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Non invasive methods for diagnosis of nonalcoholic fatty liver disease

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Abstract: Background & Aim: Non Alcoholic fatty liver disease is the coming epidemic after eradication of HCV by DAA. NAFLD is a common cause of chronic liver disease with a prevalence ranges from 17% to 33%. It is present as simple steatosis, nonalcoholic steatohepatitis, Liver cirrhosis or even HCC which can develop at NAFLD patients even without cirrhosis. The diagnosis of NAFLD was traditionally based on the histopathological changes. The fibrosis may be assessed noninvasively using serum biomarkers and (NAFLD, FIB4, APRI and BARD) scores as well as by measuring certain intrinsic physical properties of the liver parenchyma by transient elastography. TE, the most widely used and validated noninvasive technique, offers several advantages: it is user-friendly, machine-independent and painless, has a short duration of examination. Compared to liver biopsy, the technique is less prone to sampling errors as it explores a liver volume about 100 times larger. The aim of this study was to evaluate the measurement of liver stiffness by transient elastography as a noninvasive method for diagnosis of NAFLD. Methods: The study was conducted on 200 patients who had bright liver with or without elevated liver enzymes collected from the outpatient clinics and inpatients of Tropical Medicine and Infectious Diseases Department at Tanta University Hospitals. **Other causes of chronic liver disease such as** Alcoholics, Chronic HCV, Chronic HBV, Drugs induced liver injury, Schistosomal hepatic fibrosis and Metabolic diseases were excluded. diagnosis was confirmed by history taking, clinical examination, Liver function tests, Renal function tests, BMI, Lipid profile, (APRI, BARD, FIB4 NAFLD) scores. US and fibroscan. Results: Transient elastography was done and there were 71(35%) patients F0, 53(26.5%) patients F1,25(12.5%) patients F2,40 (20%) patients F3 & 11 (5.5%) patients F4 and has sensitivity 86.36, accuracy 95.4%, PPV 96 & NPV 85.1. As regard to Controlled Attenuation Parameter (CAP), There were 38 patients S1, 60 patients S2 & 102 patients S3 with sensitivity 90% & accuracy 80.7%. Conclusion: TE was found to have a very good performance in diagnosing stages of fibrosis and steatosis (CAP) in patients with NAFLD.

[Shimaa Abdeloneim Mashaal, Hanan Ahmed Elbassat, Hanan Hamed Soliman, Fathyia Elsayed Assal. **Non invasive methods for diagnosis of nonalcoholic fatty liver disease.** *Nat Sci* 2020;18(2):62-72]. ISSN 1545-0740 (print); ISSN 2375-7167 (online). http://www.sciencepub.net/nature. 10. doi:10.7537/marsnsj180220.10.

Keywords: Non invasive; method; diagnosis; nonalcoholic fatty; liver; disease

Definition of NAFLD:

Non-Alcoholic Fatty Liver disease (NAFLD) is one of the major causes of liver disease worldwide and it is estimated to range from 6.3–33%, with a median of 20% in the general population. The prevalence of non-alcoholic steatohepatitis (NASH) has been estimated to range from 3–5**% (Vernon et al., 2011).**

NAFLD is characterized by excessive hepatic fat accumulation, associated with insulin resistance (IR), and defined by the presence of steatosis in >5% of hepatocytes according to histological analysis or by a proton density fat fraction >5.6% assessed by proton magnetic resonance spectroscopy (1HMRS) or quantitative fat/water selective magnetic resonance imaging (MRI**) (Ratziu et al., 2010).**

Practice guidelines by the American Association for the Study of Liver Diseases, American College of Gastroenterology and the American Gastroenterological Association categorized NAFLD histologically into nonalcoholicfatty liver (NAFL) and NASH. NAFL is defined as the presence of hepatic steatosis with no evidence of hepatocellular injury, namely, hepatocytes ballooning. NASH is defined as the presence of hepatic steatosis and inflammation with hepatocyte injury (ballooning) with or without fibrosis **(Chalasani et al., 2012).**

NAFL is generally benign, where as NASH can progress to cirrhosis, liver failure and hepatocellular carcinoma. Thus, it is necessary to detect early stages of NASH and quickly initiate treatment **(Chalasani et al., 2012).**

Increased fat accumulation in liver or fatty liver disease can also be secondary to number of causes, including:

- Excessive alcohol consumption.

