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Vienna, Austria
21–24 June

POSTGRADUATE COURSE

From NAFLD to Liver Cancer

The International Liver Congress™

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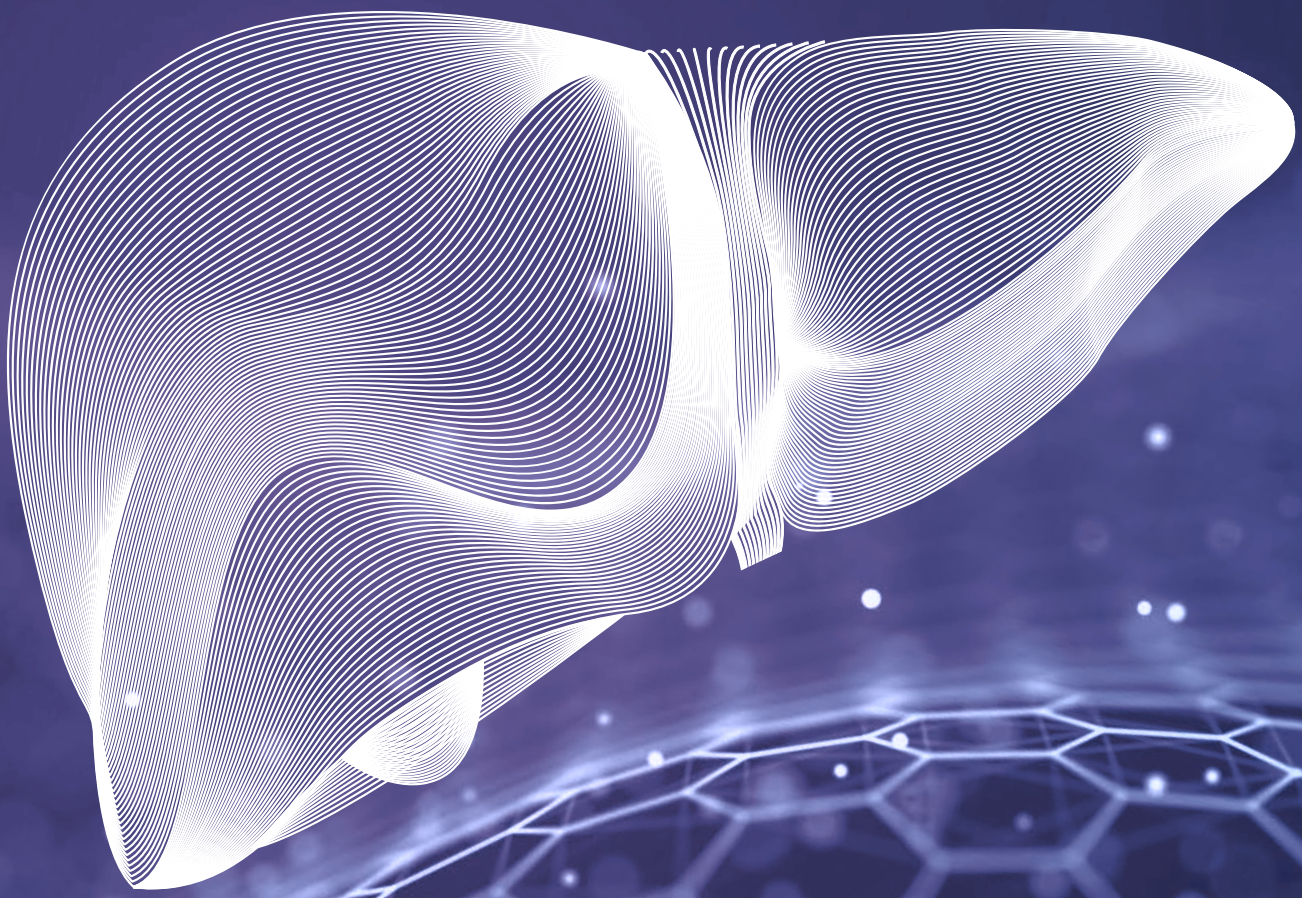

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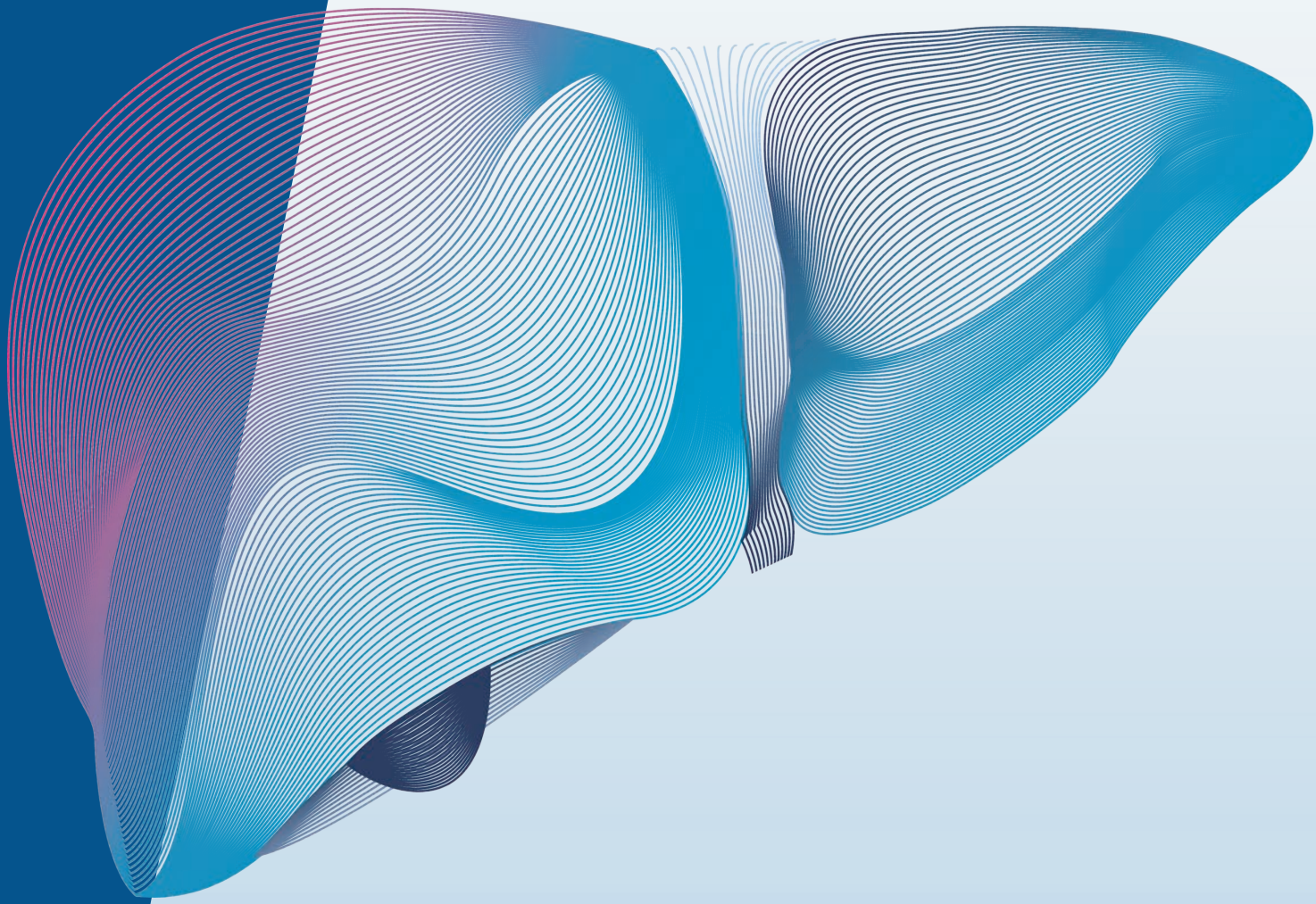

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Welcome message from the course organisers

On behalf of the European Association for the Study of the Liver (EASL), we are delighted to welcome you to EASL Congress 2023 and especially to this Postgraduate Course (PGC) on "***From NAFLD to liver cancer***".

Non-alcoholic fatty liver disease (NAFLD) has a global prevalence of 20-25% and is a major public health problem. Its incidence is increasing in parallel to the rise in obesity, diabetes, and metabolic syndrome. Progression from NASH cirrhosis to NASH-related hepatocellular carcinoma (HCC) occurs at a rate of ~1-2% of cases per year, and management of these patients is hampered by the presence of comorbidities.

The course will provide updates on the molecular pathogenesis, epidemiology, and management of NAFLD, NASH and NASH-HCC. We will first address the role of microenvironment in the development of these diseases, and the current impact of preventive strategies and drug therapies in NASH. Then, we tackle the main strategies of surveillance and diagnosis of liver cancer in patients with NASH cirrhosis and the criteria to be taken into account when treating HCC and cholangiocarcinoma with surgery, loco-regional, and systemic therapies.

The PGC is divided into four sessions on NAFLD, NASH, and the development and management of liver cancer, including hepatocellular carcinoma and cholangiocarcinoma. The course concludes with a State-of-the-Art lecture on improving liver health in Europe. As delegates, you will then be able to reply to questions from the chairs and live voting will be available. Take part and share your experience on Twitter, using the hashtags EASLCongress, #LiverTwitter, and #NAFLD.

The organisers and the faculty wish you an enjoyable time at EASL Congress 2023, and we hope you find the course stimulating and informative. We look forward to seeing you in person in Vienna.

Elisabetta Bugianesi
Italy

Josep M Llovet
Spain & United States

Philip N Newsome
United Kingdom

Programme

Postgraduate Course: From NAFLD to liver cancer

Organisers

Elisabetta Bugianesi, *Italy*

Josep M Llovet, *Spain & USA*

Philip N Newsome, *UK*

WEDNESDAY, 21 JUNE 2023

Session 1: Molecular pathogenesis of NAFLD, NASH and liver cancer

Chairs:
Scott FRIEDMAN, *USA*
Jessica ZUCMAN-ROSSi, *France*

08:35-08:55	**Overview of molecular pathogenesis of NAFLD and NASH-HCC** Scott FRIEDMAN, *USA*
08:55-09:10	**Molecular predisposition in NAFLD and NASH-HCC** Jessica ZUCMAN-ROSSI, *France*
09:10-09:25	**Immune microenvironment in NAFLD, NASH and NASH-HCC** Mathias HEIKENWALDER, *Germany*
09:25-09:40	**Molecular Pathogenesis of CCA in NAFLD patients** Jesús BAÑALES, *Spain*
09:40-10:00	**Panel discussion**

Session 2: Non-alcoholic fatty liver disease (NAFLD)

Chairs:
Elisabetta BUGIANESI, *Italy*
Philip NEWSOME, *UK*

11:45-12:00	**NAFLD – not always that clear-cut: presentation of cases** Manuel ROMERO GOMEZ, *Spain*
12:00-12:15	**Epidemiology, natural history & risk stratification of NAFLD and NASH** Hannes HAGSTRÖM, *Sweden*
12:15-12:35	**Role of lifestyle in prevention and treatment of NAFLD/NASH** Elisabetta BUGIANESI, *Italy*
12:35-12:55	**Drug therapy for NASH: present and future prospects** Philip NEWSOME, *UK*
12:55-13:15	**Panel discussion**

Session 3: Surveillance and diagnosis of HCC in NAFLD patients

Chairs:
Helen REEVES, *UK*
Augusto VILLANUEVA, *USA*

14:45-14:53	**Case presentation-surveillance for NASH-HCC** Anna SABOROWSKI, *Germany*
14:53-15:07	**Epidemiology, surveillance & early detection of HCC in NAFLD** Augusto VILLANUEVA, *USA*
15:07-15:21	**Pathology & artificial intelligence in the diagnosis of HCC** Valérie PARADIS, *France*
15:21-15:35	**Imaging for diagnosis and staging of HCC** Maxime RONOT, *France*
15:35-15:49	**Assessment of co-morbidities and impact in the management of NASH-HCC** Helen REEVES, *UK*
15:49-16:00	**Panel discussion**

Session 4: Non-alcoholic fatty liver disease (NAFLD)

Chairs:
Josep M. LLOVET, *Spain & USA*
Patrizia BURRA, *Italy*

17:15-17:30	**Surgical resection and transplantation: results in NAFLD-HCC** Patrizia BURRA, *Italy*
17:30-17:45	**Loco-regional therapies for HCC in NAFLD patients** Jean-Charles NAULT, *France*
17:45-18:05	**Systemic therapies for HCC and role of etiology** Josep M. LLOVET, *Spain & USA*
18:05-18:25	**Overview of management of Cholangiocarcinoma** Arndt VOGEL, *Germany*
18:25-18:45	**Panel discussion**

Abbreviations and Acronyms

15-PGDH	15-hydroxyprostaglandin dehydrogenase
AASLD	American Association for the Study of Liver Diseases
ACSL5	acyl-CoA synthetase long chain family member 5
AFP	Alpha-fetoprotein
aHCC	Advanced hepatocellular carcinoma
AI	Artificial intelligence
ALT	Alanine aminotransferase
AMPK	AMP-activated protein kinase
APHE	Arterial phase hyperenhancement
AST	Aspartate aminotransferase
AUC	Area under the curve
AUROC	Area under the receiver operating characteristic
BCLC	Barcelona Clinic for Liver Cancer
BMI	Body mass index
CAP	controlled-attenuation parameter
CCA	Cholangiocarcinoma
CDCA	Chenodeoxycholic acid
cfDNA	Cell-free DNA
CI	Confidence interval
CT	Computed tomography
CYP7A1	Cholesterol 7-α-hydroxylase
DAMPs	Damage-associated molecular patterns
dCCA	Distal cholangiocarcinoma
DFS	Disease-free survival
DNA	Deoxyribonucleic acid
DNL	*de novo* lipogenesis
EASL	European Association for the Study of the Liver
ECOG	Eastern Cooperative Oncology Group
EFX	Efruxifermin
ELF	Enhanced Liver Fibrosis
EMA	European Medicines Agency
EMEA	European Medicines Evaluation Agency
ENSCCA	European Network for the Study of CCA
ER	Endoplasmic reticulum
EVs	Extracellular vesicles
FABP5	FA-binding protein 5
FAO	FA β-oxidation
Fas	Fatty acids
FDA	Food and Drug Administration
FFA	Free fatty acid
FGF21	Fibroblast growth factor 21
FGFR	Fibroblast growth factor receptor
FOLFOX	FOLinic acid, Fluorouracil and OXaliplatin
FXR	Farnesoid X receptor
GIP	Glucose-dependent insulinotropic polypeptide
GLP-1	Glucagon-like peptide-1
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HDAC6	Histone deacetylase 6
HIF-1	Hypoxia-inducible factor 1
HIR	Hepatic insulin resistance
HOMA	Homeostasis model assessment

HPB	Hepatobiliary phase
HR	Hazard ratio
iCCA	Intraintrahepatic cholangiocarcinoma
ICI	Immune checkpoint inhibitor
ICU	Intensive care unit
IDH1	Isocitrate dehydrogenase 1
IGF	Insulin-like growth factor
IL-1β	Interleukin-1 beta
IL6	Interleukin-6
ILCA	International Liver Cancer Association
IRS-1	Insulin receptor substrate 1
JAK	Janus kinase
LI-RADS	Liver Imaging Reporting and Data System
LPS	Lipopolysaccharides
MELD	Model for End-Stage Liver Disease
mOS	Median overall survival
mPFS	Median progression-free survival
MRE	Magnetic resonance elastography
MRI	Magnetic resonance imaging
NAFL	Non-alcoholic fatty liver
NAFLD	Non-alcoholic fatty liver disease
NAS	NAFLD Activity Score
NASH	Non-alcoholic steatohepatitis
NAT	Neoadjuvant therapy
NIDDKD	National Institute of Diabetes and Digestive and Kidney Diseases
OLT	Orthotopic liver transplantation
OR	Odds ratio
ORR	Objective response rate
OS	Overall survival
PNPLA3	Patatin-like phospholipase domain-containing 3 gene
PAD	Arterial pressure (diastolic)
PAMPs	Pathogen-associated molecular patterns
PAS	Arterial pressure (systolic)
pCCA	Perihilar cholangiocarcinoma
PD1	Programmed cell death protein 1
PDFF	Protein density fat fraction
PD-L1	Programmed cell death protein 1 ligand
PFS	Progression-free survival
PPARs	Peroxisome proliferator activated receptors
PPAR-γ	Peroxisome proliferator-activated receptor-γ
PSC	Primary sclerosing cholangitis
PST	Performance Status
PUFAs	Polyunsaturated fatty acids
RCTs	Randomised controlled trials
RFA	Radiofrequency ablation
RNAs	Ribonucleic acids
RNAseq	RNA sequencing
ROS	Reactive oxygen species
RR	Relative risk
SAF	Steatosis, activity, and fibrosis
SFAs	Saturated fatty acids
SGLT2	Sodium/glucose transport protein 2
SIRT	Selective internal radiation therapy
SPHK1	Sphingosine kinase 1
STAT	Signal transducer and activator of transcription
T2DM	Type-2 diabetes mellitus
TACE	Transarterial Chemoembolisation

TE	Transient elastography
TGF-β	Transforming growth factor beta
THR-β	Thyroid hormone receptor β
TKI	Tyrosine-kinase inhibitor
TNF-α	Tumour necrosis factor alpha
VCTE	Vibration-controlled transient elastography
VEGF	Vascular endothelial growth factor
VEGFA	Vascular endothelial growth factor A
WHO	World Health Organization
WSI	Whole-slide imaging

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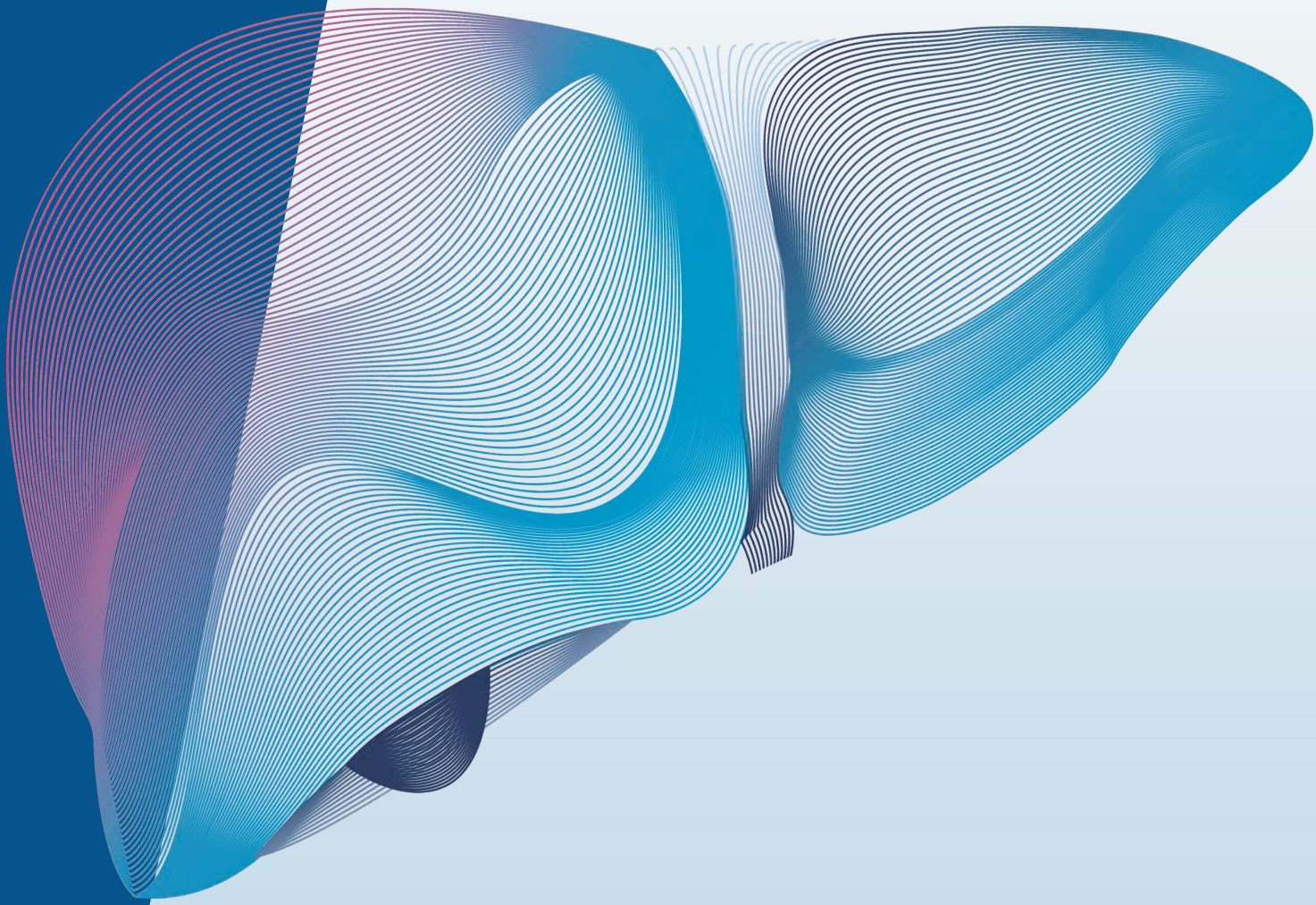
SESSION 1

MOLECULAR PATHOGENESIS OF NAFLD, NASH AND LIVER CANCER

WEDNESDAY 21 JUNE
08:30-10:00



Overview of molecular pathogenesis of NAFLD and NASH-HCC

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Take-home messages

- Non-alcoholic fatty liver (NAFL) is highly prevalent, yet only a fraction of patients develop NASH, for unclear reasons.
- NASH is a rising public health threat whose outcomes are determined by the severity of fibrosis and/or development of hepatocellular cancer (HCC).
- NASH pathogenesis is likely multifactorial, but the dominant disease drivers are not established and may differ among patients.
- Fibrosis pathways in NASH are increasingly clear and targetable.
- NASH-HCC has both shared and unique pathogenic determinants compared with other aetiologies.

Background and pathogenic drivers of NASH

The rising prevalence of obesity and type 2 diabetes has been accompanied by an astonishing increase in non-alcoholic fatty liver (NAFL) and its more advanced stage, non-alcoholic steatohepatitis (NASH). Currently affecting up to 40% of all individuals in many countries worldwide, a subset (~20% of those with NAFL) progress to NASH, yet underlying determinants of why some are protected from progression are not clear. Also unclear are the drivers of NASH in lean individuals who do not have obesity or type 2 diabetes. Regardless, recent data suggests that the world is not prepared for non-alcoholic fatty liver (NAFLD), with very few countries enacting public health measures to identify and manage the massive numbers of anticipated patients as we await effective therapies.

NASH is associated with many pathways of metabolic dysregulation both in liver and systemically in extrahepatic tissues, especially in adipose, muscle, and pancreas. Within the liver, dozens, if not hundreds of pathways are dysregulated, especially those controlling lipid homeostasis and carbohydrate metabolism. The net result of this dysregulation is increased fat accumulation from *de novo* lipogenesis as well as impaired lipolysis and hepatic export, combined with increased hepatic glucose uptake and impaired glucose utilisation. Overall, the changes in lipid and glucose homeostasis reflect an imbalance of energy metabolism with excess energy entering the liver relative to its capacity to oxidise these substrates or export them. Multiple inputs to the liver affect these pathways, especially derived from the microbiome, visceral adipose, muscle, the immune system and the CNS.

Upstream drivers of hepatocyte injury in NASH are not well identified, but include dysregulated circulating adipokines, increased circulating insulin and insulin-like growth factor (IGF), as well as signals derived from the gut microbiome, which is abnormal in NASH patients and animal models. In particular, gut dysbiosis, defined as an abnormal or unbalanced composition of the gut microbial community, is a compelling candidate for both initiating and/or perpetuating fatty liver disease and NASH fibrosis, as well as HCC. Among the effects of dysbiosis are increased gut permeability allowing intestinal products too traverse the portal vein where they lead to hepatocellular damage and sterile inflammation. Gut bacteria or their products may additionally stimulate senescence and accelerate

damage to hepatocytes, while activating stellate cells. Studies even suggest that under defined conditions, a NASH phenotype in mice may be transmissible by faecal transplantation. Recent findings also reinforce the possibility that specific gut bacteria in NASH can generate ethanol that traverses the portal vein to stimulate liver injury, referred to as 'autobrewery syndrome' – its prevalence and overall contribution to NASH are unclear. Remarkably, the composition of the gut microbiome may also influence responsiveness to medical therapies for NASH or other diseases. In general, mouse models tend to be 'optimistic' in predicting drug efficacy, in part because they are inbred and genetically identical, and their microbiota are less complex than those of outbred mice, which better predict drug responses that translate into human efficacy.

Collectively, these events associated with hepatocyte damage are thought to stimulate the release of lipotoxic molecules that both injure hepatocytes to provoke inflammation, and enhance fibrosis (Fig. 1). The specific identity of these hepatocyte-derived species is a major unanswered question, and likely includes bile acids, pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs), and products of oxidant stress and endoplasmic reticulum (ER) stress, especially those derived from mitochondrial injury. How these signals are integrated, which are the most dominant, and why they only occur in a subset of patients with fatty liver remain critical unanswered questions in clarifying NASH pathogenesis.

Once hepatocytes are injured, a cascade of signals driven by intercellular crosstalk stimulate activation, or transdifferentiation, of hepatic stellate cells into myofibroblasts, leading to enhanced fibrogenesis. These stellate cell-derived myofibroblasts are the primary fibrogenic cell in liver injury from NASH and other causes of parenchymal cell damage (*e.g.* viral hepatitis). Derived from the cardiac mesoderm/septum transversum during development, they are similar to pericytes in other tissues, and reside in the subendothelial space, with foot processes surrounding the sinusoid where they can regulate sinusoidal blood flow. More information about pathogenesis of fibrosis is detailed below (see the section 'Fibrosis pathogenesis').

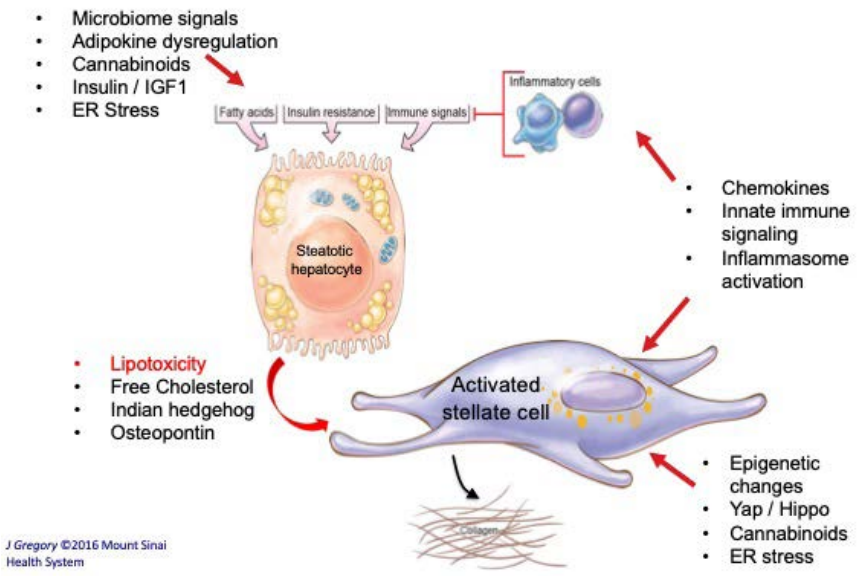

Fig. 1. Hepatic drivers of NASH and fibrosis.
A number of upstream signals converge on hepatocytes to induce injury, combined with dysregulated immunity and insulin resistance. These lead to hepatocyte-derived molecules that activate hepatic stellate cells and amplify inflammation. Within activated stellate cells, a number of intracellular changes contribute to progressive fibrosis. NASH, non-alcoholic steatohepatitis.

Genetic contributions to NAFLD pathogenesis are the subject of a separate lecture in this course. However, a growing list of single-nucleotide DNA polymorphisms, many of which involve lipid handling within hepatocytes, have been identified as potential risk factors and therapeutic targets in NASH. The extent to which these genetic factors contribute to the onset and severity of NASH is not clear, but most likely is one of several multifactorial determinants of disease. Genetic contributions to NASH are also underscored by the high frequency of NASH fibrosis among first-degree relatives, although familial clustering of disease may also reflect a shared microbiome, especially for those living in the same household.

Emerging therapeutic targets are organised according to their points of attack in this pathogenic sequence (Fig. 2). Although not the focus of this lecture, general classes of therapeutics include those aimed at reducing fat accumulation in hepatocytes, improving insulin signalling and glucose homeostasis, antagonising inflammation elicited by hepatocellular injury, reducing oxidant stress and restoring metabolic and structural integrity of a hepatocytes, and directly antagonising fibrogenic signalling by activated stellate cells/myofibroblasts.

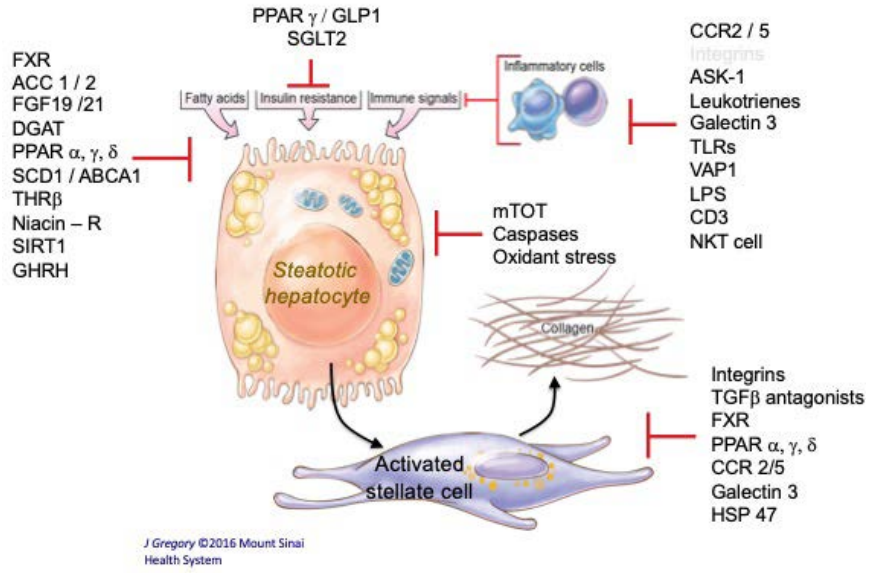

Fig. 2. NASH pathogenesis and related therapeutic targets.
Pathways related to NASH and fibrosis are illustrated here, with candidate therapeutic targets organised based on their loci/mechanisms of action. NASH, non-alcoholic steatohepatitis.

Diagnosis and risk factors for liver-related events in NASH

The definitive diagnosis of NAFLD and NASH still requires liver biopsy of sufficient size and containing adequate numbers of portal tracts to classify and stage the disease. Many studies initially relied on the NAFLD activity score comprised of three main components: steatosis, lobular inflammation, and ballooning. Fibrosis is scored separately using either a 0–4 (Brunt Kleiner score or SAF [steatosis, activity, and fibrosis] scores) or 0–6 scale (Ishak score). As data have accumulated, limitations of the NAFLD score have become evident, in particular the marked sampling variability of its three features, combined with the relative difficulty defining and quantifying ballooning within a liver section. However, *fibrosis has consistently emerged as the single most important histologic feature predicting clinical events*. This conclusion was further underscored by an important longitudinal study from the NIDDK (National Institute of Diabetes and Digestive and Kidney Diseases) NASH Clinical Research Network in almost 1,800 patients followed for 10 years. The data have clearly established the importance

of fibrosis as a risk factor for death from any cause, hepatic decompensation, and hepatocellular carcinoma. As a result, both the disease pathologic scoring systems and therapeutic efforts are both increasingly focused on defining the exact amount of fibrosis, and therapeutically targeting those pathways that directly or indirectly enhance fibrogenesis or stimulate matrix degradation. There is now also some data reinforcing the expectation that reducing fibrosis will improving outcomes, based on findings from clinical trials in which a subset of patients had fibrosis improvement – these patients had a markedly reduced incidence of clinical events compared with those in whom fibrosis progressed.

Recent data also support the superiority of digital pathologic methods to more accurately quantify fibrosis content along a continuous scale, and as these methodologies are validated in longitudinal trials they will supplant or complement conventional scoring systems. More importantly, efforts are intensifying to replace biopsy altogether with non-invasive disease staging in hopes of widening enrolment in clinical trials and enhancing the eligibility of patients for effective therapies once they are approved by the EMEA (European Medicines Evaluation Agency) or FDA (Food and Drug Administration).

Fibrosis pathogenesis

Because fibrosis is the key determinant of clinical outcomes in NASH, a more detailed review of its pathogenesis is described here. Activation of hepatic stellate cells has been recognised as a central event in fibrosis pathogenesis for decades. Typically, activated stellate cells express alpha smooth muscle actin and a whole panoply of cell surface and intracellular molecules, that collectively drive the cells to become more fibrogenic (Fig. 3).

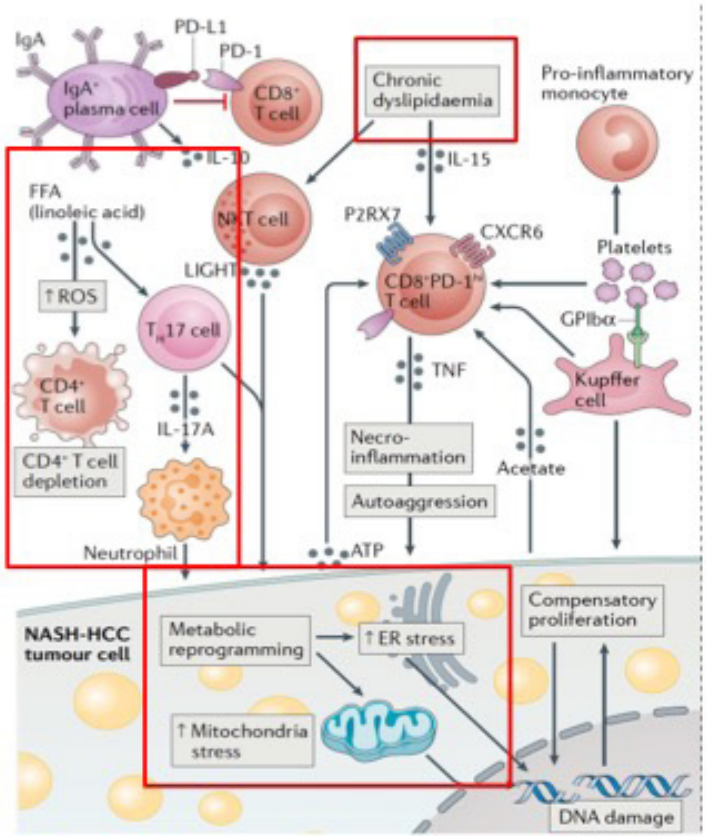

Fig. 3. NASH dependent immune responses in HCC.
Dysregulate immune signalling events are depicted. NASH, non-alcoholic steatohepatitis; HCC, hepatocellular carcinoma. Reprinted from Llovet et al.[5]

With the advent of single-cell sequencing methods, the general concept of stellate cell activation has remained valid; however, these methods have also uncovered significant heterogeneity of the stellate cell phenotype. The full consequences of stellate cell heterogeneity are not yet appreciated, but in addition to canonical 'activated' cells, stellate cell subtypes may include those that have been previously activated and later maintain an inactivated but 'primed' state to reactivate quickly following repeated liver injury. There are also senescent hepatic stellate cells, which are stimulated by signals from the microbiome to generate a senescence-associated secretory phenotype that is pro-inflammatory and contributes to fibrosis progression. This senescent subpopulation has been the target of therapeutic efforts to selectively clear them from injured liver using chimeric antigen receptor T cells, demonstrating improved fibrosis and injury in experimental NASH models in mice. There are other stellate cell subtypes whose function is not yet elucidated, but these additional populations may contribute to regional and individual differences in the extent and rate of fibrosis in patients with underlying NASH.

A vital consequence of stellate cell heterogeneity may be its divergent contributions to HCC pathogenesis. Elegant studies have identified subpopulations of stellate cells that can either promote or prevent HCC, depending on their secretory and genetic phenotypes. These important findings, made possible through single-cell sequencing methods, help reconcile longstanding debates and contradictory findings about whether fibrogenic cells prevent tumour growth in part by their encapsulation of cancer cells, or promote tumours through direct stimulation of cancer cell growth. The findings further illustrate the marked complexity of the tumour microenvironment not only among immune cells, but also among stromal cells that include activated stellate cells.

An important feature of advanced NASH fibrosis not previously recognised is that as disease progresses, hepatic stellate cells expand and become elongated, developing a dense network of autocrine cell–cell interactions that are driven by a unique repertoire of ligand receptor combinations. The findings imply that as fibrosis advances, the therapeutic targets evolve, and thus treatment may also need to account for these changes, necessitating different drugs than those administered when there is mostly fat and inflammation with less fibrosis. This 'cold' fibrosis stage driven by autocrine interactions likely explains why fibrosis continues to progress in advanced patients whose livers have lost fat and other classic histologic features of NASH.

