THE INTERNATIONAL LIVER CONGRESS™

Postgraduate course

The Future of Clinical Hepatology

22–23 June 2022

EASL™
The Home of Hepatology

easl.eu/pgc2022

Table of contents

Session 4: Big data and personalised medicine

Session 5: Digitalised medicine in practice

GENERAL INFORMATION

EASL Governing Board

Secretary General

Prof. Thomas Berg
Germany

Vice-Secretary

Prof. Aleksander Krag
Denmark

Treasurer

Prof. Francesco Negro
Switzerland

Scientific committee members

Prof. Emmanuel Tsochatzis
United Kingdom

Dr Tobias Böttler
Germany

Dr Virginia Hernández-Gea
Spain

Dr Jean-Charles Nault
France

Prof. Saskia van Mil
The Netherlands

Prof. Luca Valenti
Italy

Educational councillor

Prof. Ulrich Beuers
The Netherlands

EU policy councillor

Prof. Maria Buti
Spain

ILC 2021 Honorary President

Prof. Mario Rizzetto
Italy

Welcome message from the course organisers

Dear colleagues, dear friends,

After 2 years of COVID pandemy, we have built up for you this postgraduate course oriented to our future in Hepatology. It is important for our community to enter hepatology and the field of liver diseases in the Future. We will review with international experts the new developments in endoscopy, interventional radiology, liver surgery and liver transplantation. We will develop the new possibilities and limits in liver support and liver failure. This programme will show the place of new biomarkers in diagnosis and prognosis of liver diseases. The use of big data and the place of personalised medicine in liver diseases and liver cancer will be reviewed. Finally, the development of digital health in liver diseases diagnosis and management will be developed.

We hope you will enjoy this exciting program

Andres Cardenas
Spain

Didier Samuel
France

Norah Terrault
United States

Programme

Postgraduate Course: The future of clinical hepatology

Organisers

Andres Cardenas, *Spain*

Didier Samuel, *France*

Norah Terrault, *United States*

WEDNESDAY 22 June 2022

Session 1: Innovations in radiology, endoscopy and robotic surgery

Chairs:
Andres CARDENAS, *Spain*
Julie HEIMBACH, *United States*

12:00-12:05 **Case presentation**
Andres CARDENAS, *Spain*

12:05-12:35 **Non-invasive-testing for chronic liver disease**
Annalisa BERZIGOTTI, *Switzerland*

12:35-13:05 **Role of endoscopy in hepatology**
Wim LALEMAN, *Belgium*

13:05-13:35 **Future of radiological intervention**
Riad SALEM, *United States*

13:35-13:00 **Robotic surgery and liver transplantation**
Julie HEIMBACH, *United States*

Session 2: Liver support and regeneration

Chairs:
Anil DHAWAN, *United Kingdom*
Didier SAMUEL, *France*

14:30-14:40 **Case presentation: Artificial support in patient with ACLF awaiting Liver transplantation**
Audrey COILLY, France

14:40-14:55 **Clinical applications of liver support**
Faouzi SALIBA, France

14:55-15:10 **Hepatocyte transplantation in acute liver failure**
Anil DHAWAN, United Kingdom

15:10-15:25 **Progress and limits in the use of liver cells in clinical practice**
Fotis SAMPAZIOTIS, United Kingdom

15:25-15:40 **How to stimulate liver regeneration in liver transplantation and surgery?**
Pierre CLAVIEN, Switzerland

15:40-16:00 **Discussion and Q&A**

Session 3: Frontiers in biomarkers

Chairs:
Pierre-Emmanuel RAUTOU, *France*
Norah TERRAULT, *United States*

16:30-17:00 **Prognostic biomarkers of non-malignant liver disease – leaving the biopsy behind**
Pierre-Emmanuel RAUTOU, *France*

17:00-17:30 **Improving on MELD: New biomarkers to improve risk stratification for wait-listed patients**
Elizabeth VERNA, *United States*

17:30-18:00 **Predictive biomarkers for the optimisation of liver cancer diagnosis and therapy**
Laura GRAMANTIERI, *Italy*

THURSDAY 23 June 2022

Session 4: Big data and personalised medicine

Chairs:
Peter GALLE, *Germany*
Rohit LOOMBA, *United States*

08:00-08:10 **Case presentation: personalised medicine in the treatment of liver cancer**
Charlotte COSTENTIN, *France*

08:10-08:30 **How personalised medicine may affect our strategies in liver diseases: the example of liver cancer**
Peter GALLE, *Germany*

08:30-08:50 **Personalised approaches to NAFLD**
Rohit LOOMBA, *United States*

08:50-09:10 **Modelling the course of cirrhosis**
Gennaro D'AMICO, *Italy*

09:10-09:30 **Discussion and Q&A**

Session 5: Digitalised medicine in practice

Chairs:
Norah TERRAULT, *United States*
Maja THIELE, *Denmark*

10:00-10:20 **Social media as a tool for patient engagement and education**
Maja THIELE, *Denmark*

10:20-10:40 **E-health: Optimising remote management of patients with liver disease**
Vijay SHAH, *United States*

10:40-11:00 **Translating AI into better medical care: the example of medical imaging**
Anima ANANDKUMAR, *United States*

State of the Art: The future of omics and big data

11:00-11:30 Manimozhiyan ARUMUGAM, *Denmark*

Abbreviations and Acronyms

2D-SWE	Two-dimensional shear wave elastography
AASLD	American Association for the Study of Liver Diseases
ACLF	Acute-on-chronic liver failure
ADVOS™	Advanced Organ Support system
AEs	Adverse events
AFM	Accelerated failure model
AFP	Alpha-foetoprotein
AI	Artificial intelligence
AKI	Acute kidney injury
ALD	Alcohol-related liver disease
ALF	Acute liver failure
ALT	Alanine aminotransferase; auxiliary liver transplantation
A-PHPBA	Asian-Pacific Hepato-Pancreato-Biliary Association
APRI	AST to platelet ratio
ArLD	Alcohol-related liver disease
ASO	Anti-sense oligonucleotide
AST	Aspartate aminotransferase
BCLC	Barcelona Clinic Liver Cancer
BEST	Biomarkers, EndpointS, and other Tools
BO	Bayesian Optimisation
cACLD	Compensated advanced chronic liver disease
CAR	Constitutive androstane receptor
CKD	Chronic kidney disease
CLD	Chronic liver disease
CLIF	Chronic liver failure
CRISPR	Clustered regularly interspaced short palindromic repeat
CSPH	Clinically significant portal hypertension
CT	Computed tomography
CTLA-4	Cytotoxic T-Lymphocyte-Associated protein 4
CUSA	Cavitron ultrasonic surgical aspirator
DCP/PIVKA-II	Des-γ-carboxy prothrombin/ prothrombin induced by vitamin K absence-II
DL	Deep learning
EASL	European Association for the Study of the Liver
EBL	Endoscopic band ligation
ECAD	Extracorporeal albumin dialysis
ECHO	Extension for Community Healthcare Outcomes
ECM	Extracellular matrix
ECOG	Eastern Cooperative Oncology Group
eGFR	Estimation of GFR
EHPVO	Extrahepatic portal vein occlusion
EHRs	Electronic health records
ELAD	Extracorporeal cellular therapy
ELF	Enhanced liver fibrosis
EL-FIT app	Exercise and Liver FITness app
ELPA	European Liver Patient Organisation
ERCP	Endoscopic retrograde cholangiopancreaticography
ESMO	European Society for Medical Oncology
EUS	Endoscopic ultrasound
FAST score	FibroScan-AST score
FDA	Food and Drug Administration
FIB-4	Fibrosis-4
FISH	Fluorescence in-situ hybridisation
FLR	Future liver remnant

FNA	Fine-needle aspiration
GF	Growth factor
GFR	Glomerular filtration rate
GI	Gastrointestinal
GMP	Good medical practice
GOV	Gastroesophageal varices
GP3	Glypican 3
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HLC	Hepatocyte-like cell
HMB	Hepatocyte microbead
HOPE	Hypothermic oxygenated perfusion
HPB	Hepatopancreaticobiliary
HPVE	High-volume plasma exchange
HT	Hepatocyte transplantation
HVPG	Hepatic venous pressure gradient
ICAM-1	Intercellular adhesion molecule 1
ICU	Intensive care unit
IGV	Isolated gastric varices
ILLS	International Laparoscopic Liver Society
iPSC	Induced pluripotent stem cell
IRI	Ischaemia–reperfusion injury
KC	Kupffer cell
L3%	Lens Culinaris Agglutin-3
LASSO	Logistic regression with least absolute shrinkage and selection operator
LB	Liver biopsy
LI-RADS	Liver Imaging Reporting and Data System
LLS	Left lateral donor segmentectomy
LNCRNAs	Long non-coding RNAs
LSAM	Liver Simulated Allocation Model
LSM	Liver stiffness measurement
LTx	Liver transplantation
MAFLD	Metabolic fatty liver disease
MAST	MRI-AST
MDSCs	Myeloid-derived suppressor cells
MEFIB	MRE plus FIB-4
MELD	Model for end-stage liver disease
MeSH	Medical Subject Heading
ML	Machine learning
MRE	Magnetic resonance elastography
MRI	Magnetic resonance imaging
MRI-PDFF	Magnetic resonance imaging-proton density fat fraction
MSC	Mesenchymal stem cell
NAFLD	Non-alcoholic fatty liver disease
NAS	NAFLD Activity Score
NASH	Non-alcoholic steatohepatitis
NFS	Non-alcoholic fatty liver disease fibrosis score
NIH	National Institutes of Health
NIT	Non-invasive test
NK	Natural killer
NMP	Normothermic machine perfusion
NNs	Neural networks
NSBB	Non-selective B blocker
OGD	Oesophagogastroduoscopy
OLT	Orthotopic liver transplantation
ORR	Overall response rate
OS	Overall survival
PALF	Paediatric acute liver failure
PD-1	Programmed Death 1
PDFF	Protein density fat fraction
PE	Plasma exchange
PFS	Progression-free survival

PHLF	Post hepatectomy liver failure
PHT	Portal hypertension
PNPLA3	Patatin-like phospholipase domain containing 3
PPV	Positive predictive value
PSC	Primary sclerosing cholangitis
pSWE	Point shear wave elastography
PVE	Portal vein embolisation
PVE-HPE	PVE with hepatic vein deprivation
PVL	Portal vein ligation
PVT	Portal vein thrombosis
RCT	Randomised controlled trial
RNN	Recurrent neural network
RPM	Remote patient monitoring
SCAN-ECHO	Specialty Care Access Network-ECHO
SFSS	Small-for-size syndrome
sHx	Standard hepatectomy
SMT	Standard medical treatment
SOC	Standard of care
SOFA	Sequential organ failure assessment
SoMe	Social media
SVMs	Support vector machines
TACE	Transarterial chemoembolisation
TAMs	Tumour-associated macrophages
TCGA	The Cancer Genome Atlas
TE	Transient elastography
TIME	Tumour immune microenvironment
TIPS	Transjugular intrahepatic portosystemic shunt
TKI	Tyrosine-kinase inhibitor
TM6SF2	Transmembrane 6 superfamily member 2
TME	Tumour microenvironment
TRE	Transarterial radioembolisation
Tregs	Regulatory T cells
TSH	Two-stage hepatectomy
TTP	Time to progression
US	Ultrasound
VAS	Visual analogue scale
VCAM-1	Vascular cell adhesion molecule 1
VCTE	Vibration-controlled transient elastography
VEGF	Vascular endothelial growth factor
VHA	Veterans Health Administration
vWF	von Willebrand factor

EASL™ | CAMPUS

easlcampus.eu

Open-access eLearning anytime, anywhere

START LEARNING

U.E.M.S.

2,000+ resources available
21 CME-accredited courses

- 19 CPG slide decks
- 20,300 registered users
- 4,100 ePosters
- 2,200 webcasts

EASL™
The Home of Hepatology

Young Investigators

The future of hepatology

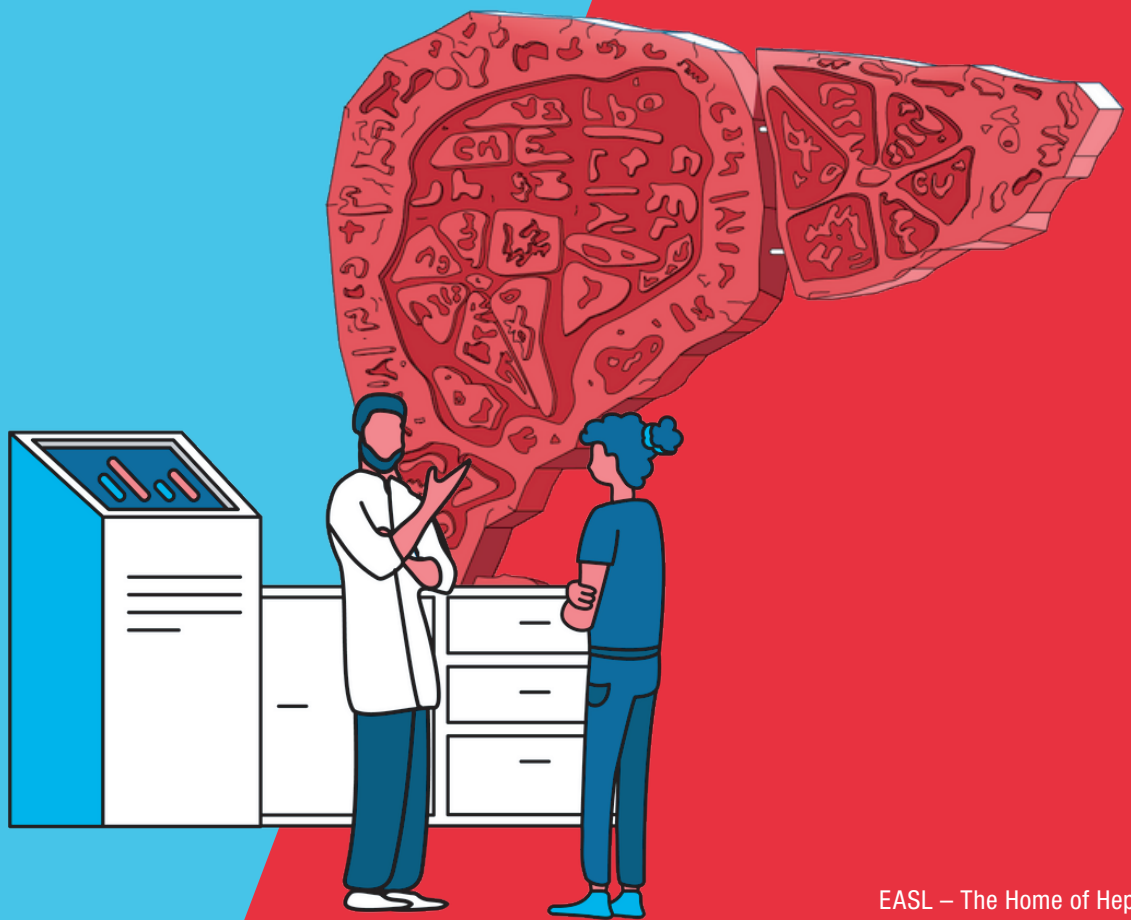
SCAN FOR MORE INFO

+ YIs Task Force
+ YIs webinars
+ EASL Schools & Masterclasses
+ Fellowships & Mentorships
+ EASL Emerging Leader Award
+ Abstracts & Bursaries at events
+ YIs newsletter

SESSION 1

INNOVATIONS IN RADIOLOGY, ENDOSCOPY AND ROBOTIC SURGERY

WEDNESDAY 22 JUNE
12:00 - 14:00



Non-invasive-testing for chronic liver disease

Annalisa Berzigotti

Department of Visceral Surgery and Medicine, Bern University Hospital, University of Bern, *Switzerland*

E-mail address: annalisa.berzigotti@insel.ch

Take-home messages

- Non-invasive tests (NITs) are currently widely used in clinical practice and allow compensated advanced chronic liver disease to be ruled out.
- Liver stiffness measurement and simple laboratory tests allow identification of patients at high risk of clinically significant portal hypertension and clinical events, who are candidates for therapy with non-selective beta-blockers, and/or trials with novel therapies.
- The dynamic use of NITs improves the prediction obtained at baseline, as it mirrors liver fibrosis progression and regression.

Non-invasive tests in 2022

Chronic liver disease (CLD) is characterised by a long asymptomatic compensated stage, lacking specific clinical signs.[1] A large amount of data showed that severity of fibrosis stage 2 or greater, and in particular presence of cirrhosis on histology, and presence of clinically significant portal hypertension (CSPH, as defined by a hepatic venous pressure gradient [HVPG] ≥10 mmHg), are major prognostic factors in patients with compensated CLD of any aetiology. This also applies to non-alcoholic fatty liver disease (NAFLD). However, based on a merely clinical assessment, identifying patients at risk of developing clinically relevant events (clinical decompensation, hepatocellular carcinoma, liver-related death) is challenging. Liver biopsy remains the reference standard method to stage liver fibrosis, but carries risks, is expensive, and is subject to sampling error and intra- and inter-reader variability. HVPG measurement is not available in the majority of non-university centres, owing to a lack of specific expertise. Endoscopy is the reference standard for the diagnosis and grading of gastroesophageal varices, but interobserver variability is high.

NITs have been largely tested as diagnostic methods for identifying significant liver fibrosis and candidates for antiviral therapy among patients with chronic hepatitis B and C. Importantly, some NITs showed a high prognostic value, comparable to that of liver fibrosis on histology. NITs have been also studied as screening tools for fibrotic liver disease in patients in primary care and carrying risk factors for NAFLD/metabolic fatty liver disease (MAFLD) or alcohol-related liver disease (ArLD).

Ideally, NITs should be simple, inexpensive, reproducible, and repeatable. They should be quantitative and should be used as continuous tests reflecting the probability of the condition under investigation. In clinical practice, they are often applied in an oversimplistic way, using a single cut-off value to rule-in or rule-out the condition under investigation. The current guidelines suggest to use at least 2 cut-offs optimised to >90% sensitivity and >90% specificity, respectively, to identify 3 categories of patients: at very low risk (not requiring additional testing), at very high risk (in whom the condition under investigation is very likely, so not necessarily requiring additional testing) and intermediate risk patients, requiring further testing.

NITs should be validated against the reference standard for diagnostic use, but they should ideally be able to identify changes in the underlying liver disease (worsening or improvement; dynamic use of NITs), and correlate with clinically relevant endpoints (clinical decompensation, risk of hepatocellular carcinoma, risk of cardiovascular events – particularly in NAFLD, death).

Types of NITs in hepatology

NITs in hepatology include a large number of different categories of tests such as blood tests, elastography, and imaging biomarkers.[1] In general, NITs are more useful for ruling-out liver fibrosis and cirrhosis, than ruling them in.

Blood tests

Blood tests are a favourable option, as they can be easily performed, and in principle, are suited to population screening, for instance in a primary care setting. For this aim, a negative result of NITs could rule-out advanced liver disease/cirrhosis with high confidence, avoiding referral to a specialist setting.

Serum markers of fibrosis include in NAFLD:

- routine tests, indirect markers of fibrosis: aspartate aminotransferase (AST), alanine aminotransferase (ALT), platelet count, used individually or in combination, for example AST to platelet ratio (APRI).
- simple combinations of routine tests and clinical variables (*e.g.* age, BMI), such as the FIB-4 (age, AST, ALT, and platelet count) and NAFLD fibrosis score (NFS, comprising age, BMI, albumin, impaired fasting glucose/diabetes, AST, ALT, platelets). In a large meta-analysis in patients with histology-proven NAFLD, FIB-4 and NFS showed a good discriminative ability for fibrosis stage ≥3 (AUROC 0.84 for both scores).2 In addition, a FIB-4 <1.3 excludes F3–F4 with >90% negative predictive value, and is recommended as a first screening test in primary care.1,3
- patented combination of direct and indirect markers of fibrosis; this category includes the Enhanced Liver Fibrosis (ELF) score and the Fibrometer. In NAFLD, ELF <7.7 excludes significant fibrosis and >10.18 rules in F3–F4.[4]
- mac-2 binding protein glycosylation isomer (M2BPGi), N-terminal propeptide of type 3 collagen (Pro-C3) and type 4 collagen are emerging as novel markers of fibrosis, but are not yet recommended in clinical practice.

Novel patented tests based on lipidomics to diagnose NASH have been developed, but are not yet used in practice.

Elastography

Elastography measures the elastic properties of liver tissue. Fibrosis is stiffer than the healthy liver tissue, and can be quantified by measuring the shear waves or the strain waves generated into the tissue by applying an external force. There are different methods to generate the tissue movement and to measure the velocity of waves in the tissue. The first method that was available and is now well validated is transient elastography (TE; FibroScan, Echosens), which is a point-of-care method, and requires a self-standing device. Liver stiffness measurement (LSM) using this technology has an accuracy of >90% for diagnosing cirrhosis. The cut-offs suggested to rule-in and rule-out cirrhosis vary according to the aetiology of CLD. However, based on a large amount of data, and considering that liver fibrosis and risk are a continuum, recent guidelines suggested to use the pragmatic term ‘compensated advanced chronic liver disease’ (cACLD), aimed at stratifying the risk of CSPH and decompensation at the bedside, irrespective of the histological stage.[5]

Importantly, liver stiffness using TE is the single best NIT to identify cACLD across aetiologies and in NAFLD.[6] Irrespective of the technique used for its measurement, it has prognostic value in patients with cACLD, both at baseline, and at follow-up.

According to the current definition and using TE,[5] patients with LSM <10 kPa can be considered at low risk (the lower the LSM, the lower the risk), with an overall risk of decompensation and liver-related death <1% at 3 years, whereas patients above this threshold are at a progressively higher

risk and should be referred to a specialist centre if LSM has been performed in primary care. LSM >25 kPa is highly predictive of CSPH. Values of LSM >20 kPa in combination with platelet count <150 G/L are also strongly associated with CSPH, and values of LSM <20 kPa in patients with normal platelet count allow safely ruling-out high-risk oesophageal varices (<5% missed high risk varices), in all the main aetiologies of liver disease. Importantly, in patients with hepatocellular carcinoma, NITs can identify patients at higher risk of adverse outcomes after surgery.[7]

The Baveno VII consensus conference on portal hypertension[5] has suggested the use of a simple 'rule of five' to identify patients with compensated CLD at risk of clinically relevant events is summarised in Fig. 1.

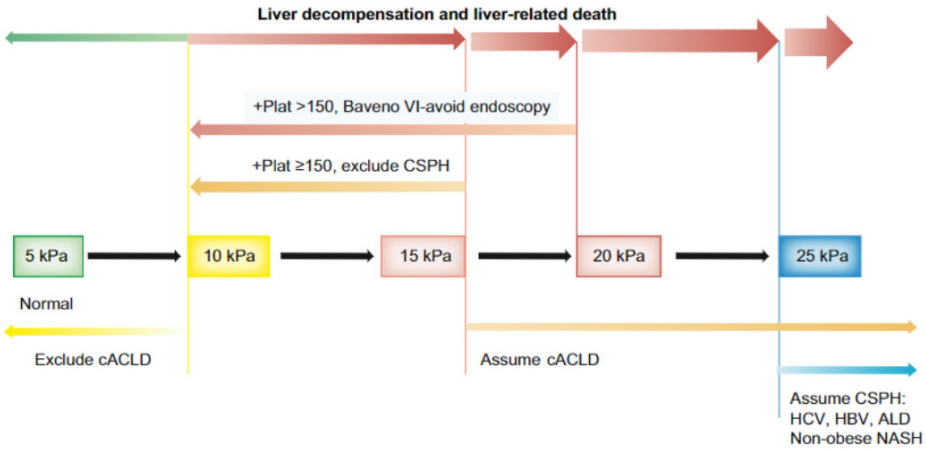


Fig. 1. Algorithm for the non-invasive determination of cACLD and CSPH according to the Baveno VII Consensus Conference. ALD, alcohol-related liver disease; cACLD, compensated advanced chronic liver disease; CSPH, clinically significant portal hypertension; NASH, non-alcoholic steatohepatitis.5

In patients with NASH CLD and obesity, taking into account the BMI helps in refining this rule.[8]

Newer elastography techniques include ultrasound elastography methods (point shear wave and 2D shear wave elastography), and magnetic resonance elastography (MRE). Overall, the accuracy of these methods to identify cirrhosis and to stratify the prognosis of compensated patients seems high, but data are much less abundant than those available for TE.9 As for the diagnosis of fibrosis and cirrhosis in patients with NASH, in a large meta-analysis, MRE showed an accuracy higher than TE for F2 (AUROC 0.91 *vs.* 0.83) and F3 (0.92 *vs.* 0.85), but is only marginally better at diagnosing, and F4 (0.90 *vs.* 0.89).10

Given its higher cost, MRE is still not used routinely in clinical practice in Europe.

Imaging

Imaging methods to assess cACLD range from simple morphologic assessment using grey scale ultrasound to Doppler-based parameters (flow presence and assessment), to complex contrast-enhanced based techniques on computed tomography (CT) and magnetic resonance imaging (MRI).[11]

As for methods to assess patients with NAFLD, in an individual patient meta-analysis of 543 patients with NAFLD proven on histology, corrected T1 (cT1) on MRI, combined with MRI quantification of liver fat, allowed identification of NASH (NAFLD activity score ≥4) with an area under the ROC curve of 0.82 (95% CI 0.78–0.85); in addition, cT1 discriminated between patients with or without fibrosis stage ≥2 with good accuracy (AUROC = 0.78; 95% CI, 0.74–0.82).[12]

Among simple, available imaging signs of cirrhosis, the nodularity of liver surface on ultrasound or on CT (which can be quantified with a specific software) has a high accuracy.

Dynamic testing

Changes of NITs in chronic hepatitis C, ArLD, and NAFLD (FIB-4[13,14]; ELF[15]; LSM by TE[16] and by MRE[17]) over the course of liver disease mirror changes in liver fibrosis and in prognosis. In particular, patients with cACLD, using TE-based LSM, a decrease in LSM of >20% with a final value below <20 kPa, or any decrease to an LSM <10 kPa is associated with a large reduction in the risk of clinical decompensation and liver-related death.[5]

Testing–retesting remains challenging in NITs because of the high rate of false-positive results attributable to different factors (unspecific inflammation for blood markers and for TE), and is being studied in large international cohorts.

Current and future approach

The current use of NITs has been recently outlined in the 2021 update of the European Association for the Study of the Liver (EASL) clinical practice guidelines.[1] The suggested algorithm for the use of NITs in the setting of a compensated patient is provided in Fig. 2[1].

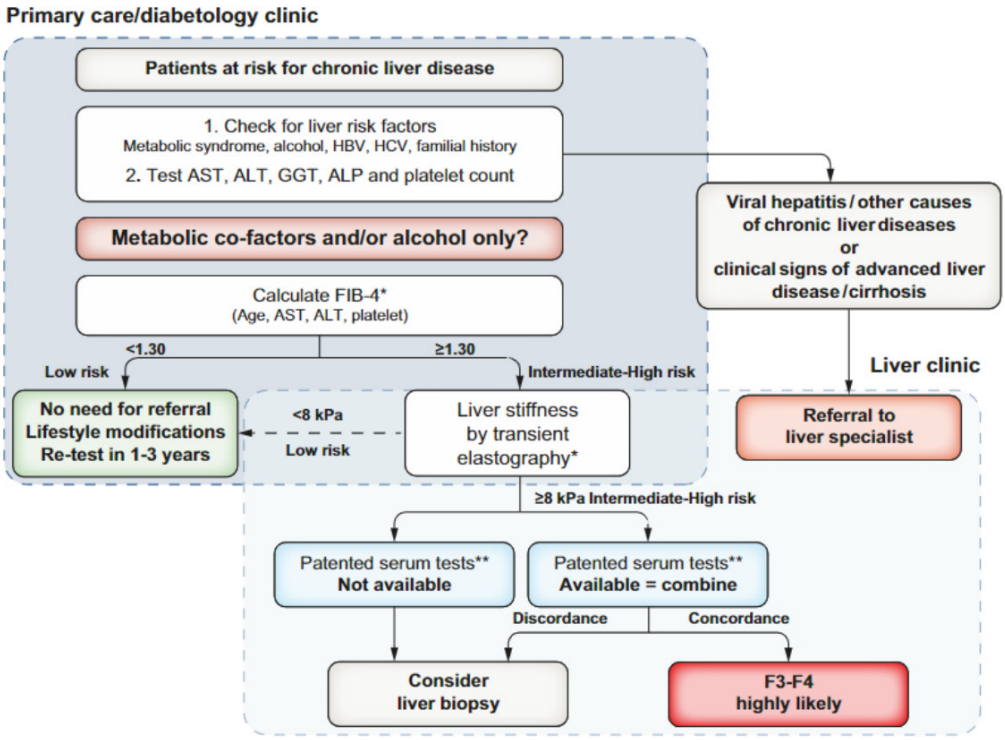


Fig. 2. Algorithm for the use of non-invasive tests in a compensated patient.

For future use, large consortia in Europe (LITMUS consortium) and in the USA (NIMBLE consortium)[18] are exploring the value of NITs as diagnostic and prognostic biomarkers to be used in clinical trials in patients with NAFLD, not only to diagnose and stage NASH to identify and prioritise patients for therapy, but also to monitor treatment response. The availability of well-validated NITs, accepted as biomarkers by the regulatory authorities would be a major advantage for the management of patients with NAFLD.

It is likely that artificial intelligence methods applied to NITs, and in particular to imaging (radiomics) will provide better identification of patients with cirrhosis and portal hypertension, beyond the existing simple algorithms.

Conclusions

In conclusion, NITs are currently simple and in part point-of-care tools allowing risk stratification in patients with or suspected of CLD. Integration of NITs, clinical history and variables, and invasive tests is key to improve outcomes in cACLD.

References

1. European Association for the Study of the Liver, Clinical Practice Guideline Panel. EASL Clinical Practice Guidelines on non-invasive tests for evaluation of liver disease severity and prognosis – 2021 update. J Hepatol 2021;75:659–89.
2. Xiao G, Zhu S, Xiao X, Yan L, Yang J, Wu G. Comparison of laboratory tests, ultrasound, or magnetic resonance elastography to detect fibrosis in patients with nonalcoholic fatty liver disease: a meta-analysis. Hepatology 2017;66:1486–501.
3. Brandman D, Boyle M, McPherson S, Van Natta ML, Sanyal AJ, Kowdley K, *et al.* Comparison of clinical prediction rules for ruling out cirrhosis in nonalcoholic fatty liver disease (NAFLD). Aliment Pharmacol Ther 2022; in press. doi:10.1111/apt.16874
4. Vali Y, Lee J, Boursier J, Spijker R, Loffler J, Verheij J, *et al.* Enhanced liver fibrosis test for the non-invasive diagnosis of fibrosis in patients with NAFLD: a systematic review and meta-analysis. J Hepatol 2020;73:252–62.
5. de Franchis R, Bosch J, Garcia-Tsao G, Reiberger T, Ripoll C; Baveno VII Faculty. Baveno VII – renewing consensus in portal hypertension. J Hepatol 2022;76:959–74.
6. Mozes FE, Lee JA, Selvaraj EA, Jayaswal ANA, Trauner M, Boursier J, *et al.* Diagnostic accuracy of non-invasive tests for advanced fibrosis in patients with NAFLD: an individual patient data meta-analysis. Gut 2021; in press. doi:10.1136/gutjnl-2021-324243
7. Berzigotti A, Reig M, Abraldes JG, Bosch J, Bruix J. Portal hypertension and the outcome of surgery for hepatocellular carcinoma in compensated cirrhosis: a systematic review and meta-analysis. Hepatology 2015;61:526–36.
8. Pons M, Augustin S, Scheiner B, Guillaume M, Rosselli M, Rodrigues SG, *et al.* Noninvasive diagnosis of portal hypertension in patients with compensated advanced chronic liver disease. Am J Gastroenterol 2021;116:723–32.
9. Vuille-Lessard E, Rodrigues SG, Berzigotti A. Noninvasive detection of clinically significant portal hypertension in compensated advanced chronic liver disease. Clin Liver Dis 2021;25:253–89.

10. Selvaraj EA, Mozes FE, Jayaswal ANA, Zafarmand MH, Vali Y, Lee JA, *et al.* Diagnostic accuracy of elastography and magnetic resonance imaging in patients with NAFLD: a systematic review and meta-analysis. J Hepatol 2021;75:770–85.
11. Qi X, Berzigotti A, Cardenas A, Sarin SK. Emerging non-invasive approaches for diagnosis and monitoring of portal hypertension. Lancet Gastroenterol Hepatol 2018;3:708–19.
12. Andersson A, Kelly M, Imajo K, Nakajima A, Fallowfield JA, Hirschfield G, *et al.* Clinical utility of magnetic resonance imaging biomarkers for identifying nonalcoholic steatohepatitis patients at high risk of progression: a multicenter pooled data and meta-analysis. Clin Gastroenterol Hepatol 2021; in press. doi:10.1016/j.cgh.2021.09.041
13. Siddiqui MS, Yamada G, Vuppalanchi R, Van Natta M, Loomba R, Guy C, *et al.* Diagnostic accuracy of noninvasive fibrosis models to detect change in fibrosis stage. Clin Gastroenterol Hepatol 2019;17:1877–85 e5.
14. Rinella ME, Dufour JF, Anstee QM, Goodman Z, Younossi Z, Harrison SA, *et al.* Non-invasive evaluation of response to obeticholic acid in patients with NASH: results from the REGENERATE study. J Hepatol 2022;76(3):536–48.
15. Sanyal AJ, Anstee QM, Trauner M, Lawitz EJ, Abdelmalek MF, Ding D, *et al.* Cirrhosis regression is associated with improved clinical outcomes in patients with nonalcoholic steatohepatitis. Hepatology 2021; in press. doi:10.1002/hep.32204
16. Petta S, Sebastiani G, Vigano M, Ampuero J, Wai-Sun Wong V, Boursier J, *et al.* Monitoring occurrence of liver-related events and survival by transient elastography in patients with nonalcoholic fatty liver disease and compensated advanced chronic liver disease. Clin Gastroenterol Hepatol 2021;19(4):806–15 e5.
17. Tamaki N, Kurosaki M, Huang DQ, Loomba R. Noninvasive assessment of liver fibrosis and its clinical significance in nonalcoholic fatty liver disease. Hepatol Res 2022; in press. doi: 10.1111/hepr.13764
18. Sanyal AJ, Shankar SS, Calle RA, Samir AE, Sirlin CB, Sherlock SP, *et al.* Non-invasive biomarkers of nonalcoholic steatohepatitis: the FNIH NIMBLE project. Nat Med 2022;28(3):430–2.

Role of endoscopy in hepatology

Wim Laleman

Department of Gastroenterology and Hepatology, Section of Liver and Biliopancreatic disorders, University Hospitals Leuven, Leuven, *Belgium*

E-mail address: wim.laleman@uzleuven.be

Take-home messages

- ***'Endo-hepatology'*** integrates different endoscopic ultrasound (EUS)-applications for advanced diagnosis and therapeutic intervention in hepatobiliary disease, including EUS-guided transgastric liver biopsy (EUS-LB), EUS-guided portal pressure gradient (EUS-PPG) measurement and EUS-guided intervention for gastroesophageal varices (GOV).
- **EUS-LB** represents a valid alternative for traditional percutaneous or transjugular liver biopsy, in particular when combined with other endoscopic procedures such as screening for GOV (so called 'one-stop clinic'). EUS-LB confers several advantages including the ability to target both lobes of the liver, increased patient comfort, decreased apprehension to repeat LB, and shorter recovery time.
- Hepatic venous pressure gradient (HVPG) has laid the foundations for 'precision medicine' in portal hypertension but lacks broad dissemination. **EUS-PPG** holds the potential to become a valuable alternative, given the widespread availability of endosonography in gastrointestinal (GI) practice. However, EUS-PPG remains to be validated in direct comparison with HVPG before it can be considered a standard of care.
- Bleeding gastrofundal varices remain an arduous and challenging clinical condition for which medical-endoscopic treatment has remained substandard. **EUS-guided intervention for gastric varices** allows detailed assessment of the vascular anatomy and might prove superior – when using glue combined with coils – in terms of variceal obliteration and rebleeding rate.
- **Single-operator cholangioscopy** (SOC) adds a novel diagnostic and therapeutic dimension to hepatobiliary disease by direct visualisation which can aid in optically targeted biopsies and intervention, in particular in the context of primary sclerosing cholangitis and post-liver transplant biliary complications.

Endo-hepatology

'Endo-hepatology' refers to a novel concept integrating different endoscopic ultrasound (EUS)-applications for the assessment of liver diseases and portal hypertension. These involve EUS-guided transgastric liver biopsy (EUS-LB), EUS-guided portal pressure gradient measurement (EUS-PPG) and EUS-guided intervention for gastroesophageal varices (GOV). Peroral cholangioscopy might also be considered part hereof as it adds a novel diagnostic and therapeutic dimension to hepatobiliary disease by direct visualisation which can aid in optically targeted biopsies and intervention.

Considering the growing interest and potential impact in endo-hepatology, the future liver-driven physician is ideally a hybrid hepatologist–endoscopist or alternatively teams up with therapeutic endoscopists, provided they are familiarised with the 'hepatological' mindset.

EUS liver biopsy

Although non-invasive testing such as serum fibrosis markers and elastography are gradually gaining interest and applicability, liver biopsy still remains one of the hepatologist's basic traits as it represents an important adjunct in terms of confirming/obtaining tissue diagnosis of (unexplained) parenchymal disease and focal liver lesions or upon need of grading liver injury and fibrosis. Ways to perform a liver biopsy include percutaneous (introduced in 1883), transjugular (first described in 1967) and the recently evolving option via EUS. EUS-LB was first reported in 2009 but was immediately toned down because of disappointing tissue yield given the lack of appropriate tissue acquisition needles. The introduction of 19G needles for fine needle aspiration (FNA) in 2012 re-fuelled the interest in EUS-LB. Meanwhile, ample evidence substantiates technical *standardisation, appropriate tissue yields*, and *procedural safety* for EUS-LB.[1]

A step-by-step overview of the author's current **technical standard operating procedure** (SOP) for EUS-LB is described in Table 1 and is based on evolving insights over the last years. Pivotal issues involve the preference of a 19G FNA needle as a smaller size needle (such as f.e. 22G core needles) only resulted in an adequate sample in 60% of patients compared with over 90% with a 19G FNA needle. The use of a 19G EUS core needle increases this yield even further and seems the best possible means of avoiding fragmentation and securing longer tissue specimens.2 Other important elements are the implementation of the 'wet suction technique' and specific sample handling (for details see Table 1).1 In contrast to percutaneous or transjugular biopsies, tissue cores from EUS-LB should not be dropped on a tissue gauze because of the high risk of iatrogenic fragmentation. It is advised to extrude the specimen from the needle onto a tissue sieve either with a flush of saline or with the stylet. This allows washing away obscuring blood and evaluating the acceptability of the LB. Thereafter, the sample can be 'floated' off the sieve into formalin or saline, depending on local practices.

Table 1. Step-by-step overview of an EUS-LB

1. Check procedure indication and need for additional procedures (screening varices, diagnostic EUS, EUS-PPG, …)
2. Check contraindications upfront
1. Inability to consent
2. INR >1.5
3. Platelet count <50,000
4. Haemophilia or use of anticoagulants or antiplatelet medications
5. Large volume ascites interposing between the GI tract and the liver
3. Select biopsy needle: 19G (FNB > FNA) > 22G FNB needle
4. EUS-LB procedure
4.1. Prior to biopsy, the stylet is removed, 2–3 ml of heparin (100 units/ml) is flushed through the needle
4.2 Apply the 'wet suction'-technique. The suction syringe is filled with 1–2 ml of water, the stopcock is turned to off, after which the suction syringe is set at a full suction setting. The syringe is then mounted on the primed needle
4.3 The needle is introduced into the working channel of endoscope
4.4 Identify a safe trajectory for needle travel into the liver that avoids sizable vessels, usually a 2–3 cm trajectory can be used.

1. A transgastric approach is used to obtain samples from the left lobe of the liver, a few centimetres below the gastroesophageal junction. Avoid inadvertent splenic biopsy as the location of the EUS-scope is similar.
2. A transduodenal approach, with the linear echoendoscope placed in the duodenal bulb, is utilised to acquire biopsies from the right liver lobe.

4.5 Once liver parenchymal penetration is achieved with the needle (1–2 cm), full suction is applied with the vacuum syringe by turning the stopcock to 'open'

4.6 One pass consists of a total of 3–4 to-and-fro slow and steady needle motions using the fanning technique applied, under direct and continuous endosonographic visualisation of the tip of the needle. A 3 cm course of the needle is ideal

4.7 After the biopsy, and before the needle is removed from the liver parenchyma, the suction is turned off by using the stopcock on the vacuum syringe

4.8 Visualisation of the puncture site with Doppler post biopsy is advisable

4.9 If the sample that is obtained appears inadequate in terms of specimen length or excessive fragmentation, then a second pass could be considered (see 6.)

5. Specimen retrieval, handling, and quality evaluation

5.1 The liver sample is pushed out of the needle with the stylet or flushed out using saline directly onto a small nylon mesh sieve (a histopathology cassette can be used)

5.2 Excessive handling of the specimen should be avoided

5.3 Do not be surprised to have a sample admixed with blood (clots are usually avoided by using heparin).

5.4 The sample can be rinsed gently with saline to separate blood from the actual tissue specimen and to allow rapid macroscopic assessment of the obtained sample length

5.5 The sample can then be 'floated' off the sieve into formalin or transported freshly on saline, depending on local practices. Beware not to flush the needle with formalin and use this needle back into the patient.

5.6 Inform your pathologist about the concept of EUS-guided liver biopsy samples

6. Post-procedural surveillance of the patient

6.1 Observation of vital parameters and clinical condition of the patient for 60 min. Thereafter, discharge and return to normal diet is possible

6.2 Pain is noticed in 30–40% of patients, usually self-limiting.

6.3 Bleeding and intrahepatic hematoma are rare.

In terms of ***tissue yields***, EUS-LB is able to deliver comparable quality demands as requested for percutaneous or transjugular LB. The prerequisites for a high-quality LB remain a preferable length >25 mm and minimally 11–15 portal tracts/biopsy which are toned down to 15–20 mm and 6–10 portal tracts by other guidelines. A retrospective study comparing all percutaneous, transjugular and EUS-LB, showed quantitative equivalence in-between all methods when considering complete portal tracts (EUS-LB: n = 14 on average [range 9–27]) and total specimen length (EUS-LB: n = 38 mm on average [range 24–81]).[3] Of note, total specimen length and complete portal triads were even higher

for EUS-LB when bilobar EUS-guided biopsies were performed which might reduce sample variation. However, classical interobserver variability is not solved by EUS-LB.

Procedural safety is guaranteed with 0.9% complications being reported (1/110, self-limiting subcapsular hematoma).

Advantages of EUS-LB over conventional approaches involve the more human nature of the procedure given the need for sedation, lower postprocedural discomfort and apparent shorter recovery and monitoring. Additional benefits of EUS-LB relate to the possibility to procure bilobar liver biopsy, perform same-session diagnostic oesophagogastroduoscopy (OGD)/EUS/EUS-PPG ('one-stop clinic'), and lower apprehension in respect of repeat LB. The shortened recovery time associated with EUS-LB (certainly when performed in a 'one-stop clinic') represents an important advantage and potential for real cost savings compared with prolonged outpatient procedure recovery.

EUS-guided portal pressure gradient

Clinically significant portal hypertension (CSPH) drives the development of GOV and other liver-related decompensations. The current '*pragmatic*' approach in terms of primary prevention of GOV bleeding is based on clinical, biochemical, endoscopic, and elastography findings (Fig. 1).

However, clinical-haemodynamic correlations clearly subscribe an *imperative* need for quantifying or measuring portal hypertension (PHT) given its impact on risk stratification and individualised care[4–7] (Fig. 2). Hepatic venous pressure gradient (HVPG)-measurement has laid the foundation for 'precision medicine' or an '*à la carte*' approach in PHT and remains therefore the golden standard to pursue this concept. Yet, practical implementation and broad dissemination of HVPG in non-academic clinical practice has proven difficult. Therefore, any additional tool that could expand the horizon in quantitative PHT assessment should be explored and objectively tested.

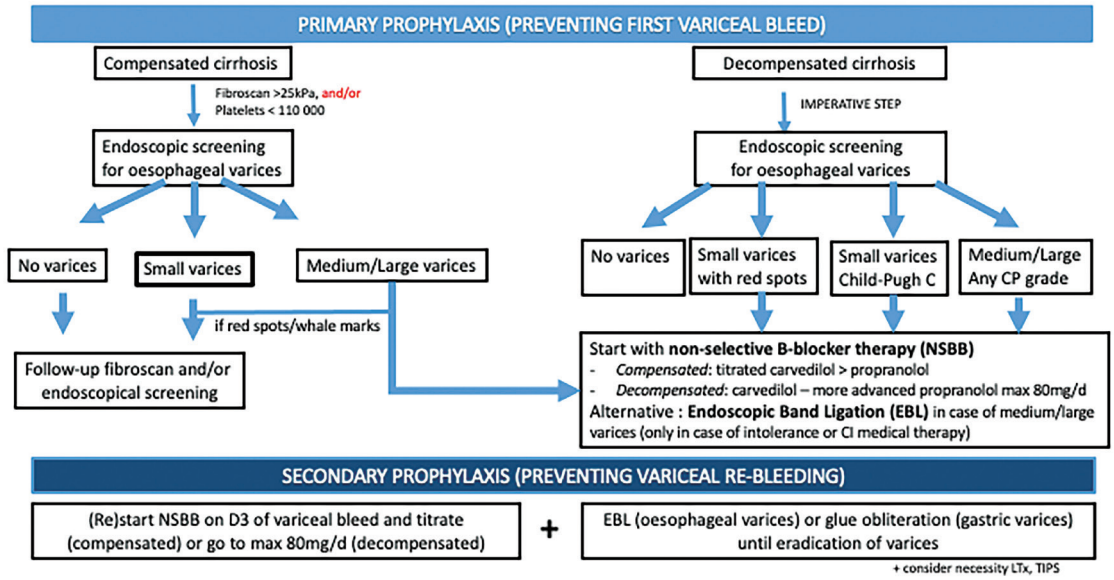


Fig. 1. Current pragmatic pathway in daily clinical practice.