- Drugs (especially chemotherapeutic agents e.g. methotrexate, tamoxifen), hepatic toxins (e.g. arsenic, carbon tetrachloride).
- Chronic viral hepatitis (e.g. hepatitis B and hepatitis C viral infection).
- Congenital storage diseases (e.g. Wilson disease, hemochromatosis**) (Hamer et al., 2006).**

The diagnosis of NAFLD requires the exclusion of both secondary causes and of a daily alcohol consumption of 30 g per day for men and 20 g per day for women (**Ratziu et al., 2010).**

Prevalence and incidence:

NAFLD is the most common liver disorder in Western countries, affecting 17–46% of adults, with differences according to the diagnostic method, age, sex and ethnicity **(Vernon et al., 2011).**

NAFLD increases the risk of more advanced disease, both in adults and in children. NAFLD is also present in 7% of normal-weight (lean) but metabolically obese persons due to insulin resistance **(Younossi et al., 2012)** more frequently in females, at a younger age and with normal liver enzymes. Their liver disease may or may not be progressive **(Fracanzani et al., 2008).**

NAFLD incidence has rarely been measured. It was 20-86/1000 person-years based on elevated liver enzymes and/or on ultrasound (US), and 34/1000 per year by 1H-MRS (**Marchesini and Mazzotti, 2015).**

However, the progressive form of NAFLD (i.e. NASH), particularly when associated with advanced fibrosis, should be identified in patients at risk (age >50 years, type 2 diabetes mellitus [T2DM] or Metabolic Syndrome**) (Chalasani et al., 2012).**

Pathogenesis of NAFLD:

- Lifestyle and genes.
- A high-calorie diet, excess (saturated) fats.
- Refined carbohydrates.
- Sugar-sweetened beverages.
- A high fructose intake and diet **(Chalasani et al., 2012).**

They are associated with weight gain and obesity, and more recently with NAFLD. High fructose consumption may increase the risk of NASH and advanced fibrosis, although the association may be confounded by excess calorie intake or by unhealthy lifestyles and sedentary behavior **(Chiu et al., 2014)**, which are more common in NAFLD **(Gerber et al., 2012).**

Several genetic modifiers of NAFLD have been identified (**Anstee et al., 2013).** The best-characterized genetic association is with PNPLA3, initially identified from genome-wide association studies and confirmed in multiple cohorts and ethnicities as a modifier of NAFLD severity across the entire histological spectrum **(Valenti et al., 2010).**

Recently, the TM6SF2 gene has been reported as another disease modifier **(Liu et al., 2014)** and may have clinical utility assisting risk stratification for liver-related vs. cardiovascular morbidity. The PNPLA3 rs738409 variant also confers susceptibility and affects the histological pattern of NAFLD and fibrosis in obese children and adolescents **(Valenti et al., 2010).**

A NASH risk score based on four polymorphisms has been validated in obese children with increased liver enzymes **(Nobili et al., 2014)**

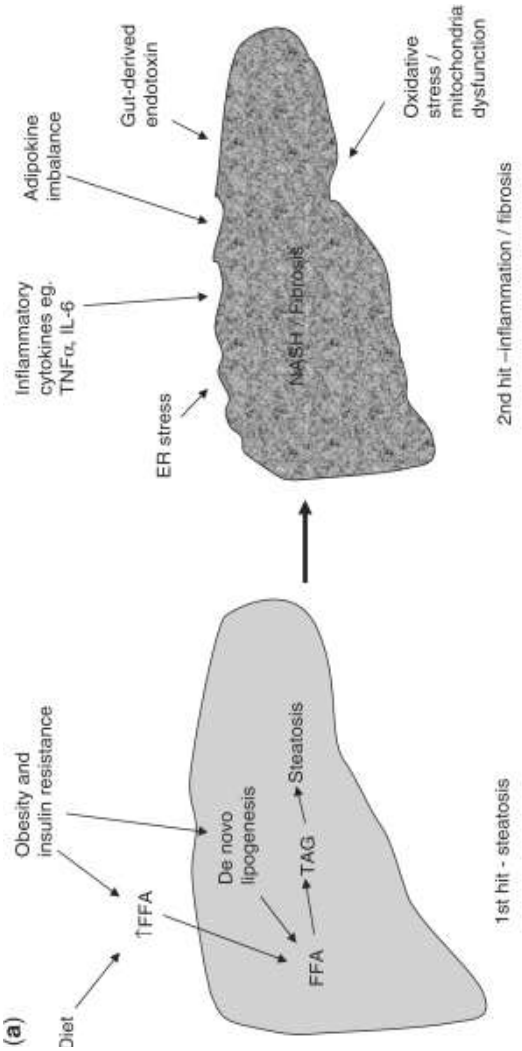

Figure (1a): The traditional 2-hit hypothesis of NAFLD: steatosis represents the 'first hit', which then sensitizes the liver to injury mediated by 'second hits', such as inflammatory cytokines, adipokines, oxidative stress and mitochondrial dysfunction, leading to steatohepatitis and fibrosis. The presence of high levels of oxidative stress reduces the ability of mature hepatocytes to proliferate, resulting in reduced endogenous liver repair **(Jou et al., 2008)**.