NASH-HCC, general features and NASH-specific drivers

The global incidence of NASH-HCC is rising dramatically, albeit with significant geographic variability. Nonetheless, this neoplasm has the fastest rising rate of increase among the several aetiologies of chronic liver disease and HCC. A critical and unique clinical feature of NASH-HCC is a higher propensity for tumours to emerge before cirrhosis is established. In about one-third of all patients with NASH-HCC, tumours arise in livers that are non-cirrhotic. In contrast, viral hepatitis-associated cancers arise only in liver that is cirrhotic in 95% of patients. Nonetheless, cirrhosis is still the strongest risk factor for HCC in NAFLD, and thus efforts to regress fibrosis are still an important mainstay in potential protection from liver cancer in NASH.

A number of features of NASH may account for the unique clinical behaviour if HCCs that arise in this disease. First, obesity in general confers a higher risk of all cancers, especially liver, because it is linked to a chronic inflammatory state with more oxidant stress, DNA damage, and genomic mutations. Also associated with obesity are higher circulating levels of mitogenic signals including IGF and hepatocyte growth factor, as well as dysregulation of adipokines. Combined with potential genetic determinants unique to HCC risk and an altered gut microbiome, emergence of cancers in this dysregulated tumour microenvironment may no longer depend strictly on cirrhosis, although advanced

fibrosis is usually present. More generally, obesity can generate systemic immune alterations that affect the liver, for example increased Th17-related inflammation.

A number of molecular differences between NASH-HCC and non-NASH-HCC have been summarised recently and are depicted in Table 1 (reprinted from Llovet *et al.*[5]). Among these differences, attention has focused on the specific role of linoleic acid accumulation leading to enhanced reactive oxygen species, CD4 T cell depletion, auto-aggressive CXCR6 CD8 T cells, metabolic reprogramming and increased DNA damage. These changes are further amplified by chronic dyslipidaemia, ER stress and other immune inflammatory changes associated with obesity.

An important consequence of these NASH-HCC-specific changes in hepatic immunity includes reduced efficacy of immunotherapy regimens using checkpoint blockade. Interestingly, hepatic stellate cells may contribute to immunotolerance and resistance to checkpoint inhibition, thereby linking fibrogenic responses directly to a dysregulated immune microenvironment. This is a rapidly evolving area, with optimism engendered by the success of some checkpoint inhibitors in both treating primary HCC and reducing the recurrence in NASH in the adjuvant setting.

In summary, uncovering the pathogenic features of NAFLD and NASH-HC is among the highest priorities in hepatology, with remarkably rapid advances, there are many unanswered questions that future studies must address. These include:

- Why do only a fraction of patients with NAFLD develop NASH and what are those NASH-specific signals? Do they reflect differences in genetics, microbiome, diabetes, lipotoxicity, immune dysregulation, or a combination thereof?
- Is NASH really more than one disease with different subgroups in which different pathways or abnormalities predominate, either insulin resistance, lipotoxicity, microbiome, or the immune system?
- Will metabolic, anti-inflammatory, or antifibrotic therapy alone be sufficient or will combinations be required? And will they improve outcomes and reduce HCC in the majority of patients? Currently even successful therapies only benefit a minority of patients, so there is much work to be done.

References

(References in **BOLD** are required reading.)

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Molecular pathogenesis of CCA in patients with NAFLD

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Background

Cholangiocarcinoma (CCA) comprises a heterogeneous group of malignant tumours that originate along the bile ducts. It represents the second most common primary liver cancer, after hepatocellular carcinoma (HCC), and ~3% of all gastrointestinal malignancies.[1] The silent growth of CCAs leads to a late diagnosis, which combined with their highly aggressive nature, chemoresistance and the limited available therapeutic options markedly contribute to high mortality rate (2% of cancer-related deaths yearly worldwide).

Based on the potential differences in aetiopathogenesis, incidence and prognosis, the World Health Organization (WHO) has approved (2021) a new classification of these tumours based on their anatomical origin, into intrahepatic (iCCA), perihilar (pCCA) and distal (dCCA) (Fig. 1). The aetiology of most CCAs are unknown; however, different risk factors have been established with different degrees of predisposition.[1,2] For instance, the presence of choledochal cysts, bile duct stones, cirrhosis, chronic biliary diseases (*e.g.* primary sclerosing cholangitis [PSC] or Caroli disease), hepatitis B or C viruses (HBV, HCV), liver fluke parasites such as *Opisthorchis viverrini* and *Clonorchis sinensis*, and certain toxins (asbestos, dioxins, or nitrosamines) highly increase the risk of CCA development. However, alcoholic liver disease, diabetes, tobacco, and non-alcoholic fatty liver disease (NAFLD) induce moderate risk but are highly prevalent.

The global geographical distribution of CCA is asymmetrical, being considered a rare cancer (<6 cases per 100,000 people/year) in most Western countries, but with significantly higher incidence in Southeast Asian countries, such as China, South Korea, Thailand and Japan.[1] This discrepancy is likely because of differences in exposure to specific risk factors, particularly to the high prevalence of liver fluke parasites and HBV/HCV infections in Southeast Asia.[3–6] Nevertheless, the global pandemic of obesity-related NAFLD may markedly change this distribution. In fact, a clear shift in the aetiology of CCA is being actually experienced, with NAFLD being now predicted as the main predisposing condition for liver cancer in the coming years.

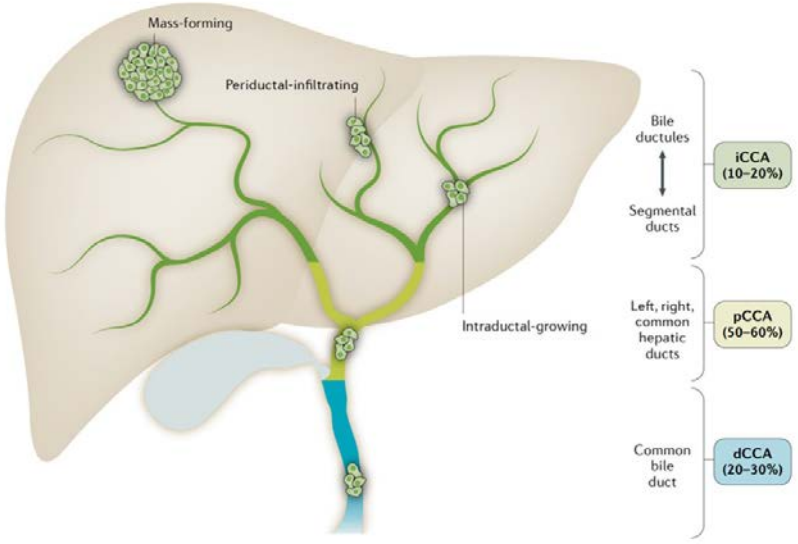

Fig. 1. Anatomical classification of cholangiocarcinoma.[1]

NAFLD is highly associated with obesity, insulin resistance, dyslipidaemia, and hypertension, which commonly lead to altered lipid metabolism and chronic inflammation, establishing a pro-carcinogenic environment and setting the ground for liver cancer development. In this regard, alone or in association with obesity, NAFLD has been proven to be a major risk factor not only for HCC, but also for CCA. The association between NAFLD and CCA have been highlighted in the past decade. A recent systematic review including case-control and cohort studies, clinical trials, and meta-analysis confirmed the association of NAFLD and CCA (odds ratio [OR] = 1.88). A subsequent subanalysis based on the CCA anatomical origin showed a significant association between NAFLD and iCCA (OR = 2.22), whereas the link with extrahepatic CCA (eCCA) was less evident.[7] Importantly, the presence of non-alcoholic steatohepatitis (NASH), but not simple steatosis, was shown to be associated with iCCA development, being an independent prognostic factor (Fig. 2).

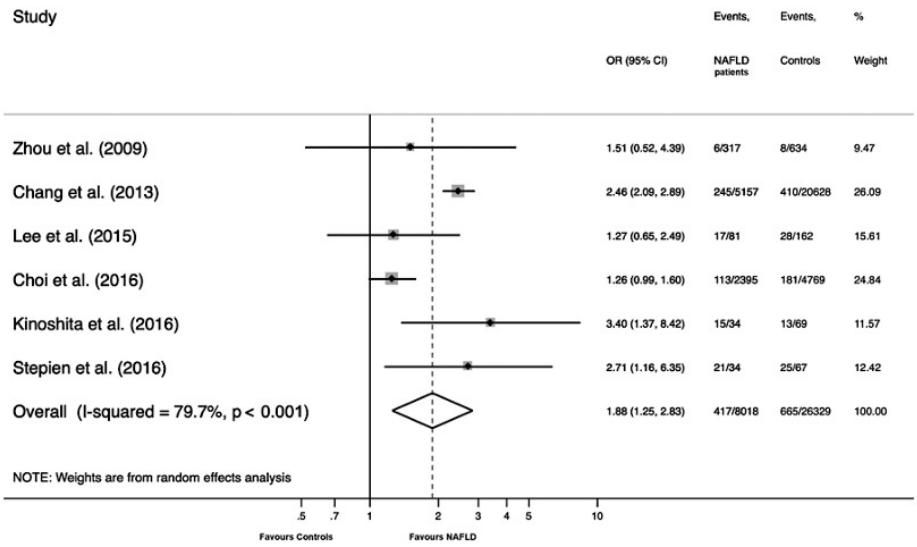

Fig. 2. Forest plot highlighting the association of NAFLD with CCA, independently of CCA subtype in a metanalysis including seven studies.[7]

CCA, cholangiocarcinoma; NAFLD, non-alcoholic fatty liver disease.

In line with this, data from the European Network for the Study of CCA (ENSCCA) registry database, including detailed demographic and clinical information from 2,234 patients with CCA, suggest a considerable prevalence of NAFLD in patients with CCA; thus, at diagnosis, more than half were found to exhibit overweight (35.7%) or obesity (19.4%), and a significant proportion had diabetes (22.5%)[8] or concomitantly presented with obesity and diabetes (15%). Of note, as severe weight loss is a common unspecific symptom of patients with CCA, the proportion of patients with a history of obesity might be underestimated. Similar findings were reported in a retrospective study conducted in a Brazilian cohort of patients with CCA, where metabolic disorders (obesity and type 2 diabetes) were common and associated with higher overall survival, relapse-free survival and survival after surgery, compared with non-metabolic cases.[9]

Cholangiocarcinogenesis and molecular subtypes

CCA cells have traditionally been considered to originate from the malignant transformation of cholangiocytes. However, recent evidence from experimental models has shown that CCA cells can also originate from hepatic progenitor cells or progenitor cells present in peribiliary glands, as well as from hepatocytes under transdifferentiation, although this has not yet been demonstrated to occur in humans.

Cholangiocarcinogenesis is a complex and heterogenous process that requires the combination of genomic, epigenetic, and molecular alterations.[1,2] Multiple signalling networks participating in biliary development during embryogenesis, including Notch, Wnt/β-catenin, Hedgehog, or Hippo/YAP, re-activate during chronic biliary inflammation and regeneration, and contributes to CCA development and progression. In addition, receptor tyrosine kinase signalling activation, including EGFR1, ERBB2, MET, RAS-MAPK, and PI3K pathways, is a common phenomenon among all CCA subtypes (Fig. 3).

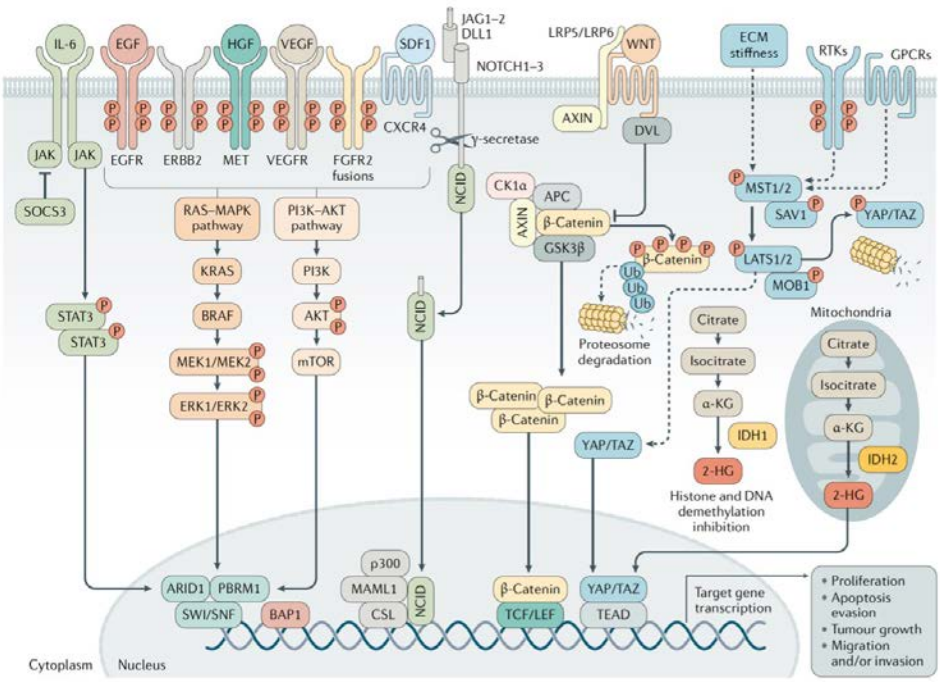

Fig. 3. Signalling pathways involved in cholangiocarcinoma development and progression.[1]

Given the high heterogeneity of CCAs, different molecular tumour classifications have been proposed.[1,2] Integrative transcriptomic analysis proposed the existence of two biologically distinct subclasses of iCCA tumours: the 'inflammatory' subtype (38%), primarily characterised by the activation of signalling pathways associated with the immune system, and the 'proliferative" subtype (62%), which presents enrichment of oncogenic pathways and *KRAS* mutations, and it was related to worse outcomes. Likewise, whole-genome expression data from eCCA tumours identified four distinct molecular classes: 'proliferative', 'metabolic', 'mesenchymal' and 'immune' tumours.[3] Of note, 18.7% of cases were grouped in the 'metabolic' subclass, characterised by gene expression profiles suggestive of activation of metabolic pathways such as fatty acid and bile acid metabolism. Increased infiltration of gamma-delta (γδ) T cells, known to be involved in the recognition of lipid antigens, was characteristic of this subclass. These tumours were shown to also display bivalent phenotypical traits of hepatocholangiocytes, owing to their positivity to the markers Hep Par-1 and CK19, respectively. In fact, one of the major regulators of a broad cluster of hepatocyte genes, *HNF4A*, was among the top increased genes in these tumours, suggesting again their biphenotypic traits. Although no clear association of the metabolic subclass was found with known risk factors of CCA, more in-depth studies should be conducted to understand if this subset of tumours most likely develop in patients with NAFLD/NASH, obesity, diabetes, and/or metabolic syndrome.

CCA development in NAFLD

The molecular mechanisms involved in the progression from NAFLD/NASH towards CCA remain mostly unknown. Nevertheless, the inflammatory milieu associated with obesity, insulin resistance, and NASH, together with changes in the hepatic and serum lipidome profiles of patients with NAFLD, as well as the increased circulating and hepatic bile acid levels of patients with NASH, could provide a favourable setting for tumour development. Indeed, CCAs often arise in the context of persistent biliary inflammation and cholestasis, which provide a rich environment of pro-inflammatory cytokines, growth factors, and toxic bile acids that can promote cholangiocarcinogenesis. The increased levels of saturated fatty acids (SFAs) characteristic of patients with NASH may result in biliary damage since palmitate and stearate were shown to rapidly trigger cholangiocyte lipoapoptosis *in vitro*.[4] At the molecular level, SFAs promote the phosphorylation and activation of p38-MAPK and ERK in cholangiocytes, leading to the accumulation of FoxO3 in the nucleus and consequent upregulation of the pro-apoptotic BH3-containing protein PUMA. This phenomenon might be linked to a reported cholestatic presentation of some patients with NAFLD, with bile duct injury, inflammation, swelling, and bile duct loss. Besides, bridging fibrosis and cirrhosis have been more commonly associated to biliary injury, which pinpoints for a potential involvement of bile duct injury and ductular reaction as a contributor to NAFLD or NASH severity. In addition, NAFLD was recently shown to exacerbate cholangitis in experimental *in vivo* models (E-cadherin gene [*CDH1*] knockout mice, $CDH1^{\Delta Liv}$) as well as favouring the development of CCA in $LSL\text{-}Kras^{G12D}/CDH1^{\Delta Liv}$ mice.[5] Although it still needs confirmation, these findings supports the hypothesis that biliary injury in patients with NASH may be a primer for CCA development, that would be sustained and aggravated by the NAFLD background in a vicious cycle of biliary injury and tumorigenesis. Apart from direct lipid-induced biliary injury, although still incompletely understood, the presence of gallstone disease may also be a predisposing factor for CCA in NAFLD. In fact, more severe stages of fatty liver were independently associated with higher risk of presenting bile duct stones, which are well-known factors of cholangiocarcinogenesis. Nevertheless, future studies should be conducted to ascertain the relationship between NAFLD and gallstone disease in the development of CCA.

Although yet to be proved, some metabolic players may be involved in the progression of NASH towards CCA. For instance, sphingolipids have been involved in NAFLD pathogenesis and progression, and also to concentrate in distinct CCA cell lines. Importantly, sphingosine-1-phosphate (S1P), a bioactive lipid mediator involved in lymphangiogenesis and lymph node metastasis in different types of cancer, was found elevated in biliary tract tumour samples (gallbladder cancer and CCA), when compared with normal biliary tract tissue. Additionally, the levels of sphingosine kinase 1 (SPHK1), which phosphorylates sphingosine to form S1P, were increased in biliary cancer tissues from patients with lymph node metastasis and positively correlated with the expression levels of the ABCC1 transporter, suggesting that the generated S1P is exported via ABCC1 and contributes to lymphatic metastasis in these tumours. In contrast, because patients with NAFLD display an increased ratio of hepatic ω-6/ω-3 polyunsaturated fatty acids (PUFAs), which is associated with a more pro-steatotic and pro-inflammatory sate, the consequent decreased levels of ω-3 PUFAs may potentially favour a more tumorigenic environment by promoting the activation of oncogenic c-Myc and allowing biliary carcinogenesis. In this line, in CCA cells *in vitro*, ω-3 PUFAs were shown to suppress c-Myc transcriptional activity, reducing the expression of miR-26a/b and promoting the expression of 15-hydroxyprostaglandin dehydrogenase (15-PGDH), thus inhibiting tumour cell growth *in vitro*. Similarly, as a result of gut dysbiosis, patients with NAFLD usually display decreased hepatic and circulating levels of short-chain fatty acids, such as butyrate. In fact, decreased hepatic butyrate levels in patients with NASH may result in cholangiocyte deciliation, thus favouring cholangiocarcinogenesis. In this regard, butyrate was recently proved to induce cilia formation in CCA cells *in vitro*, thus reducing cell proliferation. As histone deacetylase 6 (HDAC6) promotes deciliation in CCA cells, the combination of butyrate and HDAC6 inhibitors was shown to have additive anti-cancer effects in CCA. Of note, *HDAC6* was particularly overexpressed in the 'metabolic' subclass of CCA, which may indicate a role in this subset of tumours. Lastly, protein NEDDylation, which is a reversible ubiquitin-like post-translational modification upregulated in many diseases – including liver fibrosis, NAFLD, HCC, and CCA – may also be involved. Pharmacological inhibition of protein NEDDylation has been shown to decrease lipid accumulation by increasing fatty acid oxidation and to ameliorate steatosis through the DEPTOR-mTOR axis, reducing oxidative stress, lipid peroxidation and inflammation in mouse models of diet-induced NAFLD. Moreover, protein NEDDylation has been described as a relevant process in CCA initiation and progression and its inhibition markedly decreased CCA growth in distinct experimental models. Therefore, further studies specifically focused on the impact of protein NEDDylation in CCA development in the context of NAFLD should be carried out.

Lipid-rich environment in CCA progression

Not only NAFLD may promote CCA development, but also the hepatic microenvironment and lipid-rich milieu may also impact on CCA growth and progression. Metabolic reprogramming is an important hallmark of cancer. Indeed, tumour cells need large amounts of energy and biomaterials (including different nutritional sources, such as lipids, amino acids, or nucleotides) to maintain their uncontrolled growth.[6] To meet this demand, malignant cells acquire metabolic reprogramming in response to a wide variety of cell extrinsic and intrinsic signals and remodel their nutrient absorption, energy production, and biomolecule synthesis mechanisms. Lipid metabolism rewiring is a common metabolic modification in cancer, as it generates biomaterials, signalling molecules, and energy supplies to sustain tumour growth (Fig. 4). In the context of CCA, an enrichment of proteins involved in lipid and lipoprotein metabolism has been observed.

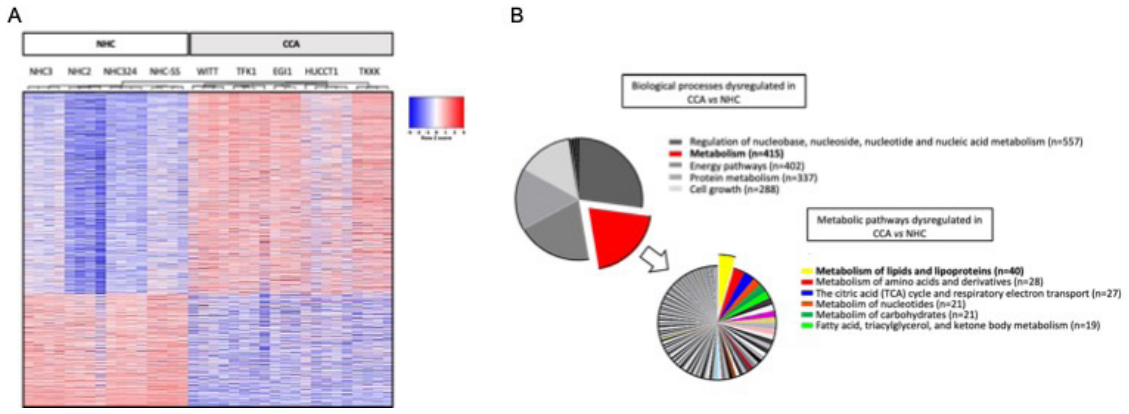

Fig. 4. Proteomic profile associated with lipid metabolism in CCA.
(A) Heatmap showing the differentially expressed proteins between CCA cells and NHCs *in vitro*. (B) Enrichment analysis of the biological processes linked to the differentially expressed proteins between NHC primary cultures and CCA cell lines. The number of proteins categorised in each process/pathway is displayed next to the name.[10] CCA, cholangiocarcinoma; NHCs, normal human cholangiocytes.

For instance, levels of the acyl-CoA synthetase long chain family member 5 (ACSL5), which participates in the activation of fatty acids (FAs) to acyl-CoA and levels of the FA-binding protein 5 (FABP5), relevant for the malignant progression of CCA and involved in FA uptake, intracellular transport, and metabolism were increased in CCA cells when compared with normal human cholangiocytes *in vitro*.[10] Moreover, human iCCAs have been reported to display high expression of FA uptake-related proteins and abundant long-chain FA uptake. Interestingly, CCA cells show greater uptake of free FAs and lipoproteins that favour energy storage in the form of triglycerides in comparison with normal cholangiocytes *in vitro*. Of note, the most proliferative CCA cell line presented greater uptake of FAs, VLDL, and HDL, elevated levels of cholesterol ester, and greater mitochondrial FA β-oxidation (FAO) as the main source of energy generation. In line with this, the pharmacological blockade of the FAO inhibited the tumorigenic capacity of different CCA cell lines *in vivo*, and this effect was more pronounced on the most proliferative CCA lines (Fig. 5). On the contrary, the less proliferative CCA cell lines show an increase in glucose uptake and glutamine consumption as the main sources of energy. This evidence suggests that a lipid-rich environment, such as the one observed in patients with NAFLD, sustain and promote the growth of CCA tumours, thus contributing to tumour progression. In this regard, four different NASH subtypes were proposed based on specific serum metabolomic profiles, which might have a role in the pathogenesis of NAFLD and/or in the progression to cancer.

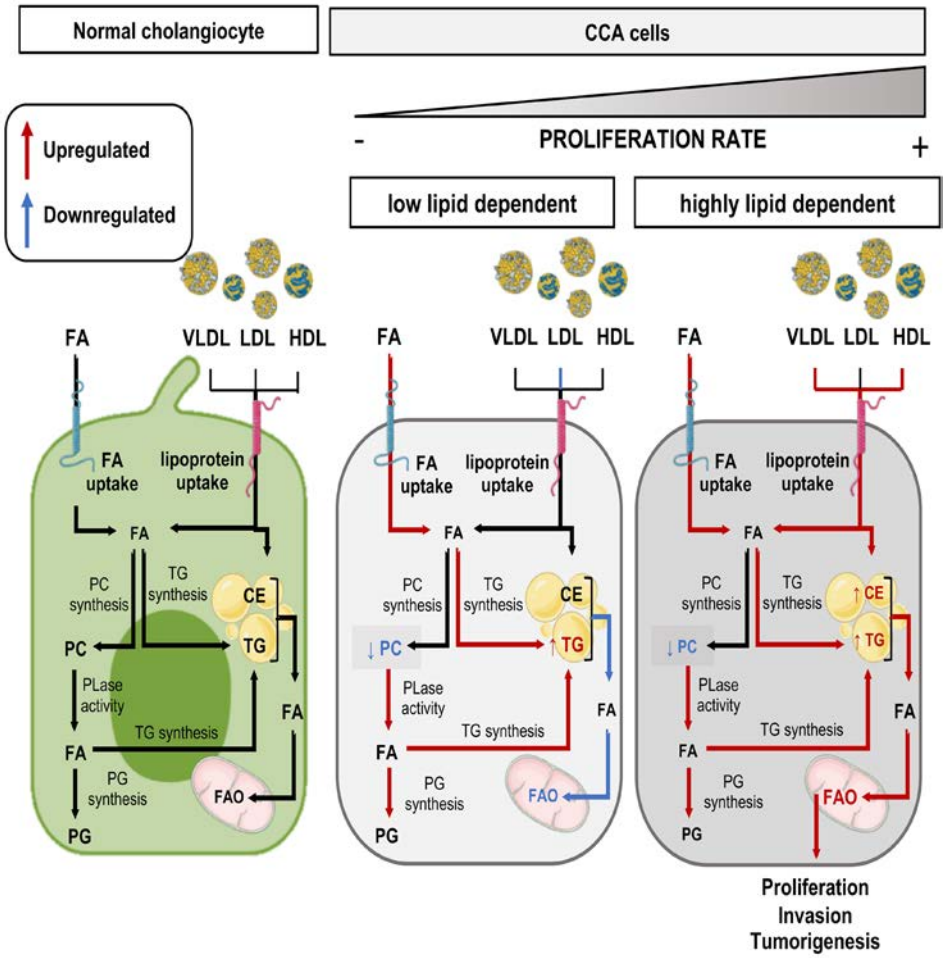

Fig. 5. Summary chart of the effect of a lipid environment in CCA.
Highly proliferative CCA cells are greatly lipid-dependent, showing increased uptake of extracellular FA and lipoproteins (VLDLs and HDLs) that results in the synthesis and storage of TGs and CEs. Moreover, increased catabolism of PC promotes the release of precursors for PG and for the synthesis of other lipids. The increased lipid availability sustains the FAO rate contributing to proliferation, tumorigenicity, and invasiveness.[10] CCA, cholangiocarcinoma; FA, fatty acid; FAO, fatty acid oxidation.

Future perspectives

The molecular pathogenesis of NAFLD-CCA remains misunderstood. Although some hints have been disclosed in previous years, there is much yet to discover. Considering the alarming NAFLD pandemic, along with its comorbidities such as obesity, type 2 diabetes, and metabolic syndrome, and knowing that all these conditions may increase the odds for cholangiocarcinogenesis, a worrying increase in the incidence of NAFLD-CCA in the next decades is anticipated. In fact, the incidence of CCA has been increasing during the past years and may exponentially rise because of the current NAFLD pandemic. Therefore, public awareness and education programmes are critical to address this growing problem. By increasing awareness, we may try to change lifestyles and use diet as a prevention and/or therapeutic strategy. In this regard, future studies should also determine the role of specific diets in the development, progression, and response to therapies in CCAs.

As the mechanisms governing biliary carcinogenesis in NAFLD are mostly unknown, developing models for the study of NAFLD-CCA progression is of utmost importance to understand disease progression. Extensive integrative studies on cellular, molecular, and metabolic processes in NAFLD-CCA are warranted. A better understanding of the molecular events leading to the transition from NAFLD to CCA will allow the discovery of new targets for preventive and therapeutic interventions. Moreover, since ω-3 PUFA, butyrate, and NEDDylation inhibitors (*i.e.* pevonedistat) were shown to ameliorate obesity and NASH, and also to reduce CCA cell proliferation in experimental models, their clinical study is required. In addition, the search of liquid biopsy biomarkers for the prediction and early diagnosis of CCA in patients in NAFLD, as well as to discriminate from patients with NAFLD-HCC, is urgently needed. We are currently conducting international collaborative studies to identify diagnostic biomarkers for patients with NAFLD-CCA and NAFLD-HCC using different multi-OMIC strategies. Combined efforts from multidisciplinary teams should be done to advance the prevention, diagnosis, and treatment of NAFLD-CCA, decipher the complexity of these tumours and determine novel key players that may improve the welfare and outcome in these patients.

References

(References in **BOLD** are required reading)

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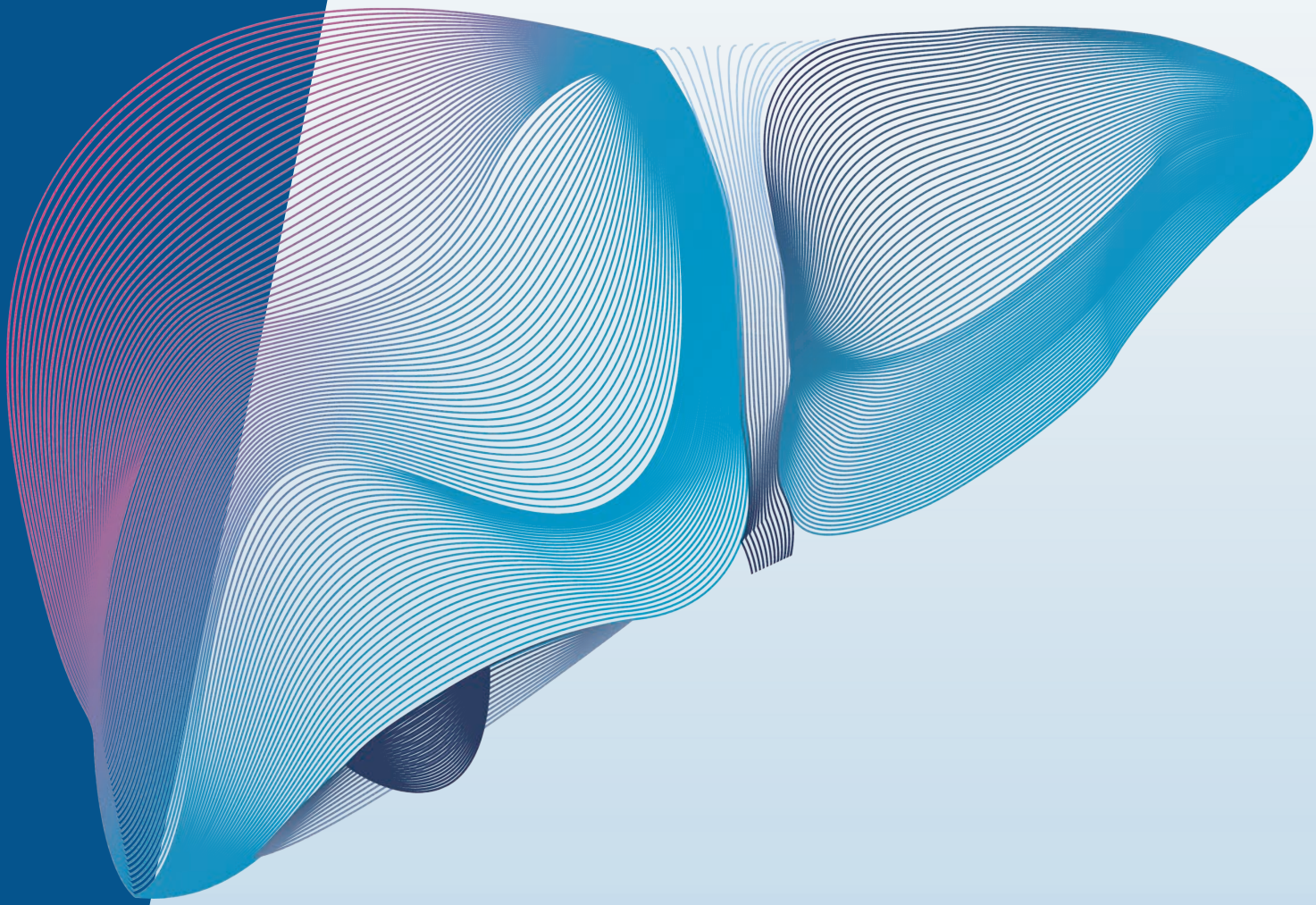
SESSION 2

NON - ALCOHOLIC FATTY LIVER DISEASE (NAFLD)

WEDNESDAY 21 JUNE
11:45-13:15



NAFLD – not always that clear cut

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Take-home messages

- NAFLD is a complex, dynamic, heterogeneous, and multifactorial disease with several oxymorons: Diet is cause and solution of NAFLD; PNPLA3 I148M promotes NAFLD progression and improvement by lifestyle intervention; HSD17B13 variant TA protects against NAFLD but was associated with outcomes in patients with advanced fibrosis.
- Stratification based on the pathophysiology of NAFLD from insulin resistance state, lipid metabolism including *de novo* lipogenesis, bile acids, ammonia metabolism and microbiome, and genetic variants should be translated into clinical practice together with a lead-in phase diet and exercise intervention.
- Each NAFLD patient is unique, personalised and precision medicine constitutes the most complete tool to allocate patients into lifestyle interventions or therapeutic management.

Introduction

Pathogenic mechanisms for the development and progression/regression of non-alcoholic fatty liver disease (NAFLD) are complex, play a dynamic role, and are highly heterogeneous. Insulin resistance as a prediabetic condition plays a major role in the development and progression of NAFLD. Insulin resistance is strongly related to metabolic derangement and could be the consequence of degradation of insulin receptor substrate 1 (IRS-1) that precludes any effect of insulin in the hepatocyte. The hyperinsulinaemic state is responsible for fibrosis progression. Lipid metabolism alteration mainly *de novo* lipogenesis (DNL) and lipid peroxidation, together with mitochondrial dysfunction, are involved in the appearance of steatosis hepatocytes and fibrosis progression linking metabolic disease with a raised risk of developing liver cancer. The gut microbiome, the gut–liver axis, and ammonia metabolism play a major role in the development and progression of NAFLD. Studying the microbiome is a difficult task with regard to detection, stratification, and intervention. Ammonia from glutaminolysis or an altered urea cycle could activate hepatic stellate cells and promote liver disease progression. Lastly, genes are the cause and solution of liver disease and progression. The PNPLA3 genotype GG is strongly related to more severe steatosis, inflammation, fibrosis, and risk of liver cancer, but in patients bearing this genotype implementing lifestyle changes should be more successful than in patients without this genotype. Moreover, a variant of HSD17B13 that protects against fat deposition at early stages seems to increase the risk for liver cancer and cirrhosis decompensation in patients with advanced chronic liver disease. Lastly, lifestyle intervention could promote disease regression when weight reduction and alanine aminotransferase (ALT) normalisation reach the threshold. Diet and exercise could be a lead-in phase to better stratify patients as responders or non-responders to lifestyle interventions.[1]

Case presentation

Herein, we describe a case of a 49-year-old female with no significant history of alcohol consumption who presented with persistent alteration of liver enzymes for a total of 12 months: ALT (62 IU/L) and aspartate aminotransferase (AST) (57 IU/L) since May 2021. Arterial pressure was 120/80 mmHg. Blood tests revealed no past infections with hepatitis C or B viruses and other causes of liver disease were ruled out. No significant metabolic comorbidities other than Grade 1 obesity (BMI 31.5 kg/m^2) and dyslipidaemia were observed. Concomitant medications included metformin 850 mg/day, simvastatin 20 mg/day, and omeprazole 20 mg/day. Ultrasound was performed to exclude hepatocellular carcinoma (HCC) signs and a hyperechogenic liver was described, suggesting liver steatosis of moderate grade moderate. The Enhanced Liver Fibrosis test (ELF®) was 10.4. Vibration-controlled transient elastography (VCTE) was 12 kPa, indicative of significant liver fibrosis, and the controlled-attenuation parameter (CAP) was 328 dB/m, suggesting intense steatosis. Magnetic resonance elastography (MRE) scored 3.32 kPa, DeMILI® 0.67, and the protein density fat fraction (PDFF) was 10.6% (Fig. 1).

Considering these preliminary results, ultrasound-guided liver biopsy under local anaesthesia was indicated and revealed non-alcoholic steatohepatitis (NASH) and significant liver fibrosis. After informed consent was signed, stool samples were obtained and extra fasting Vacutainer tubes were used to acquire serum, EDTA-plasma, and DNA for evaluation. The genetic risk score of NAFLD and metagenomics were assessed. The lipid profile and insulin resistance index were also determined.

