Metabolic Liver Diseases

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List of Abbreviations

ALT- Alanine aminotransferase

ATP III- Adult treatment panel III

AST- Aspartate aminotransferase

BMI- Body mass index

CI- Confidence interval

CT- computed tomography

FFA- Free fatty acid

FPG- Fasting plasma glucose

GGT- gamma-glutamyl transferase

HDL- high-density lipoprotein

HR- Hazard ratio

IL- Interleukin

LDL- Low-density lipoprotein

MRI- Magnetic resonance imaging

NAFLD- Nonalcoholic fatty liver disease

NAFL- Nonalcoholic fatty liver

NASH- Nonalcoholic hepatic steatosis

NHANES- National health and nutrition examination survey

OR- Odd ratio

US- Ultrasound

USA- United States of America

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Summery

Nonalcoholic fatty liver disease (NAFLD) is a spectrum of fatty changes in the hepatocytes that range from hepatosteatosis with or without inflammation and fibrosis. The prevalence of NAFLD increases with a close correlation to the increase in obesity among the population in developed countries.

As of today, NAFLD has become a major if not the number one chronic liver disease in developed countries and carries a significant risk for deterioration of liver function and may lead to an increased risk for morbidity and mortality.

The increasing awareness among the medical society for screening and detection of high-risk populations has an essential role in preventing mortality and improving liver functions. This overview will focus on the current knowledge regarding NAFLD epidemiology, pathogenesis, diagnostic workup, clinical course, and management.

Sažetak

Nealkoholna masna bolest jetre (NAFLD) je spektar masnih promjena u hepatocitima koje variraju od hepatosteatoze sa ili bez upale i fibroze. Prevalencija NAFLD-a raste u korelaciji s porastom pretilosti među stanovništvom u razvijenim zemljama.

U danasnje vrijeme je NAFLD postao česta ako ne i vodeća kronična bolest jetre u razvijenim zemljama i nosi značajan rizik za pogoršanje jetrene funkcije te može dovesti do povećanog rizika za morbiditet i smrtnost.

Povećana svijest zdravstvenog osoblja o probiru i otkrivanju visokorizičnih skupina ima ključnu ulogu u prevenciji smrtnosti i poboljšanju funkcija jetre. Ovaj pregled će se usredotočiti na trenutno znanje o epidemiologiji NAFLD-a, njezinoj patogenezi, dijagnostičkoj obradi, kliničkom tijeku i liječenju.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is a clinical pathohistological entity with a histological appearance that resembles alcohol induce liver damage, but by definition, it occurs in patients with no significant alcohol consumption history. NAFLD represents a spectrum of hepatic injuries like hepatic steatosis with or without inflammation and fibrosis (1). The prevalence of NAFLD is increasing in developed countries, and the median prevalence is estimated to be 10 to 24 percent(2).

This broad spectrum of histologic changes and hepatic damage is now recognized as a major cause of cryptogenic liver cirrhosis and decompensated liver failure (1,3). Most of the patients are asymptomatic and accidentally get into attention due to other laboratory workup or imaging.

This overview will focus on the common knowledge regarding NAFLD, and will cover the epidemiology, diagnosis, pathogenesis, clinical manifestations, clinical course, and current practices in treatment.

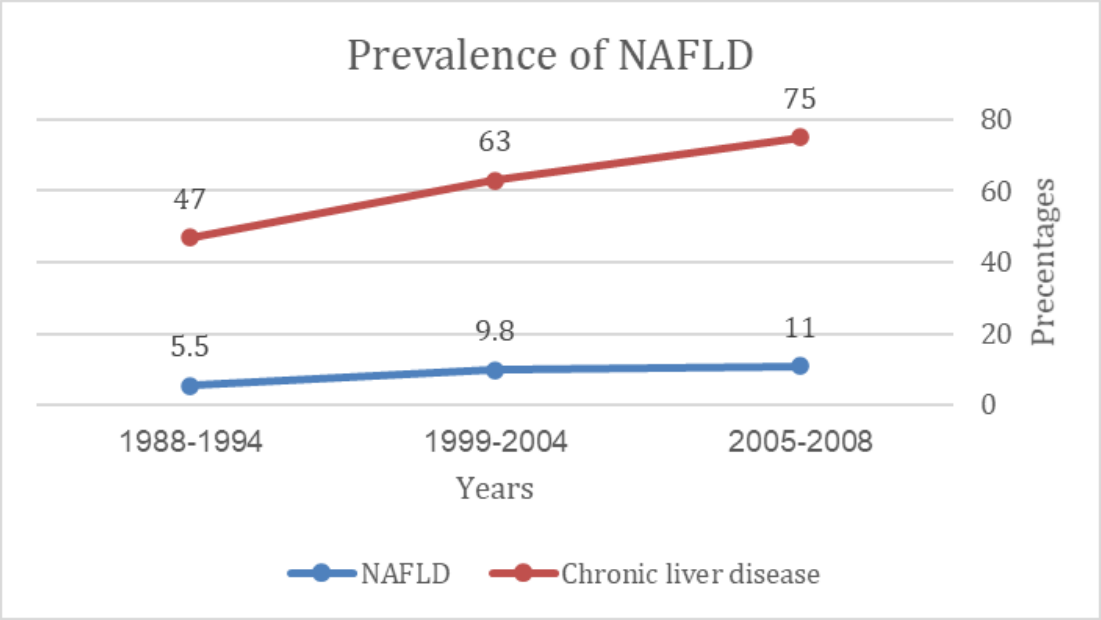
2. Epidemiology

prevalence- nonalcoholic liver disease (NAFLD) is the most common liver disease in the world and NASH is a very common indication for liver transplantation (4).

The worldwide prevalence of NAFLD ranges from 6 to 35 percent, with a median of 20 percent (5,6).