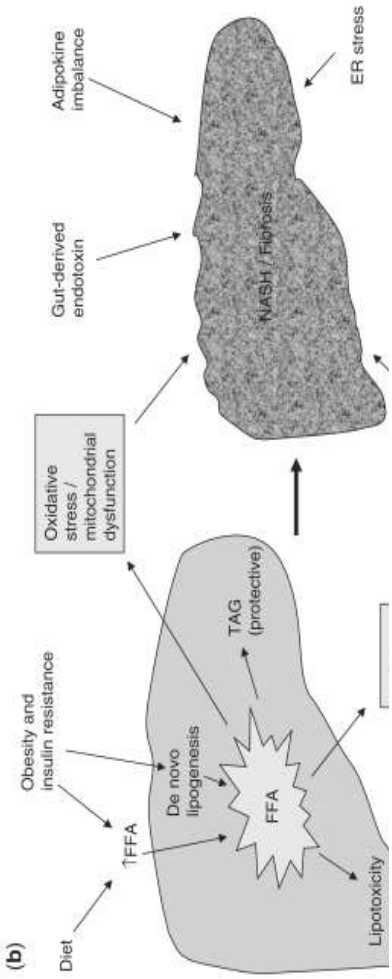

Figure (1b): Modified 2-hit hypothesis of NAFLD: the accumulation of FFA alone has been suggested to be sufficient to induce liver damage, without recourse for a second hit. Indeed, rather than being harmful, triglyceride accumulation in the form of steatosis may actually be protective by preventing FFA-induced inflammation and oxidative stress **(Jou et al., 2008)**.

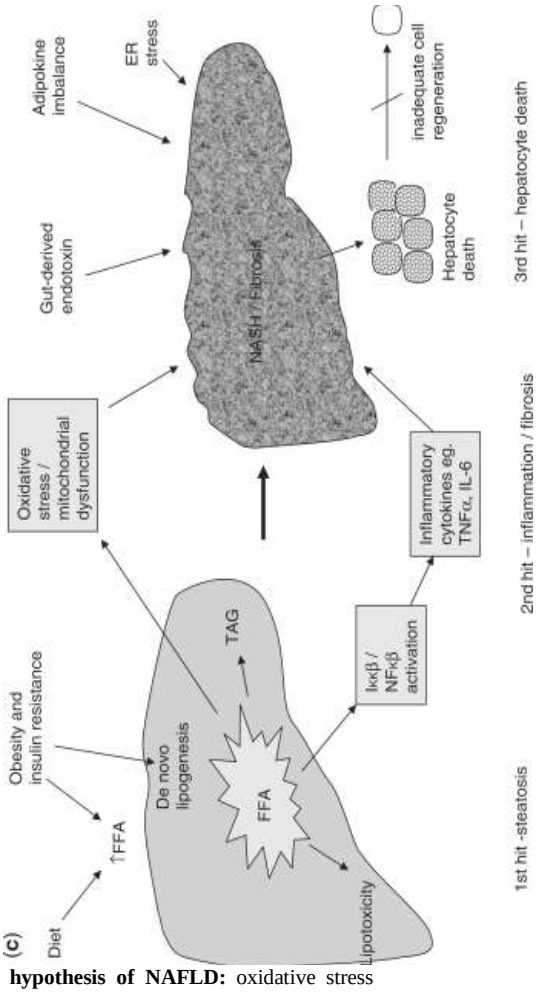

Figure (1c): The 3-hit hypothesis of NAFLD: oxidative stress reduces the ability of mature hepatocytes to proliferate, resulting in the recruitment of other pathways of liver regeneration, such as HPCs. These cells have the capability of differentiating into both cholangiocytes and hepatocytes and contributing to liver repair. It has been suggested that an inability to mount such a ductular response, as is seen in patients transplanted for NASH who have denervated livers, may be responsible for a more progressive pattern of liver damage. Thus, impaired proliferation of hepatocyte progenitors represents the proposed 'third hit' in NAFLD pathogenesis **(Jou et al., 2008).**

Common Pathogenic Mechanisms of NAFLD:

1- Adipose Tissue Inflammation:

What exactly initiates adipose tissue inflammation in obesity is uncertain; but hypoxia and death of rapidly expanding adipocytes are believed to play a role **(Johnson et al., 2012).**

Adipocytes under inflammation secrete cytokines and chemokines, particularly tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and CC chemokine ligand-2 (CCL2) **(Matherly and Puri, 2012).**

Circulating adiponectin regulates hepatic fatty β-oxidation through AMP-activated protein kinase (AMPK) and acetyl-Co Acarboxylase (ACC) signaling **(Hasenour et al., 2013)**.

2- De Novo Lipogenesis (DNL).

TNF-α was the first proinflammatory cytokine detected in adipose tissue and is involved in the regulation of insulin resistance **(Barbuio et al., 2007).**

At the same time, extra hepatic adipocytes are compromised in their natural ability to secrete adiponectin, an anti-inflammatory adipokine that facilitates the normal partitioning of lipid to adipocytes for storage, so imbalanced gut microbiota and adiponectin involved in the pathogenesis and progression of NAFLD **(Tilg, 2010).**

A number of prior studies have shown that diets enriched in both saturated fat and simple sugar carry a high risk of hepatic steatosis, at least in part, through enhanced DNL **(Ouyang et al., 2008)**.

