Current Solutions for Obesity-Related Liver Disorders: Non-Alcoholic Fatty Liver Disease and Non-Alcoholic Steatohepatitis

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Obesity is a worldwide epidemic with more than 1 billion overweight adults and at least 300 million obese patients [1]. According to recent statistics from the United States, 16.9% of children and adolescents aged 2–19 years were obese in 2009–2010 and 31.8% were either overweight or obese [2]. The rise in childhood obesity has been accompanied by an increase in pediatric liver diseases: 70%–80% of obese children and adolescents have liver diseases [3]. A review of the epidemiology of non-alcoholic fatty liver disease (NAFLD) found that in studies of bariatric surgery patients, 76% had NAFLD, 37% had non-alcoholic steatohepatitis (NASH), 23% had fibrosis, and 5.8% had cirrhosis [4]. NAFLD is defined by hepatic fat infiltration of >5% hepatocytes, as detected by liver biopsy, with no evidence of excessive alcohol intake, or viral, autoimmune or drug-induced liver disease. It constitutes a spectra of liver disease ranging from intrahepatic fat accumulation (steatosis) to various degrees of necrotic inflammation and fibrosis (NASH) [5]. In predisposed individuals, NAFLD can evolve to cirrhosis and hepatocellular carcinoma, with the consequent need for liver transplantation [6]. NAFLD is associated with abdominal obesity and severe metabolic impairments such as insulin resistance, type 2 diabetes, dyslipidemia and hypertension. It is a risk factor for metabolic syndrome and cardiovascular disease [7]. In view of the obesity and liver disease epidemic, the diagnosis and treatment of this population should become a priority for health care systems.

• PROGRESSION OF NAFLD TO NASH
The incidence of progression from NAFLD to NASH is variable (15%–40%, depending on the population and geographic location of the study), with the larger values attributed to the combined effect of metabolic syndrome and obesity [14]. Disease progression from steatosis to hepatic lipotoxic liver disease and steatohepatitis is associated with mitochondrial dysfunction, endoplasmic reticulum stress, free radical formation, and activation of inflammatory pathways by toxic lipid metabolites such as diacylglycerols and ceramides [15].

DIAGNOSIS
The gold standard for NAFLD and NASH diagnosis and monitoring is histology, with indicators such as steatosis, ballooning, lobular and portal inflammation. The severity of the disease depends on the incremental increase of symptoms. Different histological subtypes have different prognoses: liver-related mortality is limited to NASH (especially that associated with advanced fibrosis). On the other hand, cardiovascular mortality associated with liver damage...
is independent of the stage of the disease, whether simple steatosis or NASH.

However, the procurement of tissue for histologic evaluation is, by nature, invasive and associated with morbidity and, rarely, mortality. It is therefore inapplicable as a screening procedure and a non-invasive, simple and reliable alternative is required.

A myriad of non-invasive modalities such as measuring serum markers (plasma cytokeratin-18 fragments), transient elastography (liver stiffness measurement) and classical imaging techniques (MRI, CT, and ultrasound) can provide potential substitutes or be used in combination with liver biopsies.

Steatosis induces substantial changes in liver hemodynamics even in the absence of fibrosis and can significantly increase portal pressure. Metabolic syndrome parameters such as visceral adiposity assessment and insulin resistance are indicators for portal hypertension and are directly related to the degree of steatosis. Liver enzyme elevation – particularly alanine aminotransferase (ALT) and gamma-glutamyl transpeptidase (GGT) – are sometimes misleading since GGT, associated with increased cardiovascular risk, is a good marker for steatosis but probably indicates vascular oxidative stress rather than increased liver fat content. ALT, on the other hand, is more liver-specific but less sensitive for steatosis [16]. The combination of several markers with/without ultrasonography might constitute a better diagnostic tool. Several tests were designed to assess the extent of fibrosis. The BARD score, designed for patients with low risk of fibrosis [comprising body mass index (BMI), aspartate transaminase (AST)/ALT ratio and presence/absence of diabetes] generates a score of 0–4, providing a simple tool for excluding advanced liver disease. A recent study published by NASH Clinical Research Network (NASH CRN) found that the AST/ALT ratio was able to predict cirrhosis with high confidence and, if combined with demographic data and comorbidities, can increase the AUROC (area under the receiver operating characteristic, a common summary statistic for the significance of a predictor in a binary classification task) to 0.79 for NASH and 0.96 for cirrhosis [17]. The FIB-4 test combines three standard biochemical values (platelets, ALT and AST) with age to determine the extent of fibrosis and has shown high sensitivity in detecting advanced fibrosis [18]. FibroMeter combines age, weight, fasting glucose, AST, ALT, ferritin and platelet count, and has shown 78.5% sensitivity and 95.9% specificity in detecting significant fibrosis [19]. The NAFLD fibrosis score (NFS) is a panel composed of age, hyperglycemia, BMI, platelet count, albumin and AST/ALT ratio, which was constructed using a large multicenter group of 733 biopsy-proven NAFLD patients. The NFS demonstrates excellent accuracy at excluding fibrosis in morbidly obese subjects with NAFLD undergoing bariatric surgery [16]. On the other hand, this method fails in 20%–58% of patients with intermediate or advanced fibrosis [16]. FIB-4 and NFS have proven to be the more accurate tests in this group [16,18].