Studies report a prevalence of NAFLD of 10 to 46 percent and 3 to 5 percent of NASH based on biopsies-based studies(5,6).

The prevalence of NAFLD is increasing over time with Asia leading the rise and USA is following closely behind with increasing from 15% in 2005 to 25% within 5 years (4). Study in USA comparison of three cycles of the National Health and Nutrition Examination Survey (NHANES), the study showed that the prevalence between 1988 to 1994 was 5.5 percent, between 1999 to 2004 was 9.8 percent and between 2005 to 2008 was 11 percent which accounts for 47, 63 and 75 percent of the chronic liver disease during that period respectively. The increase in prevalence is related to the increasing prevalence of metabolic syndrome and its component (7).

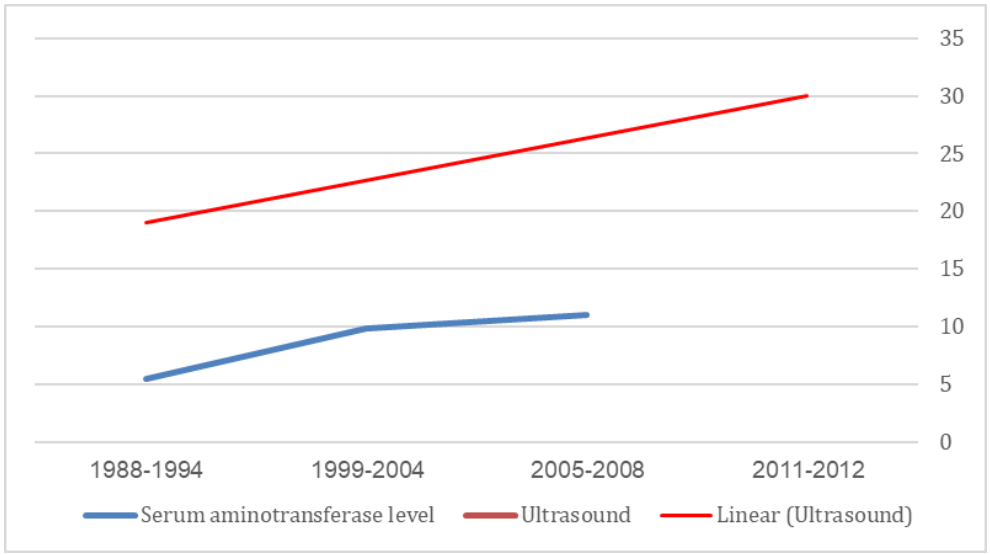


(The relation between prevalence of NAFLD to chronic liver disease) (7).

The definition that has been used in the study (elevated serum aminotransferase level in the absence of alternative explanation) can lead to an underestimation of the true prevalence of NAFLD since patients with NAFLD can present with normal serum aminotransferase level.

As shown in a subsequent study from NHANES using ultrasound collected data from patients between 1988 to 1994 estimate that the prevalence is 19 percent (8).

Another study that was done in USA used the fatty liver index estimated the prevalence of NAFLD between 2011 to 2012 of 30 percent(8).



(The difference between the diagnosis of NAFLD based on serum aminotransferase level and ultrasound finding)(8,9).

In a study of 304 patients with NAFLD who had not previously been diagnosed with diabetes, biopsies of 163 patients confirmed the presence of NASH in 120 of them (74 percent). Metabolic syndrome was diagnosed in 53 percent of patients who did not undergo biopsy, 67 percent of NAFL on biopsy, and 88 percent from the confirmed NASH on biopsy. After the correction of gender, age, and BMI, metabolic syndrome were found to be associated with an increased risk of severe fibrosis (10).

In a population-based study, patients who underwent cholecystectomy were more than twice as likely to develop NAFLD as those who did not. The results are calculated after the correction of gender, age, BMI, and cholesterol level. In patients with gallstone who did not undergo cholecystectomy, there was no increase in the prevalence of NAFLD (11).

3. Association of NAFLD with other disorders

Risk factors to develop NAFLD include-

- Central obesity
- Diabetes mellitus type 2
- Dyslipidemia
- Metabolic syndrome

NAFLD is associated with metabolic syndrome and increases the risk for NASH. Metabolic syndrome is defined according to ATP III criteria from 2005 as the presence of any **three** of the following five traits:

1. Abdominal obesity, defined as a waist circumference ≥102 cm (40 in) in men and ≥88 cm (35 in) in females.
2. Serum triglycerides ≥150 mg/dL (1.7 mmol/L) or drug treatment for elevated triglycerides.
3. Serum high-density lipoprotein (HDL) cholesterol <40 mg/dL (1 mmol/L) in males and <50 mg/dL (1.3 mmol/L) in females or drug treatment for low HDL cholesterol.
4. Blood pressure ≥130/85 mmHg or drug treatment for elevated blood pressure.
5. Fasting plasma glucose (FPG) ≥100 mg/dL (5.6 mmol/L) or drug treatment for elevated blood glucose (12).

4. Pathogenesis

Nonalcoholic fatty liver disease (NAFLD) is a term based on histologic characteristics of liver hepatocytes that are identical to alcohol-induced liver injury in patients without or with insufficient alcohol intake history. The histologic features of NAFLD range from simple fat accumulation in the hepatocytes without inflammation to accumulation of fat in the hepatocytes with inflammation (NASH) with or without fibrosis. The prevalence to progress from nonalcoholic hepatosteatosis to cirrhosis is about 20 percent (1).

The exact mechanism behind NAFLD is not fully understood, but the most accepted theory these days is insulin resistance as a significant cause for NAFLD. Other theories are genetic predisposition, gastrointestinal hormones, iron, antioxidant deficiency, and intestinal bacteria(13).

4.1 Insulin resistance

The mechanism by which insulin resistance leads to the accumulation of fat inside the hepatocytes is due to the shift from carbohydrates metabolism to free fatty acids (FFA) beta-oxidation. Insulin resistance increased lipolysis, increased hepatic intake of FFA, and increased synthesis of fatty acid all of this contribute to the accumulation of fat in the hepatocytes (14).