Simple sugars are converted to fatty acids more readily than complex carbohydrates **(Lecoultre et al., 2013)**, and fructose is a more potent inducer of DNL than glucose **(Stanhope et al., 2011).**

3. Insulin Resistance.

IR means need for 200 or more units of insulin per day to reach the glycemic control. There are many soluble mediators derived from immune cells and/or adipose tissue, such as TNF-α and IL-6 affect IR **(Tilg and Moschen, 2008).**

Serine phosphorylation of insulin receptor substrates by inflammatory signal transducers such as c-jun N-terminal protein kinase 1 (JNK1) or inhibitor of nuclear factor-κB kinase-β (IKK-β) is considered one of the key aspects that disrupts insulin signaling **(Tilg and Moschen, 2010).**

On the other hand, insulin resistant subjects with NAFLD show reduced insulin sensitivity, not only at the level of the muscle, but also at the level of the liver and adipose tissue **(Lomonaco et al., 2012).**

4. Lipotoxicity.

Studies have indicated that certain lipids can be harmful to hepatocytes in NAFLD. This is particularly true of the long-chain saturated fatty acids (SFAs) such as palmitate (C16:0) and stearate (C18:0), which are abundant in animal fat and dairy products and produced in the liver from dietary sugar. Under physiological conditions, SFAs are transported to mitochondria for β-oxidation or esterified for either excretion in the form of VLDL (very low density lipoproteins) or storage as lipid droplets **(Fuchs and Sanyal, 2012)**.

FC accumulation leads to liver injury through the activation of intracellular signaling pathways in Kupffercells (KCs), hepatic stellate cells (HSCs), and hepatocytes. The activation of KCs and HSCs promotes inflammation and fibrogenesis **(Arguello et al., 2015)**.

These lipids, including FC, SFA, and certain lipid intermediates from excessive SFA, can activate a variety of intracellular response such as JNK1 and a mitochondrial death pathway, resulting in lipotoxic stress in the endoplasmic reticulum and mitochondria, respectively **(Fuchs and Sanyal, 2012).**

In addition, the toll like receptor 4 (TLR4) is a pattern recognition receptor that activates a pro inflammatory signaling pathway in response to excessive SFAs. This pathway is initiated by recruiting adaptor molecules such as toll/IL-1 receptor domain containing adaptor protein (TIRAP) and myeloid differentiation factor 88(MyD88) that ultimately lead to activation of nuclear factor κB with production of TNF-α **(Sharifnia et al., 2015).**

5. Mitochondrial Dysfunction.

Mitochondria are the most important energy suppliers of the cell and play a pivotal role in fatty acid metabolism **(Jiang et al., 2011).** This discrepancy implicates mitochondrial dysfunction in the state of liver fat overload that is characteristic of NAFLD. Although the mechanisms responsible for the mitochondrial dysfunction remain poorly understood in NAFLD, reduced enzymatic activities of mitochondrial electron transport chain (ETC) complexes may be attributed to increased generation of reactive oxygen species (ROS) as a result of ETC leakage during mitochondrial β-oxidation in energy production (in the form of ATP) **(Day, 2002)**.

Studies have found that ROS can damage the ETCH and even cause mutations in the mitochondria DNA **(Kujoth et al., 2005)**.

6. Oxidative Stress.

In the context of increased supply of fatty acids to hepatocytes, oxidative stress can occur and be attributable to raised levels of reactive oxygen/nitrogen species (ROS/RNS) and lipid peroxidation that are generated during free fatty acid metabolism in microsomes, peroxisomes, and mitochondria **(Koek et al., 2011)**. Peroxidation of plasma and intracellular membranes may cause direct cell necrosis/apoptosis **(Novo et al., 2011).**

7. Endoplasmic Reticulum (ER) Stress.

The ER is a vast dynamic and tubular network responsible for the synthesis, folding/repair, and trafficking of a wide range of proteins. Under pathological and/or stressful conditions such as NASH, it has been observed that ER efficiency in the protein folding, repairing, and/or trafficking machinery is decreased while the demand of protein synthesis and folding/repair is increased **(Hotamisligil, 2010)**.

Such an imbalance between the load of needed protein-folding and the response-related capability of the ER is termed ER stress, which can lead to the accumulation of unfolded and/or misfolded proteins within the ER lumen **(Lee and Ozcan, 2014).** hepatic ER stress seems to play an important role in regulating the composition and size of lipid droplets as well as lipid synthesis, including cholesterol metabolism **(Zambo et al., 2013).**

The likelihood of progression to advanced NASH/cirrhosis results from a complex interplay between genetic predisposition and the mechanisms described earlier **(Feldstein et al., 2004).**

Clinical manifestation of Steatosis:

NAFLD is not driven by clinical manifestations, since most patients are asymptomatic while Symptomatic patients present unspecific complaints such as fatigue, abdominal discomfort and, only seldom, manifestations of advanced liver disease. There are, high-risk populations in whom the prevalence is so high to raise the hypothesis of

NAFLD such as obesity and T2DM, present with hepatic steatosis **(Targher et al., 2007)**.

