The IDEA Journal
International Digestive Experts Articles

Year I - N. 2, 2019

Special iGLOBE 2019

The international Gastro Liver Oesophagus and Bowel Event

Current Developments in NAFLD

Sinergie
Edizioni scientifiche

The IDEA Journal

International Digestive Experts Articles

QUARTERLY SCIENTIFIC UPDATE IN GASTROENTEROLOGY

Year I - N. 2, 2019

PUBLISHER

SINERGIE S.r.l., *Edizioni Scientifiche*

Via Morimondo, 26 - 20143 Milan, Italy

Ph. +39 02 58118054 - Fax +39 02 8322004

E-mail: redazione@edizionisinergie.com

www.edizionisinergie.com

PUBLISHING DIRECTOR

Mauro Rissa

EDITORIAL SECRETARIAT

SINERGIE S.r.l., *Edizioni Scientifiche*

GRAPHIC LAYOUT

SINERGIE S.r.l., *Edizioni Scientifiche*

PRESS

Galli Thierry Stampa S.r.l.

Via Caviglia, 3 - 20139 Milan, Italy

Registration at Court of Milan

n. 260, 09/12/2019

Special iGLOBE 2019

The international Gastro Liver Oesophagus and Bowel Event

Current Developments in NAFLD

Editorial

Current developments in NAFLD

Tom Hemming Karlsen

Nonalcoholic fatty liver disease (NAFLD) is the most common liver disease seen in patients with obesity, diabetes, and metabolic syndrome. Metabolic syndrome is an important predictor of the severe form of NAFLD, nonalcoholic steatohepatitis (NASH), and NASH patients with diabetes have an increased risk of liver cirrhosis and hepatocellular carcinoma.

With the prevalence of obesity, diabetes and cardiovascular diseases around the world, NAFLD has become a global public health problem representing one of the most important causes of liver-related disability and mortality. The effective prevention and treatment of NAFLD is expected to reduce the burden of liver disease and cardiovascular disease.

Several advances have been made recently in the understanding of the natural history, diagnosis, pathogenesis, and treatment of NASH. The first are non-invasive algorithms such as the FIB-4 score or the NAFLD fibrosis score. The most promising developments have focused on composite non-invasive tests or blood tests that might help distinguish NASH from NAFLD. An example is cytokeratin 18, which is a biomarker of hepatocyte apoptosis. Other non-invasive fibrosis staging systems also have started to gain broader recognition and are now being validated in large-scale studies.

Biopsy has remained the gold standard to differentiate advanced from early-stage fibrosis, and noninvasive fibrosis staging systems have been studied. However, active research is going on regarding staging, including transient liver elastography using FibroScan, the use of which is widely established in patients with hepatitis C virus infection. This tool will probably play a greater role in the future, in the ability to stage fatty liver disease avoiding liver biopsy.

Lifestyle interventions such as diet and exercise are the crux of treatment for the vast majority of patients with NAFLD. With regard to the particular type of diet that should be implemented, reduction in carbohydrate content, especially simple sugars, is important because it is increasingly recognized that fatty liver disease is associated with insulin resistance, which is preceded by hyperinsulinemia. Another aspect of diet is the role of saturated fat, particularly cholesterol. Animal studies have demonstrated that a diet rich in saturated fat and cholesterol has a proinflammatory effect on the liver in addition to causing steatosis. There are unequivocal data that even a 10% weight loss can result in significant improvement in and resolution of NASH. Exercise is equally important as diet. Either exercise or weight loss through diet alone can independently improve insulin and glucose metabolism, and the effects of exercise are synergistic. However, lifestyle modification is difficult to achieve and to sustain.

The remarkable progress that has been made in previous years in understanding disease pathogenesis has led to medical therapies targeting various aspects of the fat accumulation and injury pathways. These therapies can be classified according to four general groups: 1. Targeting metabolism, oxidative stress (Antioxidants and Lipid-lowering agents) and insulin resistance (bile-acid based insulin sensitization); 2. Targeting inflammation and apoptosis, 3. Targeting fibrosis, 4. Targeting the gut and obesity.

There are currently a number of drugs undergoing pivotal trials as potential therapy for NASH. The ideal treatment will lead, in the short term, to a reduction in liver inflammation and fibrosis as well as an improvement in insulin sensitivity and metabolic complications but, in the long term, will need to reduce cardiovascular and liver outcomes.

A case of non-alcoholic fatty steatohepatitis in a patient with morbid obesity, hypertension, diabetes, obstructive sleep apnea and knee osteoarthritis

Raluca Pais

INTRODUCTION

With the growing epidemic of obesity and metabolic syndrome, nonalcoholic fatty liver disease (NAFLD) has become the most common cause of chronic liver disease worldwide and will become one of the leading causes of cirrhosis.

This article presents the case of a patient with morbid obesity and suspected NAFLD undergoing instrumental and laboratory tests in order to individuate the correct management and treatment.

THE CASE

A 57 years old female patient first addressed by the nutritionist for altered LFT (>2.5 N), high ferritin, and morbid obesity (Body Mass Index - BMI - 47.83 kg/m^2) was visited at our institute (Institute of Cardiometabolism and Nutrition, ICAN, Pitié Salpetriere Hospital, Paris, France) for suspected NAFLD.

The woman presented the following characteristics of medical history:

- High Blood Pressure (HBP) since 2000
- Diabetes Mellitus T2 (T2DM) since 2010; no macro/microvascular complication. On treatment with metformin 1000 mg
- Dyslipidemia (on statins treatment - irbesartan/HCT 150/12.5 mg)
- Obstructive Sleep Apna (OSA); Apnea-hypopnea index (AHI) = 16/h
- Significant knee osteoarthritis
- Hypothyroide (lthyroxin 75 µg)
- Alcohol (wine) daily intake inferior to 10 g/day
- Former tobacco user (previous 10 years).

DIAGNOSTIC PROCEDURES

We conducted laboratory and instrumental tests in order to confirm the suspect of NAFLD.

In particular, the following parameters were investigated with laboratory tests: transaminases (AST/ALT/GGT), occult blood (FG) glicate emoglobine (HbA1C), creatinine, albumin, ferritin, transferrine salturation (TraSat Coef), clotting factors (CT), triglicerids (TG) and cholesterol (HDL/LDL). A non-invasive evaluation of liver fibrosis through liver ultrasound was also performed. Results from the tests are shown in the table 1.

The ultrasound test revealed there was steatosis but no sign of Portal hypertension (PHT). At this point we wondered whether a liver biopsy was necessary in this patient or not in order to assess the histological presence and fibrosis grade.

It is important to consider all the relevant factors that limit the reliability of non-invasive tests: age is a confounding factor for the fibrosis 4 (FIB4) score

Test	Results
Laboratory	
AST	48 IU/L
ALT =	56 IU/L
GGT	82 IU/L
FG	7.2 mmol/l
HbA1C	8%;
Creatinin	62 µmol/l
Albumin	36g/l
Ferritine	441 µg/l
TraSat Coef	34%
LDL	1.46 g/l
CT	2.25
TG	2.09
HDL	0.37
Instrumental (non-invasive ultrasound)	
FT	0.63
AT	0.45
NAFLD Fibrosis Score	1.855
FIB4	1.66

Table 1. Results from diagnostic tests

with 82% false positive results by increasing age, the presence of diabetes and factors influencing liver stiffness (portal pressure, acute inflammation, heart failure, amyloidosis, etc.).

However, there are also many factors influencing the use of liver biopsy in patients with NAFLD, as shown in Figure 1 (1).

We decided to perform the liver biopsy which showed the presence of macro-vesicular steatosis amounting to 90% together with a portal and perisinusoidal fibrosis. So, non-alcoholic fatty steatohepatitis (NASH) was diagnosed in a patient with morbid obesity, HBP, T2DM, OSA and knee osteoarthritis.

The treatment of this patient with many co-morbidities should be based on lifestyle modifications, that can be very useful in terms of fibrosis regression (2), but are hard to achieve. Another treatment option is the bariatric surgery, which can contribute to ameliorate NASH in many cases, though not in all (Figure 2).

The evaluation of cardiovascular risk is mandatory. Results showed a high risk for the presence of several important co-morbidities and the estimated 10-year risk of coronary ischemic disease (CHD), including coronary calcium, was also very high, amounting to 19.7% (3).

The control of CV risk factors requires physical

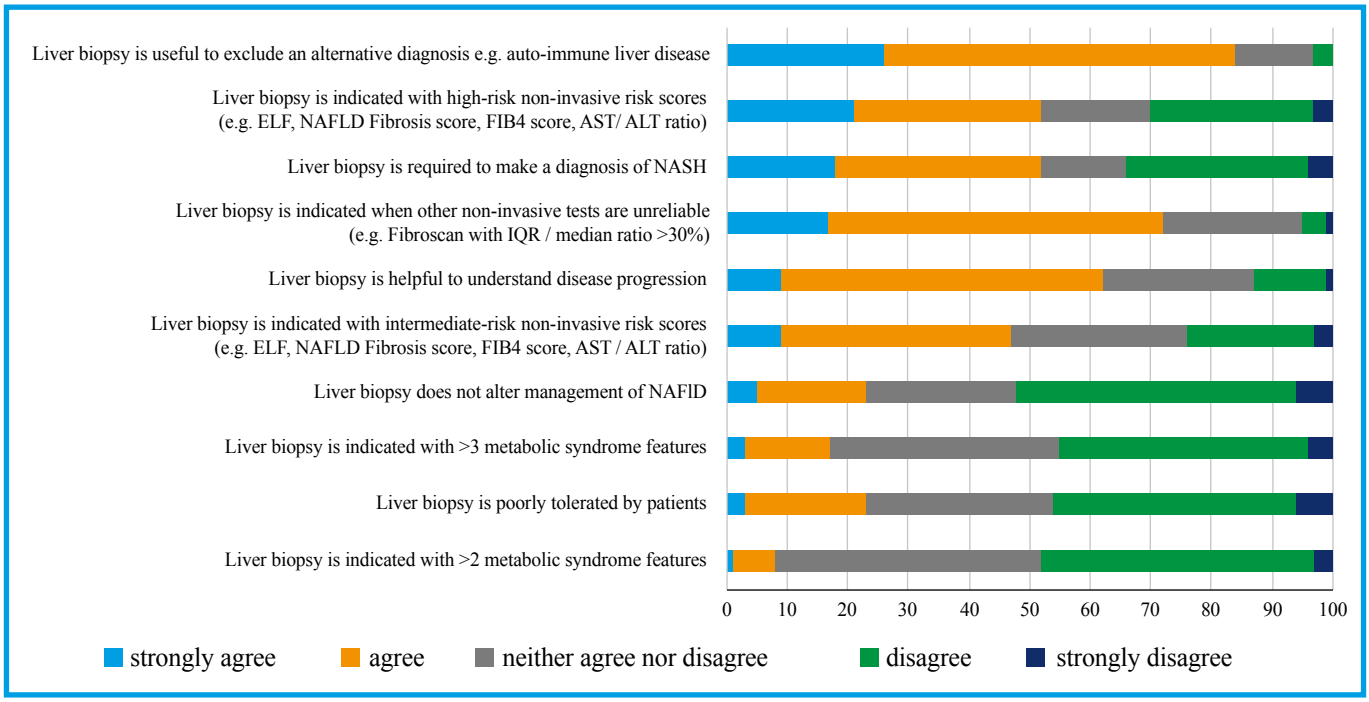
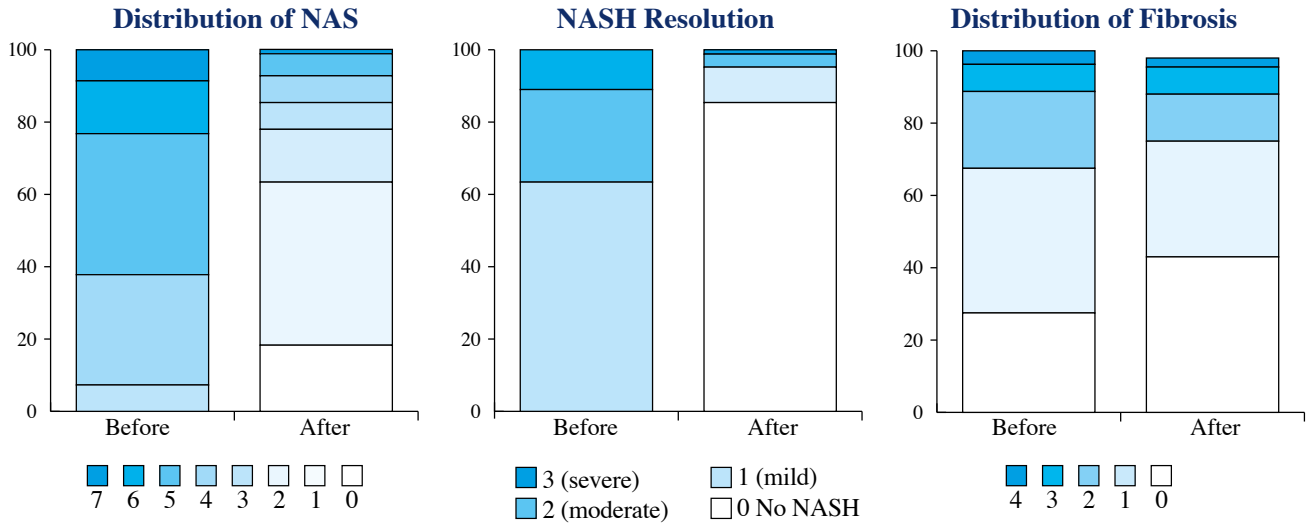


Figure 1. Factors influencing the use of liver biopsy in NAFLD (mod. from Sheridan DA et al, Frontline Gastroenterology 2017)



✔ NASH disappeared in 85.4% of cases
✔ Fibrosis improved in 46%
✔ The rate of disappeared of NASH was higher in patients with mild NASH than in those with moderate or severe NASH
✔ 14.6% of patients has persistent NASH 1 year after bariatric surgery. These patients had significantly lower weight loss, higher NAS and refractory IR profile

Figure2. Bariatric surgery in NASH patients

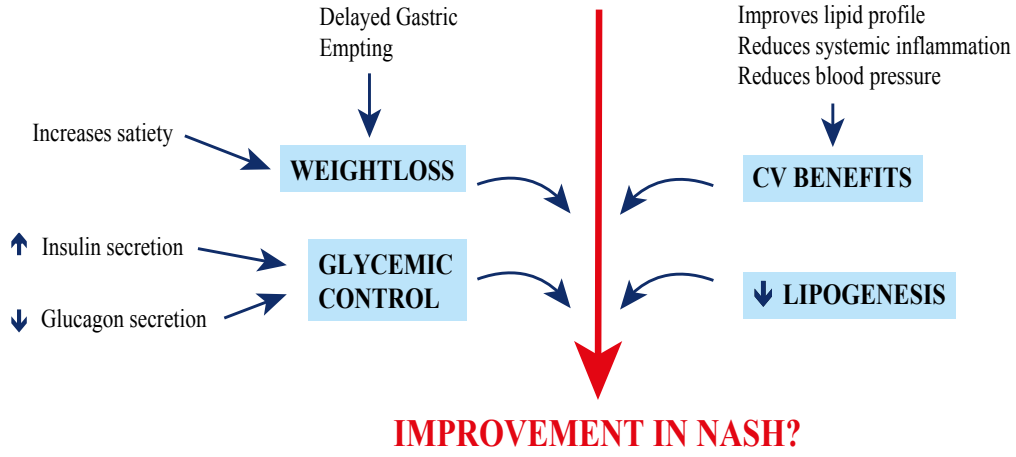
✔ **Physical activity/Weight control**
✔ **Smoking cessation**
✔ **Control for HBP**
✔ **Lipids control**