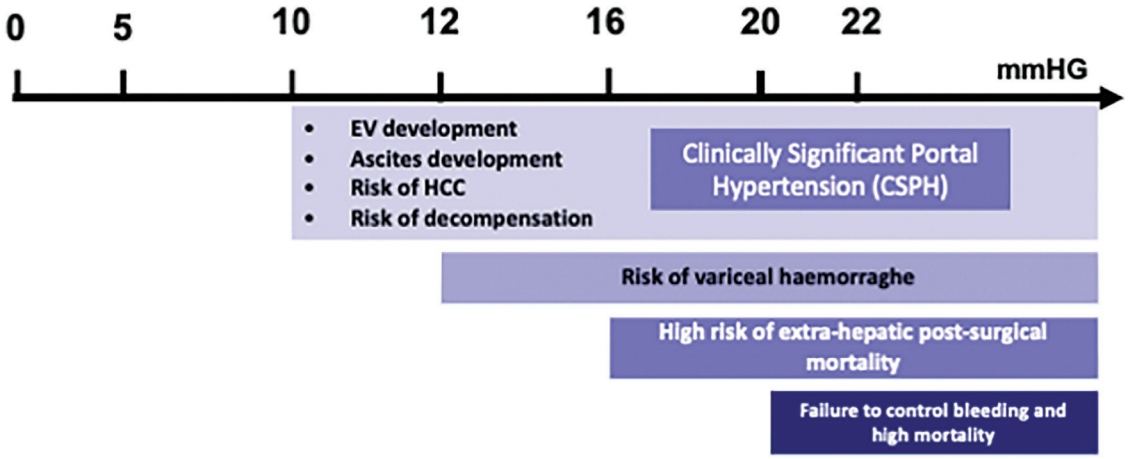


Fig. 2. Prognostic relevance of different portal pressure (hepatic venous pressure gradient) thresholds.

EUS-guided portal pressure gradient (EUS-PPG) measurement, in contrast to HVPG, represents a tool which assesses hepatic venous and portal pressure directly by puncturing these vessels transgastrically under EUS-guidance with a 25G FNA needle coupled to a digital pressure transducer (schematic representation in Fig. 3). By subtracting the hepatic venous pressure from the portal pressure, the EUS-portal PPG is determined.

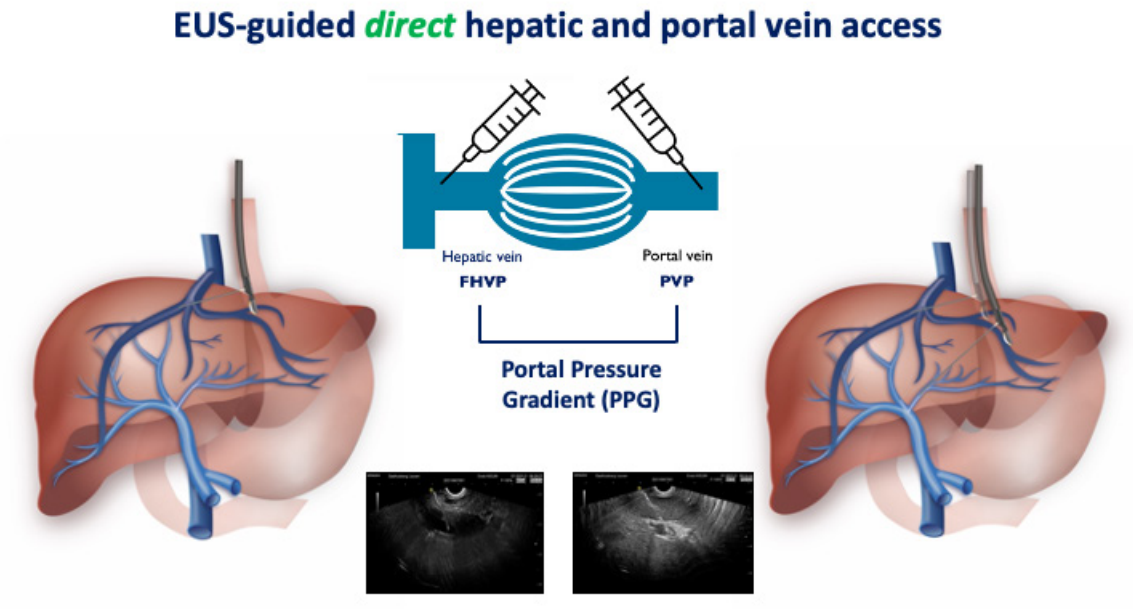


Fig. 3. Schematic representation of endoscopic ultrasound-guided portal pressure gradient.

Potential conceived differences and similarities between HVPG and EUS-PPG are summarised in Table 2.

Table 2. Comparison of different aspects of hepatic venous pressure gradient (HVPG) and endoscopic ultrasound-guided portal pressure gradient (EUS-PPG)

	HVPG	EUS-PPG
Current position	Golden standard	Novel platform
Portal pressure measurement	Indirect	Direct
Basic type of procedure	Angiography (Hepatic vein catheterisation)	Endoscopy (Endoscopic ultrasound)
Equipment	Dedicated X-ray machine	Conventional EUS-platform
Performing physician	Interventional radiologists, dedicated hepatologists	Gastroenterologists Dedicated hepatologists
Setting	More typical for tertiary units	Secondary and tertiary units
Training	Yes	Yes
Types of portal hypertension assessed	Sinusoidal	Sinusoidal Presinusoidal
Contra-indications	Allergy to iodinated contrast Platelets <20´109/L or PT <30%	Interposed ascites in the puncture path Anatomic anomalies preventing vessel access Platelets <50,000 or PT <50% Contraindications for upper GI endoscopy
Additive procedures in same intervention	Transjugular liver biopsy	Transgastric liver biopsy and screening of gastroesophageal varices
Patient sedation	Local anaesthesia or mild sedation	Mild sedation
Procedure time	comparable	
Feasible as outpatient procedure	Yes	
Grade of evidence	Validated	Remains to be validated against HVPG

The first preclinical experience was published in 2016. In a healthy porcine model, Huang *et al.*[8] established clinical feasibility of EUS-PPG and correlation with HVPG. One year later, the same authors[9] published the first human pilot study (n = 28) assessing feasibility, safety, and correlation with clinical parameters. Following this study, the only currently available platform, EchoTip Insight (Cook Medical), was FDA-approved. Meanwhile real-life cohort studies have confirmed safety and feasibility.

Except for 1 study which included 12 patients with non-cirrhotic portal hypertension (sinusoidal obstruction syndrome [n = 10], Budd Chiari syndrome [n = 2]),[10] no data are available correlating EUS-PPG with HVPG in real-time which leaves the question in terms of applicability unanswered. To answer this particular issue, we recently initiated the ENCOUNTER study (NCT04987034). The primary study objective is to evaluate the correlation of EUS-PPG obtained using the EchoTip® Insight™ and HVPG in a prospective manner. Patients will serve as their own controls, with both measurements obtained during the same procedure. In a subgroup undergoing transjugular intrahepatic portosystemic shunts (TIPS), direct portal pressure measurement will be compared with EUS-guided portal pressure. Results are awaited in 2022–2023.

Provided that EUS-PPG can show adequate correlation to the considered golden standard HVPG, further follow-up studies will need to be performed under minimal sedation.

If all of these prerequisites can be realised, EUS-PPG may hold the potential to become an alternative valuable tool given the wide availability of endosonography in GI practice. A potential future diagnostic algorithm, incorporating EUS-PPG, is depicted in Fig. 4.

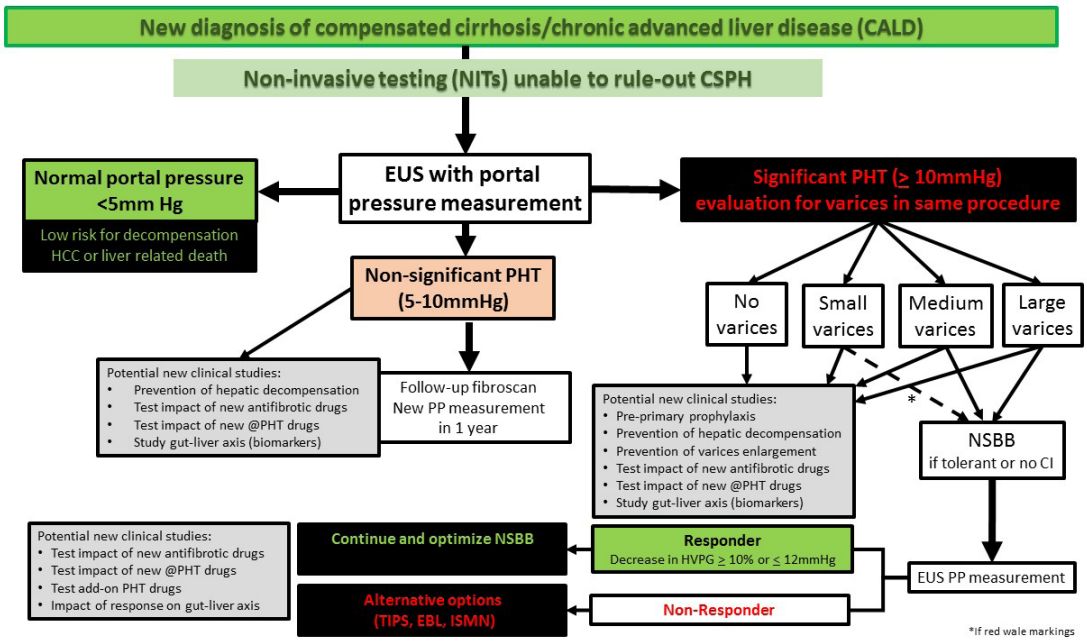


Fig. 4. Potential future algorithm incorporating EUS-PPG. CSPH, clinically significant portal hypertension; EBL, endoscopic band ligation; EUS-PPG endoscopic ultrasound-guided portal pressure gradient; HVPG, hepatic venous pressure gradient; NSBB, non-selective B blocker; TIPS, transjugular intrahepatic portosystemic shunts.

EUS-guided intervention for GOV

Bleeding GOV remain a devastating and life-threatening clinical condition that requires immediate and urgent medical and endoscopic intervention. Gastric variceal bleeding, more specifically, accounts for 10–15% of all variceal haemorrhages related to cirrhotic PHT but can also occur in the context of pre-hepatic PHT (so-called 'left-sided PHT') caused by portal or splenic vein thrombosis or schistosomiasis amongst others. Irrespective of the type of GOV bleeding, suspicion of an acute variceal bleeding should instantly trigger the roll out of an algorithm containing intensified monitoring of vital functions,

initiation of prophylactic antibiotics, careful replacement of volaemia (with restrictive transfusion policy), and the immediate administration of vasoactive drugs before endoscopic haemostasis is pursued (within a 12-h window after onset), per proposed BAVENO VII guidelines.[7,11]

Bleeding gastric varices, irrespective of their aetiology, tend to bleed less frequently than oesophageal varices. However, when they do bleed, the clinical situation is vastly more disheartening. The reasons for this apparent discrepancy relate to greater difficulties in achieving haemostasis and, thus, greater propensity to rebleed, which culminate in increased bleeding-related mortality. Multiple factors are considered essential including the large submucosal component typical of gastric varices, the vascular anatomy feeding and draining the gastric varix, and most important – but amenable – lack of widespread treatment options and expertise available for this condition. Gastric varices are classically divided into GOV or isolated gastric varices (IGV), according to Sarin.12 For the purpose of this section, we will focus specifically on fundal type varices (GOV2 and IGV1, Fig. 5), as they represent the *true* endoscopic stalemate compared with other types of upper varices which are efficiently treated by endoscopic band ligation.

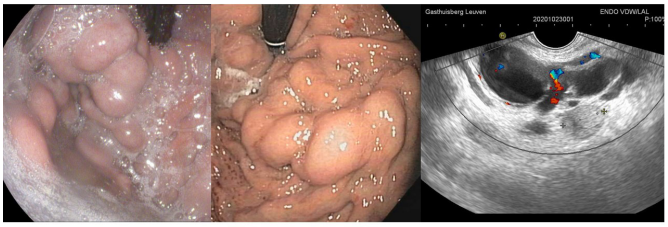


Fig. 5. Gastro-fundal varices (IGV-1) shown on conventional endoscopy (left and middle) and upon endoscopic ultrasound (right).

Indeed, whereas endoscopic band ligation is the undisputed standard of care for oesophageal and GOV1-type varices, it is not when applied for bleeding gastric varices given the lower rate of immediate haemostasis and higher rebleeding rates (2.6–4.1-fold higher risk), in particularly when compared with endoscopic cyanoacrylate ('glue') injection.11 For this reason, cyanoacrylate injection applied via standard endoscopy is now considered the 'gold standard' haemostatic treatment for gastric variceal haemorrhage and secondary prophylaxis.[7,11] However, it carries several potentially severe flaws and would therefore perhaps be better categorised as 'the best available gold standard'. Primarily, and most strikingly, efficacy data are scarce, with only half of the patients included in the few published randomised controlled trials (RCTs) having cardiofundal varices. Notably, initial haemostasis was reported in over 90% of patients, but rebleeding rates were still over 15–20%, with a clear impact on morbidity and mortality. The rate of variceal obliteration is unclear and varies from 44% to 100%, the latter figure being untrustworthy. Secondly, the procedure is not without risks, including systemic glue emboli (0.5–4.3%), ulcerative extrusion of glue with bleeding (4.4%), and sepsis with or without thrombophlebitis. These latter phenomena might relate to the combination of a rather untargeted approach, the size and complexity of the underlying portosystemic shunts, and the aliquot(s) of glue injected per injection. In view of these limitations, endoscopic haemostasis of bleeding gastric varices or secondary prophylaxis has remained challenging and far from satisfactory compared with oesophageal variceal haemorrhage. Attempts to improve this stalemate are almost non-existent except for EUS-guided glue and/or coil injection. Binmoeller *et al.*[13] were the first to report on this technique, which aims to improve the efficacy of the direct glue injection technique using standard endoscopy and to reduce the risk of systemic complications. More specifically, EUS not only allows precise targeting of the vessels responsible for feeding the gastric varix but also directly monitors, via Doppler, the effect of therapy on variceal flow in real time, as well as the theoretical risk of embolisation.

Robles-Medranda *et al.*[14] substantiated the superiority of combining coils with glue *vs.* coils alone under EUS guidance in terms of variceal obliteration (86.7% *vs.* 13.3%), rebleeding rate (3.3% *vs.* 20%) in a single procedure (83.3% *vs.* 60%). Notably, these data have been further confirmed and reinforced by a systematic review by Mohan *et al.*,[15] meanwhile endorsed by others. Mohan *et al.*[15] compared EUS with coil, EUS with glue, EUS with coil and glue *vs.* glue via the standard endoscopy approach. EUS-guided therapy with combined coil and glue was the best modality, with a pooled treatment efficacy rate of 97%, obliteration rate of 86%, recurrence rate of 5%, and early and late rebleeding rates of 8% and 9%, respectively. These data provide clear reasons to be optimistic about progress in the endoscopic management of gastric varices but also underscore the indispensable and growing therapeutic impact of EUS in hepatobiliary disease.

Nevertheless, our enthusiasm to unleash this technique temerariously on all gastric varices should be somewhat restrained, as major issues remain. First, high quality confirmatory RCTs, although difficult to perform, should be pursued to validate the currently reported outcomes and further delineate the patient groups most likely to benefit from this kind of procedure, as the current data include a mixture of patients in the setting of active bleeding and secondary prophylaxis but also primary prophylaxis. Especially the benefit of endoscopic intervention in the latter group is still under debate, as the latest BAVENO consensus report advises against any endoscopic gastric variceal obliteration (by means of endoscopic cyanoacrylate injection) in primary prophylaxis owing to insufficient data and thus, unclear risk/benefit. Second, as an endoscopist, one should remember that endoscopic haemostasis for variceal bleeding is not the end but merely the beginning, as gastric variceal bleeding represents a symptom of the much larger syndrome of PHT, most frequently in the context of cirrhosis. Management of these patients should therefore incorporate a multidisciplinary approach to consider the best possible option, which does not necessarily extend to EUS-assisted coil and glue delivery alone, but might also involve pharmacological (*i.e.* non-selective beta-blockers), radiological (including transjugular intrahepatic portosystemic shunt and balloon-occluded retrograde transvenous obliteration) or surgical intervention (*e.g.* shunt surgery or liver transplantation).

Therefore, overall and in conclusion, sticky stuff might just not be enough when treating gastric varices but, at least from an endoscopic point of view, the EUS-assisted delivery of combined coil and glue represents a genuine and promising step forward in endoscopic haemostasis for bleeding gastric varices and should be explored further.[16]

Single-operator cholangioscopy

Hidden deep in the abdomen, almost inaccessible and banned to oblivion by the mighty liver and GI tract, the biliary system retains its enigmatic nature and therefore shallow and cursory characterisation. It could have been a phrase from *Plato's allegory of the Cave* in which the philosopher tells the tale of prisoners trapped in a cave and whose only perception of the outside world is related to shadows on the wall, which they perceive and believe as reality, albeit indirect and manufactured based on reflections of the true reality. The analogy with the current endoscopic approach of biliary disorders could not be more striking.[17]

The prospect of visualising the biliary tree has allured endoscopists for decades. However, the combined endoscopic and fluoroscopic approach by means of endoscopic retrograde cholangiopancreaticography (ERCP) has been – and still is – applied as the primary tool for biliary intervention. The downside of this approach lies in its indirect visualisation of the biliary system by fluoroscopy and ditto application of therapy. Although most clinical problems can be solved in this manner, some arduous and challenging situations remain, both diagnostically as therapeutically. More specifically from a hepatologist's perspective, indeterminate biliary strictures associated with *primary sclerosing cholangitis (PSC)* and

post-transplant biliary problems. In these circumstances, *direct* visualisation of the bile duct, that is *cholangioscopy*, might not only shape our reality more proficiently by direct macroscopic evaluation but may also facilitate visually targeted biopsies or enable optically assisted therapy such as electrohydraulic or laser lithotripsy for difficult stones or casts.[17]

Single-use, single-operator cholangioscopy digitalised systems (*e.g.* SpyGlassä DS) are being used with increasing efficiency, ease of use and set-up, and image quality.

PSC is a major risk factor for the development of cholangiocarcinoma (20%). Because of the stricture-forming nature of the disease (30–50% of PSC patients develop dominant strictures) (Fig. 6), it can be hard to differentiate between fibro-inflammatory *vs.* malignant strictures. Traditionally, tissue sampling through ERCP via brushing cytology and fluoroscopy-guided intraductal biopsies are still being performed but lack sensitivity (27–30%) and accuracy (39–54%).17 Adding fluorescence *in-situ* hybridisation (FISH) or next generation sequencing (SS 73% to SP 100%) might improve diagnostic yield but remains far from satisfactory and is even still questionable for the specific context of PSC.18 Early detection of cholangiocarcinoma in PSC therefore remains a clinical challenge with an often delayed and protracted diagnostic course.

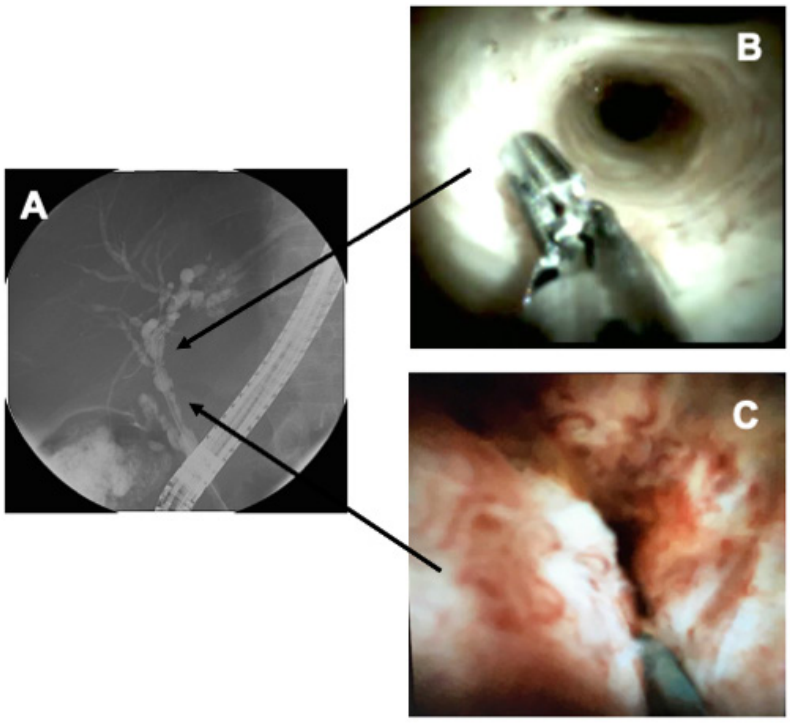


Fig. 6. Dominant stricture in primary sclerosing cholangitis on cholangiography via endoscopic retrograde cholangiopancreaticography (A) with single-operator cholangioscopy images of the hilum and micro-forceps (B) and the stenosis (C) which shows spider-vascularity as a suggestive sign of tumoral angiogenesis.

Over the past decade, several endoscopic techniques, including endoscopic ultrasound, confocal laser endomicroscopy and most of all SOC have been proposed as potential auxiliary tools. Data on the usefulness of SOC, defined as the combination of successful procedural completion, clinical success, and incidence of procedure-related adverse events, have been reported in the context of PSC but remain scarce and limited to case-series or cohort-studies. In terms of *procedural completion*, a retrospective study of 165 patients[19] undergoing SOC (including a small cohort with PSC) reported

that although SOC appeared useful for the evaluation of indeterminate biliary lesions and difficult biliary stones in patients without PSC, the technique was associated with a lower procedure success rate (59% *vs.* 92%) and lower rate of bile duct cannulation (82% *vs.* 97%) in patients with PSC compared with patients without PSC. With regard to *clinical success*, biliary stones were detected by cholangioscopy in nearly 1 of 3 patients with PSC which were missed by cholangiography. However, a study by the European Cholangioscopy Group showed low sensitivity and specificity for blinded (74% and 46.9%, respectively) and unblinded (72.7% and 62.5%, respectively) visual appraisal of indeterminate biliary strictures, especially in PSC patients, which significantly toned down the initial over-enthusiasm. Therefore, optimising visually directed biopsy sampling may be the most important contribution of cholangioscopy in biliary stricture assessment in PSC for now.[20] SOC with biopsy sampling was found the most cost-effective diagnostic modality for cholangiocarcinoma in PSC strictures.[21,22]

Future innovation in timely and accurate diagnosis of a (pre-)malignant stenosis might be expected with the incorporation of modern molecular biomarkers such as DNA methylation and proteome analysis.

Procedure-related adverse events related to SOC in PSC, despite usage of substantial rinsing fluid, remain within range with a risk of cholangitis of 0–11%, acute pancreatitis 2–8.9%, bleeding 0–3.3%, and perforation 0.4–1%.

The last condition to be discussed for SOC are ***post-liver transplant biliary*** complications. In case of strictures, not suitable for standard cannulation, cholangioscopy enables guidewire insertion in typically tight, angulated strictures under visual control as such improving stricture cannulation rate and overall technical and clinical success. The implementation of cholangioscopy in stricture management could spare the need for percutaneous drainage and surgical re-interventions.

Alternatively, desquamated epithelial cells (as a result of ischaemia), infection, and cholesterol supersaturation predispose to formation of clots, casts ('biliary cast syndrome'), and stones which easily can cause bile obstruction facilitated by either anastomotic or non-anastomotic strictures. The reported incidence of obstructive casts, stones, etc., after LT ranges widely between 4% and 18%. In most cases, a conventional ERCP with sludge/stone extraction and additional stenting (in case of stenosis) is sufficient as definitive treatment with a success rate over 90%. In case cholestasis or jaundice persists nevertheless, cholangioscopy should be considered as it provides visual confirmation on the absence/presence of obstructive content and allows visually controlled fragmentation of large biliary stones if present, with little risk of biliary injury. Advanced intraductal techniques such as electrohydraulic lithotripsy achieve outstanding results in difficult cases.[17]

References

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

1. **Diehl DL. Endoscopic ultrasound-guided liver biopsy. Gastrointest Endosc Clin N Am 2019;29:173–86.**
2. Ching-Companioni RA, Diehl DL, Johal AS, Confer BD, Khara HS. 19G aspiration needle versus 19G core biopsy needle for endoscopic ultrasound-guided liver biopsy: a prospective randomized trial. Endoscopy 2019;51:1059–65.
3. Pineda JJ, Diehl DL, Miao CL, Johal AS, Khara HS, Bhanushali A, Chen EZ. EUS-guided liver biopsy provides diagnostic samples comparable with those via the percutaneous or transjugular route. Gastrointest Endosc 2016;83:360–5.

4. Vanderschueren E, Van der Merwe S, Laleman W. Novel approaches & disease modifiers to alter the course of cirrhotic portal hypertension. In de Franchis R, editor. Portal Hypertension VII. Proceedings of the 7th BAVENO consensus workshop. In press 2022.

5. Garcia-Tsao G, Abraldes JG, Berzigotti A, Bosch J. Portal hypertensive bleeding in cirrhosis: risk stratification, diagnosis and management: 2016 practice guidance by the American Association for the Study of Liver Diseases. Hepatology 2017;65:310–35.

6. Bureau C, Péron JM, Alric L, Morales J, Sanchez J, Barange K, *et al.* "A la carte" treatment of portal hypertension: adapting medical therapy to hemodynamic response for the prevention of bleeding. Hepatology 2002;36:1361–6.

7. de Franchis R, Bosch J, Garcia-Tsao G, Reiberger T, Ripoll C; Baveno VII Faculty. BAVENO VII – renewing consensus in portal hypertension. J Hepatol 2022;76:959–74.

8. Huang JY, Samarasena JB, Tsujino T, Chang KJ. EUS-guided portal pressure gradient measurement with a novel 25-gauge needle device versus transjugular approach: a comparison animal study. Gastrointest Endosc 2016;84:358–62.

9. Huang JY, Samarasena JB, Tsujino T, Lee J, Hu KQ, McLaren CE, *et al.* EUS-guided portal pressure gradient measurement with a simple novel device: a human pilot study. Gastrointest Endosc 2017;85:996–1001.

10. Zhang W, Peng C, Zhang S, Huang S, Shen S, Xu G, *et al.* EUS-guided portal pressure gradient measurement in patients with acute or subacute portal hypertension. Gastrointest Endosc 2021;93:565–72.

11. Garcia-Pagan JC, Barrufet M, Cardenas A. Management of gastric varices. Clin Gastroenterol Hepatol 2014; 12: 919–28.

12. Sarin SK, Lahoti D, Saxena SP. Prevalence, classification and natural history of gastric varices: a long-term follow-up study in 568 portal hypertension patients. Hepatology 1992;16:1343–9.

13. Binmoeller KF, Weilert F, Shah JN, Kim J. EUS-guided transesophageal treatment of gastric fundal varices with combined coiling and cyanoacrylate glue injection (with videos). Gastrointest Endosc 2011;74:1019–25.

14. Robles-Medranda C, Oleas R, Valero M, Puga-Tejada M, Baquerizo-Burgos J, Ospina J, et al. Endoscopic ultrasonography-guided deployment of embolization coils and cyanoacrylate injection in gastric varices versus coiling alone: a randomized trial. Endoscopy 2020;52:268–75.

15. Mohan BP, Chandan S, Khan SR, Kassab LL, Trakroo S, Ponnada S, Asokkumar R. Adler DG. Efficacy and safety of endoscopic ultrasound-guided therapy versus direct endoscopic glue injection therapy for gastric varices: systematic review and meta-analysis. Endoscopy 2020;52:259–67.

16. Laleman W. Endoscopic ultrasound-guided intervention for gastric varices: sticky stuff might not (yet) be enough. Endoscopy 2020;52:244–6.

17. Derdeyn J, Laleman W. Current role of endoscopic cholangioscopy. Curr Opin Gastroenterol 2018;34:301–8.

18. Kaura K, Sawas T, Bazerbachi F, Storm AC, Martin JA, Gores GJ, *et al.* Cholangioscopy biopsies improve detection of cholangiocarcinoma when combined with cytology and FISH, but not in patients with PSC. Dig Dis Sci 2020;65:1471–8.

19. Kalaitzakis E, Sturgess R, Kaltsidis H, Oppong K, Lekharaju V, Bergenzaun P, *et al.* Diagnostic utility of single-user peroral cholangioscopy in sclerosing cholangitis. Scand J Gastroenterol 2014t;49:1237–44.

20. Stassen PMC, Goodchild G, de Jonge PJF, Erler NS, Anderloni A, Cennamo V, *et al.*; European Cholangioscopy Group. Diagnostic accuracy and interobserver agreement of digital single-operator cholangioscopy for indeterminate biliary strictures. **Gastrointest Endosc 2021;94:1059–68.**

21. Deprez PH, Garces Duran R, Moreels T, Furneri G, Demma F, Verbeke L, *et al.* The economic impact of using single-operator cholangioscopy for the treatment of difficult bile duct stones and diagnosis of indeterminate bile duct strictures. Endoscopy 2018;50:109–18.

22. Njei B, McCarty TR, Varadarajulu S, Navaneethan U. Cost utility of ERCP-based modalities for the diagnosis of cholangiocarcinoma in primary sclerosing cholangitis. Gastrointest Endosc 2017;85:773–81.

Future of interventional radiology

Riad Salem

Section of Vascular and Interventional Radiology, Department of Radiology, Northwestern University, Chicago, IL, *USA*

E-mail address: Rsalem1@nm.org

Take-home messages

- Imaging is critical for the proper diagnosis of HCC.
- LI-RADS is been studied a tool for response assessment.
- Interventional techniques for HCC/CCA include ablation and embolisation.
- Portal vein recanalisation TIPS permits conversion of patient to transplant candidature in patients with cirrhosis.
- In patients without cirrhosis, portal vein recanalisation TIPS is a novel way of addressing this unmet need.

Screening

International guidelines recommend surveillance of populations at high-risk for hepatocellular carcinoma (HCC) with 6-monthly abdominal ultrasound with or without alpha-foetoprotein (AFP).[1,2] In addition to a better implementation of surveillance for patients at risk, novel surveillance tests are needed,[3] particularly for the increasing number of patients with NAFLD, in whom ultrasound performance is impaired in the setting of obesity.[4]

Diagnosis

HCC is diagnosed by validated imaging criteria (computed tomography [CT]/magnetic resonance imaging [MRI]) in cirrhotic livers or by tissue biopsy. The former criterion involves arterial enhancement followed by delayed venous washout.[5]

Because the presentation of nodules can vary, the Liver Imaging Reporting and Data System (LI-RADS) system was developed. LI-RADS incorporates tumour size, contrast enhancement, and the capsule, and categorises nodules as: LR-NC: non-categorised as a result of inadequate imaging; LR-1: definitely benign; LR-2: probably benign with 0% likelihood of being malignant; LR-3: intermediate risk of HCC (12–50%); LR-4: probably HCC (47–80%); LR-5: definitely HCC (93–96%) and LR-M indicated a probably malignant lesion but not definitely HCC.[6]

Treatment

The treatment options for patients with HCC are determined by Barcelona Clinic Liver Cancer (BCLC) staging.[7]

Interventional treatments

Ablation

Ablation is currently recommended for early-stage HCC patients (≤2 cm – this involves inducing thermal injury to the tumour tissue, resulting in tissue necrosis.[8] Ablation is considered curative[9] in most guidelines. Microwave ablation represents the next generation technology with advantages over radiofrequency ablation including higher temperatures and shorter times.

Intra-arterial therapies

HCC tumours are hypervascular. This physiology represents the main rationale for arterial therapies, including bland particle embolisation, chemoembolisation or drug-eluting beads, and radioembolisation. Bland embolisation involves the injection of 100–500 micron-sized particles until stasis is reached. Chemoembolisation involves the injection of chemotherapeutics directly into the tumour bed. Drug-eluting beads involve a slow release approach to chemotherapy directly into the tumour tissue. Radioembolisation also involves the arterial route, but involves injection of beta-emitting micron-sized particles, and was recently adopted into guidelines.[7]

Response

RECIST 1.1 is the gold standard method of assessing response.[10] This approach sums the maximum length of all target tumour diameters at baseline, and subsequently measures changes at follow-up, where specific criteria are used to determine response status.

Portal vein recanalisation – TIPS

Extrahepatic portal vein occlusion (EHPVO) from portal vein thrombosis (PVT) affects 1–2% of the world population.[11-14] Portal hypertension caused by EHPVO is associated with significant mortality and morbidity, owing mainly to variceal bleeding, hepatorenal syndrome, ascites, and other downstream effects.[11,12,15] EHPVO has been shown to arise from numerous aetiologies (cirrhosis, hypercoagulable state) or acquired (post-operative, malignancy, etc.).[13-15]

Several studies have demonstrated the efficacy of transjugular intrahepatic portosystemic shunts (TIPS) in patients with PVT.[16-18] However, these studies have mostly studied the use of TIPS in patients with acute PVT and cirrhosis, while little scientific attention has been paid to the potential value of this procedure in individuals without cirrhosis.[16-19] In fact, isolated PVT with cavernomatous transformation has long been thought to represent a contraindication to TIPS. There exist few comprehensive studies to date focused on implementing TIPS in chronic, EHPVO patients without cirrhosis with cavernomas, and little consensus regarding appropriate treatment for these individuals.[20] We will present data on outcomes utilising novel approaches in converting the ‘unTIPSable’ to ‘TIPSable’ in a patient population without cirrhosis.

References

1. Vogel A, Cervantes A, Chau I, Daniele B, Llovet JM, Meyer T, *et al.* Hepatocellular carcinoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2019;30:871–3.
2. European Association for the Study of the Liver. EASL Clinical Practice Guidelines: management of hepatocellular carcinoma. J Hepatol 2018;69:182–236.
3. Adeniji N, Dhanasekaran R. Current and emerging tools for hepatocellular carcinoma surveillance. Hepatol Commun 2021;5:1972–86.
4. Singal AG, El-Serag HB. Rational HCC screening approaches for patients with NAFLD. J Hepatol 2022;76:195–201.
5. Khatri G, Merrick L, Miller FH. MR imaging of hepatocellular carcinoma. Magn Reson Imaging Clin N Am 2010;18:421–50.
6. Kim YY, Kim MJ, Kim EH, Roh YH, An C. Hepatocellular carcinoma versus other hepatic malignancy in cirrhosis: performance of LI-RADS Version 2018. Radiology 2019;291:72–80.
7. Reig M, Forner A, Rimola J, Ferrer-Fàbrega J, Burrel M, Garcia-Criado A, *et al.* BCLC strategy for prognosis prediction and treatment recommendation: the 2022 update. J Hepatol 2022;76:681–93.
8. Lencioni R, Crocetti L. Radiofrequency ablation of liver cancer. Tech Vasc Interv Radiol 2007;10:38–46.
9. Llovet JM, Kelley RK, Villanueva A, Singal AG, Pikarsky E, Roayaie S, *et al.* Hepatocellular carcinoma. Nat Rev Dis Primers 2021;7:6.
10. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, *et al.* New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer 2009;45:228–47.
11. Cagin YF, Atayan Y, Erdogan MA, Dagtekin F, Colak C. Incidence and clinical presentation of portal vein thrombosis in cirrhotic patients. Hepatobiliary Pancreat Dis Int 2016;15:499–503.
12. Cool J, Rosenblatt R, Kumar S, Lucero C, Fortune B, Crawford C, *et al.* Portal vein thrombosis prevalence and associated mortality in cirrhosis in a nationally representative inpatient cohort. J Gastroenterol Hepatol 2019;34:1088–92.
13. Ogren M, Bergqvist D, Bjorck M, Acosta S, Eriksson H, Sternby NH. Portal vein thrombosis: prevalence, patient characteristics and lifetime risk: a population study based on 23,796 consecutive autopsies. World J Gastroenterol 2006;12:2115–9.
14. Rajani R, Bjornsson E, Bergquist A, Danielsson A, Gustavsson A, Grip O, *et al.* The epidemiology and clinical features of portal vein thrombosis: a multicentre study. Aliment Pharmacol Ther 2010;32:1154–62.
15. Sacerdoti D, Serianni G, Gaiani S, Bolognesi M, Bombonato G, Gatta A. Thrombosis of the portal venous system. J Ultrasound 2007;10:12–21.
16. Valentin N, Korrapati P, Constantino J, Young A, Weisberg I. The role of transjugular intrahepatic portosystemic shunt in the management of portal vein thrombosis: a systematic review and meta-analysis. Eur J Gastroenterol Hepatol 2018;30:1187–93.

17. Han G, Qi X, He C, Yin Z, Wang J, Xia J, *et al.* Transjugular intrahepatic portosystemic shunt for portal vein thrombosis with symptomatic portal hypertension in liver cirrhosis. J Hepatol 2011;54:78–88.

18. Thornburg B, Desai K, Hickey R, Kulik L, Ganger D, Baker T, *et al.* Portal vein recanalization and transjugular intrahepatic portosystemic shunt creation for chronic portal vein thrombosis: technical considerations. Tech Vasc Interv Radiol 2016;19:52–60.

19. Rodrigues SG, Sixt S, Abraldes JG, De Gottardi A, Klinger C, Bosch J, *et al.* Systematic review with meta-analysis: portal vein recanalisation and transjugular intrahepatic portosystemic shunt for portal vein thrombosis. Aliment Pharmacol Ther 2019;49:20–30.

20. Hall TC, Garcea G, Metcalfe M, Bilku D, Dennison AR. Management of acute non-cirrhotic and non-malignant portal vein thrombosis: a systematic review. World J Surg 2011;35:2510–20.

Robotic surgery and liver transplantation

Julie K. Heimbach

Department of Surgery, William von Liebig Center for Transplantation, Mayo Clinic, Rochester, MN, *USA*

E-mail address: Heimbach.julie@mayo.edu

Take-home messages

- Minimally invasive techniques in liver resection and donor hepatectomy have been adopted more slowly that other types of minimally invasive surgical techniques, but are becoming more common.
- Robotic liver resection and robotic donor hepatectomy have only recently been performed and there is limited amount of comparative data.
- The primary role of robotic surgery int the field of liver transplantation will likely be in living donor hepatectomy, in which there are clear benefits for improved wound healing complications, reduced pain, and reduced length of stay compared with donor hepatectomy.

Minimally invasive techniques in liver surgery

Minimally invasive techniques in liver surgery have been evolving over the past 2 decades. Laparoscopic liver resection was first described in 1993, although it was not until a seminal paper established feasibility in a series of 30 patients published by Cherqui and colleagues that it began to be considered by more centres.[1,2] Still, compared with other surgical procedures which rapidly transitioned to a laparoscopic approach, laparoscopic liver surgery has been adopted in a much more measured approach. In 2008, an international consensus conference reviewed the available evidence and concluded that for selected patients, especially those with solitary lesions in peripheral segments that laparoscopic had advantages.[3] This conference also outlined the indications for conversion to open surgery, the classification of minor and major hepatectomies, and the areas where more information was needed. The authors noted, in particular, that living donor hepatectomy 'will remain the most controversial application of laparoscopic liver surgery and should only proceed in the confines of a worldwide registry'.

Laparoscopic donor hepatectomy was first described in 2002, with a left lateral segmentectomy, and this was followed by the description of a hybrid, hand-assisted technique, and then subsequent descriptions of fully left- and right-donor hepatectomies.[4-9] A recent survey performed by the joint initiative of the International Laparoscopic Liver Society, laparoscopic techniques and Asian-Pacific Hepato-Pancreato-Biliary Association reported on 2,370 minimally invasive donor hepatectomies which have been performed worldwide.[10] This survey was able to identify 30 centres using minimally invasive techniques, with the most common procedure being purely laparoscopic donor right hepatectomy (n = 772) followed closely by laparoscopic-assisted right-donor hepatectomy (n = 708). More than 70% of the experience is focused in Asia, which is not unexpected given this is where the majority of living donor liver transplantation activity is occurring (Fig. 1). Giulianotti *et al.* from the University of Chicago reported the first right lobe robotic living donor hepatectomy in 2012,[11] and this survey demonstrated that interest in robotic donor hepatectomy has also been developing since then, with 130 of total 2,370 cases being performed utilising robotic techniques.

Fig. 1. Centres implanting minimally invasive donor hepatectomy around the world, from Rotellar *et al.* Transplantation 2022;106:96–105.

Robotic liver resection

There are no randomised trials comparing robotic liver resection with laparoscopic liver resection or open liver resection. However, robotic techniques can generally be considered as an extension of laparoscopic techniques. Both techniques utilise a minimally invasive approach and therefore will share the same advantage of smaller incisions which provides less pain, reduced hernia risk, reduced length of stay, and faster return to full function (although the liver specimen still needs to be removed through an incision). The robotic platform allows for more precise instrumentation and ease of techniques such suturing and allows for greater magnification and a more optimal 3D view compared with laparoscopic techniques. Laparoscopic instrumentation is more widely available (hospitals may have a limited number of robotic devices available which are often shared between different surgical specialties). It is associated with a lower cost *vs.* robotic instrumentation, whereas open techniques are lower than both. However, a recent economic meta-analysis of 38 published studies reporting on 3,847 patients (1,783 open liver resection [OLR]; 1,674 laparoscopic liver resection [LLR]; 390 robotic liver resection [RLR]) concluded that higher operative costs 'are offset by lower hospitalisation costs compared with OLR leading to no statistically significant difference in total costs, while RLR appears to be a more expensive alternative approach'.[12] Another difference between lap and robotic hepatectomy is that the cavitron ultrasonic surgical aspirator (CUSA), which is widely used for the parenchymal division in open liver resection is also available for laparoscopic resection, but not for robotic resection. In robotic surgery, the enhanced magnification (up to 10´) affording identification of vascular structures allows utilisation of the harmonic scalpel, and additional instrumentation is being developed for parenchymal dissection.

A recent systematic review of robotic surgical resection versus open or laparoscopic resection (all cases, not just liver resection) was published by Muaddi *et al.*[13] This study identified 336 studies and 18 randomised controlled trials reporting on patient outcomes after robotic compared with laparoscopic or open procedures. According to the randomised controlled trials, robotic prostatectomy, and robotic surgery for endometrial cancer offered advantages compared with laparoscopic or open techniques. Otherwise, the robotic procedures were found to be similar to open or laparoscopic techniques. Specifically for liver resection, the authors identified 13 retrospective studies, and no prospective studies or RCTs comparing robotic liver resection to laparoscopic or open liver resection.

The authors also highlighted the regulatory differences for approval of robotic techniques compared with pharmaceutical products allow for approval of robotic techniques for a broad range of procedures including thoracic, gynaecology, urology, and general surgery without the requirement of prospective studies or RCTs demonstrating benefit to patients.

Robotic living donor hepatectomy

Concerns over donor safety as well as for donor morbidity has slowed the widespread adoption of living donor liver transplantation (LDLT) outside of Asia, where limited access to deceased donor liver transplantation in conjunction with a high prevalence of liver disease has fostered broad acceptance of LDLT. As noted above, laparoscopic donor hepatectomy has also been rising slowly but steadily both in Asia and around the world, with a more recent interest also being developed in robotic hepatectomy. The most active groups performing robotic donor hepatectomy are in South Korea and in Saudi Arabia. Similar to the advantages described for robotic liver resection, the advantages for robotic donor hepatectomy include greater dexterity and visualisation (10´ magnification and more optimal 3D view). The trade-offs when compared with laparoscopic donor hepatectomy or open hepatectomy are highlighted in Fig. 2 and include safety concerns related to un-docking and slower changing of instrumentation, lack of CUSA availability for parenchymal transection, and logistical considerations including higher cost and lack of availability of robotic instrumentation.

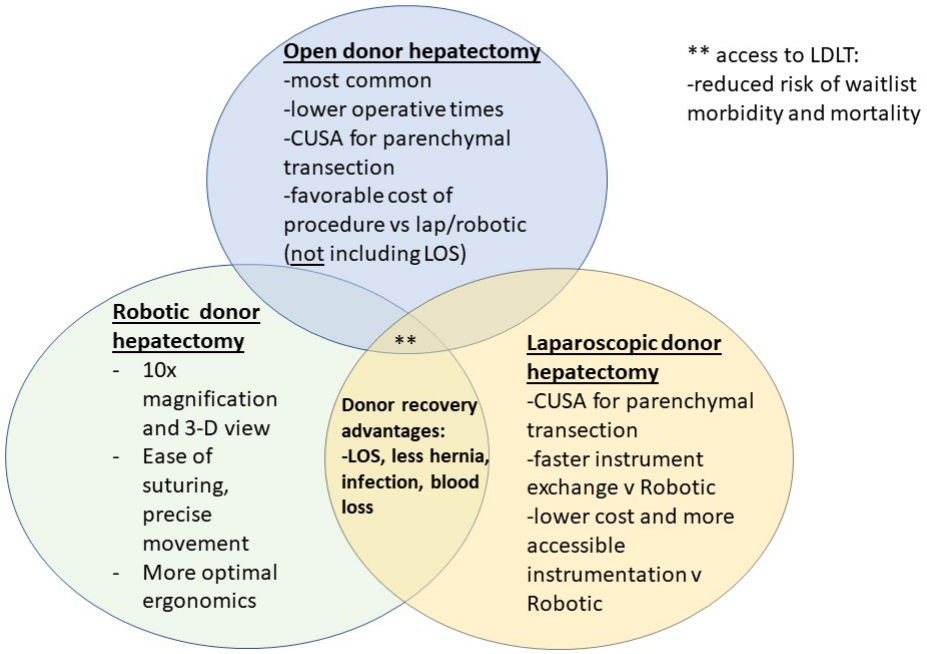


Fig. 2. Comparison of the advantages of robotic hepatectomy to open donor hepatectomy and laparoscopic donor hepatectomy, modelled after a similar figure by Broering et al. 2021. CUSA, cavitron ultrasonic surgical aspirator; LDLT, living donor liver transplantation; LOS, length of stay.