Studies on medication that increase insulin sensitization like rosiglitazone and pioglitazone demonstrate a reduction in expression of acute-phase reactant (C-reactive protein and serum amyloid A), suggesting that the reduction in inflammation improve insulin sensitivity (15).

Single nucleotide polymorphism in peroxisome proliferator-activated receptor gamma coactivator 1-alpha gene (*PPARGC1A*) promotes insulin resistance and increases the risk of developing NAFLD (16). This support by clinical trial showed more resolution of NASH and improvement in fibrosis and systemic inflammatory markers in patients who took elafibranor (an agonist of peroxisome proliferator-activated receptors alpha and delta) daily for 52 weeks versus placebo (17).

An increase in visceral adipose tissue and intrahepatic fat induce gluconeogenesis, increase free fatty acid (FFA) level, and insulin resistance (18). The visceral adipose tissue may cause an increase in the level of IL-6 (a proinflammatory cytokine) (19). Increased

expression of hepatic IL-6 plays a role in insulin resistance. As with IL-6 other proinflammatory cytokines like tumor necrosis alpha are shown to increase insulin resistance in patients with NASH (19,20).

4.2 Hepatocellular injury

The accumulation of FFAs in the hepatocytes induces cytochrome p-450 microsomal lipooxygenases that produce oxygen-free radicals species (21). The shift to beta-oxidation FFA in patients with pre-existing defects in mitochondrial phosphorylation increase the production of oxygen free radicals, hepatocellular inflammation, and fibrosis (22). Patients with NASH are shown to have mitochondrial structural abnormalities under electron microscopy in comparison to patients with NAFL (22).

The activation of nuclear factor kappa beta and increased cytokine production enhance the hepatocytes inflammation. Proinflammatory cytokines like tumor necrosis factor-alpha, complement (23), and myeloperoxidase play roles in hepatic inflammatory injury (24). Studies compared the effect of estrogen on hepatocellular injury in men and postmenopausal women to premenopausal women and found that estrogen has protected anti-inflammatory and antifibrotic effects (25).

4.3 Antioxidant

In patients with NASH, the high level of lipids peroxidation and free oxygen radical formation can lead to depletion in the available level of antioxidants like vitamin E, vitamin C, beta carotene, and glutathione enzyme, thus increasing the susceptibility for oxidative injury(26,27). Data showed significant histologic improvement in both inflammatory and fibrosis scores in patients after six months of vitamin E and vitamin C (28).

4.4 Iron

The mechanism by which iron causes necroinflammation is unknown but can be due to oxidative stress and radical formation in the reduction from Fe 3+ to Fe 2+ (29). Iron plays a role in determining insulin sensitivity, it is found that patients with glucose intolerance and fatty liver disease have 2.5 times hyperinsulinemia at baseline compared to a patient with glucose intolerance without fatty liver disease, and those with fatty liver disease both

hyperinsulinemia and aminotransferase levels improve after iron reduction therapy even though their iron level were normal (30).

4.5 Adiponectin

It is a hormone produced by adipocytes, it affects fat metabolism by decreasing plasma concentration of lipids, lowering the production of tumor necrosis factor-alpha by hepatocytes, and increasing beta-oxidation in muscles (31). One study showed a correlation between low adiponectin level and the presence of NAFLD, hepatic fibrosis, the severity of the metabolic syndrome, and hepatic insulin sensitivity. On the animal model, administration of adiponectin leads to significant improvement of hepatic steatosis, hepatomegaly, and aminotransferase levels. Administration of pioglitazone increases adiponectin level and improvement in hepatic steatosis necroinflammation and fibrosis (32).

4.6 Lipids accumulation disease

Genetic diseases in the production of VLDL result in the accumulation of intracellular fat (33). Abetalipoproteinemia autosomal recessive disease, a mutation in microsomal triglyceride transfer protein (MTP) that leads to deficiency in Apo B secretion and accumulation of lipids inside the hepatocytes. Inhibition of microsomal triglyceride transfer protein is thought to be the mechanism behind drug-induced NAFLD like amiodarone and tetracycline (34).

4.7 Genetic

NAFLD is a complex disease that influence by both the environment and heredity components. Twin studies show a strong genetic component (about 50%) of both liver fat content and liver fibrosis(35). At least four gene variations in four different genes involved in the coding of regulatory proteins of hepatic lipid metabolism are associated with the onset and progression of NAFLD (36,37). In addition, gene polymorphisms involved in insulin signaling are associated with liver fibrosis (38). Interestingly, the success of therapeutic intervention with weight loss surgery showed partial reversibility of insulin signaling gene methylation. This process has been shown to play a role in the development of NAFLD (39).

4.8 Incretins

Incretins such as glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide 1 (GLP1) are intestinal hormones that enhance postprandial insulin secretion and play an important role in glucose regulation (40). GLP1 receptor agonists have been shown to improve glucose and lipid metabolism, reduce liver fat accumulation, and improve liver enzymes (41,42). The use of GLP1 receptor agonists in patients with diabetes and non-alcoholic steatohepatitis (NASH) will be discussed below.

4.9 Bile acid

Bile acids are cholesterol-derived carboxylic acids synthesized in the liver that promote the absorption of lipids in the small intestine. Bile acids play a role in lipid and glucose metabolism by binding to the farnesoid X receptor (43) and prevention of gut bacterial growth (44). In an interim analysis of a large cohort of patients with nonalcoholic steatohepatitis (NASH), obeticholic acid (25 mg daily) resulted in a significant improvement in liver fibrosis compared to placebo (23% vs. 12%) (45).