Classification of physical activity

Absolute intensity		
Intensity	**MET**	**Examples**
Light	1.1-2.9	Walking <4.7 km/h, light household work
Moderate	3-5.9	Walking briskly (4.8–6.5 km/h), slow cycling (15 km/h), painting/decorating, vacuuming, gardening (mowing lawn), golf (pulling clubs in trolley), tennis (doubles), ballroom dancing, water aerobics
Vigorous	≥6	Race-walking, jogging or running, bicycling >15 km/h, heavy gardening (continuous digging or hoeing), swimming laps, tennis (single)

Smoking	No exposure to tobacco in any form.
Diet	Low in saturated fat with a focus on wholegrain products, vegetables, fruit and fish.
Physical activity	At least 150 minutes a week of moderate aerobic PA (30 minutes for 5 days/week) or 75 minutes a week of vigorous aerobic PA (15 minutes for 5 days/week) or a combination thereof.
Body weight	BMI 20–25 kg/m^2. Waist circumference <94 cm (men) or <80 cm (women).
Blood pressure	<140/90 mmHg[a]
Lipids[b] LDL[c] is the primary target	**Very high-risk: <1.8 mmol/L (<70 mg/dL),** or a reduction of at least 50% if the baseline is between 1.8 and 3.5 mmol/L (70 and 135 mg/dL)[d] **High-risk: <2.6mmol/L (<100 mg/dL),** or a reduction of at least 50% if the baseline is between 2.6 and 5.1 mmol/L (100 and 200 mg/dL) **Low to moderate risk: <3.0 mmol/L (<115 mg/dL).**
HDL-C	No target but >1.0 mmol/L (>40mg/dL) in men and >1.2 mmol/L (>45 mg/dL) in women indicate lower risk.
Triglycerides	No target but <1.7 mmol/L (<150 mg/dL) indicates lower risk and higher levels indicate a need to look for other risk factors.
Diabetes	HbA1c <7%. (<53 mmol/mol)

Figure 3. Control for CV risk factors (according to National/international Guidelines)



	Liraglutide	Placebo	Relative risks or mean changes (95% CI) from baseline to 48 weeks (liraglutide vs placebo)	p value*
Primary outcome				
Number of patients with paired liver biopsies	23	22	..	..
Patients with resolution of non-alcoholic steatohepatitis	9 (39%)	2 (9%)	4·3 (1·0 to 17·7)	0·019
Changes from baseline in histopathological parameters				
Total NAFLD activity score				
Change in score	-1·3 (1·6)	-0·8 (1·2)	-0·5 (-1·3 to 0·3)	0·24
Patients with improvement	17 (74%)	14 (64%)	1·2 (0·8 to 1·7)	0·46
Hepatocyte ballooning score				
Mean change	-0·5 (0·7)	-0·2 (0·6)	-0·3 (-0·7 to 0·1)	0·15
Patients with improvement	14 (61%)	7 (32%)	1·9 (1·0 to 3·8)	0·05
Steatosis				
Change in score	-0·7 (0·8)	-0·4 (0·8)	-0·2 (-0·6 to 0·2)	0·32
Patients with improvement	19 (83%)	10 (45%)	1·8 (1·1 to 3·0)	0·009
Lobular inflammation				
Change in score	-0·2 (0·6)	-0·2 (0·5)	-0·01 (-0·3 to 0·3)	0·97
Patients with improvement	11 (48%)	12 (55%)	0·9 (0·5 to 1·6)	0·65
Kleiner fibrosis stage				
Change in score	-0·2 (0·8)	0·2 (1·0)	-0·4 (-0·8 to 0·1)	0·11
Patients with improvement	6 (26%)	3 (14%)	1·9 (0·5 to 6·7)	0·46†
Patients with worsening	2 (9%)	8 (36%)	0·2 (0·1 to 1·0)	0·04†

Figure 4. GLP1 antagonists and NAFLD

activity, weight loss, smoking cessation, reduction of HBP and normalization of lipids, according to the well-known international guidelines, as shown in Figure 3.

The treatment with statins is recommended in this patient because it can reduce the CV risk and may perhaps contribute to improve liver histology (4). A large clinical trial on 346 patients with T2DM and histological proven NAFLD showed that statin users were protected from severe forms of NAFDL compared with statin non-users (5). So, atorvastatin 10 mg/die was prescribed.

In order to optimize T2DM in NASH patients, glucagon-like peptide (GLP) 1 analogues have been shown to be better than metformin in improving pathophysiological parameters of liver fibrosis (6), as displayed in Figure 4. The drug simtuzumab has resulted to be ineffective for patients with bridging fibrosis or compensated cirrhosis caused by NASH (7).

A screening to assess the presence of esophageal varices

Plt = 175 000
Albumin = 36
Creatinin = 86µmol/l
HbA1c = 7.5 mmHg
BiliT = 29 µmol/l
CT =4.01mmol/l ; TG = 1.70 mmol/l;
AST = 36 IU/l, ALT = 41 IU/l, GGT = 95 IU/l
LSM = 27kPA

CHILD = A5
MELD = 11

TREATMENT :
METFORMIN 1000 mg,
IRBESARTAN/HCT 150/12.5 mg,
LTHYROXIN 75 µg

LIRAGLUTIDE
ATORVASTATIN 10 MG/D
PREVISCAN
CARVEDILOL 12.5 MG/D
VITAMIN E 500 IUX2/D

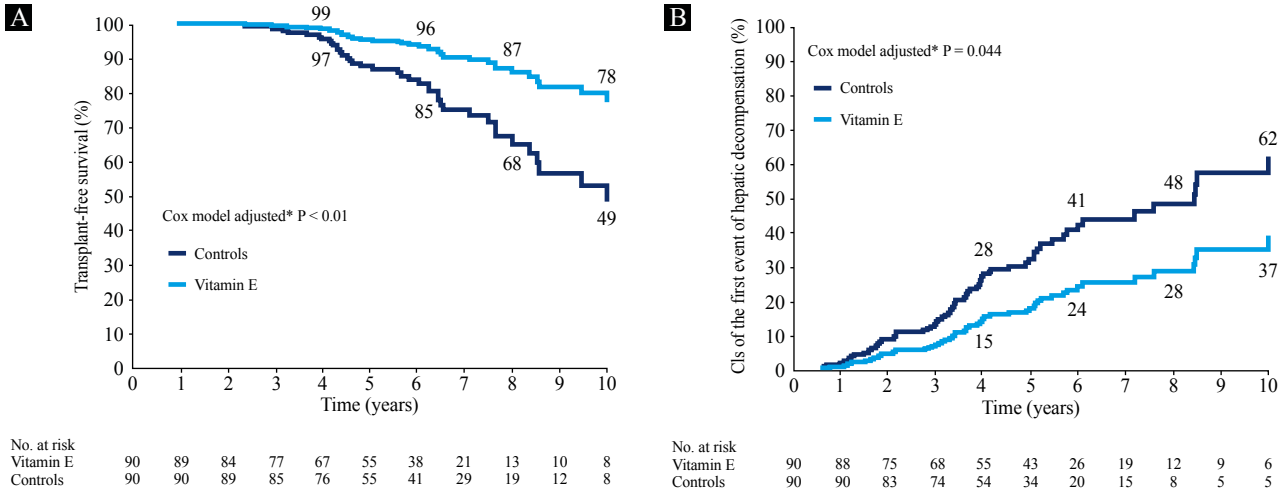


Figure 5. Difference between prescribed treatments and the initial therapy

would be necessary in this patient, due to the high liver stiffness measurement (LSM = 24 kilo Pascal) and to the very high hepatic venous pressure gradient (HPVG) = 18 mmHg.

As esophageal varices were detected at endoscopy, therefore we had to decide about the best therapy among band ligation, propranolol and carvedilol. Data from medical literature indicate that carvedilol is significantly superior to both band ligation (8) and propranolol (9) in decreasing HPVG and therefore, it was preferred in this case.

So, the treatment of this patient was deeply reviewed and changed from her initial therapy and the new prescribed drugs were liraglutide, atorvastatin, previscan, carvedilol and vitamin E. The difference between the two treatments is visible in Figure 5.

References

1. Sheridan DA, Aithal G, Alazawi W, Allison M, Anstee Q, Cobbold J, Khan S, Fowell A, McPherson S, Newsome PN, Oben J, Tomlinson J, Tsochatzis E. . Care standards for non-alcoholic fatty liver disease in the United Kingdom 2016: a cross-sectional survey. FrontlinGastroenterol. 2017 Oct;8(4):252-259.

2. Vilar-Gomez E, Martinez-Perez Y, Calzadilla-Bertot L, Torres-Gonzalez A, Gra-Oramas B, Gonzalez-Fabian L, Friedman SL, Diago M, Romero-Gomez M. Weight Loss Through Lifestyle Modification Significantly Reduces Features of Nonalcoholic Steatohepatitis. Gastroenterology. 2015 Aug;149(2):367-78.

3. Budoff MJ, Young R, Burke G, Jeffrey Carr J, Detrano RC, Folsom AR, Kronmal R, Lima JAC, Liu KJ, McClelland RL, Michos E, Post WS, Shea S, Watson KE, Wong ND. Ten-year association of coronary artery calcium with atherosclerotic cardiovascular disease (ASCVD) events: the multi-ethnic study of atherosclerosis (MESA). Eur Heart J. 2018 Jul 1;39(25):2401-2408.
4. Dongiovanni P, Petta S, Mannisto V, Mancina RM, Pipitone R, Karja V, Maggioni M, Kakela P, Wiklund O, Mozzi E, Grimaudo S, Kaminska D, Rametta R, Craxi A, Fargion S, Nobili V, Romeo S, Pihlajamaki J, Valenti L. Statin use and non-alcoholic steatohepatitis in at risk individuals. J Hepatol. 2015 Sep;63(3):705-12
5. Nascimbeni F, Aron-Wisnewsky J, Pais R, Tordjman J, Poitou C, Charlotte F, Bedossa P, Poynard T, Clément K, Ratziu V; LIDO study Group. Statins, antidiabetic medications and liver histology in patients with diabetes with non-alcoholic fatty liver disease. BMJ Open Gastroenterol. 2016 Mar 18;3(1):e000075.
6. Armstrong MJ, Gaunt P, Aithal GP, Barton D, Hull D, Parker R, Hazlehurst JM, Guo K; LEAN trial team, Abouda G, Aldersley MA, Stocken D, Gough SC, Tomlinson JW, Brown RM, Hübscher SG, Newsome PN. Liraglutide safety and efficacy in patients with non-alcoholic steatohepatitis (LEAN): a multicentre, double-blind, randomised, placebo-controlled phase 2 study. Lancet. 2016 Feb 13;387(10019):679-90.
7. Harrison SA, Abdelmalek MF, Caldwell S, Shiffman ML, Diehl AM, Ghalib R, Lawitz EJ, Rockey DC, Schall RA, Jia C, McColgan BJ, McHutchison JG, Subramanian GM, Myers RP, Younossi Z, Ratziu V, Muir AJ, Afdhal NH, Goodman Z, Bosch J, Sanyal AJ; GS-US-321-0105 and GS-US-321-0106 Investigators. Simtuzumab Is Ineffective for Patients With Bridging Fibrosis or Compensated Cirrhosis Caused by Nonalcoholic Steatohepatitis. Gastroenterology. 2018 Oct;155(4):1140-1153.
8. Tripathi D, Ferguson JW, Kochar N, Leithead JA, Therapondos G, McAvoy NC, Stanley AJ, Forrest EH, Hislop WS, Mills PR, Hayes PC. Randomized controlled trial of carvedilol versus variceal band ligation for the prevention of the first variceal bleed. Hepatology. 2009 Sep;50(3):825-33.
9. Reiberger T1, Ulbrich G, Ferlitsch A, Payer BA, Schwabl P, Pinter M, Heinisch BB, Trauner M, Kramer L, Peck-Radosavljevic M; Vienna Hepatic Hemodynamic Lab. Carvedilol for primary prophylaxis of variceal bleeding in cirrhotic patients with haemodynamic non-response to propranolol. Gut. 2013 Nov;62(11):1634-41.

What's New in Pathophysiology and Disease Mechanisms of NAFLD

Frank Tacke

ABSTRACT

Non-alcoholic fatty liver disease (NAFLD) is rapidly becoming the most common chronic liver disease as well as the leading indication of liver transplantation. The treatment of NAFLD is a key point to prevent NASH progression to advanced fibrosis and cirrhosis and the development of its extra-hepatic complications. This review provides a panoramic sight on the current attempts to cure NAFLD patients.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a negative definition of a very common disease that refers to the presence of hepatic steatosis when no other causes for secondary liver fat accumulation (e.g. heavy alcohol consumption, hypothyroidism, drugs, etc.) are present. It affects about 25% of the general population in western countries and is considered to be the hepatic manifestation of the metabolic syndrome (MS).

It is subdivided into non-alcoholic fatty liver (NAFL) and non-alcoholic steatohepatitis (NASH). In the former hepatic steatosis is present without any evidence of inflammation, whereas in the latter, hepatic steatosis is associated with inflammation, which is histologically undistinguishable from alcoholic steatohepatitis.

The clinical reality of NAFLD is characterized by the fact that there is a substantial proportion of patients who are completely asymptomatic (silent disease), the age is older, the body mass index (BMI) is increased, many co-morbidities can be present (diabetes, chronic kidney disease, cardiovascular disorders, etc.) and a high rate of malignancies has been observed.

However, despite the common frequency of the above-mentioned features, a great heterogeneity from patient to patient can be found in clinical practice.

The pathogenesis of NAFLD is complex and involves crosstalk among various organs, such as the adipose tissue, pancreas, gut and brain, as displayed in Figure 1.

NAFLD is an example of ectopic fat accumulation, that is lipid deposit occurs in sites other than adipose tissue, and this is usually associated with increased secretion of hepatokines, increased gluconeogenesis, decreased glucagon synthesis and inhibition of insulin signaling. There is also increasing evidence that dysbiosis of the gut microbiota plays a key role in regulating several intrahepatic metabolic and inflammatory pathways that contribute to the development and progression of NAFLD.

It is important to stress that NAFLD pathophysiology is associated with many extra-hepatic diseases with their manifestations and complications (1). The natural history of NAFLD with the estimated progression rates is displayed in Figure 2, which shows that the evolution to cirrhosis from NASH ranges from 0.25% to 3.0% and that from cirrhosis to hepatocellular carcinoma (HCC) varies from 0.3% to 2.6%. However, it is also possible a direct passage from NASH to HCC in 0.04-0.3% of cases. A regression of cirrhosis to NASH and to NAFLD has also been observed, depending on patient lifestyle modifications and control of obesity and diabetes, genetic factors, etc.