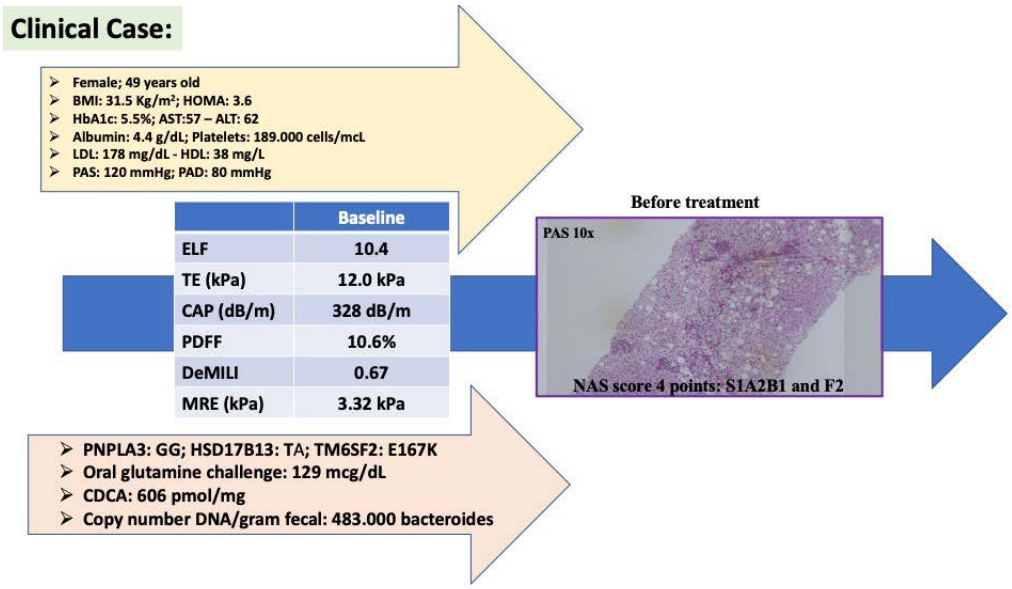

Fig 1. Schematic representation of a clinical case.
ALT, alanine aminotransferase, AST, aspartate aminotransferase; CAP, controlled-attenuation parameter; CDCA, chenodeoxycholic acid; ELF, Enhanced Liver Fibrosis; HOMA, homeostasis model assessment; MRE, magnetic resonance elastography; NAS, NAFLD Activity Score; PAD, arterial pressure (diastolic); PAS, arterial pressure (systolic), PDFF, protein density fat fraction; TE, transient elastography.

Mechanisms of progression

NAFLD has been strongly associated with loss of metabolic flexibility. Hepatic insulin resistance (HIR) enhances both gluconeogenesis and DNL, two key steps in the progression of NAFLD. Indeed, gluconeogenesis promotes uncontrolled fatty acid synthesis through fructose metabolism by DNL, closing a vicious circle. Moreover, glucagon resistance exacerbates gluconeogenesis and gluconeogenesis promotes glutaminolysis that together with a reduced urea synthesis yield in AMP-activated protein kinase (AMPK) inhibition and increased intracellular ammonia production that could activate GLS-2 and hepatic stellate cells. Glutaminolysis promotes DNL by oxidative metabolism and carboxylation of 2-oxoglutarate. In summary, excessive gluconeogenesis drives NAFLD initiation and progression.[2]

In patients gaining weight, adipose tissues and the liver can store triglycerides as a benign form of storage to avoid metabolic disturbances (Fig. 2). However, when adipose tissues show dysfunction, metabolic inflexibility emerges. Low-grade systemic inflammation as caused by aberrant adipokine secretion promotes insulin resistance. Indeed, increased circulating free fatty acid (FFA) caused by lipolysis in adipose tissues diverts FFA into the liver. Metabolically healthy obesity is resilient to NASH, type 2 diabetes mellitus, or dyslipidaemia by expanding healthy adipose tissues.[3] Lastly, factors such as cytokines, adipokines, hepatokines, and exosomes secreted from adipose tissues play a crucial role in developing NASH. Extracellular microvesicles open a new and fascinating field searching for the major compartment where mediators of disease progression and regression are located. In some patients, extracellular vesicles CD133+EpCAM+ have been involved in steatosis to steatohepatitis transition.[4] Early intervention preventing obesity-induced adipose tissue dysfunction could be a therapeutic approach for NAFLD.

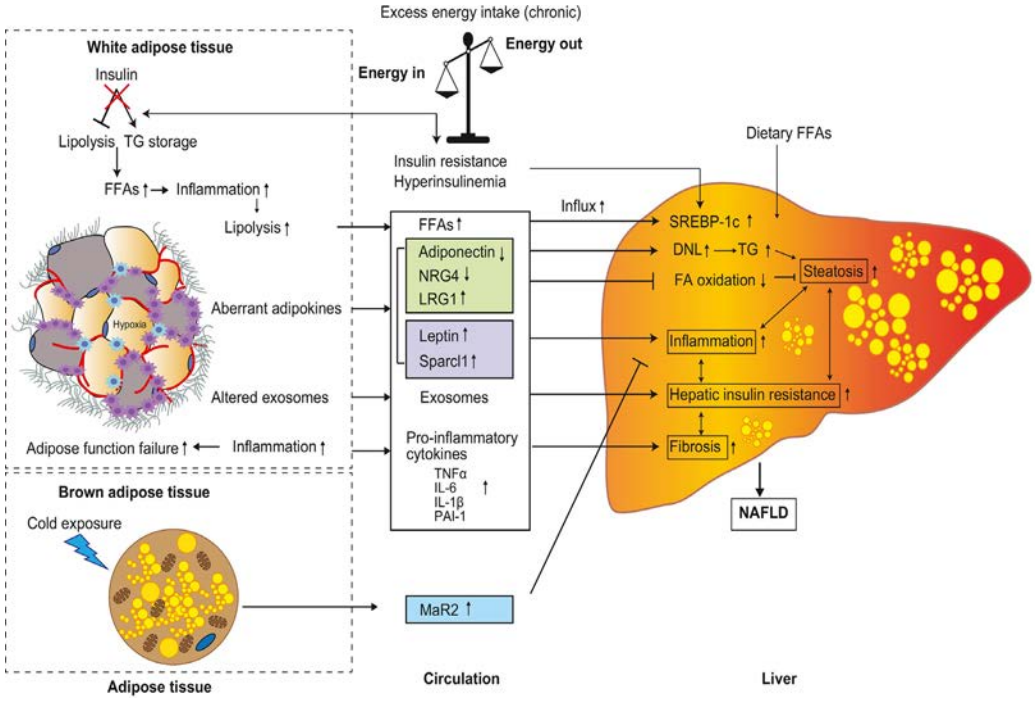

Fig 2.
The mechanism of triglyceride accumulation in the liver. DNL, *de novo* lipogenesis; FA, fatty acid; TG, triglycerides. Reproduced from Lee E, Korf H, Vidal-Puig A. An adipocentric perspective on the development and progression of non-alcoholic fatty liver disease. J Hepatol 2023, doi: 10.1016/j.hep.2023.01.024.

Gluconeogenesis, DNL, and glutaminolysis promoted NAFLD development and progression. Mitochondrial dysfunction modifies enzymatic expression, REDOX status, and ATP production. Oxidative stress starts with the hydroxide radical ($OH^{\bullet -}$) production from a superoxide anion ($O_2^{\bullet -}$) as final step in electron transference to the respiratory chain to produce hydrogen superoxide ($HOO^{\bullet}$) able to promote protein or DNA oxidation together with lipid peroxidation when glutathione levels are scarce. Oxidative stress is responsible for starting apoptosis, pyroptosis, necroptosis, or paraptosis. Moreover, it could be responsible for proteins, histones, and DNA methylation to modify immune response enhancing inflammasome by NLRP3 and increasing expression of Toll-like receptors and natural killer cells that are responsible for cytokine production such as transforming growth factor beta (TGF-β), hypoxia-inducible factor 1 (HIF-1) and vascular endothelial growth factor (VEGF) (Fig. 3).

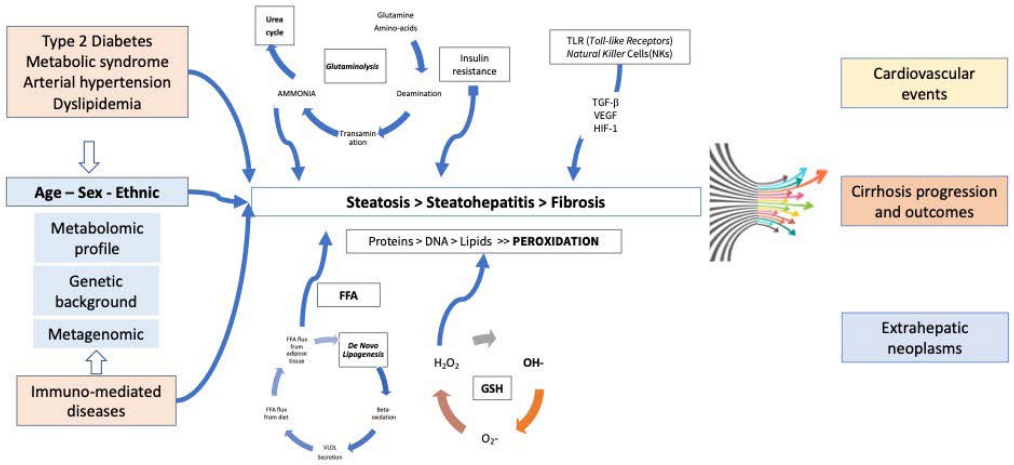

Fig 3.
Reproduced from Romero-Gómez M. Non-alcoholic steatohepatitis. Med Clin (Barc) 2022;159:388–95.

Genetics in NAFLD

Recent evidence has revealed associations between epidemiological factors (such as geographic location, race, and environment) underlying different genetic backgrounds with the prevalence of NAFLD. Several genetic variants have been identified that are associated with, and play a significant role in, the development of NAFLD. These mutations usually lead to structural, functional, or changes in expression in the protein they code for, influencing metabolic pathways involved in the metabolism of fats and sugars in the body, which are differentially activated leading to an accumulation of fat in the liver and, therefore, disease progression. Some of these genetic factors include variants of the *PNPLA3*, *TM6SF2*, *GCKR*, *MBOAT7*, and *HSD17B13* genes. Nevertheless, although genetics can play a role in the development and progression of NAFLD, lifestyle factors such as diet, exercise, and alcohol consumption also play a significant role. Therefore, individuals with a family history of NAFLD or other liver diseases should be particularly mindful of these lifestyle factors to help reduce their risk of developing NAFLD. However, the liver, being the most metabolically active and adaptable organ, can go through different metabolic stages throughout liver disease progression to maintain hepatic regeneration and homeostasis. In addition, the physiopathology of NAFLD is not exclusively related to the liver but to the features of metabolic syndrome, and the functional impact of these variants in peripheral tissues also accounts for potential risks, although they have not been properly

evaluated yet. However, the link between the clinical course of patients with cirrhosis and different circulating metabolites, which manifest different metabolic statuses as predisposing factors in end-stage liver disease, has been recently demonstrated. In this context, we have observed that the variant rs72613567:TA located in *HSD17B13*, which has been shown to protect from liver disease and reduce the risk for fibrosis and HCC, indeed negatively impacts the prognosis for patients with cirrhosis.[5] In agreement with previous studies, we observed a similar frequency of the variant in our cohort, and we confirmed the protection against cirrhosis compared to the general population, with a trend towards lower ALT levels. However, after adjusting for confounding factors, we found that patients harbouring the variant showed an increased risk of liver transplantation, hepatic decompensation, and mortality. This suggests that the loss of function of HSD17B13 may have a negative impact once cirrhosis is established. Currently, the natural substrate(s) and metabolite(s), biological roles, and underlying mechanisms of HSD17B13 in hepatic lipid metabolism remain incompletely understood. Similarly, previous studies from our lab associated the length of a microsatellite in the promoter region of the glutaminase (GLS) gene, which potentially affects the expression of the enzyme, with a greater risk of decompensation in patients with cirrhosis. In recent work, we showed increased hepatic expression and activity of GLS along with the progression of NAFLD. Curiously, this polymorphism (microsatellite length) was associated with a reduced risk of steatohepatitis, being more frequently present in patients with bland steatosis, an effect that presumably is opposed to that observed in patients with cirrhosis. The modulation of GLS-1 activity by hyperammonaemia and microsatellite methylation could explain, at least in part, this controversy. It is well known that the effect of a polymorphism may be modified by various physiological factors, such as age, sex, diet, exercise, hormonal status, and medication use, processes that can affect gene expression and function. Also, the metabolic needs of the organ may vary according to the stage of the disease, so different genotypes may have singular effects. Therefore, it is important to consider not only the physiological/metabolic status, but also the degree of the disease of an individual when evaluating the impact of a polymorphism, as it can affect the interpretation of genetic data and the design of personalised interventions. Lastly, patients bearing the PNPLA3 genotype GG showed an increased risk of progression of fibrosis to liver cirrhosis and HCC.[6] However, in patients with PNPLA3 GG, a lifestyle intervention would be more effective than in patients who are non-GG shifting the risk associated with the genetic variant.[7]

Gut–liver axis in NAFLD

A disrupted gut–liver axis contributes to NAFLD development and the progression of NASH through alterations in the gut microbiota, which may imply changes in microbial-derived metabolites, the appearance of translocation and endotoxaemia as a result of gut barrier damage, changes in hormones and bile acid signalling, and the production of proinflammatory cytokines. These changes lead to immune and metabolic disturbances inducing steatosis, inflammation, and fibrosis, key events in the progression of NAFLD. The metabolic inflammation observed in patients with NAFLD can be sterile: derived from non-infectious factors. Indeed, the gut microbiota contributes significantly to the pool of metabolites present in the human systemic circulation (up to 10%), featuring a systemic bioactive effect with both inflammatory and metabolic functions. Because of the anatomical characteristics of the gut–liver axis, the liver is continuously challenged by the metabolic stress induced by bacteria and their metabolites. In this sense, the gut microbiota are involved in the metabolism of dietary lipids and can convert them into toxic metabolites that contribute to the development of NASH. Nevertheless, the microbiota can also contribute to liver fat accumulation through indirect effects on the host, including appetite regulation, energy extraction from the diet, energy expenditure, and lipid handling through effects on insulin sensitivity. Other mechanisms that could be involved in the progression of the disease are the production of secondary bile acids from primary bile acids that are toxic to the liver

and can lead to inflammation and oxidative stress, or the alteration of gut hormone secretion, including incretin hormones such as glucagon-like peptide-1 (GLP-1), which play a role in regulating glucose and lipid metabolism. Altered gut hormone secretion can contribute to insulin resistance development, a hallmark of NASH.[8] Also, this disruption can be a consequence of gut dysbiosis and increased gut permeability which leads to increased delivery of bacterial products, including lipopolysaccharides (LPS), to the liver. LPS is a component of the outer membrane of Gram-negative bacteria and can trigger low-grade inflammation. In this sense, the gut microbiota can produce proinflammatory cytokines, such as tumour necrosis factor alpha (TNF-α) and interleukin-1 beta (IL-1β), that can contribute to the progression of NASH by inducing inflammation and oxidative stress in the liver. The complex relationship between the host, the microbiota, and the external environment limits the impact of every study and must be considered along with other limitations in the experimental design; unified research standards are needed. Promising therapeutics targeting the gut–liver axis for NAFLD are underway which, together with non-invasive predictive biomarkers obtained by current multi-omics approaches, will establish the basis of future precision medicine for NAFLD. Further research is needed to better understand the precise mechanisms by which the gut microbiota contributes to NASH progression and to determine the potential diagnostic and therapeutic implications of these findings.[9]

Lifestyle intervention

To complete the stratification process, patients diagnosed with NAFLD should undergo lifestyle interventions with a hypocaloric Mediterranean diet and aerobic physical activity for 3 h per week.[10] After an intervention of between 12 and 48 weeks patients could be classified as responders or not according to the NASH resolution calculator that includes ALT normalisation and percentage of body weight lost.[11] Indeed, Promrat *et al.* showed that a lifestyle intervention for 48 weeks promoted improvement in steatosis, inflammation and ballooning, but not in fibrosis stage. However, losing 10% of body weight could promote fibrosis regression. A Mediterranean diet designed by nutritional geometry is highly recommended (Fig. 4).[12]

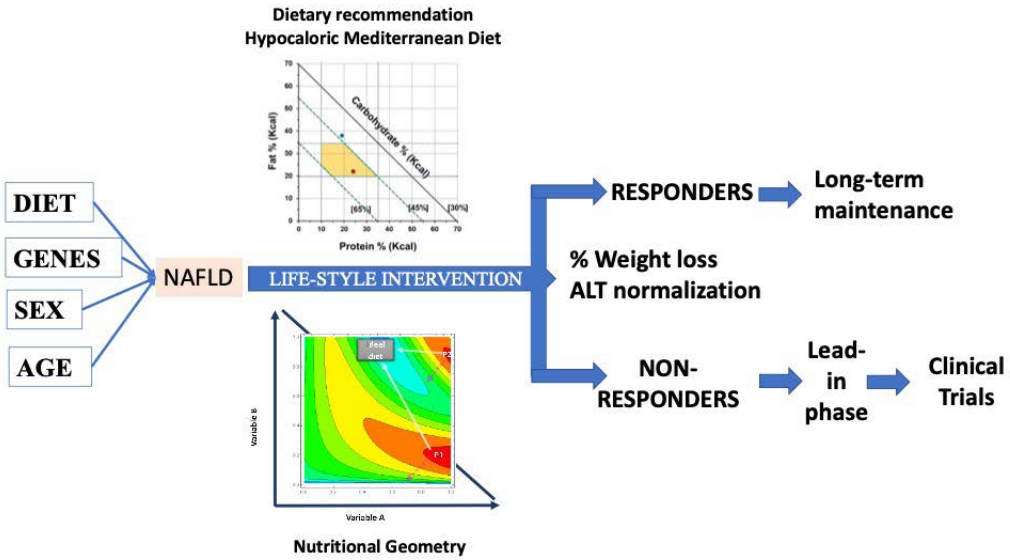

Fig 4.
Lifestyle intervention for non-alcoholic fatty liver disease. Reproduced from Romero-Gómez M, Aller R, Martín-Bermudo F. Dietary recommendations for the management of non-alcoholic fatty liver disease (NAFLD): a nutritional geometry perspective. Semin Liver Dis 2022;42:434–45.

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Epidemiology, natural history, and risk stratification of NAFLD and NASH

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Take-home messages

- NAFLD is the most common liver disease with a global prevalence of approximately 38%. This estimate is dependent on the background prevalence of the dominant risk factors: obesity and insulin resistance.
- Biopsy studies suggest 10–20% of patients with NAFLD might have NASH.
- Approximately 3–5% of patients with NAFLD might progress to cirrhosis.
- The stage of fibrosis is the key determinant of liver-related outcomes and overall mortality.
- Estimating fibrosis stage is therefore the main objective when assessing people with NAFLD.

As most societies gradually have adopted lifestyles with less physical activity and have more access to food, the body mass index (BMI) in most parts of the world has steadily increased over recent decades. For instance, the proportion of people with a BMI of ≥30 kg/m^2 in the United States has increased from around 11% in 1980 to 42% in 2020 (www.cdc.gov/obesity/data/adult.html). It is now well known that in an anabolic state with excess calories, the body stores most surplus energy in the adipose tissue. However, when the adipose tissue is overloaded, often in the insulin-resistant state, it will start to export free fatty acids to primarily the liver, where they are stored as triglycerides. *De novo* lipogenesis in the setting of high insulin and glucose levels in the blood, and increased amount of nutrients from the gut, also contributes to build up of liver fat, or non-alcoholic fatty liver disease (NAFLD). Given the close association with obesity and type 2 diabetes, NAFLD has become highly prevalent in most societies. Recent meta-analyses estimate that around 38% of the global population has NAFLD, with even higher estimates in countries with higher prevalence of obesity and type 2 diabetes, with these estimates being approximately 50% higher than at the turn of the millennium.[1,2] However, most data on NAFLD prevalence using imaging comes from Asian countries such as Korea and China, and there are few high-quality studies from representative cohorts in most other countries. Fig. 1 shows the estimated increase in NAFLD prevalence globally between 1990 and 2019. The prevalence of NAFLD is higher in settings with a higher prevalence of obesity and metabolic syndrome.

Some individuals with NAFLD will develop hepatic inflammation, termed non-alcoholic steatohepatitis (NASH). The reasons for why this occurs are still being uncovered, but a higher prevalence of NASH in people with more advanced obesity and more severe metabolic syndrome suggests a primarily environmental effect, although there are known genetic risk modifiers such as the G/G genotype of the *PNPLA3* gene.[3] The prevalence of NASH in NAFLD is difficult to estimate because it requires a liver biopsy, and most studies using liver biopsy suffer from some degree of selection bias. From the available data, it can be estimated that 10–20% of people with NAFLD might have NASH, and that in the global population, approximately 3–5% have NASH.[2] This is affected by the background prevalence of obesity and type 2 diabetes. In an study from Texas, USA, the authors found that in people referred for colonoscopy screening, 38% had NAFLD, and of the total cohort, 14% had NASH.[4] In this cohort however, 35% had diabetes, which is not generalisable to many other settings.

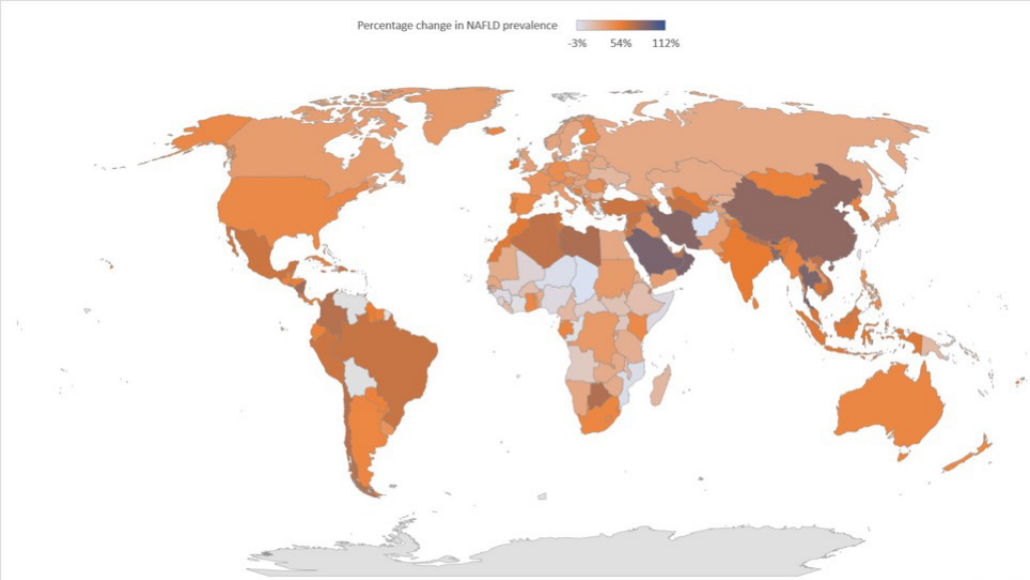

Fig. 1. Relative increase in NAFLD between 1990 and 2019.
Adapted from Wong *et al.* (J Hepatol, 2023, submitted).

As for most chronic liver diseases, the resulting inflammation eventually result in tissue scarring; fibrosis. The build-up of hepatic fibrosis takes time, and it has been estimated that it will take at least 20 years to develop cirrhosis in patients with NAFLD, and in most instances even longer.[5] As most persons gain weight later in life, the majority of persons with NAFLD will never develop cirrhosis, with some studies suggesting that around 3–5% might progress to cirrhosis, and of these many will die from non-hepatic causes.[6] Given the prolonged time needed to develop cirrhosis, there is some concern with many populations presenting a shift in *when* people develop obesity or type 2 diabetes. In many countries, BMI is now increasing also in younger age groups, and childhood and adolescent obesity prevalence estimates are becoming higher. According to the World Health Organization, more than 340 million persons aged 5–19 were obese in 2016 (www.who.int). As overweight and obesity early in life is associated with a higher risk of developing cirrhosis,[7] there is concern that these figures will eventually result in a considerable higher burden of NAFLD-related cirrhosis. Given that modern treatment for cardiovascular risk have improved life expectancy, there is also more time available for susceptible persons to develop cirrhosis. Indeed, cardiovascular disease is the main cause of death in patients with NAFLD without cirrhosis, whereas cirrhosis becomes the major cause of death after development of cirrhosis.[8]

The consequences of a higher prevalence and clinical significance of NAFLD is already manifest, best seen perhaps in the number and proportion of cases with advanced liver disease such as decompensated cirrhosis and hepatocellular carcinoma (HCC). Several studies have recently shown that NAFLD is becoming a more common cause for needing liver transplantation and admission to hospital care as a result of end-stage liver disease, across different countries. Further, HCC is another common reason for needing liver transplantation, and NAFLD is now among the top aetiologies of HCC requiring liver transplantation, although figures differ between countries.

Unfortunately, it is still difficult to predict which patients with NAFLD might develop cirrhosis or HCC. Current practices are based on identifying the subset of patients that have already started to develop pre-cirrhotic fibrosis. These patients are at a higher risk for further progression to cirrhosis, and to accurately determine the stage of hepatic fibrosis is therefore an important diagnostic procedure.

Advanced fibrosis is usually defined as stage 3–4 on liver biopsy, although also persons with fibrosis stage 2 have a higher rate of liver-related outcomes than those with stage 0–1. Several studies have consistently shown that the stage of fibrosis is the key determinant of poor outcomes in NAFLD (Fig. 2). A first-line test in primary care to exclude advanced fibrosis is recommended in most international guidelines (e.g. EASL[9]). Markers of advanced fibrosis reflect both the risk for prevalent fibrosis, and therefore also risk for incident liver-related outcomes.

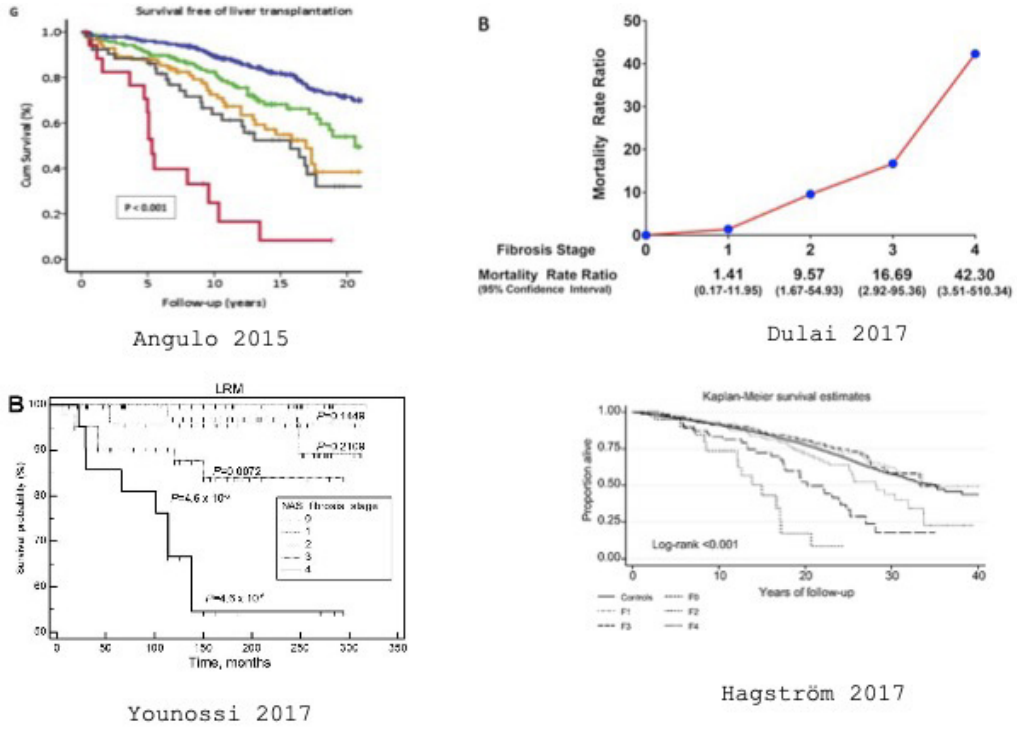

Fig. 2. Selected studies showing associations between fibrosis stage on liver biopsy and risk for overall mortality.

Usually, these scores, such as the commonly used Fibrosis-4 (FIB-4) score, have a high negative predictive value in the setting of a low prevalence population, therefore further investigation with more advanced and expensive methods can be preserved for those with an increased risk on the first test. However, the sensitivity of most scoring systems is poor, and repeated measurements within 3–5 years is therefore usually recommended, as this can identify a further subset of those individuals that might progress to cirrhosis.[10] There is currently no guidance on when repeated measurements should stop. Following first-line testing, patients with elevated risk for having or developing advanced fibrosis according to non-invasive scores should be further tested with methods with a higher specificity. Most guidelines currently recommend secondary testing with vibration-controlled transient elastography or an alternative, as locally available.[9] These approaches, although not perfect, can give some risk prediction estimates for individual patients.

Apart from fibrosis stage, the main risk factors for a more severe disease phenotype include older age, which could be a proxy for the duration of NAFLD; type 2 diabetes and other components in metabolic syndrome; genetic polymorphisms, in particular the G/G genotype of *PLPLA3*; and, perhaps controversially, alcohol consumption. Although alcohol consumption in individuals with NAFLD has previously been considered to already have been excluded as part of making the diagnosis, recent studies using alcohol biomarkers have shown that around 10–15% of patients with NAFLD in

these studies have a higher alcohol consumption than reported in a clinical interview. There is now considerable evidence from preclinical, epidemiological. and clinical studies that there is an interaction between NAFLD and alcohol on the risk for development of cirrhosis. Therefore, detailed clinical interview regarding alcohol consumption, ideally supported by validated questionnaires such as the AUDIT and at least once together with specific alcohol biomarkers such as phosphatidyl ethanol in blood, could be part of the evaluation and risk assessment.

Of note, patients with NAFLD have a higher rate of extrahepatic outcomes, such as cardiovascular disease and extrahepatic malignancies.[6] Active investigation of cardiovascular risk factors such as blood pressure measurement, smoking status history, blood lipids, and diabetes detection are therefore important in this population. Importantly, there seems to be a two-way association between NAFLD and other parameters of metabolic syndrome, for example patients with more advanced metabolic syndrome and more poorly controlled diabetes type 2 have a worse NAFLD phenotype with higher stages of fibrosis, and *vice versa*.

Epidemiological studies have consistently found associations between NAFLD and a higher risk of developing extrahepatic cancers compared with people without NAFLD. In particular, risk estimates have found to be higher for cancers in the colorectum and upper gastrointestinal tract, pancreas, breast, gynaecological system, and urinary system. These risk estimates are on a relatively modest scale, approximately 1.1–1.5 times higher than people without NAFLD. This implies that specific screening for non-hepatic cancers in NAFLD is currently not indicated, but can be a further motivation for patients affected by NAFLD to pursue and maintain lifestyle changes needed to resolve NAFLD and also other features of metabolic syndrome. However, there are few high-quality studies showing that informing patients with NAFLD about their disease state leads to meaningful and lasting lifestyle changes.

The risk for liver cancer, in particular HCC, is highly increased in individuals with NAFLD compared with the general population. A considerable difference in HCC risk compared with many other chronic liver diseases is that in NAFLD, a higher proportion of people develop HCC without pre-existing cirrhosis. Around one-third of patients with NAFLD and HCC do not have underlying cirrhosis, which can be compared with around 10% of non-cirrhotic HCC in other liver diseases. Patients with non-cirrhotic HCC in the setting of NAFLD are usually older and frequently have diabetes, again having considerable risk factors for poor outcomes in NAFLD. Current guidelines suggest surveillance for HCC in patients with cirrhosis and NAFLD, but there is controversy regarding surveillance of other potential risk groups, such as those with F3 fibrosis. In conclusion, the burden of NAFLD-HCC is increasing considerably.

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Role of lifestyle in prevention and treatment of NAFLD/NASH

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Take-home messages

- NAFLD affects one-third of the global population in every region of the world, but it continues to remain secondary on national and global health agendas.
- Obese children have a higher risk of liver-related mortality later in life, making the promotion of a healthy lifestyle essential.
- In the absence of approved drug therapy, healthy lifestyle modifications based on diet and physical activity are the mainstay in the management of NAFLD for both adults and children.
- NAFLD requires a multidisciplinary assessment in which modifying the patient's nutrition, based on a Mediterranean dietary pattern, and physical activity are a key component to improve efficacy and compliance with treatment.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is the most frequent chronic liver disease worldwide being independently associated with increased risk for type-2 diabetes mellitus (T2DM) and cardiovascular disease. NAFLD encompasses a spectrum of severity, ranging from non-alcoholic fatty liver (NAFL) to non-alcoholic steatohepatitis (NASH) and hepatic fibrosis, which is the leading cause of progression to cirrhosis and hepatocellular carcinoma. Patients with NAFLD are frequently obese and/or have diabetes. Obesity and type 2 diabetes may impact on the progression of NAFLD thus patient stratification is important to define and implement new therapeutic interventions that can improve liver disease.[1] Up to now, no pharmacological therapy has been approved, making lifestyle correction, in both adults and children, a mandatory approach in these patients.

Importance of disease awareness to improve compliance

The clinical burden of NAFLD is not limited to liver-related morbidity and mortality but includes a wide range of other prevalent non-communicable diseases that may increase its occurrence. It is well known that NAFLD is a strong predictor of metabolic syndrome, T2DM, cardiovascular disease, and certain extrahepatic cancers.

NAFLD prevalence has increased by +50%, from 25% between 1990 and 2006, to 38% between 2016 and 2019.[2] The highest NAFLD prevalence has been found in Latin America (44%), then in the Middle East and North Africa (36%), South Asia (33%), South East Asia (33%), North America (31%), East Asia (29%), Asia Pacific (28%), and Western Europe (25%).[2] Moreover, approximately one out of five people with NAFLD develop NASH.[3] Despite these rates and its close link with obesity and metabolic syndrome, NAFLD seems to remain in the background of global health agendas.

All the NAFLD-related complications can result in significant health, economic, and experiential burden on patients, their families, and society. In addition, there is no single global strategy for the treatment of NAFLD and NASH requiring an increase in healthcare resources dedicated to this disease. Likewise,

multidisciplinary teams that include procedures to support patient motivation and adherence are essential, as most patients with NAFLD do not perceive their condition as a disease.[4]

Role of prevention in childhood

NAFLD is an early onset disease, which has also become more prevalent in children and adolescents because of the increasing incidence of overweight and obesity in this population. Children who are obese have an increased risk of liver-related mortality later in life.[5] Therefore, the first step in NAFLD prevention is to counteract obesity by promoting a lifestyle based on physical activity and a healthier diet. In addition, it is important to monitor childhood obesity throughout life; in fact, current children's diets favour unhealthy dietary patterns that consist of an increased intake of ultra-processed foods rich in sugar at the expense of whole or fibre-rich foods such as fruit and vegetables. An important role is played by marketing, to which children are exposed on a daily basis, as it promotes the intake of energy-dense foods, rich in saturated fats, trans fats, added sugars and salt, which provide little, if any, nutritional contribution.[5]

Nowadays, there are very few randomised controlled trials studying the impact of lifestyle as a treatment for paediatric NAFLD, although there is evidence that the combination of diet and increased physical activity shows beneficial effects. In a systematic review and meta-analysis of 19 studies including 923 subjects (477 boys and 446 girls) aged 6–18 years with a predominantly ultrasound diagnosis of NAFLD, the intervention included aerobic exercise and diet.[6] Lifestyle changes did not have a significant impact on BMI (pooled RR = -0.82; 95% CI: -1.26 to -0.37) whereas they did on steatosis by reducing the risk by 61% (pooled RR = 0.39; 95% CI: 0.27–0.56). Thus, lifestyle changes lead to an improvement in NAFLD markers even in patients without significant weight reduction.

Special attention should be given to raising awareness of the preventable and treatable nature of many liver diseases, early diagnosis, and treatment in children. Prevention and public health promotion initiatives aimed at tackling unhealthy diets, physical inactivity, and alcohol consumption are very important to achieve beneficial outcomes. Similarly, investment in schools, to provide healthy school lunches and necessary physical activity, and in communities, to develop well-equipped and safe neighbourhoods with competent staff, will help in the prevention of these diseases.

Evidence of histological benefit

Histologically, NAFLD is typically classified into two categories: NAFL, also referred as simple steatosis, and NASH, where the presence of hepatic steatosis is accompanied by inflammation with hepatocyte injury and with or without hepatic fibrosis. In this sense, weight reduction has also shown benefits on attenuating both the histological activity and fibrosis. A study of 293 biopsy-proven patients with NAFLD enrolled in a tertiary medical centre in Havana, Cuba, showed that lifestyle modifications for 52 weeks led to a weight loss of more than 5% and 10% and to a consequent improvement of NASH by 58% and 90%, respectively. Specifically, in those who lost more than 10% of weight, 45% had regression of fibrosis.[7]

Indeed, in a meta-analysis of 43 studies including 2,809 participants, it was shown that 1 kg of weight lost was associated with a 0.03-point reduction in steatosis assessed by histology or ultrasound (95% CI: 0.02–0.04, p <0.0001, I^2 = 77%, n = 12. Each kilogram of weight lost was associated with a 0.83 unit reduction in alanine aminotransferase (ALT) (U/L) (95% CI: 0.53–1.14, p <0.0001, I^2 = 92%, n = 18), 0.56 unit reduction in aspartate aminotransferase (AST) (U/L) (95% CI: 0.32–0.79, p <0.0001, I^2 = 68%, n = 11) and 0.77 percentage points of steatosis assessed by radiology or histology

(95% CI: 0.51–1.03, p <0.0001, I^2 = 72%, n = 11). Furthermore, a dose–response relationship with liver inflammation, ballooning, and resolution of NAFLD or NASH emerged, with little evidence of a dose–response relationship with fibrosis and NAFLD activity score.[8] Resolution of NASH can also be achieved after massive weight loss following bariatric surgery. For instance, in a study of 109 morbidly obese patients, after 1 year of bariatric surgery, 85% of patients showed resolution of NASH according to the Brunt score. Specifically, there was a greater NASH resolution in those patients with mild NASH before surgery (94%) compared with those with severe NASH (70%).[4] Consequently, a lifestyle-based treatment approach aimed at significant weight loss appears to benefit these patients.