5. Clinical manifestations

Most of the patients with nonalcoholic fatty liver disease (NAFLD) are asymptomatic and come to attention after an incidental laboratory finding an increase in liver enzymes or abnormal imaging. Patients with nonalcoholic steatohepatitis (NASH) may have some non-specific symptoms like malaise, fatigue, and right upper quadrant abdominal pain(46).

5.1 Physical finding

Some patients with NAFLD have hepatomegaly, and maybe the initial finding leads to the diagnosis. The prevalence of hepatomegaly among patients with NAFLD is highly variable (1,47,48).

In a population-based study of 1168 patients, NAFLD was detected on ultrasound in 19 percent of those older than 20 years old, and among them, 5 percent had hepatomegaly (49). However, this study does not differentiate between patients with NAFLD and patients with NASH, and from another study, it is estimated that there is a correlation between the severity of the inflammation and the presence of hepatomegaly.

5.2 Laboratory findings

Patients with NAFLD may show mild or moderate elevations of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (50), but normal aminotransferase levels do not rule out NAFLD (51–53). The true prevalence of abnormal transaminase in NAFLD patients is unknown, as many patients have been diagnosed with NAFLD because they have been found to have abnormal aminotransferases. The use of laboratory findings in patients with NAFLD will be discussed below.

6. Diagnosis approach

The diagnosis of NAFLD is made by radiologic finding and exclusion of all other possible etiology of hepatic steatosis (54):

- Exclusion of excessive alcohol consumption
- Exclusion of other causes of hepatic steatosis
- Absent of coexisting chronic liver disease

6.1 Rule out other disorders

To make a proper diagnosis of NAFLD, the physician should take a complete history from the patient to identify other causes of hepatic steatosis like alcohol consumption, medications, risky behavior for viruses, and severe starvation.

Based on the prevalence of the differential diagnosis for NAFLD, it is recommended to obtain (46):

- Anti-hepatitis C virus antibody.
- Hepatitis A antigen.
- Hepatitis B surface antigens, surface antibodies and core antibodies.
- Plasma iron, ferritin and total iron binding capacity.
- Serum gammaglobulin, antinuclear antibody, anti-smooth muscle antibody, and anti-liver/kidney microsomal antibody-1.

Other disorders must be ruled out based on the patient's history, symptoms, or family history like Wilson disease, celiac disease, and alpha-1 antitrypsin deficiency.

6.2 Laboratory finding

The liver enzymes previously were part of the diagnosis (50), but it is noted that not all patients with NAFLD have abnormal liver enzymes. Normal liver enzymes do not exclude NAFLD (51–53).

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) may be elevated. The elevation is typically two to five times the standard limit with a ratio of less than one (unlike alcoholic liver steatosis with a ratio of greater than two) (55–57). The true prevalence of abnormal liver enzymes among patients with NAFLD is unclear because the accidental abnormal liver enzymes usually proceed by radiologic imaging that confirms the diagnosis. However, the diagnosis can be made by incidental radiologic findings and normal liver enzymes. Furthermore, the degree of aminotransferase elevation does not predict the degree of liver damage, inflammation, or fibrosis (50,51).

Serum alkaline phosphatase may be elevated twice the standard upper limit. Albumin and bilirubin are typically normal but may become abnormal in patients with cirrhosis. Other laboratory abnormalities in patients who progress to cirrhosis are prolonged prothrombin time, thrombocytopenia, and neutropenia (46).

Patients with NAFLD may have elevated serum ferritin concentration of transferrin saturation (48,50). Evidence shows that elevated serum ferritin is 1.5 times the upper standard limit in patients with NAFLD, more likely to have higher nonalcoholic fatty liver disease activity scores and more advanced hepatic fibrosis (58).

6.3 Radiologic imaging

Many radiologic methods can detect NAFLD, but none can differentiate between nonalcoholic fatty liver (NAFL) and nonalcoholic steatohepatitis (59).

If fatty changes are detected by radiologic imaging is sufficient to make the diagnosis if other causes of liver disease are excluded, and there are no signs and symptoms of cirrhosis.

- Ultrasound- fatty infiltration is seen on ultrasound as bright liver or hyperechoic liver (60). Compared to liver biopsy (the gold standard), ultrasound has a sensitivity of 85 percent and specificity of 94 percent (61). The sensitivity was found to be lower in obese patients (62,63). In a study of 187 grade 2 obesity patients undergoing weight loss surgery, fatty changes were detected in 94 percent of the patients by liver biopsy and only 49 percent detected by ultrasound (63).
- Vibration controlled transient elastography- a method used routinely to grade liver fibrosis is also being developed to grade liver steatosis. There is insufficient data about the sensitivity and specificity of this method (64–66).

- CT and MRI- both methods can detect steatosis but not inflammation and fibrosis (67). The difficulty in obtaining the sensitivity and specificity of CT and MRI in NFLD diagnosis is that not all patients diagnosed will have confirmation by biopsy (68). One study of 131 patients that undergo partial hepatectomy, usually for malignancy, used biopsy as a gold standard and found that sensitivity of non-contrast CT was 33 percent, contrast-enhanced CT 50 percent, and MRI 88 percent for detecting hepatic steatosis. The specificity was 100, 83, and 63 percent, respectively. Moreover, the accuracy of non-contrast CT is decreased with an increase in BMI.
- Liver biopsy- liver biopsy is the gold standard method for diagnosing NAFLD. It is an invasive method; therefore, the diagnosis can be made by radiologic imaging, careful exclusion of other etiology, and laboratory tests. Patients with unclear diagnoses should undergo liver biopsy (46). Currently, this is the only way to determine the severity of the disease and differentiate between NAFL and NASH. Liver biopsy plays a role in guiding the treatment and may help increase patients motivation. For example, patients with mild liver inflammation and potentially reversible disease will have greater motivation for making dramatic lifestyle changes than patients with severe end-stage cirrhosis (69–71). The indication for liver biopsy are signs and symptoms of cirrhosis (e.g., splenomegaly, ascites, cytopenia), serum ferritin >1.5 times the upper normal limit (suggestive for NASH and advanced fibrosis), and patients >45 years of age with diabetes and obesity (increased risk for advanced fibrosis) (46).
- Histologic finding- NAFLD represents a spectrum of clinical and histological findings from nonalcoholic fatty liver (NAFL) to nonalcoholic steatosis (NASH). The minimum criterion to diagnose NAFLD is >5 percent steatosis hepatocytes in a segment of liver tissue (72). We can defrentiat between NAFL to NASH according to histological features. The diagnosis of NAFL is made when any of the following findings are present in the liver biopsy (72):
    1. Steatosis alone
    2. Steatosis with lobular or portal inflammation, without hepatocytes ballooning.
    3. Steatosis with hepatocytes ballooning without inflammation.

