI >31.1 kg/m^2 in males, >32.3 kg/m^2 in females), NIDDM, and an aspartate transaminase (AST)/ALT ratio >1 were independent predictors of fibrosis.[17] Clearly, the conclusions of these studies in selected groups may not be applicable to all patients with NAFLD and similar studies in unselected patients are required. However, given this proviso, at present it would seem sensible to restrict liver biopsy to patients with some, if not all, of the following: (a) ALT greater than twice normal; (b) AST >ALT; (c) at least moderate "central" obesity; (d) NIDDM or impaired glucose tolerance; (e) hypertension; and (f) hypertriglyceridaemia. With respect to liver biopsy reporting, the recent publication and general acceptance of a standardised scoring system for NAFLD[18] should lead to more consistency in the reporting of histological features in studies on NAFLD. This is particularly important for studies concerned with histological predictors of prognosis and will also be vital in the interpretation of studies assessing the efficacy of treatment interventions.

The choice of drug, or other intervention, to be used in clinical trials depends on a detailed knowledge of the mechanisms involved in disease pathogenesis. In 1998, the "two hit" hypothesis of disease pathogenesis was proposed whereby the first "hit"—steatosis—sensitises the liver to a variety of second "hits" which lead to necroinflammation and fibrosis.[19] The principal candidates for the second hit at that time were oxidative stress and abnormal cytokine production. With regard to the first hit, several recent studies have reported that both peripheral and hepatic insulin resistance is present in almost all patients with NAFLD, irrespective of the coexistence of impaired glucose tolerance or obesity.[6 20] This observation, together with the associated hypertension, hypertriglyceridaemia, central/visceral obesity, and family history of diabetes or hypertension has resulted in NAFLD being considered as the liver manifestation of the metabolic syndrome.[6] This has led to the hypothesis that a combination of, as yet largely unknown, genetic and known acquired factors (for example, development of central obesity) responsible for insulin resistance are the true first hit, leading to the development of steatosis through increased lipolysis and delivery of free fatty acids (FFA) to the liver.[6] A primary role for insulin resistance is supported by the high frequency of steatosis in inherited syndromes of severe insulin resistance[21] and amelioration of steatosis in the ob/ob leptin deficient mouse model of steatosis with metformin, an agent that improves hepatic insulin resistance.[22] There is however no doubt that whatever its primary aetiology, steatosis per se will contribute to insulin resistance by reducing insulin clearance,[23] setting up a vicious cycle linking steatosis and insulin resistance.

Importantly, all of the recent studies examining insulin resistance in patients with NAFLD have shown that patients with NASH are more insulin resistant than patients with fatty liver alone.[6 8 20] Again, this may be an effect rather than a cause of the liver pathology but raises the possibility that insulin resistance may be a "second" as well as a "first" hit, contributing to the development of progressive disease (discussed below). While this would be consistent with the clinical data associating diabetes with an increased risk of NASH and fibrosis,[6 16 17] it is clear that only a proportion of insulin resistant patients (either with NAFLD or NIDDM) ever develop advanced liver disease, suggesting that other factors or "hits" must play a role.

> "Insulin resistance may be a "second" as well as a "first" hit, contributing to the development of progressive disease"

With respect to these "hits", oxidative stress maintains its prominent role. A recent study, staining liver biopsies for 3-nitrotyrosine as a marker of lipid peroxidation, reported that compared with controls, patients with fatty liver and NASH had significant staining, with the highest levels observed in those with NASH.[20] A role for oxidative stress is supported by

different animal models of NASH which show either increased reactive oxygen species formation[24] or evidence of extensive lipid peroxidation.[25] The most likely source of the reactive oxygen species (ROS) leading to lipid peroxidation in patients with NASH are the mitochondria, the cell's principal source of ROS (reviewed by Pessayre and colleagues[26]). An increase in mitochondrial ROS production seems likely to be a response to an increased hepatic supply of FFA, arising due to insulin resistance and visceral obesity, resulting in a compensatory increase in the rate of mitochondrial β oxidation.[20 26] The resulting lipid peroxidation products alter mitochondrial DNA and also react with mitochondrial proteins to inhibit the transfer electrons along the respiratory chain, further increasing ROS production and resulting in a self perpetuating cycle of oxidative stress and lipid peroxidation. In support, humans with NASH exhibit ultrastructural mitochondrial lesions and have decreased activity of respiratory chain complexes.[26] Support for a critical role of FFA in this process comes from studies showing that incubating hepatocytes with FFA increases ROS formation and from gene knockout studies demonstrating that blocking peroxisomal FFA oxidation induces NASH.[27] Other potential sources of oxidative stress that have been suggested to play a role in NASH include the cytochrome P450 enzymes CYP2E1 and CYP3A4[25] and an increase in liver iron observed in some patients.[28]