Impact of different diets

The usual diet of the patient with NAFLD follows a Western dietary pattern and has been often associated with the development of this disease independently of physical activity. This diet is high in saturated fat, trans fat, and high carbohydrate consumption, which have been shown to induce obesity, metabolic syndrome, NAFL, and potentially NASH. Thus, current management for NAFLD includes diet and lifestyle changes for achieving weight loss.[1] Caloric restriction inducing a negative energy balance has been associated with improvement of NAFLD and resolution of hepatic steatosis, regardless of the macronutrient composition of the diet. However, achievement of this weight loss could be difficult to obtain and maintain over the years. Currently, the Mediterranean diet (MedDiet) is the dietary pattern recommended for NAFLD patients by the recent EASL–EASD–EASO Clinical Practice Guidelines.[1] Specific compounds present in the MedDiet, such as polyphenols, fibre carotenoids, and omega-3 PUFAs have been proposed as responsible for the beneficial effects of this dietary pattern on liver health. A systematic review and meta-analysis involving 22 studies showed that weight-loss interventions were significantly associated with improvements in biomarkers, including ALT (-9.81 U/L; 95% CI, -13.12 to -6.50; I^2 = 97%), histologically or radiologically measured liver steatosis (standardised mean difference: -1.48; 95% CI, -2.27 to -0.70; I^2 = 94%), histologic NAFLD activity score (-0.92; 95% CI, -1.75 to -0.09; I^2 = 95%), and presence of NASH (OR, 0.14; 95% CI, 0.04–0.49; I^2 = 0%).[9]

However, alternative approaches for NAFLD management have been suggested. The low-fat diet and the low-carbohydrate diet are the most commonly compared diets. Depending on the percentage of carbohydrates intake, a low-carbohydrate diet could be classified on a ‘moderate-carb or reduced-carb diet’ (26–45% of carbohydrates intake to the total calorie intake per day) or a low-carb diet (<26%). Additionally, the ‘ketogenic diet’ or ‘very-low carb diet’ is characterised by a carbohydrate intake <10%.[10] Recent studies have also proposed time-restricted eating as an option to reduce hepatic steatosis and related metabolic disturbances.[1,10] However, long-term safety has not been tested and because of the small number of trials and the lack of liver biopsy, no convincing results have yet been reported to determine the superiority of any of these diets. As there is strong evidence of improvement NAFLD outcomes when patients adhere to a dietary pattern based on healthy eating patterns of minimally processed foods, low in sugar and saturated fat, the latest recommendations lead to the conclusion that the MedDiet should represent the basis on which other types of diets should be modelled.[10]

Interestingly, NAFLD can also develop in individuals with a BMI within the ethnic-specific cut-off of 25 kg/m^2 BMI in Caucasian and 23 kg/m^2 in Asian subjects, so-called ‘lean’ NAFLD. In these patients, current European guidelines state that follow-up is also mandatory because of possible disease progression. Regular physical activity should also definitely be indicated, as it can specifically reduce visceral fat.[11]

Dietary patterns are therefore associated with the risk of NAFLD, and this association could be modified by genetic background. In this context, *PNPLA3* is the most widely studied gene related to NAFLD which has been shown to interact with the environment. Hence, modification of dietary patterns in genetically predisposed individuals by the influence of gene–diet interactions, could modulate specific clinical outcomes, so personalised nutrition therapy should be speculated in the near future.

Impact of exercise *per se*

Exercise is a planned and structured physical activity, which, in general, becomes mandatory during the weight loss maintenance phase. Population-based studies have shown that exercise can promote a 20–30% reduction in hepatic fat content, by regulating triglyceride turnover and liver fat, with similar effects of different exercise modalities (aerobic, resistance, or high-intensity intermittent), even in low-volume, low-intensity aerobic exercise and without significant weight loss.[1] In fact, guidelines recommend over 150 min/week of moderate intensity physical activity over three to five sessions including a combination of aerobic and resistance exercises.[12] Exercise is therefore able to produce significant changes in liver fat, albeit modest compared with weight loss, which is able to reduce >80% liver fat.[1]

Many things are unknown in the field of exercise and NAFLD. In most studies, exercise was always accompanied by diet, so it was not clear whether exercise had an isolated effect. It should also be noted that all exercise trials remain small and have a high degree of variability. More recently, better controlled studies have been able to demonstrate that physical exercise has been shown to have little effect on hepatic insulin sensitivity, but to improve peripheral insulin sensitivity, reducing hepatic *de novo* lipogenesis. Direct effects on lipid flux have also been seen, increasing the clearance of very low-density lipoproteins which contributes to the reduction of hepatic fat with exercise. A meta-analysis of 16 RCTs on exercise found that exercise alone significantly reduced liver fat compared with the control group.[10] Indeed, although studies on patients with fibrosis are limited, in a 12-week intervention study with aerobic exercise, a one-stage reduction in fibrosis according to repeat liver biopsy was demonstrated in about 60% of patients.[10]

Importantly, many people with NAFLD find it difficult to increase physical activity/exercise levels as a treatment for their liver disease, so strategies to improve adherence to physical activity/exercise interventions are needed. In addition, further studies should take into account the genetic background of patients and its influence on the response to physical activity. In fact, *PNPLA3* appears to influence the response to lifestyle intervention, with patients with GG genotype responding better compared with CC or CG genotype. It is therefore important to have as many options as possible to respond to patients' preferences and needs, while also taking into account genetic and environmental factors.

Conclusions

The promotion and acquisition of healthy lifestyle habits from early childhood is essential as a preventive strategy for NAFLD. Any form of healthy diet, based on a Mediterranean dietary pattern, that leads to weight reduction has an established and confirmed effect on liver health. Exercise should be routinely recommended to patients with NAFLD to prevent and treat complications, improve liver-related outcomes and increase quality of life.

Patients with NAFLD require multidisciplinary intervention approaches to ensure that their treatment is as comprehensive and personalised as possible, reducing the severity, associated comorbidities and the impact on healthcare systems of this disease.

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Drug therapy for NASH: present and future prospects

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Take-home messages

- The prevalence of NASH is increasing, with close association to metabolic syndrome
- There are currently no approved medications for the treatment of NASH – the best evidence so far is for lifestyle modifications and weight reduction.
- Drugs approved to treat associated comorbidities may be considered in relevant clinical settings for patients with NASH.
- Non-invasive predictive biomarkers that reliably predict histological and clinical response are being developed.
- Targeted and personalised therapy is required with many new NASH therapies on the horizon.

Introduction

Non-alcoholic steatohepatitis (NASH) is one of the most prevalent causes of chronic liver disease worldwide. It is set to be the major cause of liver transplantation and liver cancer, and in the USA, NASH accounts for more than 25% of transplant cases, compared with 5% in 2002.[1] Notably, in the recent European Liver Transplant Registry study, a greater number of patients who underwent transplantation for NASH (39.1%) had hepatocellular carcinoma than patients without NASH (28.9%).[2]

Non-alcoholic liver disease (NAFLD) is strongly linked to metabolic syndrome, however the pathophysiology of NAFLD is not yet fully understood and is likely driven by a combination of lipotoxicity, insulin resistance, and activation of inflammatory and immune pathways, which are often linked to metabolic disorders. Various genetic polymorphisms have also been identified in NAFLD that affect the lipid pathway.[3] More recently, there is increasing evidence of a link between the gut microbiome and the development of insulin resistance in NASH, and thus far, eight species of gut microbes have been identified in patients with advanced fibrosis.[4]

Despite progress being made in understanding the aetiology and pathophysiology of NAFLD, there are currently no licensed treatments for NAFLD. Currently, physical activity and weight loss strategies are the main treatments in NASH, with aerobic and resistance training having been shown to reduce hepatic steatosis and NAFLD-associated cardiovascular risks (Fig. 1).[5] Current therapeutic options are focused on improving steatosis, inflammation, and fibrosis, with the ideal drug therapy for NAFLD also having effects on glucose metabolism, lipid regulation, insulin resistance, and obesity (Table 1).

Therapeutic strategies for NAFLD

Antioxidants

Vitamin E is an antioxidant that inhibits the production of reactive oxygen species (ROS), which contribute to liver injury and the development of steatohepatitis. In the PIVENS trial (Pioglitazone versus Vitamin E versus Placebo in non-diabetic NASH), vitamin E at a dose of 800 mg/day improved the NAFLD activity score (NAS) histologically (43% *vs.* 19%, $p = 0.001$), whereas pioglitazone did not (34% *vs.* 19%, $p = 0.04$). Both agents significantly improved hepatic steatosis, lobular inflammation, and hepatocellular ballooning, but neither had an effect on fibrosis in this trial.[6]

Moreover, in the TONIC trial, both metformin and vitamin E improved liver histology in terms of hepatocellular ballooning and NAS in children and adolescents with NASH, as compared with placebo. However, neither metformin nor vitamin E reduced the alanine aminotransferase (ALT) level nor ameliorated steatosis, inflammation, or fibrosis in patients with NASH.[7] A retrospective study evaluating the use of vitamin E in patients with biopsy-proven NASH and bridging fibrosis or cirrhosis, found that vitamin E was associated with improved clinical outcomes – higher adjusted transplant-free survival and lower rates of decompensation.[8]

However, various meta-analyses raised concerns regarding the use of vitamin E long term because of increased all-cause mortality, prostate cancer, and haemorrhagic stroke.[9] Nonetheless, vitamin E is one of the two treatments recommended for patients without diabetes and with biopsy-proven NASH in both European Association for the Study of the Liver (EASL) and American Association for the Study of Liver Diseases (AASLD) guidelines.

Farnesoid X receptor agonists

The farnesoid X receptor (FXR) is a ligand-activated transcription factor that is critical in maintaining hepatic homeostasis. Its activation reduces lipotoxicity, increases mitochondrial oxidation, and increases hepatic cholesterol excretion which results in improved insulin resistance, inflammation, and fibrosis. When activated by appropriate agonists, FXR reduces bile acid synthesis by inducing expression of small heterodimer partners in the liver which in turn reduces cholesterol 7-α-hydroxylase (CYP7A1) thereby impacting on bile acid synthesis. Additionally, in the gastrointestinal tract, FXR stimulates fibroblast growth factor which activates the mitogen-activated protein kinase (MAPK) pathway, reducing CYP7A1 expression and leading to further reductions in bile acid synthesis. FXR also plays a key role in triglyceride metabolism and lipogenesis. FXR agonists have been found to upregulate peroxisome proliferator-activated receptor-γ (PPAR-γ) expression and decrease activation markers in hepatic stellate cells *in vitro*, as well as reduce hepatic fibrosis in vivo.[10,11]

Obeticholic acid (OCA) is a selective FXR agonist that met its primary endpoint in the phase II FLINT trial and is currently being tested in a regulatory phase III trial (REGENERATE). At the 18-month interim analysis of 1,968 patients with NASH and F2–F3 fibrosis, OCA met the primary endpoint of improvement by at least one stage of fibrosis with no worsening on NASH-fibrosis improvement endpoint was achieved by 37 (12%) patients in the placebo group, 55 (18%) in the OCA 10 mg group, and 71 (23%) in the OCA 25 mg group, although it did not meet the endpoint on NASH resolution.[12] A re-review of liver histology by two pathologists also confirmed histological efficacy.[13] A correlation between the dose of the drug and pruritus was seen with OCA, which resulted in 19 (1.6%) patients discontinuing therapy as a result of grade 3 pruritis.[14]

A reduction in high-density lipoprotein cholesterol HDL-C levels, and an increase in low-density lipoprotein cholesterol LDL-C levels has also been described,[15] although a recent interim 18-month analysis of safety data from the REGENERATE trial was reassuring as regards cardiovascular events.[13]

Peroxisome proliferator-activated receptor agonists

Peroxisome proliferator activated receptors (PPARs) are ligand-activated transcription factors of nuclear hormone receptors and comprise three subtypes. PPAR-α activation reduces triglyceride levels, PPAR-β/δ improves fatty acid metabolism and PPAR-γ sensitises insulin, resulting in enhanced glucose metabolism.[16]

Pioglitazone, a PPAR-$\alpha\gamma$ agonist, was studied in patients with NASH and without diabetes in the PIVENS study. A group of 247 patients with NASH and without diabetes received pioglitazone 30 mg or vitamin E 800 IU daily, or placebo for 96 weeks, but there was no beneficial effect of pioglitazone found compared with the placebo as regards the histological primary endpoint (34% *vs.* 19%). Serum ALT and aspartate aminotransferase (AST) were reduced with vitamin E and pioglitazone when compared with placebo. Both agents were associated with reductions in hepatic steatosis ($p = 0.005$ for vitamin E and $p < 0.001$ for pioglitazone) and lobular inflammation ($p = 0.02$ for vitamin E and $p = 0.004$ for pioglitazone) but not with improvement in fibrosis scores ($p = 0.24$ for vitamin E and $p = 0.12$ for pioglitazone).[7]

In a randomised controlled trial with 101 patients with prediabetes or type 2 diabetes mellitus (T2DM) biopsy-proven NASH, patients were prescribed a hypocaloric diet (500 kcal/day deficit from weight-maintaining caloric intake) and then randomly assigned to pioglitazone *vs.* placebo in an 18-month open label trial. A total of 58% of patients achieved the primary outcome of at least a 2-point reduction in NAFLD without worsening of fibrosis compared with 17% in the placebo arm. Secondary outcomes were also met with improvement in histological NAS, reduced hepatic triglyceride content, and improved insulin sensitivity. Weight gain was greater in the pioglitazone group attributable to the known expansion in adipose tissue.[17] A recent meta-analysis suggested that pioglitazone can significantly improve liver histology, including steatosis, inflammation, ballooning, and fibrosis. Furthermore, pioglitazone has been shown to improve insulin sensitivity and significantly reduce fasting blood glucose, glycosylated haemoglobin, AST, and ALT.[18]

Pioglitazone increases the risk of adverse events such as fluid retention, weight gain, and osteoporosis, but in an updated meta-analysis, the much greater beneficial effects of pioglitazone on cardiovascular risk factors and NASH, outweighed the small prevalence of bladder cancer in patients with diabetes and exposed to pioglitazone (<0.3%).[19] To avoid some of these side effects, clinical trials with dual PPAR agonists are ongoing.

Elafibranor, a dual PPAR-α and PPAR-β/δ agonist, improved serum lipid profile, insulin resistance, and NASH without worsening fibrosis in the phase IIb GOLDEN trial.[20] In the elafibranor group 120 mg *vs.* placebo – NASH resolved without worsening fibrosis in 19% *vs.* 12% patients. However, in a phase III trial (RESOLVE-IT) the drug failed to meet either of its primary endpoints of resolution of NASH without worsening of fibrosis and secondary endpoint of fibrosis improvement[21] and was discontinued for this indication.

Lanifibranor is a pan-PPAR agonist that has successfully completed a 24-week phase IIb trial with 247 participants. It met its primary endpoint of reduction of 2 points or more on the SAF score (steatosis, activity, and fibrosis) with no increase in fibrosis – achieved by 49% of patients on 1,200 mg lanifibranor compared with 27% receiving placebo.

It also met its secondary endpoint of reducing fibrosis by at least one stage without worsening NASH.[22] Currently a phase III trial is underway (NATIV3). Lanifibranor was generally well tolerated with the most common adverse events being diarrhoea, fatigue, and nausea.

Saroglitazar, a dual PPAR-α and PPAR-γ agonist, improved diet-induced NASH by upregulating β-oxidation and fatty acid desaturation.[23] Saroglitazar has been shown to improve liver-related histology in preclinical NASH models by reduction in hepatic steatosis, inflammation, and ballooning, and prevented the development of fibrosis.[23] It was recently approved for treatment in India following the EVIDENCES II phase III trial, in which it met its primary and secondary endpoints with reductions in liver fat, liver enzymes, and disease activity.[24] There is a lack of phase III data for saroglitazar in the USA and Europe.

Fibroblast growth factor 21 agonist

Fibroblast growth factor 21 (FGF21) is an important regulator of metabolism that has been shown to improve metabolic status in preclinical models of obesity, NASH, and diabetes. Moreover, FGF21 has been shown to increase energy expenditure, reduce sugar intake, stimulate β-oxidation, increase production of adiponectin, and improve insulin resistance.[25]

Efruxifermin (EFX) is a fusion protein of human IgG_1 Fc domain linked to a modified human FGF21 (Fc-FGF21), which in patients with type 2 diabetes demonstrated improvement in lipoprotein profiles and glycaemic control.[26] In a phase IIb trial (HARMONY), the primary endpoints for per treatment analysis were met for both the 50 mg and 28 mg EFX dose groups, with 41% and 39% of EFX-treated patients, respectively, experiencing at least a one-stage improvement in liver fibrosis with no worsening of NASH by week 24, compared with 20% for the placebo arm. The study also met its secondary endpoint with 76% and 47% of patients treated with 50 mg and 28 mg, respectively, achieving NASH resolution without worsening of fibrosis, compared with 15% for the placebo group.[27]

Pegbelfermin, a pegylated-FGF21 analogue was studied in a phase IIb trial (FALCON 1) in patients with NASH and bridging fibrosis and in FALCON 2 in those with NASH and compensated cirrhosis. Neither studies demonstrated histological efficacy with pegbelfermin which may reflect tachyphylaxis and/or the time required to treat patients with advanced fibrosis/cirrhosis.

Glucagon-like peptide-1 agonists and gut hormone combinations

Glucagon-like peptide-1 (GLP-1) helps in regulating plasma glucose levels by stimulating glucose-dependent insulin secretion and inhibiting glucagon secretion.[28] GLP-1 also increases satiety and reduces gastric emptying time by activating GLP-1 receptors in the hypothalamus.

In a 72-week phase II trial with 320 participants, daily subcutaneous semaglutide resulted in resolution in NASH with no worsening of fibrosis for 56% patients on the 0.4 mg dose compared with 20% on placebo.[29] Although there were no significant differences in fibrosis improvement, there was less fibrosis progression in semaglutide-treated patients, in keeping with the data from the LEAN trial.[30] A phase III registration trial (ESSENCE) of weekly semaglutide is underway with oral agonists also in advanced development. The main reported adverse effects of semaglutide were nausea, constipation, and vomiting which led to 7% of patients discontinuing the drug.[29]

Tirzepatide, a dual agonist of glucose-dependent insulinotropic polypeptide and glucagon-like petite 1 receptor, showed significant reduction in NASH-related biomarkers and increased adiponectin in patients with T2DM.[31] The phase III trial (SURMOUNT-1) involved 2,539 adults in patients with a BMI >30 with no history of T2DM, or BMI of 27 or more and at least one weight-related complication, excluding diabetes – it showed that once weekly tirzepatide provided substantial and sustained

reductions in body weight.[32] The mean reduction in total body fat mass was 33.9% with tirzepatide, as compared with 8.2% with placebo. At week 72, most (95.3%) of the participants with prediabetes at baseline in the tirzepatide groups had reverted to normoglycaemia, as compared with 61.9% of participants in the placebo group.[33]

In the SURPASS-3 phase 3 trial in type 2 diabetes, tirzepatide showed a 30% liver-fat content reduction from baseline when compared to degludec, with its main adverse effects being mild and gastrointestinal in nature.[34] Dose-dependent decreases from baseline in ALT and AST were observed with tirzepatide, but these decreases were not greater than with placebo.[33] These data provide additional evidence on the metabolic effects of this novel dual glucose-dependent insulinotropic polypeptide GIP and GLP-1 receptor agonist and support further evaluation of tirzepatide in the NASH population.

In a phase IIb study with cotadutide, a dual receptor agonist with GLP-1 and glucagon activity, there was improved glycaemic control and weight loss in participants.[35] After 54 weeks of treatment, significantly more participants achieved target weight loss of ≥5% with cotadutide 100 µg (40%), 200 µg (30%), and 300 µg (47%) and liraglutide 1.8 mg (31%) *vs.* placebo (10%).[36] Improvements in lipid profiles, Fibrosis-4 score and NAFLD fibrosis score were also noted. Cotadutide was associated with a higher incidence of gastrointestinal disorders across all treatment doses when compared with liraglutide or placebo.[36]

Sodium/glucose transport protein 2 inhibitors

Sodium/glucose transport protein 2 (SGLT2) inhibitors increase urinary glucose excretion, which induces weight loss and reduction in body fat. Dapagliflozin reduced hepatic fat content (–3.7%) albeit without any major effects on tissue-specific insulin-sensitivity,[37] whereas empagliflozin reduced hepatic fat content in patients with type 2 diabetes alongside excellent glycaemic control.[36] In the EMPA-REG OUTCOME trial, the adjusted mean difference in ALT change was -3.15 U/L with empagliflozin *vs.* placebo, and -4.88 U/L in empagliflozin *vs.* glimepiride in patients with type 2 diabetes. Similar reductions were seen in AST levels, but at a lower rate.[38]

Thyroid hormone receptor β agonist

Activation of hepatic thyroid hormone receptor β (THR-β) is associated with systemic lipid lowering, increased bile acid synthesis, and fat oxidation.[39] Because of the specificity for the THR-β receptor, such agents avoid the adverse effects seen with THR-α agonism such as atrial arrhythmias and osteoporosis. In patients with NASH, treatment with the THR-β agonist resmetirom in a 36-week phase II trial with 125 participants showed a reduction in liver fat by 30% compared with baseline at 12 weeks.[40] Preliminary results for a phase III trial with resmetirom (MAESTRO-NASH) demonstrate that it met its primary histological endpoints of NASH resolution with ≥2-point reduction in NAS score and no worsening of fibrosis (24% 80 mg resmetirom *vs.* 28% 100 mg resmetirom *vs.* 8% placebo). This was also significant for fibrosis – one or more stage improvement in fibrosis with no worsening of NAS (24% 80 mg resmetirom *vs.* 26% 100 mg resmetirom *vs.* 12% placebo).[41] There were also positive results for imaging measurements of liver fat, liver stiffness, and a reduction in LDL-cholesterol and apolipoprotein B (ApoB).

Combination therapy / future direction

Given the heterogeneity of NASH, combination therapy is likely to be of greater effectiveness in the treatment of patients with NASH rather than monotherapy alone. The histological resolution of NASH in monotherapy trials currently does not exceed 32% of the signal with placebo across a range of drugs

with different mechanisms of action[42], which highlights the complex pathophysiology of NASH and the various pathways involved. Combination therapies are likely to require multiple mechanistic pathways – antimetabolic, anti-inflammatory and antifibrotic pathways – to achieve an optimal histological response. Indeed, an increasing number of agents are being investigated in combination for NASH, for example GLP-1 RAs and SGLT2 inhibitors, which may help improve both liver-related outcomes and diabetes-related outcomes. Current combinations being studied are summarised in Table 2.

Non-invasive predictive biomarkers that reliably predict histological and clinical response need to be developed and qualified to allow for more continuous monitoring of patients with NASH that are receiving therapies.[43,44] Current phase III trials are listed in Table 3. Combination therapy potentially allows for targeted and personalised therapy and could have potential effects beyond the liver such as weight loss, cardiovascular optimisation, insulin sensitisation. It may also reduce dose-dependent side effects by allowing lower doses to be used, to improve tolerability without compromising on efficacy. The side effect and safety profiles need to be better than monotherapy.

The extensive activity in the field for both therapeutics and biomarkers bodes well for patients and it is likely that licensed therapies will be in use within the next 1–2 years.

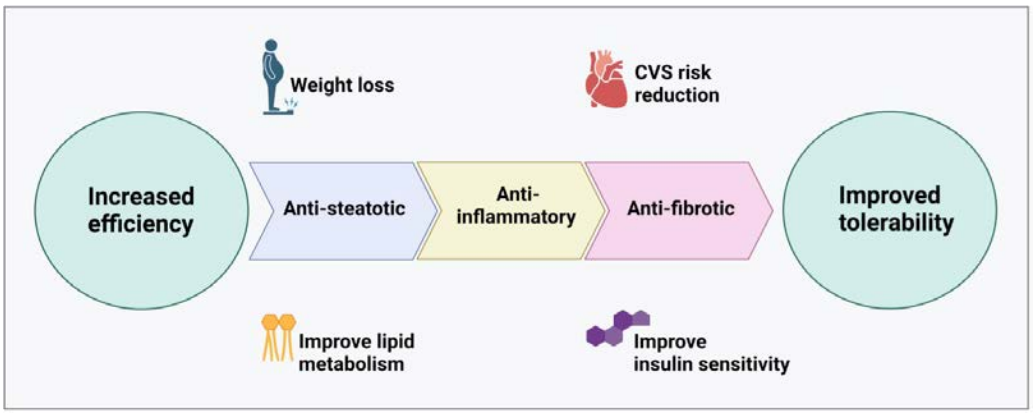

Fig. 1. Therapeutic strategies in non-alcoholic steatohepatitis.

Table 1. Therapeutic strategies in non-alcoholic fatty liver disease.

Pathway	Mechanism of action	Drug
Metabolism	Farnesoid X receptor (FXR) agonists	Obeticholic acid Topifexor Cilofexor
	Peroxisome proliferator-activated receptor (PPARs) agonists	Pioglitazone Lanifibranor Elafibranor
	Fibroblast growth factor 21 (FGF21) agonist	Aldafermin
	Glucagon-like peptide-1 (GLP-1) agonists	Liraglutide Semaglutide
	Sodium/glucose transport protein 2 (SGLT2) inhibitors	Dapagliflozin Empagliflozin
	Thyroid hormone receptor β (TRβ) agonist	Resmetirom
Inflammation	CCL receptor type 2 (CCR 2) and type 5 (CCR5)	Cenicriviroc
Cell death	Antioxidant	Vitamin E
Gut–liver axis	Probiotics Symbiotics	VSL3 Multi-strain probiotic (14 bacteria including: *Bifidobacterium, Lactobacillus, Lactococcus*) Synbiotic (7 strains including: *Lactobacillus casei, L. bulgaricus, L. rhamnosus, L. acidophilus, Bifidobacterium breve, B. longum, Streptococcus thermophiles*)

Table 2. Current combination trials in non-alcoholic steatohepatitis.

Name	Trial phase	First drug	Second drug	Population	Primary endpoints	Secondary endpoints
CONTROL NCT02633956	Phase II	Obeticholic acid	Atorvastatin	NASH F1–F3 F4 no decompensation	LDL cholesterol	Safety, tolerability, lipoproteins
TANDEM NCT03517540	Phase II	Tropifexor	Cenicriviroc	NASH F2/F3	Safety profile	Improvement in fibrosis Resolution of NASH
ELIVATE NCT04065841	Phase II	Tropifexor	Licogliflozin	NASH F2/F3	Resolution of NASH and no worsening of fibrosis or improvement of fibrosis by at least one stage without worsening of NASH	Improvement of fibrosis by two stages Reduction in body weight Change in liver fat content on MRI-PDFF Improvement in liver tests
ATLAS NCT03449446	Phase II	Cilofexor	Firsocostat Selonsertib	NASH F3/F4	Adverse events One stage improvement in fibrosis without worsening of NASH	
NCT03987074	Phase II	Cilofexor	Semaglutide Firsocostat	NASH F2/F3	Safety profile	
NCT03776175	Phase IIa	PF-05221304 ACC inhibitor	PF-06865571, DGAT2 inhibitor	NAFLD	Steatosis	Safety and tolerability

Table 3. Current phase III trials in non-alcoholic steatohepatitis.

Trial name	Medication	Mechanism of action	Participants	Duration	Inclusion criteria
REGENERATE NCT02548351	Obeticholic acid	FXR agonist	2,480	18 months	NASH with fibrosis
REVERSE NCT03439254	Obeticholic acid	FXR agonist	919	12 months	NASH with compensated cirrhosis
MAESTRO-NASH NCT03900429	Resmetirom	THR-β agonist	2,000	52 weeks	F2/F3 NASH
MAESTRO-NAFLD1 NCT04197479	Resmetirom	THR-β agonist	700	52 weeks	NAFLD/NASH
NATiV3 NCT04849728	Lanifibranor	Pan-PPAR agonist	2,000	72 weeks	F2/F3 NASH
ARMOR NCT04104321	Aramchol	SCD1 inhibitor	2,000	72 weeks	F2/F3 NASH
ESSENCE NCT04822181	Semaglutide	GLP1 agonist	1,200	72 weeks	F2/F3 NASH
NAVIGATE NCT04365868	Belpectin	Galectin 3 inhibitor	1,010	78 weeks	NASH cirrhosis
DEAN NCT03723252	Dapagliflozin	SGLT2 inhibitor	100	52 weeks	NASH with stable glycaemic control

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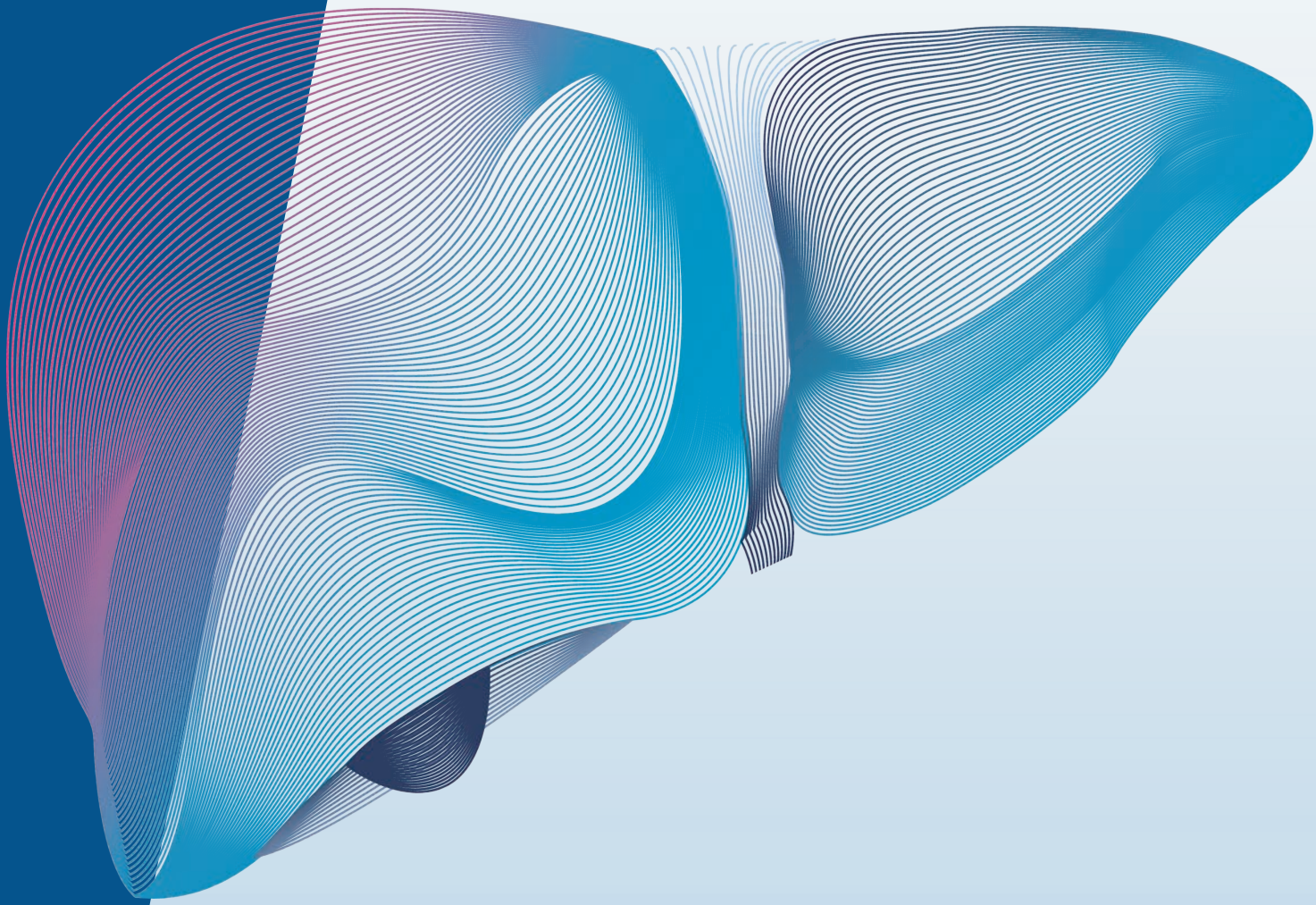
SESSION 3

SURVEILLANCE AND DIAGNOSIS OF HCC IN NAFLD PATIENTS

WEDNESDAY 21 JUNE
14:45-16:00



Epidemiology, surveillance, and early detection of hepatocellular carcinoma in non-alcoholic fatty liver disease

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Take-home messages

- NAFLD is the fastest growing cause of HCC in Western countries.
- Although HCC can develop in patients with non-cirrhotic NAFLD, the magnitude of the risk is still unclear but likely very low. Thus, HCC surveillance is not cost-effective in patients with NAFLD without advanced fibrosis.
- Patients with NAFLD-cirrhosis should be enrolled in biannual surveillance with abdominal ultrasound and AFP.
- Detection performance of ultrasound in NAFLD is frequently suboptimal. These patients should undergo MRI as an alternative strategy for HCC surveillance.
- Evaluation of minimally-invasive biomarkers for early HCC detection (*e.g.* liquid biopsy) or alternative imaging technologies (AMRI) are promising, but still investigational.

Epidemiology of HCC in NAFLD

There is increasing clinical concern on the rise of non-alcoholic fatty liver disease (NAFLD) and its more advanced stage, non-alcoholic steatohepatitis (NASH). In the United States, NAFLD cases are projected to expand from 83.1 million in 2015 (~25% of the population) to 100.9 million in 2030, with an increased proportion with NASH, rising from 20% to 27% of adults with NAFLD during this interval.[1] The predicted increase of patients with NASH who will develop end-stage liver disease will impose a significant economic burden and lengthen the liver transplant waiting lists. Globally, the prevalence of NAFLD is ~25%. Another major consequence of the NASH epidemic is the dramatic increase in hepatocellular carcinoma (HCC). NASH is the fastest rising cause of HCC in the United States.[2] Unlike other aetiologies of HCC such as HCV infection or alcohol use disorder, a significant fraction of HCC arises in patients without cirrhosis. This poses a significant clinical challenge as these patients are not enrolled in surveillance programmes and when diagnosed with HCC, are generally less amenable to curative therapies.[3]

There are notable clinical differences between HCC in NASH when compared with other aetiologies. Patients with NAFLD-HCC tend to be older, have higher BMI, more metabolic risk factors but less prevalence of advanced liver disease.[4] When compared with HCV-HCC, a larger proportion of patients with NAFLD-HCC are diagnosed outside surveillance programmes, which leads to diagnosis at more advanced clinical stages. For instance, a prospective analysis of 756 newly diagnosed patients with HCC found that 54% of patients with HCV-HCC were diagnosed at Barcelona Clinic for Liver Cancer (BCLC) stages 0/A as opposed to 42% in those with NAFLD-HCC. A similar study including 1,419 patients with HCC reported rates of 60% surveillance in HCC attributable to alcohol use disorder compared with only 43% in those with NAFLD-HCC. Given this, it is very difficult to estimate the actual incidence of HCC in NAFLD. The largest study to assess this included 296,707 patients with

NAFLD and 296,707 matched controls.[5] The incidence of HCC was significantly higher among patients with NAFLD *vs.* controls (hazard ratio 7.62). Among patients with NAFLD, those with cirrhosis had the highest annual incidence of HCC. The overall annual HCC risk was 1.06% but it ranged from 0.2% to 2.4%. In patients with NAFLD cirrhosis, HCC risk ranged from 1.6 to 23.7 per 1,000 person-years. With this heterogeneity, it is difficult to provide a unique recommendation in terms of need for HCC surveillance among patients with NAFLD.