Another non-invasive method for liver fibrosis evaluation is based on fibrosis biomarkers in serum. The European Liver Fibrosis (ELF) test combines detection of N-terminal peptide of pro-collagen III (P3NP), hyaluronic acid and tissue inhibitor of metalloproteinase 1 (TIMP1) with/without combination with age [20]. When this test is expanded to include other metabolic related factors such as BMI, impaired fasting glucose, AST/ALT ratio, platelets and albumin, it can serve also as a prognostic tool of disease progression [21]. FibroTest combines the detection of biochemical markers of haptoglobin, α2-macroglobulin, apolipoprotein A1, total bilirubin and GGT, with a correction accounting for gender and age. This test is sensitive also in intermediate cases of fibrosis [22].

A different type of fibrosis detection is transient elastography (Fibroscan®, Echosens, France), used for the detection of advanced fibrosis. This is based on the principle that propagation of ultrasound vibrations in tissue is directly proportional to its stiffness (i.e., faster propagation through stiffer tissue). Fibroscan measures liver stiffness in a cylindrical volume of 1 x 4 cm, between 25 and 65 mm below the skin surface. In obese patients, the measurement is compromised by attenuation of overlying fat. A pilot study using modified equipment showed improved detection in obese patients [23].

Enzyme-linked immunosorbent assay (ELISA)-detected serum CK-18 fragment is the only non-invasive marker differentiating NASH from simple steatosis which has been validated to provide a sensitivity/specificity ratio of 0.78/0.87, precluding its use as a single screening test but providing good prediction of liver disease when combined with other methods [16].

**TREATMENT**

- **LIFESTYLE CHANGES**

Lifestyle changes (diet and exercise) are the best preventive and therapeutic methods for NASH and the associated metabolic syndrome, but are, unfortunately, difficult to implement in the majority of patients. This calls for other curative methods like pharmacological and surgical therapies. Since lipid accumulation in hepatic tissue is the main identifier for patients in different stages of NAFLD, both clinically and histologically, it is clear that reduction of this lipid buildup is a key factor in treating the disease. A lifestyle intervention study involving weight loss and physical activity initiated early or later within the program resulted in clinically significant weight loss in significantly obese patients, and positive changes in cardiovascular risk factors (body fat mass determined by CT, blood
pressure and levels of fasting glucose, insulin, hepatic enzymes, and lipids including cholesterol and triglycerides) [24]. Longer engagement in physical activity resulted in greater reductions in hepatic fat content and waist circumference.

- **PHARMACOLOGICAL TREATMENT**
  Most current treatment strategies are summarized in the literature [25]. We will briefly describe the different methodologies, and add some recent findings.

  - **Statins, fibrates, and omega-3 polyunsaturated fatty acids (PUFAs).** It is well established that statins (HMG-CoA reductase inhibitors) are efficient in primary and secondary prevention of cardiovascular diseases, with the highest beneficial effect noted in patients with diabetes mellitus. The impact of statins on NAFLD is unclear. A decrease in plasma liver aminotransferase levels in patients receiving these medications might also be attributed to weight loss. The anti-inflammatory and antioxidant properties of statins have also been proposed as the mechanism for hepatic improvements as they can reduce plasma levels of detrimental cytokines related to NASH. Large studies have shown the safety of statins in patients with NAFLD and hyperlipidemia.

  - **Metformin.** Metformin has long been used to lower insulin resistance. Patients with NASH, who commonly have insulin resistance, can benefit from this drug. Recent epidemiological studies propose metformin for prevention of hepatocellular carcinoma (similar to the effect of statins).

  - **Pentoxifylline.** Inflammation is an important factor in NAFLD progression, with TNFα as a possible culprit. Pentoxifylline is a TNFα antagonist with an established safety profile. Its impact on NAFLD patients has been studied in several small trials evaluating improvement of steatosis by histology. The findings demonstrated amelioration of steatosis, inflammation and ballooning.