Several studies in both animal models and more recently in humans with NASH have provided evidence that disordered cytokine production is likely to play a role in the pathogenesis of NASH.[29] Abnormal cytokine production has been variously attributed to: (a) abnormal macrophage function, (b) an effect of oxidative stress through nuclear translocation of the transcription factor nuclear factor κB, (c) direct release by adipose tissue (of tumour necrosis factor α), and (d) bacterial overgrowth.

> "Disordered cytokine production is likely to play a role in the pathogenesis of NASH"

Thus far the majority of this work has been performed in animal models although a recent report using ^{14}C-D-xylose and lactulose breath tests demonstrated evidence of small intestinal overgrowth in 50% of patients with NASH compared with 22% of control subjects.[30] More studies in both animal models and patients are required to define both the source and consequences of disordered cytokine production in NASH. The role of cytokines and other factors, including leptin, in the pathogenesis of the fibrosis seen in NASH seems an area particularly in need of further study.

Although many issues remain to be resolved, this emerging information provides a clear basis for the design of studies aimed at understanding disease susceptibility and a rationale for the testing of novel treatment strategies. With respect to susceptibility, a recent report of NASH and cryptogenic cirrhosis occurring within kindreds suggested that genetic factors may be important.[31] For the development of the first stage of disease, fatty liver, these genetic factors are likely to be those that influence the development of insulin resistance and FFA supply, including genes that play a role in determining body mass and distribution. Polymorphisms in genes encoding proteins involved in hepatic lipid metabolism and storage may also be important, as illustrated by a recent study showing an association between a low activity promoter polymorphism in the gene encoding microsomal triglyceride transfer protein and transaminitis in patients with NIDDM.[32] Susceptibility to NASH and fibrosis is likely to be determined by genetic factors influencing the magnitude of the putative second hits. Potential candidates would include: genes encoding proteins involved in the generation of ROS and antioxidant defences, genes encoding cytokines and their receptors, and genes encoding proteins determining the adverse adaptive effects to high levels of FFAs, including the transcription factors peroxisomal proliferator activated receptors α and γ (reviewed by Day and Daly[33]). Candidate gene, case control studies are awaited with interest although investigators will need to tackle a variety of potential pitfalls in such studies.[33]

A recent study demonstrating the presence of mitochondrial paracrystalline inclusions in patients with NASH but not steatosis led the authors to hypothesise that a primary mitochondrial abnormality leading to increased ROS production is responsible for the progression of steatosis to NASH.[20] However, it seems more likely that these lesions are an effect, rather than a cause, of oxidative stress.[26] In respect of environmental factors leading to increased susceptibility to NASH, diet and lack of exercise would seem to be the most likely candidates in view of their influence on fat distribution, FFA supply, and oxidative stress.

Until now there have been no randomised biopsy controlled trials of treatments for patients with simple fatty liver or NASH. Clearly the pathogenic mechanisms outlined above suggest a variety of potential treatment strategies and already reports of uncontrolled pilot studies have begun to appear in the literature, predominantly in abstract form (reviewed by Angulo and Lindor[34]). Treatments have included physical exercise and weight reducing diets, antioxidant therapy (vitamin E), the inevitable ursodeoxycholic acid, and agents directed at the treatment of the associated hyperlipidaemia. Only one of these studies included follow up liver biopsies and this failed to confirm the improvement suggested by the liver blood tests.[35]

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Perhaps the most exciting report thus far is a study of metformin treatment of non-diabetic patients with NASH prompted by the beneficial effect of metformin in insulin resistant ob/ob mice.[21] Four months of treatment led to a reduction in serum transaminase and liver volume compared with no changes in non-compliant patients.[36] Clearly, a randomised controlled trial is now urgently required.

In summary, the field of NAFLD/NASH has come a long way in a relatively short space of time. Information on disease prevalence and natural history however remains sparse and is urgently needed to better inform management strategies. Advances in our understanding of disease pathogenesis suggest a variety of potential treatment modalities and randomised controlled trials with follow up liver biopsies are now eagerly awaited.

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