Overview of surveillance in HCC: target population and surveillance tools

In 2030, more than 1 million people will die from liver cancer worldwide.[6] Most HCC patients are diagnosed at advanced stages, and they live less than 2 years on average. This contrasts with those diagnosed at an early stage, who can be cured with surgery. Therefore, it is crucial to increase early detection rates of HCC among patients at-risk (mostly those with cirrhosis). Survival in patients enrolled in HCC surveillance doubles that of those not enrolled in surveillance.[7] Clinical practice guidelines agree that patients with cirrhosis, regardless of aetiology, should be enrolled in HCC surveillance programmes. This applies for patients that would otherwise be eligible for potentially curative therapies, based on liver function and comorbidities. However, given that HCC can arise in patients with NAFLD without cirrhosis, there is some debate on whether these patients should also be enrolled in surveillance programmes. The main question is if the risk of HCC in patients without cirrhosis is high enough to render HCC surveillance cost-effective. Importantly, not all patients with NAFLD are the same, and it is well established that the degree of liver fibrosis is the key factor to determine poor outcomes including liver-related deaths and HCC. This was specifically evaluated in a large prospective study including 1,773 patients with NAFLD followed for 4 years, for whom fibrosis stage was available via baseline liver biopsy.[8] All-cause mortality and incidence of liver-related complications (*e.g.* ascites, encephalopathy, bleeding, and HCC) increased with increasing fibrosis stages. The incidence of cardiac events and extrahepatic cancers were similar across fibrosis stages. In terms of HCC development, the risk was significantly higher in patients with advanced fibrosis (defined as stages F3 or F4) compared with those without significant fibrosis (stages F0 to F2). Patients with F4 and F3 fibrosis had hazard ratios of 4.2 and 9.1 for the development of HCC compared with patients with F0–2 fibrosis. Thus, patients with advanced fibrosis but not yet cirrhosis (*e.g.* F3), are still at a significantly higher risk than those with F0–2 fibrosis. Although fibrosis scoring based on histology is the gold standard, it is not commonly used in clinical practice. Accurate estimation of fibrosis, particularly for F2–3 stages, using non-invasive methods is quite challenging. Thus, the decision on whether patients with NAFLD F3 should be enrolled in HCC surveillance is controversial. The American Gastroenterology Association guidelines on HCC surveillance recommend considering surveillance in patients with NAFLD and two non-invasive tests showing evidence of advanced fibrosis, specifically a Fibrosis-4 score higher than 2.67 and a vibration-controlled transient elastography value higher than 12.[9]

There are significant disparities in the prevalence and severity of NAFLD, with the highest burden in Hispanics. Unequal distribution of known single-nucleotide polymorphisms (SNPs) associated with NAFLD (*e.g. PNPLA322, TM6SF223, MBOAT23*) could contribute to these differences. Furthermore, specific variants in PNPLA3 (rs58542926) and TM6SF2 (rs58542926), two genes involved in lipid metabolism, are associated with HCC. Although there are not much data on the racial distribution of these SNPs, a new variant of HSD17B13 associated with chronic liver disease shows different distribution across race/ethnicity.[10] These data suggest race-specific differences of genetic traits associated with risk of chronic liver diseases and potentially HCC.

The recommended surveillance tools (abdominal ultrasound and serum alpha-foetoprotein [AFP]) have a modest sensitivity of 63% to detect early-stage HCC.[11,12] This fact, added to the low implementation

rate (25%) of HCC surveillance,[13] underscores the need for new strategies to detect curable HCC (Fig. 1). Ultrasound has known limitations, mainly being operator-dependent and showing significant variability across centres. In patients with NAFLD, there is an added problem with ultrasound, related to suboptimal sonic window in patients with obesity, which decreases its performance to detect early-stage HCC. Poor exam quality has been reported in up to 20% of ultrasounds, mainly in patients with high BMI and NAFLD. For patients with poor visualisation, alternative techniques include cross-sectional imaging such as CT scanning and MRI, but given radiation exposure with CT scan, MRI is safer for repeated testing. There is modelling evidence that MRI could be cost-effective for HCC surveillance in patients at high risk of HCC development.[14]

There are other imaging modalities currently under investigation for HCC. One of the most promising is abbreviated MRI (AMRI), which follows a simplified protocol for image capture that decreases exam time and cost. Preliminary data on AMRI shows improved performance when compared with ultrasound and it may solve the problem of low-quality readings of ultrasound in patients with NAFLD and elevated BMI. The role of AFP in HCC surveillance has been controversial. European guidelines do not recommend the use of AFP for surveillance because of its poor sensitivity for the detection of early-stage HCC.[15] The American guidelines still endorse the use of AFP as it increases the detection performance of ultrasound as per a large meta-analysis.[12] Most false positives for AFP were in patients with active HCV infection, where AFP fluctuations were common. In fact, most of the performance data of AFP came from the pre-DAA era. One could argue that the performance of AFP could be better in patients with NAFLD than with viral-related HCC, but additional data are still needed.

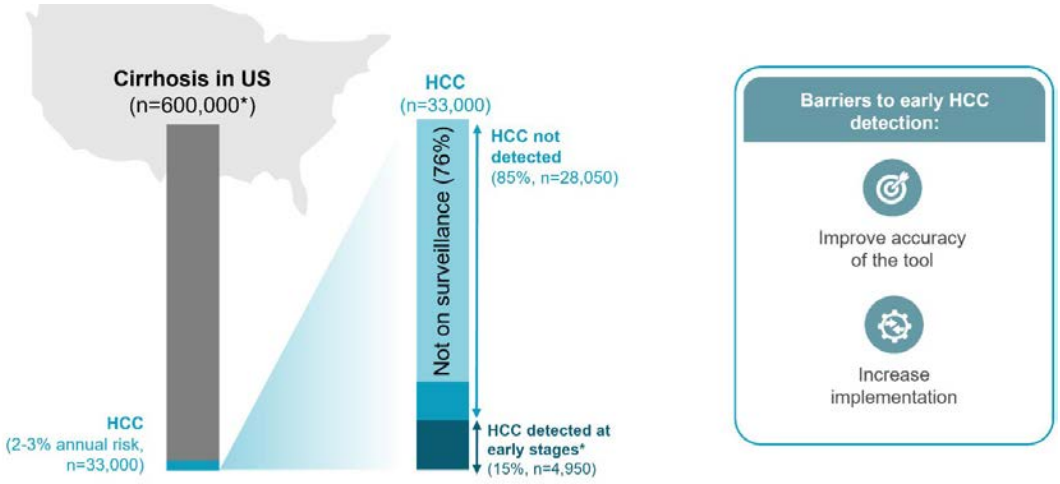

Fig. 1. Limitations of HCC surveillance in the United States.
With a low implementation rate (<25%) and considering the suboptimal performance of ultrasound and AFP for early detection of HCC (63% sensitivity for early stages), there is an urgent need to develop new tools for HCC surveillance and to increase implementation. AFP, alpha-foetoprotein; HCC, hepatocellular carcinoma.

The future of biomarker development for early detection of HCC

Given the drawbacks of ultrasound and AFP for early detection, there have been many attempts to develop new approaches for HCC surveillance. Most of them have focused on developing new approaches using blood-based biomarkers. Increasing the accuracy of early HCC detection with a blood biomarker will effectively remove the need for ultrasound as part of HCC surveillance and transform the current paradigm for HCC surveillance. This will have a dramatic impact in the implementation

of surveillance, as patients at risk will be able to get tested using a point-of-care device rather than having to go to a tertiary medical centre to undergo an ultrasound performed by a skilled operator. A blood-only HCC surveillance test has been an aspiration in the field for more than 20 years. One of the most advanced and promising blood-only biomarkers is the GALAD score, which combines age, sex, AFP, AFP-L3% and des-gamma-carboxy prothrombin (DCP). Data from a phase II biomarker study (case-control) reported a sensitivity of 68% and specificity of 95% for the detection of early-stage HCC. In patients with NAFLD, the AUROC curve for the detection of early-stage HCC with GALAD was 0.85, with sensitivity of 68% and specificity of 95% were achieved at the common cut-off of -0.63.

There have been parallel efforts to use liquid biopsy technologies to improve HCC surveillance. Liquid biopsy defines a wide array of technologies that aim at detecting tumour components released to the bloodstream (as well as other body fluids), thus providing an easy access to molecular tumour information. Initially developed for non-invasive prenatal testing, analysis of cell-free DNA (cfDNA) is increasingly used for cancer screening and monitoring, including detection of minimal residual disease and identification of novel therapeutic targets. cfDNA fragmentation is not random and reflects the basic patterns of chromatin organisation in the nucleus of origin if the DNA that is leaked into circulation after apoptosis, necrosis or immune-cell mediated destruction. Thus, the analysis of cfDNA 'fragmentomes' permits the evaluation of the size distribution and frequency of millions of naturally occurring cfDNA fragments across the genome. As a cfDNA fragmentome can comprehensively represent both genomic and chromatin characteristics, it has the potential to identify many tumour-derived changes in the circulation. The use of cfDNA fragments has shown promising performance for the detection of early-stage HCC.[16] Besides fragment analysis, there is evidence of the role of DNA methylation markers for HCC surveillance. Studies from China[17] and the USA[18] show how a set of DNA methylation markers detected on cfDNA can accurately detect early-stage HCC with sensitivity and specificity higher than 80% and 90%, respectively. Besides cfDNA, extracellular vesicles (EVs) have also been evaluated for early HCC detection. EVs are nanoparticles whose nucleic acid payload is capable of engaging receptor cells to modify key functions. Although larger EVs such as apoptotic bodies mostly contain fragmented DNA, smaller EVs such as exosomes are enriched in small RNAs. The identification of different extracellular RNA carriers such as EV, lipoprotein complexes, and ribo-nucleoprotein complexes, challenged the dogma that extracellular RNAs were quickly degraded by RNAses in body fluids. Most studies characterising extracellular RNA have focused on annotated genomic regions, disregarding the non-trivial percentage of human reads known to map to unannotated regions in exRNA carriers. There are recent studies reporting on an in-depth analysis of the content of EVs in plasma from patients with HCC and controls using RNAseq.[19] Expression of a set of three small RNAs in EVs closely correlates with the presence of early-stage HCC when compared with controls at high risk of HCC development. The study reported a sensitivity of 86% and specificity of 91% for early-stage HCC, when these three small RNAs were combined with AFP. Most of these studies were phase II biomarker studies. However, to be recommended in guidelines and introduced in clinical practice, we still need data from phase III or ideally phase IV biomarker studies. A recent white paper from the International Liver Cancer Association (ILCA) summarises the singularities of biomarker research in HCC and proposes a set of best practices to academia and industry on how to better design, execute, and interpret these studies.[20]

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Pathology and artificial intelligence in the diagnosis of HCC

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Take-home messages

- Pathology allows the diagnosis of HCC but also additional prognostic information.
- Different subtypes of HCC are recognised according to morphological and molecular features.
- The steatohepatitic type of HCC is more often observed in patients with NAFLD.
- Artificial intelligence and deep learning methods provide relevant algorithms for HCC diagnosis.

Pathology diagnosis of HCC

Histological diagnosis of hepatocellular carcinoma (HCC) relies on cytological characteristics and architectural patterns of the hepatocellular proliferations. In difficult cases (*i.e.* differential diagnosis with dysplastic nodules, cholangiocarcinoma, or combined hepatocholangiocarcinomas), additional immunohistochemical analysis is required, including a panel of markers reflecting hepatocytic or cholangiocytic differentiation, malignancy, and stem cell features. According to the latest WHO classification, in addition to HCC NOS (not otherwise specified), eight different morphological types of HCC are recognised (fibrolamellar, scirrhous, clear cell type, steatohepatitic, macrotrabecular massive, chromophobe, neutrophil-rich, and lymphocytic-rich). In parallel, high-throughput multi-omics studies have provided a comprehensive molecular landscape of HCC allowing a definition of two main molecular HCC classes (proliferation and non-proliferation), further subdivided into subclasses, correlated with clinical factors and outcome as well as histopathological signatures.[1] Additional analysis integrating immune information identified HCC distinct classes as immune (including active and exhausted subclasses) and immune-excluded, the latter being primarily resistant to immune checkpoint inhibitors.[2] A pathomolecular classification has been further proposed linking histological pathological features to molecular classes according to the G1–G6 classification.[3] It identifies four main subtypes (progenitor phenotype, macrotrabecular/massive, steatohepatitic and microtrabecular/pseudoglandular), the macro- and microtrabecular subtypes being the most frequent.[3] Importantly, different subtypes may be present within a tumoural nodule, illustrating the great intratumour heterogeneity of HCC, that may impact prognosis and clinical outcome.[4] Pathological diagnosis of HCC also provides information on its differentiation. Two main grading systems are used in practice, the Edmondson and Steiner system subdividing HCC into four grades and a three-tier grading system (well-, moderately, and poorly differentiated HCC), the worst grade tending to drive prognosis.

Although HCC diagnosis in patients with cirrhosis is based on non-invasive imaging, tumour biopsy is indicated in atypical nodules lacking the specific vascular pattern ('wash-in, wash-out'). Biopsy performance in the differential diagnosis between high-grade dysplastic nodules and well-differentiated HCC is increased with the use of the three-antibody panel (glypican-3, heat shock protein, and glutamine synthetase), reaching a 100% specificity and 72% sensitivity.[5] Beyond diagnosis, biopsy has shown its performance for identifying macrotrabecular/massive and microtrabecular subtypes.[6]

HCC related to NAFLD

In patients with non-alcoholic fatty liver disease (NAFLD), liver carcinogenesis presents some specificities: (i) an increased proportion of HCC developing in the absence of cirrhosis compared with other aetiologies, and (ii) an increased proportion of the steatohepatitic subtype (SH-HCC). Although this subtype has been identified more than 10 years ago, its morphological definition varies across studies resulting in variable prevalence.[7] Basically, all features diagnostic for NAFLD, among steatosis, ballooning, Mallory–Denk bodies, inflammation, and fibrosis may be present throughout the tumour.

Molecularly, according to the G1–G6 classification, SH-HCC is more frequently associated with the G4 group which does not display specific mutations.[3] From a transcriptomic level, SH-HCC frequently exhibits activation of *IL6/JAK/STAT* and hedgehog pathways as well as inhibition of carnitine palmitoyl transferase 2, which may suggest the role of inflammation and lipotoxicity in its carcinogenesis.

Artificial intelligence in the diagnosis of HCC

Artificial intelligence (AI) through computational approaches including deep learning methods enables computers to provide predicting algorithms for dataset classification using artificial neural networks. Imaging and histopathology are particularly well-suited to AI, as they present a massive number of features (visible and invisible to the human eye). Several studies have applied AI in the diagnosis of liver tumours. As examples, using haematein and eosin-stained whole-slide imaging (WSI), AI tools have shown their performance for distinguishing: (i) HCC from healthy liver tissue, and (ii) HCC from cholangiocarcinoma with accuracy higher than 0.85.[8,9] More recently, Cheng *et al.*[10] evaluated the performance of four deep neural networks to classify different types of hepatocellular nodules including benign, dysplastic, and malignant proliferations from surgical and biopsy specimens. Overall, the optimal model performed very well (AUC of 0.935) in the independent external validation cohort, and contributed to enhancing the diagnosis rate of early HCC.[10] Beyond diagnosis, and because morphological features are closely related to molecular signatures and clinical outcome, several studies were able to build deep learning models to predict outcome of HCC patients from WSI, and identify histological features associated with high and low risk of survival.[11] Interestingly, some of these models outperformed composite scores including clinical, biological and pathological features. Finally, the authors observed that the combination of the model and the pathologist outperformed both the model alone and the pathologist alone, suggesting that AI tools should be used to augment, rather than replace, the conventional histological diagnosis. Further ongoing studies, as for other kinds of cancers, aim to apply deep learning methods to predict molecular signatures/genomic alterations directly from the histopathological features.

Conclusions

Although pathology might provide deeper characterisation of HCC, its role remains restricted to its diagnosis in inconclusive cases by imaging. The input of molecular information and the development of new approaches, such as deep learning methods based on WSI, will probably modify our management of patients with HCC in the near future.

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Imaging for diagnosis and staging of HCC

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Take-home messages

- The concept of non-invasive diagnosis of HCC corresponds to the possibility of reaching a definitive diagnosis without invasive procedures. It has been formalised in numerous guidelines, the most comprehensive and recent one being the LI-RADS.
- The main imaging features used for the non-invasive diagnosis of HCC are the arterial phase hyperenhancement, the washout, the presence of an enhancing capsule, and features of venous invasion.
- The non-invasive diagnosis of HCC can be performed in NAFLD-cirrhosis but does not apply to patients with non-cirrhotic NAFLD.
- HCC developed in patients with non-cirrhotic NAFLD are typically large, heterogeneous tumours with typical imaging features of HCC.
- Steatohepatitic HCC is a rare subtype characterised by a steatohepatitic component associated with NAFLD or metabolic syndrome. On imaging, steatohepatitic HCCs typically appear as small, well-delineated fat-containing tumours displaying typical imaging features of HCC and developed on a background steatotic liver.

The concept of non-invasive diagnosis of HCC

A general rule in oncology requires that the diagnosis of malignancy be confirmed by tissue sampling. Hepatocellular carcinoma (HCC) is one of the rare exceptions. In 2001, the concept of imaging-based non-invasive diagnosis of HCC was introduced by the Barcelona-2000 European Association for the Study of the Liver (EASL) conference. It refers to the possibility of reaching a definitive diagnosis without invasive procedures. Imaging-based diagnosis relies on depicting vascular modifications occurring during hepatocarcinogenesis in patients with a high pre-test probability of HCC. Twenty years, several updates, and refinements later, virtually all national and international guidelines have endorsed this approach.

In 2011 the American College of Radiology introduced the Liver Imaging Reporting and Data System (LI-RADS; LR) to provide a standardised lexicon and stepwise algorithm to characterise liver observations in patients at high risk of HCC.[1] The LI-RADS is a categorical and algorithmic system where liver observations are assigned a category corresponding to the estimated probability of HCC or malignancy. The LI-RADS has undergone several updates to clarify definitions and concepts, address limitations, and incorporate new evidence, and has gained worldwide acceptance, at least in academic centres and scientific studies. The latest version (released in 2018) has been endorsed by the American Association for the Study of Liver Disease and is aligned with the Organ Procurement and Transplant Network.[1]

One of the main benefits of the LI-RADS over pre-existing systems is to categorise liver observations according to the risk of HCC or malignancy and to endorse management suggestions. To be valid, this approach requires that this risk be known for each LI-RADS category. Many studies have been

published aiming to assess the performance of the LI-RADS, and all consistently validated the excellent specificity of the LR-5 category (definitely HCC) and the value of the LR-M category (malignancy but not specific for HCC). Notably, the proportion of HCC and malignancy across categories is not affected by the imaging modality (*i.e.* computed tomography [CT] or magnetic resonance imaging [MRI]) or contrast agent used (*i.e.* extracellular or liver-specific).

Of course, the system is not perfect. Although systematic reviews have confirmed that the likelihood of an observation being an HCC corresponds to the ordinal LI-RADS category,[2,3] the recent meta-analysis by Lee *et al.* (49 studies, 9,620 patients with 11,562 observations, comprising 7,921 HCCs, 1,132 non-HCC malignancies, and 2,509 benign entities) reported relative spread of the proportion of HCC for LR-2 (probably benign, with 2% to 18% of HCC), LR-3 (intermediate probability of malignancy, with 32% to 60% of HCC), and LR-4 (probably HCC, with 67% to 93% of HCC), which calls for improvements.[3] Finally, the agreement for LI-RADS categorisation is moderate, as recently shown by Rimola *et al.*,[4] despite recent updates and clarification in the LI-RADS lexicon.

Major imaging features of HCC across guidelines

EASL 2018 clinical practical guidelines

According to the 2018 EASL guidelines, the diagnosis of HCC can be reached non-invasively in patients with cirrhosis if a lesion >10 mm in size shows typical features, namely a combination of arterial phase hyperenhancement (APHE, *i.e.* enhancement in arterial phase more than the liver) and washout (reduction in enhancement from earlier to later phase resulting in hypoenhancement relative to the liver.[5] This corresponds to changes in hepatic microcirculation, which occur during the development of HCC, that is, the progressive development of impaired arterial vessels and the deprivation of portal venules.

LI-RADS

The LI-RADS can be used in patients with cirrhosis or chronic hepatitis B viral infection or current or prior HCC, regardless of lesion size.[1] It combines more major features than the EASL, that is, APHE, washout, an enhancing capsule, and tumour growth. APHE is refined into rim (confined at the observation's periphery) or non-rim APHE. Similarly, the washout is refined into peripheral and non-peripheral washout (not mainly in the observation's periphery). The capsule corresponds to a smooth, uniform, sharp border on CT or MRI around most or all of the observation, and the threshold growth ≥50% in ≤6 months. A definite HCC (LR-5) is present if a lesion ≥20 mm shows non-rim APHE and at least one additional major feature among a non-peripheral washout, an enhancing capsule, and threshold growth. The LR-5 category also applies to lesions 10–19 mm in size, showing non-rim APHE and either non-peripheral washout or threshold growth.[1]

Notably, the LI-RADS also considers the presence of unequivocal enhancing soft tissue in a vein, regardless of visualisation of a parenchymal mass as highly specific of HCC, and reports it as 'tumour-in-vein' (LR-TIV). Finally, the LR-M category (probably or definitely malignant, but not necessarily HCC) is introduced, mainly for lesions displaying a target-like morphology, that is, concentric arrangement of internal components that likely reflects peripheral hypercellularity and central stromal fibrosis or ischaemia.

For the EASL and LI-RADS, the washout can be analysed on portal venous and/or delayed phases on CT or MRI if extracellular contrast agents are used, but on the portal venous phase only if liver-specific MR contrast agents are used. These liver-specific agents are gadolinium chelates taken up by functioning hepatocytes via organic anionic transporting polypeptides expressed on the sinusoidal

membrane of hepatocytes. The level of expression of these proteins is significantly decreased in impaired hepatocytes. Therefore, their injection allows for the acquisition of dedicated T1-weighted images obtained when the liver is markedly enhanced (the so-called 'hepatobiliary phase' [HBP]). On HBP images, non-hepatocellular tumours, tumours containing impaired hepatocytes (such as HCC), and vessels and cysts appear hypointense. Notably, the loss of hepatocellular function occurs early during the carcinogenesis of liver tumours, even before tumour neoangiogenesis. This is why 80% to 90% of HCCs are hypointense on HBP, whereas most non-HCC, cirrhosis-associated regenerative or dysplastic nodules appear iso- or hyperintense. Therefore, liver-specific MR agent-enhanced MRI is expected to offer higher sensitivity for detecting nodules. A recent meta-analysis focusing on the diagnostic performance of MRI for diagnosing HCC up to 2 cm has shown that gadoxetic acid enhanced-MRI had significantly increased sensitivity compared with extracellular contrast agent MRI (92% and 67%, respectively). Therefore, it may be tempting to consider this new feature (hypointensity on HBP) as a new imaging hallmark of HCC and to expect an improvement in diagnostic performance, the same way introducing the criterion of washout did. However, other studies have shown that the hypointensity on HBP used as an alternative to washout led to a significant increase in sensitivity for the diagnosis of HCC, but at the cost of an unacceptable decrease in specificity. Finally, recent prospective multicentric studies comparing extracellular and liver-specific contrast agents reported a better accuracy and specificity of extracellular agents over liver-specific ones for the non-invasive diagnosis of HCC.[6]

Asian guidelines

Asian guidelines also use APHE and washout but allow hypointensity on the transitional and/or HPB when using liver-specific contrast agents. In more detail, the Korean Liver Cancer Association practice guidelines consider a nodule to be a HCC with liver-specific-enhanced MRI if it measures ≥10 mm and displays APHE and washout on portal venous phase or hypointensity on transitional phase and/or hepatobiliary phase. For the Asian Pacific Association for the Study of the Liver and the Japanese Society of Hepatology, a nodule is a HCC with liver-specific-enhanced MRI regardless of size when displaying APHE and washout on portal venous phase and/or hypointensity on HBP.

Non-invasive diagnosis of HCC in patients with NAFLD

A critical (and often overlooked) element is that the non-invasive diagnosis of HCC can be applied to patients who are considered at high risk of developing HCC only. The definition of high-risk patients is needed to maintain a high specificity for the diagnosis of HCC because of several HCC mimickers in patients without risk factors. Indeed, the accuracy of a diagnostic test (*e.g.* imaging) is affected by the pre-test probability of the disease. In a population that does not have a sufficiently high pre-test probability of having HCC, typical imaging features can be observed in other benign and malignant non-HCC lesions, leading to an unacceptable number of false-positive diagnoses and a reduced specificity for HCC.

The definition of high-risk patients differs in medical societies. It reflects the estimated risk of developing HCC in specific populations. For instance, the CT/MRI and contrast-enhanced ultrasound LI-RADS algorithms can only be applied to patients ≥18 years old with cirrhosis, chronic hepatitis B (regardless of the presence of cirrhosis), or with a prior or current history of HCC.[1] The LI-RADS diagnostic categories cannot be applied to patients without the above-defined risk factors, with congenital hepatic fibrosis, cirrhosis as a result of vascular disorders, or in the paediatric population. According to the EASL guidelines, a non-invasive imaging diagnosis of HCC can only be applied to patients with cirrhosis. A direct consequence is that the non-invasive diagnosis of HCC cannot be made in patients with non-alcoholic fatty liver disease (NAFLD) without cirrhosis, and a biopsy is

recommended. Indeed, despite an unequivocally increased risk of HCC in non-cirrhosis-related NAFLD, the pre-test probability in these populations has not yet been precisely established.

Data regarding the performance of the non-invasive diagnosis in patients with NAFLD patients without cirrhosis is scarce because the vast majority of studies addressing the performance of the non-invasive diagnosis of HCC adhere to the definition of high-risk populations. Ludwig *et al.* specifically focused on the performance and reliability of the LI-RADS for distinguishing HCC from non-HCC primary liver carcinomas in patients who did not meet strict LI-RADS high-risk criteria.[7] They included 131 patients, including 25 (19%) with steatosis without fibrosis, 10 (7%) with steatosis and fibrosis, eight (6%) with NASH but without fibrosis, and 33 (25%) with NASH and fibrosis. In the entire cohort, the specificity of LR-5 as a predictor of HCC was 97–100%, and the combination of LR-5 or LR-TIV as a predictor of HCC did not change the specificity. However, the authors did not provide the result for the subgroup of NAFLD patients. The same group published another study focusing on non-HCC malignancies.[8] They suggested that non-HCC malignancies were more likely to mimic HCCs on CT and MRI in the LI-RADS target population than in patients without LI-RADS-defined HCC risk factors. However, here again, no subgroup analysis in patients with NAFLD was provided. Kim *et al.* also focused on patients without LI-RADS-defined HCC risk factors, but no patients with NAFLD patient were included.[9]

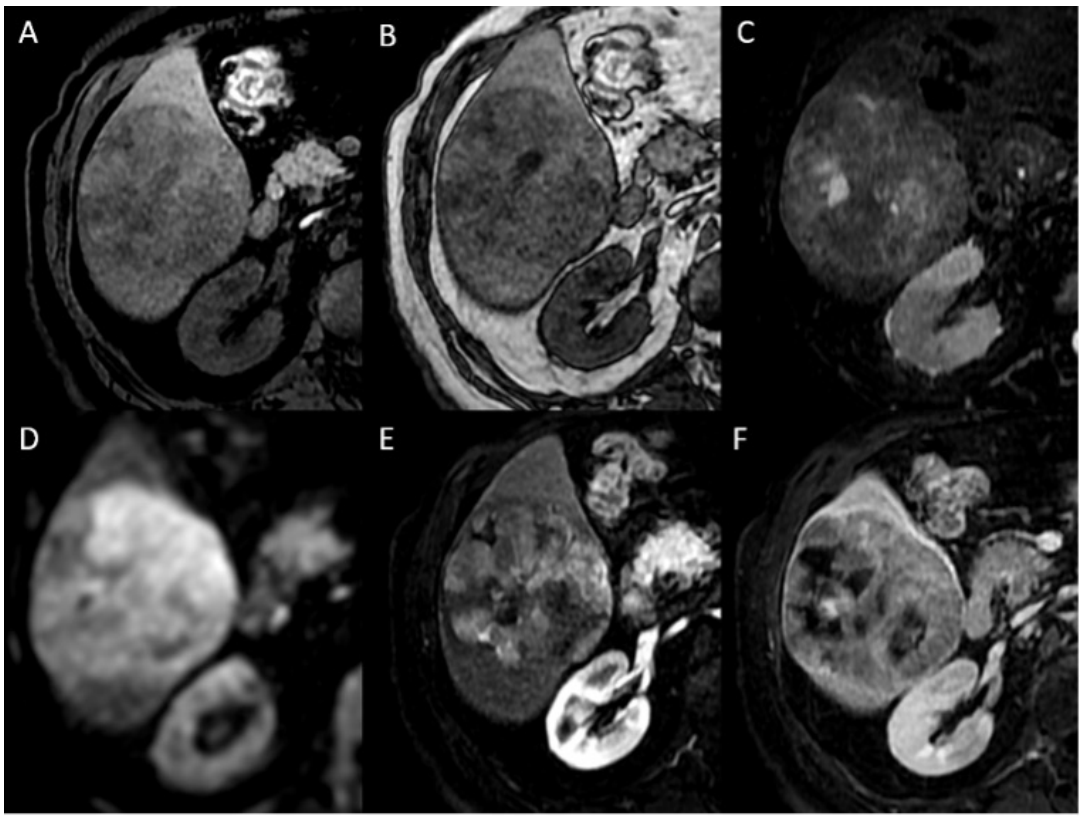

Fig. 1. Non-otherwise specified HCC containing fat in a 65-year-old man with metabolic syndrome.
(A) T1 in phase. (B) T1 opposed phase. (C) T2 with fat suppression. (D) Diffusion-weighted imaging (DWI). (E) Contrast-enhanced fat-suppressed T1 on arterial phase. (F) T1 contrast-enhanced fat-suppressed T1 on portal venous phase. The lesion is heterogeneous with a mosaic appearance. It contains fat. It shows intermediate T2 signal intensity, DWI signal hyperintensity, non-rim arterial phase hyperenhancement, and with non-peripheral washout and an enhancing capsule on the portal venous phase. In a high-risk patient, the lesion would be graded as LR-5 (definite HCC).

Imaging features of HCC in patients with NAFLD

Imaging appearance in patients with non-cirrhotic NAFLD

Evidence suggests that the imaging appearance of HCC developed in patients with NAFLD-cirrhosis is similar to other aetiologies (see earlier). However, knowledge of imaging presentations of HCC in patients with non-cirrhotic NAFLD is limited. The main reason is that very few studies, including small series of patients, have described the clinical, pathological, and imaging features of HCC developed in the non-cirrhotic liver65 years]. Interestingly, these studies consistently reported that the main imaging features of HCC are present in most patients. However, those studies did not differentiate HCC developed in patients with advanced fibrosis from those in the non-fibrotic liver and did not specifically separate non-alcoholic fatty liver (NAFL) from non-alcoholic steatohepatitis (NASH) or NAFLD from other possible causes of liver disease.

Most of the HCC developed in patients with non-cirrhotic NAFLD present as solitary lesions or as a dominant mass with satellite nodules65 years]. Infiltrating forms are anecdotal. The vast majority of HCCs present with non-rim APHE and non-peripheral washout (Fig. 1). No evidence suggests any differences between patients with cirrhosis and without cirrhosis, except for larger tumour size in patients without cirrhosis, probably because of surveillance programs that patients with advanced chronic liver disease are encouraged to follow. Park *et al.* published a systematic review and meta-analysis (five studies, 170 patients with 193 HCC) in NAFLD patients. The pooled percentages of APHE, washout, and enhancing capsule were 94.0% (95% CI 89.1–96.7%), 72.7% (95% CI 63.3–80.4%), and 57.5% (95% CI 45.1–69.1%), respectively. The percentages of these three major features did not significantly differ between NAFL and NASH ($p \geq 0.21$). MRI showed similar pooled percentages of APHE (94.3% *vs.* 93.4%, $p = 0.82$) and washout (70.4% *vs.* 77.2%, $p = 0.38$) to CT, but a higher pooled percentage of enhancing capsule (67.1% *vs.* 44.7%, $p = 0.02$).[10] The better ability of MRI to depict an enhancing capsule was also shown by Cannella *et al.*[11]

Influence of hepatic steatosis

The detection and characterisation of focal liver lesions are modified by steatosis. It may lead to underestimating the tumour burden, particularly with CT. It can also make the characterisation of the lesion more difficult. MRI is the most appropriate imaging examination to address this limitation. Thompson *et al.* assessed the effect of hepatic steatosis on major features of HCC at MRI in patients with NAFLD. They reported an 18% and 22% increase in the odds of absent washout and capsule appearance for every 1% increase in hepatic fat fraction[12]

Steatohepatitic HCC

Steatohepatitic HCC (sh-HCC) is one of many variants of HCC listed in the World Health Organization classification. It was described in 2010 as a HCC presenting histological features of steatohepatitis (*i.e.* ballooning, steatosis, fibrosis, inflammatory infiltrates, Mallory-Denk bodies). It is the most frequent subtype, accounting for about 20% of HCC. It has been initially described in HCV patients, but its association with metabolic syndrome and NAFLD is now well established. The diagnosis relies on depicting a steatohepatitic component ≥50% of the total viable tumour surface on pathology. A less than 50% component will classify the tumour as classic HCC (non-otherwise specified HCC) with a steatohepatitic component.

There are still few radiological descriptions of sh-CHC. Steatohepatitic HCCs are usually smaller than other HCCs and classically develop in a background of hepatic steatosis. Therefore, tumours may be difficult to distinguish from the surrounding liver parenchyma in patients with severe hepatic steatosis.

On CT and MRI, fat in mass is significantly more frequent in sh-HCCs than in other subtypes. This is depicted as a diffuse or low focal attenuation on CT and intralesional signal loss on opposed-phase MR images. However, the presence of fat in mass is insufficient to reliably predict the sh-HCC subtype because this feature is also observed in other HCC subtypes, in early HCC and other fat-containing liver lesions. In high-risk patients, most sh-HCCs are categorised as LR-5 because most tumours show APHE, washout, and a capsule.[13] The majority of tumours also show hypointensity in the HBP. Steatohepatitic HCC uncommonly exhibits tumour-in-vein. Notably, the possible non-invasive diagnosis of sh-HCC should be considered in the appropriate clinical context only (*e.g.* HCC showing fat in mass in patients with steatosis, metabolic syndrome) (Figs 2 and 3).

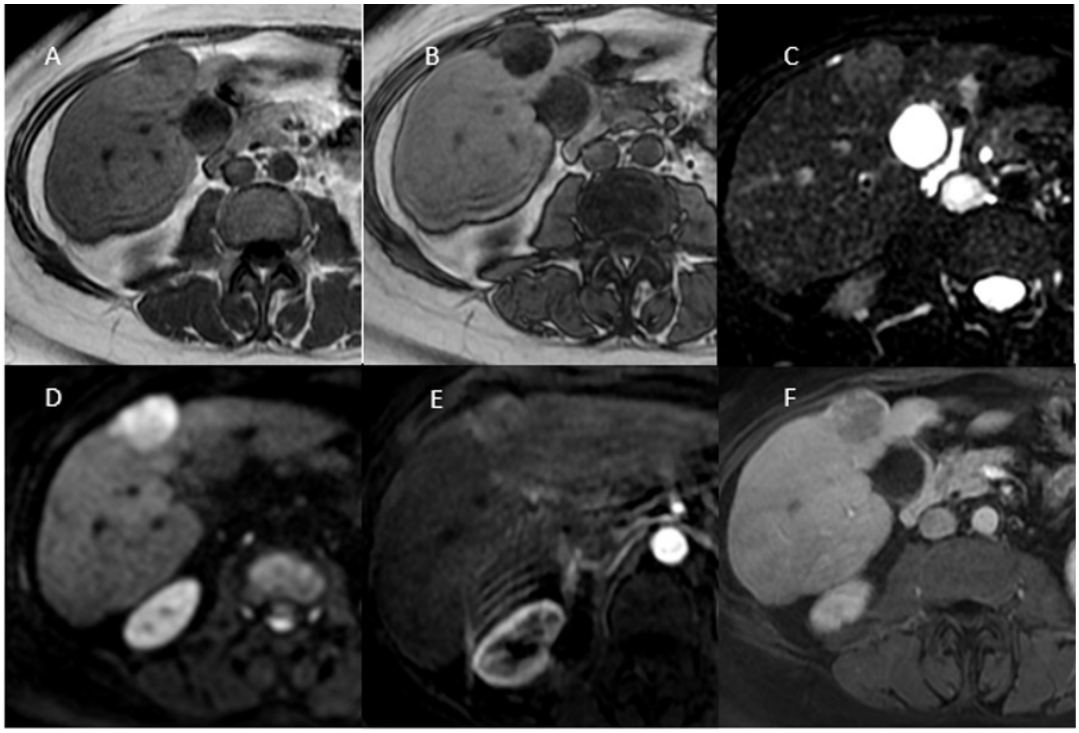

Fig. 2. Steatohepatitic HCC in segment 4 in a 68-year-old woman with metabolic syndrome.

(A) T1 in phase. (B) T1 opposed phase. (C) T2 with fat suppression. (D) diffusion-weighted imaging (DWI). (E) Contrast-enhanced fat-suppressed T1 on arterial phase. (F) T1 contrast-enhanced fat-suppressed T1 on portal venous phase. The lesion contains fat, appears homogeneous, and presents with intermediate T2 signal intensity, DWI signal hyperintensity, non-rim arterial phase hyperenhancement, and non-peripheral washout in the portal phase. In a high-risk patient, the lesion would be graded as LR-5 (definite HCC).