The therapeutic targets in patients with NASH are multiple and therefore many different drugs have been proposed and proven in order to interfere with the pathophysiological mechanisms and improve liver fibrosis, as shown in Figure 3. They include metabolic regulators, anti-inflammatory, anti-apoptotic and anti-fibrotic factors.

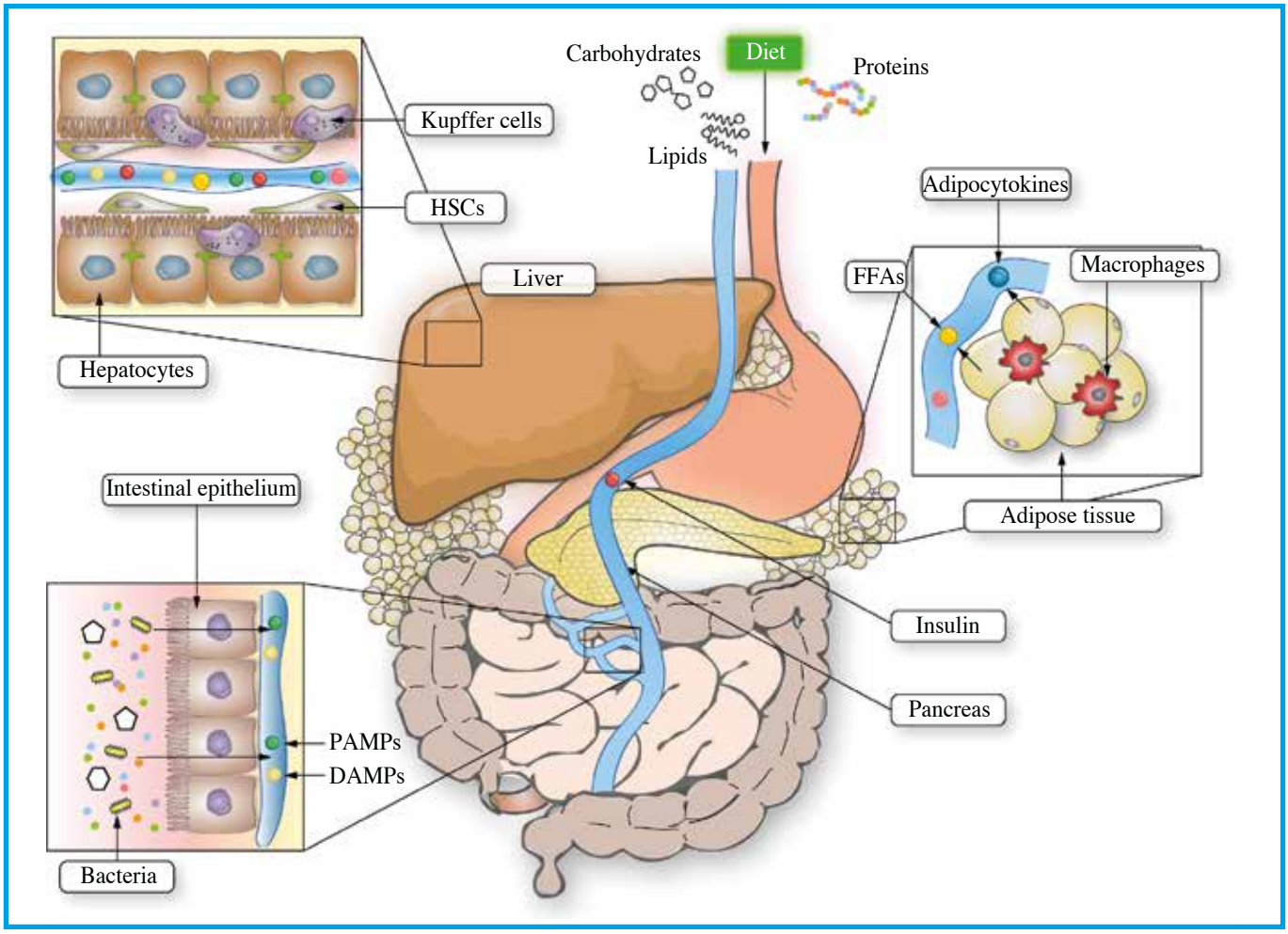


Figure 1. Pathogenesis of NAFLD involves inter-organ crosstalk – adipose tissue, pancreas, gut, brain (mod. from Nobili V et al. J Hepatol 2013)

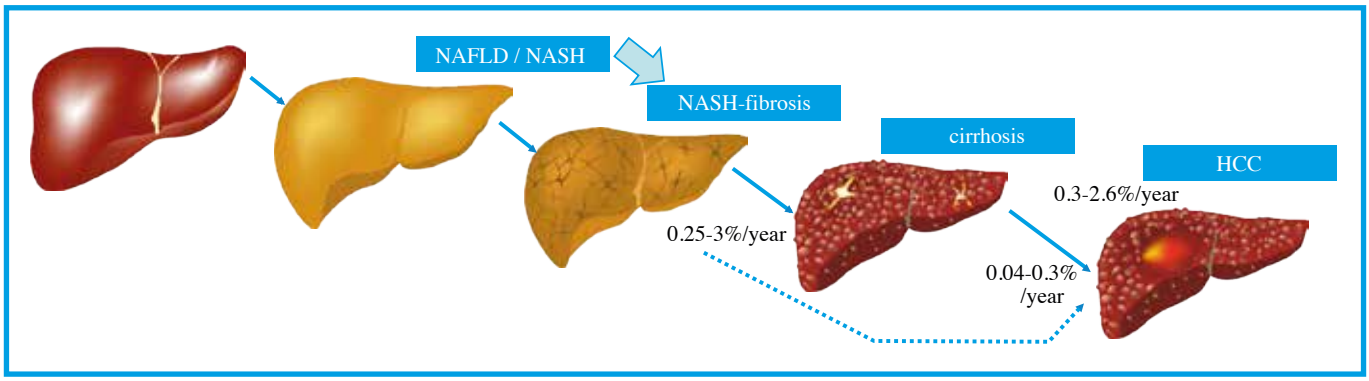


Figure 2. Natural history of fatty liver disease: estimated progression rates

METABOLIC REGULATORS

Various pharmacological interventions target different aspects of aberrant metabolism. Such metabolic targets include insulin resistance and subsequent lipolysis, free fatty acid generation and lipotoxicity; excessive triglyceride accumulation in hepatocytes and subsequent disturbances in autophagy and mitochondrial functions together with the excess of free fatty acids and subsequent oxidative and endoplasmatic reticulum stress.

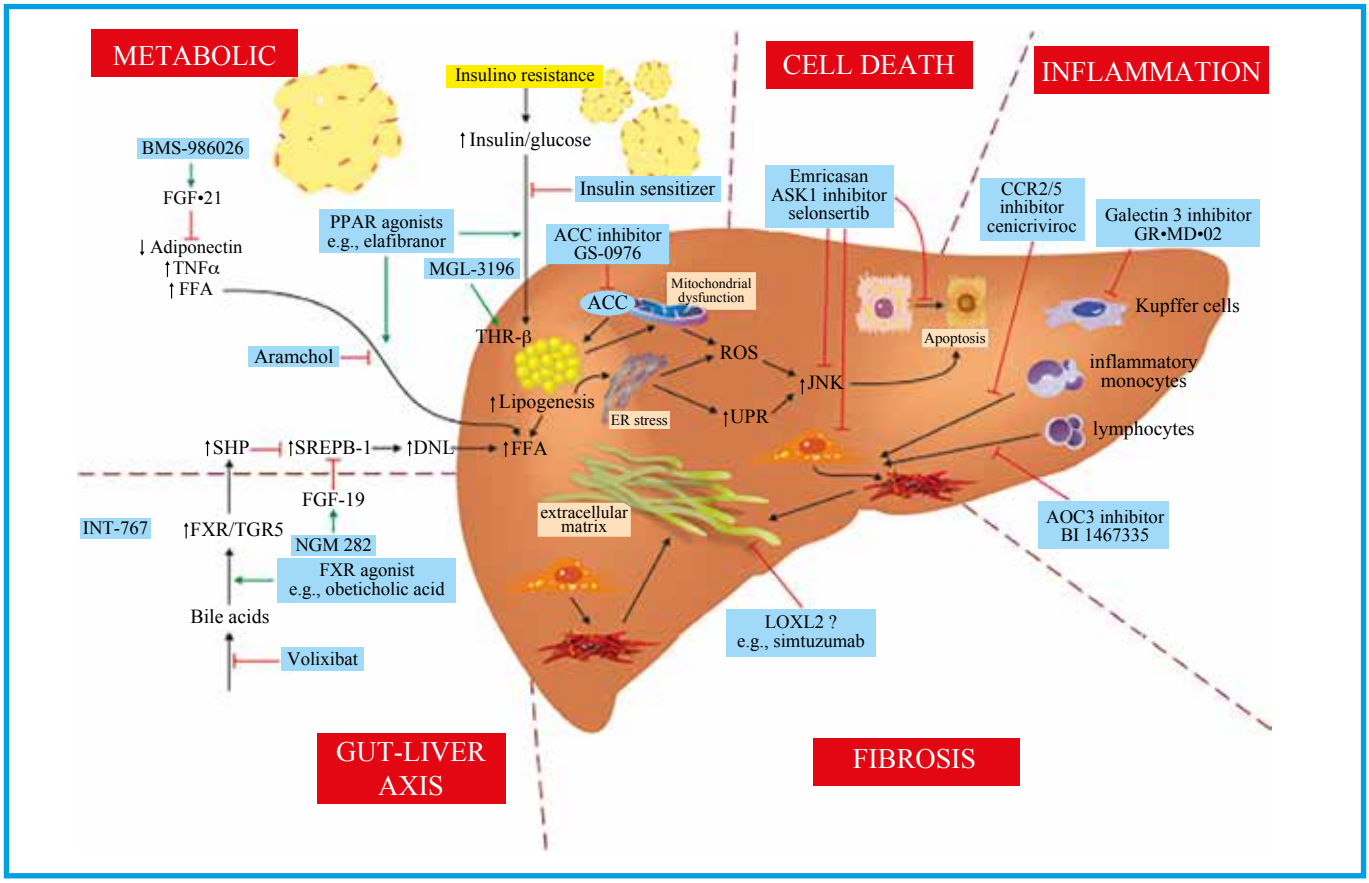


Figure 3. Therapeutic Targets in Steatohepatitis and fibrosis (mod. from Konerman MA, et al. J Hepatol 2018; Tacke F & Weiskirchen R. Exp Rev Gastroenterol Hepatol 2018)

Farnesoid X receptor (FXR) Agonists

One key mechanism of counter-balancing some of these metabolic stress pathways are bile acid receptors, especially the nuclear bile acid receptor FXR, farnesoid X receptor. The semi-synthetic bile acid derivative obeticholic acid (OCA; 6-ethoxy-chenodeoxycholic acid) has very potent FXR agonistic activity (2).

The results of 18 months interim analysis of the phase 3 REGENERATE trial (3) were shown during the last International Liver Congress 2019. The population included 931 patients (placebo = PBO (n = 311), OCA 10 mg (n = 312) or OCA 25 mg (n = 308)), with histological NASH and fibrosis F2-F3. The improvement of fibrosis with no worsening of NASH was met by 11.9% PBO, 17.6% OCA 10 mg and 23.1% OCA 25 mg patients, as visible in Figure 4. The improvement of NASH without worsening of fibrosis was not statistically significant. Increases in LDL were also observed by week 4. Pruritus incidence peaked within first 3 months before declining.

NGM282 (known as M70) is a non-tumorigenic, engineered variant of fibroblast growth factor (FGF)-19 that binds FGF receptors 1c and 4 to reduce lipotoxicity due to lipid accumulation. It was studied in a randomized, double-blind, placebo-controlled phase 2 trial (NCT02443116) in 82 patients with histological-proven NASH with ≥8% absolute liver fat content by MRI proton density fat fraction (MRI-PDFF). It was administrated subcutaneously, once-daily, for 12 weeks (4). It induced marked relative reduction from baseline in steatosis with a 57% and 45% at the 6- and 3-mg doses, respectively. Improvements in ALT, AST were also observed, but not in glycemic control. Injection site reactions were reported in 54% and 41% of subjects in the 6- and 3-mg dose groups relative to 7% in the PBO group. Others adverse events were: diarrhea in 36% and 41% with the 6- and 3-mg doses, respectively) and 22% with PBO, nausea, abdominal pain, and distension.

In an exploratory, non-placebo-controlled, 12-week

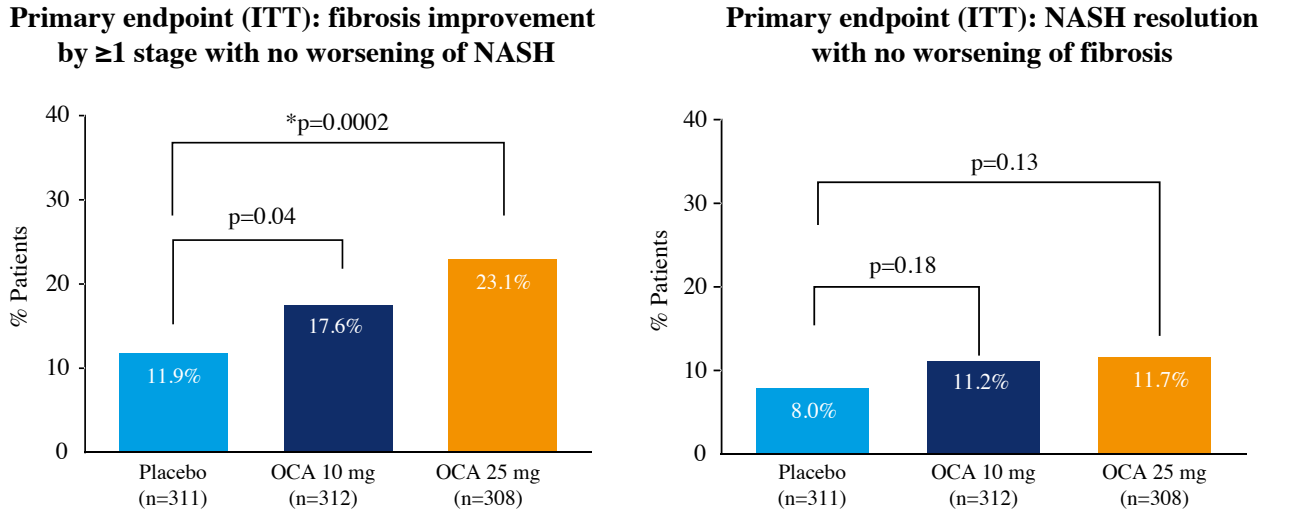


Figure 4. Obeticholic acid (OCA) in NASH (REGENERATE): First Phase III trial results - primary endpoints (mod. from Younossi Z, et al. ILC 2019)