A recent outstanding review by Broering *et al.* describes in detail the challenges limiting the adoption of laparoscopic donor hepatectomy including the steep learning curve, unfavourable ergonomics, and instrumentation which in not optimised for the procedure.[14] They also performed an updated systematic review of minimally invasive donor hepatectomy and by including only studies with comparative data, they identified 5 relevant publications which compared outcomes of laparoscopic with open donor hepatectomy, as well as 3 studies which compared robotic hepatectomy with open hepatectomy, and 1 study which compared robotic left lateral donor segmentectomy (LLS) to laparoscopic LLS (summarised in Table 1). In this review the authors also report their results with 318 robotic donor hepatectomies (132 right lobes, 113 left lateral lobes, and 73 left lobes, in which they note 2 conversions and an overall donor mortality of 0 and morbidity of 5.9%. They compared these results with their prior experience with open donor hepatectomy including 639 open donor hepatectomies and found overall a higher rate of donor complications in the open group including a higher rate of Clavien grade 3 and 4 complications (p <0.01.) Importantly, the series began with open living donor hepatectomy, followed by a transition to laparoscopic and finally robotic hepatectomy and thus there may impact on the learning curve in the earliest part of this impressive series.

The International Laparoscopic Liver Society (ILLS) and the Asian-Pacific Hepato-Pancreato-Biliary Association (A-PHPBA) recently expert consensus guidelines on the safe development and implementation of minimally invasive donor hepatectomy.[15] A systematic review formed the basis of recommendations developed by 12 experts and their associates who utilised the Delphi method to attain agreement. There were 18 key questions assessed and 44 recommendations developed which were primarily based on laparoscopic approach to donor hepatectomy, with the most important being:

- Donor safety: they identified a similar or favourable rate of complications for minimally invasive donor hepatectomy *vs.* open donor hepatectomy with a level of evidence graded as 1-, and a strong recommendation.
- Donor recovery and wound healing complications: the summarised data concluded improved recovery and wound healing complications using minimally invasive techniques compared with open surgery, with level of evidence graded as 1-, and a strong recommendation.
- Recipient impact: the authors concluded that recipient outcomes are probably not inferior when minimally invasive donor hepatectomy is used compared with open approach for both paediatric and adult patients, with a grade of evidence at 2++ and a conditional recommendation.
- Robotic technique: the authors reviewed the evidence in support of the robotic approach and concluded 'RADH is still in its early phase compared to PLDH' and that the robotic approach appears as safe as the conventional open procedure, and offers similar advantages inherent to minimally invasive techniques, with the grade of evidence 2+ and a strong recommendation.

The consensus statement also presents considerable technical detail regarding optimal management of vascular and biliary structures as well as techniques for parenchymal division.

Areas of future research

As further experience with robotic liver resection and robotic donor hepatectomy grows, more comparative evidence between the robotic and laparoscopic approach will become available, providing important guidance on the optimal techniques to use for selected donor procedures or potentially with specific anatomic variants as well as the optimal methods of providing adequate training and experience to surgeons to master these techniques. An area of future research where there may be significant advantages to robotic donor hepatectomy over laparoscopic approach may be in creating synergy or merging the images from the 3D view obtained by the robotic camera and those obtained from 3D reconstructions obtained from cross-sectional imaging to allow for a more precise and safe

dissection. Although techniques to facilitate intraoperative navigation have been more readily adopted in neurosurgery, orthopaedics and ENT, where a rigid structure facilitates accurate synergy between intraoperative real-time images and preoperative imaging data, the deformation of the liver and surrounding tissues in the abdomen are problematic, and require more innovative techniques which are under development, as nicely summarised by Saito *et al.* in their recent review.[25]

A novel case report published in 2021 describes laparoscopic-assisted liver transplantation, with the recipient hepatectomy for a patient with a neuroendocrine tumour being performed laparoscopically, followed by implantation of the liver allograft through a reduced size midline incision.[26] The procedure included the unconventional step of dividing of the native liver (with extensive intrahepatic metastasis) into separate right and left lobes and removing them sequentially through an upper midline incision, followed by implantation of a right lobe living donor allograft via the same midline incision. The large size of a whole liver allograft as well as the size of an explanted native liver will remain a significant barrier to implementation of robotic or laparoscopic techniques. Combining this with coagulopathy and the importance of minimising warm ischaemia time, as well as the fact that reducing wound healing complications would likely have a limited clinical impact on the spectrum of issues experienced by liver transplant recipients, makes it unlikely that robotic techniques, at least as currently used may significantly impact the recipient operation. However, as identified in 1931 by Charles H. Mayo, one of the founders of the Mayo Clinic, 'Today the only thing that is permanent is change'.[27]

Table 1. Review of minimally invasive donor hepatectomy

Author	N	Donor morbidity (all)	Major morbidity (grade III and IV)	Hospital LOS (in days)
Rhu *et al.*[15]	Lap RH = 171 *vs.* open RH = 171	Lap 19.3% *vs.* open 20%	Lap 7.6% *vs.* open 10%	8.8 *vs.* 11.2
Hong *et al.*[16]	Lap RH = 198 *vs.* open RH = 198	Lap 6.2% *vs.* open 10.6%	Lap 2.5% *vs.* open 2.5%	7.5 *vs.* 8.6
Rhu *et al.*[17]	Lap RH = 64 *vs.* open RH = 64	Lap 15.6% *vs.* open 23%	Lap 6.2% *vs.* open 4.6%	8 *vs.* 10
Park *et al.*[18]	Lap RH = 53 *vs.* open RH = 53	Lap 15.7% *vs.* open 5.5%	Lap 15.7% *vs.* open 9%	11.3 *vs.* 11.2
Cho *et al.*[19]	Lap RH = 90 *vs.* open RH = 90	Lap 7% *vs.* open 3.3%	Lap 7% *vs.* open 1%	8.2 *vs.* 10.4
Chen *et al.*[20]	Robotic RH = 13 *vs.* open RH = 54	Robotic 7.7% *vs.* open 9.3%	Robotic 0% *vs.* open 1.4%	5.03 *vs.* 5.8
Broering *et al.*[21]	Robotic RH = 35 *vs.* open RH = 70	Robotic 6% *vs.* open 17%	Robotic 3.9 % *vs.* open 1.6%	
Rho *et al.*[22]	Robotic RH = 52 *vs.* open RH = 62	Robotic 23% *vs.* open 35%	Robotic 3.8% *vs.* open 1.6 %	9 *vs.* 10
Troisi *et al.*[23]	Robotic LLS = 25 *vs.* Lap LLS = 50	Robotic 0% *vs.* Lap 10 %	Robotic 0% *vs.* Lap 2 %	3 *vs.* 4

References

1. Gagner M, Rheault M, Dubuc J. Laparoscopic partial hepatectomy for liver tumor. Surg Endosc 1992;6:99.
2. Cherqui D, Husson E, Hammoud R, Malassagne B, Stéphan F, Bensaid S, *et al.* Laparoscopic liver resections: a feasibility study in 30 patients. Ann Surg 2000;232:753–62.
3. Buell JF, Cherqui D, Geller DA, O'Rourke N, Iannitti D, Dagher I, *et al*. The international position on laparoscopic liver surgery: the Louisville Statement, 2008. Ann Surg 2009;250:825-30.
4. Cherqui D, Soubrane O, Husson E, Barshasz E, Vignaux O, Ghimouz M, *et al*. Laparoscopic living donor hepatectomy for liver transplantation in children. *Lancet.* 2002;359:392–6.
5. Koffron AJ, Kung R, Baker T, Fryer J, Clark L, Abecassis M. Laparoscopic-assisted right lobe donor hepatectomy. Am J Transplant 2006;6:2522–5.
6. Han H-S, Cho JY, Yoon Y-S, Hwang DW, Kim YK, Shin HK, *et al.* Total laparoscopic living donor right hepatectomy. Surg Endosc 2015;29:184.
7. Troisi RI, Wojcicki M, Tomassini F, Houtmeyers P, Vanlander A, Berrevoet F, *et al.* Pure laparoscopic full-left living donor hepatectomy for calculated small-for-size LDLT in adults: proof of concept. Am J Transplant 2013;13:2472–8.
8. Chen K-H, Siow TF, Chio U-C, Wu J-M, Jeng K-S. Laparoscopic donor hepatectomy. Asian J Endosc Surg 2018;11:112–7.
9. Samstein B, Griesemer A, Halazun K, Kato T, Guarrera JV, Cherqui D, *et al*. Pure laparoscopic donor hepatectomies: ready for widespread adoption? Ann Surg 2018;268:602–9.
10. Rotellar F, Ciria R, Wakabayashi G, Suh KS, Cherqui D, WS-MIDH collaborative group. World survey on minimally invasive donor hepatectomy: a global snapshot of current practices in 2370 cases. Transplantation 2022;106:96–105.
11. Giulianotti PC, Tzvetanov I, Jeon H, Bianco F, Spaggiari M, Oberholzer J, *et al*. Robot-assisted right lobe donor hepatectomy. Transpl Int 2012;25:e5–9.
12. Ziogas IA, Evangeliou AP, Mylonas KS, Athanasiadis DI, Cherouveim P, Geller DA, *et al*. Economic analysis of open versus laparoscopic versus robotic hepatectomy: a systematic review and meta-analysis. Eur J Health Econ 2021;22:585–604.
13. Muaddi H, Hafid ME, Choi WJ, Lillie E, de Mestral C, Nathens A, *et al.* Clinical outcomes of robotic surgery compared to conventional surgical approaches (laparoscopic or open): a systematic overview of reviews. Ann Surg 2021;273(3):467–73.
14. Broering D, Sturdevant ML, Zidan A. Robotic donor hepatectomy: a major breakthrough in living donor liver transplantation. Am J Transplant 2022;22:14–23.
15. Rhu J, Choi G-S, Kwon CHD, Kim JM, Joh J-W. Learning curve of laparoscopic living donor right hepatectomy. Br J Surg 2020;107:278–88.
16. Hong SK, Tan MY, Worakitti L, Lee J-M, Cho J-H, Yi N-J, *et al*. Pure laparoscopic versus open right hepatectomy in live liver donors: a propensity score-matched analysis. Ann Surg 2022;275:e206–12.
17. Rhu J, Kim MS, Choi GS, Kim JM, Kwon CHD, Joh JW. Laparoscopic living donor right hepatectomy regarding the anatomical variation of the portal vein: a propensity score-matched analysis. Liver Transpl 2021;27:984–96.

18. Park J, Kwon CHD, Choi G-S, Lee S-K, Kim JM, Oh J, *et al.* One-year recipient morbidity of liver transplantation using pure laparoscopic versus open living donor right hepatectomy: propensity score analysis. Liver Transpl 2019;25:1642–50.

19. Cho HD, Kim KH, Yoon YI, Kang WH, Jung D-H, Park G-C, *et al*. Comparing purely laparoscopic versus open living donor right hepatectomy: propensity score-matched analysis. Br J Surg 2021;108:e233–4.

20. Chen P-D, Wu C-Y, Hu R-H, Ho C-M, Lee P-H, Lai H-S, *et al*. Robotic liver donor right hepatectomy: a pure, minimally invasive approach. Liver Transpl 2016;22:1509–18.

21. Broering DC, Elsheikh Y, Alnemary Y, Zidan A, Elsarawy A, Saleh Y, *et al*. Robotic versus open right lobe donor hepatectomy for adult living donor liver transplantation: a propensity score-matched analysis. Liver Transpl 2020;26:1455–64.

22. Rho SY, Lee JG, Joo DJ, Kim MS, Kim SI, Han DH, *et al*. Outcomes of robotic living donor right hepatectomy from 52 consecutive cases: comparison with open and laparoscopy-assisted donor hepatectomy. Ann Surg 2022;275:e433–42.

23. Troisi RI, Elsheikh Y, Alnemary Y, Zidan A, Sturdevant M, Alabbad S, *et al.* Safety and feasibility report of robotic-assisted left lateral sectionectomy for pediatric living donor liver transplantation: a comparative analysis of learning curves and mastery achieved with the laparoscopic approach. Transplantation 2021;105:1044–51.

24. Cherqui D, Ciria R, Kwon CHD, Kim KH, Broering D, *et al.* Expert consensus guidelines on minimally invasive donor hepatectomy for living donor liver transplantation from innovation to implementation: a joint initiative from the International Laparoscopic Liver Society (ILLS) and the Asian-Pacific Hepato-Pancreato-Biliary Association (A-PHPBA). Ann Surg 2021;273:96–108.

25. Saito Y, Shimada M, Morine Y, Yamada S, Sugimoto M. Essential updates 2020/2021: Current topics of simulation and navigation in hepatectomy. Ann Gastroenterol Surg 2021;6:190–6.

26. Dokmak S, Cauchy F, Sepulveda A, Choinier P, Dondéro F, Aussilhou B, *et al.* Laparoscopic liver transplantation. Ann Surg 2020;272:889–93.

27. Mayo, C.H. The Doctors Mayo quotes. https://history.mayoclinic.org/toolkit/quotations/the-doctors-mayo.php.

EASL™
The Home of Hepatology

Nurses & AHPs

On the frontline of hepatology

SCAN FOR MORE INFO

+ Nurses & AHPs Task Force
+ Nurses & AHPs webinars
+ Nurses & AHPs Forum at ILC
+ Rising Star Award
+ Abstracts & Bursaries at events

SESSION 2

LIVER SUPPORT AND REGENERATION

WEDNESDAY 22 JUNE
14H30 - 16H00

Clinical applications of liver support

Faouzi Saliba[1,2,3,*], Didier Samuel[1,2,3]

[1]AP-HP, Hôpital Paul Brousse, Centre Hépato-Biliaire, Villejuif, *France*; [2]Université Paris Saclay, Paris, *France*; [3]Unité INSERM 1193, Villejuif, *France*

*E-mail address: faouzi.saliba@aphp.fr

Take-home messages

- Extracorporeal albumin dialysis using artificial liver support therapies has been mostly used for a short period of time (a few days and rarely a few weeks), mostly as a rescue therapy or a bridge to liver transplantation (LT).
- Extracorporeal albumin dialysis with the MARS™ system showed a short but not long-term survival benefit, respectively, at 21 and 15 days in acetaminophen-induced acute liver failure (ALF) and acute-on-chronic liver failure (ACLF).
- Plasma exchange allows a bridge to LT and improved transplant-free survival in patients with ALF.
- Plasma exchange is currently under evaluation in a large RCT in patients with ACLF.
- Extracorporeal albumin dialysis should be limited to centres experienced in the management of advanced liver disease and to patients with a transplant project.
- Technical improvement in liver support devices and controlled clinical trials are still needed to evaluate the impact of liver support therapies in various clinical settings.

Introduction

Among liver support therapies, the artificial liver support system using dialysis machines is the only one currently used worldwide. Considering bioartificial liver support, the Vital Therapies extracorporeal cellular therapy (ELAD) failed to show, in two randomised controlled trials (RCTs), a beneficial effect on survival in patients with severe alcoholic hepatitis. Since, and to our knowledge, there are no ongoing clinical trials on bioartificial liver support systems.

In the mid-1990s, the concept of albumin dialysis appeared as a new revolution in the concept of dialysis with the great capacity of removal of toxins, drugs, and molecules strongly bound to albumin. Albumin dialysis devices, linked to a dialysis machine, allowed removal of both hydrosoluble and albumin-binding molecules, drugs, and toxins which are often increased in patients with advanced liver disease. **Among the three currently available artificial liver support devices, the Molecular Adsorbent Recirculating System (MARS™, Baxter International Inc., Deerfield, IL, USA), the fractionated plasma separation and adsorption system Prometheus™** (Fresenius Medical Care, Bad Homburg, Germany), **the single-pass albumin dialysis – MARS™ is the most studied and used system worldwide**.[1]

Extracorporeal albumin dialysis (ECAD) has been applied in various types of liver failure: acute liver failure (ALF), decompensated cirrhosis, acute-on-chronic liver failure (ACLF), liver dysfunction, and primary liver non-function after liver transplantation (LT), liver failure after major hepatectomy, refractory pruritus, drug overdose, and secondary liver dysfunction in intensive care unit (ICU) patients. ECAD has been used in most situations of liver failure as a rescue therapy or a bridge to transplantation.[1] The aim of ECAD is to provide a local and systemic best environment for liver regeneration.

Therapeutic plasma exchange with fresh frozen plasma and various forms of plasma adsorption have been explored as extracorporeal liver support therapies in ALF and in eastern countries in ACLF. The mechanism of action of therapeutic plasma exchange is mainly based on the removal of plasma cytokines and adhesion molecules, replacement of plasma factors, and immune modulation.[2]

Several reviews and meta-analyses on the effect of ECAD on short- and medium-term survival has been published. These meta-analyses faced serious limitations, as they pooled together data from studies testing different devices, artificial and bioartificial, they mixed different indications with very heterogenous population, and currently many of these devices are no longer on the market. Two recent meta-analysis clearly distinguish the devices and their effect on short- and mid-term survival in ALF and ACLF.[3,4]

Acute liver failure

Plasma exchange

A multicentre RCT of high-volume plasma exchange (HVPE) included 182 patients with ALF: 92 patients treated with HVPE *vs.* 90 patients treated with standard of care (SOC). The mean time to transplantation following listing was 4.6 ± 0.6 days in the HVPE-treated group and 3.7 ± 1.5 in the SOC group ($p = 0.75$). The study showed a significant improvement in overall hospital survival; 58.7% (HVPE) *vs.* 47.8% (SOC) (HR: 0.56; 95% CI 0.36–0.86; $p = 0.008$) and mainly transplant-free survival. In patients who were transplanted, HVPE before transplantation did not improve survival compared with patients who received SOC alone. The survival of those patients who fulfilled poor prognostic criteria but were not listed for transplantation owing to contraindications was significantly higher in those who received SOC plus HVPE (n = 28) as compared with those in receipt of SOC alone (n = 36).[2]

Molecular Adsorbent Recirculating System (MARS™)

An RCT was carried out with the use of MARS™ in patients with ALF. Patients had to be listed for liver transplantation at time of randomisation. The study could not demonstrate a survival benefit with MARS™ (n = 53) compared with a control group (n = 49) that received the standard medical therapy, at 6 months and 1 year (*i.e.* 85% in the MARS™ arm *vs.* 76% in the control arm at 6 months, and 83% *vs.* 76% at 1 year, respectively). In patients with paracetamol-related ALF, the 6-month survival rate was 68.4% (CI: 43.5–86.4%) with conventional treatment and 85.0% (CI: 61.1–96.0%) with MARS ($p = 0.46$). The limitation of the study was related to the short time between randomisation and transplant (fast availability of the graft median 16 h).[5]

In a recent multicentre study from the US Acute Liver Failure Study Group registry, 104 patients with ALF who received MARS™ were propensity-scored matched to 416 controls. The multivariable conditional logistic regression showed that MARS™ was significantly associated with increased 21-day transplant-free survival (OR: 1.90; 95% CI: 1.07–3.39; $p = 0.030$). Treatment with MARS™ has been associated with significant improvements (post *vs.* pre) in haemodynamics, creatinine, lactate, and ammonia particularly in acetaminophen-ALF.[6]

Decompensated cirrhosis and ACLF

Albumin dialysis

The clinical effects of albumin dialysis in ACLF patients have been evaluated in several RCTs and meta-analyses.

The HELIOS RCT (*The Fractionated Plasma Separation and Adsorption, Prometheus® [FPSA] system*) recruited 145 patients with ACLF, defined as Child-Pugh score >10 and bilirubin >5 mg/dl. Patients were randomized to either FPSA treatment or SOC. The survival did not differ among the 2 groups; on Day 28, 66% in the FPSA group and 63% in the control group ($p = 0.70$); on Day 90, they were 47% and 38%, respectively ($p = 0.35$).[7]

The RELIEF trial involved 189 patients with ACLF who were randomised to receive either MARS™ plus standard medical treatment (SMT, n = 95) or SMT alone (n = 94). Inclusion criteria were bilirubin >5 mg/dl and at least 1 of the following: hepatic encephalopathy grade II–IV, hepatorenal syndrome or bilirubin >20 mg/dl. The mean number of MARS™ sessions was 6.5. The study did not show an improvement of 28-day survival, which was comparable in both groups in intention-to-treat and per protocol population analyses (60.7% *vs.* 58.9% and 60% *vs.* 59.2%, respectively)[8]

The above 2 large studies did not use the current definition of ACLF. Two recent studies have evaluated MARS™ considering retrospectively the new ACLF definition.

Gerth *et al.* assessed the impact of MARS™ in a case-control study in patients with ACLF using the CANONIC study definition. The study suggested that MARS™ may improve short-term survival (14-day mortality in patients with ACLF-Organ Failure >1: 9.5% *vs.* 50%).[9] Bañares *et al.* evaluated, in a meta-analysis of individual patient data from 3 RCTs, showed that the number of MARS™ sessions independently predicted survival, indicating that the intensity of the MARS™ therapy may influence clinical outcomes.[10]

We recently reported (AASLD 2018, ILTS 2019) that in patients with ACLF who received MARS™, CLIF-SOFA score prior to MARS™ was the main predictive factor of 28-day mortality. Patients who received ≥3 MARS™ sessions had a significantly high short-term (28-day) survival. Factors associated with absence of response to 1–3 session treatments and futility to pursue treatment were: history of previous decompensation, high lactates before MARS™, long interval between ICU admission and initiation of MARS™ treatments, and short duration of MARS™ sessions.

Plasma exchange

Plasma exchange (PE) has been evaluated in few retrospective trials in the East[16,17]:

Yue Meng *et al.* evaluated the efficacy of PE in patients with ACLF secondary to HBV decompensated cirrhosis. Patients were enrolled into either a PE group (n = 38) or control group (n = 120). All patients were treated with entecavir along with the SOC. Patients in the PE group received 2–5 sessions of PE therapy. The cumulative survival rate at Week 4 and Week 12 in the PE group and control group were, respectively, 37% and 18%, and 29% and 14% ($p < 0.001$, by log rank test). On multivariate analysis, hepatic encephalopathy, ascites, PE treatment, and model for end-stage liver disease (MELD) scores were independent factors for liver-related mortality at Week 12.

Mao *et al.* analysed 62 patients with ACLF related to HBV reactivation who received PE treatment and compared them with 131 patients treated with SOC. The 30-day survival rate of the patients who received PE *vs.* controls was 41.9% *vs.* 25.2% ($p < 0.05$). The 30-day survival benefit was not seen for patients with MELD scores >30.

A systematic review was performed and included 4 studies that compared plasma-exchange to SMT in a non-randomized models and showed improvement in survival in the non-transplanted patients.

A large international RCT to determine the efficacy and safety of plasma exchange in patients with ACLF meeting the definition of CLIF-EASL criteria is currently ongoing in patients with ACLF (NCT03702920).

Overall, artificial liver support as to be integrated within the global critical care management of ACLF patients in the liver intensive care unit.

Refractory pruritus

MARS™

In patients with cholestatic pruritus, MARS™ has beneficial effects in relieving itch for a prolonged period. In a series of 20 patients with resistant pruritus, MARS™ resulted in a significant decrease of

itch assessed using a visual analogue scale (VAS).[13] Compared with baseline values, the VAS decreased by 72% immediately after treatment, and by 51% after 1 month. Pruritus decreased in all but 1 patient. There was a significant decrease of circulating bile acids after treatment and after 1 month.

In another series of 15 patients, treatment with MARS™ was associated with immediate and complete response in 11 patients (2 patients had a partial response, and 2 patients had no response). Mean VAS values decreased significantly while patients' quality of life improved. The median duration of acceptable relief in responders was three months[14].

Other artificial organ support under clinical trials

The *DIALIVE™* is a novel liver dialysis device, that replaces dysfunctional albumin and removes pathogen and damage associated molecular patterns in patients with liver failure. A phase II RCT, in patients with ACLF, has been presented at the EASL 2021 congress and showed improvement in ACLF grading and safety of the device (NCT03065699).

The Advanced Organ Support system (ADVOS™) allows elimination of water-soluble and albumin-bound substances and correct acid–base abnormalities. The ADVOS™ showed comparable clearance results to MARS™ in a retrospective comparative analysis with good safety[15].

Plasma bilirubin adsorption (Jafron Biomedical Co.), used in eastern countries in patients with ACLF as a result of HBV infection, alone or combined to 2 or 3 different dialysis systems that included continuous veno-venous hemodiafiltration (CVV-HDF), plasma exchange, MARS™ as well as other devices (Cytosorb®) have been reported, but not evaluated in prospective trials.

Conclusions

Artificial liver support has shown in numerous trials efficacy in clearance of bilirubin, biliary acids, ammonia, creatinine, and other toxins commonly increased in patients with ALF and ACLF. Their use should be limited to ICUs experienced in the management of advanced liver disease and in the setting of LTx. Their efficacity relies on the intensity of treatment and could allow a bridge to LTx in certain situations. They should be used at an earlier stage of the disease in both ALF and ACLF. Appropriate timing, patient selection, and survival benefit still needs to be shown prospectively in well-designed clinical trials.

References

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

1. Saliba F, Samuel D. Artificial liver support: a real step forward. Minerva Med 2015;106:35–43.
2. Larsen FS, Schmidt LE, Bernsmeier C, Rasmussen A, Isoniemi H, Patel VC, *et al.* High-volume plasma exchange in patients with acute liver failure: an open randomised controlled trial. J Hepatol 2016;64:69–78.
3. Kanjo A, Ocskay K, Gede N, Kiss S, Szakács Z, Párniczky A, *et al.* Efficacy and safety of liver support devices in acute and hyperacute liver failure: a systematic review and network meta-analysis. Sci Rep 2021;11:4189.
4. **Ocskay K, Kanjo A, Gede N, Szakács Z, Pár G, Eröss B, *et al.* Uncertainty in the impact of liver support systems in acute-on-chronic liver failure: a systematic review and network meta-analysis. Ann Intensive Care 2021;11:10.**

5. Saliba F, Camus C, Durand F, Mathurin P, Letierce A, Delafosse B, *et al.* Albumin dialysis with a noncell artificial liver support device in patients with acute liver failure: a randomized, controlled trial. Ann Intern Med 2013;159:522–31.
6. MacDonald AJ, Subramanian RM, Olson JC, Speiser JL, Durkalski-Mauldin VL, Abraldes JG, *et al.* Use of the molecular adsorbent recirculating system in acute liver failure: results of a multicenter propensity score-matched study. Crit Care Med 2022;50:286–95.
7. Kribben A, Gerken G, Haag S, Herget-Rosenthal S, Treichel U, Betz C, *et al.* Effects of fractionated plasma separation and adsorption on survival in patients with acute-on-chronic liver failure. Gastroenterology 2012;142:782–89 e783.
8. Banares R, Nevens F, Larsen FS, Jalan R, Albillos A, Dollinger M, *et al.* Extracorporeal albumin dialysis with the molecular adsorbent recirculating system in acute-on-chronic liver failure: the RELIEF trial. Hepatology 2013;57:1153–62.
9. Gerth HU, Pohlen M, Thölking G, Pavenstädt H, Brand M, Wilms C, *et al.* Molecular adsorbent recirculating system (MARS) in acute liver injury and graft dysfunction: results from a case-control study. PLoS One 2017;12:e0175529.
10. Banares R, Ibanez-Samaniego L, Torner JM, Pavesi M, Olmedo C, Catalina MV, *et al.* Meta-analysis of individual patient data of albumin dialysis in acute-on-chronic liver failure: focus on treatment intensity. Therap Adv Gastroenterol 2019;12:1756284819879565.
11. Tan EX, Wang MX, Pang J, Lee GH. Plasma exchange in patients with acute and acute-on-chronic liver failure: a systematic review. World J Gastroenterol 2020;26:219–45.
12. **Bernal W, Karvellas C, Saliba F, Saner FH, Meersseman P. Intensive care management of acute-on-chronic liver failure. J Hepatol. 2021;75(Suppl. 1):S163–77.**
13. Parés A, Herrera M, Avilés J, Sanz M, Mas A. Treatment of resistant pruritus from cholestasis with albumin dialysis: combined analysis of patients from three centers. J Hepatol 2010;53:307–12.
14. Leckie P, Tritto G, Mookerjee R, Davies N, Jones D, Jalan R. 'Out-patient' albumin dialysis for cholestatic patients with intractable pruritus. Aliment Pharmacol Ther 2012;35:696–704.
15. Falkensteiner C, Kortgen A, Leonhardt J, Bauer M, Sponholz C. Comparison of albumin dialysis devices molecular adsorbent recirculating system and ADVanced Organ Support system in critically ill patients with liver failure – a retrospective analysis. Ther Apher Dial 2021;25:225–36.
16. Yue-Meng W, Yang LH, Yang JH, Xu Y, Yang J, Song GB. The effect of plasma exchange on entecavir-treated chronic hepatitis B patients with hepatic de-compensation and acute-on- chronic liver failure. Hepatol Int 2016;10(3):462-9.
17. Mao W, Ye B, Lin S, Fu Y, Chen Y, Chen Y. Prediction value of model for end-stage liver disease scoring system on prognosis in the acute on chronic liver failure patients with plasma exchange treatment. ASAIO J 2010;56(5):475–478.

Hepatocyte transplantation in acute liver failure

Anil Dhawan

Paediatric Liver, GI and Nutrition Center and Mowat Labs, King's College Hospital, London, *UK*

E-mail address: Anil.dhawan@kcl.ac.uk

Take-home messages

- Acute liver failure (ALF) carries high mortality without liver transplantation; however, the procedure is limited by its timely availability and need for long-term immunosuppression.
- Auxiliary liver transplantation for ALF demonstrates that up to 70% of the children were able to regenerate their native liver with discontinuation of immunosuppression.
- Hepatocyte transplantation as a functional unit of liver can provide synthetic and detoxification function for period while native liver can regenerate.
- Hepatocyte transplantation in the large and small animal models of ALF have shown efficacy of this procedure.
- Initial human experience with allogenic human hepatocytes embedded alginate beads have shown native liver recovery in 50% of children who fulfilled criteria for liver transplantation.
- Currently several labs, including ours, are working on mechanistic insights, creating a source of human hepatocytes from embryonal or iPSC sources, use of mesenchymal stromal cells and cryopreservation of the alginate beads will be major developments in advancing the field.

Summary

Acute liver failure (ALF) in children and newborns is associated with high mortality without liver transplantation (LT). LT poses challenges of timely availability of a suitable donor liver, the need for a complex and expensive surgery and the need for life-long immunosuppression. The non-availability of size-matched donor livers can lead to considerable delay in LT, especially in neonates. The decision to proceed with LT in a child is compounded by the limitations of existing prognostic systems in reliably identifying those who may not recover spontaneously.[1] Auxiliary transplantation in paediatric ALF (PALF), when feasible in a limited number of cases, allows for discontinuation of immunosuppression in up to 65% children in the 23 months post-LT where the native liver regenerates.[2] As an extension to the concept, a modality to support liver function, such as hepatocyte transplantation (HT), could be a bridge to liver transplantation or spontaneous recovery with native liver regeneration. The advantages of HT include the possibility of (i) cryopreserved, off-the-shelf availability, (ii) precluding the need for a complex and expensive surgery, (iii) withdrawal of immunosuppression in case of native liver regeneration or total avoidance when alginate encapsulation technology is used, (iv) reinfusion of hepatocytes if required, and (v) the use of a single donor liver for multiple recipients or the use of stem cell derived hepatocytes.[3]

HT has been studied for long in animal models before being attempted in metabolic liver diseases and ALF.[3-5] Improved understanding of isolation and storage of hepatocytes, delivery modes, cell engraftment, allogenic rejection, hepatocyte survival and role of other cell types such as mesenchymal stromal cells offer opportunities of better outcomes with HT.[6] The context of ALF poses unique challenges for HT such as (i) the acuity of the condition that necessitates the immediate availability of preserved hepatocytes, (ii) the presence of coagulopathy that limits the mode of delivery of hepatocytes, (iii) the need to limit the use of immunosuppression for HT in an acutely unwell child with immunoparesis while awaiting transplantation or spontaneous recovery.

Cell types and source

HT currently uses mature hepatocytes from donor livers. In view of the experimental nature of therapy, the donor livers available for HT are of low quality with either severe steatosis, prolonged ischaemia, older or non-heart beating donors. Unused liver segments I or IV are also used. Neonate liver-derived hepatocytes are an attractive source of liver cells. The isolation of hepatocytes from liver is by a 3-step collagenase perfusion technique under aseptic precautions.[7] Exploration into new sources of hepatocytes include reprogramming of differentiated somatic cells to induced pluripotent stem cells (iPSCs), which can potentially differentiate into hepatocyte-like cells (HLCs).[8] These cells are functionally more akin to foetal hepatocytes with possible tumorigenic potential. The addition of mesenchymal stromal cells to hepatocytes provides the potential of immunoregulation and regeneration of hepatocytes.[6]

Hepatocytes, if not used for transplantation immediately, are often cryopreserved for future use with University of Wisconsin solution with 10% dimethyl sulfoxide with 5% glucose as a solution with 10^6–10^7 cells/ml at a temperature of -140°C.[9] The free-thaw cycle before usage may induce mitochondrial damage and apoptosis.[10] Hepatocyte quality assessments include the Trypan blue exclusion test and microbial analysis. These tests do not evaluate for early apoptosis or the functional status of hepatocytes.[11]

When HT is delivered through hepatocyte microbeads (HMBs), the hepatocytes are cryopreserved within alginate microbeads. The cell behaviour, protein release, and biological response following implantation of microbeads is dependent on the physical properties of the microbeads.[12] The protocols for HMB production have been optimised using good medical practice (GMP). Storage in HMBs could protect hepatocytes from the damage of cryopreservation with improved cell survival and functional viability. Uniform size (583.5 ± 3.3 mm) HMBs with the optimal polymerisation time (15 min) for microbead mechanical stability with the optimal cell density ($3.56´10^6$ cells/ml) for cell viability have been produced.[5] The purity of alginate in microbeads influences biocompatibility, as impurities tend to induce an immunological response to the beads.[13] The addition of cytoprotectants, especially a pan-caspase inhibitor (benzyloxycarbonyl-Val-Ala-DL-Asp-fluoromethylketone), improves the ultrastructure of encapsulated hepatocytes with a lower degree of cell apoptosis.[14]

Hepatocytes in alginate microbeads

Delivery of cells in HT in PALF is compounded by coagulopathy. Hence intrasplenic or intraperitoneal delivery is preferred over intraportal infusion through the portal vein.

Unlike in orthotopic liver transplantation (OLT), where the liver is an immunologically privileged organ with a potential for allogenic immune tolerance in the long term, hepatocytes infused in HT have high propensity for immune clearance when exposed to the recipient immune system. In hepatocyte infusions, this is mediated by an initial phase of phagocytic immunological clearance. This includes granulocyte/monocyte-mediated cytolysis as a result of exposed surface adhesion proteins and also coagulation/complement-mediated instant blood-mediated inflammatory reaction because of exposed tissue factors.[15,16] This is followed by T-cell mediated allogenic immune rejection. This immunological clearance of hepatocytes in overcome by HMB, which by virtue of being encapsulated are protected from the immune system leading to better cell survival. No significant activation of PBMCs co-cultured with HMB is observed when compared with PMBC co-cultured with empty beads.[5] Thus HMB can be used without the need for immunosuppression in PALF. The advantage of encapsulation was demonstrated by the fact that rat hepatocyte microbeads retrieved from the peritoneal cavity in animal studies were intact and free of immune cell adherence and contained viable hepatocytes with preserved function.[5] Although early cell destruction in HMB is less compared with that during infusion

of bare cells in tissues, the HMB technique of HT for ALF may still require higher hepatocyte mass in view of the impaired cellular function following cryopreservation and thawing.[5]

HMBs allow diffusion of nutrients, oxygen, and metabolic products while avoiding immunological activation. Laboratory studies have shown permeation of vital proteins such as albumin, coagulation factor VII, and also metabolites such as urea across HMBs.[5]

The use of HMBs in animal studies has shown superior survival to controls.[17] In a named patient, off-licence use of HMB in 8 children with PALF (median age of 14.5 days, range 1 day to 6 years), intra-peritoneal transplantation was well tolerated without complications. Staggered multi-session administration may be required to avoid large volume infusion so as to avoid abdominal compartment syndrome. Four of the 8 patients avoided LT, while 3 were successfully bridged to LT following HT with HMBs. The HMBs retrieved by irrigation during LT or by laparoscopy post-recovery were structurally intact without host cell adherence and contained viable hepatocytes with preserved functions. HMBs retrieved in one patient, 6 months post-HT, did show HMB enmeshed in fibrovascular tissue thus stressing the need for early retrieval once the therapeutic aim is achieved.[18]

Further research on intraperitoneal HMB infusion in PALF has focussed on improving cell viability and function by the addition of mesenchymal stromal cells to hepatocytes within the microbeads.[6]

Conclusion

HT, especially intraperitoneal delivery using HMB, is possibly a safe and feasible option in the management of PALF as a bridge to spontaneous recovery or liver transplantation.

References

1. Jagadisan B, Dhawan A. Emergencies in paediatric hepatology. J Hepatol 2022; in press. doi:10.1016/j.jhep.2021.12.027
2. Faraj W, Dar F, Bartlett A, Melendez HV, Marangoni G, Mukherji D, *et al.* Auxiliary liver transplantation for acute liver failure in children. Ann Surg 2010;251:351–6.
3. Forbes SJ, Gupta S, Dhawan A. Cell therapy for liver disease: from liver transplantation to cell factory. J Hepatol 2015;62(Suppl. 1):S157–69.
4. Matas AJ, Sutherland DE, Steffes MW, Mauer SM, Sowe A, Simmons RL, *et al.* Hepatocellular transplantation for metabolic deficiencies: decrease of plasms bilirubin in Gunn rats. Science 1976;192:892–4.
5. Jitraruch S, Dhawan A, Hughes RD, Filippi C, Soong D, Philippeos C, *et al.* Alginate microencapsulated hepatocytes optimised for transplantation in acute liver failure. PLoS One 2014;9:e113609.
6. Wang YH, Wu DB, Chen B, Chen EQ, Tang H. Progress in mesenchymal stem cell-based therapy for acute liver failure. Stem Cell Res Ther 2018;9:227.

7. Mitry RR. Isolation of human hepatocytes. Methods Mol Biol 2009;481:17–23.
8. Roy-Chowdhury N, Wang X, Guha C, Roy-Chowdhury J. Hepatocyte-like cells derived from induced pluripotent stem cells. Hepatol Int 2017;11:54–69.
9. Terry C, Dhawan A, Mitry RR, Lehec SC, Hughes RD. Optimization of the cryopreservation and thawing protocol for human hepatocytes for use in cell transplantation. Liver Transpl 2010;16:229–37.
10. Stéphenne X, Najimi M, Ngoc DK, Smets F, Hue L, Guigas B, *et al.* Cryopreservation of human hepatocytes alters the mitochondrial respiratory chain complex 1. Cell Transplant 2007;16:409–19.
11. Gramignoli R, Tahan V, Dorko K, Venkataramanan R, Fox IJ, Ellis EC, *et al.* Rapid and sensitive assessment of human hepatocyte functions. Cell Transplant 2014;23:1545–56.
12. Moya ML, Morley M, Khanna O, Opara EC, Brey EM. Stability of alginate microbead properties in vitro. J Mater Sci Mater Med 2012;23:903–12.
13. Orive G, Ponce S, Hernández RM, Gascón AR, Igartua M, Pedraz JL. Biocompatibility of microcapsules for cell immobilization elaborated with different type of alginates. Biomaterials 2002;23:3825–31.
14. Jitraruch S, Dhawan A, Hughes RD, Filippi C, Lehec SC, Glover L, *et al.* Cryopreservation of hepatocyte microbeads for clinical transplantation. Cell Transplant 2017;26:1341–54.
15. Olszewski WL, Interewicz B, Durlik M, Rudowska A, Mecner B. Early loss of transplanted autologous hepatocytes-lysis by leukocytes in vivo and in vitro. Transplant Proc 2001;33:651–3.
16. Gustafson EK, Elgue G, Hughes RD, Mitry RR, Sanchez J, Haglund U, *et al.* The instant blood-mediated inflammatory reaction characterized in hepatocyte transplantation. Transplantation 2011;91:632–8.
17. Mei J, Sgroi A, Mai G, Baertschiger R, Gonelle-Gispert C, Serre-Beinier V, *et al.* Improved survival of fulminant liver failure by transplantation of microencapsulated cryopreserved porcine hepatocytes in mice. Cell Transplant 2009;18:101–10.
18. Dhawan A, Chaijitraruch N, Fitzpatrick E, Bansal S, Filippi C, Lehec SC, *et al.* Alginate microencapsulated human hepatocytes for the treatment of acute liver failure in children. J Hepatol 2020;72:877–84.

Progress and limits in the use of liver cells in clinical practice

Teresa Brevini[1] and Fotios Sampaziotis[1,2,3,*]

[1]Wellcome-MRC Cambridge Stem Cell Institute; Cambridge, *UK*; [2]University of Cambridge, Department of Medicine, Cambridge, *UK*; [3]Cambridge Liver Unit; Cambridge University Hospitals NHS Foundation Trust, Cambridge, *UK*

*E-mail address: fs347@cam.ac.uk

Take-home messages

- Organoid-based regenerative medicine is an attractive alternative to liver transplantation.
- Multiple platforms have been developed giving rise to functional hepatocyte, cholangiocyte and multicellular liver organoids *in vitro.*
- The main challenges to clinical translation include the development chemically defined hydrogels, and addressing issues of immunocompatibility and safety.

Introduction

The liver has a remarkable regenerative capacity. Humans can tolerate up to 70% hepatectomy and still regenerate their liver. However, in disease, the extent or rate of damage exceeds the regenerative capacity of the liver resulting in liver failure. Despite recent advances in the management of liver disorders, transplantation remains the only treatment option for end-stage liver disease. However, transplantation is limited by organ availability. Regenerative medicine aims to address this challenge by using 2 complementary strategies. First, it aims to promote endogenous tissue regeneration. This approach is discussed in a different section of this syllabus and will not be the focus of this chapter. The second strategy aims to replace damaged tissue/cells with healthy ones via transplantation of bioengineered tissues or cells, which are not limited by organ availability. This chapter will cover the progress and limitations of regenerative cellular therapies for liver disease, with a particular focus on the organoid field.

Sources of cellular therapies for liver disease

In the past 30 years cellular therapies for liver disease have flourished. Historically, regenerative medicine in hepatology has been mainly based on primary hepatocyte transplantation or bone marrow-derived mesenchymal stem cell (MSC) infusion. Both strategies have shown encouraging results and advanced the field of regenerative hepatology. Primary hepatocyte transplantation has been one of the first effective cellular therapies for acute liver disease and is extensively discussed elsewhere in this syllabus. MSC infusions have shown promising systemic anti-inflammatory effects and improved liver function in acute-on-chronic liver disease and decompensated cirrhosis patients in a phase II clinical trial. However, both approaches also have some limitations. Primary hepatocyte transplantation is hampered by tissue availability and engraftment efficiency. Increased risk of thrombosis and cell engraftment in the lung has been reported with MSC infusions. Therefore, further studies are needed to fully address the safety and efficacy of these therapies.

Since the development of these approaches, recent advances in the stem cell and organoid field have resulted in new cell sources for regenerative medicine applications. The next sections summarise this progress.

What is an organoid?

Organoids are 3D cellular structures which are suspended in hydrogels composed of extracellular matrix (ECM) proteins and self-organise in an organotypic fashion and resemble their *in vivo* counterparts in terms of marker expression and function. Organoids have been developed both from induced pluripotent stem cells (iPSCs) or from adult primary cells to recapitulate most of the organs of the human body, including liver, lung, kidney, heart, brain, and gut. Organoids have been used for mechanistic studies on organ biology, disease modelling, drug testing, and regenerative medicine applications.[1] Organoids provide several advantages over conventional 2D culture conditions, the comparison between 2D and 3D culture is summarised in Table 1.

Table 1. Comparison between 2D and 3D culture conditions

	2D culture	3D culture
Cell properties	Limited expansion and functionality of primary cells, *e.g.* hepatocytes.	Primary cells can be cultured for long-term and show increased functionality and maturity compared to 2D.
Cell niche	Cells grow in 2D, resulting in a different spatio-conformational relationship between cells and ECM than *in vivo.* Limited apico-basal polarisation.	Cells self-organise in 3D space allowing to better recapitulate the 3D *in vivo* niche, e.g. lumen formation. Cells are highly polarised.
Clinical translation	Compatible with large scale manufacture. Cells have been grown in GMP-compatible conditions Already used in the clinical setting.	Challenges remain with large-scale production. The capacity to grow primary cells, which undergo minimal manipulation and show genetic stability, offers an attractive option for transplantation. Non-chemically defined culture conditions (Matrigel)/non-GMP. The regulatory landscape remains to be defined.

	2D culture	3D culture
	Inexpensive and time effective.	Expensive and more labour intense.
	Relatively little tissue culture training required.	Specific training is required.
Cost and practical aspects	Well-established assays without the need for further optimisation.	Assays need further optimisation for 3D.
	More homogeneous cell population, easy to characterise.	Culture is more heterogeneous, requires higher resolution techniques, *e.g.* single-cell RNA sequencing.

2D, 2-dimensional; 3D, 3-dimensional; ECM, extracellular matrix proteins; GMP, good manufacturing practice.

Hepatic and biliary organoids

Over the past few years, several organoid platforms have been developed in hepatology with overlapping and different characteristics. The next section summarises the available hepatic and biliary organoid platforms.