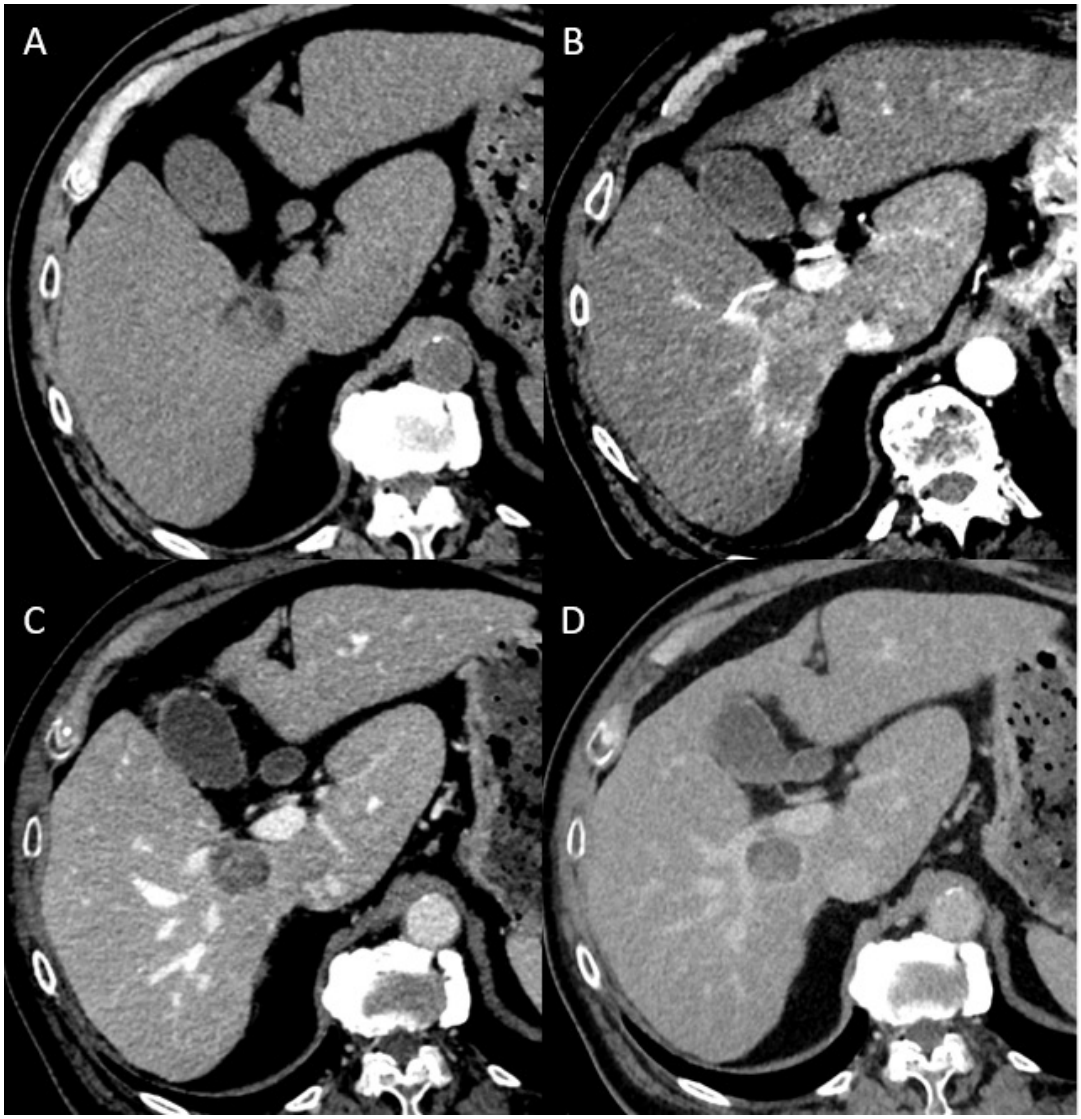

Fig. 3. CT appearance of a steatohepatitic HCC in segment 9 in a 77-year-old man with metabolic syndrome.
(A) Precontrast. (B) Arterial phase. (C) Portal venous phase. (D) Delayed phase. The lesion is small, contains fat, appears heterogeneous, and presents with non-rim arterial phase hyperenhancement, non-peripheral washout on the portal and delayed phases, and an enhancing capsule on the delayed phase. In a high-risk patient, the lesion would be graded as LR-5 (definite HCC).

Conclusions

The concept of a non-invasive diagnosis of HCC corresponds to the possibility of reaching a definitive diagnosis without invasive procedures. It has been formalised in numerous guidelines, the most comprehensive and recent one being the LI-RADS. The main imaging features used for the non-invasive diagnosis of HCC are the arterial phase hyperenhancement, the washout, the presence of an enhancing capsule, and features of venous invasion. This non-invasive diagnosis of HCC can be performed in NAFLD-cirrhosis but does not apply to patients with non-cirrhotic NAFLD. HCC developed in patients with non-cirrhotic NAFLD are typically large, heterogeneous tumours with typical imaging features of HCC. A recently described variant, the steatohepatitic HCC, is characterised by a steatohepatitic component and is more frequent in patients with NAFLD or metabolic syndrome. On imaging, steatohepatitic HCCs typically appear as small, well-delineated fat-containing tumours displaying typical imaging features of HCC developed on a background of steatotic liver.

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Assessment of co-morbidities and impact in the management of NASH-HCC

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Take-home messages

- Patients with NASH-HCC are older and have comorbidities associated with metabolic syndrome.
- The BCLC algorithm should guide care, but age, comorbidities, and available evidence for this patient group should be considered when discussing their therapeutic options.
- Management within a multidisciplinary team, considering the impact on the individual patient with different therapies, is advised.
- Taking part in clinical trials is encouraged, but the accessibility of these to patients and local expertise should also be considered.
- In those who are fit despite age, outcomes with surgical or locoregional therapies can be excellent and should be considered.
- Those who are fit despite age can benefit from medical therapies and these should be considered as per standard guidelines.
- A clinical nurse specialist, alongside lifestyle advice centred on activity and diet, should be a part of these patients' standard care.

Description of prevalent co-morbidities associated with NASH-HCC

Patients with non-alcoholic fatty liver disease-hepatocellular carcinoma (NAFLD-HCC) are typically older, with a higher prevalence of comorbidities associated with metabolic syndrome.[1] These are summarised in Table 1, along with common medications these patients take.

Table 1. Metabolic syndrome co-morbidities common in NAFLD-HCC patients

Metabolic disease	Type 2 diabetes, dyslipidaemia, hypertension
Complications of metabolic disease	Renal disease, cataracts, neuropathy, 'diabetic foot', urinary tract infections
Cardio- and cerebrovascular disease	Ischaemic heart disease, stroke, aortic aneurysm, peripheral vascular disease
Musculoskeletal	Osteoarthritis, gout, sarcopaenia, impaired mobility
Pulmonary disease	Obstructive sleep apnoea
Other gastrointestinal disease	Gallbladder disease, pancreatitis, altered bowel habit
Other cancers	Breast, colon, prostate, bladder
Common medications to consider	Metformin, insulin, aspirin, clopidogrel, apixaban, warfarin, antihypertensives

The management of the metabolic syndrome in patients with NAFLD-HCC is not dissimilar to that for patients with NAFLD and no HCC. The development of HCC should not distract from the management of their underlying disease.[2]

Impact of comorbidities in the applicability of therapies in NASH-HCC compared with other aetiologies

Patient assessment

Comorbidities are essential to consider, alongside tumour stage, liver function, and performance status (PST), when assessing patients. Ideally, this should be within the setting of a multidisciplinary team – including hepatologists, surgeons, radiologists, oncologists, and clinical nurse specialists. At the outset, simple things such as mobility and distance from an expert centre are important considerations. Repeated face-to-face visits may be challenging and input from a specialist nurse – to communicate with the patient and co-ordinate investigations and optimal care in regional hospitals aligned with a specialist centre, will be of benefit to many patients. Objective liver function assessment is essential, but look at other blood tests – such as renal function. Impaired renal function may influence imaging decisions, as contrast agents can impact this. These patients are often on medications that need to be omitted before a scan (metformin) or an invasive procedure (those that impair clotting). Remembering these things are cost effective and will save the patients the inconvenience of rescheduled appointments and hospital visits.

From the outset, it is essential not just to know a patients' past medical history, but also to fully appreciate the impact chronic comorbidities and their cancer diagnosis have on their physical performance and quality of life. For many years in oncology, this has been captured by the Eastern Cooperative Oncology Group (ECOG) Performance status scale,[3] summarised in Table 2.

Table 2. ECOG Performance Status Scale

Grade	ECOG Performance Status
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, *e.g.* light house work, office work
2	Ambulatory and capable of all selfcare but unable to carry out any work activities; up and about more than 50% of waking hours
3	Capable of only limited selfcare; confined to bed or chair more than 50% of waking hours
4	Completely disabled; cannot carry on any selfcare; totally confined to bed or chair
5	Dead

The assessment of PST is a key part of the European Association for the Study of the Liver (EASL) management guideline for patients with HCC, incorporated into the Barcelona Clinic for Liver Cancer (BCLC) Algorithm.[4] However, a patient with NASH-HCC may well have a performance status of 0, but be older with a history of stroke or cardiovascular disease which may influence treatment choice. Subjectively, some practitioners may class such a patient as having a PST of '1'. Within the ECOG PST, there may also be subjective overlap between what practitioners class as categories '1' and '2'. A patient who has previously suffered a stroke, or has arthritis, may be 'up and about' for the entire day and able to perform light duties (PST 1), but use a mobility aid such as a stick in the house or a wheelchair on leaving it. Some may class this ECOG PST 2. As the allocation of a PST category

influence treatment choices, considering the individual patient and the likely impact on their PST is key. A nurse specialist can help patients consider these options.

Applicability of therapies

Patients with NAFLD-HCC are not well represented in the clinical trials on which guidelines are based. Older age, distance from trial centres, being less mobile, comorbidities, and PST assessments have all likely contributed to this. However, the BCLC algorithm has been widely validated as a prognostic tool – across all aetiologies of disease – and 'real world' literature confirms acceptable outcomes for many with NAFLD. Thus, the EASL management guidelines BCLC algorithm should be followed, alongside review of the individual patient and the likely impact for them personally, within a multidisciplinary setting. For these patients in particular – with lifestyle related liver disease – lifestyle advice to improve their fitness, as well as potentially their subsequent prognosis and benefit from therapies, should be available (www.livingwithlivercancer.co.uk). In a patient considered 'borderline' for palliative therapies, it may be appropriate to adopt 'active monitoring', to focus on optimal supportive care while observing the rate of change of a tumour with a follow-up scan. In a patient with 'stable disease', their quality of life and survival may be better without active therapy.

Surgical therapies

Resection should be considered, especially for the 30% or so patients who do not have cirrhosis, but input from an anaesthetist is essential, possibly with cardiopulmonary exercise testing.[5] A cardiology assessment may also be advisable. The residual liver is always critical, but for patients with NAFLD or metabolic syndrome, it is not just size that matters. A biopsy of the background liver – to assess the degree of steatosis, non-alcoholic steatohepatitis (NASH), and fibrosis which influence regenerative capacity – can be helpful. For those with cirrhosis and tumour criteria meeting eligibility for transplantation, an expert anaesthetic assessment is essential. Prehabilitation – with dietician and physiotherapy input to encourage healthy diet and exercise should be part of these patients' holistic care – to try to improve both short- and longer-term outcomes.

Locoregional therapies

For patients with early-stage disease and preserved liver function, microwave ablation is well tolerated and potentially curative – including for patients with NAFLD and metabolic syndrome.[6] Transarterial chemoembolisation (TACE) and selective internal radiotherapy (SIRT) can also be well tolerated in NAFLD patients with preserved liver function,[7] with intermittent treatments avoiding daily side effects of medical therapies often preferable in this patient group. Combination TACE and ablation may also be considered for those unamenable to ablation at the outset.[8] Stereotactic radiotherapy (SABR) has yet to find a place within guidelines, but for patients with a single lesion <5 cm, unsuitable for other therapies (*e.g.* impaired renal function, ECOG PST 1/2), this also has a place within a multidisciplinary team setting (NHS England: Stereotactic ablative radiotherapy (SABR) for hepatocellular carcinoma (adults): https://www.england.nhs.uk/publication/stereotactic-ablative-radiotherapy-sabr-for-hepatocellular-carcinoma-adults/).

Medical therapies

From 2008 to 2017, only the multikinase inhibitor sorafenib was available as a first-line medical therapy for patients with advanced HCC. As a group, NAFLD-HCC patients may have benefitted less overall from this treatment, which may be more attributable to their fitness, comorbidities, and ability to withstand side effects rather than their underlying disease. A UK wide audit indicated benefit regardless of aetiology and age, in patients with preserved liver function and PST[9] – hence the

important of discussing these factors when considering treatment choices. In those with an ECOG PST 1/2, there may be no objective harm associated with a trial of the drug, possibly starting at a lower dose. The patient may feel healthier mentally given the opportunity. However renal function, mobility issues, skin conditions, gastrointestinal function, and appetite should be considered and the possible impact/things to look out for and discussed with the patient. A specialist nurse should help the patient in decision-making and provide ongoing support for symptom management.

In 2018, a second kinase inhibitor became available – lenvatinib, having shown non-inferiority to sorafenib. It has different targets and may be better tolerated in some patients. Although trials-based evidence is lacking, the drug is reported to be well tolerated in patients with NAFLD by oncologists using it. Similarly, in NAFLD patients who have benefitted from first-line kinase inhibitors, second-line treatment with the kinase inhibitor regorafenib should be considered for them – in keeping with standard guidelines.

In 2018, data from trials with checkpoint inhibitors (CKIs) emerged. Although as monotherapies the objective response rates were low (15%) and primary endpoints confirming superiority over sorafenib were not met, some patients clearly benefitted markedly. Patients with 'NAFLD' *per se* were not analysed as a distinct subgroup, but data indicated responses were seen in patients with both 'viral' or 'non-viral aetiologies. In 2019/2020 the combination of atezolizumab + bevacizumab (CKI in combination with a vascular endothelial growth factor receptor inhibitor) surpassed survival benefit derived from sorafenib and became the first choice first-line therapy for patients with advanced HCC. There have been later subgroup analyses suggesting that patients with NAFLD-HCC may derive less benefit from CKI immunotherapies. However, predictive biomarkers are lacking and in 'real world' clinical practice, there are NAFLD-HCC patients who benefit from these drugs. The key is to assess the patient and their fitness – particularly to withstand side effects that may be more dramatic (gastrointestinal haemorrhage, immune-mediated conditions) than those seen with kinase inhibitors. Fit patients, classed ECOG PST 0 or 1, with preserved liver function and no contraindication (varices at risk of bleeding, known autoimmune condition) should be offered this therapy in preference to others, while research to identify predictive biomarkers and tailored approaches for different aetiologies of disease is ongoing.

Impact of comorbidities in complications/outcomes in NASH-HCC treatment

In our own published series from Newcastle upon Tyne, North East UK, patients with NAFLD-HCC had a poorer prognosis compared with other common aetiologies, but this was largely attributable to having a more advanced stage at presentation.[1] Ours and a number of other studies indicate that only a relatively small proportion of patients with NAFLD-HCC are offered liver resection or orthotopic liver transplantation (OLT) (17.8% and 4.4%, respectively), and patients with NAFLD-HCC are more likely to be offered supportive care.[10-12] In our study however, in a multivariate analysis including tumour stage, liver function, and PST, NAFLD was not associated with poorer outcomes. Neither were age, diabetes, or the presence of obesity. These findings have been confirmed in other observational cohort studies.[10] In recent years, publications looking at larger series of NAFLD patients comparing outcomes, particularly regarding transplantation, have increased.

Transplantation

Although the older age of patients with NAFLD-HCC, along with presence of comorbidities, undoubtedly affect fitness for transplantation, with fewer NAFLD-HCC patients undergoing transplantation,[13] NAFLD cirrhosis is an increasingly common indication for OLT in both the USA and Europe. According to the

European Transplant Registry, NAFLD transplants increased from 1.2% in 2002 to 8.4% in 2016, with HCC more common in patients transplanted for NAFLD compared with other aetiologies.[14] Historically, the presence of NASH has been associated with early post-transplant mortality, with sepsis and cardiovascular disease the causes. Patients with NAFLD have been reported to have longer operative times, increased blood loss, and longer length of stay postoperatively. In the European Transplant Registry, however, considering 68,950 liver transplants recipients between 2002 and 2016, comparable patient and graft survival were reported comparing NAFLD and non-NAFLD patients, with and without HCC. Notably, recurrent HCC, infection and extrahepatic solid organ malignancy were the most common causes of death in patients with NAFLD-HCC, whereas infection and cardiovascular complications were the most common in patients with NAFLD without HCC. Studies in the USA have shown similar findings, although recurrence of NAFLD is reportedly common following transplantation, with both diabetes and co-existent obesity contributing to reduced 5-year survival rates.[15] A recent meta-analysis assessing predictors of survival following liver transplant for NASH, also highlighted obesity and diabetes, alongside age and the presence of HCC.[16] Overall, these studies suggest that NAFLD patients are at increased risk of short-term complications following liver transplantation, and although patients with NAFLD-HCC have comparable longer-term outcomes to those of other aetiologies underlying their HCC, age, diabetes, and obesity identify those with poorer outcomes. Prehabilitation optimising fitness, weight, and glycaemic control are therefore important to introduce pre-transplant and should remain a focus post-transplant – particularly for those with NAFLD-HCC.

Other therapies

Although data in NAFLD-HCC is limited for other therapies, a large French series has recently explored the impact of NAFLD in patients who have ablative therapies, reporting similar outcomes as compared with other aetiologies, despite advanced age and features of metabolic syndrome.[6] Although the landscape for medical therapies is changing rapidly, with personalised approaches expected, standard guidelines should be applied when considering options for patients with NAFLD – including for first-line atezolizumab and bevacizumab.[17]

Finally

Recently, in a large series of 776 patients with NAFLD-HCC diagnosed in 130 facilities in the US Veterans Administration, the clinical course and outcomes for patients were reported.[18] Older age and comorbidities were affirmed, with 1- and 3-year mortality rates 47.0% and 69.6%, respectively. The majority died of HCC, but non-cancer mortality contributed to 40% of deaths in those receiving curative therapies. Poor performance and older age were strongly associated with non-cancer mortality. Although we do not want to disadvantage our patients with NAFLD, this study highlights again the importance of assessing and managing comorbidities in these ageing patients.

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FUTURE EVENTS 2023–2024

2023

30 June–1 July	EASL School	Larissa, Greece
7–8 July	EASL School	Odense, Denmark
7–8 July	EASL School	Madrid, Spain
21–23 September	EASL NAFLD Summit	Prague, Czechia
27–29 September	EASL School	London, UK
30 Nov–2 Dec	EASL-AASLD Masterclass	Madrid, Spain

2024

22–24 February	EASL Liver Cancer Summit	Rotterdam, The Netherlands
TBC April	EASL School	TBC
TBC April	EASL School	TBC
TBC April	EASL School	TBC
5–8 June	**EASL Congress 2024**	**Milan, Italy**
TBC September	EASL NAFLD Summit 2024	TBC
TBC TBC	AASLD-EASL Endpoints	USA

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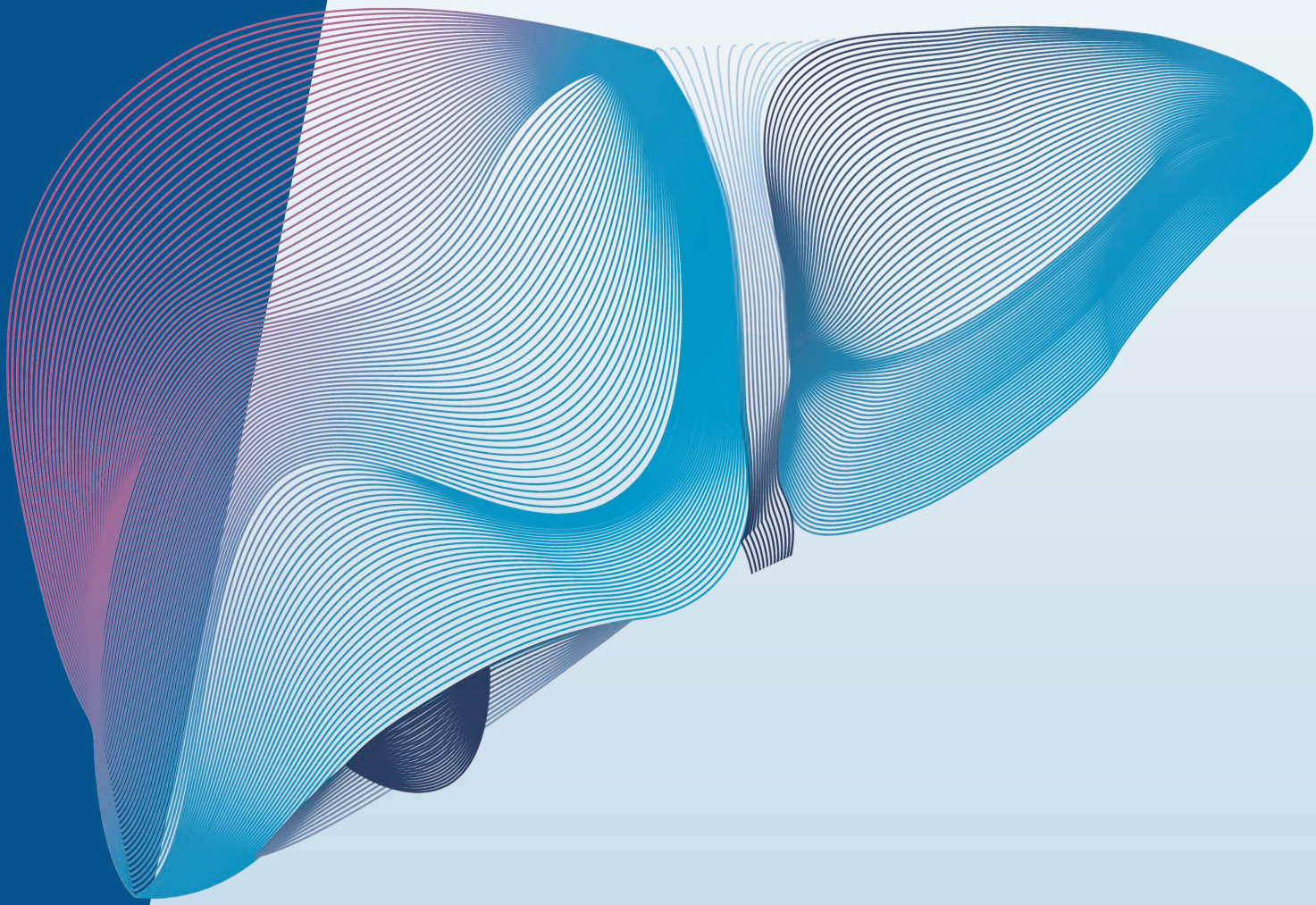
SESSION 4

MANAGEMENT OF HCC & CHOLANGIOCARCINOMA IN PATIENTS WITH NAFLD

WEDNESDAY 21 JUNE
17:15-18:45



Surgical resection and transplantation: results in NAFLD-HCC

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Take-home messages

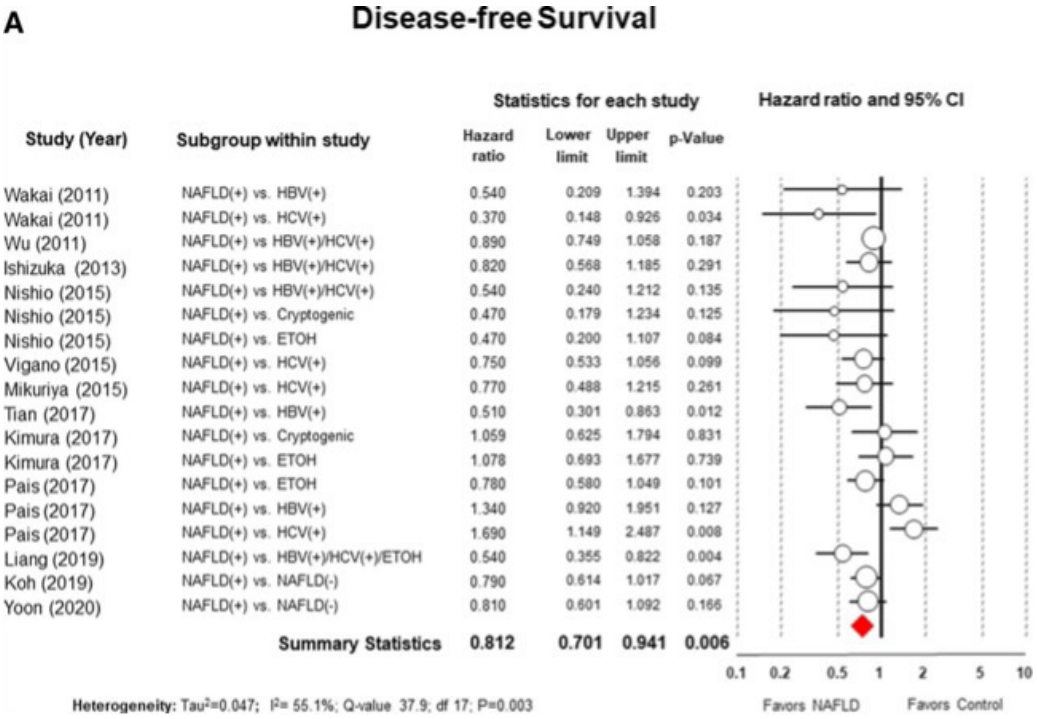
- HCC is the most common primary tumour of the liver.
- NAFLD is the fastest rising cause of HCC in the USA and parts of Europe.
- Liver transplantation has the best cure rate for NAFLD-HCC, but liver resection is the most common surgical therapy.
- With improvements in surgical techniques and postoperative care, mortality after liver resection has decreased remarkably in the most recent years, but these advances have not been translated into improved care for the patients with NAFLD-HCC.
- Contrary to previous studies that suggest that NAFLD-HCC are associated with poorer prognosis and outcomes; the most recent findings suggest that patients with NAFLD-HCC that were eligible for surgical treatment would have good outcomes.
- A deep evaluation of such cases may help to inform and guide approach to surgical management of NAFLD-related HCC.

Current management of HCC with surgical therapies

As a result of the efficacy of antiviral therapy for HBV and HCV infection, the burden of hepatocellular carcinoma (HCC) caused by viral hepatitis is declining; in contrast, the prevalence of non-alcoholic fatty liver disease (NAFLD) HCC is rising rapidly.[1] NAFLD is the fastest rising cause of HCC in the USA and parts of Europe, and is expected to rise exponentially in parallel with the global obesity epidemic.[2,3]

HCC is the most common primary tumour of the liver and the fifth most frequent malignancy worldwide.[4] Liver transplantation (LT) has the best cure rate, however, resection is the most common surgical therapy. There is evidence that there are oncological and clinical differences between HCC in the settings of NAFLD *vs.* other risk factors, such as an upregulation of signal transducer and activator of transcription 1 (STAT-1) and signal transducer and activator of transcription 3 (STAT-3), two proteins linked to hepatocarcinogenesis in the absence of cirrhosis.[5]

A recent systematic review and meta-analysis was performed to test the hypothesis that there are no significant differences in the overall survival (OS) and disease-free survival (DFS) between the two groups of patients, NAFLD-HCC *vs.* non-NAFLD-HCC, undergoing liver resections.[6] The 5-year DFS ranged from 24.4% to 66% for NAFLD patients compared with 17.4–46.9% for patients within the control group (Fig. 1A).



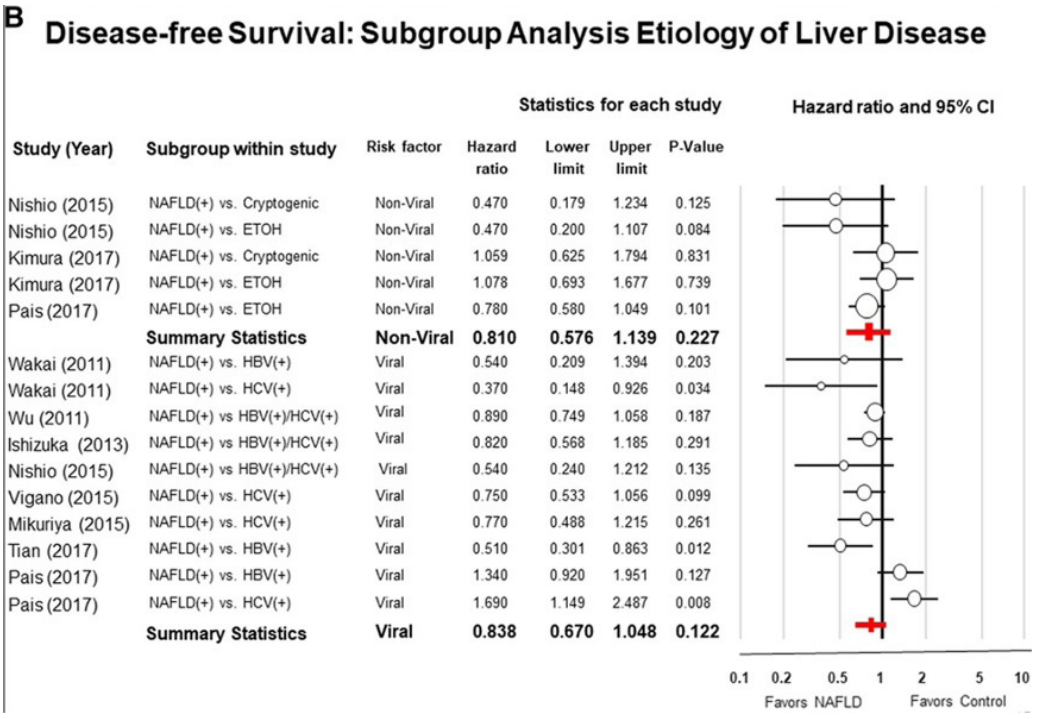


Fig. 1. Disease-free survival.
(A) Forest plot of meta-analysis with random effect model of the DFS of patients with NAFLD after hepatic resection for HCC compared with patients with other risk factors.[6]

(B) Subgroup analysis comparing NAFLD patients with patients with non-viral risk factors (alcoholic or cryptogenic cirrhosis) and with viral hepatitis (HBV or HCV).[6] DFS, disease-free survival; HCC, hepatocellular carcinoma; NAFLD, non-alcoholic fatty liver disease.

Subgroup analyses showed no differences in DFS between patients with NAFLD and patients with viral hepatis (HBV or HCV) or patients with other types of non-viral liver diseases (alcoholic or cryptogenic cirrhosis). (Fig. 1B). For patients with NAFLD, the 5-year OS ranged from 28.1% to 91.1% compared with 21.2% to 79.2% for the control group. The authors hypothesised that improvement in patient selection, surgical techniques, and perioperative care occurring over the study period might have contributed to the heterogeneity of the data of the meta-analysis. For studies published before 2015, the authors found no significant difference in the OS between patients with NAFLD and the control group, but when studies published after 2015 were included, the pooled HR for the OS favoured patients with NAFLD. The authors concluded that the main finding of their study was that the HR for the DFS and OS of patients with NAFLD was significantly lower than the HR of patients with other risk factors. Sensitivity analysis showed that the DFS favoured patients with NAFLD in Asia, while there was no significant difference between the two groups in studies from Europe and North America. Similar findings were observed for the OS that favoured Asian patients with NAFLD, while there was no survival difference between the two groups when only studies from Europe and North America were included. The current review also demonstrates that most studies on the outcomes of hepatic resections for HCC are from Asia. Therefore, there is the need for more studies from Western centres to better characterise the outcomes of non-Asian patients after hepatic resections for HCC in the setting of NAFLD.[6]

Liver resection

With improvements in surgical techniques and postoperative care, mortality after hepatectomy has decreased remarkably from 58% to 10% and overall morbidity similarly decreased.[7] However, these advances have not been translated wholly into improved care for the specific group of patients with NAFLD-related HCC. In an interesting study from Singapore on 996 patients who underwent liver resection for HCC,[8] patients were categorised into subgroups of 152 NAFLD *vs.* 844 non-NAFLD-HCC based on histological evidence of hepatic steatosis. Overall morbidity for NAFLD-related HCC post hepatectomy was over 50%. The most common postoperative complication was liver failure (49.6%), followed by cardiorespiratory complications (11.8%) and pulmonary embolism (2%). It is important to note that preoperative comorbidities were significantly more common in the NAFLD group ($p < 0.0001$). The NAFLD group had greater amount of intraoperative blood loss, postoperative complications, and length of stay, however 5-year OS rates were significantly better in the NAFLD group ($p = 0.0355$). Significant factors that contribute to a poorer survival outcome include age, congestive cardiac failure, Child B status, cirrhosis, tumour size, and multi-nodularity. For the NAFLD group, patients with abnormal parenchyma showed poorer survival and 5-year OS, 64.8% *vs.* 75.6% ($p = 0.2291$), which could be related to the underlying hepatocellular dysfunction attributable to steatosis and the pro-inflammatory state. The authors concluded that NAFLD-related HCC was associated with greater surgical morbidity and post-hepatectomy liver failure, but despite this, the long-term survival outcomes were favourable compared with non-NAFLD aetiologies.[8]

Liver transplantation

Non-alcoholic steatohepatitis (NASH) has become the leading indication for LT in many countries, with a growing rate in the Western world. NASH patients are older and share a higher risk of comorbidities and cancer than patients with viral and/or alcoholic aetiologies. In our centre, we evaluated waiting list (WL) registration and LT rates in patients with NASH-related cirrhosis in the past 15 years and

compared clinical characteristics and indications for LT between patients with and without NASH, as well as the WL survival and post-transplant outcome.[9] All adult patients with cirrhosis listed for LT at Padua University Hospital between January 2006 and June 2020 were retrospectively reviewed using a prospectively updated database; patients with NASH-related cirrhosis were divided by indication for LT (decompensated cirrhosis–NASH *vs.* HCC–NASH) and compared with patients with other aetiologies of liver disease. The outcomes in terms of waiting-list survival and post-transplant outcome were assessed. A total of 1,491 adult patients with cirrhosis were wait-listed during the study period. NASH patients accounted for 12% of all WL registrations, showing an increasing trend over time (from 2.5% in 2006 to 23% in 2020). In the past 5 years, NASH was the third, but most rapidly growing, indication for LT at our centre. This trend was confirmed both for patients with decompensated cirrhosis (from 1.8% to 18%) and HCC as the leading indication for transplantation (from 4% to 30%). Patients with NASH were older than those without NASH (mean ± SD age 59 ± 9 *vs.* 56 ± 9 years; $p < 0.01$), whereas no difference was found in terms of gender, Child–Pugh or model for end-stage liver disease (MELD) score at WL registration. A majority (60.9%) of NASH patients underwent LT, showing 1-, 5-, and 10-year post-transplant survivals of 86%, 73%, and 60%, respectively. Therefore, we confirmed that NASH cirrhosis has become a rapidly growing indication for LT at our centre both for HCC and decompensated disease, (Figs 2 and 3A and B), with good post-transplant survival.