    On the other hend the histologic features require for diagnosis NASH is hepatic steatosis with hepatocytes ballooning degeneration and hepatic lobular inflammation (most commonly acinar zone 3). Fibrosis can be present but not require to meke the diagnosis. Based on histological features alone, NASH and alcoholic steatohepatitis cannot be distinguished from one other.

    Histological findings in NASH may include (73,74):

1. Apoptotic (acidophilic) bodies
2. Mild chronic portal inflammation (in case of severe inflammation disproportionate to the acinar lesion is more characteristic of hepatitis C virus)
3. Accumulation of perisinusoidal collagen deposition in zone 3 gives the typical "chicken wire" appearance.
4. Mallory-Denk bodies.
5. Cirrhosis, which is typically macronodular or mixed.
6. Portal fibrosis without perisinusoidal or pericellular fibrosis.
7. Megamitochondria.
8. Lobular lipogranulomas.
9. Glycogenated nuclei in periportal hepatocytes (rarely seen in alcoholic steatohepatitis).
10. PAS-diastase-resistant Kupffer cells.
11. Mild hepatic siderosis involving periportal hepatocytes or panacinar reticuloendothelial cells.

As NASH progress to cirrhosis, the inflammation subsides, and the cause cannot be determined ("Cryptogenic cirrhosis") (75). Isolated portal fibrosis (may represent a variant of NASH) can be seen more commonly than in adults in children with NASH (46).

Nonalcoholic fatty liver disease activity score (NAS)- The nonalcoholic fatty liver disease activity score (NAS) is a system that sums the possible histological changes in the spectrum of nonalcoholic liver disease (76). It enables the physician to categorize the patient according to the findings. The microscopic changes include:

1. Steatosis
2. Lobular inflammation
3. Ballooning

The scoring system ranges from 0 to 8. In the study that derived the NAS, scores 0-2 largely considered not diagnostic of NASH; scores 3-4 are equally divided between not diagnostic, borderline, and diagnosis of NASH; scores 5-8 largely consider a diagnosis of NASH. In practice, NAS is mainly used to grade the activity of NASH and help guide the treatment. Furthermore, diagnosis of NASH should not be made solely on the NAS score as the values are not always correlated with the diagnosis of NASH (77).

NAS scoring table:

Microscopic finding	Points	Criteria
1.steatosis	0	<5%
	1	5-33%
	2	>33-66%
	3	>66%
2. Lobular inflammation	0	Non
	1	<2 foci per 200X field
	2	2 to 4 foci per 200X field
	3	>4 foci per 200X field
3.Ballooning	0	Non
	1	Few ballooning cells
	2	Many cells/prominent ballooning

7. Management

As nonalcoholic fatty liver disease is mainly a consequence of secondary lifestyle and obesity, the primary treatment focuses on changing lifestyle and reducing fatty tissue. The treatment is further divided into non-pharmacologic and pharmacologic interventions.

7.1 General measures that should be applied to all patients

- Abstain from alcohol- alcohol cessation is recommended to all patients with NAFLD, and especially heavy alcohol use should be avoided (>14 drinks per week or >4 drinks per day for men, and >7 drinks per week or >3 drinks per day for women). Heavy alcohol use in patients with NAFLD is associated with hepatic steatosis, hepatic injury, and fibrosis progression. A study was followed of 71 patients with NAFLD for a mean of 14 years, 24 percent (17 patients) had fibrosis progression. In this study, the definition for heavy episodic drinking was 60g of alcohol on one occasion for a man and 48g for a woman. Heavy episodic drinking was more common in patients with fibrosis progression (47 versus 11 percent) (78).
  The data regarding mild to moderate alcohol consumption is controversial; some studies suggest that alcohol consumption slows down the resolution of the

inflammatory process, and other studies show improvement of hepatic ballooning and fibrosis among patients with moderate alcohol consumption (79).

- Immunization- Patients with chronic liver disease who contract viral hepatitis are at increased risk for hepatic decompensation. For this reason, they should receive vaccination against hepatitis A and B unless they have documented evidence of immunity. Pneumococcal vaccination with the 23-valent pneumococcal polysaccharide vaccine (PPSV23) is recommended for all adults age ≥65 and for those age <65 with certain comorbid conditions that increase risk of pneumococcal disease (eg, chronic liver, lung, heart disease; diabetes mellitus; smoking). The influenza vaccine is recommended annually for all adults. The intramuscular inactivated influenza vaccine appears to be more effective than the live attenuated intranasal vaccine and is preferred. All adults should receive a tetanus vaccine every 10 years, either with the tetanus-diphtheria toxoids vaccine (Td) or the tetanus-reduce diphtheria-acellular pertussis vaccine (Tdap). For adults who have not received Tdap, at least one dose of Tdap should be given (80).
- Patients with NAFLD are at increase risk for cardiovascular diseases and often have risk factors for cardiovascular disease (e.g., hypertension hyperlipidemia). Patients with other comorbidities like diabetes or dyslipidemia should be treated to maintain normal glucose and lipids levels, respectively (80).