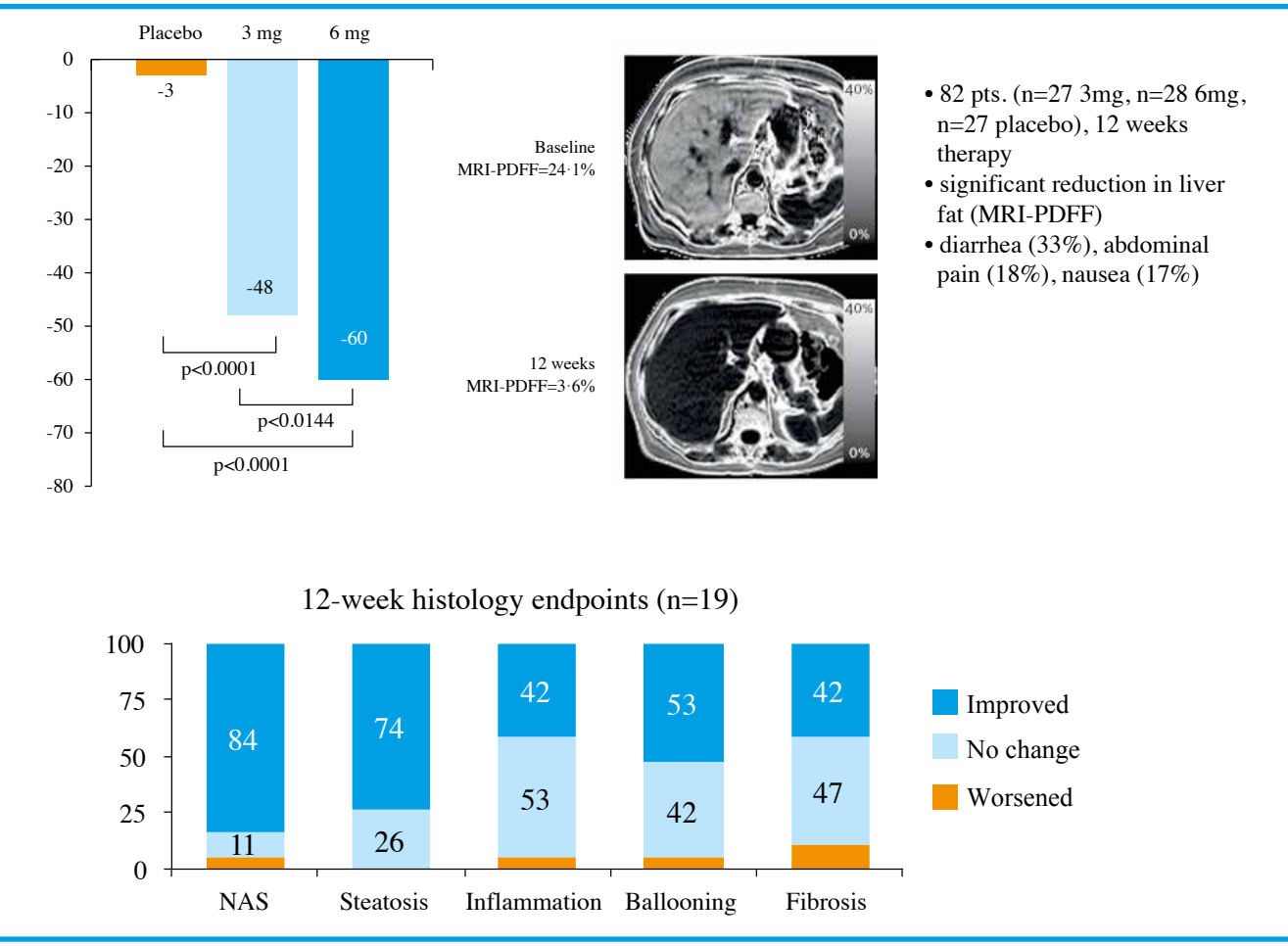


Figure 5. FGF-19 analogue NGM282: Reduction in liver fat and trends in histology (mod. from Harrison SA, et al., Lancet 2018; Harrison S, et al. ILC 2018)

study, 67% (1 mg) and 74% (3 mg) of subjects showed an improvement in steatosis, 33% (1 mg), and 42% (3 mg) improvement in inflammation, 42% (1 mg) and 53% (3 mg) improvement in ballooning, and 25% (1 mg) and 42% (3 mg) improvement in fibrosis, as assessed by liver histology (5). The results of the above studies are displayed in Figure 5.

Peroxisome proliferator-activated receptors (PPARs) Agonists

PPARs are ligand activated transcription factors and represent a subfamily of the NR1C nuclear receptors, including three isotypes: PPARα (NR1C1), PPARδ (also called PPARβ or NR1C2), and PPARγ (NR1C3). Each isotype has different tissue distribution patterns and functions, playing a key role in glucose and lipid metabolisms and inflammation. Elafibranor (GFT505) is an agonist of both PPARα and PPARδ, that was initially evaluated in animal models and showed protective effects on steatosis, inflammation and fibrosis. It has also shown beneficial effects on NASH histological features in a trial with 276 patients (GOLDEN-505) (6), without an overall improvement of fibrosis for the whole patient cohort, as shown in Figure 6. Importantly, treatment of NASH patients with elafibranor was associated with beneficial changes in lipids, glucose profiles, liver enzymes, and inflammatory markers, which has been the basis to move forward with the clinical development of elafibranor for NASH in a phase III trial (RESOLVE-IT).

Agonist of Thyroid Hormone Receptor

Thyroid hormones play a key role in the regulation of homeostasis, lipid and carbohydrate metabolism, regulation of body weight and adipogenesis through the effects on thyroid hormone receptor β (THR β), which represents also the predominant THR isoform in the liver. MGL-3196 (resmetirom) is a highly selective THR β agonist that showed positive effects on reduction of LDL cholesterol and triglycerides in phase I studies (7). It was assessed in a multicenter double-blinded RCT versus placebo in patients with NAS score ≥ 4 and fibrosis stage F1-F3. MGL-3196

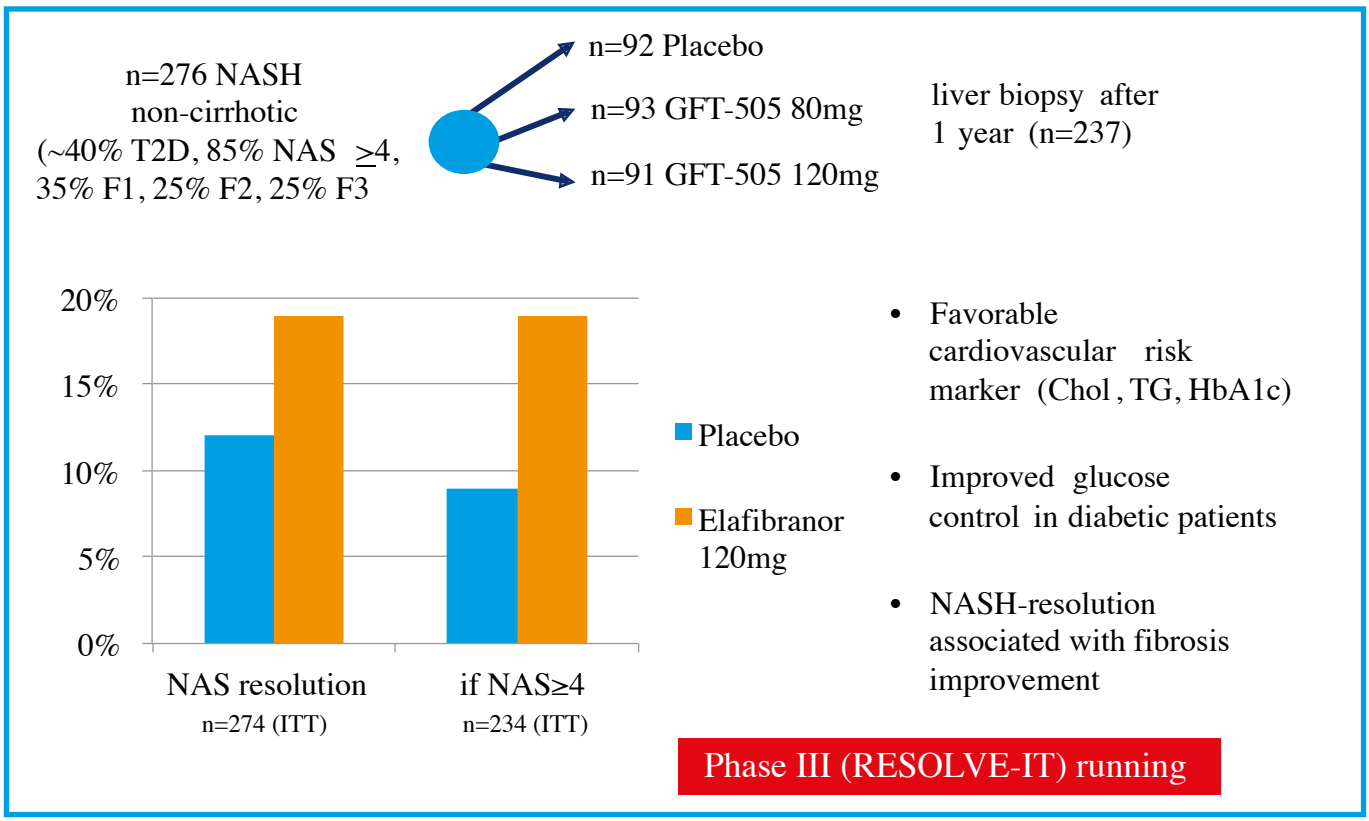
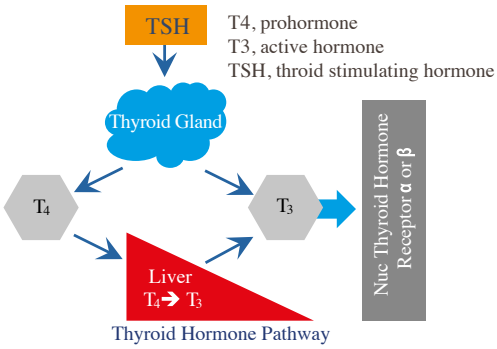


Figure 6. Elafibranor (PPARα/δ agonist) in NASH (GOLDEN-trial): Histological endpoints (mod. from Ratziu V, et al. Gastroenterology 2016)



Competitor: VK2809 (THR-β agonist)
small Phase II trial reported (n=47, three arms)
12 weeks treatment
- Mean absolute decrease in liver fat content about 10%
- Mean relative decrease in liver fat content about 55%

125 patients, 2:1 MGL-3196 vs. placebo
NASH, fibrosis F1-3, ≥10% liver fat
12 wks. MRI-PDFF, 36 wks. MRI + biopsy

- Positive effects on liver fat (MRI)
- Positive effects on blood lipids, blood pressure, ALT and fibrosis-biomarkers
- no T3/T4 side effects

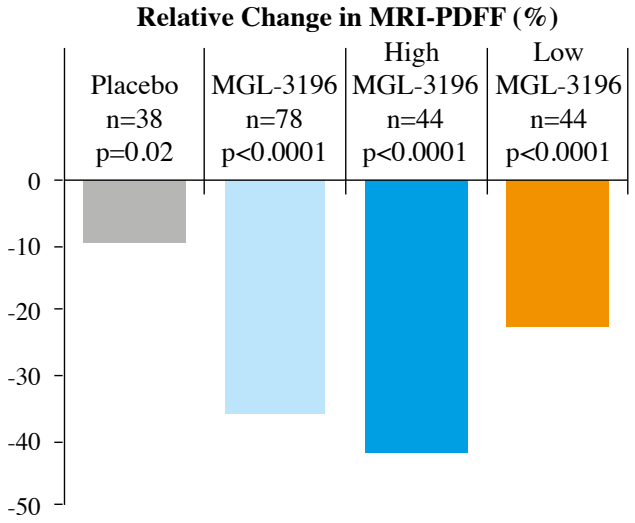


Figure 7. Thyroid hormone receptor β analogue MGL-3196: Reduction in liver fat (mod. from Harrison A, et al. ILC 2018)

showed efficacy in achieving NASH resolution (27% vs. 6%) and in decreasing significantly ALT levels, fibrosis biomarkers and lipids, with an acceptable safety profile, as displayed in Figure 7. A phase III trial has recently started and is ongoing.

Aramchol

Aramchol, a novel, liver-targeted steroyl-CoA desaturase-1 (SCD1) modulator, was associated with liver fat reduction, as well as improved histology, in a dose-response pattern after 52 weeks. Ratziu explained that SCD1 is "a key enzyme in hepatic lipogenesis," and downregulation of this enzyme in high-fat or high-carbohydrate dietary models may result in resistance to obesity or decreased adiposity, reduced hepatic lipogenesis, and enhanced insulin sensitivity. In a phase IIa trial, the drug showed a significant reduction in liver fat, that suggested the possibility for a phase III trial for aramchol at 600 mg among advanced therapeutic candidates for NASH (8).

ANTI-INFLAMMATORY AND ANTI-APOPTOTIC FACTORS

Inhibition of apoptosis signal-regulating kinase 1, a serine/threonine kinase, leads to improvements in inflammation and fibrosis in animal models of nonalcoholic steatohepatitis. A clinical study showed the safety and efficacy of selonsertib, a selective inhibitor of apoptosis signal-regulating kinase 1, alone or in combination with simtuzumab (humanized monoclonal antibody against Lysyl Oxidase-like-2), in patients with NASH and stage 2 or 3 liver fibrosis. In this multicenter phase 2 trial (9), 72 patients were randomized to receive 24 weeks of open-label treatment with either 6 or 18 mg of selonsertib orally once daily with or without once-weekly injections of 125 mg of simtuzumab or simtuzumab alone. After 24 weeks of treatment, the proportion of patients with one or more stage reduction in fibrosis in the 18-mg selonsertib group was 13 out of 30; in the 6-mg selonsertib group, 8 out of 27; and in the simtuzumab-alone group, 2 out of 10.

Improvement in fibrosis was associated with reductions in liver stiffness on magnetic resonance elastography, collagen content and lobular inflammation on liver biopsy, as well as improvement in serum biomarkers of apoptosis and necrosis. Simtuzumab did not meet the designated primary end point of fibrosis regression and the trial was terminated early.

ANTIFIBROTIC FACTOR

Another treatment for NASH is cenicriviroc (CVC), a dual antagonist of C-C chemokine receptor types 2 and 5. A randomized, double-blind, multinational phase 2b study (10) enrolled subjects with NASH, a nonalcoholic fatty liver disease activity score (NAS) ≥4, and LF (stages 1-3, NASH Clinical Research Network) at 81 clinical sites. After 1 year of CVC treatment, twice as many subjects achieved improvement in fibrosis and no worsening of NASH compared with placebo. Given the urgent need to develop antifibrotic therapies in NASH, these findings warrant phase 3 evaluation.

CONCLUSION

In the next few years the pandemic of NAFLD will lead to an increase of NASH patients and those with fibrosis at higher risk of developing liver-related complications. A number of drugs targeting different pathogenetic pathways are under investigation: a few of them already confirmed their efficacy in phase III trials, others failed to prove their superiority compared with placebo, while some others are still under investigation.