Hepatocytes

Hepatocytes constitute 95% of the liver parenchyma and perform most of the functions of the liver. Regardless of aetiology, all liver diseases result in hepatocyte damage and end-stage organ failure. Primary hepatocyte transplantation has been proven to be an effective cellular therapy; however, issues related to cell availability remain. Therefore, the development of hepatocytes for regenerative medicine applications remains a priority for modern hepatology. The organoid platforms summarised below have been developed to address his challenge.

iPSC-derived hepatocytes and hepatocyte organoids

iPSC-derived hepatocytes and hepatocyte organoids have been designed to mimic key steps of liver development *in vitro*. During development, hepatocytes derive from the bipotent liver progenitors (hepatoblasts) and commit to the hepatic or biliary lineage based on Notch or TGF-β signalling. This process has been reproduced *in vitro* through approaches performing the whole differentiation in monolayer, 3D culture or a combination of a 2D and 3D process, in which hepatoblasts differentiated in 2D are transferred in 3D hydrogels to complete hepatic maturation. The latter system generates relatively homogeneous populations of hepatocytes which are best suited for mechanistic studies; whereas the former leads to more heterogeneous organoids comprising hepatocytes and cholangiocytes or mesenchymal cells. Nevertheless, these platforms still displayed a foetal phenotype, with limited

functionality and engraftment *in vivo*. This has been at least partially addressed by the development of more mature multicellular tissue-like organoids, known as liver-buds.[2] These organoids combine iPSC-derived hepatocytes, endothelial cells, and mesenchymal cells, which self-organise in liver tissue-like structures. These liver-buds display enhanced hepatic functions, such as albumin secretion, and readily integrate in the host vasculature following transplantation. However, until recently these systems did not contain biliary structures required to drain bile from the liver. Several groups have tried to address this challenge and recently, a mixture of foregut and midgut organoids were used to simulate early endoderm morphogenesis and hepatobiliary development and give rise to multi-organ structures incorporating liver, pancreas, intestinal, and biliary progenitors.[3] This system could provide precious insights into liver development and set the stage for developing fully functional 'mini-liver' units *in vitro* compatible with transplantation.

Primary tissue derived organoids

Multiple strategies to propagate primary tissue-based hepatocyte organoids have been developed. The first generation of liver-derived organoids originated from adult liver stem cells of organs with end-stage liver disease. These adult liver stem cells were able to differentiate towards the hepatic lineage following treatment with a combination of growth factors such as EGF, HGF, and FGF10 with the Wnt activator R-spondin.[4] The resulting cells showed hepatocyte-like functions, such as albumin secretion; however, they still maintained some differences in terms of function and maturation compared to primary cells. To address this, the second generation of hepatic organoids was based on direct propagation of primary hepatocytes and was designed based on physiological mechanisms of liver regeneration and injury.[5,6] The resulting organoids displayed markers and functions comparable to primary cryopreserved hepatocytes, including albumin secretion and CYP3A4 activity. These systems resulted in successful propagation of primary hepatocytes from foetal or adult liver; however, adult hepatocytes showed limited expansion potential, posing issues related to access to tissue and manufacturing.

Hepatocyte organoids use for regenerative medicine

Both iPSC-derived, adult stem cell, and primary hepatocyte organoids have been transplanted in immunocompromised mice, either through splenic injection[4–6] or via ectopic transplantation.[2]

All systems have shown some degree of engraftment and functionality, as shown by detection of human albumin up to 90 days following transplantation.[5] Nevertheless, challenges remain for the clinical translation of these systems including large-scale expansion and engraftment efficiency. This could be addressed by generating bioengineered liver tissue *in vitro* which could enhance hepatocyte function and engraftment following transplantation.

Cholangiocytes

Biliary epithelial cells (cholangiocytes) represent only 3–5% of the liver parenchyma, however, bile ducts diseases (cholangiopathies) account for 25–30% of adult and 70% of paediatric liver transplants, creating a pressing need for the development of alternative treatment options. To address this, several cholangiocyte organoid systems have been developed and are summarised below.

iPSC-derived organoids

To achieve cholangiocyte differentiation of iPS cells, protocols based on this technology recapitulate key stages of bile duct development *in vitro*. Developmentally, in humans around Day 45 of gestation

we observe the formation of the ductal plate, which is a monolayer of cholangiocytes around the portal mesenchyme. Remodelling of the ductal plate into 3D ducts is driven by the Jagged1-Notch2 signalling between the ductal plate and the portal mesenchyme. This process has been recapitulated *in vitro* using different strategies, including monocellular or multicellular approaches. In multicellular systems hepatoblasts are co-cultured with OP9 cells, an irradiated stromal cell line, which recapitulates the portal mesenchyme; this crosstalk led to cholangiocyte-like cells showing key biliary features, such as marker expression and flow-sensing cilia. Nevertheless, the paracrine signalling between cell types remains difficult to disentangle. On the contrary, monocellular systems are chemically defined, as they are solely based on small molecules activating key biliary pathways such as Notch, Wnt, TGF-β and FGF, reducing culture variability and making them more amenable for clinical translation.[7,8] Monocellular iPSC-derived cholangiocyte organoids express key biliary markers, show typical morphological features, and respond to secretory stimuli.

Similarly to their hepatocyte counterparts, iPSC-derived cholangiocyte organoids address issues related to access to tissue but retain some foetal characteristics and show reduced functionality compared with primary tissue, making these cells more suitable for developmental studies than for regenerative medicine applications.

Primary tissue derived organoids

Organoid platforms based on primary cholangiocytes were rapidly developed following iPSC-derived biliary organoids. Primary systems are based on two complementary approaches revolving around Wnt signalling. Wnt is a master regulator of the stem cell *vs.* mature phenotype in the biliary tree, but also in other organs. Platforms based on canonical Wnt signalling are used to propagate adult liver stem cells with a biliary phenotype.[4] These cells express basic cholangiocyte markers and are able to differentiate both towards the biliary and the hepatic lineage. This bipotency comes at the cost of maturity, as these cells do not fully recapitulate the function of primary cholangiocytes. Conversely, systems based on non-canonical Wnt signalling promote the expansion of mature cholangiocytes which are committed to the biliary lineage and show increased functionality, making these cells highly suitable for regenerative medicine application.[9]

These mature organoids also capture the diversity and plasticity of primary cholangiocytes and can give rise to different cholangiocyte populations (intrahepatic, common bile duct or gallbladder cells), when exposed to appropriate environmental stimuli, such as bile.[10]

Importantly, primary organoids can be derived from liver biopsies, endoscopic retrograde cholangiopancreatography (ERCP) brushings and excised biliary tissue. Both adult stem cell and mature organoids have also been successfully derived from bile; however, the origin, health, and viability of cholangiocytes shed in bile remains variable. This must be taken into consideration and may pose an obstacle for mechanistic studies.

Cholangiocyte organoids use for regenerative medicine

Similarly to hepatocytes, both iPSC-derived, adult stem cell, and mature cholangiocyte organoids have been used to regenerate the biliary tree either via splenic injection or through retrograde infusion in the bile ducts. iPSC-derived and adult stem cell cholangiocyte organoid engrafted in the liver of immunocompromised mice but were not shown to rescue animal models of biliary injury. On the contrary, mature cholangiocyte organoids have been shown to regenerate up to 50% of the biliary epithelium following injury in mice.[10] Importantly, these organoids have also rescued ischaemic cholangiopathy in human livers perfused *ex-situ*; this was the first proof-of-principle demonstration of the use of organoid-based regenerative medicine in human organs.[10]

In case of extensive injury, where cellular therapy is not adequate, surgical intervention may be required. To address this, bioengineered bile ducts have been generated combining mature cholangiocyte organoids and densified collagen scaffolds.[9] These constructs have successfully reconstructed the extrahepatic duct in immunocompromised mice; nevertheless, several challenges remain to be addressed to upscale this technology to humans.

In summary, organoids have been derived from iPSC or adult cells, both with their advantages and disadvantages. Indeed, iPSC organoids can be derived from minimally invasive tissue sources, such as skin biopsies or blood, addressing issues related to tissue access and facilitating the development of patient's autologous cell therapies. Nevertheless, iPSC organoid protocols are based on iPSC reprogramming and recapitulate developmental stages, resulting in highly manipulated cells which express some markers of mature cells but often retains foetal characteristics. In addition, iPSC organoid derivation is a lengthy process, with significant variation in efficiency between users and experiments and is accompanied by the iPSC characteristic risk of genetic instability. On the contrary, primary cell organoids offer several advantages over iPSC organoids, including increased maturity, ease, and efficiency of organoid derivation and limited risk of genetic instability as the cells do not undergo reprogramming. Nevertheless, primary organoids are still limited by access to primary tissue. A comparison between iPSC and primary tissue derived organoids can be found in Table 2.

Table 2. Comparison between iPSC and primary tissue derived organoids

	iPSC-derived organoids	Primary tissue organoids
Cell source	Easy to derive autologous cells from virtually any patient with minimally invasive procedures, *e.g.* skin biopsy or blood sample.	Requires direct access to primary tissue. Autologous cells can be derived from moderately invasive procedures, such as ERCP.
Function	Retain some foetal characteristics showing limited functionality and maturity.	Closely recapitulate their *in vivo* counterpart.
Genetic stability	Cells undergo reprogramming and subsequent differentiation, resulting in a higher risk of genetic instabilities.	Cells are already differentiated. Require minimal manipulation and have a lower risk of genetic instabilities.
Culture efficiency	Lengthy process with variable efficiency. Culture requires highly experienced personnel.	Time-efficient process, as cells are already differentiated. Higher culture efficiency. Derivation is less labour intense and requires basic training.

ERCP, endoscopic retrograde cholangiopancreatography.

Challenges in translating organoids into clinical practice

Although several advances have brought organoids closer to clinical translation, multiple challenges remain to be tackled. A major limitation is linked to the use of poorly defined ECM hydrogels, such as Matrigel which is derived by a mouse sarcoma and present significant batch-to-batch variability. The development of chemically-defined hydrogels compatible with good manufacturing practice (GMP)

which can be used in combination with bioreactors could allow the upscaling required to use organoids in clinical practice and increase reproducibility, as it reduces operator-dependent variations.

Another challenge to overcome is immunocompatibility. The use of autologous cellular therapy is attractive as it could spare patients the need for immunosuppression; however, it would not be feasible in the case of acute liver failure or to treat genetic disorders. In these contexts, using allogeneic cellular therapies, which have been genetically engineered to escape recognition from the immune system, for example by HLA knockout, could represent a valuable strategy. Alternatively, organoid HLA-matching could be used in combination with immunomodulatory therapy as for solid-organ transplantation.

Finally, safety remains the first concern when exploring organoid clinical translation. Although proof-of-principle of the use of organoids has been shown in human organs and in small animal models, their employment in clinical trial is still very limited, with the only example coming from the use of salivary gland organoid for patients with radiotherapy-induced xerostomia. The regulatory framework which is developing around organoids will play a critical role on their future employment in clinical trial.

Conclusion

Hepatobiliary organoids have already shown to be powerful tools for regenerative medicine and will most likely shape the future of cellular therapies in hepatology.

References

1. Rossi G, Manfrin A, Lutolf MP. Progress and potential in organoid research. Nat Rev Genet 2018;19:671–87. **This review discusses advances and future directions of organoids and their applications for basic and translational science.**
2. Takebe T, Sekine K, Enomura M, Koike H, Kimura M, Ogaeri T, *et al.* Vascularized and functional human liver from an iPSC-derived organ bud transplant. Nature 2013;499:481–4**. This article reports the generation of vascularised liver-buds *in vitro*.**
3. Koike H, Iwasawa K, Ouchi R, Maezawa M, Giesbrecht K, Saiki N, *et al.* Modelling human hepato-biliary-pancreatic organogenesis from the foregut–midgut boundary. Nature 2019;574:112–6.
4. Huch M, Gehart H, van Boxtel R, Hamer K, Blokzijl F, Verstegen MM, *et al.* Long-term culture of genome-stable bipotent stem cells from adult human liver. Cell 2015;160:299–312.
5. Hu H, Gehart H, Artegiani B, Löpez-Iglesias C, Dekkers F, Basak O, *et al.* Long-term expansion of functional mouse and human hepatocytes as 3D organoids. Cell 2018;175:1591–606.e19. **This article reports the expansion of functional primary human hepatocytes as organoids.**
6. Peng WC, Logan CY, Fish M, Anbarchian T, Aguisanda F, Álvarez-Varela A, *et al.* Inflammatory cytokine TNFα promotes the long-term expansion of primary hepatocytes in 3D culture. Cell 2018;175:1607–19.e15.
7. Sampaziotis F, de Brito MC, Madrigal P, Bertero A, Saeb-Parsy K, Soares FAC, *et al.* Cholangiocytes derived from human induced pluripotent stem cells for disease modeling and drug validation. Nat Biotechnol. 2015;33:845–52.
8. De Assuncao TM, Sun Y, Jalan-Sakrikar N, Drinane MC, Huang BQ, Li Y, *et al.* Development and characterization of human-induced pluripotent stem cell-derived cholangiocytes. Lab Investig 2015;95:684–96.

9. Sampaziotis F, Justin AW, Tysoe OC, Sawiak S, Godfrey EM, Upponi SS, *et al.* Reconstruction of the mouse extrahepatic biliary tree using primary human extrahepatic cholangiocyte organoids. Nat Med 2017;23:954–63. **This article describes the generation of bioengineered bile ducts using primary human organoids.**

10. Sampaziotis F, Muraro D, Tysoe OC, Sawiak S, Beach TE, Godfrey EM, *et al.* Cholangiocyte organoids can repair bile ducts after transplantation in the human liver. Science 2021;371:839–46. **This article reports the first proof-of-principle demonstration of the efficacy of using organoids to repair marginal human organs.**

How to stimulate liver regeneration in liver transplantation and surgery?

Pierre-Alain Clavien*, Bostjan Humar, Richard Xavier Sousa da Silva

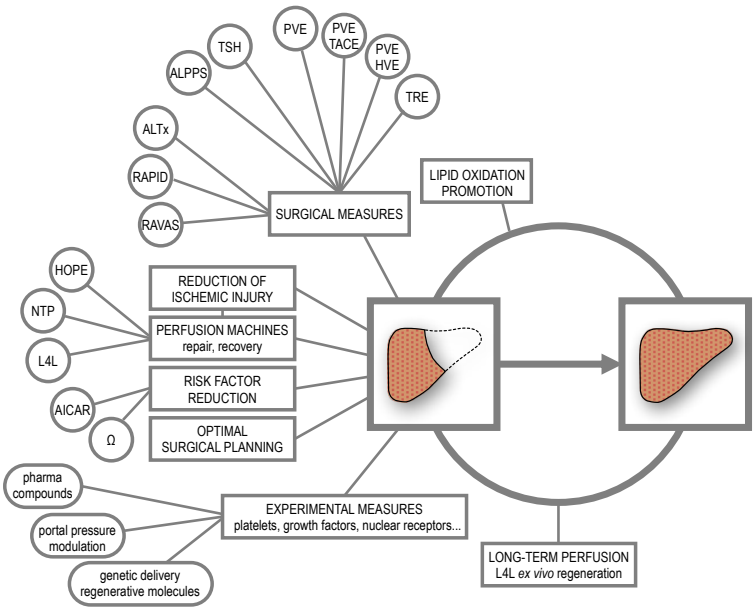
Department of Visceral & Transplant Surgery, University Hospital Zurich, Zurich, *Switzerland*

*E-mail address: clavien@access.uzh.ch

Take-home messages

- Measures to enhance the regenerative response after resection or transplantation basically cover preoperative strategies to increase the functional volume of the future remnant/graft, or approaches that exploit the physiological triggers and promoters of liver regeneration.
- Functional volume can be increased by the reduction of risk factors (steatosis, fibrosis a.o.) or ischemic injury, and includes proper surgical planning to minimize complications.
- The increase in portal pressure post surgery is perhaps the most important regenerative trigger and comes to play e.g. in two-stage hepatectomies that divert portal blood from diseased to healthy liver parts to increase the volume of the latter.
- Experimental measures relate to physiological promoters of regeneration, for example platelets, growth factors and other components of the regenerative machinery.
- Metabolic factors are increasingly being recognized as drivers of liver regeneration, such as the provision of sufficient regenerative energy via lipid oxidation.
- Novel options include ex vivo perfusion devices that can act to mitigate ischemic injury or to maintain liver alive for prolonged periods, enabling preoperative organ repair or potentially the generation of new, transplantable liver mass.

Synopsis



Measures to enhance the regenerative response after resection or transplantation basically cover preoperative strategies to increase the functional volume of the future remnant/graft, or approaches that exploit the physiological triggers and promoters of liver regeneration. Functional volume can be increased by the reduction of risk factors (steatosis, fibrosis, etc.) or ischaemic injury, and includes

proper surgical planning to minimise complications. The increase in portal pressure post surgery is perhaps the most important regenerative trigger and comes to play, for example in 2-stage hepatectomies that divert portal blood from diseased to healthy liver parts to increase the volume of the latter. Experimental measures relate to physiological promoters of regeneration, for example platelets, growth factors, and other components of the regenerative machinery. Metabolic factors are increasingly being recognised as drivers of liver regeneration, such as the provision of sufficient regenerative energy via lipid oxidation. Novel options include *ex vivo* perfusion devices that can act to mitigate ischaemic injury or to maintain liver alive for prolonged periods, enabling preoperative organ repair or potentially the generation of new, transplantable liver mass.

Clinical need for liver resection and transplantation

The application of both major liver resection and liver transplantation depends on the liver's ability to regenerate after tissue loss. Prime indications for resections are primary and secondary liver tumours of any kind, with transplantation remaining an option for unresectable (*e.g.* large, multiple) tumours.

Colorectal liver metastasis remains the most frequent indication for resection, followed by primary liver cancer. Incidences for hepatocellular carcinoma (HCC) and cholangiocarcinoma however are rising, mostly attributable to poor lifestyle (sugar-rich diet, physical inactivity) but also to changes in primary care leading to the appearance of more advanced disease stages. Besides benign hepatic tumours, other frequent indications are symptomatic cysts or extensive echinococcosis.

In liver transplantation, indications have expanded massively over the past 2 decades despite the maintenance of very strict selection criteria. Entities such as perihilar and intrahepatic cholangiocarcinoma, colorectal liver metastases, neuroendocrine tumours, and chronic liver failure (mostly as a result of liver cirrhosis caused by alcoholic steatohepatitis or the steadily increasing non-alcoholic steatohepatitis) have been added to the established curative treatment of HCC and acute liver failure. A component of liver transplantation distinct from resection is ischaemia–reperfusion injury (IRI), particularly relevant in donation after circulatory death. IRI occurs to a lesser extent in any other transplantation, but is avoidable in resection, even though vessel clamping for the prevention of blood loss is standard for many hepatectomies. IRI is characterised by mitochondrial dysfunction and parenchymal necrosis, ultimately reducing the functional liver volume.

Liver failure

Liver failure can occur after both resection or transplantation, with the key cause being a too small functional volume of the liver remnant or graft, respectively. As the liver needs to maintain vital metabolic function also after surgery, a small remnant/graft must balance the metabolic pressure with the need to regenerate. If too small, metabolic pressure is overwhelming, and the remnant/graft cannot recover volume anymore. As a result, metabolic tasks cannot be maintained over time, and eventually the liver fails, reflected in hyperbilirubinaemia, prolonged coagulation, hypoalbuminaemia, increased lactate, and hepatic encephalopathy. No specific treatment exists for surgical liver failure, and preventive measures largely centre around the securing of a sufficient functional liver volume. The risk for posthepatectomy liver failure (PHLF) is increased if the volume of the remnant is <20% of the original volume (corresponding to <0.5% remnant-to-body weight ratio) for healthy liver, <30% for chemotherapy-exposed, or <40% for cirrhotic liver. For transplantations, the small-for-size syndrome (SFSS, failure post transplant) may develop if the graft-to-recipient body weight ratio is <0.8%. The higher volume requirements for transplantations are explained by the inevitable presence of IRI, reducing the functional graft volume. Functionality of the remnant/graft can be assessed by specific liver function tests, including ICG, HIDsnA, or LiMAx.

Extensive IRI of grafts leads to early allograft dysfunction (usually transient), or to primary graft non-function requiring re-transplantation with high mortality and morbidity. Indeed, PHLF/SFSS remains the most common cause of death as a result of liver surgery.

Experiments in our and other laboratories have demonstrated that both PHLF and SFSS can be efficiently prevented through measures that are potent promoters of liver regeneration. According approaches that are or could be used in the clinic will be presented in the following sections.

Liver regeneration

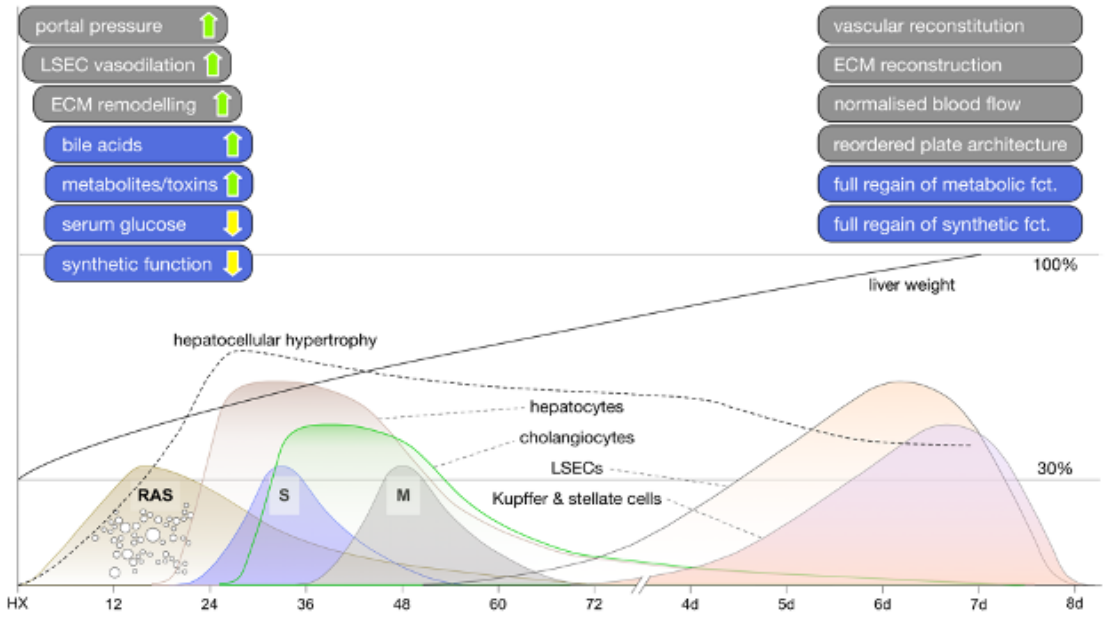


Fig. 1. Temporal course of key processes during liver regeneration following tissue loss. Boxes indicate processes marking the proximal and distal events during regeneration. Percentages (right Y-axis) refer to percentage of liver weight regain after 70% hepatectomy. Time is indicated in hours followed by days and refers to liver regeneration in mice. The dashed line indicates the extent of hepatocellular hypertrophy occurring, while solid lines indicate the main proliferative phases of liver parenchymal and non-parenchymal cells. RAS indicates the peak of regeneration-associated steatosis occurring in hepatocytes after resection. S and M refer to the replication and division peaks of hepatocytes.

The liver is the only mammalian organ capable of complete recovery following major tissue loss. Research in particular on standard hepatectomy (sHx, 68% vol. removed) in mice has revealed the profound complexity behind liver regeneration. In mice, the original volume is restored within 7–10 days after sHx, whereas this process takes about 2–3 weeks in humans. In general, liver regeneration (LR) starts from mature hepatocytes (*dissociating it from cancerous growth which depends on stem cells*) that increase their size (*cellular hypertrophy, slow growth*) and enter proliferation (*hyperplasia, rapid growth*). LR following tissue loss can be subdivided into 3 major phases, **priming** (*preparation for imminent growth*), **progression** (*cells commit to growth and progress through the cell cycle*), and **termination** (*re-entry of hepatocytes into quiescence*, not discussed here). Altogether, these phases run in an orderly, interdependent fashion, where initiation of 1 phase requires completion of the previous. An overview of the regenerative process is given in Fig. 1.

Key events priming phase

The most profound event immediately after sHx is the increase of portal inflow into the remnant, roughly tripling without a change in arterial supply. As a result, portal pressure rises, providing key signals to trigger the regenerative process: (i) to cope with the increased inflow, the sinusoids dilate and become mechanically activated to produce 2 mitogens, HGF and WNT2, which stimulate hepatocytes to grow and divide (Fig. 2); (ii) the elevated portal inflow provides the remnant with additional nutrients and circulating growth factors, in particular EGF (together with HGF & WNT2 the central mitogens driving LR); (iii) shear stress further leads to the activation of the urokinase system, which remodels the extracellular matrix (ECM), a prerequisite for the repopulation and reorganisation of the liver. Likewise, shear stress (together with injury) also activates platelets that release their contents to foster LR.

The hepatic mitogens engage their respective receptors (HGF>MET, WNT2>FZ, EGF>EGFR) to elicit various signalling pathways that promote hepatocyte hypertrophy, cell cycle entry, and the metabolic adaptations (Fig. 3). Besides sinusoids, a series of other cell types contribute to regenerative signals. Kupffer cells (KCs) have a prominent role here: activated through gut endotoxins (via increased portal inflow), complement components (via injury and platelet activation), and reactive oxygen species (via injury, portal blood), KCs secrete IL-6, TNFA, and WNTs to amplify downstream signalling events in hepatocytes. Collectively, the sum of these events prepares the remnant to enter the major growth phase.

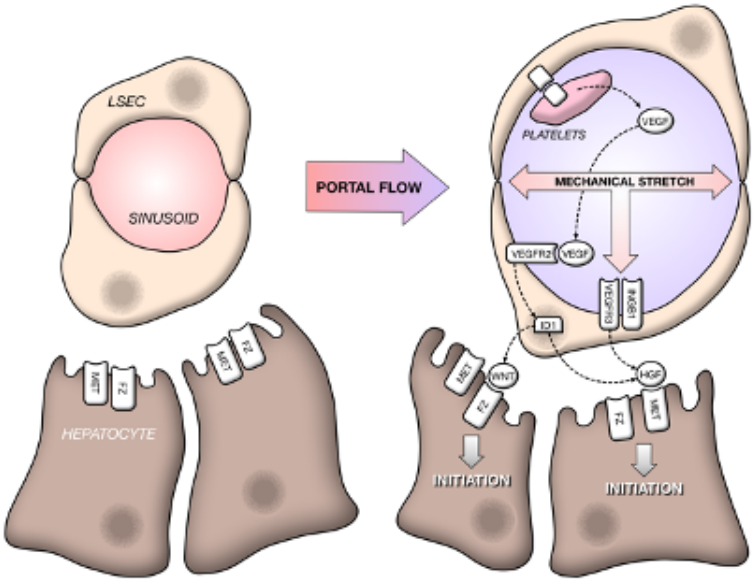


Fig. 2. The angiocrine niche provides central triggers for the initiation of liver regeneration. Mechanical stretch and the ongoing vasodilation of sinusoids in response to the increased portal inflow induce via INGB1/VEGFR3 the release of the core hepatic trophogen HGF. In parallel, increases in circulating VEGF (through blood import and platelet activation) stimulate the VEGFR2-ID1 axis to provide WNT2 and further HGF. Activation of MET and FZ receptors then initiates the regenerative process in nearby hepatocytes.

The importance of portal inflow. In general, the increases in portal inflow can be considered as the most important trigger behind LR. In 2-stage hepatectomies, for example, diseased liver parts are first portally ligated to induce growth of the remaining unligated parts, enabling a safe resection of diseased liver at a later stage. Portal ligation simply diverts portal blood to the unligated liver part, increasing portal inflow locally. Thus, an elevation in portal flow is sufficient to induce LR. However, if the increase is excessive, such as after extended resection, liver may fail.

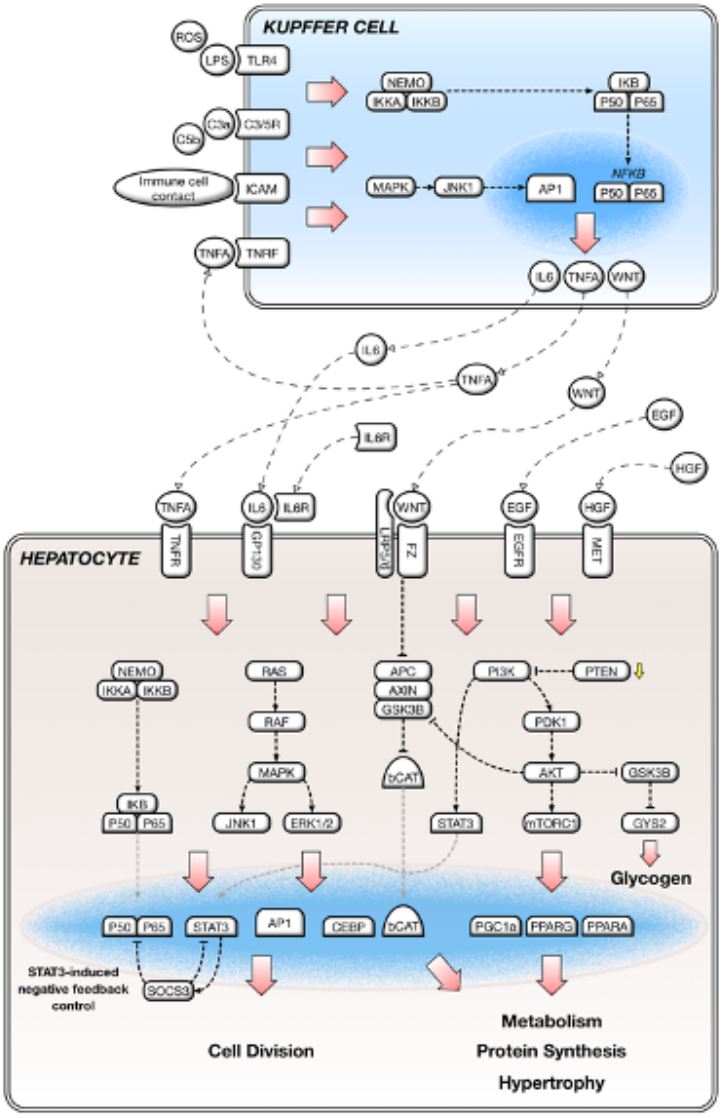


Fig. 3. Main signalling pathways related to the priming phase and the entry of hepatocytes into the cell cycle. Kupffer cells translate inflammatory signals associated with resection into regenerative stimuli acting on hepatocytes. Liver sinusoidal endothelial cells and other non-parenchymal cells likewise contribute to the priming and progression phases. For graphical clarity, these contributions have not been depicted.

Key events progression

How priming segues into progression is less clear. Rather than specific events, the mutual fortification and precise orchestration of pathways to an overall activity peak seems to constitute a plausible trigger point. Cytokine-induced NFKB/STAT3 and MET/EGFR-induced STAT3 activities, however, are considered central for the initiation of hepatocyte proliferation. Combined *vs.* single deletion of given pathways further suggests that cell cycle entry depends on full cooperation between main mitogenic pathways, whereas sustained activity of a lesser number of pathways is needed for further progression. To note, many additional pathways participate in LR (*e.g.* Notch, Hedgehog, FGFs, PDGFs, nuclear receptors, and all the usual pathways regulating cell division and growth). Most single pathways act in redundant ways (*i.e.* their deficiency causes delay, but not complete halt) as to provide LR with the stability required to restore full liver function also under suboptimal conditions.

Metabolic control of LR

As the liver is our metabolic headquarter, the loss of liver tissue leads to metabolic insufficiencies which are sensed by the body to generate systemic metabolic responses that aid LR.

The liver provides bodily glucose, and resection hence leads to hypoglycaemia. In response, peripheral fat stores are mobilised and lipids accumulate in the remnant (regeneration-associated steatosis, see Fig. 1), where they are oxidised to provide fuel for LR. Therefore, systemic hypoglycaemia constitutes a trigger to provide the growing liver with sufficient energy.

Bile released by the liver is re-absorbed by the gut and re-enters the liver via the portal vein. Following resection, bile acids thus accumulate in the remnant and activate the nuclear receptor FXR, which in turn induces growth genes in the liver and FGF15 in the gut. FGF15 then signals back to the liver to enhance growth signals, and to downregulate bile acid production to avoid toxic overload.

Pathophysiology behind posthepatectomy liver failure. Work from our lab provides a novel concept for PHLF development. Extended resection induces a hyperafflux of O2-poor portal blood. In response, early hypoxia develops in the remnant, HIF2A becomes activated, and suppresses lipid oxidation (via PPARA/PGC1alpha downregulation). As a result, energy provision is insufficient for hepatocytes to progress through the cell cycle and to meet their metabolic functions. RAS persists, the remnant cannot grow, and eventually fails under the metabolic pressure.

The liver is equipped with a plethora of nuclear receptors that each react to a range of metabolites. Thus, derangements in bile acids, lipids, xeno/endobiotics etc. are sensed by specific nuclear receptors to induce growth promoting genes as well as metabolising/synthetic genes needed for the liver to cope with the growing metabolic pressure. Constitutive androstane receptor (CAR) is a classic example; activated by various xeno/endobiotics, bilirubin, or steroid hormones, the transcription factor induces metabolic genes in for example drug/bilirubin clearance, but likewise proliferative genes. Activation of many of the nuclear receptors in resting liver can lead to hepatomegaly, illustrating how metabolic strain adapts liver volume to request – properties that are likewise essential for the regenerating liver.

Regeneration and liver volume stimulation in resection

Clinical measures

Surgical manoeuvres

As mentioned in the previous chapter (green box), modulation of portal inflow is the basis for a series of surgical approaches aiming at increasing preoperative liver volume to minimize PHLF risks. **Pretreatments** have been introduced in the 1980s and mostly rest on **portal vein embolisation** (PVE, where the portal veins supplying diseased liver parts are occluded by embolisation), which induces compensatory liver growth (via diverting portal flow) of healthy liver parts that will serve as future liver remnant (FLR). PVE can increase the FLR volume by up to 40% within a period of 4–8 weeks. Thereafter, a safe resection of the diseased liver part is feasible also for patients that otherwise (*i.e.* in the case of a straight hepatectomy) would be at PHLF risk. A pretreatment variant is **PVE preceded by transarterial chemoembolisation** (TACE, chemotherapy), which is applied particularly for HCC to reduce the risk of tumour progression between PVE and later resection. Compared with PVE alone, this approach has a higher FLR growth rate but also higher morbidity. A related approach is **transarterial radioembolisation** (TRE, 'radiation lobectomy'), where radioembolisation of the diseased part causes portal vein thrombosis (with effects akin to PVE) and a FLR growth comparable to PVE, however with reduced tumour progression rates. A recent variant combines **PVE with hepatic vein deprivation** (PVE-HVE), thus the embolisation of both portal and hepatic veins to reduce the development of collateral vessels that counteract the portal inflow reduction. As a result, portal inflow should be elevated in the healthy liver parts, leading to improved volume gains as confirmed in initial studies.

If the FLR however also is diseased, the above techniques can be combined with a cleansing resection of the tumour from the FLR, followed by tumour resection from the embolised part at a second stage. These approaches are collectively coined **two-stage hepatectomies** (TSH). For bilobar tumour involvement, TSH was adapted as early as 2000. Here, in a first stage, the FLR is surgically freed from tumour nodules and a portal vein ligation (PVL, with effects akin to PVE) is performed on the contralateral (diseased) side. After a waiting period of 4–8 weeks, hepatectomy of the tumour-involved liver lobe follows in a second stage. However, the long latency between the first and the second stage (up to 3 months) comes with a high risk of tumour progression. In 2012, Schnitzbauer *et al.* introduced a novel TSH now called **ALPPS (associating liver partition and portal vein ligation for staged hepatectomy)**. In stage 1, any tumours are resected from the FLR, the contralateral portal vein is ligated, and additionally a parenchymal transection along the demarcation line between the ligated and unligated part is done. The transection disrupts intrahepatic shunts, thus portal flow is much more efficiently diverted towards the FLR. More so, transection adds injury that triggers regenerative signalling. As a result, the regeneration of the FLR is massively accelerated, enabling stage 2 resections of diseased liver already after a mere 1–2 weeks. Although ALPPS decreases the chances of tumour progression, it is a high-risk surgery with elevated morbidity and mortality. To counteract, several adaptations have been made to reduce morbidity and mortality, including partial ALPPS, mini-ALPPS, and tourniquet ALPPS. Meanwhile, ALPPS is reasonably safe and offered by many university hepatopancreaticobiliary (HPB) centres.

Preoperative measures that improve liver quality

Steatosis and other pathologies (*e.g.* fibrosis) reduce the functional liver volume and thus deteriorate LR. Recently, a strong reduction in hepatic lipid content was reported for coronary artery patients treated with the AMPK activator AICAR. In animals undergoing liver surgery, AICAR likewise reduced

liver fat, suggesting this compound may be beneficial for preoperative defattening. Other compounds with antisteatotic effects include W3 fatty acids, which were shown to accelerate LR and recovery in living donor liver transplantation.

3D printing of patient liver models

An emerging option to improve surgical outcomes is the preoperative 3D printing of patient liver models based on MRI, CT, and 3D angiography. 3D models enable more precise definition of liver volume/size, complex vascular/biliary structures, and of tumour localisation. Thus, surgeries can be planned to fit each patient (including better donor/recipient matching), minimising surgical complications that may interfere with optimal LR. *Outlook:* 3D printing may be used for bioartifical liver scaffolds that can be colonized by cells (*e.g.* mesenchymal stem cells that can differentiate into both hepatocytes and endothelial cells), yielding implantable material to aid LR.

Promotion of lipid oxidation

Several compounds known to foster lipid oxidation have been shown to reduce IRI, promote LR and prevent PHLF in animals. These include AICAR (an AMPK activator), and the widely used W3 fatty acids and L-carnitine. Although W3 fatty acids had no effects in a hepatectomy trial (likely as a result of suboptimal dosing) but were beneficial in transplantation, L-carnitine did improve survival after transplantation and reduce PHLF occurrence after resection in patients. *Caveat: glucose infusion is standard for liver surgeries, however it may hinder LR as it counteracts lipid oxidation.*

Experimental measures

Platelet infusion

Platelets promote LR, and low platelet counts correlate with low volume gain and high complication rates post surgery. Pre-/perioperative infusion of platelets (and perhaps of other haemostatic factors) may improve LR.

Growth factors

Given the full dependence of LR on mitogens, perioperative supplementation of growth factors (GFs, particularly HGF, EGF, and WNTs) is efficient in enhancing LR and preventing/counteracting PHLF in animals. Supplementation may be via recombinant protein, modern genetic approaches such as mRNA delivery, or by pharmacological compounds (ligand mimics, receptor activators, or agents that activate respective downstream signalling such as T3 thyroid hormone inducing the WNT endpoint b-catenin). One key limitation is the oncogenic role of GFs: HGF, EGF, WNTs all can foster neoplasia such as occult microtumours left after resection. Thus, the use of GFs would require molecular patient profiling to identify tumour-specific GF addictions. Many HCCs, for example, do not depend on b-catenin and hence could be compatible with WNT supplementation to support LR upon their resection.

Nuclear receptors

Activation of a series of nuclear receptors (CAR, FXR, PXR, PPARs, ER, etc.) by natural or synthetic ligands induces a gene expression program that promotes (i) parenchymal growth and (ii) metabolic capacity, often associated with spontaneous hepatomegaly. Nuclear Receptors (NR) activators potently stimulate LR after resection/transplantation and prevent PHLF in animals. Activation of CAR by TCPBOP is highly efficient in rodents, however human CAR induces another set of genes than mouse CAR and might be unsuited for clinical settings. By contrast, several agents currently being tested in the clinic are potent activators of human nuclear receptors and could be promising targets to support human LR. To mention are ligands of FXR (to mimic and potentiate the regenerative, but not the toxic effects of bile acids), PPARA (ligand fenofibrate, also a strong inducer of lipid oxidation), or ER (oestradiol, to promote LR in postmenopausal women or men). Again, oncological risks need consideration.

Splanchnic vasoactive compounds

Increased portal inflow triggers LR, whereas excessive inflow may provoke PHLF. Pharmacological modulation of portal flow shows desired effects in animals. Perioperative monitoring of portal pressure may guide the use of splanchnic vasodilators (*e.g.* substance P) to enhance portal inflow and the regenerative response, while splanchnic vasoconstrictors (*e.g.* terlipressin) may serve to lower portal inflow and PHLF risks in settings of extended resections.

Future options

Novel methodologies that transiently alter levels of a given molecule (*e.g.* mRNA delivery by nanoparticles) may be handy tools to manipulate regenerative capacities in the clinic. Besides the supplementation with GFs or other molecules mentioned above, such technology could be used to suppress inhibitors of LR or foster other key events occurring with successful regeneration. Genetic overexpression of for example the urokinase system might enhance ECM remodelling to support hepatocyte proliferation. Systemic screens in animal models are identifying novel proteins with crucial roles in LR; intriguing examples include MKK4 (identified by a RNAi-screen), or BAZ2 bromeodomain transcription factors (identified by a CRISPR screen), all potent inhibitors of mouse LR the suppression of which markedly accelerates regeneration. Of note, pharmacological antagonists of these proteins exist and have similar benefits on LR as the genetic suppression of the inhibitors. The CRISPR screen further revealed that promotion of general protein synthesis (*e.g.* by overexpression of ribosomal subunits) is sufficient to enhance the regenerative response after injury.

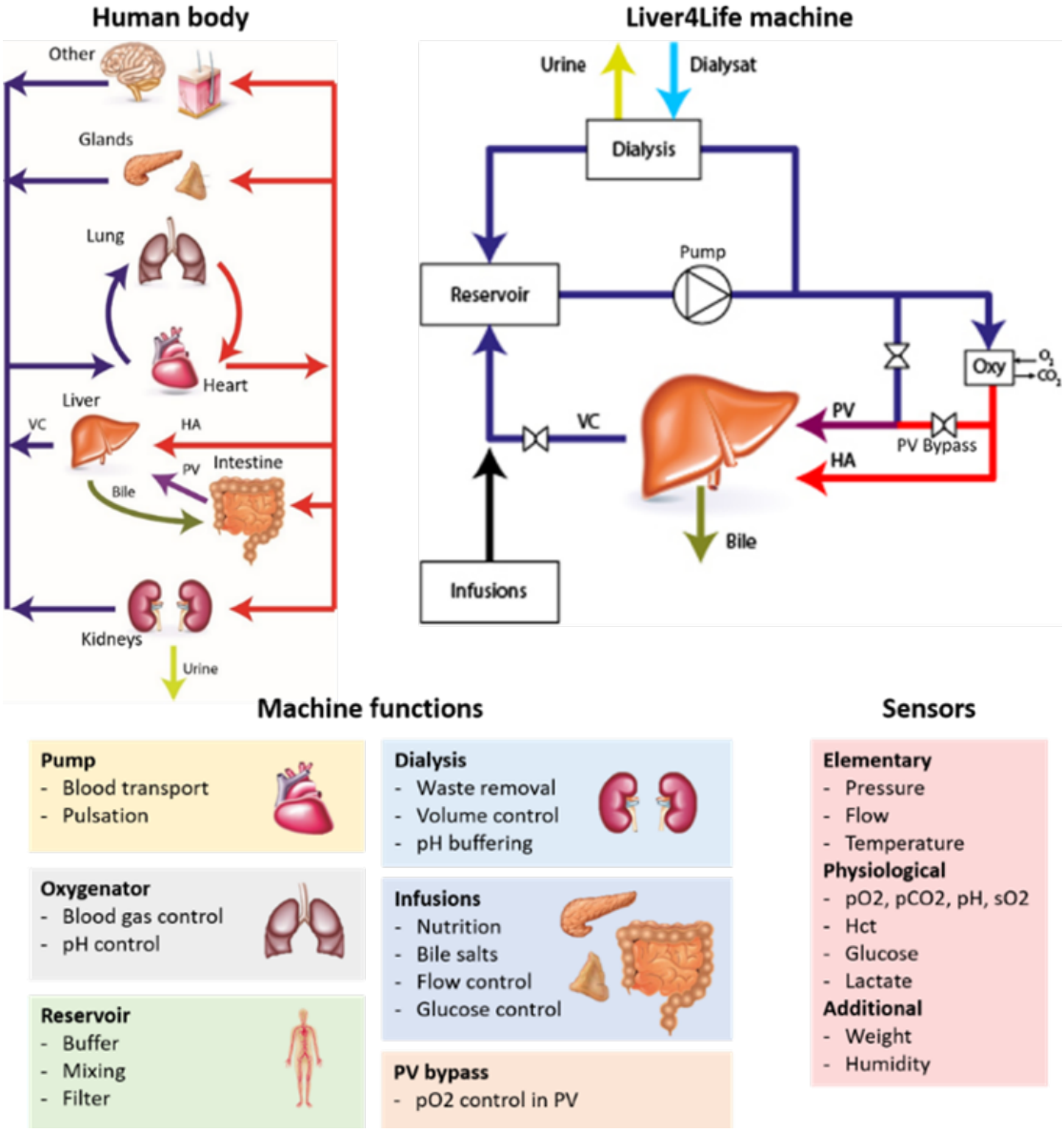


Fig. 4. The Liver4Life machine. The circulatory system (heart and lungs) is provided by an integrated pump, oxygenator, reservoir, and tubing system. This maintains pulsatile blood circulation, portal and hepatic artery flow and pressure, blood gas control, and blood pH levels. Filtration (kidney) is provided by an integrated dialysis unit that clears waste products and maintains electrolytes and bicarbonates at physiologic levels while controlling the blood haematocrit. Blood glucose levels are measured continuously and maintained via controlled infusions of insulin and glucagon (pancreas). The blood is supplemented with nutrients (alimentary tract) following a circadian pattern, and infusion of vasoactive hormones provides control over the hemodynamic resistance of the liver. The machine is also equipped with several sensors and a control system that enables autonomous operation for the entire perfusion duration. Liver function (biomarkers, histology, bile flow) is monitored.

Wyss Liver4Life long-term perfusion machine: a platform to test proregenerative measures

Sponsored by the Wyss Translational Institute Zurich, a collaborative effort between ETH engineers, surgeons, and biologists was set up to develop a perfusion device able to maintain *ex vivo* human and pig liver alive for prolonged periods. The principle behind the perfusion technology is to mimic critical body functions, thereby providing near-physiologic conditions for the liver outside of the body. Currently, liver can be kept vital *ex vivo* for up to 10 days. A modified design of the perfusion device further enables long-term storage of partial livers. See Fig. 4 for a description of the 'Liver4Life' machine.