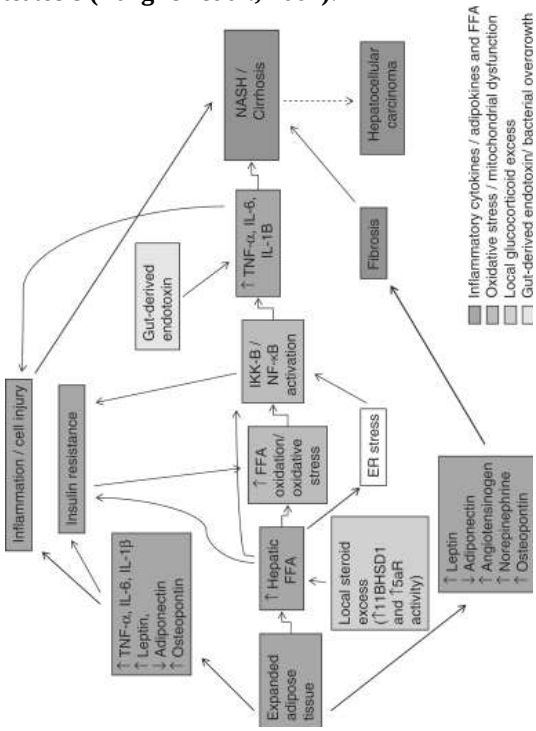

Figure (2): Proposed pathogenesis of NASH.

Also, liver tests, namely aminotransferases, are usually normal, and when increased, typically present mild elevation with a fluctuant pattern **(Fracanzani et al., 2008)**.

Isolated increase in alkaline phosphatase is not frequent, but it has been reported in 10% of patients referred to tertiary care **(Pantsari and Harrison, 2006).**

Some do not recommend it, advocating that traditional scores should assess cardiovascular risk, while others consider diagnosis and evaluation of NAFLD as part of the management of DM being an indication for more intense monitoring and therapeutic intervention **(Obika and Noguchi, 2012).**

Steatohepatitis, NASH:

The diagnosis of NASH provides important prognostic information and indicates an increased risk of fibrosis progression, cirrhosis and possibly hepatic comorbidities (HCC). It may also prompt a closer follow-up and possibly a greater need for more intensive therapy. Clinical, biochemical or imaging measures cannot distinguish NASH from steatosis **(Machado and Cortez-Pinto, 2015).**

Cytokeratin-18 fragments (CK-18), which are generated during cell death (M65fragments) or apoptosis (M30 fragments), have modest accuracy for the diagnosis of NASH (66% sensitivity, 82% specificity) (**Cusi et al., 2014).**

CK-18 changes parallel histological improvement but do not perform better than alanine transaminase (ALT) in identifying histological responders to life style modification and diet **(Vuppalanchi et al., 2014).**

Common metabolic disorders related to NAFLDis tightly associated with IR not only in the liver, but also in muscle and adipose tissues **(Gaggini et al., 2013).**, and also with the Metabolic Syndrome, defined as the cluster of any three of the following five features associated with IR:

- impaired fasting glucose (IFG)<or =110 mg/dl.
- hypertriglyceridemia<150mg/dl.
- low high-density lipoprotein (HDL)-cholesterol (gender-adjusted) less than 40 mg/dl at male & 5040 mg/dl at female.
- increased waist circumference (ethnicity adjusted) more than 102 cm at male and 88cm at female.
- high blood pressure more than 110/ 85mmHg **(Alberti et al., 2005).**

As all components of Met S correlate with liver fat content, independently of BMI, the presence of Met S in any given patient should lead to an evaluation of the risk of NAFLD, and vice versa. The presence of NAFLD should lead to an assessment of all components of Met S. Hepatic triacylglycerol accumulation is accompanied by abnormal hepatic energy metabolism **(Koliaki et al., 2015).** And impaired insulin mediated suppression of hepatic glucose and very low-density lipoprotein production **(Yki-Jarvinen, 2014).**

Liver disease progression has been associated with persistence or worsening of metabolic abnormalities, including HOMA-IR **(McPherson et al., 2015).**

Obesity, BMI and waist circumference, a measure of visceral adiposity, are positively related to the presence of NAFLD and predict advanced disease, particularly in the elderly **(Bedogni et al., 2005).**

A large proportion of patients with cryptogenic cirrhosis have a high prevalence of metabolic risk factors **(Caldwell and Crespo, 2004).**

Suggesting that the majority of cases of cryptogenic cirrhosis are ‘‘burned-out” NASH. Common comorbidities of obesity, such as T2DM, and sleep apnoea **(Aron-Wisnewsky et al., 2012).**

Polycystic ovary syndrome and other endocrine disorders (hypogonadism), further drive NAFLD prevalence and severity (**Gaggini et al., 2013).** Importantly, patients with BMI <30 kg/m2 (or even <25kg/m2) but with visceral fat accumulation or dysfunctional adipose tissue can exhibit NAFLD with/without abnormal liver enzymes (**Koliaki et al., 2015)**.