References

1. Byrne CD, Targher G. NAFLD: a multisystem disease. J Hepatol. 2015 Apr;62(1 Suppl):S47-64.
2. Tacke F, Weiskirchen R. An update on the recent advances in antifibrotic therapy. Expert Rev Gastroenterol Hepatol. 2018 Nov;12(11):1143-1152.
3. Ratziu V, Sanyal AJ, Loomba, R Rinella M, Harrison S, Anstee QM, Goodman Z, Bedossa P, MacConell L, Shringarpure R, et al. REGENERATE: Design of a pivotal, randomised, phase 3 study evaluating the safety and efficacy of obeticholic acid in patients with fibrosis due to nonalcoholic steatohepatitis. Contemp. Clin. Trials 2019, 84, 105803.
4. Harrison SA, Rinella ME, Abdelmalek MF, Trotter JF, Paredes AH, Arnold HL, Kugelmas M, Bashir MR, Jaros MJ, Ling L, et al. NGM282 for treatment of non-alcoholic steatohepatitis: A multicentre, randomised, double-blind, placebo-controlled, phase 2 trial. Lancet 2018;391:1174-1185.
5. Harrison SA, Rossi SJ, Paredes AH, Trotter JF, Bashir MR, Guy CD, Banerjee R, Jaros MJ, Owers S, Baxter BA, et al. NGM282 Improves Liver Fibrosis and Histology in 12 Weeks in Patients with Nonalcoholic Steatohepatitis. Hepatology 2019.
6. Ratziu V, Harrison SA, Francque S, Bedossa P, Lehert P, Serfaty L, Romero-Gomez M, Boursier J, Abdelmalek M, Caldwell,S, et al. Elafibranor, an Agonist of the Peroxisome Proliferator-Activated Receptor-alpha and -delta, Induces Resolution of Nonalcoholic Steatohepatitis without Fibrosis Worsening. Gastroenterology 2016;150:1147-1159.e5.
7. Harrison S. MGL-3196, a selective thyroid hormone receptor-beta agonist significantly decreases hepatic fat in NASH patients at 12 weeks, the primary endpoint in a 36 week serial liver biopsy study. Hepatology 2018;68:38.
8. Ratziu V. One-Year Results of the Global Phase 2b Randomized Placebo-Controlled ARREST Trial of Aramchol, a Stearoyl CoA Desaturasemodulator in NASH Patients; AASLD: Boston, MA, USA, 2018.
9. Puengel T, De Vos S, Krenkel O, Pujuguet P, Auberval M, Marsais F, Shoji KF, Saniere L, Trautwein C, Brys R, Tacke F. Pharmacological inhibition of the medium chain fatty acid receptor GPR84 reduces myeloid cell in filtration into injured liver and ameliorates steatohepatitis and fibrosis. J Hepatol April 2018; 68, Suppl 1, S338.
10. Friedman SL, Ratziu V, Harrison SA, Abdelmalek MF, Aithal GP, Caballeria J, Francque S, Farrell G, Kowdley KV, Craxi et al. A randomized, placebo-controlled trial of cenicriviroc for treatment of nonalcoholic steatohepatitis with fibrosis. Hepatology. 2018 May;67(5):1754-1767.

What's New in Diagnosis and Treatment of NAFLD

Manuel Romero-Gómez

ABSTRACT

Fatty liver disease exists on a spectrum ranging from simple non-alcoholic fatty liver disease (NAFLD) to a condition in which the liver is inflamed, called non-alcoholic steatohepatitis (NASH). Age and diabetes are the strongest clinical predictors of progressive disease. Fibrosis stage is the most important histological variable to predict mortality and liver-related complications. So far, no study has been able to show that non-alcoholic steatohepatitis at baseline predicts mortality or future liver-related complications when adjusting for fibrosis. This article shows the most recent developments in the diagnosis and treatment of this disease and its complications.

INTRODUCTION

NAFLD encompasses a histological spectrum ranging from isolated steatosis, i.e., non-alcoholic fatty liver (NAFL), to non-alcoholic steatohepatitis (NASH), cirrhosis, and hepatocellular carcinoma (HCC). NASH is characterized by hepatocellular accumulation of fat accompanied by lobular inflammation and ballooning of hepatocytes. Until recently, NAFL has been considered a benign disease and NASH the progressive disease state with risk of development of liver-related complications and increased mortality from hepatic and non-hepatic causes. Obesity entails a 3.5-fold increased risk of developing NAFLD, and the prevalence of NAFLD therefore mirrors the steady increase of obesity globally.

The prevalence of NAFLD in Europe (global population = 512 million people) is 23%-25% (105 million people), NASH amounts to 2%-3% (13.9 million people) which can cause liver fibrosis. This may eventually progress to cirrhosis (0.5%-1% that is three million people) and hepatocellular carcinoma in 0.5%-1% of the patients (Figure 1) (1,2).

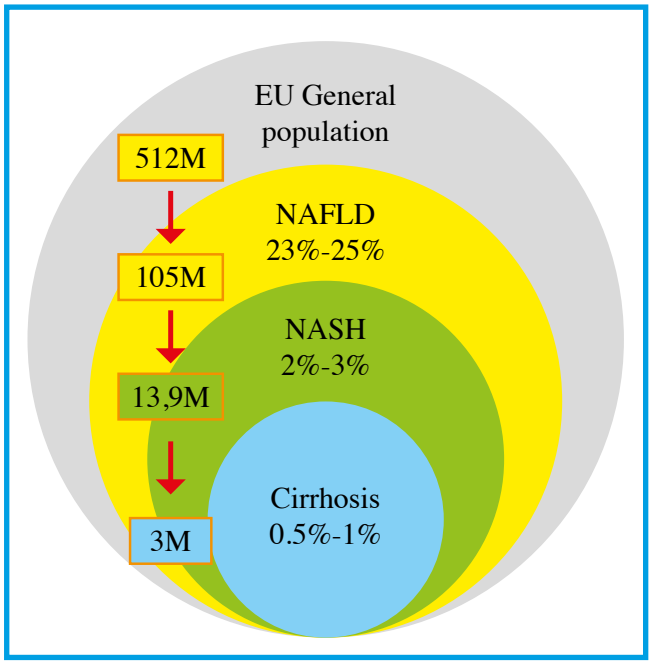


Figure 1. Prevalence of NAFLD and its complications across the EU population (mod. from da Kwok et al. GUT 2015; Anstee et al. Nat Rev 2013)

NAFLD affects a variety of extra-hepatic organ systems and therefore it is strictly associated with many systemic diseases, such as obesity, diabetes, arterial hypertension, chronic kidney disease, cardiovascular disease, extrahepatic malignancies, etc (3), as shown in Figure 2.

Natural history of the disese

NAFLD is in most cases a slowly progressive disease, and the majority of NAFLD patients will never develop end-stage liver disease. However, a significant proportion will experience liver-related complications.

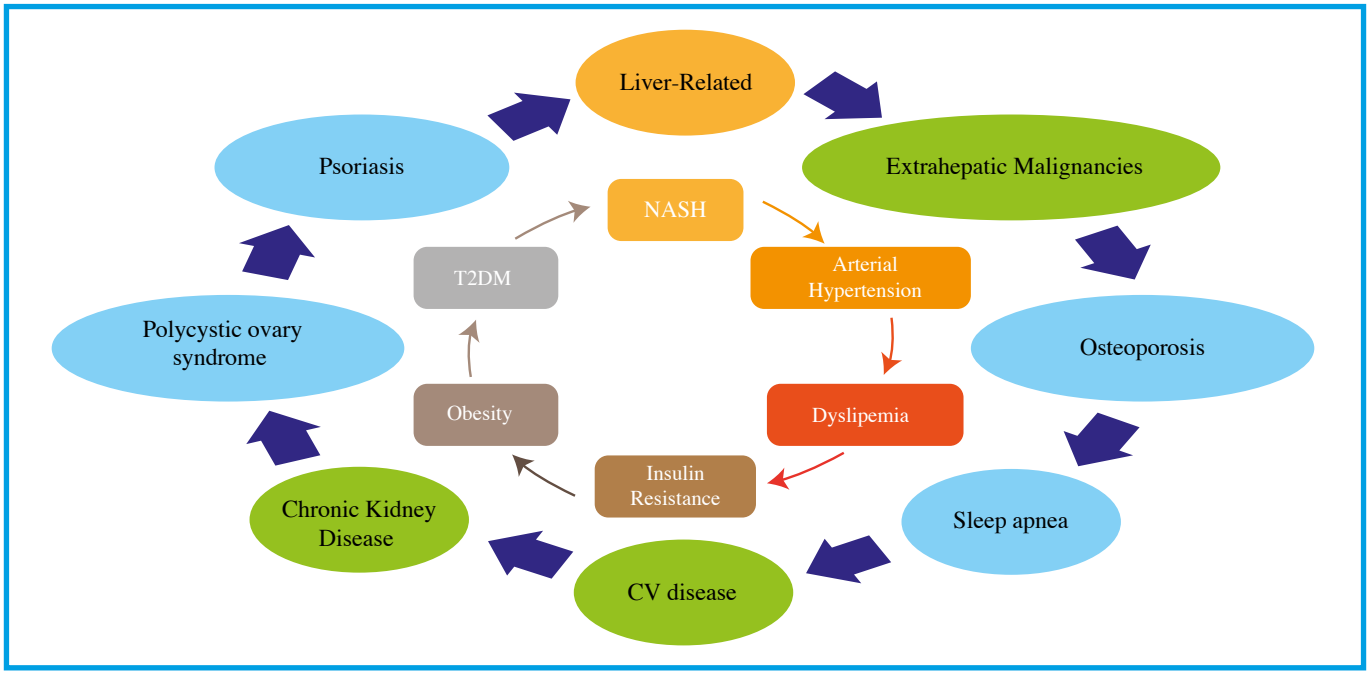


Figure 2. NAFLD related systemic diseases (mof. from Harman DJ et al. AP&T 2018; Ampuero J, Romero-Gómez M. AP&T 2018)

Predicting which NAFLD patients will develop liver-related complications in the future and when this will occur is extremely difficult.

The natural history of NAFLD is still unclear. Older studies report limited numbers of patients with histologically proven NAFLD who had been referred to specialized tertiary care centres with short and/or highly variable follow-up time. There is still a shortage of population-based studies to determine the long-term prognosis of NAFLD and it is uncertain whether previously reported morbidity rates could be generalized to the whole affected population.

Adams et al. conducted a population-based cohort study to examine the natural history of patients diagnosed with NAFLD in Olmsted County, Minnesota. During a mean follow-up of 7.6 (4.0) years, 13 (3.1%) patients developed cirrhosis-related complications, including ascites in 2%, jaundice in 2%, portosystemic encephalopathy in 2%, variceal bleeding in 1%, and HCC in 0.5%. Ekstedt et al. studied 129 subjects with biopsy-proven NAFLD. Follow-up was 13.7 (± 1.3 years) and all patients were followed for more than 10 years. Seven patients (5.4%) developed cirrhosis-related complications during follow-up including ascites in 4%, variceal haemorrhage in 0.8%, and HCC in 2%. Subgroup analysis showed that no patient with absence of fibrosis or stage 1 fibrosis at baseline had developed liver-related complications during follow-up. One of the 4 patients (25%) with cirrhosis at baseline, 3 of the 22 patients (14%) with stage 2 fibrosis at baseline, and 3 of the 12 patients (25%) with stage 3 fibrosis at baseline developed end-stage liver disease during follow-up.

In a longitudinal, international, multicentre cohort study, Angulo et al. evaluated an outcome in 619 patients with biopsy-proven NAFLD. Over a median follow-up period of 12.6 years, 44 patients (7%) developed liver-related events including 5 subjects (0.8%) that were diagnosed with HCC. Of the 44 patients that developed end-stage liver disease, 17 died from the complications.

Mortality in NAFLD patients has been investigated in a number of studies during the last two decades. The main cause of death in follow-up studies with biopsy-proven NAFLD is cardiovascular disease (HR 1.55–1.85), and liver-related complications with a marked increase in risk (HR 3.2-6.5). The most significant clinical predictors are age and T2DM. Smoking was associated with mortality in the study by Adams et al. (4).

DIAGNOSIS

Serological non-invasive methods

Detecting hepatic steatosis, often associated with fibrosis progression, has been for long a challenge and this is the hallmark of non-alcoholic fatty liver disease. Several non-invasive scoring systems have been proposed to identify patients at risk of advanced fibrosis: at present, the NAFLD Fibrosis Score (NFS) and the Fibrosis-4 (FIB-4) index are the serological non-invasive methods most widely used in clinical practice. Ampuero et al. developed the Hepamet fibrosis scoring (HFS), a non-invasive tool to predict the fibrosis stage in NAFLD patients, based on serum markers (4). The score was compared with NFS and FIB-4 and the results demonstrated that the HFS was the most accurate and had the greatest benefit. The score was not affected by patient age, body mass index (BMI), aminotransferase levels or diabetes and was also able to predict who should undergo liver biopsy analysis. The advantages of HFS in primary care are the following: it takes into consideration both the metabolic status and the fibrosis stage, has the potential for monitoring the disease overtime, its negative predictive value (NPV) is > 96% when HFS is < 0.12, that is a negative test excludes almost totally the presence of liver fibrosis, and is cost-effective, because reduces significantly the referring rate (4).

Imaging biomarkers

The ultrasound based controlled attenuation parameter (CAP) can be used to detect hepatic steatosis, however, despite its good diagnostic accuracy, the clinical use of CAP is limited due to uncertainty regarding optimal cut-offs and the influence of co-variates, such as underlying disease, BMI and diabetes (5). Based on EALS-ALEH Clinical Practice Guidelines, liver stiffness measurement (LSM) can be staged using 1-dimensional ultrasound transient elastography (TE, FibroScan), which measures the velocity of a low-frequency elastic shear wave propagating through the liver. This velocity is directly related to tissue stiffness. The stiffer the tissue, the faster the shear wave propagates. Based on the experience of Castera and colleagues (6), the liver stiffness measurements are difficult to interpret and have several limitations, which are mainly represented by obesity, waist circumference and limited operator experience.

The excess of fat accumulation is typical of liver with hepatic steatosis and this can be assessed by radiological imaging techniques, such as ultrasonography (US), computed tomography (CT), and magnetic resonance imaging (MRI). In patients with suspected NAFLD, imaging modalities confirm the diagnosis, but they have some limitations (inadequate sensitivity and specificity, radiation safety and confounding factors). These negative aspects are overcome by the use of standardized quantitative MR biomarkers: the proton density fat fraction (PDFF), T2 and stiffness.

The degree of steatosis is best measured by the molecular quantity of triglycerides: indeed, the fat is stored as triglycerides within the hepatocytes. ***The Proton density fat fraction (PDFF)*** is an unconfounded and universal measure of triglyceride concentration, defined as the fraction of mobile protons (H1) belonging to fat triglycerides relative to those of water, has been proposed as a standardized MR biomarker of steatosis. PDFF is a non-invasive imaging biomarker of steatosis and its measurement represents nowadays the gold standard method for hepatocyte steatosis, because it is reproducible, permits a whole liver analysis and is not time-consuming (7). The high sensitivity of PDFF for low-grade steatosis is advantageous, because other histological features of NAFLD, such as inflammation and fibrosis, do not necessarily follow the steatosis grade. In the magnetic resonance (MR) research community, PDFF is now generally accepted as the reference standard for liver fat. The main disadvantages of MRI-PDFFs are the high costs and the possibility of access (8).