  - **Antioxidants.** Since oxidative stress is a key player in the pathogenesis of NAFLD, antioxidants such as ursodeoxycholic acid (URSO), vitamin E, silymarin and betaine are potent therapeutic agents for NAFLD. URSO is a hydrophilic bile acid with cytoprotective and antioxidant properties. Trials using high doses demonstrated improvement in serological markers of fibrosis but did not show histological improvement. Vitamin E is a fat-soluble vitamin with potent antioxidant capacities. Vitamin E treatment results in reduced hepatocellular ballooning. A 2 year controlled study comparing vitamin E, pioglitazone and placebo in non-diabetic patients with NASH showed improvement in liver histology in 43% of the patients as a response to vitamin E, compared to 19% of patients receiving placebo. Another study showed that vitamin E benefited children and adolescents with NAFLD. Nevertheless, large doses of vitamin E (> 400 IU/day) may result in an increase in all-cause mortality. Unfortunately, this study showed that vitamin E cessation might cause elevation of transaminase levels.

  - **Drugs modulating the renin-angiotensin system (RAS).** RAS is a well-known target of antihypertensive therapy. Chronic injury increases the response to a stimulus of RAS in the liver, contributing to activation of inflammatory cells and development of fibrosis. Drugs that modulate the RAS may offer an alternative approach for the treatment of NAFLD/NASH. In experimental rodent models of NAFLD, angiotensin-converting enzyme inhibitors (ACE-I) and angiotensin receptor blockers (ARB) caused a marked reduction in fibrosis markers (hyaluronic acid and transforming growth factor-beta) as well as in the histological score of fibrosis. Human studies with ARB in NASH are few but promising. A pilot study comparing telmisartan and valsartan treatment for 20 months in patients with biopsy-proven NASH and hypertension led to amelioration of cytolysis and necro-inflammation, with telmisartan achieving better results in insulin resistance and histologic appearance.

  - **Silymarin.** Silymarin (milk thistle) is a lipophilic extract from the seeds of the *Silybum marianum* plant exhibiting antioxidant properties. A pilot study using this compound showed promising results with regard to the serological profile.

  - **Thiazolidinediones (TZDs).** Peroxisomal proliferator activated receptor-c (PPAR-c) agonists constitute a class of nuclear transcription factors that are abundant in adipose tissue. These drugs are effective in NASH patients since they reduce subclinical inflammation, improve adipose tissue and hepatic insulin sensitivity, and restore liver histology. TZDs were shown to be effective in patients with NAFLD, achieving histological improvement in liver steatosis and inflammation but not in fibrosis.

- **ALTERATION OF GUT FLORA**
  The gut microbiota has been shown to be involved in intestinal biological functions, such as defense against pathogens, immunity, development of the intestinal microvilli, and degradation of non-digestible polysaccharides [26]. NASH patients have increased gut permeability and high blood levels of bacteria endotoxin, which result in liver injury [27]. In animal studies...
probiotics have shown a profound effect on NASH. Treatment with VSL\#3 (a high concentration mixture of viable, lyophilized bifidobacteria, lactobacilli, and Streptococcus thermophilus) or anti-TNF antibodies improved liver histology, reduced hepatic total fatty acid content, and decreased serum ALT levels [28]. A high fat diet induced a depletion of hepatic NKT cells. NKT cells express both natural killer (NK) receptors and T cell receptors, thus leading to insulin resistance and steatosis. Oral probiotic treatment (VSL\#3) significantly improves hepatic NKT insulin resistance and hepatic steatosis resulting from a high fat diet [29].

Gut microbiota in combination with fructo-oligosaccharide (FOS) was administered to patients in a lifestyle-change program (diet and exercise). Patients were compared with a group not receiving food supplement. Bifidobacterium longum with FOS and lifestyle modification, when compared to lifestyle modification alone, significantly reduced TNF-α, serum AST levels, serum endotoxin, and liver steatosis and NASH markers [30]. A randomized controlled study of 28 patients with biopsy-proven NAFLD showed that a mixture of Lactobacillus bulgaricus and Streptococcus thermophilus for 3 months led to a greater reduction in ALT, AST and GGT levels, compared to controls [31]. A recent study showed that probiotic treatment with four strains of Lactobacillus and Bifidobacterium resulted in significant reduction in intrahepatic triglycerides (as monitored by MRI), and AST levels in patients with biopsy-proven NASH [32].