This machine provides an ideal tool to test proregenerative measures before clinical application. Its construction enables to adapt to the physiological changes that occur in the liver following resection: (i) an increase in the portal blood influx, (ii) increased infusion of lipids to cover the energy demands of a growing liver, (iii) increased infusion of nutrients (amino acids, nucleotides, phosphate, cholesterol, etc.) to provide building material for new tissue, and (iv) modulating oxygen levels to mimic O_2 changes post sHx, such as LR-promoting hypoxia.

Notably, *ex vivo* means that putative compounds can be tested regardless of their oncological or toxic effects. Thus, measures discussed above (growth factors, nuclear receptor activators, etc.) can be assessed for their proregenerative effects and optimised regarding dosing and duration. Likewise, delivery systems (*e.g.* mRNA nanoparticles, viral vectors) can be explored for their efficacy, penetrance, and sustainability in evoking desired effects. Should one or several measures be successful, the Liver4Life machine could be used to directly grow *ex situ* liver mass that then could be transplanted into patients (*e.g.* split liver donation for several recipients, autologous transplantation).

Regeneration and liver volume stimulation in liver transplantation

The measures that aid LR after resection all (safe for some surgical manoeuvres discussed above) should support also the recovery of a transplanted liver, because every graft will come with an impaired functional volume that needs regeneration. Therefore, preoperative improvement of liver quality, promotion of lipid oxidation, platelet infusion, and so on are valid options to stimulate regeneration of transplanted grafts.

Special surgical techniques

Special surgical techniques have been developed for the reduction of SFSS risks, which are particularly high for donor grafts after circulatory death (*i.e.* as a result of prolonged warm ischaemia). Classic **auxiliary liver transplantation** (ALT) is offered for acute liver failure, whereby the most severely damaged liver part is resected and a partial liver graft from a living donor is transplanted as an auxiliary liver. After complete recovery of the remaining native liver, the auxiliary graft is removed along with abrupt termination of immunosuppression, or immunosuppression is tapered, resulting in chronic atrophy of the graft.

More recently, two new transplantation procedures for unresectable bilobar liver tumours have been developed. Both share the transplantation of a partial liver graft in combination with PVL of the diseased liver. In the **RAPID procedure**, a partial hepatectomy is additionally performed, creating room for the partial liver graft. In contrast, the **RAVAS procedure** keeps the complete diseased liver *in situ* and transplants the partial liver heterotopically into the splenic fossa after splenectomy. Once the graft has gained sufficient volume, the diseased (ligated) liver is completely resected in both procedures. RAPID and RAVAS are currently being assessed in several clinical studies that show promising results.

Machine liver perfusion

An exciting new area is machine liver perfusion, whereby the liver graft is stored *ex vivo* before transplantation. A key factor here is the *ex vivo* perfusion (with blood or specific perfusates) through the portal vein, maintaining some portal pressure that likely helps to keep the graft functional. **Hypothermic oxygenated perfusion (HOPE)** was introduced 10 years ago and is being used by an increasing number of centres. The perfusate solution is hypothermic to minimise metabolic stress, while controlled oxygenation leads to mitochondrial recovery and hence the repair of IRI (and thus an increase in functional volume). Real-time measurement of circulating mitochondrial markers further enables the monitoring of repair progress and, consequently, can predict the success of transplant outcomes. **Normothermic machine perfusion (NMP)** of grafts with blood has gained recent attention as it enables *ex vivo* graft preservation over several days to allow repair of damaged or poor quality (*e.g.* highly steatotic) livers – again leading to a gain in functional liver volume. Therefore, grafts that normally would be discarded may be used for transplantation in the future. Indeed, a first 'discarded' graft has been successfully transplanted following treatment with the currently most sophisticated normothermic perfusion machine (**Wyss LIver4Life**, see earlier). This proof-of-concept study demonstrates the enormous potential of machine perfusion to expand the application of transplantation and to push for completely novel treatment options, such as *ex situ* chemotherapy before transplantation.

References

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

1. **Del Basso C, Gaillard M, Lainas P, Zervaki S, Perlemuter G, Chagué P, et al. Current strategies to induce liver remnant hypertrophy before major liver resection. World J Hepatol 2021;13:1629–41.**
2. **Große-Segerath L, Lammert E. Role of vasodilation in liver regeneration and health. Biol Chem 2021;402:1009–19.**
3. **Greenbaum LE, Ukomadu C, Tchorz JS. Clinical translation of liver regeneration therapies: a conceptual road map. Biochem Pharmacol 2020;175:113847.**

Further reading

1. Eshmuminov D, Becker D, Bautista Borrego L, Hefti M, Schuler MJ, Hagedorn C, *et al*. An integrated perfusion machine preserves injured human livers for 1 week. Nat Biotechnol 2020;38:189–98.
2. Lesurtel M, Graf R, Aleil B, Walther DJ, Tian Y, Jochum W, *et al*. Platelet-derived serotonin mediates liver regeneration. Science 2006;312:104–7.
3. Jia Y, Li L, Lin YH, Gopal P, Shen S, Zhou K, *et al*. In vivo CRISPR screening identifies BAZ2 chromatin remodelers as druggable regulators of mammalian liver regeneration. Cell Stem Cell 2022;29:372–85.e8.
4. Zhao P, Fan S, Gao Y, Bi H. Nuclear receptor-mediated hepatomegaly and liver regeneration: an update. Drug Metab Dispos 2022; in press. doi: 10.1124/dmd.121.000454.

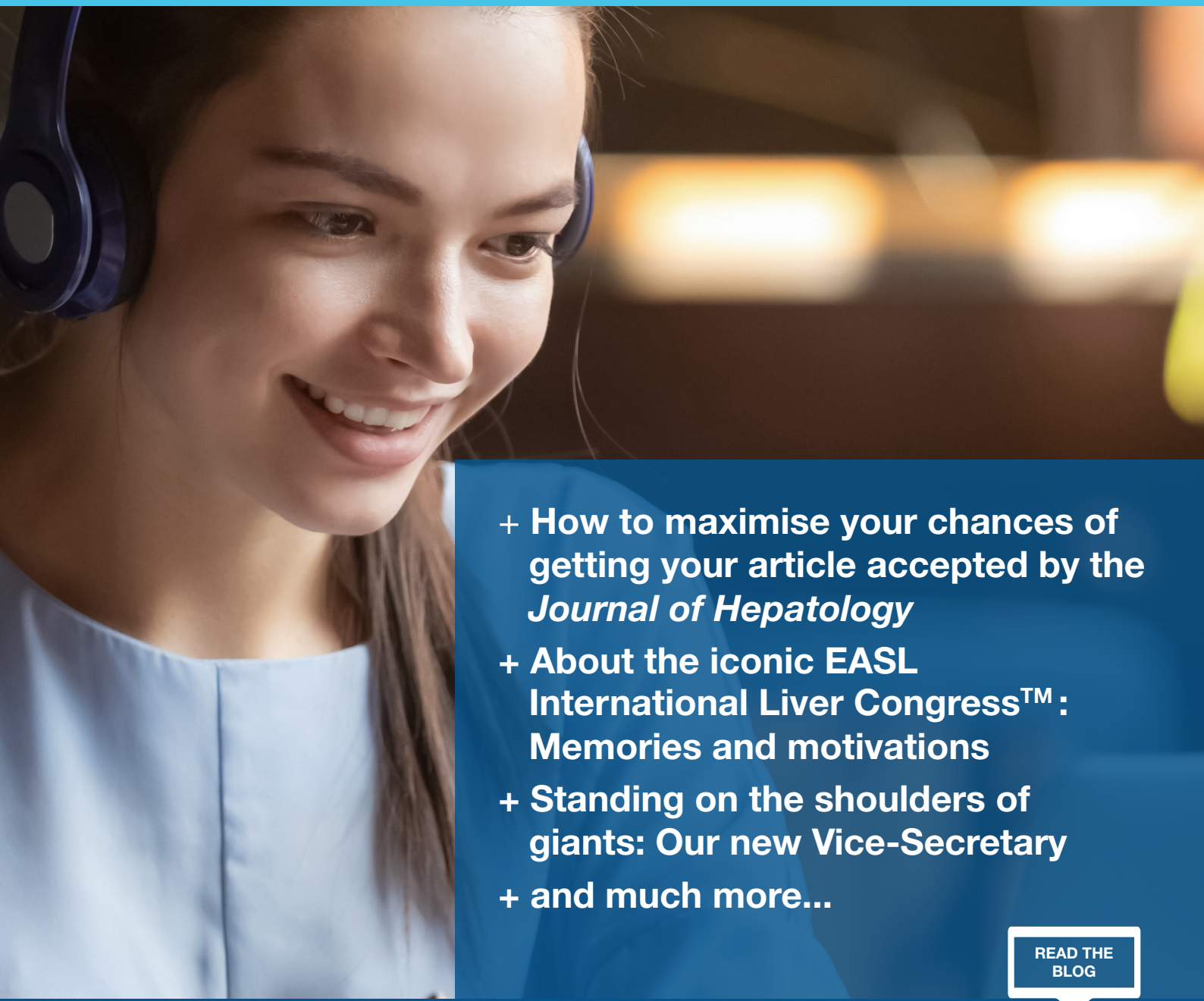
5. Zhang L, Yang Z, Zhang S, Wang W, Zheng S. Conventional two-stage hepatectomy or associating liver partitioning and portal vein ligation for staged hepatectomy for colorectal liver metastases? A systematic review and meta-analysis. Front Oncol 2020;10:1391.
6. Line PD, Hagness M, Berstad AE, Foss A, Dueland S. A novel concept for partial liver transplantation in nonresectable colorectal liver metastases: the RAPID concept. Ann Surg 2015;262:e5–9.
7. Ravaioli M, Fallani G, Cescon M, Prosperi E, De Pace V, Siniscalchi A, *et al*. Heterotopic auxiliary segment 2-3 liver transplantation with delayed total hepatectomy: new strategies for nonresectable colorectal liver metastases. Surgery 2018;164:601–3.
8. Clavien PA, Petrowsky H, DeOliveira ML, Graf R. Strategies for safer liver surgery and partial liver transplantation. N Engl J Med 2007;356:1545–59.

EASL™

BLOG

Liver Matters

Voices and views from our community



+ **How to maximise your chances of getting your article accepted by the *Journal of Hepatology***
+ **About the iconic EASL International Liver Congress™: Memories and motivations**
+ **Standing on the shoulders of giants: Our new Vice-Secretary**
+ **and much more...**

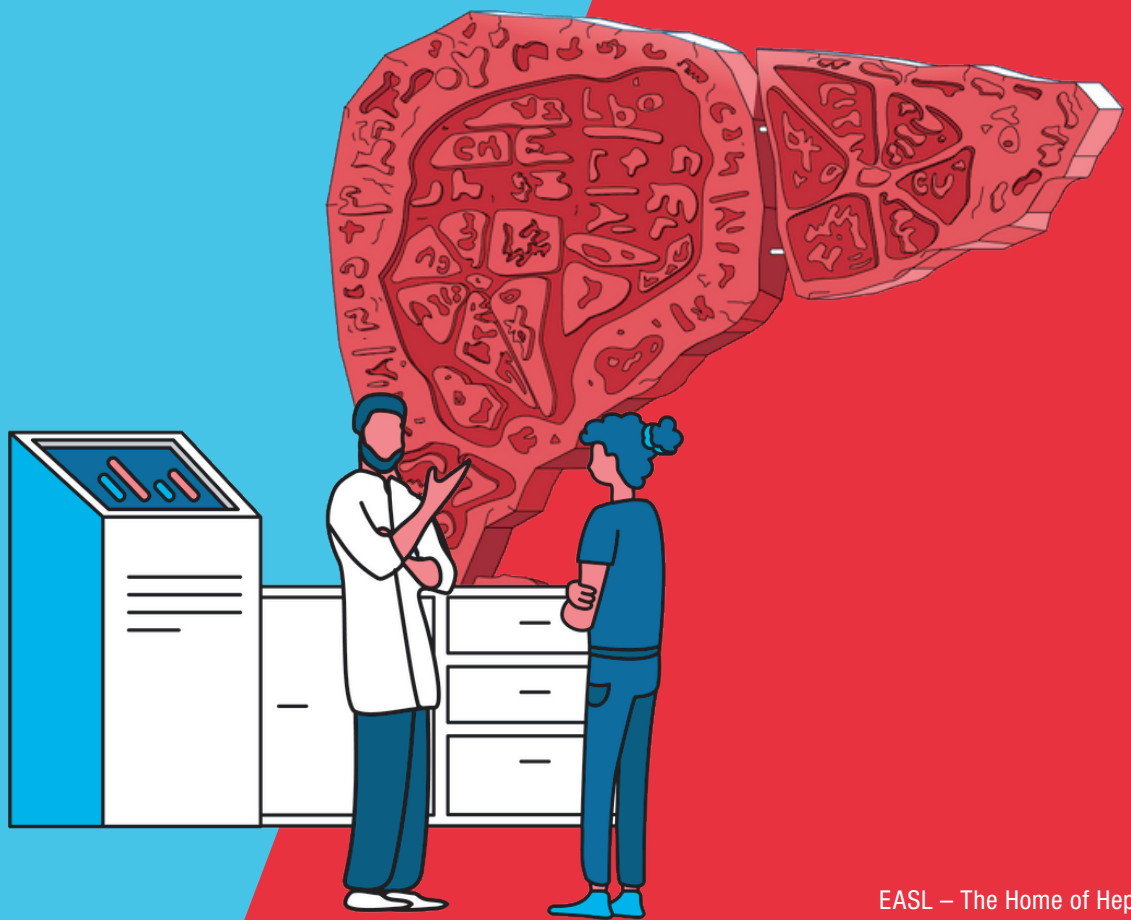
READ THE BLOG

easl.eu/easl-blog

SESSION 3

FRONTIERS IN BIOMARKERS

WEDNESDAY 22 JUNE
16:30-18:00



Prognostic biomarkers of non-malignant liver disease – leaving the biopsy behind

Pierre-Emmanuel Rautou

Service d'Hépatologie, Hôpital Beaujon, Université Paris-Cité, Clichy, *France*

E-mail address: pierre-emmanuel.rautou@inserm.fr

Take-home messages

- Although non-invasive tests were initially viewed as tools to evaluate liver fibrosis stage, they are now also seen as tools to predict patient outcome, namely decompensation of cirrhosis.
- Liver stiffness measurement by transient elastography (and to lesser extent by point shear wave elastography [pSWE] and two-dimensional shear wave elastography [2D-SWE]) is a strong and validated predictor of clinical decompensation in patients with cirrhosis.
- A rule of five for liver stiffness measurement by transient elastography (10–15–20–25 kPa) has been proposed by the Baveno VII conference to denote progressively higher relative risks of decompensation and liver-related death.

Prognosis of chronic liver diseases: why leaving the biopsy behind?

Liver biopsy remains a key tool in hepatology, as it provides extensive information on causes and activity of the disease and on its consequence, namely liver fibrosis. However, over the past 2 decades, numerous non-invasive tests have been developed, and some have been extensively validated. Their relatively low cost and repeatability has been an asset for their wide use. Although non-invasive tests were initially viewed as tools to evaluate liver fibrosis stage, they are now also seen as tools to predict patient outcome, namely decompensation of cirrhosis (development of ascites, variceal haemorrhage, and overt hepatic encephalopathy) and hepatocellular carcinoma. This chapter will only focus on prediction of decompensation of cirrhosis, as prediction of hepatocellular carcinoma is addressed in the next chapter.

Because 'cirrhosis' implies a pathological (invasive) diagnosis, the Baveno conference put forward the concept of 'compensated advanced chronic liver disease' (cACLD) based on non-invasive tests, to reflect that the spectrum of severe fibrosis and cirrhosis is a continuum in asymptomatic patients, and that distinguishing between them is often not possible on clinical grounds. Practically, Baveno consensus conferences stated that liver stiffness measurement (LSM) by transient elastography (TE) ≥10 kPa is suggestive of cACLD and ≥15 kPa is highly suggestive of cACLD.[1] Both terms 'cACLD' and 'compensated cirrhosis' are thus very close.

Because LSM by TE can lead to false-positive results, an index LSM ≥10 kPa should be repeated in fasting conditions as soon as feasible or complemented with an established serum marker of fibrosis, namely fibrosis-4 (FIB-4) ≥2.67, Enhanced Liver Fibrosis Score (ELF™) ≥9.8, FibroTestÒ ≥0.58 for alcohol-related/viral liver disease, and FibroTestÒ ≥0.48 for non-alcoholic fatty liver disease [NAFLD]).[1]

Currently available non-invasive tests to predict outcome in patients with cACLD

Several non-invasive tests hold prognostic value (Table 1), but only few of them have been extensively validated in patients with cACLD/compensated cirrhosis. Among serum markers and a combination

of blood tests, the ELF™, FibroTestÒ and von Willebrand factor (vWF) and Factor VIII/protein C ratio have been associated with the development of clinical decompensation and mortality in patients with cACLD.[2-6] Yet, data do not seem sufficient to recommend their use to base clinical decisions.

LSM by TE (and to lesser extent by point shear wave elastography [pSWE] and two-dimensional shear wave elastography [2D-SWE]) is a strong and validated predictor of clinical decompensation in patients with cACLD or cirrhosis.[1,7] A 'rule of five' for LSM by TE (10–15–20–25 kPa) has been proposed by the Baveno VII conference to denote progressively higher relative risks of decompensation and liver-related death independently of the aetiology of chronic liver disease (Fig. 1)[1]:

1. LSM by TE <10 kPa: negligible risk of decompensation of cirrhosis (≤1% at 3-year risk).
2. LSM by TE <15 kPa plus platelet count $^{3}150´10^{9}$/L: negligible risk of decompensation of cirrhosis.
3. LSM by TE ≥25 kPa: significant risk of decompensation of cirrhosis (referred to as clinically significant portal hypertension, CSPH) in patients with virus- and/or alcohol-related cACLD and non-obese (BMI <30 kg/m^2) non-alcoholic steatohepatitis (NASH)-related cACLD.
4. LSM between 15 and 25 kPa: accuracy for predicting risk of decompensation of cirrhosis is lower. Platelet count can be used to refine this risk.

In patients who are obese (BMI <30 kg/m^2) with NASH-related cACLD, these values cannot be applied.[8]

Spleen stiffness measurement has also been proposed to predict decompensation of cirrhosis.[9] However, data are still insufficient to recommend its use for prognostic assessment in cACLD.

Routine imaging procedures can also provide prognostic information. Indeed, the presence of porto-systemic collaterals on ultrasound, computed tomography (CT) or magnetic resonance imaging (MRI) is associated with an increased risk of decompensation of cirrhosis and mortality.[3]

Table 1. Advantages and disadvantages of the main non-invasive tests proposed to predict outcome in patients with compensated advanced chronic liver disease (cACLD)/cirrhosis. Adapted from EASL, 2021.[3] 2D-SWE, two-dimensional shear wave elastography; FIB-4, Fibrosis 4; NFS, non-alcoholic fatty liver disease fibrosis score; pSWE, point shear wave elastography; US, ultrasound.

	Serum markers		Transient elastography	pSWE	2D-SWE
	Non-patented	Patented			
Reproducibility	Good	Good	Good	Good	Good
Applicability	Excellent	Excellent	Good (obesity, ascites, operator experience)	Very good	Very good
Availability	Wide and free	Wide	Requires a dedicated device	Can be performed in combination with regular US if the device is provided with adequate software	

	Serum markers		Transient elastography	pSWE	2D-SWE
	Non-patented	Patented			
False-positive results	With FIB-4 and NFS in case of age >65 years	In case of extrahepatic inflammatory conditions, profibrotic, extrahepatic disease and other (*e.g.* haemolysis, Gilbert syndrome)	In case of acute hepatitis, extrahepatic cholestasis, liver congestion, excessive food intake and excessive alcohol intake		

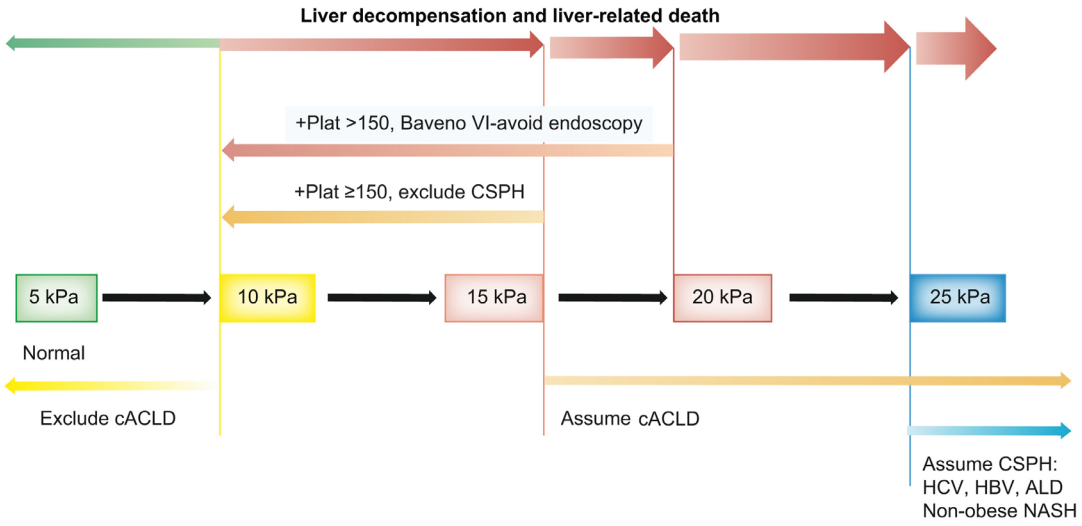


Fig. 1. Algorithm for the non-invasive prediction of cirrhosis decompensation.

ALD, alcohol-related liver disease; cACLD, compensated advanced chronic liver disease; CSPH, clinically significant portal hypertension, associated with an increased risk of decompensation of cirrhosis; NASH, non-alcoholic steatohepatitis from De Franchis *et al.*, 2022.[1]

Non-invasive tests are not only useful at diagnosis of liver diseases, but also during follow-up. The recent Baveno consensus conferences thus proposed repeating LSM every 12 months to monitor changes, in patients with cACLD/cirrhosis. A decrease in LSM associated with substantially reduced risk of decompensation and liver-related death can be defined as a decrease in LSM of ≥20% associated with LSM <20 kPa or any decrease to a LSM <10 kPa.[1]

Novel tests to predict outcome

Liver surface nodularity score on routine CT images

Recently, software was developed to quantify liver surface nodularity on routine CT images, providing quantitative measurement of irregularities of the liver surface.[10] Liver surface nodularity quantification

is associated with a high reproducibility, and increased agreement compared with visual assessment. Liver surface nodularity quantification is able to differentiate cirrhotic from non-cirrhotic livers and is associated with clinically significant portal hypertension in patients with cirrhosis and risk of cirrhosis decompensation in patients with compensated cirrhosis.[11]

Multiparametric magnetic resonance imaging

Magnetic resonance elastography (MRE) using 2D gradient recalled echo holds a high accuracy for fibrosis staging in all the main aetiologies of liver disease and is superior to LSM by TE in patients with NAFLD.[3] However, its high cost and suboptimal availability limit its use in clinical practice. Three-dimensional (3D)-MRE evaluates shear wave propagation in multiple planes and avoids mathematical assumptions inherent to 3D techniques. Although 3D-MRE has been demonstrated to be more accurate in estimating advanced fibrosis in patients with hepatitis B, C, and NAFLD than 2D-MRE, further validation is required to understand the incremental benefit of this technique.[12] MRI has the advantage of being potentially multiparametric, incorporating features representing liver fat (protein density fat fraction [PDFF]), liver iron content but also liver inflammation. Therefore, multiparametic MRI might capture not only liver fibrosis, but also liver disease activity and therefore help improve prediction of outcome of patients with cirrhosis.[12]

Extracellular vesicles

Extracellular vesicles are vesicles released outside the cells by all cell types and can be found in all biological fluids such as blood. Composition of extracellular vesicles reflects the type of activation or stress their mother cell was exposed to. Plasma concentrations of hepatocyte-derived extracellular vesicles improve prediction of decompensation of cirrhosis and patient mortality over routinely available tests.[6,13] However, the potential of extracellular vesicles for predicting cirrhosis trajectory is not just that of hepatocyte-derived extracellular vesicles. Indeed, in cirrhosis, detrimental changes occur not only in the liver, but also in many other organs and systems including vessels, immune cells, gut, muscles, and kidneys. Plasma extracellular vesicles, by capturing multiorgan involvement associated with cirrhosis, exhibit a remarkable potential improve prediction of patient outcome.[6,13]

Biomarkers of extracellular matrix formation

Liver fibrosis is characterised by excessive deposition of extracellular matrix (ECM) consisting of different collagen types that are produced by hepatic myofibroblasts derived from activated hepatic stellate cells. Fibrosis results from a dynamic process that involves both formation and degradation of the ECM and that becomes unbalanced during liver disease. During this remodelling process, markers of collagen turnover (especially collagen types III, IV, and VI) are released. Their circulating concentrations have been studied in different contexts of chronic liver disease and seem promising to predict patient outcome.[14]

Microbiome

The gut microbiome has been implicated in the genesis of liver injury and fibrosis in chronic liver diseases. Proof-of-principle studies using different sequencing technology have demonstrated that the bacterial composition in stool varies according to fibrosis stage in patients with NAFLD. Metagenomic signature of gut microbiome has been shown to detect presence of advanced fibrosis with high accuracy.[15] Gut microbiota might thus also predict cirrhosis outcome.

Genetic prediction models

Genetic variability between individuals leads to differential susceptibility towards the development of liver fibrosis, cirrhosis, and its complications. Genetic variants related to single nucleotide polymorphisms within genes or epigenetic changes such as differential DNA methylation, have been associated with fibrosis progression in chronic hepatitis C and NAFLD. More recently the development of polygenic risk scores, calculated by summing the number of trait-associated alleles carried by an individual, have been shown to identify a subset of the population at substantially increased risk of cirrhosis, who are most susceptible to the hepatotoxic effects of excess alcohol consumption or obesity.[16] We can speculate that such polygenic risk scores can also help improve prediction of complications of cirrhosis.

Conclusions

Non-invasive tests, and in particular LSM, now base daily management of patients with chronic liver diseases and cirrhosis, including decisions for endoscopy and for prevention of complications of cirrhosis based on non-selective beta-blockers.[1] Yet, prediction is far from perfect and additional complementary tools are needed to improve patient management. Numerous approaches have been proposed but their validation remains awaited.

References

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

1. **de Franchis R, Bosch J, Garcia-Tsao G, Reiberger T, Ripoll C, on behalf of the Baveno VII Faculty. Baveno VII – Renewing consensus in portal hypertension. J Hepatol 2022;76:959–74.**
2. Scheiner B, Balcar L, Nussbaumer RJ, Weinzierl J, Paternostro R, Simbrunner B, *et al.* Factor VIII/protein C ratio independently predicts liver-related events but does not indicate a hypercoagulable state in ACLD. J Hepatol 2022. doi:https://doi.org/10.1016/j.jhep.2021.12.038
3. European Association for the Study of the Liver. EASL Clinical Practice Guidelines on non-invasive tests for evaluation of liver disease severity and prognosis – 2021 update. J Hepatol 2021;75:659–89.
4. Ferlitsch M, Reiberger T, Hoke M, Salzl P, Schwengerer B, Ulbrich G, *et al.* von Willebrand factor as new noninvasive predictor of portal hypertension, decompensation and mortality in patients with liver cirrhosis. Hepatology 2012;56:1439–47.
5. Are VS, Vuppalanchi R, Vilar-Gomez E, Chalasani N. Enhanced liver fibrosis score can be used to predict liver-related events in patients with nonalcoholic steatohepatitis and compensated cirrhosis. Clin Gastroenterol Hepatol 2021;19:1292–3.e3.
6. Elkrief L, Ganne-Carrié N, Manceau H, Tanguy M, Barget N, Chaffaut C, *et al.* Hepatocyte-derived biomarkers concentrations predict liver-related events within 2-years in patients with Child-Pugh class A alcohol-related cirrhosis. J Hepatol 2022;76; in press.
7. Trebicka J, Gu W, de Ledinghen V, Aube C, Krag A, Praktiknjo M, *et al.* Two-dimensional shear wave elastography predicts survival in advanced chronic liver disease. Gut 2022;71:402–14.

8. Pons M, Augustin S, Scheiner B, Guillaume M, Rosselli M, Rodrigues SG, et al. Noninvasive diagnosis of portal hypertension in patients with compensated advanced chronic liver disease. Am J Gastroenterol 2021;116:723–32.

9. Colecchia A, Colli A, Casazza G, Mandolesi D, Schiumerini R, Reggiani LB, *et al.* Spleen stiffness measurement can predict clinical complications in compensated HCV-related cirrhosis: a prospective study. J Hepatol 2014;60:1158–64.

10. Sartoris R, Rautou PE, Elkrief L, Pollorsi G, Durand F, Valla D, *et al.* Quantification of liver surface nodularity at CT: utility for detection of portal hypertension. Radiology 2018;289:698–707.

11. Smith AD, Zand KA, Florez E, Sirous R, Shlapak D, Souza F, *et al.* Liver surface nodularity score allows prediction of cirrhosis decompensation and death. Radiology 2017;283:711–22.

12. Loomba R, Adams LA. Advances in non-invasive assessment of hepatic fibrosis. Gut 2020;69:1343–52.

13. Thietart S, Rautou PE. Extracellular vesicles as biomarkers in liver diseases: a clinician's point of view. J Hepatol 2020;73:1507–25.

14. Kerbert AJC, Gupta S, Alabsawy E, Dobler I, Lonsmann I, Hall A, *et al.* Biomarkers of extracellular matrix formation are associated with acute-on-chronic liver failure. JHEP Rep 2021;3:100355.

15. Oh TG, Kim SM, Caussy C, Fu T, Guo J, Bassirian S, *et al.* A universal gut-microbiome-derived signature predicts cirrhosis. Cell Metab 2020;32:878–88.e6.

16. Emdin CA, Haas M, Ajmera V, Simon TG, Homburger J, Neben C, *et al.* Association of genetic variation with cirrhosis: a multi-trait genome-wide association and gene-environment interaction study. Gastroenterology 2021;160:1620–33.e13.

Improving on MELD: New biomarkers to improve risk stratification for wait-listed patients

Elizabeth C. Verna

Center for Liver Disease and Transplantation, Columbia University, New York, NY, *USA*

E-mail address: ev77@columbia.edu

Take-home messages

- Model for end-stage liver disease (MELD)-based allocation significantly improved risk stratification of wait-list mortality, although ongoing improvements in this score are needed.
- MELD 3.0 has recently been proposed to address several limitations in the current MELD-Na score, including improved prioritisation for women.
- Novel approaches to risk stratification regarding kidney function are still needed, including accurate estimations of glomerular filtration rate as well as chronic and reversible forms of kidney insufficiency.
- Additional approaches to risk stratification beyond our current measures are also now being studied, including novel serum biomarkers, microbiota-related signatures and functional measures including frailty. How to incorporate these measures into allocation policies will be an important initiative.

The use of the model for end-stage liver disease (MELD) score to risk stratify patients for liver transplant was a significant advance in organ allocation systems, leading to decreased wait-list mortality without worsening post-transplant outcomes. MELD-based allocation has since been continuously refined, including changes in MELD exception points for diagnoses that impact mortality, but are not reflected in the MELD parameters, such as hepatocellular carcinoma. In addition, the components of the MELD score itself have been updated to improve risk stratification on the wait list with the addition of sodium to the MELD score (MELD-Na).[1]

However, despite these ongoing adjustments, the accuracy of MELD to predict wait-list mortality has significantly declined over time, highlighting the need for further refinement. In addition, there is increased acknowledgement that specific populations may be systematically underserved by MELD on the wait list, including women.[2–5]

This has led to renewed interest in revisiting the components of the MELD-Na score to improve wait-list risk stratification. Most recently, a new score termed MELD 3.0 has been proposed and is currently being considered in the United States to replace MELD-Na.[6] In the initial study regarding the derivation of MELD 3.0, UNOS data from the United States from 2016 to 2018 were interrogated to identify the best predictors of 90-day survival in patients on the transplant wait list. The final model included additional variables including sex, albumin, and interaction terms (bilirubin–Na and albumin–creatinine). In addition, the upper bound for creatinine was changed to 3.0. The authors specifically stated that height was not included as it was collinear with sex, and height did not impact outcomes among men, thus sex had a larger impact on the model. The final MELD 3.0 model had better discrimination than MELD-Na (C-statistic 0.869 *vs.* 0.862, $p < 0.01$) for 90-day mortality. MELD 3.0 also correctly reclassified a net of 8.8% of decedents to a higher MELD tier, affording them a higher chance of transplantation, particularly in women. In the Liver Simulated Allocation Model (LSAM) analysis, MELD 3.0 also resulted in fewer wait-list deaths compared with MELD-Na (7788 *vs.* 7850, $p = 0.02$). However, there remain controversial aspects of using MELD 3.0 clinically for

allocation prioritisation. Albumin now plays a prominent role in the model, although for hospitalised patients with significant portal hypertension and/or hepatorenal syndrome, albumin administration and artificial elevation of albumin level may become a challenge for implementation of the model. In addition, there remains controversy regarding the addition of sex to the model but not accounting for height or replacing creatinine as a measure of kidney function in cirrhosis. Thus, the debate continues at this time as to whether this new system will be implemented.

Improvement in markers of kidney function in the MELD-based allocation model also remains an important area of development. It is well established that creatinine is an inadequate refection of kidney function in cirrhosis attributable to in part sarcopaenia and significant extracellular volume changes. In addition, it is clear that the relationship between creatinine and measure of glomerular filtration rate (GFR) are not directly comparable between women and men, as well as between different races.[7] As a result, there has been a significant effort to identify the best estimation of GFR (eGFR) among patients with cirrhosis.[8,9] In a recent systematic review and meta-analysis of 25 studies with 18 different equations, all creatinine-based eGFR equations considered significantly overestimated GFR. eGFR equations with both creatinine and cystatin C were the least biased, however for patients with GFR <60 or ascites, all equations overestimated GFR.[10] It is likely that cirrhosis-specific, and perhaps cirrhosis-stage-specific estimators of GFR will be required to better represent the risk for patients on the transplant list, particularly among women.

It is also notable that there has been a shift the in the type of kidney dysfunction among patients on the transplant waiting list. As our transplant candidates get older and the aetiology of liver disease shifts towards NAFLD, there appears to be an increasing prevalence of chronic kidney disease (CKD) rather than acute kidney injury (AKI) among wait-listed patients.[11] In addition, CKD appears to portend a lower risk of wait-list mortality than AKI or AKI on CKD, highlighting the lack of differentiation of these processes in our current system.[12] This raises the concern that not only do we need to better define GFR in these patients, but perhaps also differentiate the type and reversibility of kidney dysfunction to correctly stratify patients on the list and to inform decisions regarding simultaneous liver–kidney transplant listing. Significant work has been done to better understand reversibility of kidney function following transplantation.[13] Additional novel biomarkers of that can assist in the differentiation between functional changes in GFR and actual kidney injury should also be studied.[14–17] There should perhaps be a focus on markers of repair (such as uromodulin[18], YKL-40[19]), which could aid in clinical decision-making in cirrhosis.

Finally, completely new markers of risk stratification of patients with decompensated cirrhosis on the wait list must also be considered. There remains significant debate as to whether patients with acute-on-chronic liver failure (ACLF) should be given additional prioritisation.[20,21] Novel biomarkers such as those related to the microbiome are also accumulating data.[22] Functional testing such as frailty may also significantly enhance MELD to risk stratify patients for transplant.[23,24] How to incorporate these additional markers of risk should remain an area of ongoing investigation.

References

1. Kim WR, Biggins SW, Kremers WK, Kamath PS, Benson JT, Edwards E, *et al.* Hyponatremia and mortality among patients on the liver-transplant waiting list. N Engl J Med 2008;359:1018–26.
2. Moylan CA, Brady CW, Johnson JL, Smith AD, Tuttle-Newhall JE, Muir AJ. Disparities in liver transplantation before and after introduction of the MELD score. JAMA 2008;300:2371–8.
3. Lai JC, Terrault NA, Vittinghoff E, Biggins SW. Height contributes to the gender difference in wait-list mortality under the MELD-based liver allocation system. Am J Transplant 2010;10:2658–64.

4. Verna EC, Lai JC. Time for action to address the persistent sex-based disparity in liver transplant access. JAMA Surg 2020;155:545–7.
5. Locke JE, Shelton BA, Olthoff KM, Pomfret EA, Forde KA, Sawinski D, *et al.* Quantifying sex-based disparities in liver allocation. JAMA Surg 2020;155:e201129.
6. Kim WR, Mannalithara A, Heimbach JK, Kamath PS, Asrani SK, Biggins SW, *et al.* MELD 3.0: the model for end-stage liver disease updated for the modern era. Gastroenterology 2021;161:1887–95.e1884.
7. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. Ann Intern Med 1999;130:461–70.
8. Asrani SK, Jennings LW, Kim WR, Kamath PS, Levitsky J, Nadim MK, *et al.* MELD-GRAIL-Na: glomerular filtration rate and mortality on liver-transplant waiting list. Hepatology 2020;71:1766–74.
9. Asrani SK, Jennings LW, Trotter JF, Levitsky J, Nadim MK, Kim WR, *et al.* A model for glomerular filtration rate assessment in liver disease (GRAIL) in the presence of renal dysfunction. Hepatology 2019;69:1219–30.
10. Singapura P, Ma TW, Sarmast N, Gonzalez SA, Durand F, Maiwall R, *et al.* Estimating glomerular filtration rate in cirrhosis using creatinine-based and cystatin C-based equations: systematic review and meta-analysis. Liver Transpl 2021;27:1538–52.
11. Cullaro G, Verna EC, Lee BP, Lai JC. Chronic kidney disease in liver transplant candidates: a rising burden impacting post-liver transplant outcomes. Liver Transpl 2020;26:498–506.
12. Cullaro G, Verna EC, Lai JC. Association between renal function pattern and mortality in patients with cirrhosis. Clin Gastroenterol Hepatol 2019;17:2364–70.
13. Levitsky J, Asrani SK, Abecassis M, Ruiz R, Jennings LW, Klintmalm G. External validation of a pretransplant biomarker model (REVERSE) predictive of renal recovery after liver transplantation. Hepatology 2019;70:1349–59.
14. Xu K, Rosenstiel P, Paragas N, Hinze C, Gao X, Huai Shen T, *et al.* Unique transcriptional programs identify subtypes of AKI. J Am Soc Nephrol 2017;28:1729–40.
15. Shen TH, Stauber J, Xu K, Jacunski A, Paragas N, Callahan M, *et al.* Snapshots of nascent RNA reveal cell- and stimulus- specific responses to acute kidney injury. JCI Insight 2022;7:e146374.
16. Verna EC, Brown RS, Farrand E, Pichardo EM, Forster CS, Sola-Del Valle DA, *et al.* Urinary neutrophil gelatinase-associated lipocalin predicts mortality and identifies acute kidney injury in cirrhosis. Dig Dis Sci 2012;57:2362–70.
17. Fagundes C, Pepin MN, Guevara M, Barreto R, Casals G, Solà E, *et al.* Urinary neutrophil gelatinase-associated lipocalin as biomarker in the differential diagnosis of impairment of kidney function in cirrhosis. J Hepatol 2012;57:267–73.
18. Mansour SG, Liu C, Jia Y, Reese PP, Hall IE, El-Achkar TM, *et al.* Uromodulin to osteopontin ratio in deceased donor urine is associated with kidney graft outcomes. Transplantation 2021;105:876–85.
19. Puthumana J, Hall IE, Reese PP, Schröppel B, Weng FL, Thiessen-Philbrook H, *et al.* YKL-40 associates with renal recovery in deceased donor kidney transplantation. J Am Soc Nephrol 2017;28:661–70.

20. Abdallah MA, Kuo YF, Asrani S, Wong RJ, Ahmed A, Kwo P, *et al.* Validating a novel score based on interaction between ACLF grade and MELD score to predict waitlist mortality. J Hepatol 2021;74:1355–61.

21. Bajaj JS, Verna EC. What role should acute-on-chronic liver failure play in liver transplant prioritization? A survey of US-based transplant providers. Liver Transpl 2020;26:1658–61.

22. Bajaj JS, Reddy KR, O'Leary JG, Vargas HE, Lai JC, Kamath PS, *et al.* Serum levels of metabolites produced by intestinal microbes and lipid moieties independently associated with acute-on-chronic liver failure and death in patients with cirrhosis. Gastroenterology 2020;159:1715–30. e1712.

23. Lai JC, Covinsky KE, Dodge JL, Boscardin WJ, Segev DL, Roberts JP, *et al.* Development of a novel frailty index to predict mortality in patients with end-stage liver disease. Hepatology 2017;66:564–74.

24. Lai JC, Dodge JL, Kappus MR, Dunn MA, Volk ML, Duarte-Rojo A, *et al.* Changes in frailty are associated with waitlist mortality in patients with cirrhosis. J Hepatol 2020;73:575–81.

Predictive biomarkers for the optimisation of liver cancer diagnosis and therapy

Laura Gramantieri

Division of Internal Medicine, Hepatobiliary and Immunoallergic Diseases, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, *Italy*

E-mail address: laura.gramantieri@aosp.bo.it

Take-home messages

- Surveillance, early diagnosis, and personalisation of treatment are highly recommended for hepatocellular carcinoma (HCC) and need improvement.
- Traditional biomarkers are sub-optimal for risk stratification of HCC development in high-risk populations, for early diagnosis, prognostication, and prediction of response to treatments. Treatment allocation is mainly driven by clinical instead of molecular criteria also in advanced stage.
- *Liquid biopsy and composite biomarkers hold promise as powerful tools*, however, they are still in the setting of research studies and do not guide patient management.
- Validations are ongoing to collect robust evidence of clinical utility, which are needed to recommend biomarkers introduction in the clinical practice, to optimise surveillance, early diagnosis, prognostication, and personalisation of treatments.

Rationale for biomarker research in HCC

Hepatocellular carcinoma (HCC) is a highly lethal cancer, which management needs improvement at multiple levels. **Surveillance** is recommended since HCC is the fifth cancer-related cause of death worldwide, it arises in a well-defined population at risk, which displays an estimated cumulative incidence higher than 1.5% per year, and early diagnosis improves survival. Available tools do not allow an accurate **risk stratification of HCC development** in high-risk populations. Meanwhile, new aetiologic factors such as dysmetabolic liver diseases, are gaining more and more relevance, and require a distinct assessment. No international consensus exists on surveillance biomarkers for HCC. An ideal surveillance biomarker should be easily available, cheap, reproducible. **Early diagnosis** may be challenging as well, especially in the case of small nodules without typical imaging, when biopsy is either not conclusive or not feasible. Similarly, tools helping the **prognostication and prediction of response to treatments** are not available for the routine workout, therefore treatment allocation is driven by clinical instead of molecular criteria, also in advanced cases. In other words, no molecular biomarker drives the choice of molecularly targeted agents in HCC.

Reliable biomarkers might optimise surveillance, by stratification of HCC risk in cirrhosis, and treatment efficacy, by improving early diagnosis, prognostication, and prediction of response to treatments.

A huge amount of studies has investigated tissue and circulating biomarkers (Fig. 1). Most biomarkers tested in the surveillance and diagnostic settings showed a high specificity but a low sensitivity. Similarly, biomarkers tested for prognostication and prediction of response to treatments have remained in the setting of research studies and did not enter the clinical practice because robust evidence of clinical utility has not been gathered so far. Indeed, strong evidence and specific requirements are needed to recommend the introduction of biomarkers in the clinical practice, as described in the BEST (Biomarkers, EndpointS, and other Tools) document, by the Food and Drug Administration-National

Institutes of Health (FDA-NIH) Biomarker Working Group.[1] Biomarker is a 'defined characteristic that is measured as an indicator of normal biological processes, pathogenic processes, or biological responses to an exposure or intervention, including therapeutic interventions. Biomarkers may include molecular, histologic, radiographic, or physiologic characteristics'.[1]

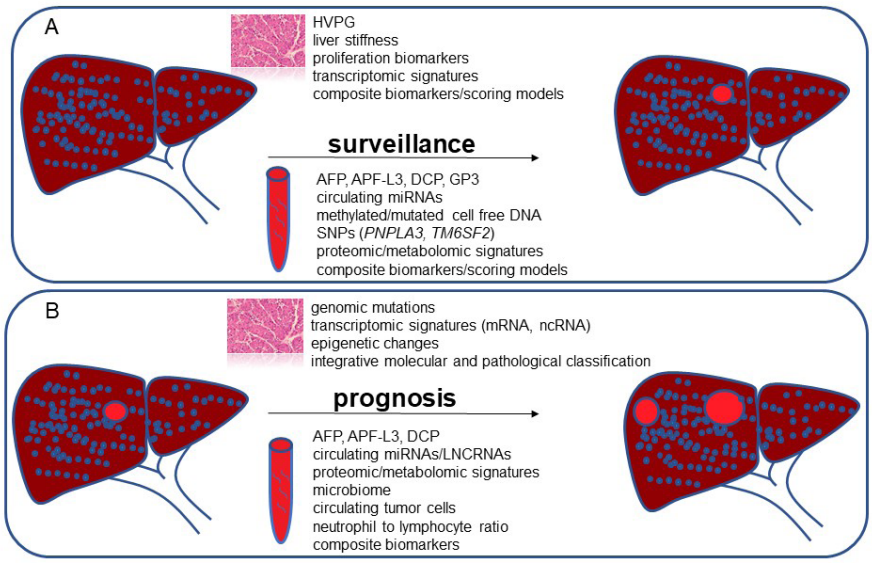


Fig. 1. Main classes of tissue and circulating biomarkers under evaluation for surveillance (A) and prognosis (B) of hepatocellular carcinoma.

Here follows a brief summary of the most studied old and novel tissue and circulating biomarkers proposed to improve the clinical management of HCC patients.