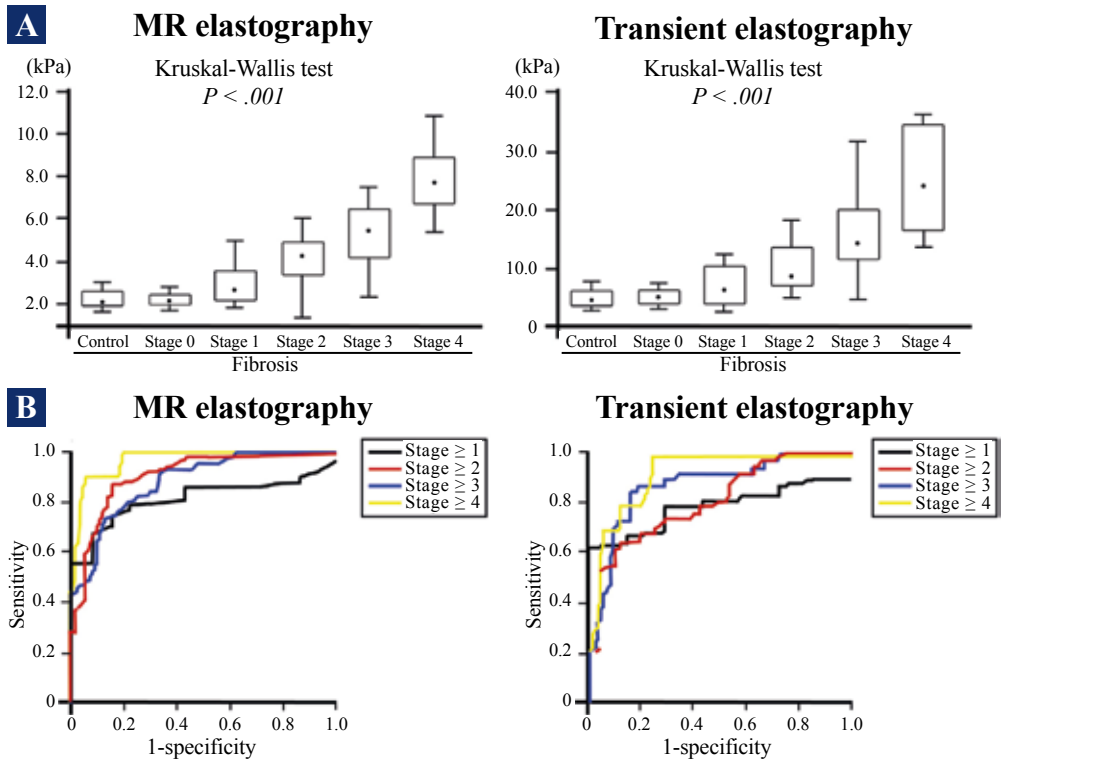
T1ρ is a novel magnetic resonance protocol for liver tissue characterization, that can be performed without intra-venous contrast on existing scanners. T1ρ detects early liver fibrosis as collagen content correlates with T1ρ. Mean liver T1ρ values in patients with liver cirrhosis have shown to be significantly higher than those in healthy subjects, thus suggesting a potential role of liver T1ρ as a MR biomarker for liver cirrhosis. T1 mapping method is reproducible and accurate, unaffected by the degree of adiposity or the presence

of ascites, in contrast with acoustic-based techniques such as elastography, and also has the potential to demonstrate which parts of the liver are affected or spared (9).

MR elastography (MRE)

Fibrosis is an important histopathological feature of advanced NASH and a precursor to cirrhosis. Extracellular collagen deposition in fibrosis alters the liver mechanical property, causing loss of its normal elasticity (or equivalently, increased stiffness), which can be quantified as a mechanical stiffness parameter using elastography techniques including MR elastography (MRE), that uses different type of sequences: Gradient-Recall Eco (GRE), Spin Echo (SE) or Echo-planar imaging (EPI). Elastography, which measures changes in mechanical properties associated with liver fibrosis, such as strain, stiffness, or visco-elasticity, is available on US and MRI (10).

Imajo et al. (11) have compared the accuracy of MRI including MR elastography (MRE) to that of transient elastography (TE) for grading steatosis and fibrosis. The differences between MRE and TE relate to the mechanism of wave propagation and the imaging reconstruction algorithm. It has been proven that MRE generally provides more reliable measurements (see Figure 3) and lower failure rate in patients with obesity or ascites. In a recent retrospective review from the Mayo Clinic, the reported failure rate was less than 6%, with no effect of BMI on it. MRE may be also a better



	MRE (n = 142)							MRE (n = 142)						
Fibrosis stage	Cut-off level, *k*Pa	AUROC	95% CI	Se	Sp	PPV	NPV	Cut-off level, *k*Pa	AUROC	95% CI	Se	Sp	PPV	NPV
≤1	2.5	0.80	0.71-0.89	75.0	85.7	99.0	84.6	7.0	0.78	0.70-0.87	61.7	100.0	100.0	86.6
≤2	3.4	0.89	0.85-0.94	87.3	85.0	88.4	83.6	11.0	0.82	0.74-0.89	65.2	88.7	88.2	66.2
≤3	4.8	0.89	0.83-0.95	74.5	86.9	74.5	81.0	11.4	0.88	0.79-0.97	85.7	83.8	75.0	91.9
≤4	6.7	0.97	0.94-1.00	90.9	94.5	58.8	99.2	14.0	0.92	0.86-0.98	100.0	75.9	73.0	100.0

NPV, negative predictive value; PPV, positive predictive value; Se, sensitivity; Sp, specificity

Figure 3. Non-invasive evaluation of fibrosis in NASH (mod. from Imajo K et al. Gastroenterology 2016)

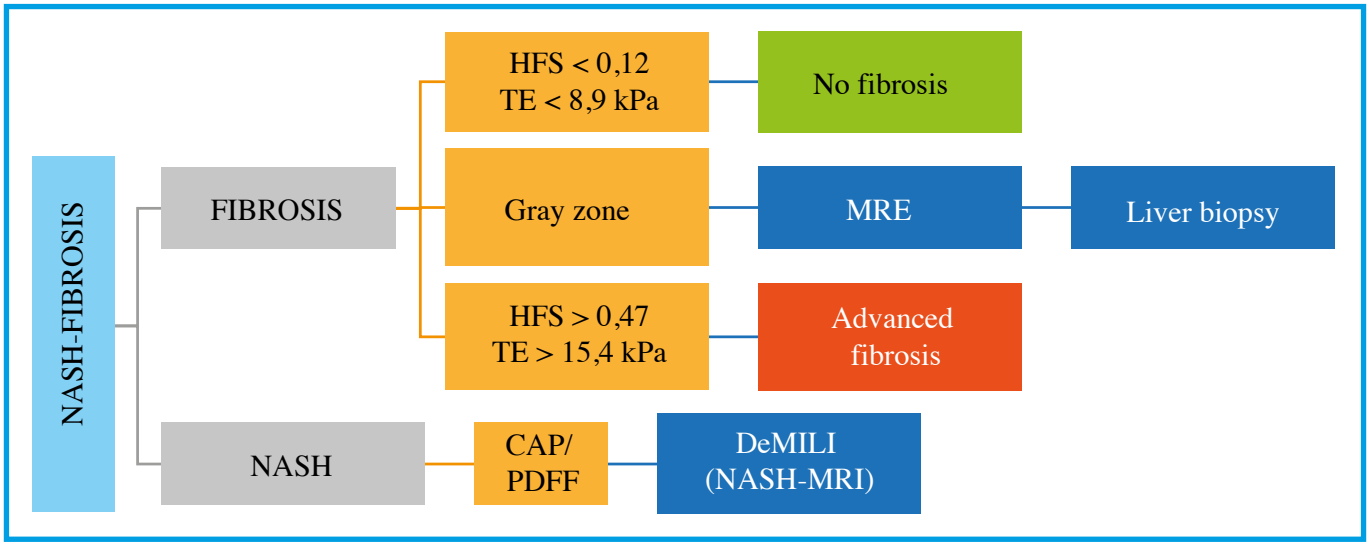


Figure 4. Diagnostic algorithm using imaging biomarkers in clinical practice (mod. from Romero-Gómez, LITMUS project proposal 2018)

candidate than elastography for assessing response to new therapies for NASH. A diagnostic algorithm using imaging biomarkers in clinical practice is visible in Figure 4.

TREATMENT

NASH therapy should combine lifestyle measures, physical activity and drugs aimed at controlling overweight and diabetes together with liver steatosis and inflammation, as reported in Figure 5.

It has been demonstrated that weight loss significantly reduces the features of NASH, particularly if it is higher than 10% compared with baseline (12). However, also a lower weight reduction of 5%-7% is able to improve steatosis and favor fibrosis regression.

Many drugs for blocking the liver fibrotic progression

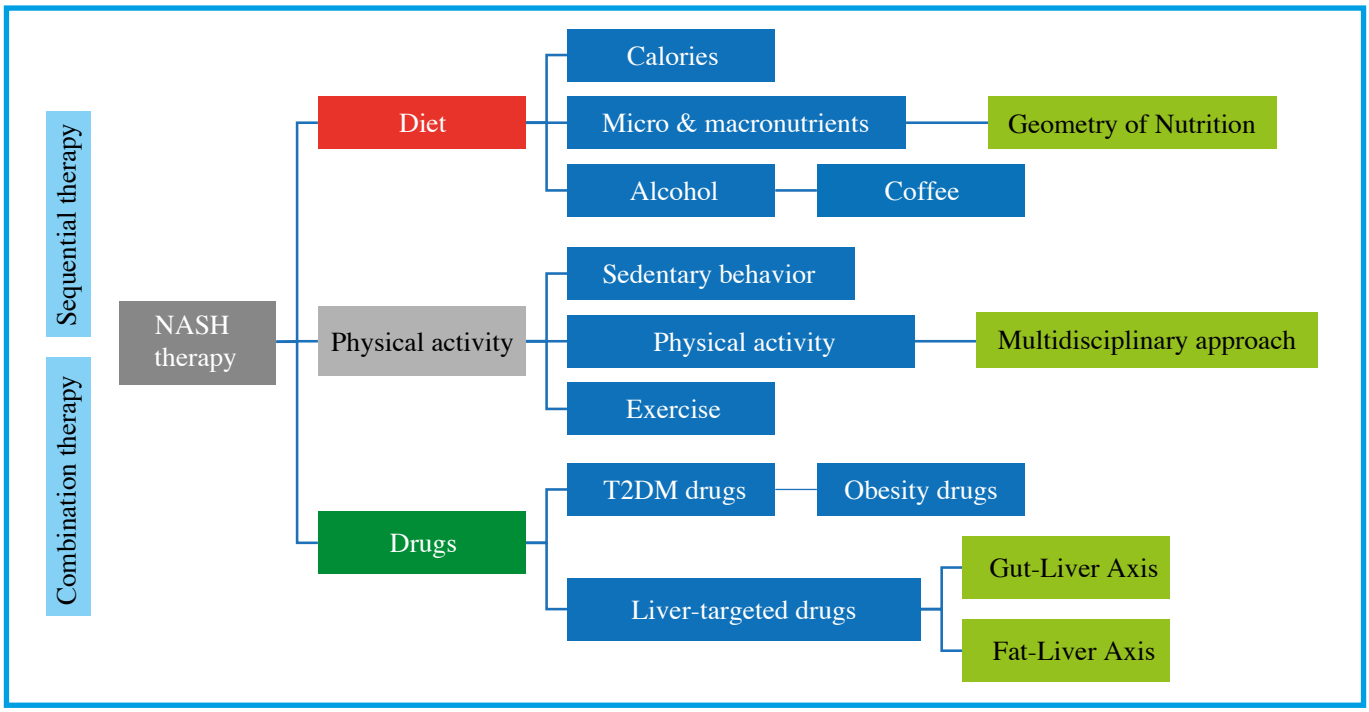


Figure 5. Treatment pathway of NASH

Positive phase 3	Positive phase 2b	Negative phase 2b/3
OCA-Regenerate	OCA - FLINT	Simtuzumab - LOXL2
	Elafibranor - GOLDEN	Selonsertib - ASK1
	Cenicriviroc - CENTAUR	Emricasam - Caspase 9
	Pioglitazone/Vit E - PIVENS	GR-MD-02 - Galectin-3
		Naloxone - TLR4
		6-ethyl-EPA - PUFA
		IMMURON - Bovine calostrum

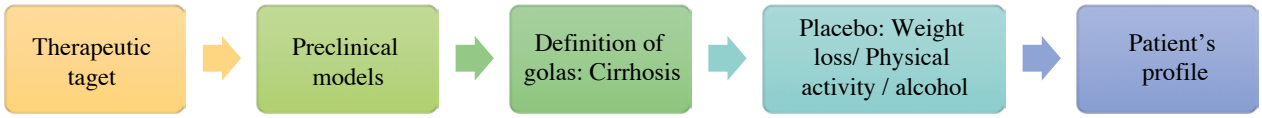


Figure 6. Drugs development for NASH fibrosis

have been developed and are under scrutiny in various clinical trials, whereas others have already shown to be ineffective, like simtuzumab and selonsertib. Elafibranor and cenicriviroc, instead, appear to be promising and OCA is the only drug which provided somewhat positive results in a phase III trial (13), using doses of 10 mg and 25 mg /daily compared with placebo (Figure 6).