\textbf{Optimal treatment of NAFLD and NASH is weight loss, achieved through change in lifestyle (diet and exercise) or by bariatric surgery}

Bariatric surgery is safe and provides sustained weight loss in most patients, accompanied by substantial improvement in quality of life and resolution or amelioration of weight-related co-morbidities (e.g., diabetes mellitus, hypertension, hyperlipidemia, obstructive sleep apnea, as well as NAFLD and NASH). Unfortunately, a specific association of bariatric surgery with NAFLD and NASH in case-controlled studies is not available and most data are retrospective [33]. Rabl and co-workers [34] reviewed several reports describing positive changes in hepatic biopsies obtained during surgery: Roux-en-Y gastric bypass (RYGB), biliopancreatic diversion without (BPD) or with duodenal switch (BPD-DS), and adjustable gastric banding (AGB), and ensuing weight loss, resulted in amelioration of NAFLD and NASH. A separate report on sleeve gastrectomy showed that NASH patients exhibited a significant weight loss and improvement of NASH status [35].

The remission of NAFLD and NASH after bariatric surgery is associated with the recovery from metabolic syndrome-associated co-morbidities such as diabetes mellitus (T2D), insulin resistance, and hyperlipidemia. The major factor of liver disease improvement is the sustained weight loss after surgery, which is also associated with the remission of T2D [36]. The other mechanisms responsible for T2D and fatty liver disease remission originate from the alternative route created for food delivery after surgery, with changes in gut and pancreatic hormones that influence lipid and carbohydrate metabolism. The anatomic changes resulting from bariatric surgery cause changes in postprandial as well as fasting levels of various gastric system hormones that lead to these positive effects. For example, glucagon-like peptide 1 (GLP-1) increases dramatically following RYGB, BPD or BPD-DS [37]. GLP-1 regulates blood glucose by stimulating insulin secretion, inhibiting glucagon secretion, decreasing hepatic glucose production, and slowing down the evacuation of the gastric contents. GLP-1 receptors were found in liver biopsies from patients with focal nodular hyperplasia or hepatic adenoma, and in patients with NASH [38]. Expression of GLP-1 receptors in human liver biopsies demonstrates that GLP-1 regulates the expression of transcription factors and enzymes participating in the hepatic metabolism of lipids [38].

Peptide YY (PYY) is a protein that is synthesized and secreted by the distal small bowel, colon and rectum. Its action is similar to that of leptin, namely, it affects hypothalamic neural circuits, stimulating hypothalamic receptors, decreasing neuropeptide/agouti-related protein and increasing α-melanocyte-stimulating hormone levels. PYY also inhibits gastrointestinal motility as well as pancreatic exocrine and endocrine secretion. Obese patients typically present low levels of PYY, which might partially explain their metabolic condition. Gastric bypass is associated with an increase in postprandial levels of PYY [39].

A recent study described the effect of bariatric surgery on liver transaminase and on ALT and AST in a large group of obese patients, with a long-term follow-up of 2 and 10 years [40]. The incidence of and the remission from high transaminase levels at both 2 and 10 year follow-up were significantly more favorable in the surgery group compared to the control group. Similarly, the incidence of an ALT/AST ratio < 1 (an index of severe liver disease) was lower in the surgery group compared to the control group at both 2 and 10 year follow-up.

\textbf{CONCLUSIONS}

Non-alcoholic fatty liver disease (NAFLD), and the ensuing NASH disease, which can extend to cirrhosis, is rising at an alarming rate in conjunction with severe obesity. The disease is partially mediated by co-morbidities of obesity such as diabetes mellitus and other diseases considered as metabolic syndrome. The disease is primarily a hepatological disease, with most early studies performed within this discipline. With the disease becoming widespread in the obese/morbidly obese population, it is important that metabolic physicians/bariatric surgeons treating these patients on a daily basis become aware of the complexity of the disease, the diagnosis and the treatments provided.
Diagnosis of NAFLD and NASH is still based on the golden standard of liver biopsy, which is obviously an extreme modality. Other blood/biochemical markers, combined with demographic data, are reasonable diagnostic tools and can be used on a daily basis, provided that their limitations are taken into consideration. Ultrasonography is currently being used to assess liver damage, with a special device designed for measurement of liver elastosis (Fibroscan), with or without MRI.

Treatment of NAFLD and NASH is best assured by weight loss, achieved through change in lifestyle (diet and exercise) or by bariatric surgery. Pharmacological approaches include treatment of the diabetes pathway (e.g., metformin), the lipid pathway (e.g., statins), and other anti-inflammatory drugs. An interesting new treatment uses probiotics to change the gut flora; this is presently in a relative preliminary stage, but looks promising.

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