The currently used concept of ‘‘metabolically healthy” obese individuals should be considered with caution, given that they may exhibit gene expression similar to those of metabolically altered obese patients, and may have altered liver tests and adverse health outcomes when longitudinally **(Gomez-Ambrosi et al., 2014).**

Diabetes mellitus and NAFLD:

T2DM patients are insulin resistant, often obese, dyslipidemic display increased liver enzymes **(Ghouri et al., 2010)** and tend to accumulate hepatic fat independently of BMI **(Kotronen et al., 2008).**

The prevalence of NAFLD is also higher in persons at risk of T2DM, defined as:

- A glycosylated haemoglobin A1c (HbA1c) of 5.7–6.4% (38.8–46.4 mmol/mol).
- IFG (fasting glucose: 100–125 mg/dl [5.55–6.94 mmol/L]) and/or
- Impaired glucose tolerance (IGT; glucose: 140–199 mg/dl [7.77–11.04 mmol/L] at 2 h of the standardized 75 g oral glucose tolerance test [OGTT]) **(Loomba et al., 2012).**

Diabetes risk and T2DM closely associate with the severity of NAFLD, progression to NASH, advanced fibrosis and the development of HCC **(Loomba et al., 2012).**

Conversely, US-defined NAFLD is associated with a2–5fold risk of developing T2DM after adjustment for several lifestyle and metabolic confounders **(Armstrong et al., 2014)**. The standardized 75 gOGTT should therefore be performed in persons with increased diabetes risk **(American Diabetes Association., 2014).**

Insulin treatment increases body fat, but it does not appear to promote or worsen NAFLD in diabetes **(Llaurado et al., 2015),** while acute insulin infusion dose-dependently increases liver fat content in T2DM **(Anderwald et al., 2002).**, chronic insulin treatment improves adipose tissue IR and therefore reduces non-esterified fatty acids flux and hepatic fat content **(Anderwald et al., 2002).**

Complications of NAFLD:

In general, NAFLD is a slowly progressive disease, both in adults and in children, but fibrosis rapidly progresses in 20% of cases **(**The rate of progression corresponds to 1 fibrosis stage every14 years in NAFL and every 7 years in NASH, and is doubled by arterial hypertension **(Singh et al., 2015).**

NASH is associated with an increased standardized mortality ratio compared with the general population **(Haflidadottir et al., 2014)** and liver disease is the third most common cause of death after CVD and cancer. US-diagnosed NAFLD is not associated with increased mortality **(Kim et al., 2013)** presumably because progression to NASH and fibrosis is rare for steatosis alone **(McPherson et al., 2015).**

Cardiovascular disease:

The prevalence and incidence of CVD is higher in NAFLD than in matched controls and driven by the association between NAFLD and MetS components (**Oni et al., 2013).**

CVD is a more common cause of death than liver disease in NAFLD **(Targher et al., 2010).**

In most studies, biochemical markers of atherosclerosis (low HDL cholesterol, high triacylglycerol) or inflammation (high-sensitive C reactive protein [CRP]), and increased levels of procoagulant/ prothrombotic factors are more common in NAFLD than in persons without steatosis **(Targher et al., 2010).**

Pre-atherogenic lesions such as increased carotid intima-media thickness; coronary artery, abdominal aortic and aortic valve calcifications; endothelial dysfunction and functional un responsiveness of the artery wall are more prevalent in NAFLD and are, in some studies, correlated with histological severity **(Bhatia et al., 2012).**

Other defects such as echocardiographic and ECG abnormalities and altered cardiac energy metabolism have also been demonstrated **(Bhatia et al., 2012).** They are largely independent of traditional risk factors, duration of diabetes, glycemic control, drug treatment and Met S components. In the general population, US-detected steatosis and its surrogate markers (e.g., FLI) are associated with a higher risk of CVD mortality in the long-term **(Calori et al., 2011)** and the risk increases further in NASH and in advanced fibrosis **(Targher et al., 2010).**

The overall consensus is that CVD should be identified in NAFLD regardless of the presence of traditional risk factors. Conversely NAFLD screening should be performed in persons at high CVD risk. An association between serum c-glutamyl transferase (GGT) and CVD incidence has been prospectively established, although it is insufficient for devising follow-up protocols. Not ably, CVD and metabolic risk factors are also reported in adolescents and children with NAFLD **(Pacifico et al., 2014).**

Hepatocellular carcinoma:

Large-scale epidemiological studies have repeatedly associated obesity and T2DM with the risk of HCC, and the occurrence of HCC has also been reported in NAFLD/cryptogenic cirrhosis. The cumulative incidence of NAFLD-associated HCC (>10-fold higher in T2DM and obesity) varies according to study population (population-based, natural history vs. clinic-based cohorts with/without fibrosis or cirrhosis) **(Dyson et al., 2014).**

Other extrahepatic disorders:

Chronic kidney disease (CKD) can be found in 20-50% of NAFLD patients, particularly in biopsy-proven NASH **(Musso et al., 2014).** US-defined

NAFLD carries a 1.5- to 2-fold adjusted risk of incident CKD in Type 1 diabetes mellitus **(Targher et al., 2010).**

NAFLD is also associated with colorectal cancer **(Kim et al., 2014),** metabolic bone disease (vitamin D deficiency, osteoporosis) **(Hazlehurst and Tomlinson, 2013).**

Treatment of NAFLD:

The resolution of the histological lesions defining NASH is now accepted as a surrogate endpoint, particularly in clinical trials. Only a few properly designed randomized controlled trials (RCTs) are available, with improvement/ regression of hepatic necro inflammation and/or fibrosis as primary outcomes (**Valenti et al., 2014).**

Diet and lifestyle changes:

Epidemiological evidence suggests a tight relationship between unhealthy lifestyle and NAFLD **(Zelber-Sagi et al., 2011).** which makes lifestyle correction mandatory in all patients Relatively small amounts of weight loss reduce liver fat and improve hepatic IR **(Petersen et al., 2005).**

In a pilot RCT of cognitive-behaviour therapy, lifestyle intervention resulted in more weight loss, more frequent resolution of NASH and a borderline higher ($p = 0.05$) reduction in the NAS score **(Promrat et al., 2010).**

In a post hoc analysis on the study of weight loss on NAFLD patients, a weight loss of 7% was associated with histological improvement **(Vilar-Gomez et al., 2015)**. In an uncontrolled, 12-month study with 261 paired biopsies, a modest lifestyle-induced weight loss was associated with NASH regression (25% of total cases) without worsening of fibrosis **(Vilar-Gomez et al., 2015).**

Pragmatic approaches combining dietary restriction and a progressive increase in aerobic exercise/resistance training (**Rodriguez et al., 2012)** are preferable and should be individually tailored. No data are available on their long-term effects on the natural history of NAFLD Drug treatment Rationale. Drug therapy should be indicated for progressive NASH (bridging fibrosis and cirrhosis) but also for early-stage NASH with increased risk of fibrosis progression (age >50 years; diabetes, MetS, increased ALT **(Adams et al., 2005)** or active NASH with high necroinflammatory activity **(Sanyal et al., 2015).**

No drug has currently been tested in phase III trials and is approved for NASH by regulatory agencies. Therefore, no specific therapy can be firmly recommended and any drug treatment would be off-label (**Younossi et al., 2014).**

Insulin sensitizers:

There is scarce evidence for a histological efficacy of metformin in NASH **(Haukeland et al., 2009).** The effect of metformin on liver fat is weak, because of its inability to restore serum adiponectin levels in the short-term **(Tiikkainen et al., 2004).** Some preclinical data support an anti-tumorigenic activity of metformin on liver cancer (**Bhalla et al., 2012)** while the demonstration of reduced rates of HCC in humans is limited to retrospective studies **(Zhang et al., 2012)**.

Thiazolidinediones:

Are peroxisome proliferator-activated receptor (PPAR) c agonists with insulin-sensitizing effects. PIVENS trial compared low dose pioglitazone vs. vitamin E vs. placebo for 2 years in patients without overt diabetes. Pioglitazone improved all histological features (except for fibrosis) and achieved resolution of NASH more often than placebo (**Sanyal et al., 2010).**

The histological benefit occurred together with ALT improvement and partial correction of IR. Similar results were reported in two smaller and shorter RCTs (**Aithal et al., 2008).**

Prolonged therapy with rosiglitazone, up to 2 years, did not result in further histological improvement (**Ratziu et al., 2010).** although this was not formally tested with pioglitazone. Side effects of glitazones are of concern: weight gain, bone fractures in women and, rarely, congestive heart failure. Despite the safety and tolerability profile, pioglitazone can be used for selected patients with NASH, particularly in T2DMwhere the drug has a registered use. Incretin-mimetics, acting on the glucose-insulin interplay have shown favorable results in pre-marketing studies on liver enzymes (**Vilsboll et al., 2012).**

A small pilot trial of daily injections of liraglutide met the histological outcome of NASH remission without worsening of fibrosis **(Armstrong et al., 2015).**

Antioxidants, cytoprotective and lipid lowering agents:

In the PIVENS trial, vitamin E (800 IU/day) improved steatosis, inflammation and ballooning and induced resolution of NASH in 36% of patients (21% in the placebo arm **(Sanyal et al., 2010).** Reduced ALT correlated with histological improvement and histological non responders did not reduce ALT (**Hoofnagle et al., 2013).**

In the pediatric TONIC trial (**Lavine et al., 2011)**, vitamin E failed to reduce amino transferases, steatosis and inflammation but improved ballooning and doubled the rate of NASH clearance vs. placebo. These results contrast with previous trials, which were mostly negative in both adults and children. Concerns about long-term safety of vitamin E exist, mainly an increase in overall mortality **(Bjelakovic et al., 2007).** In haemorrhagic stroke and prostate cancer in males older than 50 (**Schurks et al., 2010).** Vitamin E may be used in non-cirrhotic non-diabetic NASH patients

but further studies are needed before firm recommendations can be made **(Klein et al., 2011)**.