Biomarkers for HCC risk prediction in cirrhosis (optimisation of surveillance)

Tissue biomarkers

Among tissue biomarkers, several molecules were proposed a few decades ago as predictors of HCC development in cirrhosis; among these, markers of hepatocyte proliferation, such as PCNA, AgNOR, and Ki67 immunostaining. None of them ever entered the surveillance protocols, even at the time when liver biopsy was routinely performed. More recently, gene expression signatures emerged from profiling analysis and were validated in subsequent studies. In particular, a cirrhotic tissue signature associated with HCC development was identified by Hoshida *et al.* in paraffin-embedded liver biopsy specimens from patients followed in surveillance programmes.[2] This 186-gene signature was firstly identified in non-neoplastic liver tissue from patients surgically treated for HCC and subsequently validated in needle liver biopsies obtained from patients with hepatitis C-related, Child-Pugh class A cirrhosis, being able to predict clinical outcome in this independent patient population. The performance of the signature was ascribed to the fact that it reflects the 'field effect' in cirrhotic liver. Thus, it was proposed as a new tool to identify patients at highest risk of HCC among those with early-stage cirrhosis, finally helping to enable cost-effective tumour surveillance on a risk-based patient prioritisation. As for immunohistochemical tissue biomarkers of hepatocyte proliferation described above, any gene expression signature did not enter the clinical practice so far. This might be ascribed

to the difficulties in standardisation, reproducibility, homogeneous interpretation of this type of tissue test, together with concerns regarding sampling variability and aetiology-dependent variability, possibly hampering robust determinations.

Meanwhile, techniques such as hepatic venous pressure gradient (HVPG) revealed as good predictors of HCC development in cirrhosis[3] and non-invasive approaches, such as liver stiffness determination, which made liver biopsy often unnecessary for diagnostic and staging purposes, were introduced in the management of patients with chronic liver diseases.[4] Moreover, dedicated calculators such as the CAGE-B or SAGE-B scores provide simple and reliable risk scores for HCC prediction, which might help to custom surveillance.[4] Of note, the ultrasound (US)-based determination of liver stiffness easily allows early stage, non-invasive, diagnosis of cirrhosis. As a consequence, the patient population to be enrolled in surveillance has increased, making it mandatory to consider the cost-effective allocation of medical resources and the personalisation of schedules on the basis of individual risk.

Circulating biomarkers

In the absence of routine biopsy necessity for diagnostic and staging purposes, non-invasive biomarkers have gained more and more attention. Western scientific societies recommend surveillance by US with or without alpha-foetoprotein (AFP) determination. Among 'historical' biomarkers, AFP and its Lens Culinaris Agglutin-3 (L3%) fraction have been the most studied. In the surveillance setting, AFP has been assayed in heterogeneous patient populations, with varying HCC prevalence and by using different cut-offs. Consequently, the reproducibility across studies is sub-optimal, however a low sensitivity and a high specificity are common findings. Despite not strictly recommended by guidelines, AFP remains the most used non-invasive biomarker in the clinical practice. Other molecules proposed in the setting of risk stratification include AFP Lens Culinaris Agglutin-3 (AFP-L3%), Des-γ-carboxy prothrombin/prothrombin induced by vitamin K absence-II (DCP/PIVKA-II), and Glypican 3 (GP3). In some studies, AFP-L3%, DCP/PIVKA-2, and GP3 outperformed AFP, especially when used in combination or in specific subgroups of patients. Notwithstanding, neither the European Association for the Study of the Liver (EASL) nor the American Association for the Study of Liver Diseases (AASLD) guidelines adopted these additional biomarkers to help risk assessment. Behind historical biomarkers, genetic variants of the patatin-like phospholipase domain containing 3 (*PNPLA3*) and transmembrane 6 superfamily member 2 (*TM6SF2*) have been associated with higher risk of HCC development in cirrhosis.[5] Circulating microRNA were also reported as a possible tool for HCC risk assessment, however independent validations and robust evidence about their clinical utility still need to be gained. Interesting results come from detection and characterisation (by mutational and aberrant methylations analyses) of circulating cell-free DNA, which was proposed as a novel tool aiding surveillance for HCC, even though a variable performance across aetiologies was observed.[6]

Biomarkers for early diagnosis and prognostication of HCC in cirrhosis

Both tissue and circulating biomarkers have been proposed for early HCC diagnosis, to complement imaging techniques.

Tissue biomarkers improving the diagnosis of small nodules in cirrhosis, when the basal stainings are not conclusive, include HepPar-1 (HSA), Arginase-1, Polyclonal CEA, CD10, AFP. In the differential diagnosis of nodules <2 cm in diameter, immunostaining for glutamin synthetase (diffuse immunoreactivity in HCC and a patchy immunostain in HGDN), Glypican-3 (sensitivity of 70% in early HCC) and Heath Shock Protein 70 (positive in 80% in early HCC) are acknowledged diagnostic tools. Other tissue biomarkers eventually helping the diagnosis of uncertain nodules are EZH2, CD34 (which helps to recognise the sinusoidal endothelisation occurring in HCC neoangiogenesis) and reticulin stain (which makes evident the loss of reticulin framework trabeculae).

The molecular characterisation obtained by mutational, transcriptomic, epigenetic, proteomic and metabolomic signatures allows the classification of HCC,[7] and provides putative prognostic and predictive biomarkers for targeted treatments. Notwithstanding, the translation of these established knowledge into the clinics is still awaited and genetic markers are still outside the routine workout of HCC. Beside genetic and transcriptomic signatures, microRNA-guided classification of HCC identifies subgroups with biologic peculiarities. For instance, the overexpression of miR-519d, within the C19MC confers aggressiveness and resistance to treatments and the upregulation of miR-494, belonging to the DLK1-DIO3 imprinted locus, associates with a stem-like HCC subgroup with poor prognosis.[8] Similarly, the deregulated expression of long non-coding RNAs (LNCRNAs) characterises HCC with a higher recurrence propensity and modulates the response to treatments. All these findings, however, are still confined to the research setting.

The great majority of novel putative biomarkers derives from tissue studies performed on surgical or biopsy samples, with a restricted exploration of advanced HCCs. In this regard, the debate as to whether biopsy should be routinely performed in cases were imaging criteria are satisfied is still active, weighing up clinical needs *vs.* research needs. At present, HCC diagnosis and subsequent treatments are not established by histology in the majority of cases. This might be because of the limited integration of molecular and morphological subtyping in clinical decisions. Remarkably, the HCC histological characterisation itself provides some elements of prognostic relevance: the clear-cell type and lymphocyte-rich display a better prognosis while macrotrabecular massive and neutrophil-rich have a worse prognosis.

Among novel putative biomarkers proposed in the diagnostic and prognostic settings, epigenetic changes and non-coding RNAs have been extensively studied, not only in profiling analyses, but also in functional investigations, confirming the relevance of combined evaluations to assess a complex disease such as HCC.

In the cases of other tumours, the correct classification and subtyping based on integrated molecular/morphological data have greatly improved the cure. This has not been the case of HCC. Thus, many researchers claim that also HCCs diagnosed by imaging techniques should be biopsied, within research protocols. In these cases, patients should be informed regarding the reasons behind this invasive approach, its diagnostic performance and the role of histological findings in HCC management. Of note, tissue studies may suffer sampling heterogeneity especially in large/multifocal HCCs, and changes in molecular profiles may occur during disease progression or because of treatments. Biopsy remains required for diagnosis when imaging is not conclusive, always considering that sensitivity is not 100%, especially for small nodules. These issues provide the basis for investigating non-invasive biomarkers of potential aid both in the diagnostic, prognostic, and predictive settings.

Circulating biomarkers

As reported earlier in the surveillance setting, AFP, AFP-L3%, and PIVKA3 are the most studied circulating biomarkers also in the diagnostic and prognostic settings. Here again, single markers have low sensitivity, especially in the early stages, whereas their combination improves sensitivity and specificity. A wide spectrum of other molecules was studied, ranging from peptides, to modified peptides (mainly glycosylated isoforms), circulating DNA (with mutation and aberrant methylation), RNAs (mainly non-coding RNAs), and circulating tumour cells. Both single and combined biomarkers were reported as informative. They were studied either in the protein-bound fraction, free in serum/plasma, or in the extracellular vesicles compartment. A huge amount of research has been performed so far and some correlation between aberrant circulating biomarker levels and specific subtypes of HCC in terms of histotype or prognosis are emerging: DCP and neurotensin increase is suggestive for

the fibrolamellar variant of HCC also in the presence of a normal AFP, whereas high AFP levels are associated with a worse prognosis across all stages of the disease.

Liquid biopsy is a very promising source of biomarkers. It includes tumour and non-tumour components such as nucleic acids, circulating tumour cells, peptides, metabolites, released into the bloodstream. Cell-free circulating DNA can be analysed and sequenced, to detect cancer-specific mutations and epigenetic modifications such as aberrant methylation.[9,10] To this aim, commercial assays are already available. These analyses were shown to outperform traditional biomarkers, however, they need to be further validated across different aetiologies and their clinical impact still needs to be verified. Among circulating biomarkers, microRNAs were suggested as a possible diagnostic tool for HCC, displaying a high diagnostic accuracy also in the early stages.[11] Inconsistency across studies is a major criticism to be considered before suggesting any microRNA or microRNA panel for a validation study. These inconsistencies are related to analytical reasons as well as to the choice of controls and patient characteristics, including aetiology of the underlying liver disease. It is conceivable that specific subgroups of patients as defined by histotypes or specific aetiologies or presence of comorbidities or the degree of liver function impairment, might account for the heterogeneous results across different studies.

Metabolomic- and proteomic-based tools such as mass spectrometry and proton nuclear magnetic resonance spectroscopy, have identified early changes in body fluids from patients with HCC, proposing novel candidate biomarkers. Noteworthy changes occur in the levels of both metabolites and proteins, which correlate with aetiology and stage of HCC. Again, also the most promising candidates still need reproducible assays, coupled with independent and robust validation. It seems conceivable that efforts should aim at validating the proposed biomarkers in distinct populations and HCC subgroups. In this regard, the availability of public genetic datasets allows investigation of associations between tumour molecular background and clinical-pathological and laboratory features. Taking advantage from the availability these data, Ahn *et al.*[12] analysed The Cancer Genome Atlas (TCGA) cohort and identified associations between genetic subgroups (identified by driver lesions such as CTNNB1 and TP53 mutations) and increased serum levels of AFP, AFP-L3%, and DCP. This study warrants further investigation to clarify whether and to what extent circulating biomarkers might be informative of the HCC molecular background. Remarkably, the TCGA data set provided also an insight into the molecular drivers of vascular invasion in HCC, aiming to identify therapeutic targets and non-invasive biomarkers. As an example, in HCC with vascular invasion, the MYC oncogene is an upstream regulator of distinct transcriptional, epigenetic, and proteomic changes, and it trigger fibronectin expression. In turn, fibronectin is a proteomic biomarker of invasive HCC, promoting the migratory and invasive phenotype. These findings are confirmed by a significant increase of plasma fibronectin in patients with HCC and support its role as a promising non-invasive proteomic biomarker of aggressive HCCs.

Biomarkers for prediction of response to treatments

The molecularly based prediction of response to targeted treatments mainly regards the intermediate-advanced stages of disease. Although the debate on liver biopsy in the routine diagnostic workup of early HCC is still open, the consensus on the upfront biopsy at enrolment in clinical trials exploring targeted drugs is wide. Unfortunately, most trials have been performed without tissue companion biomarkers, and the only one with a companion biomarker failed to demonstrate a treatment efficacy.[13] Thus, treatment choice is mostly based on clinical and imaging assessment and local prescription rules. The molecular characterisation of HCC in the perspective of a precision medicine approach has become even more mandatory after the introduction of multiple treatment options. The lack of upfront biopsy in most HCC trials was claimed as one reason for the failure to demonstrate the effects of targeted treatment in selected subgroups. Indeed, tissue banks from treated patients would

improve the morphological and molecular subtyping, allow to go back to tumour specimens once the clinical response to drugs is known, test biomarkers emerged as informative subsequently to patient enrolment, finally realising a pharmacologically driven classification of HCC. As an example, the FGF19–FGFR4 axis was identified as a therapeutic target with driver effects in a subgroup of highly proliferating HCC with a dismal prognosis. Copy number amplification of FGF19 characterises only the 7% of HCCs, as reported in TCGA cohort, with a wider percentage of cases displaying FGF19/FGFR4 overexpression. The FGF19/FGFR4 pathway is inhibited by trametinib, whereas conflicting data were reported about sorafenib and lenvatinib inhibitory effects. Thus, this genetic lesion might be included in the panel of upfront biomarker when systemic treatments have to be performed to clarify its possible role as a predictive tool.

A huge amount of research has been performed on circulating predictive biomarkers. Findings from body fluids are thought to suffer less from tumour heterogeneity being considered representative of the most aggressive neoplastic populations. Conversely, sampling variability may undermine biopsy in large and multifocal tumours. In addition, tumour molecular features are awaited to change across the progression of the disease, and from treatment-driven clone selection. At this regard, circulating biomarkers can be tested longitudinally during the course of treatment/s to catch any clonal evolution and identify critical events of possible relevance for subsequent therapeutic decisions. Changes in AFP levels during the course of systemic treatments predict the outcome. To date, the only biomarker helping the choice of a systemic treatment is AFP >400 ng/ml, which is a prognostic and predictive factor of response to ramucirumab in the second line after sorafenib.[14] In a retrospective analysis of SHARP and AP patients, macroscopic vascular invasion, AFP >200 ng/ml and high neutrophil-to-lymphocyte ratio were strong prognostic factors of poorer survival. Consistently, a low neutrophil-to-lymphocyte ratio was predictive of sorafenib treatment benefit.[15] These analyses, which are currently performed in routine practice, are still not recommended for treatment choice, waiting for the evaluation of their clinical impact.

Among circulating biomarkers, many studies have focused on non-coding RNAs, either microRNAs and LNCRNAs. These molecules have been assessed in serum, plasma, and extracellular vesicles fractions, by using different approaches and controls. This resulted in a relevant inconsistency across studies, undermining subsequent validations. Correlations and functional roles of specific driver mutations in the variation of microRNA expression, functions and extracellular trafficking, has been defined and will help to elucidate the representativity of these circulating biomarkers with respect of molecularly defined subgroups.

A further setting to be considered is represented by immune-targeted treatments, which may require a more focused assessment of immune-related biomarkers, together with the multitude of factors influencing the mutual relationships between microenvironment and HCC. Several studies have characterised the immune infiltrate in tumours, as well as the transcriptomic signatures informative of the immune system activation and exhaustion. Interestingly, CTNNB1 mutated HCCs appear refractory to immune checkpoint inhibitors.[16] Again, these studies have not been translated into the clinic so far, as they need further validation together with a full evaluation of their role in the optimisation of patient care.

Conclusions

The need for informative biomarkers has been recognised by EASL and AASLD guidelines as well as by clinical hepatologists and onco-hepatologists. More than 32,000 papers have been published in the field according to a PubMed search. This begs the question of why they mainly remained confined to the research field. Entering the clinical practice entails to ensure quality and reproducibility of tests

and requires analytical assay standardisation. We should also keep in mind that only appropriate applications of biomarkers result in substantial benefits and most of the biomarkers studied so far have been assessed in clinical research instead of in the setting of patient care. These steps may be completed by fostering collaborative efforts among pre-clinical scientists, clinicians and pharma-companies. To add a further layer of complexity, HCC is highly heterogeneous, arising on chronic liver diseases sustained by different risk factors. Remarkably, non-tumorous factors such as the gut microbiome are emerging as relevant factors influencing HCC development and response to treatments.[17] Thus, it is conceivable that composite biomarkers are more likely to characterise patient subgroups in terms of prognostication and prediction of response to treatments, instead of having a 'one fits all' test. These types of biomarkers might be more difficult to develop, validate, and routinely apply, even though algorithms and calculators are part of the clinical practice in almost all specialties, including hepatology. Strong consortium efforts, rigorous analytical and interpretative procedures, and proper study design[18] should be encouraged to gain large multi-centre series, to tie current and new drugs to predictive biomarkers and realise the full potential of personalised HCC care.

References

1. FDA-NIH Biomarker Working Group. *BEST (Biomarkers, EndpointS, and other Tools) Resource.* Last updated: November 29, 2021. Available from: https://www.ncbi.nlm.nih.gov/books/NBK326791/
2. Hoshida Y, Villanueva A, Sangiovanni A, Sole M, Hur C, Andersson KL, *et al.* Prognostic gene-expression signature for patients with hepatitis C-related early-stage cirrhosis. Gastroenterology 2013;144:1024–30.
3. Ripoll C, Groszmann RJ, Garcia-Tsao G, Bosch J, Grace N, Burroughs A, *et al.* Hepatic venous pressure gradient predicts development of hepatocellular carcinoma independently of severity of cirrhosis. J Hepatol 2009;50:923–8.
4. Papatheodoridis GV, Sypsa V, Dalekos GN, Yurdaydin C, Van Boemmel F, Buti M, *et al.* Hepatocellular carcinoma prediction beyond year 5 of oral therapy in a large cohort of Caucasian patients with chronic hepatitis B. J Hepatol 2020;72:1088–96.
5. Yang J, Trépo E, Nahon P, Cao Q, Moreno C, Letouzé E, *et al.* PNPLA3 and TM6SF2 variants as risk factors of hepatocellular carcinoma across various etiologies and severity of underlying liver diseases. Int J Cancer 2019;144:533–44.
6. Lewin J, Kottwitz D, Aoyama J, deVos T, Garces J, Hasinger O, *et al.* Plasma cell free DNA methylation markers for hepatocellular carcinoma surveillance in patients with cirrhosis: a case control study. BMC Gastroenterol 2021;21:136.
7. Zucman-Rossi J, Villanueva A, Nault JC, Llovet JM. Genetic landscape and biomarkers of hepatocellular carcinoma. Gastroenterology 2015;149:1226–39.
8. Luk JM, Burchard J, Zhang C, Liu AM, Wong KF, Shek FH, *et al.* DLK1-DIO3 genomic imprinted microRNA cluster at 14q32.2 defines a stemlike subtype of hepatocellular carcinoma associated with poor survival. J Biol Chem 2011;286:30706–13.
9. Kisiel JB, Dukek BA, Kanipakam RVSR, Ghoz HM, Yab TC, Berger CK, *et al.* Hepatocellular carcinoma detection by plasma methylated DNA: discovery, phase I pilot, and phase II clinical validation. Hepatology 2019;69:1180–92.

10. Labgaa I, Villacorta-Martin C, D'Avola D, Craig AJ, von Felden J, Martins-Filho SN, *et al.* A pilot study of ultra-deep targeted sequencing of plasma DNA identifies driver mutations in hepatocellular carcinoma. Oncogene 2018;37:3740–52.
11. Lin X-J, Chong Y, Guo Z-W, Xie C, Yang XJ, Zhang Q, *et al.* A serum microRNA classifier for early detection of hepatocellular carcinoma: a multicentre, retrospective, longitudinal biomarker identification study with a nested case-control study. Lancet Oncol 2015;16:804–15.
12. Ahn KS, O'Brien DR, Kim YH, Kim TS, Yamada H, Park J-W, *et al.* Associations of serum tumor biomarker with integrated genomic and clinical characteristics of hepatocellular carcinoma. Liver Cancer 2021;10:593–605.
13. Rimassa L, Assenat E, Peck-Radosavljevic M, Pracht M, Zagonel V, Mathurin P, *et al.* Tivantinib for second line treatment of MET-high, advanced hepatocellular carcinoma (METIV-HCC): a final analysis of a phase 3, randomised, placebo-controlled study. Lancet Oncol 2018;19:682–93.
14. Zhu AX, Finn RS, Kang Y-K, Yen CJ, Galle PR, Llovet JM, *et al.* Serum alpha-fetoprotein and clinical outcomes in patients with advanced hepatocellular carcinoma treated with Ramucirumab. Br J Cancer 2021;124:1388–97.
15. Bruix J, Cheng A-L, Meinhardt G, Nakajima K, De Sanctis Y, Llovet J. Prognostic factors and predictors of sorafenib benefit in patients with hepatocellular carcinoma: analysis of two phase III studies. J Hepatol 2017;67:999–1008.
16. Harding JJ, Nandakumar S, Armenia J, Khalil DN, Albano M, Ly M, *et al.* Prospective genotyping of hepatocellular carcinoma: clinical implications of next-generation sequencing for matching patients to targeted and immune therapies. Clin Cancer Res 2019;25:2116–26.
17. Moreno-Gonzalez M, Beraza N. The role of the microbiome in liver cancer. Cancers 2021;13:2330.
18. Singal AG, Hoshida Y, Pinato DJ, Marrero J, Nault JC, Paradis V, *et al.* International Liver Cancer Association (ILCA) white paper on biomarker development for hepatocellular carcinoma. Gastroenterology 2021;160:2572–84.

SESSION 4

BIG DATA AND PERSONALISED MEDICINE

THURSDAY 23 JUNE
8:00-9:30

How personalised medicine may affect our strategies in liver diseases: The example of liver cancer

Simon Johannes Gairing and Peter Robert Galle*

Department of Internal Medicine I, University Medical Center of the Johannes-Gutenberg University Mainz, Mainz, *Germany*

*E-mail address: peter.galle@unimedizin-mainz.de

Take-home messages

- Despite recent advances, hepatocellular carcinoma (HCC) is still associated with a dismal prognosis and is the third leading cause of cancer-related deaths worldwide.
- Immuno-oncology heralded a new era in the treatment of advanced-stage HCC. Based on the IMbrave150 phase III trial, the combination of atezolizumab (antiPDL1 antibody) with bevacizumab (anti-VEGF antibody) is the current standard-of-care first-line therapy for systemic therapy-naïve patients with advanced-stage HCC.
- Targeting VEGF signalling has synergistic effects with immune checkpoint blockade: normalisation of tumour vasculature and endothelium improves transport and extravasation of restored immune effector cells. Additionally, direct VEGF-driven immunosuppressive effects on immune cells such as regulatory T cells (Tregs), tumour-associated macrophages (TAMs), or myeloid-derived suppressor cells (MDSCs) are inhibited.
- Biomarkers that guide therapeutic decision-making are still lacking and are urgently needed to improve the individual choice of systemic treatment in the context of personalised oncology.
- Ongoing clinical trials are evaluating the safety and efficacy of immunotherapy in earlier HCC stages, particularly in intermediate-stage HCC (BCLC B), either in combination with locoregional treatment or without.

Introduction

Globally, liver cancer is one of the most common cancer entities and represents a major public health burden. In 2020, liver cancer ranked sixth among all malignancies worldwide with 905,677 new cases and its incidence continues to rise.[1] Regarding mortality, liver cancer ranked third with 830,180 deaths in 2020.[1]

Hepatocellular carcinoma (HCC) is the most frequent type of liver cancer, accounting for approximately 90% of all cases. HCC includes a spectrum of highly heterogeneous tumours. Major risk factors include chronic hepatitis B and C, non-alcoholic fatty liver disease (NAFLD)/non-alcoholic steatohepatitis (NASH), and chronic alcohol consumption. Depending on the geographical region, the proportion of the respective risk factors varies extensively. With about 50% of cases, chronic hepatitis B represents the most frequent underlying HCC aetiology. However, although the incidence of viral-driven HCC is expected to continue to decline as a result of highly effective HCV treatment options and vaccination against HBV, non-viral driven HCC will increase further.[2] Given the global obesity and type 2 diabetes epidemics, NAFLD and NASH are already the fastest growing aetiologies of HCC in the United States, the United Kingdom, and France.[3]

Personalised medicine, more precisely personalised oncology, heralded a new era in the treatment of tumour patients. About 50–60% of HCC patients are treated with systemic therapy during their disease course. Until 2008, no systemic treatment for advanced-stage HCC had been approved indicating its distinct resistance to conventional chemotherapeutics. In 2008, the SHARP trial established the multi-kinase inhibitor sorafenib as first-line standard-of-care treatment for patients with advanced-stage HCC for more than a decade.[4] In 2017, the tyrosine-kinase inhibitor (TKI) lenvatinib was found

to be non-inferior relative to sorafenib in terms of overall survival (OS).[5] After tumour progression on first-line therapy, regorafenib (TKI), cabozantinib (TKI), and ramucirumab (anti-VEGFR2 antibody) are regularly approved as second-line regimens by the FDA.[6–8] Pembrolizumab (anti-PD-1 antibody), nivolumab (another anti-PD-1 antibody), and nivolumab plus ipilimumab (anti-CTLA-4 antibody) received accelerated approval by the FDA based on phase I/II trial results.[9–11]

The landmark IMbrave150 phase III trial established the combination of atezolizumab (anti-PD-L1 antibody) with the monoclonal VEGF-antibody bevacizumab as a new standard-of-care in the first-line setting of advanced-stage HCC.[12] The vastly improved median OS of 19 months was a breakthrough in systemic therapy of HCC and ushered in a new era in immune-based therapy for HCC.

Recently, an AstraZeneca press release reported that the HIMALAYA phase III trial testing the combination of tremelimumab (anti-CTLA4 antibody) with durvalumab (anti-PD-L1 antibody) resulted in an OS benefit relative to sorafenib in the first-line setting.[13] In addition, results of the first interim analysis of the COSMIC-312 phase III trial were recently presented at ESMO (European Society for Medical Oncology) Asia 2021. Progression-free survival (PFS) was significantly improved in the atezolizumab plus cabozantinib cohort compared with sorafenib (hazard ratio [HR]: 0.63; 99% confidence interval [CI]: 0.44–0.91; $p = 0.0012$), whereas OS was improved but without reaching statistical significance.[14]

In general, personalised oncology is based on molecular genetic tumour characteristics guiding the choice of an individual therapeutic modality. Alternatively, if not available, it could be guided by different clinical subgroups. The former is not established in current HCC trials, as they all share an 'all-comers' design because of the lack of biomarkers, except for the REACH-2 trial, which enrolled only patients with alpha-foetoprotein (AFP) levels ≥400 ng/ml. The latter is always driven by *post hoc* analysis and thus error-prone. For example, subgroup analysis of the SHARP trial showed a large median OS difference for the HBV and HCV subgroup in the sorafenib cohort (9.7 *vs.* 14 months), while the same subgroups in the sorafenib arm of the IMbrave 150 trial nearly had identical median OS outcomes (12.4 *vs.* 12.6 months). Thus, *post hoc* analyses are suitable for designing new trials but are of limited use for guiding therapeutic decisions.

As the treatment landscape broadens, clinicians now have a rapidly increasing number of therapeutic options for advanced-stage HCC patients. In the context of personalised oncology, individual patient characteristics can now increasingly be included in the decision on the respective therapy.

Mechanistic insights into HCC and therapeutic impact

HCC is characterised by a highly heterogeneous landscape of molecular genetic alterations. Hepatocarcinogenesis involves a complex and stepwise process, which is still not fully understood. However, knowing these tumour characteristics forms the indispensable basis for future developments in the context of personalised oncology.

Whole-exome sequencing revealed several mutational signatures depending on the underlying aetiology. However, only about a quarter of the identified alterations represent potential targets for currently FDA-approved drugs.[15] Somatic genomic alterations include especially mutations in the TERT promoter, CTNNB1, TP53, and AXIN1.[16] Frequencies of these mutations depend on the underlying aetiology that drives hepatocarcinogenesis.

The liver is a critical element of the human immune system. A large number of liver-resident innate and adaptive immune cells are involved in the immune surveillance of blood-derived molecules.[17] Thus, the immunological microenvironment of the liver is a finely balanced system of numerous immune system elements that ensures both an adequate immune response against pathogens and self-tolerance to non-pathogenic exogenous and host molecules.

The most common underlying aetiologies of HCC are viral hepatitis (HBV, HCV), alcohol-related liver disease, and NASH. All of them share chronic hepatic inflammation which, in turn, leads to progressive immune suppression. This immunosuppressive microenvironment impairs anti-tumour immune surveillance and, thus, promotes hepatocarcinogenesis.[18] Several elements of the HCC tumour immune microenvironment (TIME) are characterised by immune suppression such as dysfunctional CD8+ T lymphocytes and natural killer (NK) cells.[19,20]

Inhibitory checkpoint molecules such as Cytotoxic T-Lymphocyte-Associated protein 4 (CTLA-4) and Programmed Death 1 (PD-1) are membrane receptors located mainly on T lymphocytes that play an essential physiological role in self-tolerance by suppressing the immune system. Several cancer entities including HCC use these pathways for immune evasion. The rationale of immune checkpoint blockade is the precise inhibition of the receptor-ligand interaction of these molecules to restore anti-tumour CD8+ T cell function (anti-PD-1/anti-PD-L1) or to enhance the number of activated CD4+ and CD8+ T cells (anti-CTLA-4), respectively.[21]

Rationale of combination therapy

Mounting evidence suggests not only an additive but rather a synergistic effect of combining immune checkpoint blockade with anti-angiogenic antibodies or TKIs.[21] vascular endothelial growth factor (VEGF)-driven alterations of the tumour vascularisation and direct immunomodulatory effects of VEGF on immune cells support tumour immune evasion.[22] First, abnormal tumour vessel architecture impairs tumour-directed trafficking of immune effector cells, for example by inhibiting leukocyte extravasation through downregulation of different endothelial adhesion molecules such as intercellular adhesion molecule 1 (ICAM-1) or vascular cell adhesion molecule 1 (VCAM-1, Fig. 1).[23] Second, VEGF impairs tumour surveillance by direct immunosuppressive effects on immune cells such as regulatory T cells (Tregs), tumour-associated macrophages (TAMs), or myeloid-derived suppressor cells (MDSCs, Fig. 1).[24]

Thus, targeted inhibition of VEGF signalling leads to both normalisation of the tumour vascularisation, thereby facilitating the transport and extravasation of restored immune effector cells, and inhibition of the direct VEGF-driven immune evasion mechanisms.[21]

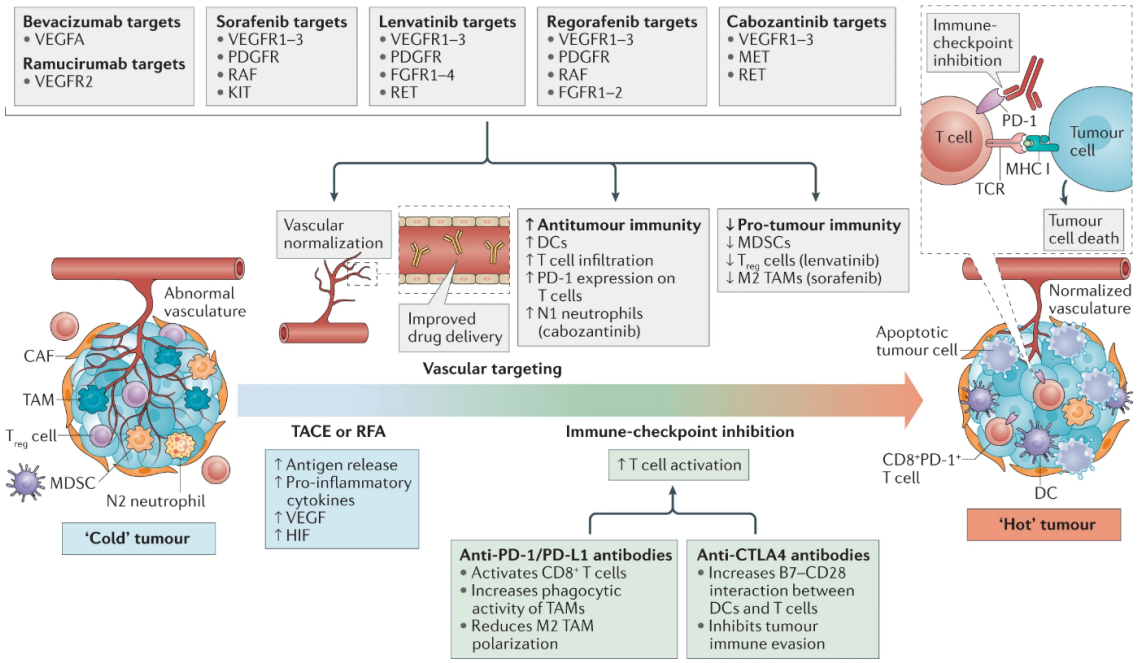


Fig. 1. Rationale of combining immune checkpoint inhibitors with antiangiogenic tyrosine-kinase inhibitors or antibodies.[21]

When to use protein-kinase-inhibitors *vs.* antiangiogenic or immunotherapy

In light of recent advances in systemic treatment of HCC, the European Association of the Study of the Liver (EASL) as well as the Barcelona Clinic Liver Cancer (BCLC) group have published updated recommendations that provide evidence-based therapeutic algorithms for HCC patients depending on their BCLC stage.[25,26] All trials, which led to an (FDA) approval including median OS, median PFS, median time to progression (TTP), overall response rate (ORR), and most frequent grade ≥3 adverse events (AEs), respectively, are summarised in Table 1. Importantly, all trials enrolled primarily patients with BCLC stage C disease, who were fit (Eastern Cooperative Oncology Group [ECOG] performance status 0–1), and had preserved liver function (Child-Pugh class A).

First-line treatment

The IMbrave150 phase III trial randomised a total of 501 patients without prior systemic HCC treatment in a 2:1 ratio to atezolizumab plus bevacizumab (atezolizumab 1,200 mg i.v. every 3 weeks, bevacizumab 15 mg/kg body weight i.v. every 3 weeks) or sorafenib (400 mg b.i.d.). Median OS was 19.2 months in the atezo/bev cohort *vs.* 13.4 months in the sorafenib group (HR, 0.66 [95% CI, 0.52, 0.85]; $p = 0.0009$).[27] Thus, atezo/bev represents the current standard-of-care first-line systemic treatment for systemic therapy-naïve HCC patients. However, patients must be thoroughly screened for contraindications to atezo/bev such as a history of clinically severe autoimmune disease or a high risk of bleeding.

In the case of contraindications to atezo/bev, either lenvatinib or sorafenib are the therapy of choice in the first-line setting based on the results of the SHARP[4] and REFLECT trial.[5] The REFLECT phase III trial enrolled 954 systemic treatment-naïve patients in a 1:1 ratio to receive either lenvatinib (12 mg/day if body weight ≥60 kg or 8 mg/day if body weight <60) or sorafenib (400 mg b.i.d.). The study met its primary endpoint, indicating non-inferiority of lenvatinib relative to sorafenib with a trend towards an improved median OS in the lenvatinib cohort (13.6 months *vs.* 12.3 months, HR: 0.92, 95% CI: 0.79–1.06).[5]

No clear recommendation can be made for the question of whether lenvatinib or sorafenib should be preferred. Lenvatinib was superior relative to sorafenib in several secondary endpoints (higher ORR, longer TTP and PFS). However, AEs that led to treatment discontinuation (40% *vs.* 32%) and serious treatment-related AEs (43% *vs.* 30%) were more frequent in the lenvatinib cohort. In addition, approved second-line regimens are only tested after progression on sorafenib, not lenvatinib, and patients with >50% liver tumour involvement and main portal vein invasion were excluded in the REFLECT trial. Finally, the different frequency spectrum of AEs should be considered in the decision-making.[25]

Second-line treatment

Regorafenib (160 mg/day for the 3 initial weeks of each 4-week cycle) yielded a median OS of 10.6 months compared with 7.8 months in the placebo group (HR: 0.63, 95% CI: 0.50–0.79; $p < 0.0001$) according to the RESORCE phase III trial including a total of 573 patients who had progressed on sorafenib.[6] Importantly, only patients tolerating at least 400 mg/day sorafenib for ≥20 days of the last 28 days before discontinuation were enrolled in this trial.

The CELESTIAL phase III trial enrolled 707 patients in a 2:1 ratio to cabozantinib (60 mg/day) or placebo. Patients were in the second or third line of systemic treatment and had already been treated with sorafenib. Median OS was 10.2 months in the cabozantinib cohort *vs.* 8.0 months in the placebo cohort (HR: 0.76; 95% CI: 0.63–0.92; $p = 0.005$).[7]

The REACH-2 phase III trial included 292 patients after progression on or intolerance to sorafenib and with AFP levels ≥400 ng/ml to receive either ramucirumab (8 mg/kg body weight i.v. every 2 weeks) or placebo. Median OS was 8.5 months *vs.* 7.3 months (HR: 0.71, 95% CI: 0.531–0·949; $p = 0.0199$).[8]

The CheckMate 040 trial enrolled 6 different study cohorts. A total of 262 patients who had previously been treated with or without sorafenib received monotherapy with the anti-PD1 antibody nivolumab. Regarding the dose-expansion phase with 3 mg/kg body weight nivolumab, ORR was 20% (95% CI: 15–26; RECIST v1.1) and 9-month OS was 74% (95% CI: 67–79).[10] Based on these results, the FDA granted accelerated approval for nivolumab in patients with advanced-stage HCC after progression on sorafenib. However, the CheckMate 459 phase III trial did not meet its primary endpoint of superiority relative to sorafenib in the first-line setting.[28] As a consequence, Bristol Myers Squibb decided to withdraw nivolumab as monotherapy after previous treatment with sorafenib from the US market.

Another cohort of the CheckMate 040 trial included 148 patients who had previously been treated with sorafenib to receive 3 different dosing regimens of nivolumab plus ipilimumab in a 1:1:1 ratio. Arm A (4´ 1 mg/kg body weight nivolumab + 3 mg/kg body weight ipilimumab every 3 weeks followed by 240 mg nivolumab every 2 weeks) had an ORR of 32% (95% CI: 20–47%) with an manageable safety profile.[11] Accordingly, the FDA granted accelerated approval for the arm A combination regimen.

The Keynote-224 phase II trial enrolled 104 patients after progression on or intolerance to sorafenib to receive the anti-PD-1 antibody pembrolizumab (200 mg i.v. every 3 weeks). ORR was 17% (95% CI: 11–26) with a manageable toxicity profile.[9] Based on this trial, accelerated approval was granted by the FDA. However, the following Keynote-240 phase III trial did not meet its co-primary endpoints (OS and PFS).[29] Interestingly, a similar trial, the phase III KEYNOTE-394 study in an Asian population, according to a press release, showed statistically significant improvement in OS *vs.* placebo.[30]

To sum up, regorafenib, cabozantinib, ramucirumab, pembrolizumab, and nivolumab plus ipilimumab are current treatment options in the second-line setting after progression on, or intolerance to, previous treatment with sorafenib. However, only the former 3 are approved and reimbursed in most countries. After progression on first-line treatment with atezo/bev or lenvatinib, respectively, evidence is lacking on how sequential systemic treatment should be continued. Therefore, especially patient-related factors, such as comorbidities or tolerance to previous therapies, should be included in the decision on the selection of second-line therapy.

Table 1. Summary of efficacy and toxicity of systemic therapies approved by regulatory agencies for HCC.

Study	Accrual period	Treatment line	Sample size	Study arm	Median OS (months)	Median PFS/TTP (months)	ORR (RECIST)	Rate of CTC grade ≥3 AEs	Subsequent systemic treatment
Multi-targeted tyrosine kinase inhibitors									
SHARP[1]	Mar 05-Apr 06	First line	299 303	Sorafenib Placebo	10.7 7.9	5.5 (TTP) 2.8	2% 1%	HFSR 8%; diarrhoea 8%; fatigue 3.4%	n.a.
SHARP-AP[96]	Sept 05-Jan 07	First line	150 76	Sorafenib Placebo	6.5 4.2	2.8 (TTP) 1.4	3.3% 1.1%	HFSR 10.7%; diarrhoea 6.0%; fatigue 4%	n.a.
REFLECT[2]	Mar 13-July 15	First line	478 476	Lenvatinib Sorafenib	13.6 12.3	7.3 (PFS) 3.6	18.8% 6.5%	Hypertension 23%; increased bilirubin 7%; proteinuria 6%	32.6% 38.7%
RESORCE[3]	May 13-Dec 15	Second line (post-SOR)	379 194	Regorafenib Placebo	10.6 7.8	3.1 (PFS) 1.5	11% 4%	Hypertension 16%; HFSR 13%; increased bilirubin 11%	n.a.
CELESTIAL[4]	Sept 13-Sept 17	Second or third line (post-SOR)	470 237	Cabozantinib Placebo	10.2 8.0	5.2 (PFS) 1.9	4% <1%	HFSR 17%; hypertension 17%; diarrhoea 17%	n.a.
Anti-angiogenic antibodies									
REACH-2[5]	July 15-Aug 17	Second line (post-SOR) & high AFP	197 95	Ramucirumab Placebo	8.5 7.3	2.8 (PFS) 1.6	4.6% 1.1%	Liver failure 18.3%; hypertension 12.7%; bleeding 5.1%	26.9% 28.4%
Immune checkpoint inhibitors (monotherapy)									
Checkmate 459[19]	Jan 16-May 17	First line	371 372	Nivolumab Sorafenib	16.4 14.7	3.7 (PFS) 3.8	15% 7%	Increased AST 6%; diarrhoea 0.8%; fatigue 0.8%	39% 47%
Keynote 240[10]	Mar 16-Nov 17	Second line (post-SOR)	278 135	Pembrolizumab Placebo	13.9 10.6	3.0 (PFS) 2.8	18.3% 4.4%	Increased AST 13.3%; increased bilirubin 7.5%; increased ALT 6.1%	41.7% 47.4%
Immune checkpoint inhibitors (combinations)									
IMbrave 150[6,20]	Mar 18-Jan 19	First line	336 165	Atezolizumab + bevacizumab Sorafenib	19.2 13.4	6.9 (PFS) 4.3	30% 11%	Hypertension 15.2%; increased AST 7.0%; increased ALT 3.6%	36% 52%
Checkmate 040 cohort (Phase II)[25]	Jan 16-Sept 16	Second line (post-SOR)	50	Ipilimumab + nivolumab	22.8 m	n.a.	32%	Hepatitis 20%; rash 6%; diarrhoea/colitis 6%; pneumonitis 6%	n.a.

AE, adverse events; AFP, alpha-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CTC, Common Toxicity Criteria; HCC, hepatocellular carcinoma; HFSR, hand-foot skin reaction; n.a., not available; ORR, objective response rate; OS, overall survival; PD-1; programmed cell death protein 1; PFS, progression-free survival; SOR, sorafenib; TTP, time-to-progression.

Table 1. Trials including hepatocellular carcinoma patients with advanced-stage disease which led to a regularly or accelerated FDA approval.[25]

Rationale of the association with locoregional therapy

In the pre-sorafenib era, locoregional therapies were the only effective therapeutic modality for patients who were ineligible for surgical resection or transplantation. Over the past decades, in particular in the current era of immunotherapy, the indication spectrum for locoregional therapies has narrowed.

Currently ongoing trials explore the place of immunotherapy in intermediate-stage HCCs (BCLC B). The ABC-HCC (NCT04803994) trial investigates atezolizumab plus bevacizumab *vs.* transarterial chemoembolisation (TACE). Mounting evidence underscores the locoregional therapy-driven immunomodulatory and pleiotropic effects that exceed direct cytotoxic mechanisms.[31] For example, a recent study reported that TACE leads to a decrease of Tregs and exhausted T effector cells in the HCC tumour microenvironment (TME), while pathways that enhance inflammatory cascades are triggered.[32] Thus, locoregional therapies such as TACE could work synergistically with immunotherapy. In line, the LEAP-012 (NCT04246177), EMERALD-1 (NCT03778957) and CheckMate 74W (NCT04340193) trials compare lenvatinib + pembrolizumab + TACE, durvalumab + TACE, and nivolumab ± ipilimumab + TACE *vs.* TACE monotherapy, respectively. Another phase II trial compares the combination of durvalumab + tremelimumab + TACE or + SIRT for intermediate-stage HCC (NCT04522544).

Place of these personalised therapeutic developments in the project of liver transplantation

Downstaging of HCCs that initially exceed the Milan criteria by surgical, locoregional, or systemic treatment modalities followed by liver transplantation has been shown to improve OS.[33] Thus, systemic treatment with sorafenib as part of a neoadjuvant therapeutic concept before liver transplantation may be a valid treatment option for selected patients with preserved liver function. However, there is only limited and uncontrolled evidence supporting this approach.

The role of immunotherapy in the context of liver transplantation is controversial. Although post-transplant immunotherapy for recurrence of HCC is contraindicated because of the risk of rejection, pre-transplant immunotherapy for downstaging may be feasible, but small cohort studies reported rejection rates up to 45%.[34] Therefore, immunotherapy currently has no place in the context of liver transplantation outside clinical trials.

Conclusions and future directions

Immuno-oncology heralded a new era in the treatment of advanced-stage HCC. Atezolizumab/bevacizumab yielded a median OS of 19.2 months in the IMbrave150 phase III trial, raising the prognosis of HCC patients with advanced-stage disease to a new level. Currently ongoing trials will prove the value of immunotherapy in earlier-stage HCC with or without locoregional therapies. However, a proportion of HCC patients does not respond to immunotherapy. Subgroup analysis from different trials suggests, that in particular NASH-driven HCCs may be resistant to immune checkpoint blockade.[35] About 25% of HCCs are infiltrated by a high number of immune cells.[36] These 'immune class' tumours could be particularly suitable for immunotherapeutic treatments.[36] In addition, Foerster *et al.* established a prognostic score based on the individual HCC immune contexture, which predicts survival in HCC patients.[37] However, no biomarker has yet been identified that reliably predicts response to immunotherapy. Thus, future research should focus on identifying predictive biomarkers to improve patient selection for the upcoming broad range of systemic therapeutic options in the context of personalised oncology.

Required reading

Llovet JM, Kelley RK, Villanueva A, Singal AG, Pikarsky E, Roayaie S, *et al.* Hepatocellular carcinoma. Nat Rev Dis Primers 2021;7:6.