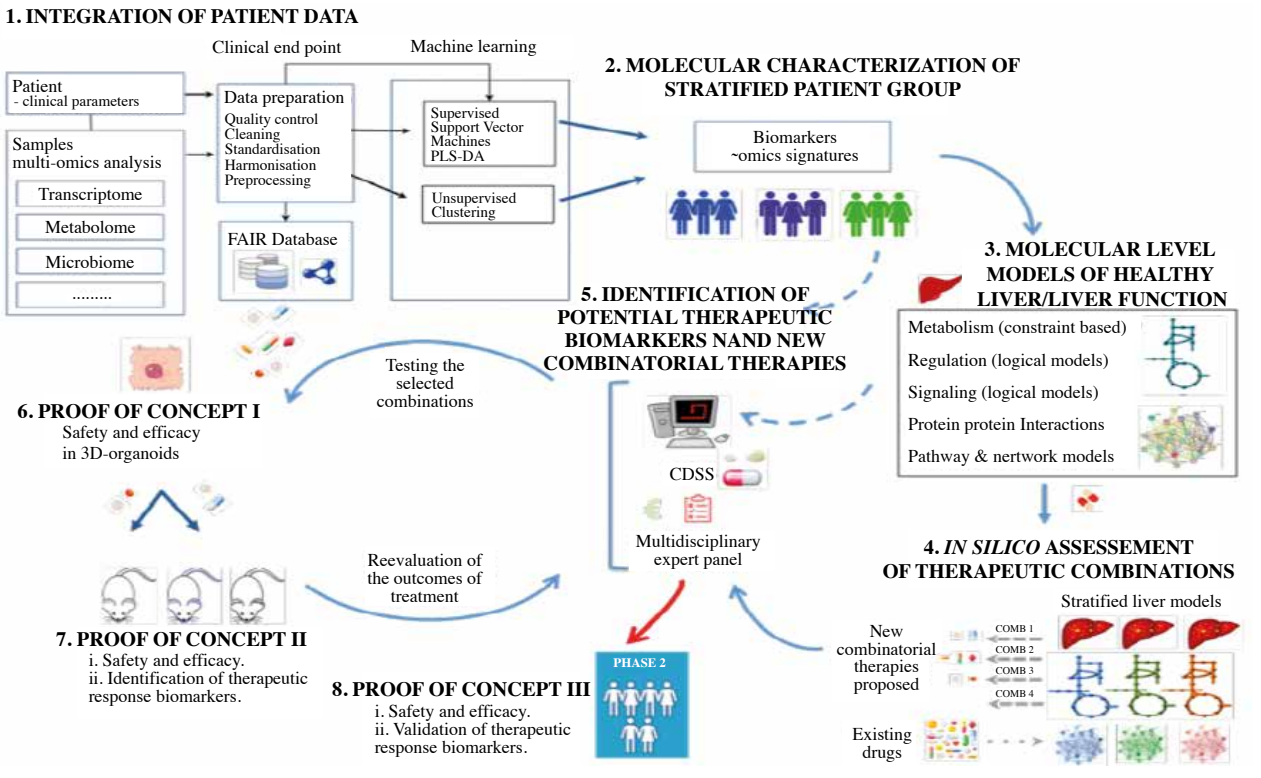


Figure 7. COMET-NAFLD: a new approach for the design of new combinatorial therapies in patients with NAFLD

Indeed, the management of co-morbidities, in particular diabetes, is necessary and the future of NASH therapy will be the combination of multiple measures, like sequential therapy, including lifestyle modifications and drugs for diabetes and obesity, as well as combination therapies involving liver-centered drugs and bariatric endoscopy.

One of the important challenges in the field of NASH is the lack of a reliable and noninvasive endpoints that can accurately serve as a surrogate for the hard outcome of mortality. Nevertheless, resolution of NASH and/or improvement of fibrosis have been the currently accepted endpoints.

CONCLUSIONS

In conclusion, a new approach for the design of combined therapies in patients with NAFLD should consider the following steps displayed in Figure 7: integration of patients data, molecular characterization of stratified patient groups, molecular level models of healthy liver/liver function, in silico assessment of therapeutic combinations, identification of potential therapeutic biomarkers and new combinatorial therapies, proof of concept I on safety and efficacy in 3D-organoids, proof of concept II with identification of therapeutic response biomarkers and proof of concept III with validation of therapeutic response biomarkers.

References

1. Kwok R, Choi KC, Wong GL, Zhang Y, Chan HL, Luk AO, Shu SS, Chan AW, Yeung MW, Chan JC, Kong AP, Wong VW. Screening diabetic patients for non-alcoholic fatty liver disease with controlled attenuation parameter and liver stiffness measurements: a prospective cohort study. Gut. 2016 Aug;65(8):1359-68.
2. Anstee Quentin M, Christopher P. The genetics of NAFL. Review Article. Day Nature Reviews Gastroenterology & Hepatology volume 10, pages 645-655(2013).
3. Harman DJ et al. AP&T 2018; Ampuero J, Romero-Gómez M. AP&T 2018
4. Ampuero J, Aller R, Gallego-Durán R, Banales JM, Crespo J, García-Monzón C, Pareja MJ, Vilar-Gómez E, Caballería J, Escudero-García D, Gomez-Camarero J, Calleja JL, Latorre M, Albillos A, Salmeron J, Aspichueta P, Lo Iacono O, Francés R, Benlloch S, Fernández-Rodríguez C, García-Samaniego J, Estévez P, Andrade RJ, Turnes J, Romero-Gómez M; HEPAmet Registry. The effects of metabolic status on non-alcoholic fatty liver disease-related outcomes, beyond the presence of obesity. Aliment Pharmacol Ther. 2018 Dec;48(12):1260-127.
5. Ampuero J, Pais R, Aller R, Gallego-Durán R, Crespo J, García-Monzón C, Boursier J, Vilar E, Petta S, Ming-Hua Z, Escudero D, Calleja JL, Aspichueta P, Diago M, Rosales JM, Caballería J, Gómez-Camarero J, Lo Iacono O, Benlloch S, Albillos A, Turnes J, Banales JM, Ratziu V, Romero-Gómez M; HEPAmet Registry. Development and Validation of Hepamet Fibrosis Scoring System A Simple, Noninvasive Test to Identify Patients With Nonalcoholic Fatty Liver Disease With Advanced Fibrosis. Clin Gastroenterol Hepatol. 2019 Jun 11. pii: S1542-3565(19)30602-0. doi: 10.1016/j.cgh.2019.05.051. [Epub ahead of print]
6. Karlas T, Petroff D, Sasso M, Fan JG, Mi YQ, de Lédinghen V, Kumar M, Lupsor-Platon M, Han KH, Cardoso AC, Ferraioli G, Chan WK, Wong VW, Myers RP, Chayama K, Friedrich-Rust M, Beaugrand M, Shen F, Hiriart JB, Sarin SK, Badea R, Jung KS, Marcellin P, Filice C, Mahadeva S, Wong GL, Crotty P, Masaki K, Bojunga J, Bedossa P, Keim V, Wiegand J. Individual patient data meta-analysis of controlled attenuation parameter (CAP) technology for assessing steatosis. J Hepatol. 2017 May; 66(5):1022-1030.
7. Castéra L, Foucher J, Bernard PH, Carvalho F, Allaix D, Merrouche W, Couzigou P, de Lédinghen V. Pitfalls of liver stiffness measurement: a 5-year prospective study of 13,369 examinations. Hepatology. 2010 Mar; 51(3):828-835.
8. Idilman IS, Keskin O, Celik A, Savas B, Elhan AH, Idilman R, Karcaaltincaba M. A comparison of liver fat content as determined by magnetic resonance imaging-proton density fat fraction and MRS versus liver histology in non-alcoholic fatty liver disease. Acta Radiol. 2016 Mar;57(3):271-278.
9. Kinner S, Reeder SB, Yokoo T. Quantitative Imaging Biomarkers of NAFLD. Dig Dis Sci. 2016 May; 61(5):1337-1347.
10. Rauscher I, Eiber M, Ganter C, Martirosian P, Safi W,

Umgelter A, Rummeny EJ, Holzapfel K. Evaluation of T1ρ as a potential MR biomarker for liver cirrhosis: comparison of healthy control subjects and patients with liver cirrhosis. Eur J Radiol. 2014 jun; 83(6):900-904.

11. Singh S, Allen AM, Wang Z, Prokop LJ, Murad MH. Fibrosis progression in nonalcoholic fatty liver vs nonalcoholic steatohepatitis: a systematic review and meta-analysis of paired-biopsy studies. Clin Gastroenterol Hepatol. 2015 Mar; 13(3):440-451.
12. Imajo K, Kessoku T, Honda Y, Tomeno W, Ogawa Y, Mawatari H, Fujita K, Yoneda M, Taguri M, Hyogo H, Sumida Y, Ono M, Eguchi Y, Inoue T, Yamanaka T, Wada K, Saito S, Nakajima A. Magnetic Resonance Imaging More Accurately Classifies Steatosis and Fibrosis in Patients With Nonalcoholic Fatty Liver Disease Than Transient Elastography. Gastroenterology. 2016 Mar;150(3):626-637.
13. Vilar-Gomez E, Martinez-Perez Y, Calzadilla-Bertot L, Torres-Gonzalez A, Gra-Oramas B, Gonzalez-Fabian L, Friedman SL, Diago M, Romero-Gomez M. Weight Loss Through Lifestyle Modification Significantly Reduces Features of Nonalcoholic Steatohepatitis. Gastroenterology. 2015 Aug;149(2):367-78.
14. Younossi Z et al. Results from REGENERATE: a phase 3 international, randomized, placebo-controlled study evaluating obeticholic acid treatment for NASH. The International Liver Congress, Vienna, abstract GS-07, 2019. Journal of Hepatology, 2019.

From genetic studies to personalized medicine in the management of NAFLD

Luca Valenti

INTRODUCTION

Over the last decade the traditional approach in the medical practice has been shifting to a targeted approach, based on the identification of selected subgroups which present better outcomes to specific therapies.

Personalized or precision medicine can be defined as a medical model that proposes the customization of healthcare, with medical decisions, practices, and/or products being tailored to the individual patient. In personalized medicine diagnostic testing are employed for selecting appropriate and optimal therapies based on the context of a patient's genetic content or other molecular or cellular analysis. The complexity of the personalized medicine approach requires appropriate tools, which may include molecular diagnostics, imaging, and analytics/software.

Ideally, these tools will be able to differentiate patients with the same diagnosis and the same prescription into four groups of patients depending on the therapy outcome:

- Safe and beneficial
- Not safe, but beneficial
- Safe, but not beneficial
- Not safe, nor beneficial.

PREVALENCE AND RISK

The role of genetics in specific pathology becomes evident when their prevalence cannot be explained by comorbidities and socioeconomical effect. In fact, large multiethnic population studies, such as the Dallas Heart Study, showed that the prevalence of steatosis differed strikingly among ethnic groups. Fatty liver was most common in Hispanics, with 45% of this subgroup demonstrating increased liver fat content. Hepatic steatosis was least common in African Americans - only 24% of this population exhibited increased liver fat. Caucasians had an intermediate prevalence (Figure 1) (1).

The higher prevalence of hepatic steatosis in Hispanics was due to the higher prevalence of obesity and insulin resistance in this ethnic group. However, the lower frequency of hepatic steatosis in blacks was not explained by ethnic differences in body mass index, insulin resistance, ethanol ingestion, or medication use. The prevalence of hepatic steatosis was greater in men than women among whites, but not in blacks or Hispanics. The ethnic differences in the frequency of hepatic steatosis in this study mirror those observed previously for NAFLD-related cirrhosis (Hispanics > Whites > Blacks). In conclusion, the significant ethnic

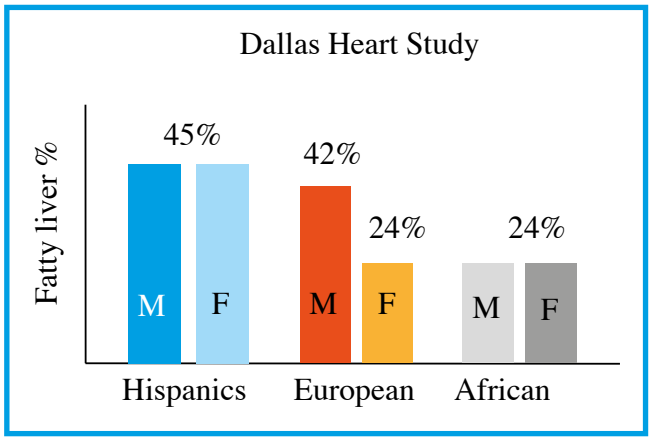


Figure 1 (Mod. from Browning 2014)

and sex differences in the prevalence of hepatic steatosis documented in this study may have a profound impact on susceptibility to steatosis-related liver disease (1).
In the general population, a positive family history has been associated with a higher risk of NAFLD, particularly when both parents were affected, and in those without cardiovascular risk factors. A phenotype familial cohort demonstrated that first-degree relatives of probands with NAFLD-cirrhosis have a 12 times higher risk of advanced fibrosis (2).

GENETICS DETERMINANTS OF NAFLD

The main common genetic determinants of hepatic fat content and NAFLD have been uncovered by genome-wide association studies (GWAS) during the last years. The one with the greatest impact is the rs738409 C>G single nucleotide polymorphism (SNP). This polymorphism encodes for an isoleucine-to-methionine substitution in the catalytic domain of patatin like phospholipase domain containing-3 (PNPLA3). The I148M variant increases susceptibility to the whole spectrum of liver damage related to NAFLD, from steatosis to inflammation and NASH, finishing with fibrosis and cirrhosis (3,4).
The most important trigger of the phenotypic expression of the mutations in those who do not drink excess alcohol is excessive adiposity. During obesity and insulin resistance the PNPLA3 protein is induced by insulin in hepatocytes and hepatic stellate cells and is localized at the surface of lipid droplets. Here, it is produced an alteration of lipid remodeling and turnover. Reduced dismissal of lipids would cause the enlargement of lipids droplets triggering steatosis and lipotoxicity (5,6). Furthermore, the I148M variant heightens the risk of hepatocellular carcinoma development independent of the effect on fibrosis, suggesting that it may have direct carcinogenic activity. Indeed, in Europeans, homozygosity for the I148M variant is enriched almost nine-fold in individuals who develop NAFLD-HCC as compared to the general population, while the absence of the variant can rule out HCC risk with a high specificity (Figure 2). Homozygosity for the variant is also associated with increased risk of hepatic decompensation in patients with fatty liver and portal hypertension. The predisposing effect of the PNPLA3 I148M variant on liver disease progression was found to be independent of the severity of liver fat accumulation. This suggest that it may influence the release of molecules that directly regulate inflammation and fibrogenesis.