7.2 Weight loss

Weight loss is recommended to all patients with NAFLD who are overweight (BMI >25 $kg/m^{2)}$) and obese (BMI>30 kg/m^2). Weight loss can lead to improvement in liver biochemical tests, liver histology, insulin level, and quality of life (80).

Initial lifestyle intervention- For patients with NAFLD, the recommended weight loss is 5 to 7 percent of body weight at a rate of 0.5to 1.0 kg per week through lifestyle modification, including diet modification and exercise. For patients with proven biopsy or suspected NASH, the weight loss goal is 7 to 10 percent of body weight (80).

For patients with abnormal serum alanine aminotransferases (ALT) (<20 for women and <30 for men) after achieving their weight loss goal, additional weight loss can be considered(80).

Meta-analysis of 373 patients, ≥5 percent weight loss results in an improvement in hepatic steatosis, and losing ≥7 percent of body weight leads to improvement in NAFLD activity score(81).

In a longitudinal study of 2793 patients, increased physical activity was linked to survival benefits in patients with NAFLD. More extended physical activity (measured by accelerometer) was associated with lower risk of all mortality causes during an average of 11 years (highest quartile of activity compared with lowest quartile: adjusted hazard ratio [aHR] 0.46, 95% CI 0.28-0.75). Furthermore, duration of physical activity was associated with lower cardiovascular disease-related mortality (highest quartile of activity compared with lowest quartile: aHR 0.28, 95% CI 0.08-0.98) (82).

7.3 Bariatric surgery

Patients with NASH and compensated cirrhosis who do not meet their weight loss goal after six months of a proper lifestyle and physical activity are candidates for bariatric surgery. Postoperative histologic improvement has been observed in obese patients with NAFLD. However, bariatric surgery can lead to fibrosis progression in some patients; it is recommended that every patient have their liver function tests monitored at six weeks, three months, and six months after the surgery (83,84).

The advantage of bariatric surgery for patients with NASH are shown in a systemic review of 21 observational studies; 18 studies report an improvement in hepatic steatosis, 11 studies report a decrease in inflammation, and six of them report improvement in fibrosis score. However, four studies report some worsening of hepatic fibrosis (85).

7.4 Pharmacologic therapies

The treatment option for patients with NAFLD depend on whether the patient has diabetes or not. Pharmacologic therapies are reserved for patients who did not achieve their weight loss target and patients with biopsy-proven NASH with fibrosis stage ≥2. Generally, liver-specific pharmacologic treatments are limited and not used in all patients (80).

7.4.1 Patient with NASH without diabetes

- Vitamin E- the mechanism of action is thought to be related to its antioxidant properties. Improvement of steatosis and inflammation was shown in some studies. However, the data are mixed, and the potential safety concern with high dose vitamin E decision is made individually for each patient. The recommended dose is 800 international units daily (86).

A randomized trial included 247 adults with NASH without diabetes to compare pioglitazone versus vitamin E versus placebo. The patient was randomly assigned to pioglitazone (30 mg/day), vitamin E (800 international units/ day), or placebo for 96 weeks. Patients treated with vitamin E were more likely to have an improvement in their histologic score than those treated with placebo (43 percent versus 19 percent). The following report from this trial found that improvement of ALT was more common in patients who received vitamin E than placebo (48 versus 16 percent). The results are consistent with observational studies that suggest improvement in aminotransferase in patients with NASH who receive vitamin E (87).

High-dose vitamin E (>4000 international units/day) has been associated with an increased mortality rate. However, the underline comorbidities or use of another supplement may have confounded the results(80).

Vitamin E is associated with an increased risk of prostate cancer. Treatment with vitamin E should be avoided in patients with a personal history or strong family history of prostate cancer (88).

7.4.2 Patients with NASH and diabetes

Patients with type 2 diabetes mellitus are usually treated with metformin as first-line therapy, metformin has no role in improving liver histology in patients with NASH and diabetes. For these patients, some other insulin-sensitizing agents can be considered, like pioglitazone and glucagon-like peptide-1 (GLP-1) receptor agonists (e.g., liraglutide).

- Pioglitazone- A drug from the thiazolidinediones class has shown to improve liver biochemical and fibrosis in patients with NASH (89,90). The impact of thiazolidinediones on histologic parameters in patients with NASH was inspected in a meta-analysis of four trials that compared placebo with thiazolidinediones treatment in 334 patients with NASH (91). The analysis found that thiazolidinediones improve hepatic histology like lobular inflammation (OR 2.6, 95% CI 1.7-4.0), ballooning degeneration (OR 2.1, 95% CI 1.3-3.4), and, hepatic steatosis (OR 3.4, 95% CI 2.2-5.3). The improvement in liver histology and biomarkers not connected with all thiazolidinediones, pioglitazone shown to be superior to other thiazolidinediones (91).

- GLP-1 receptor agonists-

  Liraglutide- in a study of 52 patients with NASH compared liraglutide and placebo following 48 weeks, end of treatment biopsy found resolution of NASH in 39 percent of patients in the liraglutide arm and 9 percent in the placebo arm (RR 4.3; 95% CI 1.0-17). As for fibrosis progression, patients in the liraglutide arm are less likely to have progression of fibrosis (9 versus 36 percent; (RR 0.2; 95% CI 0.1-1.0) (92).

  Semaglutide- A phase II study of 320 biopsy-proven patients with NASH and stage F1, F2, or F3 cirrhosis compared with placebo at 72 weeks with semaglutide (0.4 mg once daily). The histological resolution rate of NASH was higher in the semaglutide group compared to placebo (59 versus 17 percent OR 6.87, 95% CI 2.60-17.63). Low doses of semaglutide (0.1 mg or 0.2 mg once daily) were less effective but had higher histological resolution compared to placebo (40 percent; OR 3.36, 95% CI 1.29-8.86 and 36%). (OR 2.71, 95% CI 1.06-7.56) respectively. However, the rate of improvement in the stage of cirrhosis was not significantly different between the treatment group and the placebo. No statistical analysis was provided, but gastrointestinal side effects (e.g., nausea, vomiting) were reported more frequently with semaglutide than with placebo. Additional data on histological outcomes and adverse events are needed before routine use of semaglutide in NASH patients without other indications (such as type 2 diabetes) (93).