References

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

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, *et al*. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2021;71:209–49.
2. Estes C, Razavi H, Loomba R, Younossi Z, Sanyal AJ. Modeling the epidemic of nonalcoholic fatty liver disease demonstrates an exponential increase in burden of disease. Hepatology (Baltimore, MD) 2018;67:123–33.
3. Huang DQ, El-Serag HB, Loomba R. Global epidemiology of NAFLD-related HCC: trends, predictions, risk factors and prevention. Nat Rev Gastroenterol Hepatol 2021;18:223–38.
4. Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc J-F, *et al*. Sorafenib in advanced hepatocellular carcinoma. N Engl J Med 2008;359:378–90.
5. Kudo M, Finn RS, Qin S, Han K-H, Ikeda K, Piscaglia F, *et al*. Lenvatinib versus sorafenib in first-line treatment of patients with unresectable hepatocellular carcinoma: a randomised phase 3 non-inferiority trial. Lancet 2018;391:1163–73.
6. Bruix J, Qin S, Merle P, Granito A, Huang Y-H, Bodoky G, *et al*. Regorafenib for patients with hepatocellular carcinoma who progressed on sorafenib treatment (RESORCE): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet 2017;389:56–66.
7. Abou-Alfa GK, Meyer T, Cheng A-L, El-Khoueiry AB, Rimassa L, Ryoo B-Y, *et al.* Cabozantinib in patients with advanced and progressing hepatocellular carcinoma. N Engl J Med 2018;379:54–63.
8. Zhu AX, Kang Y-K, Yen C-J, Finn RS, Galle PR, Llovet JM, *et al*. Ramucirumab after sorafenib in patients with advanced hepatocellular carcinoma and increased α-fetoprotein concentrations (REACH-2): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet Oncol 2019;20:282–96.
9. Zhu AX, Finn RS, Edeline J, Cattan S, Ogasawara S, Palmer D, *et al*. Pembrolizumab in patients with advanced hepatocellular carcinoma previously treated with sorafenib (KEYNOTE-224): a non-randomised, open-label phase 2 trial. Lancet Oncol 2018;19:940–52.
10. El-Khoueiry AB, Sangro B, Yau T, Crocenzi TS, Kudo M, Hsu C, *et al.* Nivolumab in patients with advanced hepatocellular carcinoma (CheckMate 040): an open-label, non-comparative, phase 1/2 dose escalation and expansion trial. Lancet 2017;389:2492–502.
11. Yau T, Kang Y-K, Kim T-Y, El-Khoueiry AB, Santoro A, Sangro B, *et al*. Efficacy and safety of nivolumab plus ipilimumab in patients with advanced hepatocellular carcinoma previously treated with sorafenib: the CheckMate 040 randomized clinical trial. JAMA Oncol 2020;6:e204564.
12. **Finn RS, Qin S, Ikeda M, Galle PR, Ducreux M, Kim T-Y, *et al.* Atezolizumab plus bevacizumab in unresectable hepatocellular carcinoma. N Engl J Med 2020;382:1894–905.**

13. AstraZeneca press release. Imfinzi plus tremelimumab significantly improved overall survival in HIMALAYA Phase III trial in 1st-line unresectable liver cancer. 2021. https://www.astrazeneca.com/media-centre/press-releases/2021/imfinzi-and-tremelimumab-improved-os-in-liver-cancer.html.

14. Kelley RK, Yau T, Cheng AL. Cabozantinib (C) plus atezolizumab (A) versus sorafenib (S) as first-line systemic treatment for advanced hepatocellular carcinoma (aHCC): results from the randomized phase 3 COSMIC-312 trial. ESMO Asia Virtual Oncology Week 2021, VP10-2021.

15. Schulze K, Imbeaud S, Letouzé E, Alexandrov LB, Calderaro J, Rebouissou S, *et al*. Exome sequencing of hepatocellular carcinomas identifies new mutational signatures and potential therapeutic targets. Nat Genet 2015;47:505–11.

16. Caruso S, O'Brien DR, Cleary SP, Roberts LR, Zucman-Rossi J. Genetics of hepatocellular carcinoma: approaches to explore molecular diversity. Hepatology (Baltimore, MD) 2021;73(Suppl 1):14–26.

17. Jenne CN, Kubes P. Immune surveillance by the liver. Nat Immunol 2013;14:996–1006.

18. Makarova-Rusher OV, Medina-Echeverz J, Duffy AG, Greten TF. The yin and yang of evasion and immune activation in HCC. J Hepatol 2015;62:1420–9.

19. Flecken T, Schmidt N, Hild S, Gostick E, Drognitz O, Zeiser R, *et al*. Immunodominance and functional alterations of tumor-associated antigen-specific CD8+ T-cell responses in hepatocellular carcinoma. Hepatology (Baltimore, MD) 2014;59:1415–26.

20. Cariani E, Pilli M, Barili V, Porro E, Biasini E, Olivani A, *et al.* Natural killer cells phenotypic characterization as an outcome predictor of HCV-linked HCC after curative treatments. Oncoimmunology 2016;5:e1154249.

21. Llovet JM, Castet F, Heikenwalder M, Maini MK, Mazzaferro V, Pinato DJ, *et al*. Immunotherapies for hepatocellular carcinoma. Nat Rev Clin Oncol 2021;19:151–72.

22. Woller N, Engelskircher SA, Wirth T, Wedemeyer H. Prospects and challenges for T cell-based therapies of HCC. Cells 2021;10:1651.

23. Huinen ZR, Huijbers EJM, van Beijnum JR, Nowak-Sliwinska P, Griffioen AW. Anti-angiogenic agents – overcoming tumour endothelial cell anergy and improving immunotherapy outcomes. Nat Rev Clin Oncol 2021;18:527–40.

24. Fukumura D, Kloepper J, Amoozgar Z, Duda DG, Jain RK. Enhancing cancer immunotherapy using antiangiogenics: opportunities and challenges. Nat Rev Clin Oncol 2018;15:325–40.

25. Bruix J, Chan SL, Galle PR, Rimassa L, Sangro B. Systemic treatment of hepatocellular carcinoma: an EASL position paper. J Hepatol 2021;75:960–74.

26. Reig M, Forner A, Rimola J, Ferrer-Fábrega J, Burrel M, Garcia-Criado A, *et al*. BCLC strategy for prognosis prediction and treatment recommendation Barcelona Clinic Liver Cancer (BCLC) staging system. The 2022 update. J Hepatol 2021;76:681–93.

27. Finn RS, Qin S, Ikeda M, Galle PR, Ducreux M, Kim T-Y, *et al.* IMbrave150: updated overall survival (OS) data from a global, randomized, open-label phase III study of atezolizumab (atezo) + bevacizumab (bev) versus sorafenib (sor) in patients (pts) with unresectable hepatocellular carcinoma (HCC). JCO 2021;39(3 Suppl.):267.

28. Yau T, Park JW, Finn RS, Cheng A-L, Mathurin P, Edeline J, *et al.* CheckMate 459: a randomized, multi-center phase III study of nivolumab (NIVO) vs sorafenib (SOR) as first-line (1L) treatment in patients (pts) with advanced hepatocellular carcinoma (aHCC). Ann Oncol 2019;30:v874–5.

29. Finn RS, Ryoo B-Y, Merle P, Kudo M, Bouattour M, Lim HY, *et al.* Pembrolizumab as second-line therapy in patients with advanced hepatocellular carcinoma in KEYNOTE-240: a randomized, double-blind, phase III trial. J Clin Oncol 2020;38:193–202.

30. Merck press release. Merck Announces KEYTRUDA® (pembrolizumab) Met Primary Endpoint of Overall Survival (OS) in Patients with Advanced Hepatocellular Carcinoma Previously Treated with Sorafenib. 2021. https://www.merck.com/news/merck-announces-keytruda-pembrolizumab-met-primary-endpoint-of-overall-survival-os-in-patients-with-advanced-hepatocellular-carcinoma-previously-treated-with-sorafenib/.

31. Singh P, Toom S, Avula A, Kumar V, Rahma OE. The immune modulation effect of locoregional therapies and its potential synergy with immunotherapy in hepatocellular carcinoma. J Hepatocell Carcinoma 2020;7:11–7.

32. Pinato DJ, Murray SM, Forner A, Kaneko T, Fessas P, Toniutto P, *et al.* Trans-arterial chemoembolization as a loco-regional inducer of immunogenic cell death in hepatocellular carcinoma: implications for immunotherapy. J Immunother Cancer 2021;9.

33. Mazzaferro V, Citterio D, Bhoori S, Bongini M, Miceli R, de Carlis L, *et al.* Liver transplantation in hepatocellular carcinoma after tumour downstaging (XXL): a randomised, controlled, phase 2b/3 trial. Lancet Oncol 2020;21:947–56.

34. Qiao Z-Y, Zhang Z-J, Lv Z-C, Tong H, Xi Z-F, Wu H-X, *et al.* Neoadjuvant programmed cell death 1 (PD-1) inhibitor treatment in patients with hepatocellular carcinoma before liver transplant: a cohort study and literature review. Front Immunol 2021;12:653437.

35. Pfister D, Núñez NG, Pinyol R, Govaere O, Pinter M, Szydlowska M, *et al.* NASH limits anti-tumour surveillance in immunotherapy-treated HCC. Nature 2021;592:450–6.

36. Sia D, Jiao Y, Martinez-Quetglas I, Kuchuk O, Villacorta-Martin C, Castro de Moura M, *et al.* Identification of an Immune-specific class of hepatocellular carcinoma, based on molecular features. Gastroenterology 2017;153:812–26.

37. Foerster F, Hess M, Gerhold-Ay A, Marquardt JU, Becker D, Galle PR, *et al.* The immune contexture of hepatocellular carcinoma predicts clinical outcome. Sci Rep 2018;8:5351.

Personalised approaches to NAFLD

Harris Siddiqi[1] and Rohit Loomba*

NAFLD Research Center, Division of Gastroenterology and Hepatology, University of California at San Diego, La Jolla, California, *USA*

*E-mail address: roloomba@ucsd.edu

Take-home messages

- Non-invasive assessment is taking the centre stage in risk stratification.
- MRI-PDFF response defined as ≥30% relative decline is associated with 2-point improvement in NAS and NASH resolution.
- MEFIB and FAST may be used to identify 'at risk' NASH.
- Advances in microbiome and genetics = personalised risk stratification.
- Several classes of drugs are showing promise in the treatment of NASH.
- Personalised therapy is on the horizon.

Non-alcoholic fatty liver disease (NAFLD) affects nearly 100 million Americans and is the leading cause of chronic liver disease in the USA. Globally, NAFLD is present in 1 in 4 people with the highest prevalence of NAFLD among Asian Indians and Hispanics and the lowest among African Americans.[1] Risk factors for NAFLD include obesity, hypertension, hypertriglyceridaemia, insulin resistance, and diabetes. Additionally, many genetic factors have been associated with NAFLD such as risk alleles in PNPLA3, TM6SF2, and MBOAT7 as well as one protective HSD17B13 variant. A diagnosis of NAFLD is based either on biopsy or imaging evidence of hepatic steatosis (≥5% liver fat) in individuals who consume little or no alcohol without any other cause for liver disease or hepatic steatosis.

NAFLD covers a wide spectrum of diseases including steatosis, non-alcoholic steatohepatitis (NASH), and cirrhosis. It is defined by the presence of steatosis in ≥5% hepatocytes, minimal alcohol use, biopsy consistent with NAFLD, and no other aetiology for liver disease or secondary causes of NAFLD. Non-alcoholic fatty liver (NAFL) is the non-progressive form of NAFLD and is relatively benign. NASH, however, is the progressive form of NAFLD and the second leading indication for liver transplantation in the USA. Studies regarding the natural history of NAFLD have estimated the fibrosis progression rate among NASH patients to be 1 stage every 7 years. Amongst this group, 20% are estimated to be fast progressors and will likely advance to cirrhosis within the next decade.[1]

The future of clinical medicine in NAFLD involves a personalised approach to treatment. Primarily, a genetic and metabolic risk assessment encompassing genomics, epigenomics, transcriptomics, proteomics, and metabolomics will provide a comprehensive patient-oriented treatment plan that leaves no stone unturned. This personalised approach involves an initial primary risk stratification and prevention strategy, followed by diagnosis and staging, secondary risk stratification (prognosis), treatment tailored to the individual, monitoring of disease status, and response-guided combination therapy.

Currently, NASH can only be diagnosed by liver biopsy and is defined by the histological presence of steatosis, lobular inflammation, and ballooning with or without Zone 3 fibrosis.[2] Indications for liver biopsy include the presence of metabolic syndrome, high aspartate aminotransferase/alanine aminotransferase (AST/ALT) ratio, low platelet count or serum albumin level, cholecystectomy or bariatric surgery, advanced age, or diabetes. Liver biopsy and histology are considered the gold

standard for the diagnosis of NAFLD and NASH and the traditional paradigm for treatment response assessment. However, an estimated 1 billion people have NAFLD worldwide, including 100 million affected Americans and 72 million Europeans. Because of the sheer scale and magnitude of the NAFLD population, liver biopsy is impractical both as a diagnostic screening approach and for treatment response assessment. In addition, liver biopsy procedures put patients at risk for pain, infection, bleeding, perforation, and even death.

Detection of patients with 'at risk' NASH (defined as those with NASH and ≥stage 2 fibrosis) is of importance owing to the association between the stage of fibrosis and the risk of liver-related mortality.[3] Patients who are 'at risk' are candidates for pharmacologic therapies and non-invasive identification of candidates for pharmacologic therapies is a major unmet need. Given the drawbacks of performing liver biopsies, many non-invasive metrics have been developed to help identify 'at risk' patients with NASH. The FibroScan-AST (FAST) score was developed to help detection of high-risk NASH, however it suffers from a low positive predictive value (PPV).

The magnetic resonance imaging-derived proton density fat fraction (MRI-PDFF) is a quantitative imaging biomarker that enables accurate, repeatable, and reproducible quantitative assessment of liver fat across the entire organ. Furthermore, MRI-PDFF response defined as ≥30% relative decline is associated with 2-point improvement in NAFLD Activity Score (NAS) and NASH resolution.[4] A non-invasive magnetic resonance electrography (MRE) method designed by UCSD in collaboration with Yokohama University using a combination of imaging and serum markers (MRE ≥3.3 kPa and Fibrosis-4 [FIB-4] ≥1.6) yielded a high positive predictive value for a clinician to rule in clinically significant disease that needs pharmacologic treatment.[3] A new score, MEFIB (MRE plus FIB-4), was then developed and proven to be superior to FAST in detection of 'at risk' NASH patients among patients with biopsy-proven NAFLD.[5] These metrics are currently the best methods to identify which patients need to be treated without a liver biopsy.

Recently, advances have been made in utilising gut microbiota as a biomarker for advanced fibrosis. Metagenomic sequencing has yielded high diagnostic accuracy for the detection of advanced fibrosis in NAFLD.[6] Furthermore, Oh *et al.*[7] have demonstrated a universal metagenomic signature for cirrhosis across diverse aetiologies of liver disease and geographically and ethnically distinct cohorts. Genetic risk assessment has shown promise, as many genes have been found to be associated with NAFLD and lean NAFLD.[8]

The treatment landscape for NAFLD is extensive and still under development. Many drugs have been tested for their efficacy in reversal/resolution of NASH. The target mechanism for these drugs is often lipotoxicity. In NASH resolution landscape monotherapies, a large proportion of subjects observed resolution of NASH without worsening of fibrosis. The largest proportion (67%) was seen in trials with 0.4 mg daily injections of semaglutide. In terms of drug therapies for NAFLD, combination therapy has also shown promising results. Combination therapy involves the combination of 2 or more drugs to boost response rates, improve side-effect tolerability, and broaden the therapeutic index. The most effective drug combinations are still being researched today.

As discussed prior, there are many genes that have been linked to NASH. It is therefore of interest to develop genetic therapies in NASH and other metabolic diseases. Currently, 2 classes of approaches are on the horizon: gene silencing approaches and clustered regularly interspaced short palindromic repeat (CRISPR)-Cas9 approaches. Gene splicing approaches include anti-sense oligonucleotide (ASO), ASO-Lica, and small interfering RNA. CRISPR-Cas9 approaches include: CAS9 editing (proof of concept in sickle cell anaemia and cystic fibrosis), base editing (proof of concept in PCSK9 adenine base editing in familial hypercholesterolaemia), and prime editing. More research is needed in the realm of genetic therapies for NASH; however, they are seen as a crucial part of developing a personalised

medicine paradigm for NASH treatment. Ideally, when a patient with suspected NASH presents to the clinic, their genomic and metabolic risk will be assessed using FIB-4/vibration-controlled transient elastography (VCTE)/MRE/enhanced liver fibrosis (ELF) and metabolomics/proteomics/lipidomics/microbiome. This will then be followed by a genetic risk score and metabolic risk assessment to determine what is driving the risk of cirrhosis and hepatocellular carcinoma (HCC).

To create a more personalised approach to treating NAFLD, many factors must be taken into consideration (Fig. 1). Primary risk stratification should involve a comprehensive analysis of family history of cirrhosis, diabetes, and metabolic syndrome. Diagnosis and staging should consist of an efficacious non-invasive imaging strategy such as FAST/MRI-AST (MAST)/MEFIB or VCTE/MRE. Next, treatment should consist of a genetic analysis to determine whether risk alleles such as PNPLA3 are present, or the protective HSD17B13 variant or an exome wide genetic scan in selected cases such as those with lean NAFLD with significant fibrosis. Monitoring disease status can be done via MRI-PDFF/MRE, VCTE, and OMICS. Lastly, treatment should revolve around effective combination therapies that follow a response-guided approach. This combined effort will set the foundation for the future of clinical medicine in NAFLD, and allow for a patient-oriented pathway to treatment.

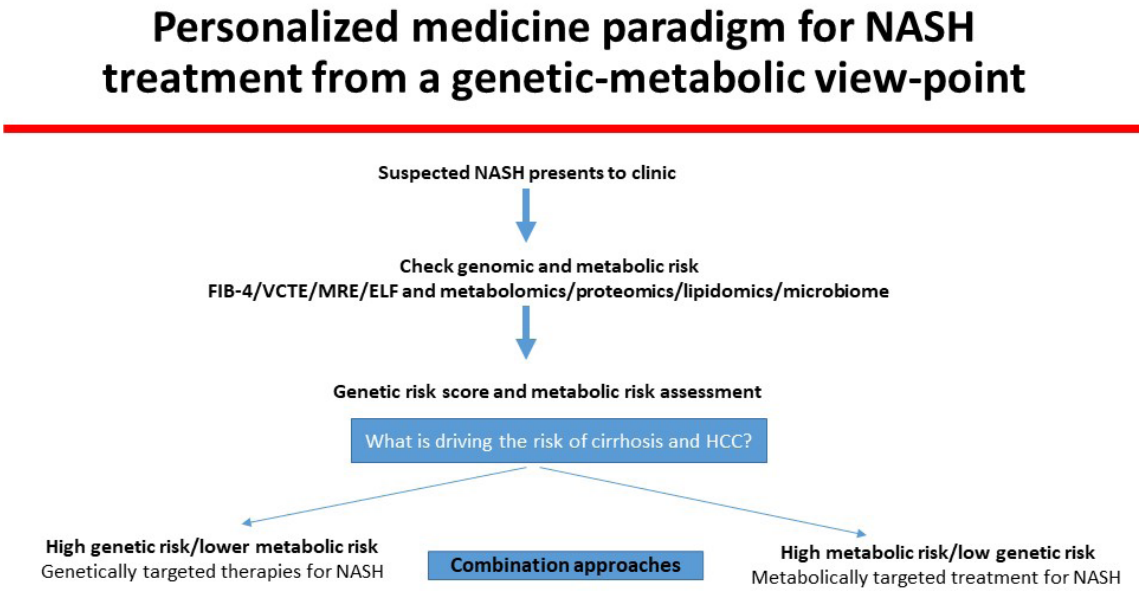


Fig. 1. Personalised medicine paradigm for NASH treatment. ELF, Enhanced liver fibrosis; FIB-4, Fibrosis-4; HCC, hepatocellular carcinoma; MRE, magnetic resonance elastography; NASH, non-alcoholic steatohepatitis; VCTE, vibration-controlled transient elastography.

References

1. Loomba R, Friedman SL, Shulman GI. Mechanisms and disease consequences of nonalcoholic fatty liver disease. Cell 2021;184:2537–64.
2. Chalasani N, Younossi Z, Lavine JE, Charlton M, Cusi K, Rinella M, et al. The diagnosis and management of nonalcoholic fatty liver disease: Practice guidance from the American Association for the Study of Liver Diseases. Hepatology 2018;67:328–57.
3. Jung J, Loomba RR, Imajo K, Madamba E, Gandhi S, Bettencourt R, *et al.* MRE combined with FIB-4 (MEFIB) index in detection of candidates for pharmacological treatment of NASH-related fibrosis. Gut 2021;70:1946–53.
4. Stine JG, Munaganuru N, Barnard A, Wang JL, Kaulback K, Argo CK, *et al.* Change in MRI-PDFF and histologic response in patients with nonalcoholic steatohepatitis: a systematic review and meta-analysis. Clin Gastroenterol Hepatol 2021;19:2274–83.e2275.
5. Tamaki N, Imajo K, Sharpton S, Jung J, Kawamura N, Yoneda M, *et al.* MRE plus FIB-4 (MEFIB) versus FAST in detection of candidates for pharmacological treatment of NASH-related fibrosis. Hepatology 2022;75:661–72.
6. Loomba R, Seguritan V, Li W, Long T, Klitgord N, Bhatt A, *et al.* Gut microbiome-based metagenomic signature for non-invasive detection of advanced fibrosis in human nonalcoholic fatty liver disease. Cell Metab 2017;25:1054–62.e1055.
7. Oh TG, Kim SM, Caussy C, Fu T, Guo J, Bassirian S, *et al.* A universal gut-microbiome-derived signature predicts cirrhosis. Cell Metab 2020;32:901.
8. Vilarinho S, Ajmera V, Zheng M, Loomba R. Emerging role of genomic analysis in clinical evaluation of lean individuals with NAFLD. Hepatology 2021;74:2241–50.

Modelling the course of cirrhosis

Gennaro D'Amico

Gastroenterology Unit, Ospedale V. Cervello, Clinica La Maddalena, Palermo, *Italy*

E-mail address: gredamico@libero.it

Take-home messages

- Deep learning (DL) is an advanced technique of machine learning (ML).
- One important characteristic of DL is the recursive updating of covariates weights.
- DL prognostic models allow for non-linear risks and may provide more granularity in knowledge of the clinical course of cirrhosis.
- In DL algorithms, optimisation of predictive performance is performed by complex analyses not understandable to physicians and patients ('black box') which limits their use.
- Big data-based DL may provide powerful decisional tools for the individual patient.
- Major limitations are the type and completeness of available big data ensuring correctness of the learning phase, satisfactory external validation methods, and understandability for physicians and patients.

Introduction

The use of electronic health records (EHRs) is continuously increasing in clinical research aiming at providing a guide to clinical practice. EHR-based studies offer several advantages based on the prompt availability of databases of very large patient samples including baseline and follow-up characteristics, time to relevant clinical events, and outcome. Therefore, these studies may answer important research questions in much less time and at much less cost than prospective studies when the risk of bias common in retrospective studies (mostly patient selection, attrition, blindness, and outcome assessment) may be adequately controlled.

The exponential expansion of computing capacity and data storage in the past 2 decades made it possible to create huge databases including any type of data produced in clinical practice, spanning from genome sequencing to high resolution imaging, vital function monitoring, sequential clinical characteristics, treatments, time to relevant clinical events, mortality, and many others. The availability of such types of stored data has vigorously prompted the research in the field of artificial intelligence (AI) to support personalised clinical management.

AI is a complex of several technologies aimed at building dynamic learning algorithms able to classify patients according to individual risks and healthcare requirement. Deep learning (DL) is one of such techniques, proposed in recent years and largely used to model survival, competing risks, and to inform clinical management.[1]

Major steps of DL algorithms and their application to personalised clinical decision-making will be summarised here.

From artificial intelligence to DL

AI uses algorithms designed to adsorb information from large volume medical data and find out their relationship with a defined condition (disease or disease stage) or outcome (time to clinical events or death) to assist clinical practice. These algorithms also include self-updating instructions to improve accuracy based on regular feedback input, thereby reducing clinical error, and offering a potential for real-time diagnostic and prognostic inferences. A very large variety of medical data are used for AI in medicine, encompassing screening, diagnosis, laboratory, imaging, histology, treatment, instrumental monitoring recording, data update along time, follow-up events, and outcome. Special techniques (natural language processing) have also been developed to convert unstructured clinical notes and intervention reports, to machine usable data. AI is therefore based on powerful computational ability (*machine*) and the capacity to find relationships between the acquired data (*learning*). The process generating AI is therefore termed *machine learning* (ML) and the relevant algorithms are subdivided into '*unsupervised*' and '*supervised*' learning, according to whether they are aimed at *disease characterisation* based on patient features, or at *predicting outcome* through identification of some relationship between patient characteristics and outcome. Because supervised learning provides results more clinically relevant, they are the most frequently used algorithms for AI in medicine. Among several suitable algorithms the most widely used in ML are support vector machines (SVMs) and neural networks (NNs).

SVMs are based on the weights to be attributed to patient characteristics to identify 2 groups of patients according to a relevant outcome variable and a decision boundary. The analysis algorithm is aimed at achieving the smallest classification error.

NNs may be thought about as extensions of linear regression to *capture non-linear relationships* between baseline features (input variables) and an outcome of interest (output). In NNs, the associations between the input variables and the output are represented through several *hidden layers* composed by *prespecified combinations* unapparent in clinical practice because they may be hidden in the massive amount of data. In fact, the weights of the associations between the input variables and the output are adjusted at any transition across such hidden layers, aiming at minimising the prediction error (Fig. 1).

DL may be considered as an extension of NNs where many more layers are used to detect more complex non-linear relationships between patient features and the outcome of interest. It is essentially the process of training a NN to perform a given task. A recurrent neural network (RNN) is among the most commonly used DL algorithms. What increases RNN precision in identifying disease patterns or in making predictions is the fact that it does not take into consideration just the actual input, but also the previous input which allows it to memorise what happened previously and to adjust the covariate weights accordingly (Fig. 2). Therefore, the algorithm is recursively repeated, and estimates adjusted at each new input.[1]

As for the other AI algorithms, the efficiency of DL critically depends on the training (*learning*) process. In fact, when the training dataset is not enough various or if it bears some inadvertent bias, the algorithm performance may be unsatisfactory. DL algorithms are particularly suitable for complex and highly dimensional data and are mostly used in the field of diagnostic imaging, although in the past few years they are increasingly used in survival modelling.

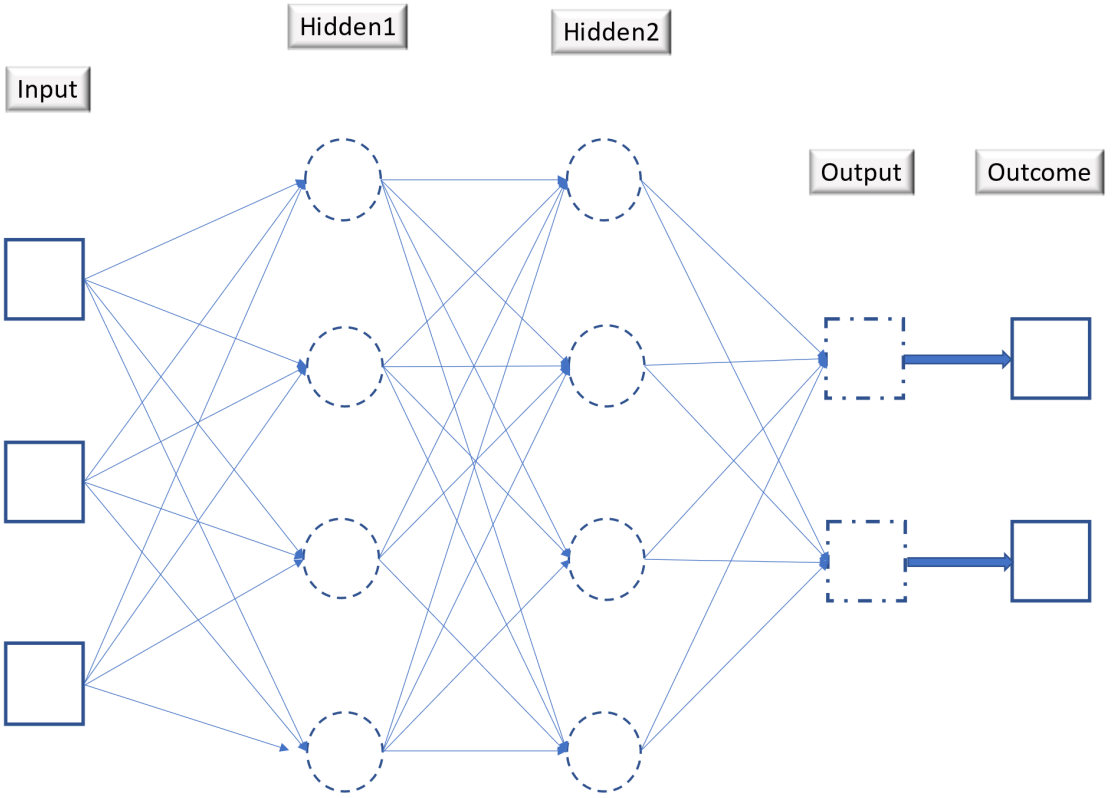


Fig. 1. Schematic representation of a neural network process with 2 hidden layers.

Towards individual medicine

The increasing availability of big data and of computational power with DL algorithms is going to change the clinical practice paradigm from an average to an individual approach. A first step in this change is personalised/individualised/precision medicine: here individuals are seen on the basis of some fixed pattern, defined on the basis of a limited numbers of factors with risk classifications and predictions based mostly on SVM or NN algorithms. However, the upcoming increasing use of ML algorithms such as RNNs, is opening the scene to so-called 'bespoke medicine'. Bespoke medicine is in fact an evolution of individual medicine based on all the available information and updates and adapts individual patterns as new information becomes available – incorporating the effects of ageing, lifestyle changes, onset and evolution of various conditions, as well as progress through a course of treatment.[2]

Survival modelling

Standard survival modelling

Survival analysis is routinely used in medicine to appraise the clinical course of diseases and the relationships between patient characteristics and time to death or relevant events. The key elements of survival analysis are the inception point or *time zero*, the occurrence of the event of interest and the time elapsed to the occurrence of the event of interest. Among several available models, the Kaplan-Meier model is the most widely used for survival analysis in clinical research. The model accounts for any single event of interest occurring in the study period and allows the survival function to be accordingly changed at any time 1 event occurs. The ideal survival analysis would terminate when all

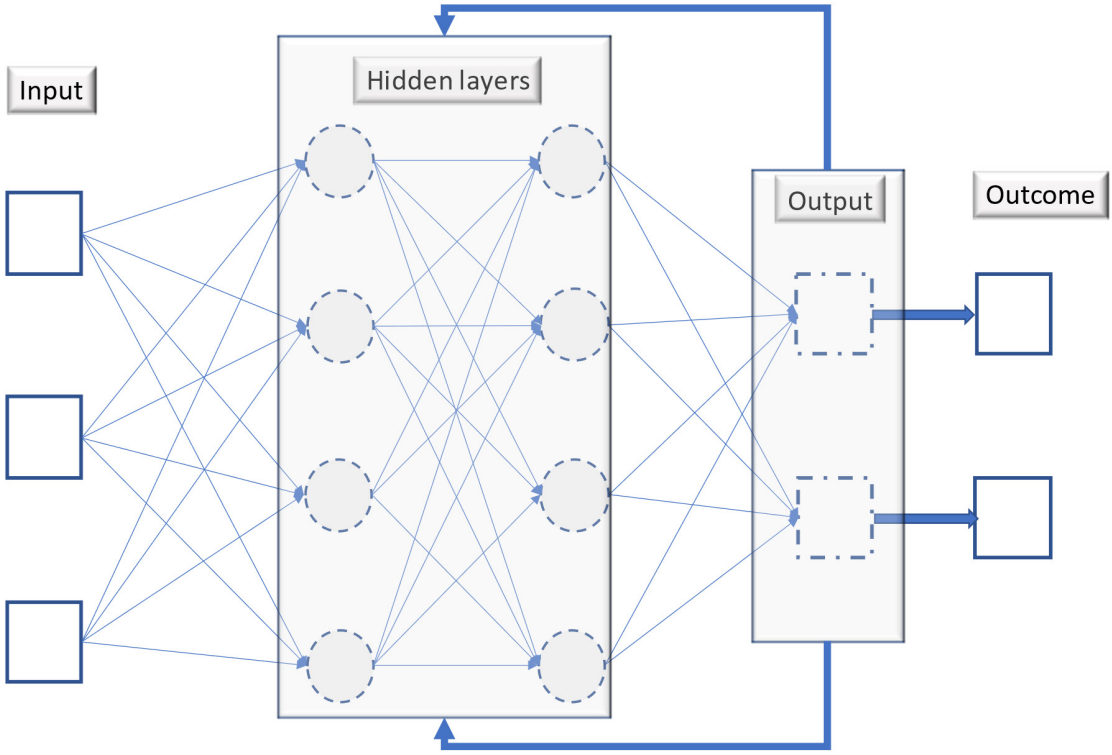


Fig. 2. Schematic representation of the recursive characteristics of a recurrent neural network algorithm applied in a deep learning process.

the patients had died or had developed the event of interest. However, this is not the case in almost all the studies because, usually, when the study terminates, a number of patients have not yet experienced the event of interest and their observation is truncated. The interruption of the observation is termed censoring and, in this specific case, '*right censoring*'. Censoring may also occur when the true time zero is not known, that is the underlying illness is present, but it is not known when it exactly started: this is termed '*left censoring*'. It is to note that each right censoring event causes a reduction of the number of patients at risk which is reflected along the rest of the curve and causes an unmeasured grade of approximation to (or separation from) the true curve. Clearly, if censoring depends on the probability of event occurrence, such approximation will be distorted (biased). Therefore, censoring must be *non-informative* or unrelated to the event of interest.[3]

Sometime 2 or more outcomes of interest may compete each other to occur first. This requires the use of competing risks analysis as the traditional Kaplan-Meier analysis should only be used in 2-state settings and in the presence of competing risks, it invariably results in upward biased estimates.

The relationship between patient baseline characteristics and the occurrence of the event of interest (*prognostic analysis*) is usually studied by multiple regression analyses where the dependent variable is the time to the event and the independent covariates are the candidate predictors. The most widely used model for such kind of analyses is the Cox model which is based on the assumption of proportionality of hazards, implying that the hazard curves of the groups should be proportional and cannot cross.[4]

In the presence of competing risks, the standard Cox model is not appropriate because, similarly to the Kaplan-Meier model, it may result in upwards biased estimates. A specific multiple-regression proportional hazards model has been developed for competing risks, by Fine and Gray. This model

measures the relationship between the candidate predictive factor and the outcome, accounting for the modifying effect of the competing events.

A different approach to prognostic analysis is that of the accelerated failure model (AFM). The principle here is that the effect of a covariate is of stretch the survival curve along time by a constant relative amount. For positive values of this amount (>1) the survival time is longer and with negative values it is shorter.

DL survival modelling

A limitation of the standard approaches to survival and prognostic analysis is the underlying assumption of linearity of risks which hampers fine tuning of risk prediction in specific subgroups where risk may assume a different form. ML techniques (NNs in particular) have been used to model non-linear representations for the relation between covariates and the risk of an event, thus weakening the proportionality assumption of the Cox model. The DL approach to survival modelling may overcome this limitation based on an RNN which may allow for time-varying risks and for competing events. The RNN allows the algorithm to be updated each time new information is added. Using such longitudinal information, the system *learns* to predict the next value of the time varying covariates and estimates the probability of the first new event to occur. The general formula of such a survival model may be written as follows:

$S(\tau^*|\mathcal{X}^*) \triangleq P(T > \tau^*|\mathcal{X}^*, T > t^*_{J^*})$ where S is the survival (at time τ \ given covariates X); P is the probability that the whole available time T is longer than the event time τ ($T>\tau$ indicates that the event occurs before T) conditioned on to the history of longitudinal measurement of the covariates X recorded at a time t_j with $T>t_j$. Whenever a new measurement is recorded for each subject at time t_j, the model is updated accounting for that information in a dynamic fashion.

One of such models (Dynamic-DeepHit) uses automated ML to combine different underlying survival models, together with endogenously determined time-varying weights to produce a well-calibrated survival function that offers high discriminative performance at different times.[5] This approach to survival modelling is known as Survival Quilts and the temporal construction is referred to as *temporal quilting*. This algorithm configures the weights sequentially over a fine grid of time intervals. A constrained Bayesian Optimisation (BO) is applied, to achieve the best possible performance ('black box'). Based on the constructed array of weights, the algorithm may provide valid predictive models.[2]

To account for competing risks, the above formula may be modified to allow not only for time to death, but also for time to a number of other events of interest. In this model, once an event has occurred, the patient is no longer considered at risk for the other events. Therefore, the output of the model is the Cumulative Incidence function of the competing events of interest.

How DL will help to understand the natural history and prognosis of cirrhosis

As with any other disease model, DL algorithms may help to have a more granular insight in the course of cirrhosis. This expectation is mostly based on the diverse approach to the analysis of predictive factors and on the huge amount of explorable data which was inaccessible until the very recent past. Besides the multitude of data, in terms of included subjects, the potential to explore the role of any type of covariate spanning from genetics to sociocultural states, may literally change the horizon of disease interpretation and management.

Great expectation lies on what would be the effect of updating the weights along time of already known prognostic variables on predictive accuracy. This has been partly explored by standard methods such

as the time-dependent Cox proportional hazards model, although its application has not produced satisfactory and generalisable prediction rules. Some work has already been done in this sense with several tenths of studies done with this aim,[6,7] failing to reach clinical practice changing conclusions. However, this research field is still at its very beginning, with analysis approach continuously evolving towards the definition of more appropriate models. As an example, one of the most promising approaches, namely the Survival Quilts model has not yet been used in hepatology.

There are several segments of the clinical course of cirrhosis, deserving more granular insight where DL may provide important new information. Compensated cirrhosis is a phase of the disease where scientific evidence is still insufficient for adequate management. We know that this is a very long and mostly silent phase of the disease with as many as 50% of patients still in a compensated stage 20 years after diagnosis. Knowledge of valid predictors of decompensation would be therefore a key element for timely use of preventative measures in individual patients at risk. The definition of decompensation *per se*, might be updated, being currently based on clinical signs, whereas the role of liver function measures should be explored either as part of the definition or as predictors. Even the paradigm of decompensation as the most important risk-stratifying feature might be abandoned if more efficacious indicators of disease progression are disclosed, or the definition of decompensation may be changed. ML approaches may help to detect reliable non-invasive markers of clinically significant portal hypertension (CSPH) in compensated cirrhosis and the definition of CSPH may be modified or abandoned in favour of such non-invasive markers. Similarly, in decompensated cirrhosis there is a need to define the risk indicators of further decompensation following the first decompensating event. The best time for liver transplantation could be re-defined and patient selection for transplant should be based on new and more efficient prognostic tools than the MELD. Another unsolved question is to what extent may non-selective beta-blockers (NSBBs) be continued in decompensated patients and when would be most appropriate for curing portal hypertension by placing a transjugular intrahepatic portosystemic shunt (TIPS).

Impact on scoring system developments

ML techniques are expected to substantially improve prognostication as the candidate predictors may span largely beyond the boundaries of what have been explored until now with standard methods. However, the complexity of covariate weighting with recursive updating of weights and their optimisation in a 'black box' to achieve the best possible performance of relevant predictions rules, make them hard to understand for clinicians and patients. The complexity of scoring rules applied in DL hinder their routine clinical implementation in all but the most advanced informatics settings. A potential way out from physicians' and patients' blindness to such complex although 'expectedly' more accurate prediction tools, is to derive some 'post-estimation' strategy which uses the strengths of ML to identify the important covariates and their weights and to convert these in simpler, clinically explainable risk scores. Such strategies should optimise the trade-off between accuracy and interpretability and also make subsequent implementation easy. This approach has been explored in a very large database from the Veterans Administration, including 107,939 patients with cirrhosis.[8] Three different statistical and ML methods were evaluated: gradient descent boosting, logistic regression with least absolute shrinkage and selection operator (LASSO) regularisation, and logistic regression with LASSO constrained to select no more than 10 predictors (partial pathway model). The predictors identified in the most parsimonious (the partial pathway) model were refit using maximum-likelihood discrete time-to-event logistic regression estimation which allowed to estimate the beta coefficient per each significant parameter detected by the more complex ML model. Beta coefficients were then used to calculate individual risks. However, given the complexity of calculations these were not shown in the article. An online calculator has instead been posted. Fig. 3 represents the risk calculation

for a patient with cirrhosis, 55 years old, with history of chronic obstructive pulmonary disease, non-African American ethnicity, Na 133 mEq/L, bilirubin 2.4 mg/dl, 151 platelets/nl, haemoglobin 8.8 g/dl, aspartate aminotransferase/alanine aminotransferase (AST/ALT) ratio >2, no encephalopathy, with ascites, no HCC.

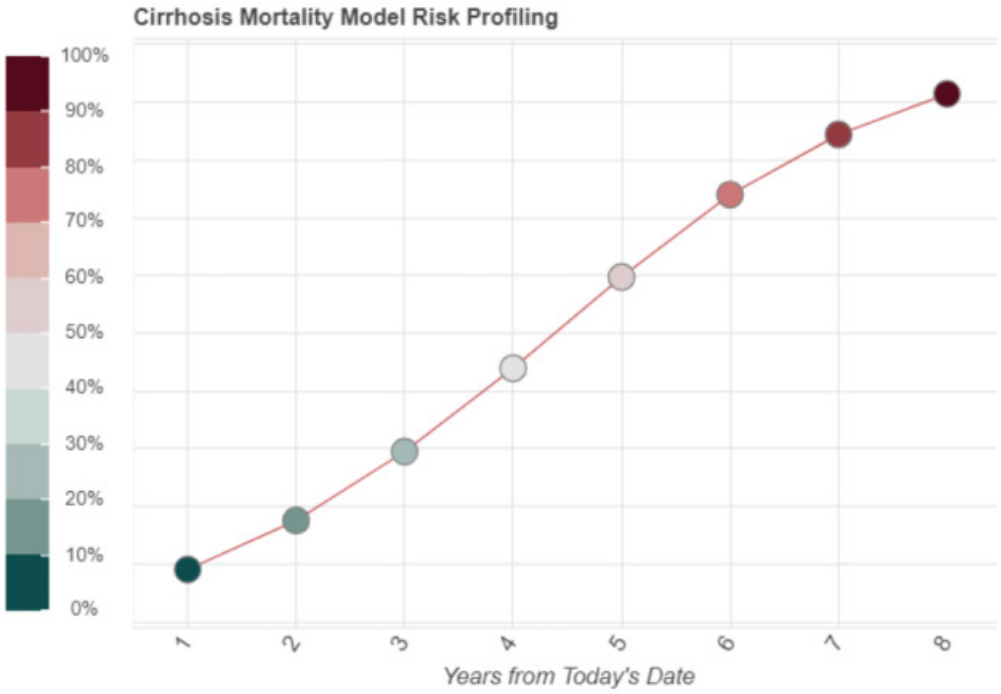


Fig. 3. Example of mortality risk diagram from 1 to 8 years for a simulated patient according to a prediction rule based on machine learning and re-fitted by a logistic analysis, calculated at http://cimm.herokuapp.com/main.

However, such a model simplification loses one most important characteristic of ML algorithms, which is its dynamicity resulting by the recursive update at each new input, implying that the mortality risk of this patient could be different when reassessed along time. Therefore, there is still probably much work to do in this field but it seems that the translation from the ML language to a physician/patient understandable one is on the horizon.

Impact on therapeutic strategies

When a DL algorithm includes treatments, the model learns the treatments given and the period the treatments were given, so that treatments are handled like any other covariate, their weights are adjusted according to subsequent input to the system and interactions with a large number of covariates are explored. A treatment recommendation function may be defined as the log of the hazard ratio of the personal risk of being given 1 treatment option over another. The analysis context recalls that of observational studies of treatment effects where to overcome the limited number of assessable interactions, comparable patient groups are selected in the population under study by propensity score matching. However, in a big data context an ML algorithm may be able to detect even small groups of patients who may benefit from a given treatment, and who would probably remain hidden in traditional observational studies.

In the context of AI, even the methodology of a randomised clinical trial might be adapted to ML techniques, by expanding at the level of daily clinical practice the data collection. While maintaining the randomisation principle to ensure comparability of the treatment groups, the expansion of collected data available for the analysis of interaction with treatment may strongly empower studies to detect even small patient subgroups who may benefit from the treatment. Clearly the sample size constraints will not change.

DL limitations

A major issue with DL models such as recurrent (multi-layer) NNs is that they tend to overfit data of a specific training dataset, which may diminish the generalisability of the model. Moreover, the learning phase of the model strictly depends on the type of input the model is given. If the input has not been predefined based on some strong and plausible hypothesis on biology, pathophysiology, or clinical grounds appropriate to answer specific relevant questions, the algorithm may lead to distorted conclusions. Moreover, certain patient subgroups may be disregarded depending on the structure of the training set and/or data missingness.

Application of DL algorithms in clinical practice will be challenging because of several inherent unsolved issues. A first consideration is that there is not yet a satisfactory validation methodology for the predicted effects on health outcomes. Given the complexity of big data, available studies have claimed validity mostly based on a split sample technique either temporal or randomised: these methods are however internal validation tools, whereas external validation should be performed in a similar big dataset collected in a different place. Clearly the paradigm of validity might be remodelled specifically for big data, but no convincing strategy has been proposed yet.

Validation may not be disregarded because although DL algorithms may be capable to uncover hidden features successfully applicable to small groups of patients, they may likewise provide spurious associations which require skilled judgement to be identified. Another issue regards the vast areas of medicine uncertainty like interobserver variability, grey zone in diagnostic or outcome assessments. Such issues will be part of the information on which the learning phase of the DL process is based and will result in unintended erroneous conclusions, which may remain undetected at least for a while.

In this regard it is also to be noted that no comparative studies have yet shown the effectiveness of ML-based decision-support systems.

Another important issue with the application of AI in clinical practice could be over-reliance on the capabilities of automation which in the long term may lead to medical de-skilling.

Therefore, although AI is most appealing, great efforts should be done to ensure transparency of the modelling process and to detect and overcome any hidden pitfall that may result in harm for the patients.