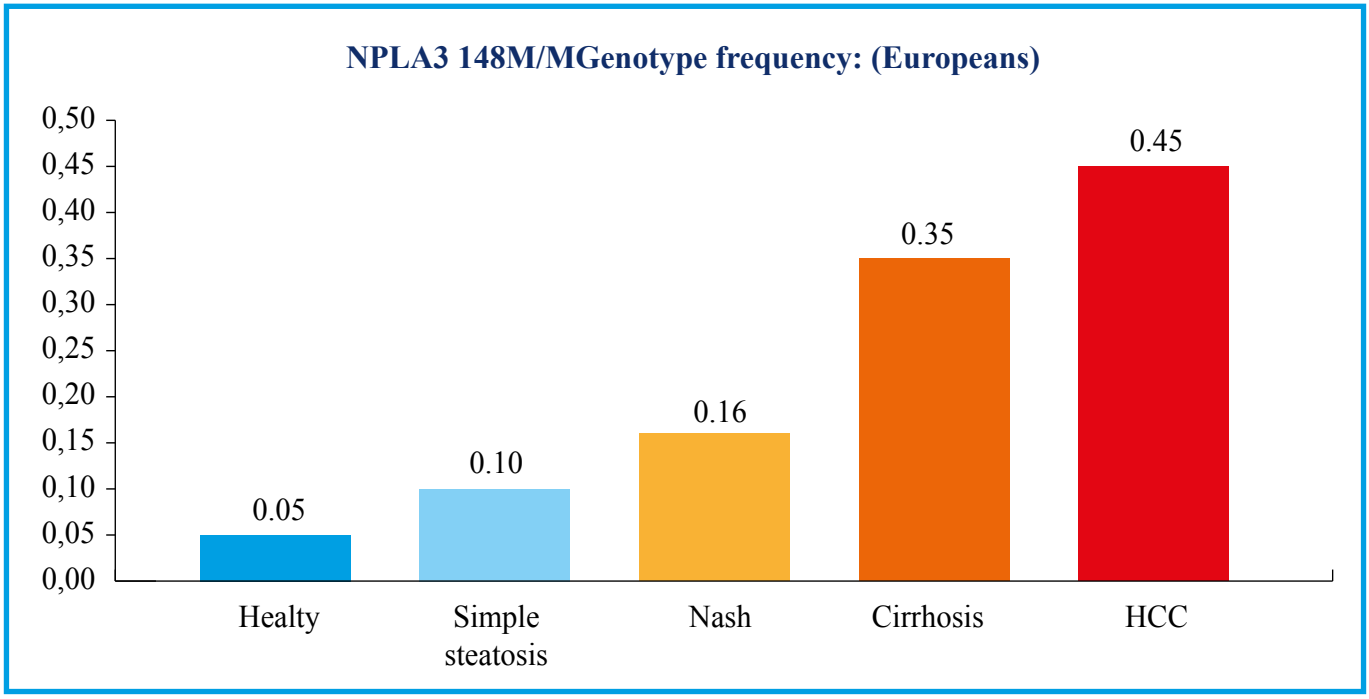


Figure 2 (Mod. from Valenti, Curr Pharm Des 2018)

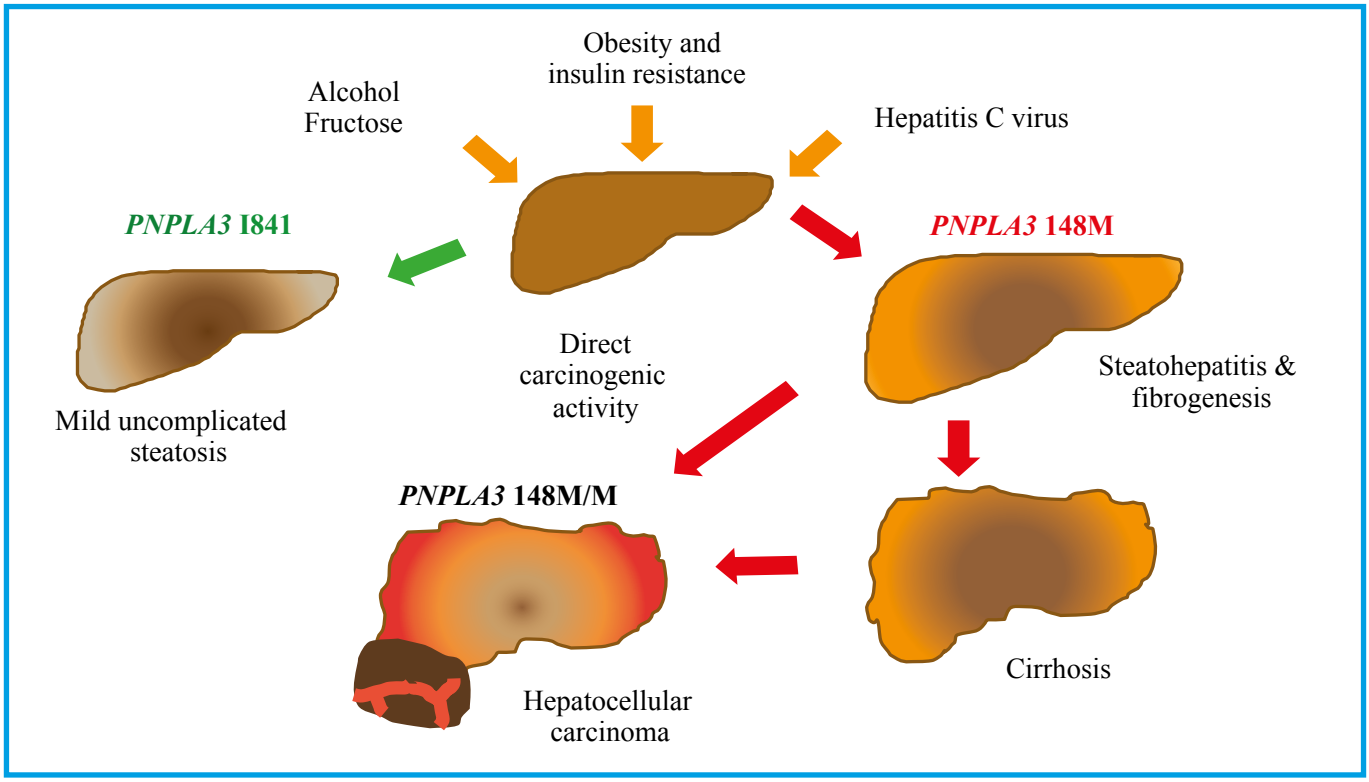


Figure 3 (Mod. from Valenti, Dig Liver Dis 2013)

Therefore, when triggering factors are present, such as obesity and insulin resistance, excess alcohol, or Hepatitis C virus, the "bad" I148M variant will favor steatohepatitis and fibrogenesis, which can lead to cirrhosis in susceptible in individuals, and especially in homozygous patients to HCC via cirrhosis or by directing favoring carcinogenesis (7) (Figure 3). The authors suggest this might be a genetic model of a common genetic variant (i.e., PNPLA3 I148M) influencing the HCC and that PNPLA3 genotype might potentially be a marker to increase clinical surveillance and whether modulation of PNPLA3 expression/ activity could be used to modify susceptibility to hepatocellular carcinoma.

Another major variant influencing NAFLD risk involves Transmembrane 6 superfamily member 2 (TM6SF2) that favors fat accumulation by decreasing lipid secretion in very low-density lipoproteins (VLDL), also leading to increased susceptibility to liver damage. This variant increase susceptibility to NAFLD and progressive NASH, while protecting from dyslipidemia and cardiovascular disease (8,9).

New gene implicated in the pathogenesis of NAFLD is Membrane bound O-acyltransferase domain-containing 7 (MBOAT7), which is involved in the remodeling of phosphatidylinositol by incorporating arachidonic acid and other unsaturated fatty acids within the so-called Lands cycle. The variant was recently associated with the risk of NAFLD, inflammation and fibrosis, as well as increased risk of alcoholic cirrhosis (10). It was recently demonstrated that the number of PNPLA3, TM6SF2, and MBOAT7 risk variants was associated with NAFLD-HCC independently of clinical factors (age, sex, T2DM, severe fibrosis), but did not significantly improve their predictive accuracy and that in non-cirrhotic patients the T allele remained associated with HCC. In a combined cohort of non-cirrhotic patients with chronic hepatitis C or alcoholic liver disease, the T allele was independently associated with HCC risk. The authors conclude that the MBOAT7 rs641738 T allele is associated with reduced MBOAT7 expression and may predispose to HCC in patients without cirrhosis, suggesting it should be evaluated in future prospective studies aimed at stratifying NAFLDHCC risk (11).

Rare mutations with a strong impact on the function of the encoded proteins may contribute to determine

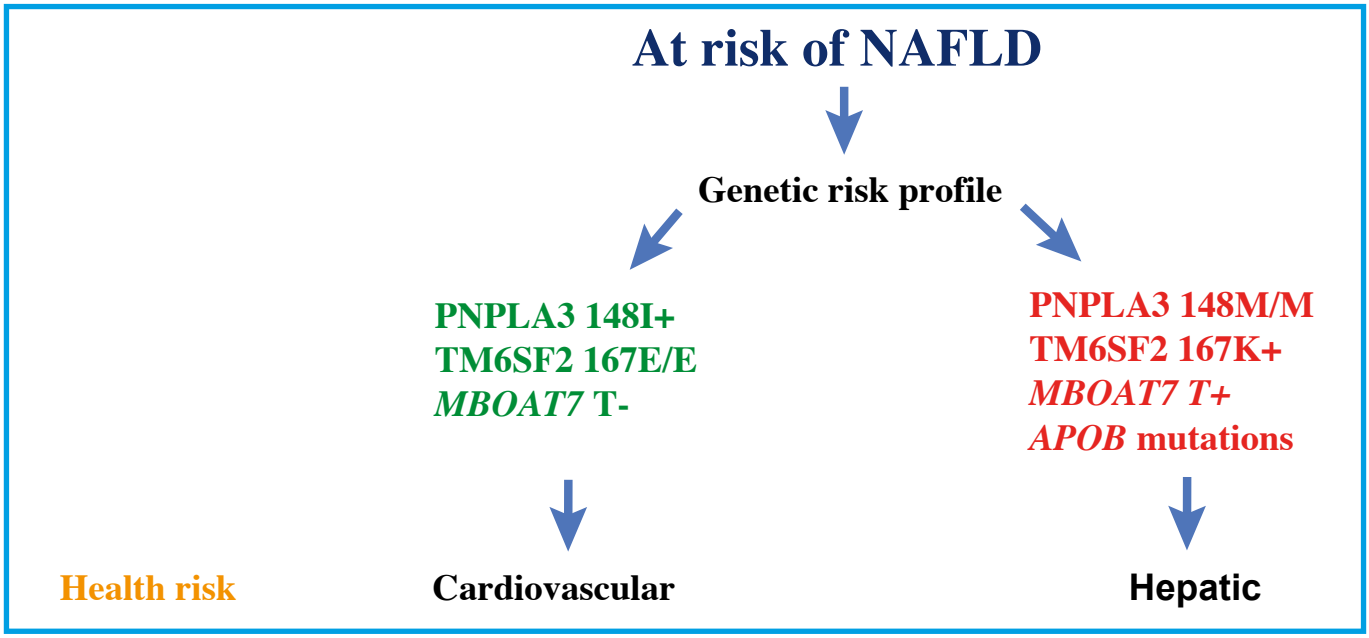


Figure 4 (Mod. Valenti, Curr Pharm Des 2018)

the predisposition to develop advanced NAFLD and disease clustering in specific families. Rare variants in Apolipoprotein B (APOB) have been associated with advanced liver disease and hepatocellular carcinoma associated with fatty liver, due to lipid compartmentalization in hepatocytes. This is related to the inability to secrete VLDL from hepatocytes, which requires ApoB100. Apo48 is also involved in the secretion of chylomicrons, and APOB mutations damage enterocytes, leading to malabsorption of liposoluble vitamins (and especially A, D, E may be relevant for the risk of NAFLD) and possibly of the intestinal barrier. APOB mutations has been related to carcinogenesis, even though the underlying mechanism is still not well understood. Hence, the identification of APOB mutations in subjects with NAFLD-HCC might to allow the diagnosis allowing to establish adequate HCC surveillance (12).

Conclusion and future application

In a precision medicine approach evaluation of genetic risk profile in patients with NAFLD would allow the identification of two prototypes of patients: those homozygous 148M, carrying TM6SF2, MBOAT7 and APOB mutations would be a higher risk of liver disease, whereas cardiovascular complication would be more likely in the others (Figure 4) (8).

This may have implications for screening, but also for treatment personalization. Carriage of specific genetic risk factors may influence the response or the side effect of drugs. For example, the PNPLA3 I148M variant has been reported to reduce the protective effect of statins on NAFLD, to reduce the beneficial impact of dapagliflozin, a sodium-glucose cotransporter-2 (SGLT2) inhibitor, on hepatic fat accumulation (12,13). Drugs directly targeting protein mutated in NAFLD may prove beneficial to prevent progressive NAFLD. For example, silencing of the mutated PNPLA3 protein may potentially revert liver damage by restoring dismissal of lipids from intracellular droplets, and possibly retinol metabolisms. A model of personalized clinical management of NAFLD patients based on genetic background, which should be tested in future studies (14).

In conclusion, the high variability of fatty liver disease has a great impact on clinical practice. Personalized medicine tools may provide useful hints since genetics play a major role in determining the severity and prevalent organ involvement; it has also, highlighted the role of hepatic fat reduction as a major therapeutic outcome.

Genetic risk scores (GRS) may improve the ability to stratify the risk of progressive disease and HCC and may predict response/side effects of specific therapeutic approaches.

References

1. Jeffrey D. Browning Lidia S. Szczepaniak Robert Dobbins Pamela Nuremberg Jay D. Horton Jonathan C. Cohen Scott M. Grundy Helen H. Hobbs Prevalence of hepatic steatosis in an urban population in the United States: Impact of ethnicity. 2004. Hepatology. Volume40, Issue6, 1387-1395.
2. Cyrielle Caussy, Rohit Loomba, the Familial NAFLD Cirrhosis Research Consortium. Nonalcoholic fatty liver disease with cirrhosis increases familial risk for advanced fibrosis. J Clin Invest. 2017;127(7):2697-2704.
3. Romeo S, Kozlitina J, Xing C, et al. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. Nat Genet 2008;40(12):1461-5.
4. Valenti L, Al-Serri A, Daly AK, Galmozzi E, Rametta R, Dongiovanni P, et al. Homozygosity for the PNPLA3 / adiponutrin I148M polymorphism influences liver fibrosis in patients with nonalcoholic fatty liver disease. Hepatology 2010;51:1209-17.
5. Mitsche MA, Hobbs HH, Cohen JC. Patatin-like phospholipase domain–containing protein 3 promotes transfer of essential fatty acids from triglycerides to phospholipids in hepatic lipid droplets. J Biol Chem 2018;293(18):6958-68.
6. Donati B, Motta BM, Pingitore P, et al. The rs2294918 E434K variant modulates patatin-like phospholipase domain-containing 3 expression and liver damage. Hepatology 2016;63(3):787-98.
7. Valenti L, Dongiovanni P, Ginanni Corradini S, Burza MA, Romeo S. PNPLA3 I148M variant and hepatocellular carcinoma: a common genetic variant for a rare disease. Dig Liver Dis. 2013;45(8):619-624.
8. Valenti LV & Baselli GA. Genetics of Nonalcoholic Fatty Liver Disease: a 2018 Update. Current Pharmaceutical Design, 2018;24:4566-4573
9. Luukkonen PK, Zhou Y, Nidhina Haridas PA, et al. Impaired hepatic lipid synthesis from polyunsaturated fatty acids in TM6SF2 E167K variant carriers with NAFLD. J Hepatol 2017;67(1):128-36.
10. Liu YL, Reeves HL, Burt AD, et al. TM6SF2 rs58542926 influences hepatic fibrosis progression in patients with non-alcoholic fatty liver disease. Nat Commun 2014;5:4309.
11. Pelusi S, Baselli G, Pietrelli A, et al. Rare Pathogenic Variants Predispose to Hepatocellular Carcinoma in Nonalcoholic Fatty Liver Disease 2019. Sci Rep 9, 3682.
12. Mancina RM, Dongiovanni P, Petta S, et al. The MBOAT7-TMC4 Variant rs641738 Increases Risk of Nonalcoholic Fatty Liver Disease in Individuals of European Descent. Gastroenterology. 2016 May;150(5):1219-1230.e6
13. Dongiovanni P, Petta S, Mannisto V, et al. Statin use and nonalcoholic steatohepatitis in at risk individuals. J Hepatol 2015;63(3):705-12.
14. Valenti L, Dongiovanni P. Mutant PNPLA3 I148M protein as pharmacological target for liver disease. Hepatology 2017;66(4):1026-8.