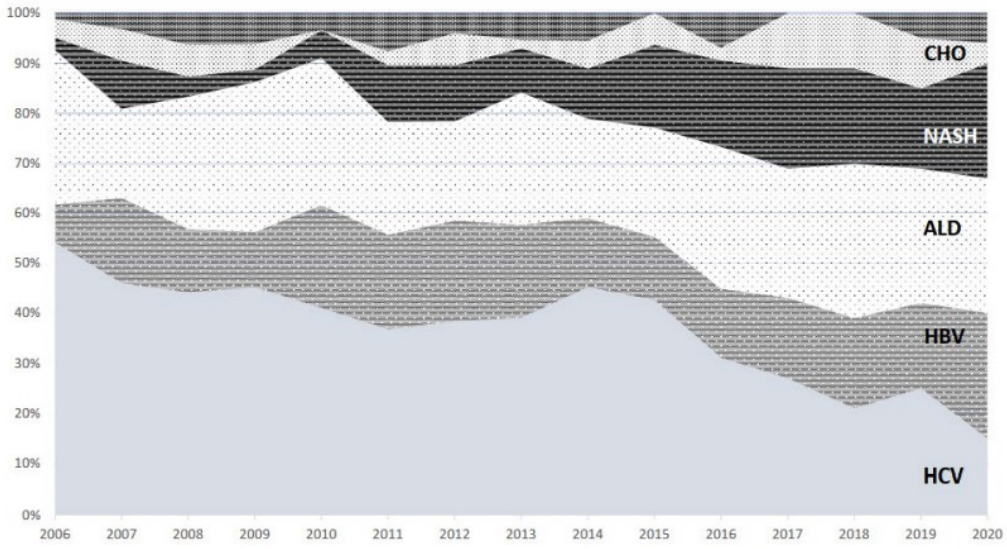


Fig. 2. Temporal trends in waiting list registrations at Padua University Hospital Liver Transplant Center between 2006 and June 2020.
The field with a vertical b/w line accounts for other indications. ALD, alcoholic liver disease; CHO, cholestatic/autoimmune disease; HBV, hepatitis B virus; HCV, hepatitis C virus; NASH, non-alcoholic steatohepatitis.[9]

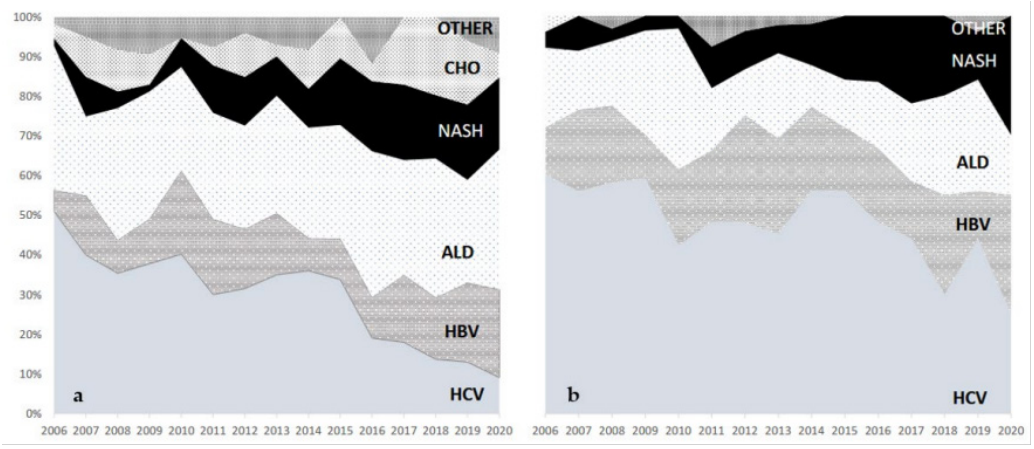


Fig. 3 Trends in waiting list registrations.
(A) Patients with decompensated cirrhosis. (B) Patients with hepatocellular carcinoma on compensated cirrhosis. ALD, alcoholic liver disease; CHO, cholestatic/autoimmune disease; HBV, hepatitis B virus; HCV, hepatitis C virus; NASH, non-alcoholic steatohepatitis.[9]

A European study aimed to determine the frequency and outcomes of LT for patients with NASH in Europe and identify prognostic factors.[10] The authors analysed data from patients who underwent transplantation for end-stage liver disease between January 2002 and December 2016 using the European Liver Transplant Registry (ELTR) database. They compared data between patients with NASH *vs.* other aetiologies. The principal endpoints were overall patient and graft survival. Among 68,950 adults undergoing first LT, 4.0% underwent transplantation for NASH – an increase from 1.2% in 2002 to 8.4% in 2016 was observed. A greater proportion of patients who underwent transplantation for NASH (39.1%) had HCC compared with patients who did not have NASH (28.9%, $p < 0.001$). NASH was not significantly associated with reduced patient and graft survival after adjusting for available recipient and donor variables. Infection (24.0%) and cardio/cerebrovascular complications (5.3%) were the most common causes of death in patients with NASH without HCC. Increasing recipient age, elevated MELD score and low or high recipient body mass index (BMI) independently predicted death in patients transplanted for NASH without HCC. Data must be interpreted in the context of absent recognised confounders, such as pre-morbid metabolic risk factors. The authors concluded that the number and proportion of liver transplants performed for NASH in Europe has increased from 2002 through 2016 and that HCC was more common in patients with NASH who underwent transplantation. They also reported that survival of patients and grafts in patients with NASH was comparable to that of other disease indications (Fig. 4).[10]

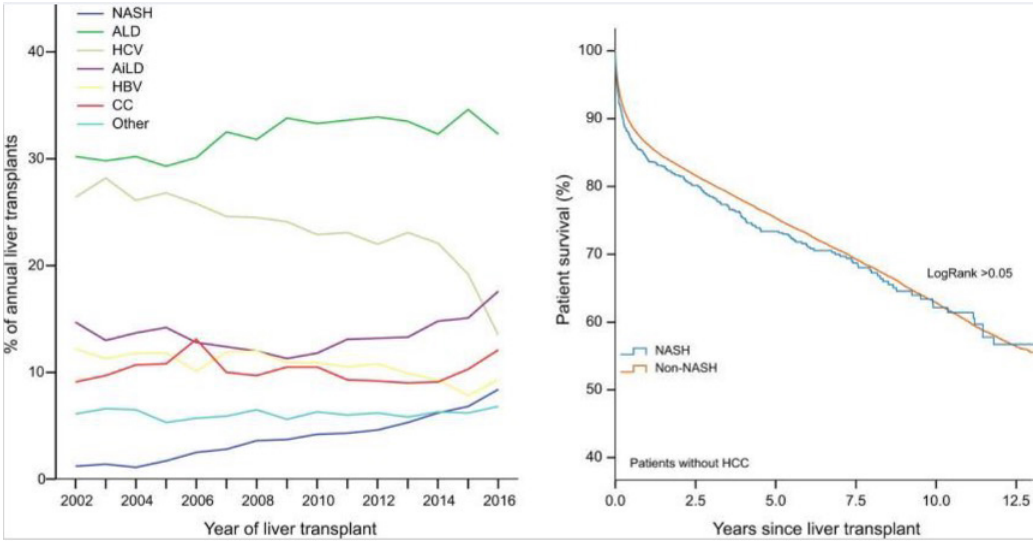


Fig. 4. An increasing proportion of patients are being transplanted for NASH in Europe. Survival in recipients with NASH is comparable to that of other disease indications.[10] ALD, alcoholic liver disease; NASH, non-alcoholic steatohepatitis.

A recent review on variables predictive of post-LT survival of patients with NASH identified 25 studies, and five studies were included in the meta-analysis.[11] The authors suggested that the following variables were predictive of post-LT survival in patients with NASH: recipient age, functional status, pre-LT hepatoma, MELD, diabetes mellitus, pre-LT dialysis, hepatic encephalopathy, portal vein thrombosis, hospitalisation/intensive care unit at LT, and year of LT. Predictors of graft survival included recipient age, BMI, pre-LT dialysis, and diabetes mellitus. Their pooled meta-analyses included five predictors of patient survival. Increased patient mortality was associated with older recipient age (HR = 2.07, 95% CI: 1.71–2.50, $I^2 = 0$, $\tau^2 = 0$, $p = 0.40$) and pre-transplant diabetes mellitus (HR = 1.18, 95% CI: 1.08–1.28, $I^2 = 0$, $\tau^2 = 0$, $p = 0.76$). Clinically, this might help to identify modifiable risk factors that can be optimised in the post-transplant setting to improve patient outcomes and optimise decision-making in the resource-limited LT setting.[11]

New approaches: laparoscopic surgery, neo-adjuvant therapies

Laparoscopic surgery

A recent study aimed to elucidate the effects of laparoscopic liver resection (LLR) *vs.* open liver resection (OLR) for major complications in individuals who are obese with HCC.[12] The clinical records of 339 and 733 patients who underwent LLR and OLR, respectively, for HCC between 2000 and 2019 were retrospectively reviewed. BMI groups were classified as: underweight group, BMI ≤ 18.4 kg/m^2 (LLR *vs.* OLR: 27 *vs.* 47); normal weight, BMI 18.5–24.9 kg/m^2 (211 *vs.* 483); overweight, BMI 25.0–29.9 kg/m^2 (85 *vs.* 181); and obese, BMI ≥ 30.0 kg/m^2 (16 *vs.* 22). The effects of obesity on major complications after LLR and OLR were investigated. Eighteen (5.3%) and 127 (17.3%) patients presented with major complications after LLR and OLR, respectively. There was no significant difference in the incidence of major complications after OLR in the four BMI groups. However, a stepwise decrease in the incidence of major complications after LLR was observed from the underweight to the obese group. In addition, a multivariate analysis revealed that increased BMI was an independent preventive factor for major complications after LLR ($p = 0.026$, OR = 0.84). The authors concluded that laparoscopic liver resection is beneficial for patients who are obese and is superior to OLR.

Neo-adjuvant therapies

Neoadjuvant therapy (NAT) is the administration of therapeutic agents before definitive surgery. A consensus has not been reached regarding the effects of NAT on HCC. As systemic therapy, particularly targeted therapy and immunotherapy, is given for HCC treatment; accumulating evidence shows that the 'spring' of NAT for HCC is imminent. In the future, HCC researchers should focus on identifying biomarkers for treatment response, explore the mechanisms of resistance, and standardise the endpoints of NAT, which includes neo-adjuvant radiotherapy, neo-adjuvant chemotherapy, immunotherapy, ablation therapy, systemic therapy, and target therapy.[13]

Impact of NAFLD in the selection of patients for surgical therapies in HCC

Metabolic liver disease in Europe: an epidemic on the rise

The European landscape on liver disease is changing and the EASL Lancet Commission proposed a framework for the principal actions required to improve liver health in Europe (Fig. 5).[14] NAFLD is becoming a leading cause of liver-related mortality in Europe and is predicted to become the leading cause of end-stage liver disease in Europe unless drastic action is taken.[15] The prevalence of NAFLD is very high in people with obesity (75–92%) or severe obesity (>90%),[16] and was 59.7% in people with type 2 diabetes mellitus.[17]

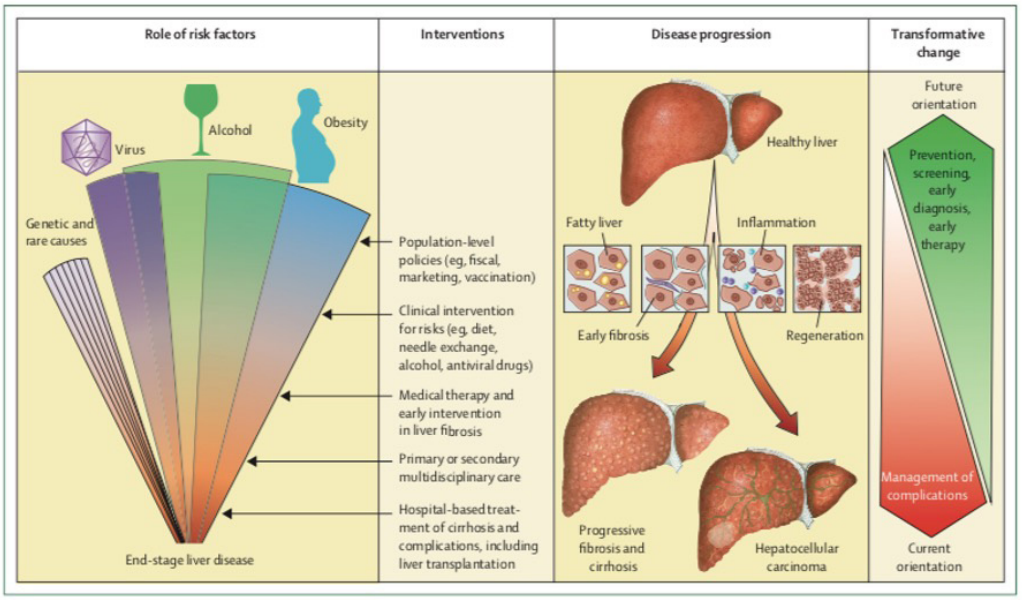


Fig. 5. Risk factors, interventions, and disease progression for different liver diseases. Progressive liver fibrosis is the single common pathway for all causes of chronic liver disease. Liver cancer mostly develops in patients with advanced fibrosis but is increasingly observed in people without cirrhosis with non-alcoholic fatty liver disease. Population-level interventions tend to be more effective and less expensive than hospital interventions. Printed with permission from Kari Toverud.[14]

Data regarding the clinical presentation and outcomes for NAFLD-HCC *vs.* HCC attributable to other causes are conflicted. Several studies reported more advanced disease at presentation and poorer survival among patients with NAFLD-HCC compared with those with HCC attributable to other causes,

whereas other studies have reported similar clinical presentation and improved survival. In addition, it is unclear what proportion of patients with NAFLD-HCC do not have cirrhosis or receive surveillance before a HCC diagnosis. Tan *et al.*[3] aimed to establish the prevalence, clinical features, surveillance rates, treatment allocation, and outcomes of NAFLD-HCC. In their systematic review and meta-analysis, the proportion of NAFLD-HCC was about 15%, with the highest proportion in the South East Asia region, followed by the Western Pacific region, European region, and the region of the Americas. There was an increase in the global proportion of NAFLD-HCC over time periods, 9.77%, for before 2000 *vs.* 16.97%, for 2010 and beyond. Patients with NAFLD-HCC were older, had higher BMI, and were more likely to present with metabolic comorbidities such as diabetes, hypertension, hyperlipidaemia or cardiovascular disease than patients with HCC attributable to other causes. They were also more likely to be non-cirrhotic, had larger tumour diameters, were more likely to have uninodular lesions, and a lower proportion of patients underwent surveillance. Interestingly, there were no significant differences in treatment allocation including curative therapy, palliative therapy, and best supportive care, between patients with NAFLD-HCC and those with HCC attributable to other causes. The percentage of NAFLD-HCC patients who received LT was 3.9%, who received resection was 33.6%, and who received ablation was 12.0%. Patients with NAFLD-HCC were less likely to undergo LT but more likely to undergo liver resection than patients with HCC attributable to other causes, whereas they had similar odds of receiving ablation. OS did not differ between NAFLD-HCC *vs.* non-NAFLD-HCC, also when compared in details to HBV, HCV, or alcohol-related HCC. A sensitivity analysis of patients who underwent curative therapy established that NAFLD-HCC was associated with longer OS than HCV-related HCC for patients receiving curative treatment, but not when compared with HBV-HCC. In a sensitivity analysis for type of curative treatment, OS did not differ between NAFLD-HCC compared with other causes in patients receiving LT or resection. NAFLD-HCC had improved DFS compared with non-NAFLD-HCC although subgroup analysis by specific causes (HBV, HCV, and alcohol) did not differ. In a sensitivity analysis, NAFLD-HCC was associated with improved DFS among patients who received curative therapy. In sensitivity analysis for specific types of curative treatment, NAFLD-HCC was also associated with improved DFS in patients who underwent liver resection, but not for LT. Meta-regression of study-level data revealed that only increased alpha-foetoprotein was associated with reduced DFS, all other assessed risk factors were not associated with DFS. However, there was publication bias in the analysis of baseline characteristics between NAFLD-HCC and non-NAFLD-HCC, notably in the analysis of BMI, diabetes, and hypertension, but not age or male gender. In conclusion, this meta-analysis provides a comprehensive global overview of the clinical presentation, surveillance rates, treatment allocation, and outcomes of NAFLD-related HCC. This study provides high-level evidence that a substantially greater proportion of patients with NAFLD-HCC do not have cirrhosis and have lower surveillance rates than have patients with HCC attributable to other causes. The proportion of HCC secondary to NAFLD is rising globally, and urgent measures are required to tackle the metabolic risk factors associated with NAFLD-related HCC. Further studies are required to improve HCC surveillance strategies for patients with NAFLD who are at high-risk of HCC without cirrhosis.[3]

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Loco-regional therapies for HCC in NAFLD patients

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Introduction

Loco-regional therapies in patients with hepatocellular carcinoma (HCC) mainly include percutaneous ablation, transarterial chemoembolisation (TACE), and selective internal radiation therapy (SIRT). Their roles in the therapeutic algorithm of HCC have evolved as new studies were published in the field. Moreover, the impact of the aetiologies of the underlying liver disease on the safety and efficacy of these treatments is still an open question. However, an accurate evaluation of the impact of aetiologies should take into account potential confounding factors: comorbidities, severity of liver fibrosis, degree of portal hypertension/liver failure, and overlap among the different aetiologies.

The increasing incidence of patients with non-alcoholic fatty liver disease (NAFLD) attributable to metabolic syndrome and the related increased in NAFLD-related HCC have highlighted the need to collect more data on the role of NAFLD in HCC treatment including loco-regional treatments. The aim of this presentation is to describe the strategy of the main loco-regional treatments in the management of patients with HCC, new advances in the field, and the impact of NAFLD in the applicability, safety, and efficacy of loco-regional treatments.

Percutaneous ablation for early HCC

Percutaneous ablation encompasses a variety of techniques including thermic methods such as radiofrequency ablation (RFA; monopolar or multibipolar), microwave ablation, or cryoablation, and non-thermic methods such as irreversible electroporation.[1] Percutaneous monopolar RFA, has replaced percutaneous ethanol injection after several randomised controlled trials (RCTs) showed a better local control rate by RFA in all trials and an increased overall survival in two RCTs. Currently, monopolar RFA is considered as the standard for percutaneous ablation and was safely used to treat HCCs of <3 cm in patients with portal hypertension and/or mild liver failure, which are classical contraindications to liver resection.

However, several limitations exist using the classical monopolar RFA in terms of efficacy (leading to the risk of treatment failure) and applicability (impossibility to perform the ablation). These limitations could be responsible of downgrading the treatment of early HCCs not amenable to usual monopolar RFA, transplantation, or resection and to a shift to non-curative treatments.

Limitations restraining applicability of usual monopolar RFA were related either to the location of the tumour or to the general condition of the patient. Limitations as a result of the location include HCC not visible at ultrasonography and at-risk locations such as subcapsular HCC (because of the risk of bleeding and tumour seeding), subdiaphragmatic HCC (because of the risk of diaphragmatic lesions), HCC close to biliary structure or gallbladder (because of the risk of thermal-induced lesions) or HCC close to the colon or stomach (because of the risk of perforation). However, several methods have been developed to bypass these limitations.

For at-risk localisation, creation of artificial ascites have proved to protect the colon/small bowel/stomach or the diaphragm from thermal injury and irreversible electroporation. A non-thermic ablation method could be used as an alternative for these at-risk localisations and also for HCC close to the biliary convergence. Moreover, subcapsular HCC could be treated using multibipolar RFA with a no-touch technique to avoid direct puncture of the tumour.[1]

The limitations related to the patients were the presence of biliary anastomosis or sphincterotomy (because of the risk of liver abscess), thrombocytopaenia (because of the risk of bleeding), liver function (that could be deteriorated by the ablation), and presence of pacemakers (because of the risk of interference). Periprocedural transfusion of platelets or use of thrombopoietin-receptor agonists are helpful to safely treat patients with significant thrombocytopaenia (<50,000/mm^3). Bipolar RFA was considered as a safe procedure in patients with a pacemaker because energy is conducted between the two needles and antibiotic prophylaxis considerably reduced the risk of biliary abscess in patients with biliary anastomosis or sphincterotomy.[1]

If patients who are designated Child–Pugh C are contraindicated for percutaneous ablation, several studies have suggested that percutaneous ablation could be performed safely in selected patients designated as Child–Pugh B. However, these results remain controversial as the populations in these studies were heterogeneous in terms of inclusion criteria, and liver failure was associated with a lower overall survival as a result of the complications of cirrhosis in most of these studies.[1]

Regarding oncological outcomes, the overall survival varies are between 50% and 70% at 5 years with 50% to 80% of tumour recurrence at 5 years in HCC within Milan criteria. However, for larger nodules (>2–3 cm), the results of RFA were less effective because of the decreased local control rate with usual monopolar RFA, owing to the difficulty to achieve a sufficient ablation area with the peritumour margin. New methods of ablation including no-touch multibipolar RFA or microwave ablation have been used to increase the ablation margin and increase the efficacy of ablation in tumours of >2 cm. A retrospective multicentric study has suggested that local recurrence was decreased in patients treated by multibipolar RFA compared with patients treated with monopolar RFA. Moreover, multibipolar RFA could be used to treat HCC efficiently in the vicinity of major vessels that are usually associated with less efficacy in monopolar RFA, which is attributable to the heat-sink effect.

In patients with HCC with tumours that are considered resectable, retrospectives studies as well as studies using the Markov model have suggested that percutaneous RFA had similar long-term outcomes compared with liver resection in HCCs of <2 cm.[1] Four RCTs have compared monopolar RFA to resection in the treatment of HCC; one of these trials showed an advantage for resection in terms of overall survival, whereas the three others, including a recent multicentric study conducted in Japan, showed no difference in terms of oncological outcomes between the two treatments.[2] RFA was associated with less morbidity than surgery in all trials. Moreover, in patients who were suitable to undergo transplant, a first-line treatment with RFA followed by salvage liver transplantation for tumour recurrence was associated with prolonged long-term survival and had the advantage of reducing the number of grafts used. Of note, one study reported that a tumour size >2 cm and serum alpha-foetoprotein (AFP) levels >100 ng/ml predict tumour recurrence outside Milan criteria.

Up to 30% of patients with small HCC evaluated for ablation were considered untreatable, mainly because the HCC was not visible at ultrasonography and the presence of obesity is associated with a higher rate of non-visible nodules at ultrasonography. Several studies showed that obesity was associated with the need of more ablation to achieve complete response without difference in terms of long-term oncological outcomes. Moreover, several technical approaches could be used to treat these patients: creation of artificial ascites or pleural effusion, fusion between ultrasonography and pretherapeutic imaging, CT scanner guidance, and lipiodol staining to guide ablation.[1]

In terms of efficacy of percutaneous treatment for HCC developed in NAFLD, a study based on the Surveillance, Epidemiology, and End Results (SEER) and Medicare data in the USA showed that aetiology was not associated with different oncological outcomes. A monocentric study reported a similar recurrence-free survival and better survival after resection and RFA in patients with HCC who also had NAFLD compared with other aetiologies (hepatitis C and chronic alcohol intake) mainly because of the lower rate of cirrhosis in NASH patients.[3] Recently, a multicentric retrospective study including 520 patients treated using multibipolar RFA for a first diagnosis of HCC showed that oncological outcomes were similar in patients with NAFLD compared with other aetiologies.[4] In patients with HCC developed on NAFLD, RFA was a safe and efficient treatment of very early/early HCC (Barcelona Clinic Liver Cancer [BCLC] 0 or A) with a median overall survival of 79 months and 59% of survival at 5 years. Interestingly, in patients with HCC developed on other aetiologies (hepatitis B, hepatitis C, and chronic alcoholic liver disease), the BMI itself or the presence of metabolic syndrome were not associated with a higher rate of tumour recurrence or with poorer survival (Fig. 1).[4]

Some studies reported a higher rate of adverse events following hepatectomy for HCC in patients with metabolic syndrome, whereas studies on RFA did not show any impact of the presence of NAFLD and metabolic syndrome on occurrence of adverse events. This fact should be taken into account when selecting the choice of treatments for early HCC developed on NAFLD.[5]

In EASL guidelines, percutaneous ablation (RFA) is recommended in uninodular HCCs of <2 cm (BCLC 0) as a first-line treatment and in uninodular HCC between 2 and 5 cm or bifocal or trifocal HCC of <5 cm in patients with tumours deemed unsuitable for resection or who are not suitable to undergo transplantation. The aetiologies of underlying liver disease including NAFLD are not used to stratify the treatment received in early-stage HCC.[6]

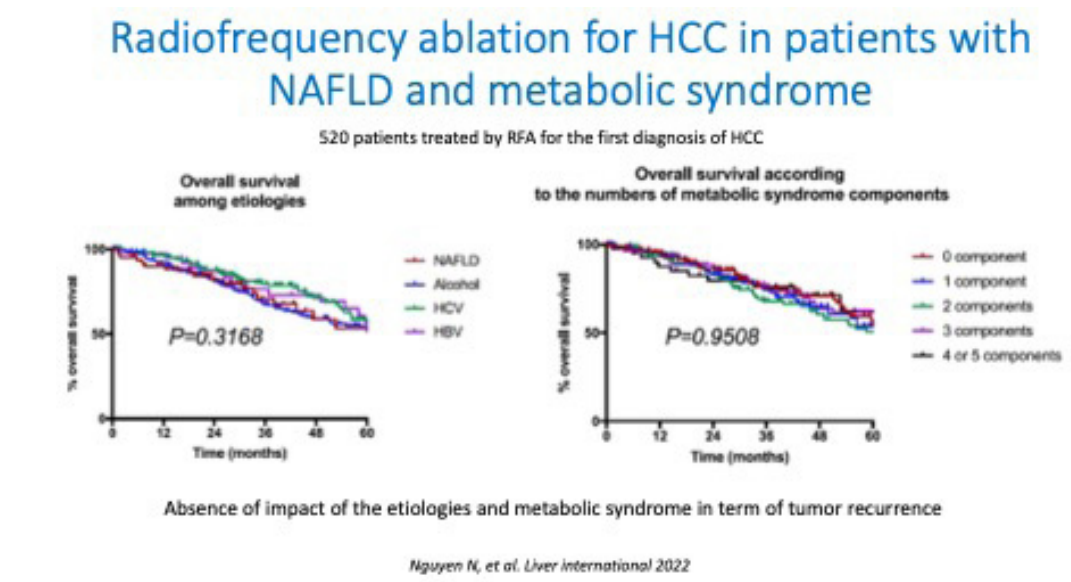

Fig. 1. Outcomes of patients with hepatocellular carcinoma treated using radiofrequency ablation according to the presence of non-alcoholic fatty liver disease and metabolic syndrome.
Adapted from Nguyen, *et al.*[4].

TACE for intermediate HCC

TACE consists of the injection of a chemotherapeutic agent into the tumour-feeding arteries (sometimes mixed with lipiodol), followed by bland embolisation with gelatine sponge cubes. No consensus exists regarding the chemotherapy and techniques used for TACE and a huge variation exists worldwide.

A meta-analysis of several randomised control trials has shown than TACE improved overall survival in patients with intermediate HCC (multifocal HCC without tumour portal vein thrombosis and without metastasis) (Fig. 2). TACE is also frequently used to downstage patients within transplantation criteria (Milan criteria, AFP score, etc.). Where efficient downstaging was obtained the survival and recurrence after transplantation was similar compared with patients within transplantation criteria at baseline.

In contrast, TACE should not be performed in cases of high tumour burden such as bilobar infiltrating HCC because of the risk of adverse events and the low efficacy of the treatment, and systemic treatment should be preferred for these patients. Selection of patients is the key to decrease the risk of complications and liver failure following TACE. This treatment should be avoided in patients with liver dysfunction (Child–Pugh B and C) and with portal thrombosis or inversion of the portal flow because of the risk of adverse events.

New guidance systems can assist the interventional radiologist in using TACE with a supra-selective approach, which is associated with an increased rate of radiological response and decreased damage to the non-tumour liver.

In contrast, new TACE methods such as drug-eluting beads have failed to improved long-term oncological outcomes. Moreover, RCTs testing a combination of TACE with tyrosine kinase inhibitors (such as sorafenib, brivanib, or orantanib) compared with TACE alone failed to improved survival. New RCTs are ongoing combining TACE with immunotherapy or immunotherapy together with anti-VEGF antibody treatment and results are awaited.

One monocentric study identified that high BMI was associated with a lower rate of complete response and higher rate of progressive disease after TACE for HCC. However, this study included mainly patients with chronic hepatitis B and C stratified according to the BMI at baseline. In contrast, one study showed than oncological outcomes and adverse events following TACE were similar in patients with HCC developed on NAFLD-related cirrhosis compared with HCC developed on other aetiologies.[7] Currently, despite a limited number of publications available in the literature, no strong data indicate an impact of NAFLD in patients treated using TACE for intermediate HCC.

According to EASL guidelines, TACE is recommended in patients with intermediate HCC (BCLC B), OMS 0 and Child Pugh A, regardless of the aetiology of the underlying liver disease.[6] No concomitant systemic treatments are currently recommended.

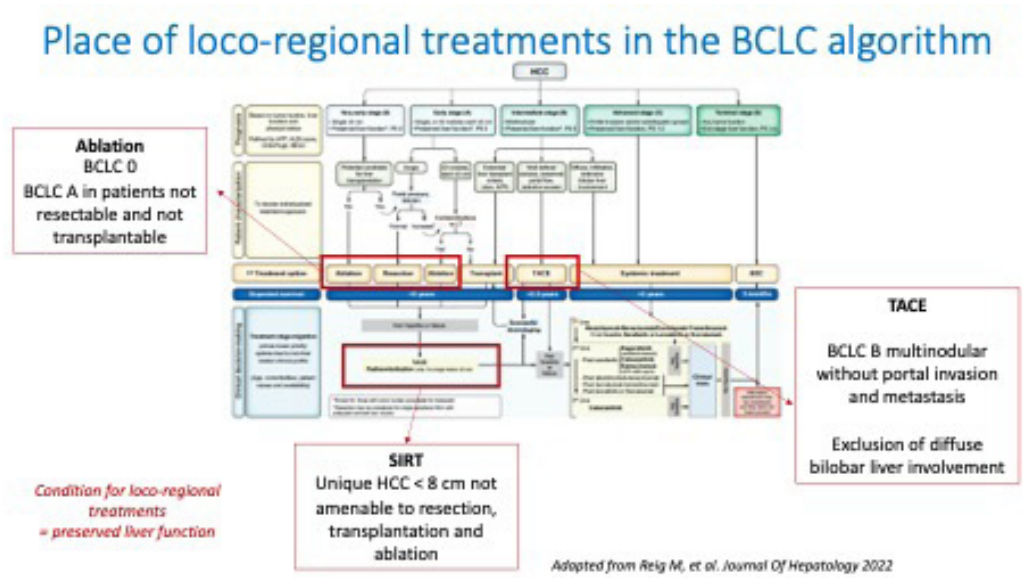

Fig. 2. Strategy of loco-regional treatments in the management of hepatocellular carcinoma according to the BCLC algorithm.
Adapted from Reig *et al.*[9]

The role of SIRT therapy in the management of HCC

SIRT, also called transarterial radioembolisation, is a technique using mostly yttrium-90 and has been widely studied for the past 10 years. SIRT requires a work-up session before treatment to assess the anatomy of the arterial supply to the liver, potential occluded small interconnections between the liver arteries and arteries to other organs, and allow personalised dosimetry to be performed. SIRT can lead to a local antitumour effect that could be delayed up to 3–6 months after the injection.

In patients with uninodular HCC, SIRT has been used to deliver a very high dose of radiation (radiation segmentectomy) in patients with tumours that are inoperable and that are not amenable to ablation. A large uncontrolled prospective study in solitary HCCs of <8 cm has reported 88.3% complete radiological response. To note, the size of the HCC in this series was small (median 2.7 cm). Moreover, the improvement of the ablation method has decreased the number of HCCs considered as not amenable to ablation in clinical practice.

Regarding intermediate stage (BCLC B), retrospective studies and monocentric prospective RCTs comparing SIRT with TACE that included limited numbers of patients have been published. These studies suggested than SIRT had a similar efficacy compared with TACE with less adverse events but the level of evidence is too limited to implement TACE in clinical practice for intermediate stage.

Finally, SIRT has been tested in advanced HCC in two multicentric RCTs comparing SIRT alone *vs.* sorafenib alone and in one multicentric RCT comparing SIRT with sorafenib compared with sorafenib alone. All these RCTs led to negative results in terms of primary endpoints with the absence of difference in term of overall survival between the experimental arm and the control arm. The results of a recent phase III RCT comparing SIRT combined with sorafenib *vs.* sorafenib alone are awaited (STOP-HCC trial). Consequently, SIRT could not be recommended to treat patients with advanced HCC.

However, all these trials have been performed with SIRT without personalised dosimetry. Personalised dosimetry allows a higher dose of radiation to be delivered to the tumour, a surrogate marker of antitumour efficacy in previous clinical trials. Recently, a phase II multicentric RCT comparing SIRT

using personalised dosimetry and SIRT using conventional dosimetry have reported an increased radiological response and prolonged overall survival in patients treated using SIRT with personalised dosimetry.[8] Currently, the best candidate for SIRT seems to be a unilobular HCC without tumour portal invasion or with a limited portal invasion (VP1 = segmental portal vein invasion, VP2 = right anterior/posterior portal vein) in Child–Pugh A patients and treated using personalised dosimetry.

In patients with NAFLD, one small monocentric cohort of patients showed that SIRT has similar toxicity and efficacy in patients who have HCC with NAFLD compared with patients with chronic HBV infection. In the three phase II and III RCTs comparing SIRT (+/- sorafenib) *vs.* sorafenib, the percentage of patients with NAFLD was not reported in two trials (the SORAMIC trial and the SIRVENIB trial) and was 21% in the SARAH trial. No subgroup analysis in patients with NAFLD was reported in these trials.

Currently, SIRT is not recommended in the EASL 2018 guidelines on HCC management because of the low level of evidence available in the literature.[6] In a recent update of the BCLC algorithm, SIRT could be considered to treat unimodular BCLC A HCC of <8 cm that is not amenable to resection or ablation, regardless of the aetiology of the underlying liver disease (Fig. 2).[9]

Conclusions

Loco-regional treatments are widely used for the management of patients with HCC. Percutaneous ablations such as RFA or microwave ablation are the standard of care in uninodular HCC of <2 cm (BCLC 0) and, for patients with tumours that are not amenable to resection or who are deemed unsuitable to undergo transplantation, in uninodular HCC of between 2 and 5 cm. Improvement in ablation techniques has decreased the rate of tumours not amenable to ablation and could increase the efficacy of ablation in tumours that are >2 cm. In intermediate HCC (BCLC B), TACE remains the standard of care except in patients with infiltrative bilobar tumours where systemic treatments should be proposed. Finally, SIRT is now used more in clinical practice, especially in large uninodular HCCs with personalised dosimetry, despite the fact that the evidence available in the literature is low. Currently, the applicability, safety, and efficacy of these loco-regional treatments are similar in patients with NAFLD-related HCC and in patients with HCC related to other aetiologies.

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Systemic therapies for HCC and role of aetiology

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Take-home messages

- The combination of atezolizumab/bevacizumab has revolutionised the treatment of hepatocellular carcinoma (HCC), resulting in unprecedented response rates of up to 30% and median overall survival of 19.2 months. It has been established as the standard of care and benchmark for the management of advanced HCC.
- Durvalumab + tremelimumab – in cases of risk of bleeding – and sorafenib and lenvatinib – in cases of contraindication for immunotherapies – are also indicated as first-line therapies, and the latter as second-line therapy in patients progressing to immune-based regimens.
- Preclinical and clinical data suggest that immunotherapies might be more effective in viral compared with non-alcoholic steatohepatitis-related aetiologies, highlighting the need for stratification of patients according to aetiology in future studies.

Introduction

Hepatocellular carcinoma (HCC) is the fourth leading cause of cancer mortality worldwide, accounting for almost 90% of the total liver cancer burden. HCC generally develops in a background of chronic liver disease, most often caused by hepatitis B (HBV) or C virus (HCV) infection, alcohol abuse and/or non-alcoholic steatohepatitis (NASH)-metabolic syndrome related to obesity and diabetes.[1-4] Early stages of the disease are eligible for potentially curative treatment options such as surgery or locoregional therapies. However, because of the high metastatic potential of the disease, 50–60% of HCC patients eventually progress to, or are diagnosed at, advanced stages of the disease (aHCC) and receive systemic therapies.[1-4]

Over the past decade, the treatment landscape of aHCC has evolved at an unnervingly fast pace and moved towards regimens combining anti-angiogenic therapies with immune checkpoint inhibitors (ICIs). This has led to an improvement in the median overall survival (OS) of patients with aHCC from 8 months – placebo arm, natural history[5]an oral multikinase inhibitor of the vascular endothelial growth factor receptor, the platelet-derived growth factor receptor, and Raf may be effective in hepatocellular carcinoma. METHODS In this multicenter, phase 3, double-blind, placebo-controlled trial, we randomly assigned 602 patients with advanced hepatocellular carcinoma who had not received previous systemic treatment to receive either sorafenib (at a dose of 400 mg twice daily – to more than 19 months with atezolizumab/bevacizumab, a combination of an anti-PDL1 ICI with a vascular endothelial growth factor A (VEGFA) inhibitor, establishing the combination as the new standard-of-care in first-line treatment.[6]open-label, phase 3 trial, patients with unresectable hepatocellular carcinoma who had not previously received systemic treatment were randomly assigned in a 2:1 ratio to receive either atezolizumab plus bevacizumab or sorafenib until unacceptable toxic effects occurred or there was a

loss of clinical benefit. The coprimary end points were overall survival and progression-free survival in the intention-to-treat population, as assessed at an independent review facility according to Response Evaluation Criteria in Solid Tumors, version 1.1 (RECIST 1.1 However, despite the unquestionable benefit of this combination (~30% of patients exhibit an objective response), there is still a need to improve outcome in the remaining 70% of patients.

Here, we provide an overview of current systemic therapies available in the management of aHCC and discuss their implementation into clinical practice. We also discuss the role of underlying liver disease in the therapeutic efficacy of ICI and underline the need for stratification according to aetiology in future studies.