7.4.3 Therapies with uncertain benefit

Other pharmacological agents are used in the treatment of NAFLD, but none of them have been studied sufficiently to be recommended.

Atorvastatin- Pilot studies fund improvement of aminotransferase level after atorvastatin in patients with NAFLD (94). The use of atorvastatin was then investigated in a secondary analysis of studies examining the effects of atorvastatin, vitamin C, and vitamin E on the development of cardiovascular events in healthy adults. Two of the exclusion criteria for this study were diabetes and serum aminotransferases above the upper limit of normal levels by 1.5 times. At baseline, 80 patients had NAFLD based on imaging criteria. After an average of 3.6 years of follow-up, patients in the treatment group had NAFLD more than in the placebo group (34 vs. 70%; adjusted OR 0.36, 95% CI 0.16-0.83) (95). However, the diagnosis of NAFLD is based on diagnostic imaging criteria rather than

histology, and exclusion criteria (diabetes or increased aminotransferase) limit generalizability.

Omega-3 fatty acid- Studies suggest the benefits of omega-3 fatty acids in patients with NAFLD (96,97). In a meta-analysis of nine studies of 355 patients, treatment with omega 3 fatty acids was associated with improved fatty liver and aspartate aminotransferase levels (96). There was also a tendency to improve alanine aminotransferase levels. Only fatty liver continued to show improvement with omega-3 fatty acid treatment when the analysis was limited to data from randomized trials (96,98).

Aspirin- Limited data suggest that taking aspirin daily may be beneficial for patients with NAFLD (99,100) During the enrollment phase of a biopsy-proven NAFLD prospective cohort study of 361 patients, daily aspirin users has less NASH (adjusted odds ratio [aOR] 0.68, 95% CI 0.37-0.89) and fibrosis (aOR 0.54, 95% CI 0.31-0.82) compared to nonuser daily aspirin (99). In addition, in 317 patients without progressive fibrosis at baseline, daily aspirin users were less likely to progress to progressive fibrosis than nonusers during the 3692 person-year follow-up period (adjusted Hazard ratio 0.63, 95% CI 0.43-0.85). These results are promising and future studies may contribute to data demonstrating the hepatoprotective effects of aspirin.

7.5 Laboratory monitoring

Levels of serum aminotransferases (ALT and aspartate aminotransferases) are measured every 3-6 months after patients have made lifestyle modifications to achieve and maintain their weight loss goals. If aminotransferase does not return to normal with weight loss, or if aminotransferase increases, evaluate the patient for another cause of liver disease (80).

Monitoring for fibrosis — The recommendation to monitor patients with advanced fibrosis relies upon whether or not they have biopsy-proven NASH and if they achieved weight loss goal and normalization of serum aminotransferases:

- Patients with biopsy-proven NASH – For patients with biopsy-proven NASH, it is recommended to achieve a noninvasive evaluation for advanced fibrosis at a time intervals depending on the clinical course:

1. For patients who have not achieved their weight loss of at least five to seven percent and/or have improved serum aminotransferases, noninvasive evaluation is recommended every three years.
2. For patients who reach their weight reduction goal and have normal serum aminotransferases, noninvasive evaluation is recommended every four years.

If the noninvasive evaluation indicates a low-risk fibrosis score (≤F1), it is recommended to monitor patients every four years (if weight reduction was achieved and maintained) or every three years (if weight reduction was not achieved or maintained). Patients with NASH and no fibrosis or minimum fibrosis have a very good prognosis and do not need close follow-up (101).

If the noninvasive evaluation indicates high-risk fibrosis (≥F2), it is recommended to proceed to liver biopsy for evaluation of advanced fibrosis. Patients with no cirrhosis on biopsy need further follow-up, as discussed above. If the biopsy identifies cirrhosis, further management of cirrhosis complications is needed (e.g., variceal hemorrhage, hepatocellular carcinoma) (80).

Patients without biopsy-proven NASH-Patients with nonalcoholic steatohepatitis do not undergo regular noninvasive testing for fibrosis. Then, if the patient's clinical condition changes (e.g., additional weight gain, expression of other features of metabolic syndrome), a noninvasive assessment of fibrosis is performed every 3-4 years (80).

Patients with cirrhosis-Treatment of cirrhosis due to NAFLD is similar to treatment of cirrhosis due to other causes, including management of portal hypertension, screening for hepatocellular carcinoma, and evaluation for liver transplantation in patients with decompensated cirrhosis (80).

8. Disease course

Progressive Fibrosis-Patients with NAFLD are at risk for progressive fibrosis, histologically defined as stage F2 or higher. Cirrhosis develops when simple steatohepatitis progresses to steatohepatitis and then to fibrosis. Fibrosis stage is the only indicator that correlates with

outcomes such as liver disease, liver transplantation, and liver-related mortality in patients with NAFLD (102–104).

The risk of disease progression in patients with NAFLD has been evaluated in several studies, but the results vary, and the risk of progressive fibrosis in patients with NAFLD is unknown (105–114). A meta-analysis of 11 studies examined the progression of NAFLD to fibrosis in 366 patients(115). Overall, the stage of fibrosis progressed in 132 patients (36%), remained stable in 158 patients (46%), and improved in 76 patients (21%). Patients with simple steatosis on biopsy appear to be at lower risk of developing progressive fibrosis, while patients with nonalcoholic steatohepatitis are at higher risk (116). In addition, some patients with fibrosis show regression of their illness (109–111).