References

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

1. **Jiang F, Jiang Y, Zhi H, Dong Y, Li H, Ma S, et al. Artificial intelligence in healthcare: past, present and future. Stroke Vasc Neurol 2017;2:e000101.**
2. *van der Schaar M, Maxfield N.* Survival analysis, competing risks and comorbidities. https://www.vanderschaar-lab.com/survival-analysis-competing-risks-and-comorbidities/.
3. Clark TG, Bradburn MJ, Love SB, Altman DG. Survival analysis part 1. Basic concepts and first analysis. Br J Cancer 2003;89:232–8.
4. Bradburn MJ, Clark TG, Love SB, Altman DG. Survival analysis part 2. Multivariate analysis – an introduction to concept and methods. Br J Cancer 2003;89:431–6.
5. **Lee C, Yoon J, van der Schaar M. Dynamic-DeepHit: a deep learning approach for dynamic survival analysis with competing risks based on longitudinal data. IEEE Trans Biomed Eng 2020;67:122–33.**
6. Ahn JC, Connell A, Simonetto DA, Hughes C, Shah VH. Application of artificial intelligence for the diagnosis and treatment of liver diseases. Hepatology 2021;73:2546–63.
7. Su TH, Wu CH, Kao JH. Artificial intelligence in precision medicine in hepatology. J Gastroenterol Hepatol 2021;36:569–80.
8. **Kanwal F, Taylor TJ, Kramer JR, Cao Y, Smith D, Allen L, et al. Development, validation, and evaluation of a simple machine learning model to predict cirrhosis mortality. JAMA Open 2020;3:e2023780.**
9. Kubota N, Fujiwara N, Hoshida Y. Clinical and molecular prediction of hepatocellular carcinoma risk. J Clin Med 2020:9;3843.
10. Cabitza F, Rasoini R, Gensini GF. Unintended consequences of machine learning in medicine. JAMA 2017;318:517–8.

SESSION 5

DIGITALISED MEDICINE IN PRACTICE

THURSDAY 23 JUNE
10:00-11:00

Social media as a tool for patient engagement and education

Maja Thiele

Department of Gastroenterology and Hepatology, Odense University Hospital and Institute of Clinical Research, University of Southern Denmark, Odense, *Denmark*

E-mail address: maja.thiele@rsyd.dk

Take-home messages

- Social media (SoMe) is an omnipresence in everyday life, including for seeking and sharing information on health and disease. Clinicians should therefore avoid dismissing social media as merely an entertainment gadget, but harvest SoMe's global potential for engagement with patients, peers, politicians, and the public.
- All SoMe platforms have interaction between networks of people at their core. Medical professionals therefore gain most from using SoMe if they are willing to interact with SoMe users, going beyond the one-way communication that is characteristic of traditional science dissemination.
- The bilateral communication on SoMe also holds specific opportunities for sharing knowledge, education, and experience among peers using the hashtags #LiverTwitter and #GITwitter. Professional use of SoMe also includes promoting your own research, with strategies for what to communicate, where, how, and with whom.
- Successful SoMe engagement requires innovative forms of communication that are uncommon in, or even contrasts, academic medicine: personal experience, storytelling, emotion, humour, personal opinion, video formats, brief messages, images, gifs, emojis.

Why should clinicians care about social media?

Social media (SoMe) have become a global presence during only 2 decades, and are now an integrated part of human life. By January 2021, Facebook alone had more than 2.7 billion users, 35% of the total world population, and according to Statista.com, the average internet user spent more than 2 h per day on SoMe in 2020.

The term social media emerged during the 1990s to describe companies who offer web-based platforms that facilitate interaction and entertainment among a network of users (https://www.forbes.com/sites/jeffbercovici/2010/12/09/who-coined-social-media-web-pioneers-compete-for-credit/). While the *social* in SoMe is arguably no more social than making a telephone call or writing a letter, registered users rapidly surged because of the online environment, ability to interact with multitudes, and the capacity for creating and consuming user-generated content. Considering their omnipresence, it is peculiar that the major SoMe companies of today are all still in their teens: LinkedIn (launched in 2003), Facebook (2004), YouTube (2005), Reddit (2005), Twitter (2006), Weibo (2009), Instagram (2010), WeChat (2011), and TikTok (2017).

Despite the young age of SoMe, a very substantial proportion of the public presently find their health information on SoMe.[1] It is therefore problematic that academic medicine and the medical community exhibited a decade-long inertia before exploring SoMe for professional purposes; it only slowly started taking off during the 2010s (Fig. 1).

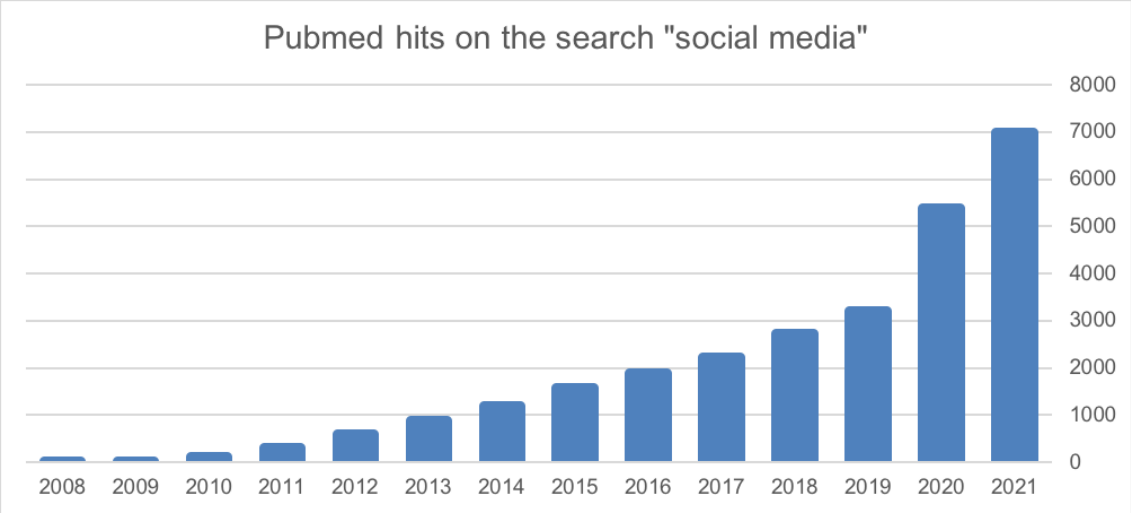


Fig. 1: PubMed counts for 'social media'. PubMed introduced social media as a MeSH (Medical Subject Heading) term in 2012.

The top health-related activities on SoMe are: (1) general knowledge seeking and sharing; (2) engagement in online communities for patients, relatives, or people with health concerns; (3) to track and share health indicators such as running routes or weight loss, albeit this is far less common than the two other top uses.[1]

In a survey of 1,000 people in October 2021 the website patientslikeme.com found that 10% of Americans looked to SoMe for reliable health information or to evaluate new treatment options. The same survey found that only 2% trusted health information on SoMe. This discrepancy is a window of opportunity for healthcare experts to step in, and assume the role of reputable source of healthcare communication on SoMe.

The public's information-seeking behaviour on SoMe has been particularly evident during the Covid-19 pandemic, where misinformation spread across SoMe platforms, and medical professionals and organisations stepped up to engage with users, correct misunderstandings, and share their own content. An example is the 'Infodemic Response Checklist'.[2] It is a 12-item list designed with Covid-19 in mind, but its measures can be adopted to curb all kinds of erroneous health-information overflow (Table 1).

Table 1. List of measures that professionals, strategists and politicians dealing with health communication may adopt, to overcome an overflow of (false) health information. Adapted from Mheidly and Fares, 2020[2]

Individual measures		Explanation
1	Exposure time	Most scientists often exclusively disseminate through peer-reviewed articles and scientific journals. However, scientists and medical professionals are uniquely positioned to empower people with facts through interviews, op-eds, townhall meetings, podcasts, blogs, and social media. This require that healthcare professionals are offered, and seek out, exposure time outside their dogma of scientific dissemination.

2	Empathetic communication	Empathy in communication is critical for engaging the public. Emotional resonance, being relatable, use of humour or taking a personal stance is far more engaging than merely sharing facts and numbers.
3	Promote dialogue	Willingness to engage in dialogue is important to understand people's perceptions and the motives behind their practices, address concerns and strengthen societal adhesion.
4	Share personal experiences	Storytelling holds enormous strength, as most humans are more prone to remember and react to a personal tale. Therefore, particular cases and patient stories can be used to highlight the numbers-based evidence.
5	Develop educational material	Professionals tend hold back on developing educational material before having a solid evidence base. However, the Covid-19 pandemic taught us that it is possible to develop materials that fills the need for reliable, easy-to-understand information while balancing the uncertainty of rapidly evolving knowledge.
6	Direct strategies towards minorities	Minorities often suffer from large health inequalities, and are therefore most in need of reliable, useful health information. However, some minority groups are not accessible via standard information pathways which tend to be uniform in language, imagery, values and cultural references. Directed efforts for minority groups are optimally delivered by someone within the community.
Legislative/ organisational measures		**Explanation**
7	Promote websites	Search engines could easily promote websites of trustworthy public health organisations by moving them up in their search result algorithms.
8	Verified accounts	Verified accounts is currently a feature reserved for those with many followers and/or publicly known figures. A 'healthcare professional' verified account would gain more traction, be more visible and make it easier to the public to know whether to trust claims from that account or not.
9	Promote posts	Similar to the search engines, social media platforms could sponsor posts by health professionals/organisations and promote their exposure via algorithms.
10	Monitor SoMe engagement	There is a growing demand by governments and a large proportion of the public for SoMe companies to monitor and review SoMe posts to ensure that false information is not spread among a wide audience.
11	Invest in health communication	Health communication by healthcare professionals will only succeed if awarded by funding bodies, university and hospital management, and scientific peers.

SoMe as a tool for bilateral engagement

Clinicians and clinical researchers will experience a multitude of missed opportunities if they dismiss SoMe as purely empty calories and trivial entertainment. Instead, we must learn to use social media as potent tools for distributing information and bilateral engagement.[3]

The power of SoMe is not that you may display yourself and your life to others, but rather that you can engage and interact with them. As long as you both have an internet connection. On SoMe, users make their own content and upload files, but this form of one-way communication only comes to life when other users engage – by clicking the link, liking, commenting, or sharing.

The Covid-19 pandemic gave rise to a multitude of examples of interactions between healthcare professionals and the broad public. One learning from these examples include how overwhelming the number of interactions on SoMe are: During a 2-h period, on the afternoon of March 11, 2020, two Emergency Department physicians from Massachusetts General Hospital received 522 active comments and questions during a Reddit coronavirus Ask Me Anything session.[4] Only 28% of the questions received an answer, and not always by one of the physicians. Users similarly answered each other's questions. This highlights the interactive, open format of SoMe. Furthermore, a comparable number of posts were labelled as 'seeking discussion', compared with 'seeking information'.[4] The interactive design consequently makes SoMe far more difficult to control than conventional media, and especially more so than scientific journals.[3]

The element of lawlessness may deter some from entering into interactions on SoMe. So will fear of trolling.[5] However, if too many healthcare professionals shy away from communicating healthcare research and science on SoMe, low-quality information rapidly takes over. In a study of the top 100 most-watched YouTube videos on erectile dysfunction, 28% of them contained direct misinformation. Misinformation correlated highly with being produced by YouTubers without a medical background, attempting to sell specific products, or promoting alternative medicine.[6]

Engaging with patients improves shared decision-making. Patient empowerment is another important argument for healthcare professionals to engage with online communities of patients: strong patients are engaged patients. The European Liver Patient Organisation (ELPA, @EuropeLiver) is followed by 10,000 on Twitter.

It is a common mistake to think that the interaction should happen directly between 2 individuals. For example, a Social Network Analysis on Twitter showed that oncology professionals communicated far better than professionals within pain management, because their choice of words were aligned with the words of patients, when tweeting about oncology.[7] This resulted in tweets by oncologists being viewed and shared between cancer patients and relatives. In contrast, pain patients were not exposed to tweets by pain physicians to the same extent, because they used different words to describe pain research and management: only 3 of patients' top 10 word pairs was repeated in pain physicians' top 10 (healthcare, mental health, and long term). Although patients used 'chronic pain' as their top word pair, it was only in 21st place for the pain professionals.

A SoMe strategy for promoting research

Although interactions between patients and hepatologists may not yet have reached its full potential, many clinical hepatologists have grasped the opportunity of SoMe to interact with peers for sharing cases, experience, and new knowledge; most notably under the hashtag #LiverTwitter.[8] For this purpose, Twitter is the SoMe place to be. In contrast, Instagram holds only 13,000 posts for the hashtag #hepatology, a bizarre mix of dodgy congresses, nurse and doctor selfies, and advertisements for alternative medicine.

SoMe are also excellent to advertise your own research.[9] In a randomised Twitter trial of 536 cardiology articles, promotion on SoMe led to a 1.43 higher rate of citations after 328 days compared with no promotion, independent of the type of article. In both randomisation arms, Altmetric score and number of users tweeting correlated positively with the number of citations.[10]

A Twitter trial from the *Journal of Hepatology* highlights the need for innovative thinking when disseminating research on SoMe. Emotions and storytelling also resonate with professionals, not just patients.[11] The journal randomised 54 newly accepted articles to either a standard tweet (graphical abstract and brief text) or 2 personalised, connected tweets, containing the corresponding authors' personal motivation for doing the study, followed by main results. The personalised tweets gathered twice the number of article downloads (median 51 *vs.* 25, $p = 0.002$) in 28 days, but a comparable number of retweets and likes.

How to use SoMe

There is an obvious need for healthcare professionals to make an effort communicating medicine and research on SoMe, to curb the poor quality, false information that everyone is also exposed to. Trust in health organisations and professionals are high, so using SoMe is an opportunity for you to spread knowledge, ideas and results to a very, very wide audience.

One note of caution: healthcare professionals should guard patient privacy and confidentiality, in real life and online. In a study of the hashtag #ShareAStoryInOneTweet, it was estimated that friends or family could likely identify the clinical scenario described in 32% of the tweets by doctors or nurses.[12] It should be obvious that one must never share potentially identifiable data on SoMe.

Professionals may need to re-learn how to disseminate science, when adding SoMe to standard scientific formats. Here are some personal experiences on how to get the best out of social media:

Be generous. Share, like, comment, question, interact. Do not limit yourself to sharing only your own research, or your own very narrow field of expertise.

Get to know your preferred SoMe channels. They differ very much on how algorithms work, type of formats, and user demography.

Journalistic tricks of the trade work far better than disseminating within the dogma of scientific reporting: begin with the conclusion/most important result, use storytelling, avoid being too abstract, use metaphors or examples.

Use your emotions. Users want to connect with real persons, who are passionate, and engaged, not a bot who only spits out facts.

Use visuals. Visual abstracts work better than key figure tweets which again work better than tweets with just a link.

Use humour, if you are actually fun and comfortable with it.

Open Access is best when linking to your own or other's results. Being met by a pay wall can be frustrating for users.

The art of communicating on SoMe can be challenging for some experienced researchers and health professionals. Consider getting help from team members who 'speak SoMe' more fluently.

When your peers and your patients all use social media, why should you not use them?

References

1. Chen J, Wang Y. Social media use for health purposes: systematic review. J Med Internet Res 2021;23:e17917.
2. Mheidly N, Fares J. Leveraging media and health communication strategies to overcome the COVID-19 infodemic. J Public Health Policy 2020;41:410–20.
3. Merchant RM, Asch DA. Protecting the value of medical science in the age of social media and "Fake News". JAMA 2018;320:2415–6.
4. Lai D, Wang D, Calvano J, Raja AS, He S. Addressing immediate public coronavirus (COVID-19) concerns through social media: utilizing Reddit's AMA as a framework for Public Engagement with Science. PLoS One 2020;15:e0240326.
5. Jamison AM, Broniatowski DA, Quinn SC. Malicious actors on twitter: a guide for public health researchers. Am J Public Health 2019;109:688–92.
6. Fode M, Nolsøe AB, Jacobsen FM, Russo GI, Østergren PB, Jensen CFS, *et al*. Quality of information in YouTube videos on erectile dysfunction. Sex Med 2020;8:408–13.
7. Kloth YM, Deutsch KM, Danielson KA, Strack J, Law C. What Twitter teaches us about patient-provider communication on pain. PLoS One 2019;14:e0226321.
8. Mikolajczyk AE, Ufere N, Breu AC, Parikh ND, Garcia-Tsao G, Tapper EB. #LiverTwitter: an emerging tool for liver education and research. Hepatol Commun 2020;4:1229–33.
9. Alasnag M, Ahmed W, Mamas M. Best practices of social media for the clinician. Curr Cardiol Rev 2021;17:118–21.
10. Ladeiras-Lopes R, Clarke S, Vidal-Perez R, Alexander M, Lüscher TF. Twitter promotion predicts citation rates of cardiovascular articles: a preliminary analysis from the ESC Journals Randomized Study. Eur Heart J 2020;41:3222–5.
11. Tapper EB, Mirabella R, Walicki JJ, Banales JM. Optimizing the use of twitter for research dissemination: the "Three Facts and a Story" randomized-controlled trial. J Hepatol 2021;75:271–4.
12. Ahmed W, Jagsi R, Gutheil TG, Katz MS. Public disclosure on social media of identifiable patient information by health professionals: content analysis of Twitter data. J Med Internet Res 2020;22:e19746.

E-health: Optimising remote management of patients with liver disease

Tiffany Wu and Vijay H. Shah

Division of Gastroenterology and Hepatology, Mayo Clinic, Rochester, MN, *USA*

E-mail addresses: Wu.Tiffany@mayo.edu and Shah.Vijay@mayo.edu

Disclaimer: This syllabus was derived from our recent review article: Wu *et al.* The digital transformation of hepatology: the patient is logged in. Hepatology 2022;75:724–39.

Take-home messages

- The rise in innovative digital health technologies has led a paradigm shift in health care toward personalised, patient-centric medicine that is reaching beyond traditional brick-and-mortar facilities into patients' homes and everyday lives.
- Novel digital solutions can monitor and detect early changes in physiological data, predict disease progression and health-related outcomes based on individual risk factors, and manage disease intervention with a range of accessible telemedicine and mobile health options.
- We discuss the unique transformation underway in the care of patients with liver disease, specifically examining the digital transformation of diagnostics, prediction and clinical decision-making, and management.
- Additionally, we discuss the general considerations needed to confirm validity and oversight of new technologies, usability and acceptability of digital solutions, and equity and inclusivity of vulnerable populations.

Introduction

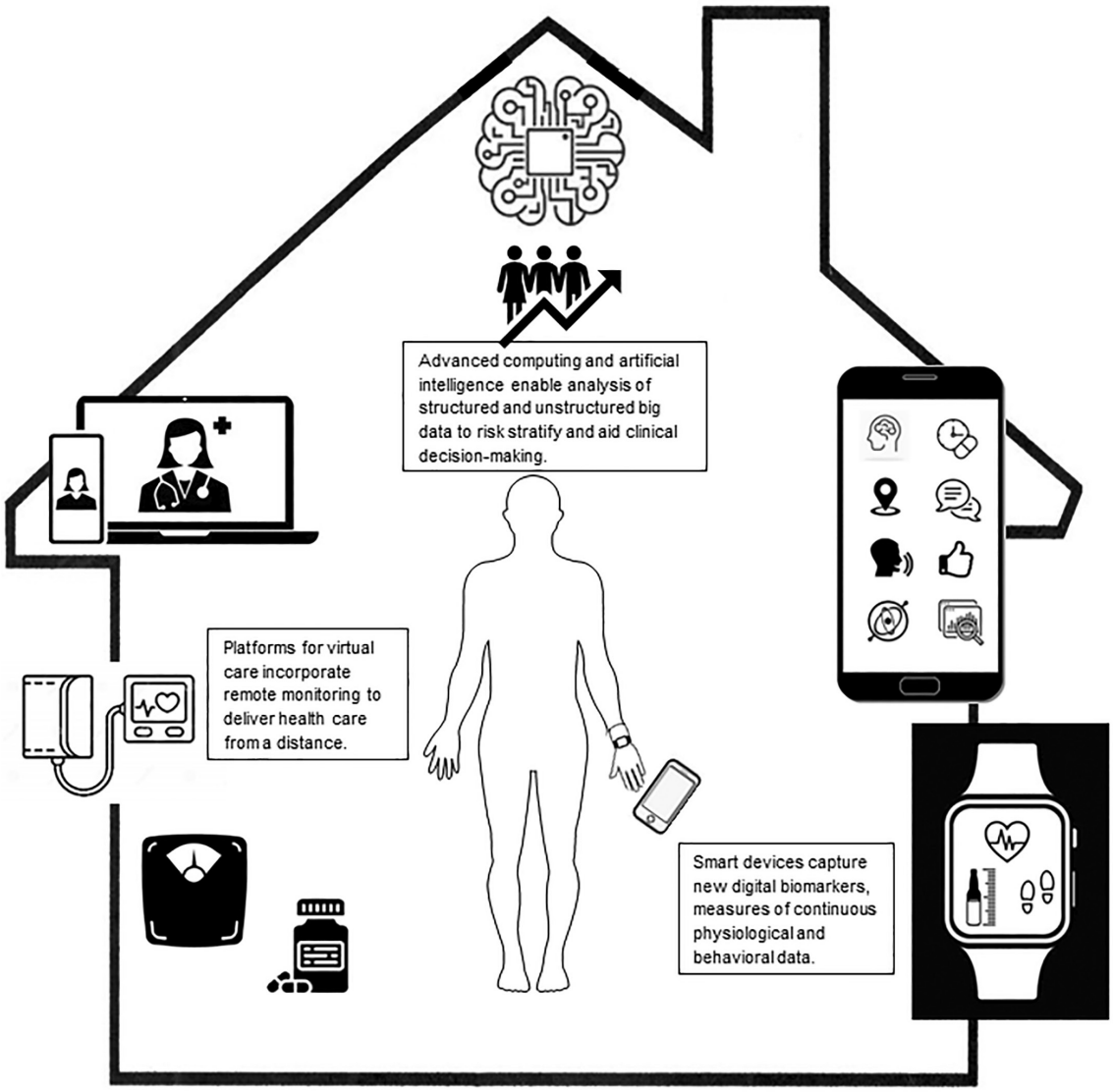
The rise in innovative digital health technologies has led a paradigm shift in health care toward personalised, patient-centric medicine that is reaching beyond traditional brick-and-mortar facilities into patients' homes and everyday lives (Fig. 1).[1] Novel digital solutions can monitor and detect early changes in physiological data, predict disease progression and health-related outcomes based on individual risk factors, and manage disease intervention with a range of accessible telemedicine and mobile health options.

Wearables and remote monitoring

The opportunity

Smartphones and wearable devices are equipped with a myriad of sensors that enable continuous measurement of individual behaviour and physiology beyond the confines of the clinical environment. These digital biomarkers have been captured for energy expenditure, cardiac arrhythmias, blood pressure, gait, and more[2–5] with adequate accuracy and predictive value of disease states or events.[6] In addition to such data generated by passive ecological sensing, smartphones also collect data requiring active engagement by answering questions or prompts on mobile devices that can be personalised for more effective use and interpretation.[7] This collective volume and variety of behavioural data from smartphones has been defined by John Torous and colleagues as digital phenotypes, 'the moment-by-moment quantification of the individual-level human phenotype *in-situ* using data from personal

Fig. 1. The digital transformation of healthcare defines an innovative model of care delivery for patients with liver disease.



devices'.[8] Digital phenotyping has been used to understand mechanisms of disease, predict outcomes of interest, and automate detection of behavioural states such as stress and depressed mood[9,10] In liver disease, application of digital biomarkers and phenotyping can be used to monitor patients remotely to diagnose clinical disease states, identify imminent complications, and guide early intervention to improve health outcomes.

Existing solutions

The immense opportunities provided by remote sensors and wearable devices have served as a unique starting point for improved diagnostics. Health monitoring outside of the clinical environment has been implemented as remote patient monitoring (RPM) systems over the years. Early use cases of RPM in liver disease were embodied by simple automated telephone monitoring. Use of interactive voice response calls to assess for symptoms such as presence of jaundice, abdominal swelling, weakness, need for paracentesis, medication changes, or weight changes were used as potential predictors of

readmission. In this context, calls indicating weakness and weight gain ≥5 pounds were associated with increased risk of hospitalisation.[11] Similarly, implementation of an outpatient telephonic transitional care strategy by a care coordinator for 30 days after hospital discharge had higher 6-month survival among patients with cirrhosis.[12] Such interventions using information technology and communications opened the door to patient monitoring at a distance.

The rise in mobile phone applications and tablets heralded the next generation of RPM. For instance, the smartphone app, Patient Buddy, was evaluated among a multi-centre cohort of patients and caregivers to evaluate feasibility and impact on 30-day readmission. Ganapathy *et al.* demonstrated app engagement and education of users on medication adherence, daily sodium intake, weight management, and cirrhosis-related signs and symptoms. Through daily monitoring of patient data by a clinical care team and the use of automated alerts regarding adherence and critical values, the app was able to prevent potential hepatic encephalopathy-related admissions and facilitate early outpatient intervention.[13] Another home monitoring strategy using 4G tablets with wireless devices to monitor vital signs, symptoms, and medication adherence to enable early intervention with phone or video visits at signs of distress also demonstrated efficacy in preventing cirrhosis-related readmissions up to 90 days.[14] In addition to preventing readmissions, home monitoring, and use of video-based educational programmes demonstrated feasibility in perioperative management of liver transplant recipients, with enhanced monitoring of postoperative vital signs. In a pilot study, programme implementation demonstrated efficacy in improving patient and caregiver understanding of postoperative care management.[15] The investigative team followed this with a randomised controlled trial comparing the home monitoring strategy to standard of care (no home monitoring) after liver transplant. The home monitoring strategy utilised electronic tablet and Bluetooth devices to support daily text messages, educational videos, and FaceTime video capability with care team providers. Adherence to digital devices was >85% for vital sign recording, and the home monitoring group showed significantly lower 90-day readmission rates as well as markers of improved quality of life compared with standard of care.[16]

Digital technologies have also been used as point-of-care testing among patients with cirrhosis. For example, the smartphone app called EncephalApp is readily available on app stores for point-of-care testing of covert hepatic encephalopathy. The app has demonstrated good face validity, external validity, and test-retest reliability in various cohorts.[17] When evaluated as a screening tool for covert hepatic encephalopathy in an independent sample of patients, the app also showed high sensitivity (86%) with overall high ratings in accessibility, convenience, and acceptability.[18] Recently, promising findings by Bloom *et al.* correlating features of human speech with neuropsychiatric scores found that history of overt hepatic encephalopathy was associated with slower speech and longer word duration.[19] These results signal the potential for application of speech biomarkers to new forms of diagnostic testing of hepatic encephalopathy among patients with cirrhosis.

The rise in wearable devices has further expanded remote monitoring into behavioural metrics such as physical activity and mental health conditions. In a study using a large sample of 96,688 prospectively recruited participants from the UK Biobank, physical activity as recorded by a wrist accelerometer showed that an additional 2,500 steps per day was associated with reduced overall risk of chronic liver disease and non-alcoholic fatty liver disease (NAFLD) development. Among patients with previously diagnosed liver disease, increased physical activity was also associated with reduced risk of disease progression and mortality.[20] Current studies in development are also exploring digital phenotypes of addiction in alcohol use disorder, which will be vital to diagnosing clinical disease and complications of chronic alcohol-associated liver disease. Existing literature in this field has focused on the young population as it relates to location-related risk factors of alcohol exposure,[21,22] and the prediction of alcohol consumption based on sensor data in a healthy population.[23] Given the critical

role of alcohol consumption in progression of liver disease, understanding the patterns of behaviour associated with increased relapse risk will provide unique opportunities to apply early interventions in treatment and prevention of alcohol use in liver disease. Similarly, digital phenotypes may be explored in other disease groups such as NAFLD to better identify environment or lifestyle factors contributing to disease progression.

Potential limitations

Although technology allows for the capture of new biomarkers that augment traditional provider-collected or patient-reported measures, we have limited understanding of which biomarkers are clinically appropriate or practical. More research is needed to understand technical test performance measures and decision frameworks in realistic scenarios for clinical use.[24] Additionally, digital health data may not easily translate to desirable outcome measures, nor be recorded and validated in a cost-effective manner.[25] Depending on the outcome measure, biosensors that are expensive or rely on a cultural shift in approach will be challenging to use. Finally, data from RPM and mobile apps will need to integrate with existing workflows and clinical platforms. Current electronic health record systems already burdened with data overload may not be equipped to merge with diverse technologies, or at least will require time-intensive and costly operative changes to adapt to new devices and applications.[25] Multimodal data generation although powerful, could add a human cost in increased time and effort for the clinician to sift through and interpret the influx of data if not managed properly. Ultimately, the widespread adoption of digital health technologies will require acceptance and approval by all stakeholders involved. From patient willingness to generate and share health data to provider comfort with learning how to incorporate such data into care plans, issues related to quality and acceptability of digital diagnostics will act as barriers to implementation.[24]

Telehealth and patient portal

The opportunity

The management of liver disease requires a multifaceted approach suitable for application of digital health technologies such as telehealth and mobile health interventions. For patients with cirrhosis, quality care metrics in disease management such as medication titration, lab monitoring, vaccine administration, or scheduling of imaging or procedures,[26] may be difficult to access in rural or underserved areas with a shortage of hepatology specialists, healthcare facilities, and resources.[27] Hepatic decompensation or development of complications often require referral to transplant hepatologists, surgeons, and other medical specialists for multidisciplinary management, which may be only available hundreds of miles away. Digital health solutions such as telemedicine (the use of telecommunications and information technology to deliver healthcare services at a distance) and mobile apps have dramatically transformed healthcare and have the ability to close existing gaps in quality of care.[27] Digital transformation of healthcare also provides opportunities to improve treatment of liver disease, facilitate access and participation in clinical trials, and advance virtual care and acute hospital care models at home.

Existing solutions

Telemedicine, in the form of teleconsultation and televisits, has been well cited in the management of chronic liver disease.[27] Early use scenarios outlined the application of teleconferencing for treatment of hepatitis C virus (HCV) in rural or underserved areas.[28,29] Beyond remote visits between patients and providers, digital technologies also facilitated provider-to-provider consultation and collaboration. One of the most established interventions was launched through the Veterans Health Administration

(VHA) in 2010. Initially designed as a care model to improve access to multidisciplinary treatment for patients with HCV in rural areas of New Mexico, the Extension for Community Healthcare Outcomes (ECHO) programme engaged primary care providers with specialists for team-based interdisciplinary education over best practices and case-based learning.[30] Evaluation of the ECHO program showed improvement in provider knowledge, satisfaction, and self-efficacy in clinical practice, building sustainable and effective local community practices for underserved populations.[30] Given the success of the ECHO program, the VHA implemented the Specialty Care Access Network-ECHO (SCAN-ECHO) to improve access to expert consultation in many chronic conditions. Specifically, the SCAN-ECHO virtual care model was studied among a regional cohort of patients with liver disease from the Ann Arbor VA, where the cohort using the virtual care consult showed improved propensity-adjusted mortality rate (HR 0.54) compared with no visit.[31]

Among care for patients with end-stage liver disease, SCAN-ECHO has also been used to facilitate case-based distance learning with real-time consultation between primary care providers and hepatologists to triage evaluations for liver transplantation (LTx).[32] The Richmond VA liver transplant program evaluated the utility of SCAN-ECHO in the referral process. Between August 2012 and September 2016, 91 patients were referred for LTx through this virtual care model. Compared with patients directly referred, patients in the SCAN-ECHO group were less likely to be deemed non-candidates at time of referral or after completion of the full work-up, and findings showed that telehealth-based triage had the potential to minimise futile testing and reduce costs.[32] From the same centre, John *et al.* analysed the effect of telehealth compared with usual care regarding wait-list time and transitioning through the care process from referral to evaluation, listing, and transplantation.[33] Patients evaluated via telehealth had reduced time from referral to evaluation and listing, especially among patients with low model for end-stage liver disease (MELD) scores, as well as shorter time on the wait list (138.8 *vs.* 249 days, $p < 0.01$). There was no association between telehealth use and time to transplantation or pretransplant mortality.[33] In the LTx care process, telemedicine has additionally been used to streamline surgical management, including remote transmission and evaluation of radiographic and histopathologic images of liver grafts to aid in transplant planning and improve chances for operative success.[34,35]

Pilot studies using mobile health apps have shown feasibility and usability of interventions to address medication and behavioural management in chronic liver disease. For patients with cirrhotic ascites, Bloom *et al.* determined feasibility of a smartphone app connected to a Bluetooth-enabled scale to facilitate outpatient management.[36] In this study, the device successfully transmitted weight data to participants' electronic health record during 71% of enrolment days, with good provider and patient engagement. Of delivered electronic alerts, providers responded to 84% and intervened in response to 57% of alerts, enabling early therapeutic intervention for ascites management.[36] Mobile apps have also been used for facilitating weight loss interventions among patients with NAFLD. Investigators in Singapore developed the Nutritionist Buddy (nBuddy) mobile app to track diet and physical activity, along with providing behavioural interventions based on real-time feedback, educational videos, and peer support.[37] Participants randomised to the nBuddy intervention program had a 5-fold higher chance of achieving ≥5% weight loss after 6 months, along with improvements in surrogate outcomes including waist circumference, systolic and diastolic blood pressure, and liver enzyme values.[37] Recently, Duarte-Rojo *et al.* presented findings of another mobile app developed for exercise training in end-stage liver disease and prehabilitation for liver transplantation.[38] The Exercise and Liver FITness (EL-FIT) app, paired with data from a physical activity tracker, was tested for feasibility, accuracy of

data transfer, and usability among participants. Based on tracker data, the EL-FIT app assigned a level of training for the participant, which was concordant with recommended levels by a physical therapist in 89% of cases. The majority of participants were able to use the app and engage with app features, such as watching educational videos, reporting measures such as perceived exertion, and surpassing recommended activity goals. The app design validated step count, recording and processing of patient data, and achievement of clinical endpoints related to physical status. Thirty-five percent of participants increased their physical performance, and this study demonstrated potential for using mobile health apps as digital therapeutics in management of liver disease.[38]

Potential limitations

Over the past several years, the exponential boom of mobile health apps has created an overcrowded market, difficult for patients and providers alike to identify the digital solutions most effective for improving targeted health outcomes. Barriers to widespread acceptance include the paucity of rigorous evidence to determine clinical effectiveness and validate meaningful improvement in health outcomes using high quality methodology. High quality studies using pragmatic trials or implementation science are needed to better understand how technology may facilitate management of liver disease.[39] To effectively utilise technology to improve quality, access, and delivery of care, we need to address certain considerations to ensure digital platforms are complementary, rather than disruptive, to patient care.

References

1. Wu T, Simonetto DA, Halamka JD, Shah VH. The digital transformation of hepatology: the patient is logged in. Hepatology 2022;75:724–39.
2. Shcherbina A, Mattsson CM, Waggott D, Salisbury H, Christle JW, Hastie T, *et al.* Accuracy in wrist-worn, sensor-based measurements of heart rate and energy expenditure in a diverse cohort. J Pers Med 2017;7:3.
3. Nachman D, Gilan A, Goldstein N, Constantini K, Littman R, Eisenkraft A, *et al.* 24-hour ambulatory blood pressure measurement using a novel non-invasive, cuff-less, wireless device. Am J Hypertens 2021; in press, doi:10.1093/ajh/hpab095
4. García-Villamil G, Neira-Álvarez M, Huertas-Hoyas E, Ramón-Jiménez A, Rodríguez-Sánchez C. A pilot study to validate a wearable inertial sensor for gait assessment in older adults with falls. Sensors (Basel) 2021;21:4334.
5. Nemati S, Ghassemi MM, Ambai V, Isakadze N, Levantsevych O, Shah A, *et al.* Monitoring and detecting atrial fibrillation using wearable technology. Annu Int Conf IEEE Eng Med Biol Soc 2016;2016:3394–7.
6. Quer G, Radin JM, Gadaleta M, Baca-Motes K, Ariniello L, Ramos E, *et al.* Wearable sensor data and self-reported symptoms for COVID-19 detection. Nat Med 2021;27:73–7.
7. Torous J, Staples P, Onnela JP. Realizing the potential of mobile mental health: new methods for new data in psychiatry. Curr Psychiatry Rep 2015;17:602.
8. Torous J, Kiang MV, Lorme J, Onnela JP. New tools for new research in psychiatry: a scalable and customizable platform to empower data driven smartphone research. JMIR Ment Health 2016;3:e16.

9. Smets E, Velazquez ER, Schiavone G, Chakroun I, D'Hondt E, De Raedt W, *et al.* Large-scale wearable data reveal digital phenotypes for daily-life stress detection. NPJ Digit Med 2018;1:67.

10. Burns MN, Begale M, Duffecy J, Gergle D, Karr CJ, Giangrande E, *et al.* Harnessing context sensing to develop a mobile intervention for depression. J Med Internet Res 2011;13:e55.

11. Thomson M, Volk M, Kim HM, Piette JD. An automated telephone monitoring system to identify patients with cirrhosis at risk of re-hospitalization. Dig Dis Sci 2015;60:3563–9.

12. Rao BB, Sobotka A, Lopez R, Romero-Marrero C, Carey W. Outpatient telephonic transitional care after hospital discharge improves survival in cirrhotic patients. World J Hepatol 2019;11:646–55.

13. Ganapathy D, Acharya C, Lachar J, Patidar K, Sterling RK, White MB, *et al.* The patient buddy app can potentially prevent hepatic encephalopathy-related readmissions. Liver Int 2017;37:1843–51.

14. Khungar V, Serper M, Peyton D, Mehta S, Norris A, Huffenberger A, *et al.* Use of an innovative telehealth platform to reduce readmissions and enable patient-centered care in cirrhotic patients. Hepatology 2017;66:94A–5A.

15. Ertel AE, Kaiser TE, Abbott DE, Shah SA. Use of video-based education and tele-health home monitoring after liver transplantation: results of a novel pilot study. Surgery 2016;160:869–76.

16. Lee TC, Kaiser TE, Alloway R, Woodle ES, Edwards MJ, Shah SA. Telemedicine based remote home monitoring after liver transplantation: results of a randomized prospective trial. Ann Surg 2019;270:564–72.

17. Bajaj JS, Heuman DM, Sterling RK, Sanyal AJ, Siddiqui M, Matherly S, *et al.* Validation of EncephalApp, smartphone-based Stroop Test, for the diagnosis of covert hepatic encephalopathy. Clin Gastroenterol Hepatol 2015;13:1828–35.e1.

18. Zeng X, Li XX, Shi PM, Zhang YY, Song Y, Liu Q, *et al*. Utility of the EncephalApp Stroop Test for covert hepatic encephalopathy screening in Chinese cirrhotic patients. J Gastroenterol Hepatol 2019;34:1843–50.

19. Bloom PP, Robin J, Xu M, Arvind A, Daidone M, Gupta AS, *et al.* Hepatic encephalopathy is associated with slow speech on objective assessment. Am J Gastroenterol 2021;116:1950–53.

20. Schneider CV, Zandvakili I, Thaiss CA, Schneider KM. Physical activity is associated with reduced risk of liver disease in the prospective UK Biobank cohort. JHEP Rep 2021;3:100263.

21. Byrnes HF, Miller BA, Morrison CN, Wiebe DJ, Remer LG, Wiehe SE. Brief report: using global positioning system (GPS) enabled cell phones to examine adolescent travel patterns and time in proximity to alcohol outlets. J Adolesc 2016;50:65–8.

22. Byrnes HF, Miller BA, Wiebe DJ, Morrison CN, Remer LG, Wiehe SE. Tracking adolescents with global positioning system-enabled cell phones to study contextual exposures and alcohol and marijuana use: a pilot study. J Adolesc Health 2015;57:245–7.

23. Bae S, Chung T, Ferreira D, Dey AK, Suffoletto B. Mobile phone sensors and supervised machine learning to identify alcohol use events in young adults: implications for just-in-time adaptive interventions. Addict Behav 2018;83:42–7.

24. Huckvale K, Venkatesh S, Christensen H. Toward clinical digital phenotyping: a timely opportunity to consider purpose, quality, and safety. NPJ Digit Med 2019;2:88.

25. Cohen AB, Mathews SC. The Digital Outcome Measure. Digit Biomark 2018;2:94–105.

26. Kanwal F, Tapper EB, Ho C, Asrani SK, Ovchinsky N, Poterucha J, *et al.* Development of quality measures in cirrhosis by the Practice Metrics Committee of the American Association for the Study of Liver Diseases. Hepatology 2019;69:1787–97.

27. Stotts MJ, Grischkan JA, Khungar V. Improving cirrhosis care: the potential for telemedicine and mobile health technologies. World J Gastroenterol 2019;25:3849–56.

28. Rossaro L, Aoki C, Yuk J, Prosser C, Goforth J, Martinez F. The evaluation of patients with hepatitis C living in rural California via telemedicine. Telemed J E Health 2008;14:1127–9.

29. Cooper CL, Hatashita H, Corsi DJ, Parmar P, Corrin R, Garber G. Direct-acting antiviral therapy outcomes in Canadian chronic hepatitis C telemedicine patients. Ann Hepatol 2017;16:874–80.

30. Arora S, Kalishman S, Thornton K, Dion D, Murata G, Deming P, Parish B, *et al.* Expanding access to hepatitis C virus treatment – Extension for Community Healthcare Outcomes (ECHO) project: disruptive innovation in specialty care. Hepatology 2010;52:1124–33.

31. Su GL, Glass L, Tapper EB, Van T, Waljee AK, Sales AE. Virtual consultations through the veterans administration SCAN-ECHO project improves survival for veterans with liver disease. Hepatology 2018;68:2317–24.

32. Konjeti VR, Heuman D, Bajaj JS, Gilles HC, Fuchs M, Tarkington P, *et al.* Telehealth-based evaluation identifies patients who are not candidates for liver transplantation. Clin Gastroenterol Hepatol 2019;17:207–9.e1.

33. John BV, Love E, Dahman B, Kurbanova N, Konjeti VR, Sundaram LT, *et al.* Use of telehealth expedites evaluation and listing of patients referred for liver transplantation. Clin Gastroenterol Hepatol 2020;18:1822–30.e4.

34. Bhati CS, Wigmore SJ, Reddy S, Mayer DA, Buckels JAC, Derek M, *et al.* Web-based image transmission: a novel approach to aid communication in split liver transplantation. Clin Transplant 2010;24:98–103.

35. Mammas CS, Lazaris A, Geropoulos S, Saatsakis G, Lemonidou C, Patsouris E. Telemedicine systems in organ transplantation: a feasibility and reliability study of the integrated teleradiological and tele-pathological evaluation of liver graft. Stud Health Technol Inform 2013;190:285–7.

36. Bloom P, Wang T, Marx M, Tagerman M, Green B, Arvind A, *et al.* A smartphone app to manage cirrhotic ascites among outpatients: feasibility study. JMIR Med Inform 2020;8:e17770.

37. Lim SL, Johal J, Ong KW, Han CY, Chan YH, Lee YM, *et al.* Lifestyle intervention enabled by mobile technology on weight loss in patients with nonalcoholic fatty liver disease: randomized controlled trial. JMIR Mhealth Uhealth 2020;8:e14802.

38. Duarte-Rojo A, Bloomer PM, Rogers RJ, Hassan MA, Dunn MA, Tevar AD, *et al.* Introducing EL-FIT (Exercise and Liver FITness): a smartphone app to prehabilitate and monitor liver transplant candidates. Liver Transpl 2021;27:502–12.

39. Serper M, Volk ML. Current and future applications of telemedicine to optimize the delivery of care in chronic liver disease. Clin Gastroenterol Hepatol 2018;16:157–61.e8.

Translating AI into better medical care: the example of medical imaging

Anima Anandkumar

Computing + Mathematical Sciences; California Institute of Technology, Passadena, California, *USA*

E-mail address: arangelf@caltech.edu

Summary

AI is making in-roads into many facets of healthcare. Among them, medical imaging has seen some of the most impressive progress recently. Core machine-learning techniques that will be essential to make AI-based medical imaging accurate and trustworthy will be reviewed. Examples presented include recent work on recognizing gestures in robotic surgery videos using deep learning and being able to assess skills of surgeons. Additionally, a comprehensive platform for AI-assisted annotations in many modalities, including CT, MRI, x-ray, and ultrasound, called NVIDIA Clara is highlighted. This platform also supports efficient AI training on GPUs including privacy-preserving federated learning and transfer learning.

EASL™

Join our dynamic & forward-thinking community

+ Best education
+ Journal of Hepatology
+ Best research
+ JHEP Reports
+ Reduced fees for events

JOIN OUR COMMUNITY

EASL™ QUIZ

+ Testing your guidelines knowledge since June 2020
+ Sharpening your skills

TEST YOUR KNOWLEDGE

Earn up to two badges with gamification on EASL Campus

EASL™ JOURNAL CLUB

SCAN FOR MORE INFO

+ Hear directly from authors and experts in the field
+ Get answers to your questions
+ Take part every month

EASL™ STUDIO

Your weekly hepatology broadcast news

Every Wednesday 18:00 CET

Topics:

+ JHEP Live
+ YIs Choice
+ Nurses & AHPs Focus
+ and more...

WATCH ON DEMAND

JOURNAL OF HEPATOLOGY

The Home of Liver Research

EASL™
The Home of Hepatology

+ Premium hepatology journal
+ International Editorial team led by Prof. Paolo Angeli
+ 2020 Impact Factor: 25.1

Submit your article now

SCAN FOR MORE INFO

EASL™
The Home of Hepatology

FUTURE EVENTS 2022–2023

2022

1–2	July	EASL School	London, UK
1–2	July	EASL School	Freiburg, Germany
15–17	September	EASL NAFLD Summit	Dublin, Ireland
15–17	September	EASL School	London, UK
10–11	December	AASLD-EASL Masterclass	Savannah, USA

2023

20–22	April	EASL Liver Cancer Summit	Estoril, Portugal
12–13	May	Monothematic Biliary Fibrosis	Florence, Italy
TBD	May	AASLD-EASL Endpoints	USA
21–25	**June**	**EASL Congress 2023**	**Vienna, Austria**
Summer		EASL Schools	Europe
7–9	September	EASL NAFLD Summit	Europe
19–21	October	EASL-AASLD Endpoints	Brussels, Belgium
30 Nov. – 2 Dec.		EASL-AASLD Masterclass	Madrid, Spain

SEE OUR CALENDAR

EASL™
The Home of Hepatology

easl.eu/pgc2022