Systemic therapies in the management of advanced HCC

Since the seminal SHARP (Sorafenib Hepatocellular Carcinoma Assessment Randomized Protocol) trial[5]an oral multikinase inhibitor of the vascular endothelial growth factor receptor, the platelet-derived growth factor receptor, and Raf may be effective in hepatocellular carcinoma. METHODS In this multicenter, phase 3, double-blind, placebo-controlled trial, we randomly assigned 602 patients with advanced hepatocellular carcinoma who had not received previous systemic treatment to receive either sorafenib (at a dose of 400 mg twice daily demonstrated clinical benefit of sorafenib compared with placebo in 2007, we have witnessed a myriad of positive phase III trials evaluating systemic therapies in aHCC. Currently, four regimens demonstrated efficacy in the first-line setting and five distinct alternatives in second-line treatment in the West.[2,3]

First-line setting

In the first-line setting, the SHARP trial[5]an oral multikinase inhibitor of the vascular endothelial growth factor receptor, the platelet-derived growth factor receptor, and Raf may be effective in hepatocellular carcinoma. METHODS In this multicenter, phase 3, double-blind, placebo-controlled trial, we randomly assigned 602 patients with advanced hepatocellular carcinoma who had not received previous systemic treatment to receive either sorafenib (at a dose of 400 mg twice daily was the first positive phase III randomised-controlled clinical trial (RCT) to demonstrate OS benefit with systemic therapies in aHCC. The SHARP trial was a placebo-controlled, double-blinded study comparing sorafenib, a multi-target tyrosine-kinase inhibitor (TKI) with anti-angiogenic and anti-proliferative effects, with placebo, demonstrating significant improvements in OS (median OS 10.7 versus 7.9 months). Since then, sorafenib has been widely used globally, and subsequent studies have suggested that it is more effective in liver-only disease, in HCV aetiology and in patients with low neutrophil/lymphocyte ratio. A second era of first-line studies started almost a decade later, with the approval of lenvatinib, a multi-kinase inhibitor blocking FGFR1–4 that was compared to sorafenib in the open-label REFLECT trial with a non-inferiority design.[7] Lenvatinib demonstrated comparable efficacy to sorafenib (hazard ratio [HR] 0.92) with median OS of 13.6 *vs.* 12.3 months. Subgroup analysis yielded better outcomes for lenvatinib in patients with high tumoural burden, aggressive disease, and HBV infection.[3]

In 2020, the pivotal IMBrave150 study marked the start of a third era in the management of aHCC, with the introduction of the first combination regimens. The combination of the atezolizumab, an anti-PDL1 ICI, and bevacizumab, an anti-VEGFA antibody significantly improved OS and progression-free survival (PFS) compared with sorafenib (HR 0.58 and HR 0.59, respectively).[6]open-label, phase 3 trial, patients with unresectable hepatocellular carcinoma who had not previously received systemic treatment were randomly assigned in a 2:1 ratio to receive either atezolizumab plus bevacizumab or sorafenib until unacceptable toxic effects occurred or there was a loss of clinical benefit. The coprimary end points were overall survival and progression-free survival in the intention-to-treat population, as

assessed at an independent review facility according to Response Evaluation Criteria in Solid Tumors, version 1.1 (RECIST 1.1 With median OS of 19.2 months (compared to 13.4 months with sorafenib) and an unprecedented objective response rate (ORR) of up to 30%, atezolizumab/bevacizumab was established as the new standard of care in first-line setting. Importantly, all patients underwent upper endoscopy prior to start of treatment and although overall all grade bleeding events were higher in the treatment arm (25.2% *vs.* 17.3%), grade 3/4 events being 35% *vs.* 45%, respectively

Data from the phase III HIMALAYA study,[8] evaluating the combination of durvalumab (anti-PDL1) with a single high dose of the anti-CTLA4 antibody, tremelimumab, also resulted in an improvement of OS compared with sorafenib (median OS 16.4 months *vs.* 13.8 months). Unlike IMBrave150, this study excluded patients with main portal vein invasion and did not require a baseline upper endoscopy. Interestingly, there was no improvement in PFS, although ORR was 20.1% with the combination *vs.* 5.1% with sorafenib. Overall, the rate of treatment-related adverse events was generally less with the combination, although as expected, there were significantly more immune-related adverse events with the combination, requiring treatment with corticosteroids in ~20% of cases. Other regimens such as camrelizumab plus rivoceranib or tislelizumab demonstrated either superior or non-inferior survival rates, respectively, compared with sorafenib in predominantly Asian trials.

Second-line setting

Three therapies have shown improved OS compared with placebo after progression under sorafenib, namely regorafenib (median OS of 10.6 *vs.* 7.8 months), cabozantinib (median OS 10.2 months *vs.* 8.0 months) and the anti-VEGFR2 antibody, ramucirumab (median OS 8.5 *vs.* 7.3 months), specifically in patients with elevated α-foetoprotein (AFP; >400 ng/ml).[3] Two phase III clinical trials (KEYNOTE-394 and KEYNOTE 240) also evaluated pembrolizumab (anti-PD1) in a second-line setting with similar ORR (14-16%). However, although KEYNOTE-394 hit its primary OS endpoint, KEYNOTE 240 did not reach statistical significance per the prespecified statistical plan. Finally, the dual checkpoint inhibitor combination of ipilimumab (anti-CTLA4) and nivolumab (anti-PD1) demonstrated highly durable responses in 32% of patients and obtained accelerated approval by FDA. However, there was a high rate (>50%) of serious treatment-related adverse events that required the use of corticosteroids.[3]

Management strategy: choice of first-line treatment and sequential strategies

There is a general consensus, supported by guidelines from major international societies in Europe and the USA, that in the absence of contraindications for immunotherapy, the standard of care in first line therapy is atezolizumab/bevacizumab. Importantly, an upper gastrointestinal endoscopy (within 6 months of the start of treatment) is required to rule out high-risk varices. For patients with a high risk of gastrointestinal bleeding, the use of tremelimumab/durvalumab is proposed. Alternatively, for patients with contraindications to immunotherapy (*i.e.* because of autoimmune diseases or liver transplantation), both lenvatinib or sorafenib are the treatment of choice in the first-line setting (Fig. 1).

The remaining question is how to sequence therapies after progression to atezolizumab/bevacizumab as there are no phase III trials assessing the efficacy of second-line therapies in this scenario. Most updated guidelines support the view that sorafenib or lenvatinib should be offered first, maintaining the previously established evidence-based hierarchy before atezolizumab/bevacizumab becoming the first-line preferred treatment.[3] Upon progression to lenvatinib or sorafenib, conventional second-line therapies can be administered. There are no head-to-head comparisons between regorafenib, cabozantinib or ramucirumab, but their reported response rates after TKIs are similar. Regorafenib is indicated in patients that tolerated sorafenib, whereas cabozantinib of ramucirumab were assessed upon progression to sorafenib, the latter indicated only in patients with AFP >400 ng/ml. Finally,

pembrolizumab is FDA approved and can be considered in second-line scenarios in the USA, particularly if adverse events and comorbidities might be detrimental with other agents.

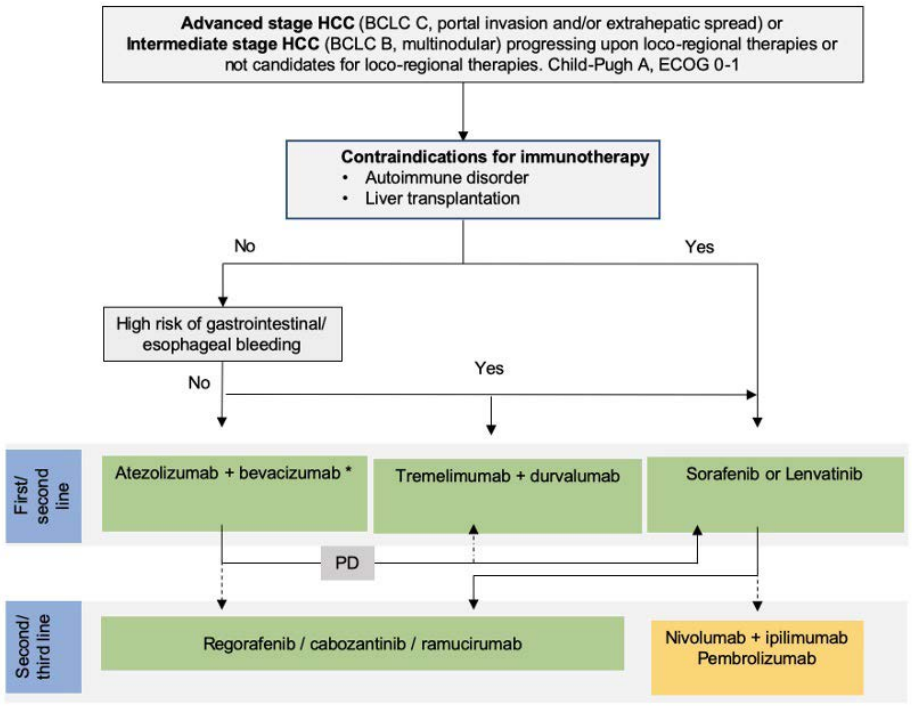

Fig. 1. Proposed treatment algorithm for the management of advanced hepatocellular carcinoma.

Green, regulatory-body-approved regimes based on phase III studies. Yellow, treatments that received FDA accelerated approval based on phase II studies. PD, progressive disease. Figure obtained with permission from Llovet *et al.*[3]

Impact of aetiology in the response to immunotherapies in HCC

As the therapeutic options have expanded, the question of how to best individualise treatment decisions has evolved.[1] Particularly, the role of various HCC aetiologies and how they relate to response and/or resistance to immunotherapy has been of great interest. Importantly, two meta-analyses have suggested that immunotherapies may be more effective in viral compared with non-viral aetiologies,[9,10] underlining the importance of understanding distinct pathogenic pathways and immune profiles associated with viral *vs.* non-viral HCC aetiologies. As per guidelines, aetiology does not directly impact the indication of therapies, but should be taken into account in terms of stratification in clinical trials. Specifically, reporting non-alcoholic fatty liver disease (NAFLD)-related HCC is considered mandatory to explore the impact of immunotherapies in this specific aetiology.

Indeed, chronic viral infections can contribute to hepatocarcinogenesis both by inducing pro-inflammatory innate immune activation and by driving aberrant adaptive immune responses.[2] In contrast, within non-viral aetiologies, several studies have highlighted that NASH-HCC is characterised by specific and unique mutational, immunological, and microenvironmental features. Genetically, NASH-HCC is characterised by an increased prevalence of *TP53* and *ACVR2A* mutations in hepatocytes[11]but its molecular features are not well defined. We aimed to identify unique molecular traits characterising

NASH-HCC compared to other HCC aetiologies. Methods: We collected 80 NASH-HCC and 125 NASH samples from 5 institutions. Expression array (n = 53 NASH-HCC; n = 74 NASH and single nucleotide polymorphisms in genes including *PNPLA3.*[4] Furthermore, from an immunological perspective, distinct innate and adaptive immune cells likely play a role in the development and progression of NASH-HCC. For example, $CD4^+$ T cells, metabolically activated $CD8^+$ T cells, platelets, and dendritic cells (e.g. $XCR1^+$cDC1 cells) have been reported to shape the liver microenvironment and influence the transition from NASH to NASH-HCC. However, neutrophils also populate the liver in various stages of NASH, suggesting a potential prominent role for the innate immune system in NASH-HCC. Strikingly, a marked increase in the abundance of intrahepatic $CD8^+PD1^+$ T cells has been identified in mouse and human NASH. This subset of T-cells has been shown to be auto-aggressive and, despite being exhausted, displays an unconventionally activated phenotype. This in turn leads to resistance to anti-PD1 treatment and enhances HCC development when given prophylactically, suggesting impaired immune surveillance.[10] Finally, in the context of NASH, 30–40% of HCCs develop in non-cirrhotic livers, whereas in viral aetiologies the majority of HCCs (>80–90%) develop at the stage of cirrhosis, further underlining the unique metabolic milieu and the likely contribution of extrahepatic drivers of cancer associated with metabolic syndrome.[4]

Rationale for trial design based on aetiologies in HCC

Despite both preclinical and clinical evidence[9,10] suggesting that aetiology-related differences in the tumour microenvironment can impact response to systemic therapies, NASH-HCC is currently treated using the same approach as other aetiologies of HCC. Indeed, historically, treatment decisions and clinical trial design did not take aetiology of the underlying liver disease into consideration. Most studies report efficacy data according to the typical stratification factors, including Eastern Cooperative Oncology Group-performance status, region, presence or absence of macrovascular invasion, and elevation of AFP. Aetiology is then explored as a clinical factor of interest and most often reported as viral (HBV, HCV, or coinfected) *vs.* 'non-viral' aetiologies, which encompasses all remaining patients, encompassing those with alcohol, NASH, and other aetiologies.[4]

Consequently, the percentage of patients with NASH-related HCC has not been disclosed in any of the ~35 phase III clinical trials reported so far in aHCC. Thus, the indirect measurement for capturing any survival effect associated with aetiology has been to explore the results of ICIs in non-viral aetiologies. A meta-analysis (including three RCTs; IMBrave150, CHECKMATE-459, and KEYNOTE-240) assessing the effect of immunotherapies on OS according to aetiology led to the conclusion that viral-related HCC responds better (HR 0.64, 95% CI 0.50–0.83) than non-viral related HCC (HR 0.92, 95% CI 0.77–1.11, $p = 0.2$).[4] Adding data from the subgroup analysis of the COSMIC-312 trial, which assessed the efficacy of atezolizumab/cabozantinib, the meta-analysis of four RCTs confirmed the difference in efficacy ($p = 0.01$). The difference was still significant, albeit less so ($p = 0.046$) when including the HIMALAYA trial (meta-analysis of five RCTs) that combined two ICIs.[4,8] These results supports the notion that immunotherapies may work better in viral-related HCC than in other aetiologies of HCC, in line with the preclinical observations that NASH-HCC tumours have dysfunctional T cells that impair immune surveillance and limit the antitumoural effect of ICI in this aetiology. Nonetheless, these subgroup observations are not powered for statistical conclusions and are not controlled for other relevant prognostic factors. Furthermore, the classification of 'non-viral aetiologies' is not specific to NASH-related liver disease but also includes alcohol-related liver cancer, idiopathic, and other metabolic causes. In addition, OS will most likely be influenced by other clinical features such as post-progression therapy and other comorbidities, which again are not accounted for.

Taken together, these data support the concept of stratification according to aetiology in future studies, although dedicated prospective studies will be required to determine the definitive role of aetiology in outcome. There is a need to specify which patients have NAFLD-related HCC aetiology in clinical trials conducted in the future, as this is the sole approach that can clarify the effect of immunotherapies on survival in patients with NASH-HCC. In the meantime, although NASH-HCC is clearly biologically distinct, the clinical management and treatment of NASH-HCC is not recommended to be different than for other non-viral aetiologies.

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Overview of management of cholangiocarcinoma

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Introduction

In 2010, the publication of the ABC-02 study in the *New England Journal of Medicine* marked a milestone in the treatment of biliary tumours: the addition of cisplatin increased median overall survival (mOS) from 8 months with gemcitabine monotherapy to 11.7 months. However, a negative phase II trial of mFOLFIRINOX, as well as negative phase III data on gemcitabine and cisplatin combined with Nab-paclitaxel subsequently underscored that further intensification of first-line chemotherapy does not necessarily translate into a therapeutic benefit.[1,2]

In addition to classical chemotherapies, two concepts, immunotherapy and targeted therapies, have become firmly established in the treatment of various cancers over the past 10 years. Indeed, immuno-oncology has recently arrived also in the treatment of biliary carcinoma: 12 years after the ABC-02 trial, the TOPAZ-1 trial, which evaluated the PD-L1 inhibitor durvalumab in combination with gemcitabine and cisplatin, provided positive phase III data in the first-line setting for the first time again. Additive treatment with the immune checkpoint inhibitor increased the mOS from 11.5 months in the control arm to 12.8 months – numerically an increase of about 6 weeks and a 24% reduction in the risk of death.[3] Progression-free survival (PFS) and objective response rate were also improved with the combination therapy compared with chemotherapy alone. In terms of safety, durvalumab was found to be well tolerated in combination with chemotherapy: the incidence of high-grade treatment-related adverse events differed little between the experimental durvalumab arm and the comparator arm, and the quality of life analysis also showed no adverse impact.

In addition, according to the January 2023 press release, the KEYNOTE-966 trial also achieved prolonged survival with the addition of PD-1 inhibitor to chemotherapy alone, meeting its primary endpoint.[4] With now two positive phase III trials, the combination of a checkpoint inhibitor with gemcitabine and cisplatin is the new standard of care in first-line treatment of biliary tumours, and the combination of gemcitabine/cisplatin with durvalumab has already been approved by the European Medicines Agency (EMA) for patients with first-line palliative biliary tumours.

However, despite the justified enthusiasm about a new standard of care, the mOS of less than 13 months in the TOPAZ-1 trial also illustrates that the prognosis for patients with locally advanced or metastatic biliary tumours remains inadequate – especially as second-line chemotherapy regimens with for example FOLFOX, analogous to the ABC-06 trial, also achieved only very moderate success.[5] Initially promising data from the Korean NIFTY trial for liposomal irinotecan in combination with 5-fluorouracil could not be confirmed in the German NALIRICC trial, so the value of irinotecan-based therapy remains unclear.[6]

However, other therapeutic options are available for individual patients, which are located in the field of 'precision oncology'. This therapeutic field has gained momentum in recent years – fueled in part by a much better understanding of tumour drivers in biliary tumours. Advanced molecular analysis is now recommended at an early stage by both the ESMO Guideline 2022, as well as in the German S3 line.[7]

However, implementing precision oncology trial designs in biliary tumours is challenging – the genetic subgroups of this tumour entity, which remains rare, are small, and the trials previously required by

regulatory authorities are often not feasible because of long recruitment times. To date, second-line therapy with the fibroblast growth factor receptor (FGFR) inhibitor pemigatinib is the only molecular therapy approved in Europe for intrahepatic cholangiocarcinomas (CCAs). However, data from small phase II trials or basket studies clearly underline the clinical benefit of targeted therapy in patients with biliary tumours. In the following, we summarise the data on clinically relevant precision oncology concepts in this tumour entity.

IDH1

IDH1 mutations occur in approximately 15% of patients with intrahepatic CCAs.[8] Mutations in the IDH1 gene alter the activity of the mutant enzyme to produce the oncometabolite (R)-2-hydroxyglutarate. Currently available data suggest that detection of an IDH1 mutation has no prognostic or predictive significance for response to first-line platinum-based chemotherapy.[9]

The ClarIDHy phase III trial evaluated the efficacy of the IDH1 inhibitor ivosidenib compared with the placebo control arm after progression on first-line therapy.[10] The primary endpoint, an improvement in PFS, was achieved with a very good hazard ratio of 0.37 with, however, a median PFS (mPFS) of only 2.7 months for ivosidenib compared with 1.4 months with placebo. The radiographic response rate of ivosidenib was very low, with disease stabilisation being the primary focus. Because of the cross over, the impact on OS can only be assessed to a limited extent, but was significantly improved when statistically 'adjusted' for the cross over. Currently, ivosidenib is only approved by the FDA for biliary tumours, so that therapy in Europe can only take place in the context of an individual curative trial with reference to the positive phase III data.

FGFR2

Approximately 10% of patients with intrahepatic CCA have FGFR2 fusions resulting from translocation between the 5' portion of FGFR2 and the 3' end of one of over 200 fusion partners now identified.[8] Several phase II trials have demonstrated consistent efficacy of the pan-FGFR inhibitors pemigatinib, infigratinib, erdafitinib, Debio1347, derazantinib, and futibatinib.[11-15] Although the phase II trials were conducted in partly heavily pretreated patients, response rates ranged from 21% to 42% with disease control rates exceeding 80%. The mPFS and mOS ranged from 7 to 9 and 12 to 22 months, respectively, in the study populations, significantly outperforming second-line chemotherapy results in cross-study comparisons. In Europe, pemigatinib has been approved in FGFR2-fused CCAs since 2022.

The rapid advancement of FGFR inhibitors provides a first indication of what precision oncology 'sequencing concepts' might look like in the future: as a result of different binding properties, sequential use of appropriate FGFR 'inhibitor classes' is already possible in individual patients even when therapy-induced 'on-target' resistance has already occurred. Repeated tumour genetic monitoring – for example by means of liquid biopsy – allows the detection of the respective resistance-inducing changes and enables the informed use of further targeted therapy lines. New generations of FGFR inhibitors, including for example the highly specific FGFR2 inhibitor RLY-4008, are thus expected to have improved activity not only with respect to fusions, but also in the context of primary and acquired FGFR2 resistance mutations.[16]

HER2

HER2-targeted therapies are already long-established in the treatment of breast carcinoma or gastroesophageal tumours.[17] Basically, two strategies can be distinguished: on the one hand, chemotherapy-free combinations of antibodies, and on the other hand, a combination of targeted therapy and chemotherapy. For the purely molecular therapies, the efficacy of dual antibody-based therapy using trastuzumab and pertuzumab has been demonstrated in biliary tumours in intensively pretreated patients in both the MyPathway and TAPUR studies.[18,19] A similar mode of action is also present with zanidatamab, a bi-specific antibody targeting the extracellular juxtamembrane domain and the dimerisation domain of HER2.[20] As a press release, positive data from the phase IIb HERIZON-BTC-01 trial were announced for zanidatamab in December 2022 with response rates above 40% in pretreated patients with biliary tumours.

Combinations of targeted- and chemotherapy are also being pursued in biliary tumours in two approaches. First, a Korean phase II trial demonstrated the efficacy of trastuzumab in combination with FOLFOX in the second-line setting.[21] In addition, the antibody–drug conjugate (ADC) trastuzumab–deruxtecan, for which approvals already exist in breast carcinoma and gastroesophageal tumours is being investigated. Central to the efficacy of ADC is likely the bystander effect exerted on surrounding cells by the cell membrane-targeting topoisomerase-I inhibitor deruxtecan, with trastuzumab 'navigating' the chemotherapy component to HER2-expressing tumour cells. In the HERB study, a phase II trial in biliary tumours also conducted in Korea, the ADC approach showed promising efficacy, particularly in patients with high HER2 expression.[22]

Although HER2 amplifications are prominent in a majority of HER2-altered tumour entities, HER2 mutations are frequently present in biliary tumours. The SUMMIT phase II basket trial specifically evaluated treatment response to therapy with the tyrosine kinase inhibitor neratinib in patients with HER2 and HER3 mutated, but not amplified, tumours.[23] With overall good tolerability, some clinical activity was demonstrated in HER2 mutated tumours, but this appears to drop off compared with the treatment response of HER2-directed therapies in HER2 amplified tumours.

BRAF

An activating V600E mutation in the BRAF gene is found in 3–5% of patients with biliary tumours.[8] In contrast to melanoma, convincing efficacy has not been achieved in gastrointestinal tumours with BRAF-directed monotherapy. However, sequential inhibition of the EGFR pathway using a combination of EGFR and BRAF or BRAF and MEK inhibitors in patients with BRAF-mutated gastrointestinal tumours is more effective, and already part of the established treatment sequence in BRAF V600E mutated colon carcinoma.

In CCA, positive data have been reported from the ROAR basket trial (combination of trametinib [MEK inhibitor] and dabrafenib [BRAF inhibitor]), and also from the TAPUR trial (combination of combimetinib (MEK inhibitor) and vermurafenib (BRAF inhibitor), with impressive response rates of over 50%.[24]

MSI

Microsatellite-instable (MSI)/mismatch-repair-deficient (dMMR) biliary tract tumours are rare, affecting approximately 1% of patients. However, microsatellite instability is one of the most well-established markers of response to immunotherapy in gastrointestinal tumours. Recently, immunotherapy has moved into the first line of therapy with the aforementioned TOPAZ-1 trial. To what extent an intensification of immunotherapy, for example by adding a CTLA4 inhibitor in the sense of a

chemotherapy-free dual checkpoint inhibition could improve the therapeutic success in patients with MSI is currently not investigated.

Other therapeutically relevant genetic alterations

Biliary tumours are heterogeneous – and the mutation profiles also differ depending on the anatomical localisation. Although the alterations discussed so far are among the most common in this tumour entity with direct therapeutic relevance, low-frequency additional alterations are found that represent promising targets for precision oncology concepts. In order not to 'miss' therapeutic options in individual cases, panels as comprehensive as possible should be used in molecular pathological diagnostics. Rare therapy-relevant alterations include other fusion proteins, including NTRK fusions, for which there is already EMA approval for the inhibitors larotrectinib and entrectinib, independent of entity.[25,26] RET fusions are known from lung carcinoma, among others, and – like NRG1 fusions – also play a role in rare cases as putative driver mutations in biliary tumours. Patients with CCA were included in basket studies of both RET inhibitors (selpercatinib and pralsetinib) and NRG1 fusions (seribantumab and zenocutuzumab). If the appropriate alterations are demonstrated, applications for individualised curative trials appear warranted with reference to the data after exhaustion of established therapies.[27-30]

Conclusions

After many years of no significant progress in the treatment of biliary tumours, a number of important studies have been successfully conducted or initiated in recent years that have provided significant insights into systemic therapeutic approaches. One clear innovation – albeit with moderate clinical benefit in the 'all-comer' population – is the introduction of durvalumab (in addition to chemotherapy with gemcitabine and cisplatin) into the first-line treatment of biliary tumours.

The comprehensive annotation of the mutational spectrum of biliary tumours has also helped to recognise the fundamental importance of patient stratification for therapy, paving the way for molecularly targeted therapies. In view of the promising results of precision oncology, early molecular genetic testing is also recommended in the guidelines of German and European professional societies. In view of the fact that biliary tumours *per se* are a 'rare' tumour entity with a broad mutation profile, so that the respective genetic subgroups are correspondingly small, it is imminently important that suitable patients are consistently included in therapy studies. Only in this way can it be ensured in the long term that new therapeutic approaches that expand the spectrum of useful therapies can be established in a targeted and timely manner.

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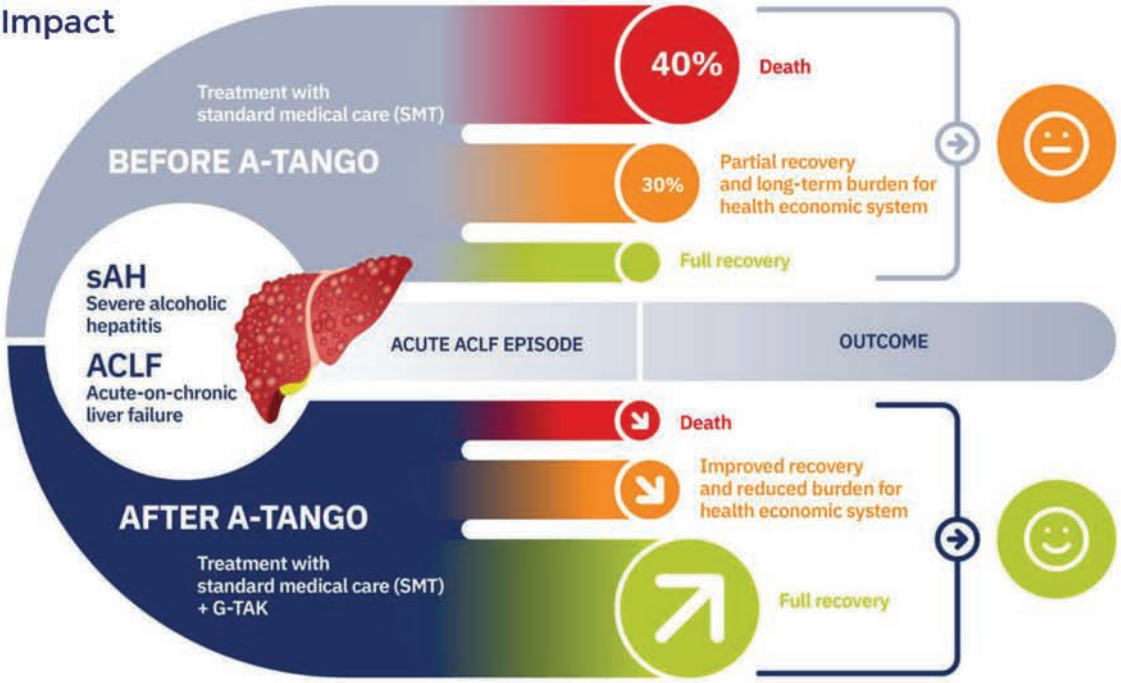
The A-TANGO project - featuring G-TAK, a novel combinatorial therapy for acute-on-chronic liver failure (ACLF)

Expected Impact



- More than **10 million people** suffer from **decompensated cirrhosis** worldwide.
- Effective treatment of ACLF is an urgent and unmet need.
- A-TANGO performs **Phase II clinical studies of G-TAK**, a novel and innovative therapeutic strategy that aims to **reduce inflammation** and **improve hepatocyte proliferation.**
- A-TANGO also strives to identify **reliable biomarkers** for better patient stratification and increased survival.

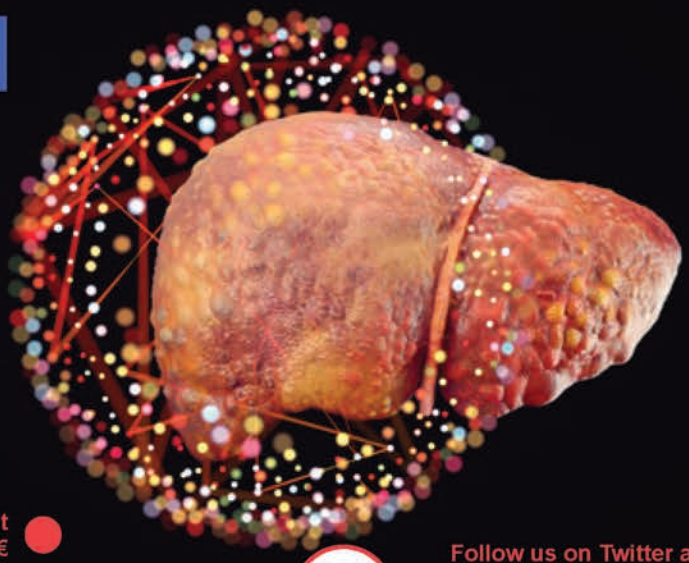
project duration	grant amount	8 countries	kick-off
5.0 years	6.0 million €	14 institutions	1st March 2021

14 partners, one goal: Helping cirrhosis patients in Europe!

www.a-tango.eu
info@atango.eu

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 945096.

DECISION



kick-off
1st April 2020

project duration
5 ½ years

10 countries
21 institutions

grant amount
6 million €

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decision-project

The objective of DECISION is to better understand the pathophysiology of decompensated cirrhosis leading to acute-on-chronic liver failure (ACLF) or death. This consortium will take advantage of already existing large and clinically well-characterised patient cohorts to develop reliable prognostic and response tests and combinatorial therapies tailored to the needs of individual patients to decrease the risk of short-term death.

- Pathophysiologic elucidation of acute decompensated cirrhosis at the systems level (genetics, epigenetics, transcriptomics, metabolomics, lipidomics, miR, and analysis of extracellular vesicles)
- Integration of existing clinical data and new multi-omics data from 2,200 patients with more than 8,600 measurements
- Development of new combinatorial therapies
- Optimization of therapies using existing and new animal models
- Development of novel and robust tests for prediction of outcome following traditional treatment versus response to new therapies
- Phase II clinical trials to test new combination therapies
- Creation of new guidelines for outcome prediction and personalized treatment of acute decompensated cirrhosis to prevent ACLF to death

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 847949.

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Understanding **G**ene **EN**vironment **I**nteraction in **AL**cohol-related hepatocellular carcinoma

A project that will use AI models to integrate genetic and non-genetic information, including digital imaging, to develop novel cost-effective strategies towards prevention and early-stage detection of ALD-HCC

AIM:

- **To portray genetic and environmental determinants promoting ALD-HCC;**
- **To evaluate how they interact at cellular level in human samples and preclinical models to get novel insights into liver carcinogenesis and identify chemopreventive targets; and**
- **To assess how they modulate the risk of ALD-HCC in several retrospective and prospective cohorts of patients included in HCC surveillance programs.**

The GENIAL Project is funded by the European Union within the Horizon Europe programme under grant agreement No 101096312.

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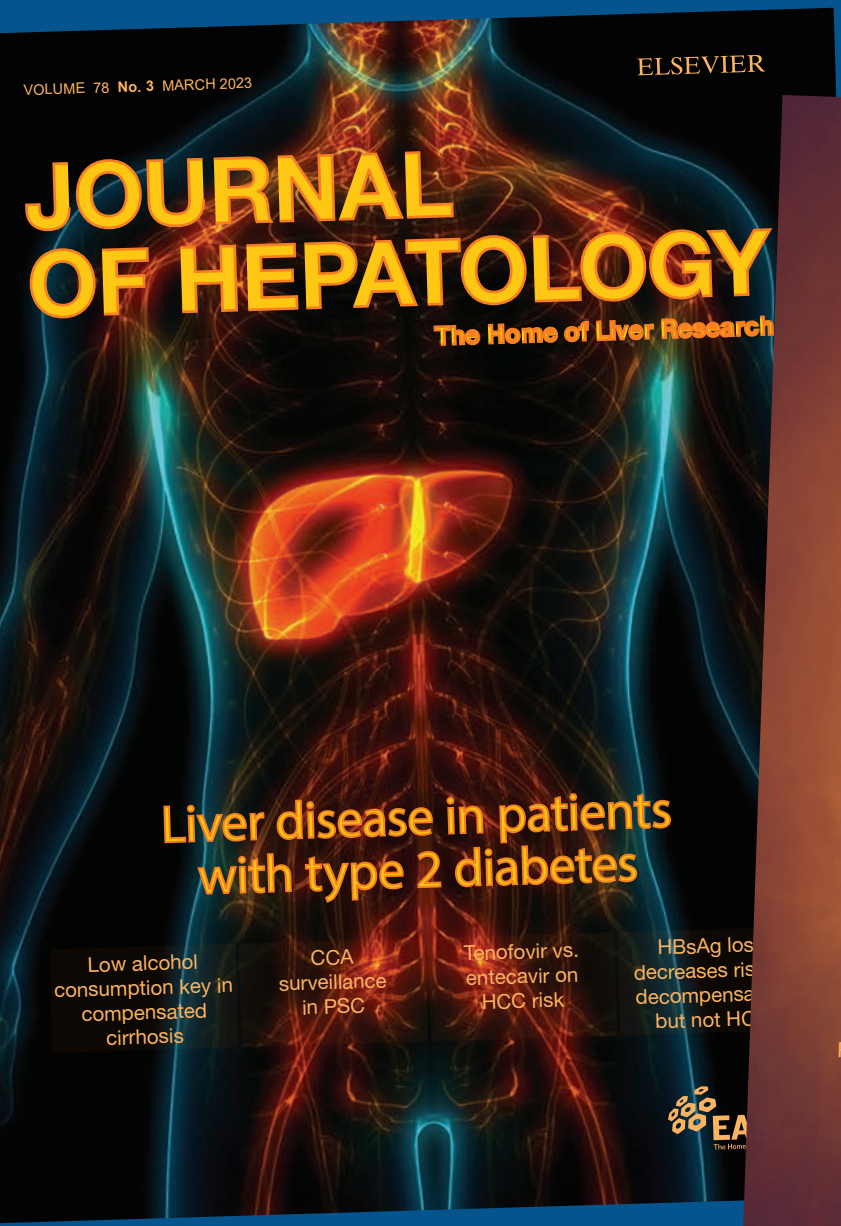

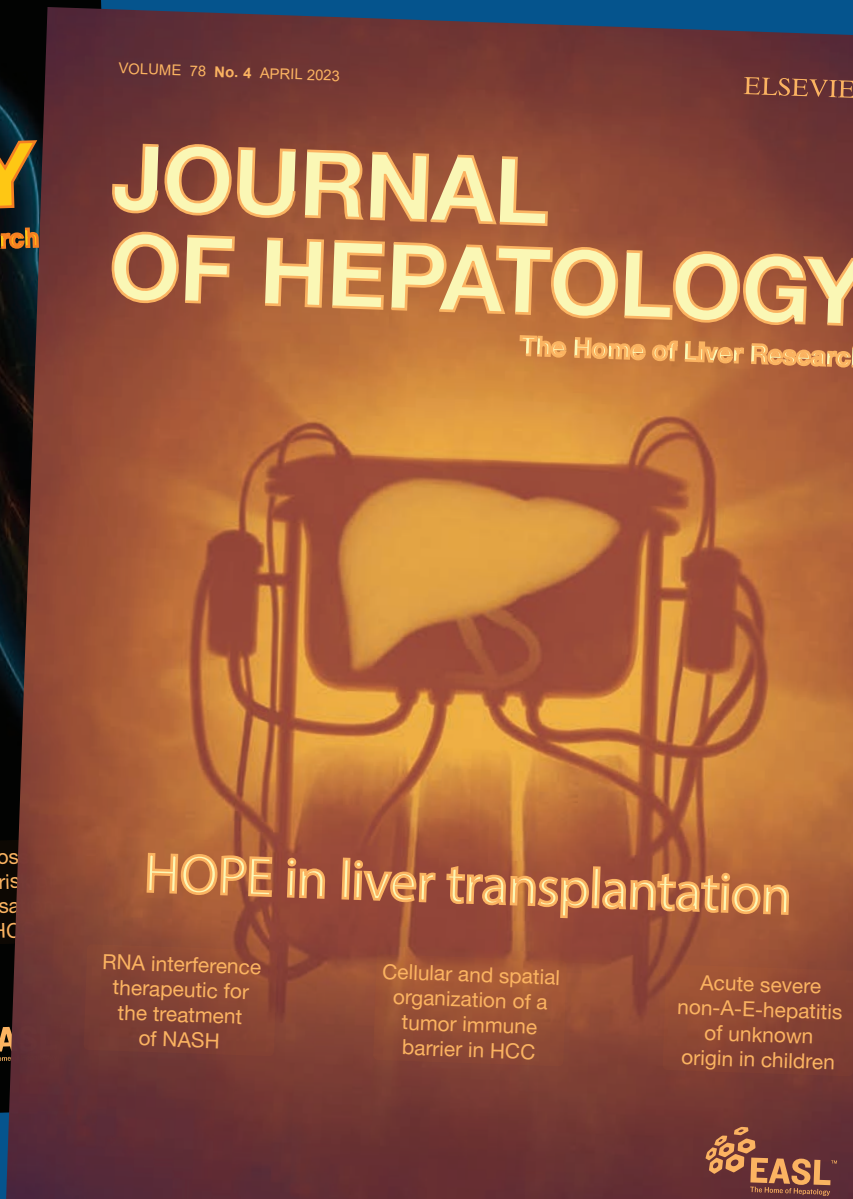

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The LITMUS project has received funding from the Innovative Medicines Initiative 2 Joint Undertaking under grant agreement No. 777377. This Joint Undertaking receives support from the European Union's Horizon 2020 research and innovation programme and EFPIA.

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Grant amount
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22 Partners

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MICROB-PREDICT will investigate

the human microbiome to identify predictors and mechanisms associated with the development of decompensation of cirrhosis and progression to acute-on-chronic liver failure (ACLF) and death.

- → New microbiome-based tests for better stratification of cirrhosis patients
- → Personalized prediction and prevention of decompensation and ACLF
- → Clinical trial to predict response to treatment
- → Modern, effective nanobiosensors as clinical tools with improved specificity
- → More personalized treatment
- → Increased survival times
- → Decreased costs for the health systems

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