Ursodeoxycholic acid (UDCA) has been investigated in several RCTs, at different doses and for up to 2 years, but only showed some biochemical but no histological improvements **(Leuschner et al., 2010).**

A synthetic farnesoid X receptor agonist, obeticholic acid, improved IR in **T2DM (Mudaliar et al., 2013).** In the phase IIb FLINT trial, a 72-week treatment with obeticholic acid in non-cirrhotic NASH patients, improved all NASH lesions while improving fibrosis **(Neuschwander-Tetri et al., 2015).**

Main issues with safety and tolerability were increased low-density lipoprotein (LDL)-cholesterol and pruritus. Preliminary data from small or uncontrolled studies suggested that n-3 polyunsaturated fatty acids (PUFA) might reduce liver fat (**Parker et al., 2012)**, but two trials testing PUFA on histological outcomes were negative (**Argo et al., 2015)** whereas statins have not been adequately tested. Their use in NAFLD is safe, with no increased risk of hepatotoxicity, and may even significantly reduce amino transferases (**Dongiovanni et al., 2015)**.

Promising novel agents with anti-inflammatory, antifibrotic or insulin sensitizing properties (dual PPARa/d agonists, dual chemokine receptor CCR2/CCR5 antagonists and fatty acid/bile acid conjugates) and antifibrotic agents (anti-lysyl oxidase-like [anti-LOXL2] monoclonal antibodies) are also being tested in late-phase RCTs in NASH (**Dongiovanni et al., 2015)**.

Iron depletion

Hepatic iron accumulation is associated with IR, and iron depletion improves IR (**Valenti et al, 2007).** In NAFLD, high ferritin levels are common, in the presence of variable transferrin saturation, independent of gene polymorphisms of familial hemochromatosis. In these patients, a phlebotomy program to reduce iron stores to near iron deficiency improved the NAS score, without worsening fibrosis **(Valenti et al., 2014**).

Bariatric Surgery:

The study by (**Mathurin et al**., **2009)** that prospectively correlated clinical and metabolic data with liver histology before and 1 and 5years after bariatric surgery in 381 adult patients with severe obesity. Gastric band, bilio-intestinal bypass, and gastric bypass were done in 56%, 23%, and 21%, respectively Compared to baseline, there was a significant improvement (**Mathurin et al**., **2009)**.

In the prevalence and severity of steatosis and ballooning at 1 and 5 years following bariatric surgery. Inpatients with probable or definite NASH at baseline (n-99), there was a significant improvement in steatosis, ballooning, and NAS and resolution of probable or definite NASH at 1 and 5 years following bariatric surgery. Most histological benefits were evident at 1 year with no differences in liver histology between 1 and 5 years following bariatric surgery. Two meta-analyses **(Chavez-Tapia et al., 2010)** evaluated the effect of bariatric surgery on the liver histology in patients with NAFLD. The meta-analysis by (**Mummadi et al., 2008)** showed that steatosis, steatohepatitis, and fibrosis appear to improve or completely resolve after bariatric surgery.

Liver transplantation:

NAFLD-associated cirrhosis is among the top three indications for liver transplantation. The 3- and 5-year survival is not different in NAFLD vs. no-NAFLD; NAFLD carries a higher risk of death from cardiovascular complications and sepsis, whereas the risk of graft failure is lower (**Wang et al., 2014)**.

The overall mortality is associated with BMI and diabetes, with 50% of cases with BMI >35 kg/m2 dying within 1-year of transplantation (**Heuer et al., 2012).** Transplant failure (10%and 45% at 10 and 20 years, respectively **(Heuer et al., 2012),** in obese patients is rarely associated with recurrent NASH cirrhosis (_2%) (**Wang et al., 2014).**

NAFLD in Patients with Other Chronic Liver Diseases:

Because of the high prevalence of risk factors for NAFLD and NASH, it is not uncommon for patients with other chronic liver diseases to exhibit co-existing histological features of NAFLD **(Brunt et al., 2003).**

Coexistent hepatic steatosis is common in chronic hepatitis C (HCV) infection and is strongly associated with more advanced liver disease **(Petta et al., 2011)**. Another large study showed high prevalence of steatosis (40.5%) and steatohepatitis (15%) in patients with primary biliarycirrhosis (PBC) **(Sorrentino et al., 2010),** In clinical practice, it is not uncommon for obese and/or diabetic patients with autoimmune liver disease to exhibit steatosis and steatohepatitis in their liver biopsies. Previous studies have shown that obesity, insulin resistance, and hepatic steatosis are associated with a lower response to pegylated interferon and ribavirin for the treatment of HCV **(Negro and Clements, 2009).**

Obesity does not have a similar negative impact on the response to newer protease-inhibitor based anti-viral regimens **(Jacobson et al., 2011).**

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