Risk Factors-Factors associated with progressive fibrosis can be classified as patient or disease related:

- Patient-related risk factors:

1. Alcohol consumption.
2. BMI ≥28 kg / m2 (117,118).
3. Diabetes (103).
4. Elderly people (eg, 50+) (111,119).

- Disease-related risk factors

1. Histological evidence of inflammation in liver biopsy.
2. Ballooning degeneration on biopsy and Mallory vitreous or fibrosis (103).
3. Elevated serum aminotransferase (eg, at least twice the upper limit of normal values) (48,117,120).

Coffee consumption is associated with a reduced risk of developing fibrosis (121).

Alcohol Ingestion-Patients are advised to avoid alcohol. In particular, it is advisable to avoid heavy drinking (>14 drinks per week or >4 drinks per day for men, and >7 drinks per week or >3 drinks per day for women).

Heavy alcohol use in patients with NAFLD is associated with hepatic steatosis, hepatic injury, and fibrosis progression. A study was followed of 71 patients with NAFLD for a mean of 14 years, 24 percent (17 patients) had fibrosis progression. In this study, the definition for

heavy episodic drinking was 60g of alcohol on one occasion for a man and 48g for a woman. Heavy episodic drinking was more common in patients with fibrosis progression (47 versus 11 percent). The data regarding mild to moderate alcohol consumption is controversial; some studies suggest that alcohol consumption slows down the resolution of the inflammatory process, and other studies show improvement of hepatic ballooning and fibrosis among patients with moderate alcohol consumption.

Hepatic inflammation- evidence of hepatitis is an important risk factor for the development of advanced fibrosis. In a systematic review of 187 patients with a paired biopsies, the median time to onset of progressive to fibrosis in patients with inflammation on the first biopsy was 4.2 years and 13.4 in patients with no inflammation (111). After adjustment of potential confounders, the presence of inflammation at the first biopsy increased the likelihood of progression to progressive fibrosis 2.5-fold compared to patients without inflammation.

Hepatocellular Carcinoma-Hepatocellular Carcinoma (HCC) monitoring is recommended for patients with NASH-related cirrhosis.

Patients with cirrhosis due to NAFLD are at increased risk of HCC compared to patients without cirrhosis (122). In a systematic review of 61 studies and case series of patients with NAFL or NASH, the risk of HCC in patients with cirrhosis ranged from 2.4% over 7 years to 12.8% over 3 years. In patients without cirrhosis, the risk of death from HCC ranged from 0 to 3 percent after a follow-up of up to 20 years.

Mortality-It is not clear whether patients with NAFLD have higher mortality from all causes compared to the general population. Although small population-based studies show the risk of death (104,123,124), the largest study in the United States suggests that mortality from all causes does not increase in the absence of fibrosis. Data from the National Health and Nutrition Examination Survey (NHANES) from 1999 to 2012 included 6000 adults with NAFLD and progressive fibrosis rates (determined by the NAFLD activity score) of 30% and 10.3%, respectively(125). Mortality in NAFLD patients without fibrosis was lower (HR 0.41, 95% CI 0.220,76) compared to patients with NAFLD and advanced fibrosis (HR 3.13, 95% CI 1.93-5.08).

Cardiovascular disease is the leading cause of death in patients with NAFLD. A previous study (NHANES III) showing similar mortality trends found that increased mortality in patients with NAFLD with fibrosis (measured by NAFLD fibrosis score) was almost entirely

due to cardiovascular causes. (HR 3.46, 95% CI 1.91-6.25)(126). Patients with NAFLD can identify and manage risk factors for cardiovascular disease (diabetes, hyperlipidemia, etc.).

Liver Decompensation-The stage of fibrosis is associated with the risk of liver decompensation. In a study of 1,773 adults with NAFLD followed up for a median of 4 years, the risk of onset of liver decompensation was higher in patients with stage F4 fibrosis (cirrhosis) and stage F3 fibrosis (bridge fibrosis) compared to pateints with stage F0 to stage F2 (2.69 and 0.99 cases per 100 man-years vs. 0.05 events per 100 man-years) (127).

9. Conclusions

NAFLD is defined by the presence of hepatic steatosis with no evidence of other causes.
It can be subclassified according to the present (NASH) or absent of inflammation (NAFL).
Most of the patients are asymptomatic but patients with NASH are more commonly present with abdominal discomfort, malaise and fatigue. Patients are commonly coming into attention with abnormal liver enzymes or accidental finding on imaging.
The pathophysiology behind NAFLD is unknown but there is a strong epidemiological association between the presence of NAFLD, metabolic syndrome and insulin resistance.
Physical activity and particularly weight loss is the number one treatment of choice with the greatest improvement potential. There are no targeted pharmacological therapy for NAFLD and some of the medications that are used for the treatment of diabetes as, part of the metabolic syndrome, have the potential to cause weight gain and thus should be use in caution.
As for today, there is an increasing number of obese people among the population and therefor the prevalence of NAFLD is increasing.
Due to the lack of efficient targeted pharmacological treatment education for a healthy lifestyle and physical activity are the most efficient way to reduce and prevent NAFLD.

10. Biography

Snir Tamari was born on September 22nd, 1992. Snir served in the Israeli Defense Forces

(IDF), in Paratroopers, between 2012 and 2015. During the years 2016-2022 Snir studied general medicine in School of Medicine University of Zagreb, Croatia. During the studies Snir spent one month in Hadassah Ein Kerem Hospital, Jerusalem in the department of Pediatric Medicine, One month in Kaplan Hospital, Rehovot, in the department of General Surgery, one month in Wolfson Hospital, Bat Yam, in the department of Internal medicine as a study experience.
Additionally worked as a Physician's assistant in Geriatric center in Jaffa and volunteered in the ambulance emergency services of the town.

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